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Application of bioactive peptides of animal origin with antihypertensive effect in processed meat products

Supevisors:
Ph.D. Lene Meinert &
Associate Prof. Michael Bom Frøst

Danish Meat Research Institute

Master Project in Gastronomy & Health
By Karen Marie Tøstesen
GSK09008
Department of Food Science
Faculty of Life Sciences,
University of Copenhagen
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Abstract

An increasing average age in the western world has resulted in higher prevalence of hypertension among the population. Traditionally problems with hypertension is alleviated by synthetic inhibitors of the angiotensin-I converting enzyme (ACE). However various side effects have been connected to these products. Hence natural occurring ACE inhibition from small peptides is desirable for functional foods.

Present study investigates four hydrolysates of animal origin HPP11, HBG, HPG1.1 and HPL, for their overall potential as agents for functional foods, by applying them into four different meat products from the Danish cuisine covering several preparation methods.

By assaying the ability of meat hydrolysates to inhibit ACE activity both as pure hydrolysates and as a part of a meat product containing 8 % hydrolysate, it was revealed that the hydrolysates was capable of inhibiting ACE. Both in pure form and as food additive in one of the four products.

Flavoring properties of meat hydrolysates when used as additives in functional meat products, was assessed by a descriptive analysis by a trained panel, as well as a regular Danish consumers (n=170). The products' containing the hydrolysates was rated with prominent bitter and off modalities by the trained panel, when compared with their respective reference product. However, when the hydrolysates is added to liver pate or wiener sausages, they are not perceived as significantly different by the consumers (*n*=170).

The attitude towards functional meat products of Danish consumers was investigated by analyzing data from two questionnaire surveys (n=1499 and n=157). The Danish consumers have a notable tendency, which divides them into three groups: i) The health and environmental concerned, which see themselves as healthy. ii) Consumers who to some extend accept additives and show interest in functional foods and iii) Consumers who compromise on taste for health and believe functional foods are healthy. Here the key finding is that forty percent of the Danish consumers are willing to compromise on taste for health if they can feel that the functional product has a beneficial effect.

When evaluating the meat hydrolysates as food additives for functional foods according to the EFSA procedure, the possibility of receiving a health claim on the basis of present evidence, it is not adequate to establish a health claim.

Resume

En stigende gennemsnitsalder i den vestlige verden har resulteret i en højere forekomst af forhøjet blodtryk blandt befolkningen. Almindeligt er forhøjet blodtryk afhjulpet af syntetiske inhibitorer rettet imod det angiotensin-I konverterende enzym (ACE). Desværre er disse produkter forbundet med en række bivirkninger, hvorfor naturligt forekommende ACE-hæmmere er blevet aktuelle som ingrediener til funktionelle fødevarer.

Dette speciale undersøger de fire hydrolysater HPP11, HBG, HPG1.1 og HPL, der alle er af animalsk oprindelse. Herved vil hydrolysaternes potentiale som bioaktive komponenter til funktionelle fødevarer undersøges ved at afprøve dem i fire forskellige traditionelle danske kødprodukter, der tilsammen omfatter adskillige tilberedningsmetoder. Hydrolysaternes evne til at inhibere ACE-aktiviten, blev undersøgt som både rent hydrolysat og tilsat i fire forskellige kødprodukter, svarende til 8 % af den totale masse. Det blev fundet, at hydrolysaterne er i stand til at inhibere ACE både som rent hydrolysat eller som et tilsætningsstof i et af de fire produkter.

Hydrolysaternes smagsgivende egenskaber er undersøgt ved hjælp af en deskriptiv analyse udført af et trænet panel, såvel som af danske forbrugere (n = 170). Resultaterne fra den deskriptive analyse, at produkter indeholdende hydrolysat havde en bitter samt afvigende smag sammenlignet med deres respektive referenceprodukt. De danske forbrugere opfattede dog ikke leverpostej eller wiener pølser med tilsat hydrolysat som markant anderledes i forhold til reference produkterne (n = 170).
De danske forbrugeres holdning til funktionelle kødprodukter blev undersøgt ved at analysere data fra to spørgeskemaundersøgelser (n = 1499 og n = 157). Hvorved det blev bestemt at de danske forbrugere kan inddeles i tre grupper: i) Forbrugere der er sundheds- og miljø orienteret og betragter sig selv som sunde. ii) Forbrugere, der til en vis grad acceptere tilsætningsstoffer og viser interesse for funktionelle fødevarer og iii) Forbrugere, der er villige til at gå på kompromis med smagen for sundhed og mener at funktionelle fødevarer er sunde. Det mest væsentlige resultat viser at 40 % af de danske forbrugere er villige til at gå på kompromis med smag for sundhed, hvis de kan mærke, at produktet har en gavnlig effekt.

Desuden er hydrolysaterne blevet evalueret i henhold til de gældende EFSA procedurer. Ved evalueringen af kød hydrolysaterne som tilsætningsstoffer i funktionelle fødevarer i henhold til EFSAs' procedure, er der på baggrund af den foreliggende data ikke fundet tilstrækkeligt bevis for at modtage et sundhedsanprisning.

Preface

Present thesis (30 ETCS) provides insight in my final work of my Master Science degree in Gastronomy and Health at Faculty of Life Sciences at Copenhagen University (KU) in the period from November 2011 to May 2012. The experimental work has been conducted at Danish Meat Research Institute (DMRI) under supervision from and Lene Meinert (DMRI) and Michael Bom Frøst at the Senory Department at Faculty of Life Sciences (KU)

During the period I had the opportunity to work with very supportive and enthusiastic people. Many have been involved in my study and I am truly thankful to all of them for sharing their great expertise and inspiring guidance. Despite the fact only a few will be mentioned here.

First of all, I would like to thank Lene Meinert (DMRI) and Michael Bom Frøst (KU) for their professional, friendly and sensible supervision through the complete length of this study. It has been important with the inputs and advises from both of them.

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Further, I would also like to thank Dat-Schaub for delivery of hydrolysates investigated in this study, Especially Erik T. Hansen for showing great interest.

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List of abbreviations

ACE	Angiotensin-I converting enzyme
Ang I	Angiotensin I
Ang II	Angiotensin II
ANOVA	Analysis of variance
APLS	ANOVA Partial least square regression
CVD	Cardiovascular diseases
DMRI	Danish Meat Research Institute
DPLS	Discriminant Partial least square regression
DVFA	The Danish Veterinary and Food Administration
EC	European Commission
EFSA	European Food Safety Authority
EV	Expectancy – Value theory
GMO	Genetically Modified Organism's
IC50	Half maximal inhibitory concentration
KU	Københavns Universitet
LPLS	L-shape Partial least square regression
LSD	Least Significance Difference
MAP	Modified atmosphere
NDA	Panel on Dietetic Products, Nutrition and Allergies
PASSCLAIM	*Process for the Assessment of Scientific Support for Claims on Foods*
PLS/PLSR	Partial least square regression
RAS	Rennin-angiotensin system
SAFE	*Selected, Available, Familiar, Exactly as expected*
SHR	Spontaneously hypertensive rats
WHO	World Health Organization

1 Introduction

Hypertension is a growing problem worldwide and a significant contributor to cardiovascular diseases (CVD) (De Leo, Panarese, Gallerani, & Ceci, 2009). CVD are among the most prevalent causes of death and the World Health Organization (WHO) has estimated that in 2020 CVD globally will be the most prevalent cause of death (Murray & Lopez, 1997). Furthermore, hypertension is a growing problem among the Danish population.

Along with the worldwide rise in dietary and lifestyle-related diseases the awareness and demand for foods with a healthy function will likewise increase. Functional foods were in 2006 estimated to represent less than 1 % of the total food and drink market in Europe (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010), but are now considered to be one of the most promising segments within the food industry (Siro, Kapolna, Kapolna, & Lugasi, 2008). From a scientific perspective these new food products containing functional ingredients could be the beginning of a new area between the food and pharmaceutical industry. The innovation of new products needs to follow this food trend but the challenges for innovation of new functional food products are the regulatory requirements. In Europe the European Food Safety Authority (EFSA) strictly regulates the requirements but the market is expected to be more liberate in the future (Bornkessel, Bröring, & Omta, 2011; K. G. Grunert, 2010).

In the last decade studies have shown examples of peptides derived from meat that process certain healthy qualities. Among those an antihypertensive affect have been discovered (Ahhmed & Muguruma, 2010; Escudero, Aristoy, Nishimura, Arihara, & Toldrá, 2012; Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011; Terashima et al., 2010) Protein hydrolysates obtained from low-value products from the slaughterhouse industry have shown to be in possession of this antihypertensive property *in vitro* by inhibition of the angiotensin-I converting enzyme (ACE). Inhibition of ACE with synthetic products is generally used as treatment against hypertension but have unfortunately several side effects (De Leo et al., 2009). In collaboration with the inSPIRe project nr. III-5: *Use of protein hydrolysates obtained from meat by-products as natural and healthy ingredients for food products,* Danish Meat Rescearch Institute (DMRI) requested for an investigation of food products containing hydrolysates and in addition an estimation of how Danish consumers would accept such products. To succeed with new food products on the market the understanding of factors affecting consumer acceptance and their attitude towards new products is pivotal. Since the market failure level for new food products is estimated to be 60-80 % it is a risky and expensive affair for the food industry to develop and innovate new food products (Siegrist, Frewer, & Trijp, 2007).

This illustrates the importance of consumer acceptance and preferences for developing new products. Traditions, habits and role models influence the choice of food where food acceptance is influenced by social norms, perceptions of taste and assimilation of variety therefore it must be SAFE: *Selected, Available, Familiar, Exactly as expected* (Wansink, 2002). Consumer acceptance and their preferences are highly correlated with good taste, which has been revealed to be one of the key issues in many consumer studies. A good taste is strongly associated to positive understandings of high quality food and healthy eating, thereby are less good taste and flavors one of the principal obstacles to consumer acceptance (Graaf, Frewer, & Trijp, 2007; Roininen & Tuorila, 1999; Urala & Lähteenmäki, 2003; Wansink, 2005)(Roininen, Lähteenmäki, & Tuorila, 1999). Other studies have although indicated that people would compromise on good taste when they see themselves within the target group for a product with certain health benefits (Verbeke, 2006).

From a gastronomic and health scientific perspective it is important to take responsibility, meaning conducting and contributing to research in improving the population's health status. Seen from this position, diet is one of the essential factors in order to increase health and prevent diseases. Sensory science is an interdisciplinary approach to understand the human responses to food product properties and can be used to link products with consumers and additionally food chemistry with psychology (M. Martens, 1999). The perception of food involves all sensory modalities; therefore sensory evaluation can be used as a tool to measure consumer perception of products and their characteristics. Another advantage of sensory evaluation is the possibility to generate and extract data, which can be used to predict or interpret consumer acceptance of new products in coherence with attitudes and responses (Tuorila, 2007).

1.1 Present study

In this study I will investigate four different hydrolysates produced from low-value material at Danish Crown slaughterhouses by Dat-Schaub for their overall potential as agents for production of functional foods. To this end, four hydrolysate containing meat products from the traditional Danish cuisine will be developed. The hydrolysates ability to inhibit ACE activity will be measured both as pure hydrolysates but also as part of the four different processed meat products. Furthermore, the application of hydrolysates in food products will be evaluated based on investigations of the attitude among Danish consumers towards functional meat products using a questionnaire. The meat products will be evaluated through a descriptive analysis as well as subjected to consumer sensory tests in order to extract the immediate sensory perception of the products. Finally, the chance of receiving a health claim from EFSA, based on the data available at present, will be evaluated according to the EFSA procedure.

The investigated hydrolysates are:

- DATPRO HPP11 (HPP11) derived from shield bloody pork meat (Batch 201108-21)
- DATPRO HBG (HBG) derived from bovine greaves (Batch 201109-22S)
- DATPRO HPG 1.1 (HPG 1.1) derived from pork greaves (Batch 201110-22S)
- DATPRO HPL-1 (HPL) derived from pork liver (batch 200901-24)

1.2 Study delimitation

The main background variables investigated are gender, age and state of health related to blood pressure. In this study antihypertensive activity will only relate to blood pressure regulating activity through inhibition of Angiotensin I-converting enzyme (ACE).

As perception of food involves all sensory modalities this study has mostly focus on flavor.

The developed product must be seen as prototypes rather than product which are ready for the market. The recipe used are conventional Danish (low-fat), and further product development is not included as a part of this study.

1.2.1 Conceptual clarification

1.2.1.1 Hypertension

Hypertension means that the blood pressure is constantly high, even under relaxing circumstances. High blood pressure is when the pressure in the arteries constantly is above the normal range, which for the systolic pressure means above 140 mmHg and diastolic above 90 mmHg (Hjerteforeningen, 2011).

1.2.1.2 Bioactive compounds

In general nutritional terms a bioactive compound has the capacity to interact with a receptor or a system *in vivo* often in relation to specify that this capacity is beneficial in some way. A dietary bioactive component is defined as: *"Food derived components (genuine or generated) that, in addition to their nutritional value exert a physiological effect in the body*" (Ryan et al., 2011). It has to comply two conditions: "*…brings about a measurable biological effect at a physiologically realistic level.*" And: "*…the bioactivity affects health in a beneficial way*" (De Leo et al., 2009).

1.2.1.3 Bioactive peptides

Bioactive peptides are short polymers usually of 2 to 20 amino acids. They are derived from the primary structure in a protein and they are not active before they are hydrolyzed from the primary protein. The hydrolysis can be generated *in vivo* by several organisms and or enzymatic *in vitro* (Zhang et al., 2010). Bioactive peptides comply with the definition of a bioactive compound (De Leo et al., 2009; Erdmann, Cheung, & Schröder, 2008; Ryan et al., 2011). The hydrolysates in this study are derived from pork and bovine proteins.

1.2.1.4 Functional foods

Various definitions regarding functional foods have been discussed but a formal definition has not yet been recognized. Any food, ingredient or compound with a positive and beneficial bioactivity can be regarded as functional. Functional foods are foods that are specially developed to promote health or prevent diseases. It can involve food in which active ingredients have been added or removed (Fødevarestyrelsen, 2009). Functional foods are: *"foods or food products that have beneficial physiological and/or psychological actions beyond the widely accepted nutritional effects"* (Aggett et al., 2005). In addition, functional foods are described as containing: "*…a component with a selective effect on one or various functions of the organism whose positive effect can be justified as functional (physiological) or even healthy*" (Zhang et al., 2010). A distinction between fortified foods and functional foods can be difficult as a food can be enriched with a nutrient such as a vitamin or a mineral and in some cases, if there is a beneficial effect, the food as will be functional (Fødevarestyrelsen, 17-02-2012).

1.3 Content outline

The first part of this study is a theoretical section dealing with three different aspects of hydrolysates. The first part is a description of protein peptides, hydrolysates and their health benefit in relation to hypertension. The second part provides insight in regulations on food additives and health claims including a description on how EFSA assess the scientifically based evidence is described. The third section deals with some of the theories on food choice, preferences and acceptance, which are important in understanding and evaluating the development of a functional meat product containing meat hydrolysates. The section ends with a description of the consumer's accept of functional foods, which will provide a framework for understanding their acceptance in relation to functional meat product further on in the study. The experimental work and data analysis will be described in details in the material and method section. Results will be discussed related to theory and previous studies in continuation with their presentation. This is chosen to enhance readability between results and discussion, as the collected data are substantial and very diverse. Finally, an overall discussion of the investigated hydrolysates followed by a main conclusion. Perspectives and suggestions for future research will end the study.

2 Background

2.1 The hypertension problem

Hypertension is a growing problem worldwide and a significant contributor to cardiovascular diseases (De Leo et al., 2009). As mentioned before WHO has estimated that cardiovascular diseases will be the most prevalent cause of death globally in 2020 (Murray & Lopez, 1997). It was estimated that 14,9 percent of the world's population had hypertension in 2007, which correspond to an increase of 27 percent within the past two decades and a further increase is expected (Ahhmed & Muguruma, 2010). The incidences in Denmark indicate that hypertension is a growing problem among the Danish population. The recently published index from the Danish Heart Association indicates that 20 percent of the Danish population has hypertension and approximately 250.000 have high blood pressure without knowing it (Hjerteforeningen, 2011).

The main cause of hypertension is still unknown but it has been discovered that ACE has a central role in regulating the blood pressure. However it is also well-known that the elasticity of the arteries wall decrease and the smaller blood vessels become narrower with age, which increase the heart rate (Ahhmed & Muguruma, 2010). It is suggested that environmental and lifestyle factors such as: smoking, alcohol, dieting, obesity, physical inactivity and stress can increase the blood pressure (Ahhmed & Muguruma, 2010; Di Bernardini et al., 2012; Vercruysse, Van Camp, & Smagghe, 2005). An unbalanced diet with a high content of salt and saturated fats are among the essential diet related factors associated with hypertension (Ahhmed & Muguruma, 2010)

Antihypertensive medications with synthetic ACE inhibitors are usually applied in treatment of hypertension. The synthetic ACE inhibitors have been reported to have several side effects such as: hypotension, angioedema, skin rashes, dizziness, tiredness, cough and headache (Di Bernardini et al., 2012), (De Leo et al., 2009). To avoid these side effects food and natural derived ACE inhibitory peptides have a growing interest in order to meet, treat and prevent the growing problem with hypertension and related cardiovascular diseases. Bioactive peptides are typically derived from plant, milk and animal proteins. This is the start of a search for an antihypertensive agent in nutraceuticals and functional foods (De Leo et al., 2009).

2.2 Mechanism of ACE and inhibitory peptides

ACE is a circulating trans-membrane dipeptidyl peptidase that is capable to cleave any peptide (Ahhmed & Muguruma, 2010). It plays an important role in the rennin-angiotensin system (RAS) and consequently on the regulation of the blood pressure as it catalyzes the changes of the inactive forms of angiotensin I (Ang I) to the active angiotensin II (Ang II) (Figure 1). Ang II act constrict directly on

vascular smooth cells. So if the RAS system is too active it has an increasing effect on the blood pressure. Furthermore ACE deactivates the vasodilator peptide, bradykinin, which among other functions has responsibility for enlarging the blood vessels hence it contributes to the decrease in the blood pressure (Ahhmed & Muguruma, 2010; De Leo et al., 2009; Erdmann et al., 2008; Escudero, Sentandreu, & Toldrá, 2010; Ryan et al., 2011).

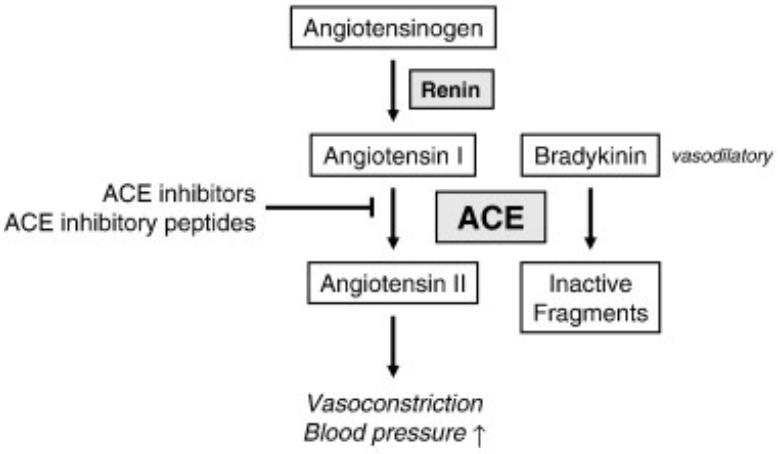

Figure 1: The renin–angiotensin system adopted from Erdmann *et al.* (2008).

With the negative impact on the blood pressure it is preferably to inactivate the ACE. Through medicine ACE can very effectively be inactivated but unfortunately medicine may bring along other strong side effects. Bioactive peptides have ability to inhibit ACE activity by acting as a competitor to the RAS since the ACE prefers ACE inhibitory peptide instead of Ang I. There are two ways a peptide can act; either it binds to the active site or to an inhibitory site of the ACE. In both ways it prevents Ang I from binding to the enzyme. Until now no harmful side effects have been registered in relation to bioactive peptides (Ahhmed & Muguruma, 2010; De Leo et al., 2009; Ryan et al., 2011).

ACE inhibitory peptides can be classified within three types: the "*true inhibitor type*", the "*substrate type*" which has a weak inhibitory activity, and the "*pro-drug type*", which is converted to "true inhibitor type". The ACE inhibitory peptides derived from meat are in general categorized as the "*true inhibitor type*"(Ryan et al., 2011). The strength of an ACE inhibitor is usually measured by the concentration that leads to 50 percent inhibition of the ACE activity and is in science expressed as the IC_{50} value (Erdmann et al., 2008). The lower the IC_{50} value is the stronger the inhibition of ACE activity.

2.2.1 Identification and measurement of ACE inhibitory peptides derived from meat

Enzymatic hydrolysis of whole meat proteins is the most frequently used technique to release ACE inhibitory peptides (Ryan et al., 2011) but it has also been revealed that curing of meat can generate such peptides (Escudero, Toldrá, Sentandreu, Nishimura, & Arihara, 2012; Zhang et al., 2010).

Following hydrolysis the hydrolysates are assayed for bioactivity. Inhibition of ACE activity resulting from hydrolysates can be evaluated both *in vitro* and *in vivo* where the peptide sequences within can still be unidentified. (De Leo et al., 2009; Ryan et al., 2011). HPP11, HBG, HPG1.1 and HPL have not been investigated before the present study.

2.2.2 Bioavailability and absorption

Bioavailability of a nutrient is partially controlled by its physicochemical characteristics such as molecular weight and size, lipophilic properties, acidity (pK_a), charge and solubility. Additionally pH, gastrointestinal mobility, transit time, intestinal permeability, present enzymes and transporters are of importance for the variability of absorption (Ryan et al., 2011).

It is important that the peptide enter the circularly system intact and remain active during the digestive process for the inhibition of ACE activity *in vivo* (De Leo et al., 2009; Ryan et al., 2011). However it is reported that the bioavailability and remained bioactivity is dependent on the peptide sequence construction and their length. To this date *in vivo* and *in vitro* studies have not shown any clear results regarding bioavailability and absorption of ACE inhibitory peptides but some essential structural characteristics of the peptide have been clarified. The overall hydrophobicity of the peptide is of importance. The hydrophobic ACE inhibitory peptides are capable to bind with the N-terminal catalytic site on ACE (De Leo et al., 2009; Hernández-Ledesma, del Mar Contreras, & Recio, 2011) whereas hydrophilic peptides are reported as incapable to bind to the active site of ACE (Ryan et al., 2011). The advantages of hydrophobic ACE inhibitory peptides have been confirmed in several studies (De Leo et al., 2009). Studies with a good result *in vitro* have not been able to demonstrate the same activity *in vivo,* which has been explained by alteration of the peptide as it may take place before reaching ACE *in vivo*. Other studies have shown higher bioactivity of peptides *in vivo*, which is suggested to be related to intestinal modification (Ryan et al., 2011).

Usually ACE inhibitory peptides consist of 2 to 12 amino acids and the most effective recognized contain tyrosine, phenylalanine, tryptophan, and Proline at the C-terminal. Shorter peptides are more resistant to degradation by the intestinal enzymes and more easily absorbed to the circularly system as well as they are more compatible in binding to the active site of ACE (Ahhmed & Muguruma, 2010; De Leo et al., 2009; Erdmann et al., 2008; Escudero et al., 2010; Hernández-Ledesma et al., 2011; Ryan et al., 2011; Terashima et al., 2010). Studies have demonstrated that ACE is incapable of binding larger peptides sequences (Hernández-Ledesma et al., 2011). Therashima *et al*. (2010) underlined this by investigation of several peptide sequences. They identified that stability and absorption *in vivo* was significantly improved by shortening the peptide length from 10 amino acids to four or two amino

acids (Terashima et al., 2010). It is important to emphasize that the knowledge of ACE inhibiting meat peptides to date has been mainly gained from *in vitro* and *in vivo* animal studies.

2.2.3 ACE inhibiting meat peptides and hydrolysates

Studies have shown realistic IC_{50} from pork and bovine muscles and unspecific meat hydrolysate have recently been under investigation. A small extract of those are shown in Table 1.

Table 1: Potency of ACE inhibiting meat peptides derived from pork and bovine and meat hydrolysate derived from pork. The IC_{50}-values corresponds to the concentration of peptide/meat hydrolysate needed to inhibit the ACE activity by half. Table values are adapted from (Ahhmed & Muguruma, 2010; Escudero et al., 2012; Ryan et al., 2011; Terashima et al., 2010).

Peptide sequence	IC_{50} *In vitro*	IC_{50} *In vitro* (µg/ml)*	*In vivo* (SHR)	Source (protein)
RMLGQTP	503 mM	388.3	-	Pork (troponin)
RMLGQTPTK	34 mM	33.9	-	Pork (troponin)
ITTNP	945.5 mM	490.3	21.0 mmHg[a]	Pork (myosin)
VKKVLGNP	28.5 mM	23.4	24.0 mmHg [a]	Pork (myosin)
KRVITY	6.1 mM	4.6	23.0 mmHg [a]	Pork (myosin)
VKAGF	20.3 mM	10.0	17.0 mmHg [a]	Pork (actin)
KRQKYDI	26.2 mM	24.1	9.9 mmHg [a]	Pork (troponin)
Meat hydrolysate	3.9 mg/ml	3900	-	Pork meat
Meat hydrolysate	3.69 mg/ml	3690	-	Pork meat
RPR	382 mM	154.9	33.21 mmHg [b]	Pork meat
KAPVA	46.56 mM	21.4	33.72 mmHg [b]	Pork meat
PTPVP	256.41 mM	124.0	25.66 mmHg [b]	Pork meat
GFHI	64.3 mg/ml	64.3	-	Bovine (muscle)
DFHING	50.5 mg/ml	50.5	-	Bovine (muscle)
FHG	52.9 mg/ml	52.9	-	Bovine (muscle)
GLSDGEWQ	117 mg/ml	117.0	-	Bovine (muscle)
Captopril**	0.022 µM	0.0048		
Carnosin***		14000		

*Calculated from the molar weight of the amino acids, see appendix X.
** Captropril is a syntetic ACE inhibitor and distributed as antihypertensive medicine under the name Accupril.
***Carnosin is a well-known dipeptide, used as control in ACE inhibitory Assay by DMRI.
[a] Measured as the maximum decrease in systolic blood pressure in spontaneously hypertensive rats proceeding oral administration of peptide at 10 mg/kg (maximum decrease between 3 to 6 h after administration).
[b] Measured as decrease in systolic blood pressure in spontaneously hypertensive rats proceeding oral administration of synthetic peptide at 1mg/kg (measured after 6 h).

The IC_{50} values of peptide sequences and meat hydrolysates with various peptide sequences have shown lower inhibiting ability compared to medications such as Accupril with synthetic ACE inhibitors. However studies with spontaneously hypertensive rats (SHR) have demonstrated an acute significant decrease in the systolic blood pressure after a single oral digestion of meat-derived peptides. The decrease is most significant from 3 to 9 hour after intake with a maximum decrease after 6 hours (Ahhmed & Muguruma, 2010; Escudero et al., 2012). Further a significant decrease in the concentration of Ang II in SHR has been verified after two weeks on a 5 % meat hydolysate diet

(Ahhmed & Muguruma, 2010). This could suggest meat hydrolysates as good potential agents for antihypertensive functional foods.

Milk peptides are the most studied and it has been documented that milk peptides are able to inhibit ACE *in vivo* on humans. Two of the identified bioactive milk peptides are IPP and VPP. Several models have showed that IPP and VPP were easily transported across the intestinal epithelium to the circularly system and further decrease in the blood pressure on hypertensive patients have been registered (Ryan et al., 2011)). Two milk products containing the antihypertensive peptides VPP and IPP are Calpis® and Evolus® available on the market in Japan and Finland respectively.

2.3 Regulations on food additives and health claims

In the continuation of that meat hydrolysates have a good potential as a substance in a functional product they need to meet the regulatory requirements for additives. The Danish Veterinary and Food Administration (DVFA) are responsible for the regulation on food additives in Denmark. DVFA have to approve all substances with nutritional or beneficial physiological effects before a product can be legally placed on the Danish market. The approval is based on risk management. The procedure is carried out on a case-to-case basis by the National Food Institute to ensure, that the given product can be consumed safely by all groups of the population. Furthermore any fortification with vitamins, minerals or other substances needs to comply with the EU Regulation (EC) No.1925/2006. (Fødevarestyrelsen, 17-02-2012).

Products containing meat hydrolysates with ACE inhibitory activity have potential to achieve a health claim, which are under the European Commission (EC) regulation. The EC legislation of health claim is the basic law in Denmark (Fødevarestyrelsen, 17-02-2012). In 2007 the European Commission (EC) began to regulate food products related to claims according to the EU Regulation (EC) 1924/2006 and compiling a active list of approved claims, which since has been updated and regulated ad hoc to be assert and legislative in all EU countries (Asp & Bryngelsson, 2008; Gilsenan, 2011; K. G. Grunert et al., 2009). The current list can be seen on EFSA's homepage. In total 19 health claims have been approved by the EC.

When food labels try to communicate certain health issues or risk reducing properties of particular ingredients, it is a health claim. Before the EC began regulating health claims was in general prohibited in Denmark (K. G. Grunert et al., 2009). A health claim is explained as: "*any claim that states, suggest or implies that a relationship exist between a food category, a food or one of its constituents and health"* (Aggett et al., 2005). In addition reduction of disease risk claims undergo the health claim classification and is explained as: "*any health claim that states, suggests or implies that the consumption of a food*

category, a food or one of its constituents significantly reduces a risk factor in the development of a human disease.'' (Asp & Bryngelsson, 2008; Gilsenan, 2011). These two types of health claims are divided in different subclasses (Figure 2) (Gilsenan, 2011).

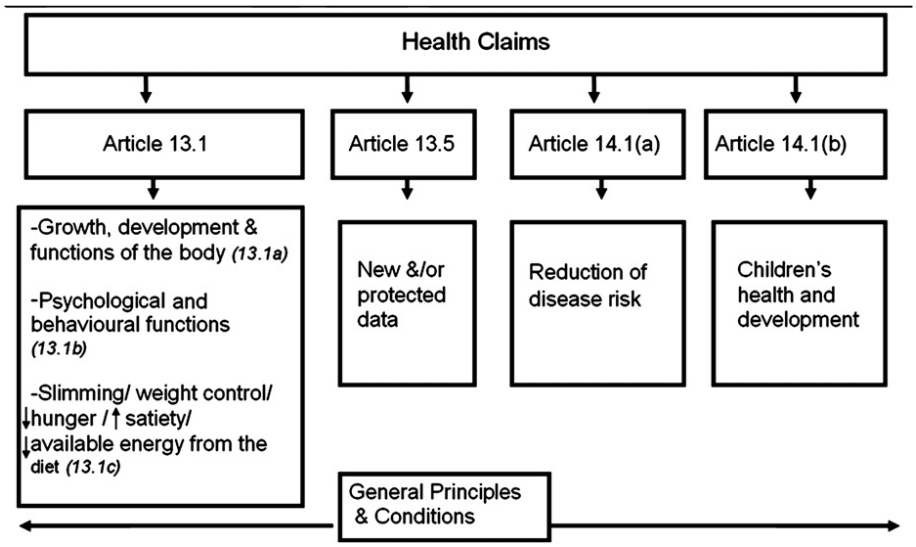

Figure 2: Schematic overview of Regulation (EC) No. 1924/2006 on nutrition and health claims made on food, with respect to health claims adopted from (Gilsenan, 2011).

Article 13.1 and 13.5 claims are: "*Health claims other than those referring to the reduction of disease risk and to children's development and health"* (Asp & Bryngelsson, 2008). Article 13.1 are divided in 3 categories of claims:

a) The role of a nutrient or other substance in growth, development, and the functions of the body
b) Psychological or behavioral functions
c) Slimming or weight control or a reduction in the sense of hunger or an increase in the sense of satiety or the reduction of available energy from the diet

Article 13.5 are claims based on newly developed scientific evidence and/or that include protection of proprietary data. Article 14 claims are divided in:

a) Reduction of disease risk
b) Children's health and development

(Asp & Bryngelsson, 2008; Gilsenan, 2011)

Products containing meat hydrolysates with ACE inhibitory peptides would presumably be evaluated to achieve an article 13.1 (b) health claim. The acute decrease in blood pressure is a psychological effect and a help to maintain a healthy blood pressure rather than a disease reduction.

2.4 Evaluation of scientific evidence for health claims

All health claims need to be substantiated by high scientific standards (Gilsenan, 2011; K. G. Grunert et al., 2009). European Food Safety Authority (EFSA) is responsible for assessing the scientifically based evidence to ensure that consumers can trust accepted claims and that the industry can get feedback on their investments in science and innovation of new functional ingredients (Gilsenan, 2011; Vero & Gasbarrini, 2012). The evidence is reviewed of EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA) who gives scientific opinions on substantiation of health claims. These scientific opinions are constructed on the basis of the *Process for the Assessment of Scientific Support for Claims on Foods* (PASSCLAIM) consensus of criteria's related to various health claims, showed in Table 2 (Aggett et al., 2005).

The PASSCLAIM approach is evaluated and considered as a valid framework in assessing a claim, as many academic experts from universities, the industry, public interest groups and regulatory organs have been involved. PASSCLAIM is sponsored by the European Commission and coordinated by ILSI Europe (Aggett et al., 2005; Asp & Bryngelsson, 2008).

Table 2: Criteria used for assessment of scientific substantiation for claims on foods. PASSCLAIM Consensus of criteria ((Aggett et al., 2005))

1. The food or food component to which the claimed effect is attributed should be characterised.
2. Substantiation of a claim should be based on human data, primarily from intervention studies the design of which should include the following considerations: (a) Study groups that are representative of the target group. (b) Appropriate controls. (c) An adequate duration of exposure and follow up to demonstrate the intended effect. (d) Characterization of the study groups' background diet and other relevant aspects of lifestyle. (e) An amount of the food or food component consistent with its intended pattern of consumption. (f) The influence of the food matrix and dietary context on the functional effect of the component. (g) Monitoring of subjects' compliance concerning intake of food or food component under test (h) The statistical power to test the hypothesis.
3. When the true endpoint of a claimed benefit cannot be measured directly, studies should use markers
4. Markers should be: - Biologically valid in that they have a known relationship to the final outcome and their variability within the target population is known. - Methodologically valid with respect to their analytical characteristics.
5. Within a study the target variable should change in a statistically significant way and the change should be biologically meaningful for the target group consistent with the claim to be supported.
6. A claim should be scientifically substantiated by taking into account the totality of the available data and by weighing of the evidence.

The PASSCLAIM consensus of criteria ranked different kinds of scientific work into a hierarchy:

***in vitro* models < animal studies < observational studies < human dietary trials** (Aggett et al., 2005)).

Certain biomarkers are considered as tools to predict psychological effects or reduction of disease risks for each of the subsequent categories: Diet related cardiovascular disease, Bone health and osteoporosis, Physical Performance and Fitness, Body weight regulation, Insulin sensitivity and diabetes risk, Diet-related cancer, Mental state and performance and Gut health and immunity (Aggett et al., 2005). Products containing meat hydrolysates inhibiting ACE activity, resulting in an acute decrease in blood pressure, will be categorized as a cardiovascular disease biomarker. Maintenance of normal blood pressure would presumably be the wording of a claim to products containing meat hydrolysates with ACE inhibitory activity. The NDA approach to provide scientific opinions on claims in relation to maintenance of normal blood pressure is approached on the basis of *Guidance on the scientific requirement related to antioxidants, oxidative damage and cardiovascular health*. In general NDA consider maintenance of normal blood pressure as a beneficial physiological effect (EFSA on Dietetic Products, Nutrition and Allergies (NDA), 2011)(EFSA on Dietetic Products, Nutrition and Allergies (NDA), 2011).

When EFSA's NDA panel examines the evidence they decide how far a cause and effect relationship is established. They evaluate the evidence of human trails and reliable biomarkers. Three superior approaches are evaluated in their assessment:

1. Characterization of the food/ constituent.
2. Relevance of the claimed effect to human health and is it a beneficial physiological effect.
3. Scientific substantiation of the claimed effect. Human intervention studies are required.

Scientific substantiation in relation to blood pressure can be obtained from human intervention studies showing a short-term reduction in the systolic blood pressure or a reduction in the diastolic blood pressure along with a reduction in the systolic blood pressure. The food or the component with the claimed effect should be tested to comparable neutrals or a control group on a normal diet. The substantiation could only be obtained from studies performed on a hypertensive study population treated with lifestyle measures including diet. Studies performed on a hypertensive study population treated with medications will be individually considered. The evidence for lack of interaction between the food or the ingredient and medications should be clear (EFSA on Dietetic Products, Nutrition and Allergies (NDA), 2011).

The Guidance have been consulted with the government, non-government organs, the industry and academic institutions (Panel on Dietetic Products, Nutrition, and Allergies (NDA). European Food Safty Authority (EFSA), 2012) and is based on the NDA Panels experience on evaluating claims on the basis of PASSCLAIM (EFSA on Dietetic Products, Nutrition and Allergies (NDA), 2011). For that reason the approach can be considered as a valid framework in assessing a claim.

2.4.1 EFSA scientific opinion on ACE inhibitory peptides

The following part will briefly review the substantiation of health claims related to two peptides and maintenance of normal blood pressure. Since meat peptides from pork and bovine are not as far along in the investigation comparative peptides will be reviewed. EFSA have conducted scientific opinions on the substantiation of health claims related to bonito protein peptide (ID 1716) and C12 peptide (Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys) (Id 1483, 3130) in respect to maintenance of normal blood pressure pursant to Article 13(1) of regulation (EC) No 1924/2006.

The opinions of the NDA are based on systematic reviews and evaluations of studies on the ACE inhibitory effects of bonito protein peptide and C12 peptide and several studies have been evaluated. Both peptides were considered as sufficiently characterized (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010a; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010b). The studies provided for the two scientific opinions involve:

- Inhibition of ACE activity in vitro.
- Antihypertensive effects on spontaneously hypertensive rats (SHR).
- Pilot acute randomized, double-blind, crossover intervention studies on humans.
- Randomized double-blind controlled trial in humans.
- Randomized crossover in humans.

The opinions from the NDA panel conclude that the results from the studies are not sufficient enough to predict the effects on humans. Only results from studies on animals have reported an effect on the blood pressure and this effect cannot be transferred to humans. Further statistical evidence and the amino acid composition was lacking in some of the submitted studies. EFSA conclude that a cause and effect relationship between the consumption of bonito protein peptide or C12 peptide and the maintenance of normal blood pressure cannot be established on the basis of the evidence provided (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010a; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010b).

Considering that only 19 claims have been approved by EC and EFSA indicate that they are very strict in their assessment and require very convincing scientific evidence. It is difficult to succeed with an application and reach a positive EFSA opinion that can lead to an approval of a claim. Very strong scientific evidence from human trails is needed to prove a cause and effect relationship between the consumption and the claimed beneficial physiological effect.

2.5 Food choice and acceptance

Insight and understanding of consumers' choice, preferences and perceptions of foods lead to knowledge about what underlying mechanisms there are operating and influencing peoples accept of new products. Applying consumer insight is beneficial in marketing strategies for functional foods. It is valuable to understand consumers' decision making as it is important to link the food attributes with the target groups health consequences in the communicating (Wansink, 2005). Besides the regulatory requirements on functional foods, it is important that the consumer accepts the product and that they understand the health claim before a product can reach the market. In other words, consumer acceptance is key for success regarding new products (K. G. Grunert, 2010; Zhang et al., 2010).

Understanding consumers food choice and accept of functional products imply many complementary scientific disciplines and theories. Whereas the fundamental biological need determines the amount of food a person needs to consume, the interplay between biological and physiological mechanisms are responsible for releasing hormones and salivation to assure a proper intake of food. Humans have conflicting tendencies in their food preferences. On one hand the food choice is governed by a neophobia expressed by reluctance to try unfamiliar foods, whereas also a subconscious attraction to seek novel food sources which ensures that the diet variety comply with the needs of the human body. This phenomenon is called the omnivore's paradox and was first introduced as *The Generalist* by Rozin in 1976 (Ogden, 2003; Rozin, 1976; van Trijp & van Kleef, 2008; Wansink, 2002) . The understanding of the psychophysiological, biological and social influence on food choice and acceptance is a broad field, ranging from the appetite system to consumer attitude and motivational influences. These can be accessed from various theories. Psycho-physiological models are used to describe the relationships between satiety, hunger and sensory properties of foods. Additionally a sociological method can contribute with information about a population's choice of food and frequency of eating particular types food. Developmental models, which are based on a more anthropological approach, can illustrate and describe how food preferences develop during a lifetime. These models describe the existence of food habits that are specifically attached to different cultures and how these constitute to the individual identity. A psychological approach can provide concepts with cognitive models of food choice that implies intentions, subjective norms, attitudes and beliefs. Furthermore, theories such as

decision-making models and neuro-economics are trying to explain food choice based on the most desired outcome of individuals (Ogden, 2003; Rozin, 2006). The following chapter will describe some of the many different theories on food choice, preferences and acceptance that are important for understanding and evaluating the development of a functional meat product containing meat hydrolysates.

2.5.1 Sensory perception

All the senses are involved in the sensory perception of food and determine whether or not people like the food. It is emphasized in several studies and theories that the combined sensory perceptions are essential in food choice and indirectly on the acceptance of food (Graaf et al., 2007; Yeomans & MacFie, 2007). This section will briefly clarify some of these studies with taste as the central focus.

The perception of attributes can be evaluated as positive or negative. The five fundamental tastes are: salt, bitter, sweet, sour and umami, which is sensed when molecules from the food interact with receptors on the tongue. Each taste have an optimum concentration for liking and perceived intensity, which differs between persons, age groups, gender, state of health, and according to different situations and circumstances (Graaf et al., 2007). In addition, taste is temperature dependent. Figure 3 illustrate that the thresholds for e.g. salt and bitter increases with the temperature.

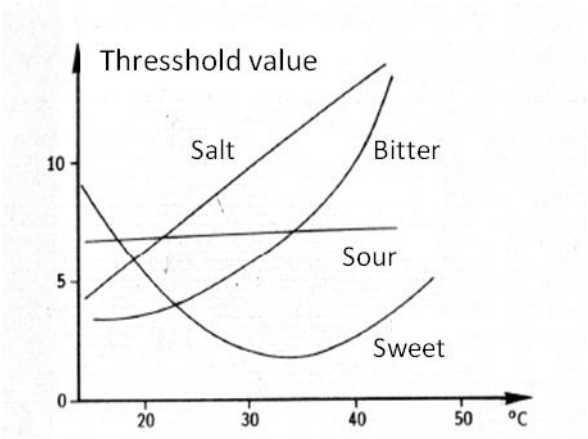

Figure 3: The threshold value of groundflavors in response to temperature. Illustration of threshold values for groundflavors are derived from (Amerine, Pangborn, & Roessler, 1965)

2.5.2 Development of preferences

We have a natural innate preference for sweet and fatty flavors and aversion for sour and bitter tastes. During the whole life especially from the infant stage during the transmission from mother to infant we develop and learn flavor preferences by different processes. Two of them are described as *Flavor-consequence learning* and *Flavor-flavor learning* (Graaf et al., 2007; Yeomans & MacFie, 2007). By *Flavor-consequence learning* the sensory properties of a food are perceived and people develop a preference or a dislike according to the post-ingestive results. By *Flavor-flavor learning* a liked flavor

will increase the acceptance of an unknown flavor. A classic example of *Flavor-flavor learning* is when people start drinking coffee they usually add milk or sugar in the beginning (Yeomans & MacFie, 2007). A third operating mechanism is development of liking by a conditional learning. In this case liking increase as the food increases in familiarity, which is dependent and affected by the frequencies of exposure. Zajonc explains it as a lack of adverse consequences in the *post-ingestive* effect of a food. It takes place with repeated exposure to a new food without any positive or negative consequences (Zajonc, 2001). The stability of food and taste preferences seems to be more long lasting as earlier the preference is obtained. Studies by Liem and Menella (Liem & Mennella, 2002; Liem & Mennella, 2003) have shown that people exposed to sour and bitter tastes in the very early stage of life acquire an acceptance earlier than those who are not. The early-obtained preferences may be able to help predict later preferences. However, it is emphasized that repeated exposures within a short time-span can lead to boredom. This result in a differentiation between two concepts: liking and wanting. Boredom decreases the wanting of a food (Graaf et al., 2007). Where preference is the choice between different products, liking is referring to the degree of pleasure derived from the sensory perceptions by intake of a certain food (Graaf et al., 2007). Consumers seldom consume a type of food they do not like whereas the consumption increases of those foods with high liking. That emphasizes liking to be dominant for food choices although there are situations in which people choose the less liked food on the background of their state of hunger or other different motives such as weight control, price or ethical reasons (Graaf et al., 2007; Yeomans & MacFie, 2007).

2.5.3 Social and psychological variables in food choice

Additionally to exposure and post-ingestive learning the social interactions during a lifetime have influence on food preferences. Many models have been developed attempting to describe food preferences. In Randall and Sanjur's (Randall & Sanjur, 1981) model of factors influencing food preferences, the variables have been divided in three groups of characteristics: individual, food and environment. The model is evolved mostly on demographic variables (Figure 4).

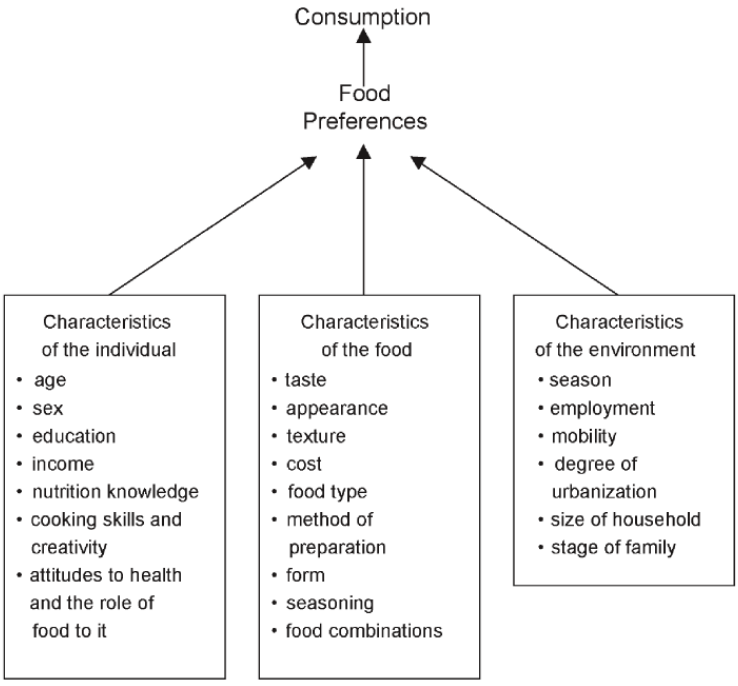

Figure 4: Factors influencing food preferences (Randall & Sanjur, 1981).

The arrows on the top of the variables designate their interrelations and how they lead to consumption. As demographic data on consumers often are incomprarable, a consumer oriented product development has to address the physiological and psychological factors (Graaf et al., 2007; Sijtsema, Linnemann, van Gaasbeek, Dagevos, & Jongen, 2002). This could indirectly be addressed through people's beliefs and attitudes (Shepherd & Sparks, 1994), where acceptance can be explored by people's perception of risk and benefits associated with a food (Wansink, 2005).

When consumers choose between different food products they will choose the product they associate most positively. The complete food attributes will in that case be weighed and summed. Depending on the beliefs and likings of the consumer the desired outcome turns out to be evaluated differently and is a result of the individual's food interaction and social communicated information (Shepherd & Sparks, 1994). A person's evaluated outcome can be weight control or environmentally friendly food whereas another person's evaluated outcome is a value for the money approach or gaining weight. One of the social psychological models used to describe food choice is through general human decision-making called Expectancy – Value theory (EV). The theory assumes that people choose to achieve the most desirable and positive outcome and prevent undesirable and negative outcomes (Shepherd & Sparks, 1994).

Food choices and evaluations reflect habitual conscious and unconscious decisions. These decisions can be categorized into series of individual defined values ((Connors, Bisogni, Sobal, & Devine, 2001;

Furst, Connors, Bisogni, Sobal, & Falk, 1996). To illustrate this process (Furst et al., 1996) developed "The food choice process model" (Figure 5) which is further investigated by Conners *et al.* in 2001. The model is based on qualitative empirical studies and reflects that consumers in the post-industrial societies are faced with many opportunities in different situations, which makes the food choice complex (Connors et al., 2001).

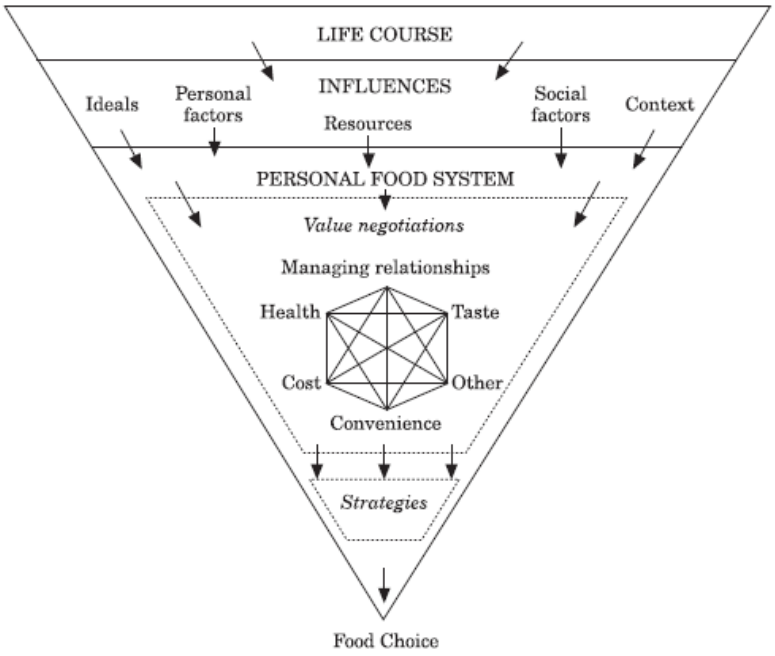

Figure 5: The food choice process model (Furst et al., 1996)**.**

The model (Figure 5) illustrates that ideals, personal factors, resources, social factors and context influence food choices during a lifetime.

The food choice process model operates with a *personal food system* consisting of two processes; value negotiations and development of experience-based *strategies*. The investigation by Connors showed that these strategies are a classification of foods, eating situations, prioritizing between conflicting values of different eating situations and balancing priorities by the individual defined time frame (Connors et al., 2001). In the value negotiation process all considerations are considered and are related to the value labels *health, taste, cost, convenience relationships, managing* and *other* in the model. These values are often in conflict with each other thereby 'forcing' the consumer to prioritize between them (Connors et al., 2001). The most prioritized values depend on the context where the food choice process takes place. Value negotiations will further be incorporated during the development of experience-based strategies. These strategies will make it easier for the individual to make a food choice in different situations (Furst et al., 1996). Furthermore the strategies can be used within one meal but also over a longer time period to handle the personal set of values through

categorizations of food and situations (Connors et al., 2001). The model thereby shows in a heuristic approach the complexity of the food choice process. Investigating consumer's accept of functional foods in a heuristic approach can be valuable as it attempts to evaluate from a consumer perspective.

2.6 Acceptance of functional foods

Consumers acceptance of foods and the measurement there of is described as: "*a phenomenological experience, best categorized as a feeling, emotion, or mood with a defining pleasant or unpleasant character. As it is a subjective construct, measurement of food acceptance relies on the use of psychometric, psychophysical, and/or behavioral methods*" (Sijtsema et al., 2002). Parameters such as nutritional knowledge, quality perceptions, purchase situation, recommendations and product expectations have influence on acceptance (Bornkessel et al., 2011; K. G. Grunert, 2010; Zhang et al., 2010). Several studies have shown that consumers perceive information about nutrition and health very differently (Wansink, Westgren, & Cheney, 2005). Acceptance of food is connected to food choice and preferences and is therefore among others dependent on situational factors such as time and place, demographic factors as well as cultural, political, social and religion variables (Siegrist et al., 2007; Zhang et al., 2010).

The following section will provide a framework for understanding consumers acceptance related to functional food. The selections of acceptance studies performed on functional meat products are limited. This framework will therefore have its origin in acceptance of functional foods in general. Bornkessel *et al.* classified three categories of consumer acceptance of functional food products and their ingredients (Figure 6). These were: *consumer characteristics, purchasing situation* and *product characteristics* (Bornkessel et al., 2011), which seems to be inspired by Randall and Sanjur's model from 1981 on factors influencing food preferences. To use different theories and studies in a clarifying way, the three key concepts adapted from Bornkessel *et al*. will outline the structure, despite that the concepts crosstalk and interact in many orders.

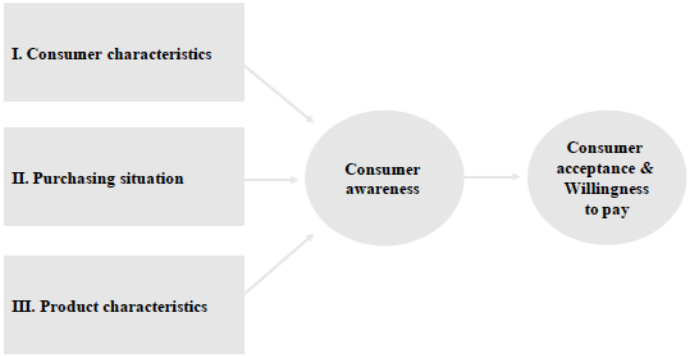

Figure 6: Influence factors of consumer acceptance (adapted from (Bornkessel et al., 2011)

2.6.1 Consumer characteristics

As mentioned, consumers have initially neophobia with regards to unfamiliar foods, even when the consumers believe the functional foods have healthy qualities. It is stressed that consumers in Europe are more critical towards functional foods as compared to North Americans. This is underlined in the market sale numbers for functional foods. In general the central and northern parts of Europe, with exception of Denmark, are paying more attention to functional foods than the Mediterranean countries. However, the Danish consumers are among the most suspicious towards functional foods in Europe and judge them as unnatural and impure foods see (Siro et al., 2008) for review, (Zhang et al., 2010).

A study examining consumer attitudes towards functional foods showed that seven factors were of importance (Urala & Lähteenmäki, 2004):

1. *Reward from using functional food.*
2. *Confidence in functional food.*
3. *Necessity for functional food.*
4. *Functional foods as medicine.*
5. *Functional food as part of a healthy diet.*
6. *Absence of nutritional risk in functional food.*
7. *Taste of functional foods.*

Of these seven factors the first "*reward from using functional foods*" had most influence on consumer readiness. Risk perceived by the consumers had no effect on the general attitude towards functional foods (Urala & Lähteenmäki, 2004)

In general there have not been identified socio-demographic variables on acceptance of functional foods. There seems to be a contradiction between studies and accept are more product specific. The type of additive and moreover the combination of product and additive are important (Lähteenmäki, Lyly, & Urala, 2007; Poulsen, 1999). Poulsen (1999) has investigated the attitude in Denmark towards functional foods. The most accepted product was vitamin D-enriched dairy product and fiber-enriched bread. However many consumer perceive calcium and vitamin D-enriched bread products positively as well. Only a minority favored fortification with omega-3 and fiber in dairy products (Poulsen, 1999), which highlights that attitudes and accept of functional foods are product dependent.

2.6.2 Gender

Gender influences food preferences and choices. A dietary study shows that men eat more red meat than women, whereas the opposite is shown regarding vegetables (Fagt et al., 2008). These gender differences have been explained by food symbolism where associations between masculine and feminine values are linked to certain foods. Meat is a symbol of strength, virility and aggressiveness, which are characters that can be associated with masculinity. Equally, vegetables are colorful and delicate, which is characteristic associated with femininity. Further men and women agreed relatively on categorizing foods as either masculine or feminine, but there is no consensus on the underlying reasons (Jensen K.O., 2008).

Health is one of the factors believed to influence food preferences between genders (Beardsworth et al., 2002; Turrell, 1997). The concept of health includes among others knowledge about health, nutrition and the state of health. (Beardsworth et al., 2002) examined gender differences in food choice, willingness to change diet, knowledge about healthy a diet and eating habits. Overall, it was concluded that women, to a greater extent than men, expressed that they better liked healthy foods, which also was reflected in food questionnaires. The women's diets generally contained more fruits and vegetables than men's, whereas men had a greater intake of fast food. Generally, men consumed more meat than women and had a different perception and understanding of eating meat. The same study showed that men were more inclined to agree with the statement about whether a healthy diet always contains meat (Beardsworth et al., 2002). In contrast women tend to follow dietary advice in greater degree than men and have been shown to be more thoughtful about food and health. The explanation was embedded in the fact that women have more knowledge about nutrition and health and their interactions. Additionally, women are more willing to change their dietary habits because they believe that recommendations have benefit to their health (Turrell, 1997; Verbeke, 2005).

2.6.2.1 Age

As previously shown, food choice is influenced by various factors during a lifetime, which affects the human cognitive development. Age can be divided into different stages of life. Certain characters of importance can define each stage and influence attitudes and perceptions related to food choice. A classic example of age classification is Eriksons Stages of Psychosocial Development. The most relevant agegroups in respect to present study population of ages 18 and above, are adolescence and the three stages of adulthood: Young, middle and late adulthood (Erikson, 1980). A study on the motivations and cognitive structures behind consumer choice, showed that young adults had more focus on increasing attributes such as vitamins and minerals, while the middle age adults was more focused on attributes preventing diseases (Krystallis, Maglaras, & Mamalis, 2008)(Murphy & Withee, 1986). It has also been demonstrated that elderly people prefers higher concentrations of salt and

sucrose compared to young people, which can be explained by the taste perception declining with age (Murphy & Withee, 1986).

2.6.2.2 Health

The concept of health has been highlighted as one of the basic explanations for how people act. E.g. European consumers perceive Genetically Modified Organism's (GMO) and irradiated products as less healthy compared with natural and low processed foods (K. G. Grunert, 2010). A consumer's view on their own state of health and degree of involvement in respect to hypertension are very important. It could be related to the before mentioned gender differences as women in general are more interested in health than men. In addition women could be interpreted as more skeptical towards functional foods as they are more interested in natural products and obtaining pleasure from eating (Roininen et al., 1999)(Roininen & Tuorila, 1999)). Consumer's personal attitude is crucial for the acceptance of functional products and it seems like it can differ between many segments (Bornkessel et al., 2011) .

Health is one of the values that may be included in the personal food system, but the perception of a healthy diet depends on the individual. It is expected that the Danish consumers will rank personal values partly depending on where they are in their life and state of health, which in theory will be expressed through their food choices. Falk *et al* (2001) studied how Danish people perceived health and managed to eat healthy. Results showed that "healthy eating" depended on the individual's perception of health and healthy eating, as a consequence of different categorizations of "healthy" and "unhealthy". The most dominant themes used to define "healthy eating" were among others: "low fat", "natural", "balanced diet" and "prevent disease"(Falk, Sobal, Bisogni, Connors, & Devine, 2001). This emphasizes that consumer perception of health in respect to functional foods is important in relation to understanding why consumers act as they do. The degree of perceived health benefits of meat products containing hydrolysate has an impact on the purchase motives. Functional foods that claim blood pressure reduction or maintenance are therefore of most interest for a consumer who are at risk or has hypertension.

2.6.2.3 Consumers' knowledge of nutrition

The link between the personal state of health and believed benefits of a product with functional ingredients are crucial for the consumer awareness and acceptance (Bornkessel et al., 2011; van Trijp & van Kleef, 2008; Wansink et al., 2005)

Consumers adapt functional foods into their diet according to their level of nutritional knowledge. Wansink et al investigated two levels of knowledge in relation to consumption of soy products; knowledge on attribute levels and knowledge on consequence level connected with benefits. The two types of

knowledge were not changing consumers' attitude or liking but rather influenced their intensions to how likely they would be to consume it. Consumers with both types of knowledge are more expected to accept and consume a new food. However there is a missing link between product attributes and self-relevant consequences and this missing link may hold back the acceptance of functional foods (Wansink et al., 2005). If a consumer cannot link their knowledge to a self-relevant consequence of eating a certain product, they will not accept buying it (Bornkessel et al., 2011)

The consumers' conclusions about a product's health qualities are created on levels of nutritional knowledge in which information can be achieved from different sources such as: nutrition labels, claims, ingredients, logos, brands, quality stamps or certificates of origin (K. G. Grunert, 2010). Studies have demonstrated that increased knowledge about food technologies and certain process methods were positively assosiated with higher acceptance of these (Siegrist et al., 2007).

2.6.3 Product characteristics

Consumers create opinions about the production and processing as it provides the product with a certain quality. Methods and products that can be associated with homemade and has as little processing as possible are the most preferred (K. G. Grunert, 2010). (Bornkessel et al., 2011) state that the quality, naturalness and the effect benefits are important factors. The Danish attitudes towards functional foods indicate that the additives should be naturally related to the product (Poulsen, 1999).

2.6.3.1 Taste

Taste and health have been examined to be among important determinants when associating the consumption of different food groups and expected outcomes with the EV (Shepherd & Sparks, 1994). Studies have emphasized that there are little willingness to compromise on taste for the benefit of health (K. G. Grunert, 2010; Verbeke, 2006). It appears that consumers associate functional foods with a bad taste. The phenomena have been investigated over time and it seems that the negative prejudgment of taste have decreased from 2001 - 2004 by Belgian consumers. This demonstrates that a consumer not necessarily believes that good taste trade-of health (Verbeke, 2006). (Verbeke, 2005) found that 50 % of Belgian consumers would accept functional foods if they taste good and only 9 % would accept if they taste less good. It was also demonstrated that the majority of Danes did not accept omega-3 in dairy products (Poulsen, 1999). The explanation may be the association between omega-3 and a "fishy" off-note in the taste (Bornkessel et al., 2011).

2.6.3.2 Taste issues with hydrolysates

For several years amino acids and peptide sequences have been used as flavorings in food and beverage products as well as nutrition contributors (Schiffman, Clark, & Gagnon, 1982). The taste

which developes during curing or fermentation of certain foods is a result of hydrolyzed proteins into peptides (Nishimura & Kato, 1988). Additionally, free amino acids are able to react with reducing sugars in the Maillard reaction and form compounds in the Strecker degradation that usefully can contribute to the taste in meat products (Zhang et al., 2010).

The chirality of amino acids as well as their side chain arrangement has different flavoring properties and the perceived intensity is concentration dependent, as the ground tastes. The perceived intensity for D-amino acid is greater than for L-amino acids. In general Cysteine, Cysteine HCl and Methionine contributes with an *unpleasant* taste because they all contain sulfur (Schiffman et al., 1982). The taste of a peptide is dependent on the type of enzyme used, the sequence of the amino acids, their composition and length (De Leo et al., 2009). It is further dependent on the duration of the hydrolyse (Aaslyng, Larsen, & Nielsen, 1999). Hydrolysates are often described as bitter and it has been shown that the intensity of bitterness increases as peptide length gets shorter and is sequence dependent, e.g. Phe-Pho has a more intense bitterness than Pho-Phe. Bitterness is related to hydrophobicity where hydrolysates of hydrophobic proteins have an intense bitter flavor whereas the hydrophilic proteins are perceived less bitter (Damodaran, Parkin, & Fennema, 2007; Nishimura & Kato, 1988).

2.6.3.3 Quality

A study on European consumer acceptance of functional foods in a quality perspective reveals that consumers identify quality different when linked to purchase motives (K. G. Grunert, 2010). According to Grunert, 2010 perception of quality can be divided in four concepts:

- Health
- Taste (and other sensory characteristics)
- Convenience
- Naturalness (degree of processing, organic production, animal welfare, GMO free)

Consumers evaluate the four concepts according to their personal values. The concepts cannot be addressed directly but must be seen from an individual standpoint and consumers may compromise on parameters from one concept to obtain higher quality in another (K. G. Grunert, 2010). A study on Danish consumers' view on food quality revealed that positive opinions about food quality generally were related to perception of taste and convenience and the more negative attitudes were related to distribution or process method (Holm & Kildevang, 1996).

2.6.3.4 Health claim

Consumers judge claimed benefits independently of the product and those who address a relevant benefit are considered as the most popular (van Trijp & van Kleef, 2008)(van Kleef, van Trijp, & Luning, 2005). Results from a study by Grunert *et al* (2009) on consumers perception of health claims in the Nordic countries (n=4.612) divided consumers in two categories: i) consumers who prefer long claims that give information on the active ingredient, the benefit and physiological function and ii) consumers who preferred short claims containing information only on the benefit. The study investigated how bioactive peptides were perceived as an active ingredient. The consumers in the first group had a higher choice probability than those in group two. In contrast both groups had a high choice probability on omega-3, which demonstrated that consumers prefer the more familiar ingredients. Ingredients that are assumed as less familiar such as bioactive peptides would be sorted out by consumers in group two, which indicates that the value of familiarity in a claim would have a stronger choice probability among consumers (K. G. Grunert et al., 2009).

2.6.4 Purchasing situation

(Bornkessel et al., 2011) states that the recommendations by health professionals, the familiarity of eating the product, the brand and its reliability influences the purchase motives among consumers. Purchasing circumstances refer to factors that have influence on purchase motives towards functional products. Within this concept the recommendations by nutritionist, media, friends or a doctor have been revealed to have influence on purchase probability (Bornkessel et al., 2011). However recommendations will not be included in the analyse of present study, as it is not a part of the main focus.

2.6.4.1 Familiarity as a trust

Perceived healthiness and acceptance of claims and functional foods increase with familiarity (Lähteenmäki, 2012). Brands are associated with fact, thoughts, feelings, perceptions, images and experiences. Consumers usually relate brands of food with a certain value, eating situations or a tradition, which assign a brand with a social attribute (Dam, Y.K. and Trijp, H.C.M., 2006). Familiar brands increase trust about the beneficial effects in functional foods (Bornkessel et al., 2011) as well as consumers' confidence with a brand and its value perception can surpass the actual quality of a product (Dam, Y.K. and Trijp, H.C.M., 2006). A study demonstrated that the Nordic consumers mainly prefer familiar ingredients in functional foods and bioactive peptides were categorized as an unfamiliar ingredient (K. G. Grunert et al., 2009)(K. G. Grunert, 2010). In a Danish survey foods with familiar brands were identified as the most positive (Holm & Kildevang, 1996). Seen in this

perspective, introducing meat products with added hydrolysates should focus on familiar Danish products in order to succeed.

2.6.5 Eating patterns

The meals consumed during a day differ from each other in their composition. This is determined by the eating patterns developed during a lifetime. These patterns are created by culture and act on traditions and ceremonies. Graaf explains the role of the eating context as appropriateness of the foods role in different meals and occasions (Graaf et al., 2007). In addition, the eating patterns are controlled by habits, where certain times of the day are associated with eating (Yeomans & MacFie, 2007). The Danish meal pattern typically consists of cold meals for breakfast and lunch and then a warm meal for evening. The evening meal seems to alternate between foreign and traditional Danish recipes such as roasted pork, meatballs and sausages. The lunch meal is characteristically consisting of Danish open sandwiches with typically Danish spreads such as salami or liver pate (Holm, 1997). Meat as a food has been identified as exceptional and very important among Danes (Holm & Kildevang, 1996).

2.6.6 Food identity in Denmark

The term "we are what we eat" was the sound of a Danish nationwide health campaign back in the 1990s. Food, in addition to contributing with energy to our bodies, is a essential part of the culture and traditional system we live in (Holm, 1997) and is therefore important for people to maintain since it expresses a part of our identity (Fürst, 1988). The cuisine in a given culture consists of a characteristic set of basic foods, preparation techniques, principles of flavor combinations, conventions for eating behavior and a distribution of infrastructure on getting the cultivated product to the table (Belasco, 2008).

> *For what is food? It is not only a collection of products that can be used for statistical or nutritional studies. It is also, and at the same time, a system of communication, a body of images, a protocol of usages, situations and behavior.*
>
> Roland Barthes – French literary theorist and philosopher (1979) in (Belasco, 2008).

Hence, the food we are eating is defining who we are and further our identity is constructed through the food we eat (Belasco, 2008; Fürst, 1988; Holm, 1997). Foods reflect a certain personal value and express a symbolic meaning in relation to identity. The traditional and especially homemade food is connected to familiarity, safeness and an individuality whereas manufactured fast food is more anonymous, unfamiliar and public (Fürst, 1988).

3 Study considerations

If the hydrolysates HPP11, HBG, HPG1.1 and HPL inhibits the ACE activity that is comparable to that of other ACE inhibitory peptides they can be assumed as good potential agents for a functional product given the inhibition of ACE activity is maintained after product processing. It is a conflicting task since both the intensity of bitterness and the inhibition of ACE activity increases as peptide length decreases. The challenges for the innovation of new functional food products are that they have to meet the regulatory requirements and furthermore reach consumer acceptance. This is key for success with new products on the market (Bornkessel et al., 2011; Zhang et al., 2010).

When introducing new functional ingredients the right combination of substituent and the product must be perceived as natural (K. G. Grunert, 2010). With this in mind, the obvious choice of a food for introduction of peptides would be meat products as the peptides are derived from meat. Based on previous findings, it can be expected that different meat products are consumed with different frequencies, depending on whether they are perceived as appropriate in the meals. Some products are perceived more appropriate in cold meals and others in warm. Therefore it could be useful to introduce different types of meat products with added hydrolysates, also to vary the intake and prevent boredom. As perceived healthiness and acceptance of functional foods increase with familiarity (Lähteenmäki, 2012), the products have to be traditional Danish and support the fact that Danish food culture must be seen as a strong part of the Danish identity. As meat in general is perceived as an important part of a typically Danish meal, the traditional Danish meat products, liver pate, salami, meatball and sausages is assumed to be appropriate targets for introduction of meat hydrolysates towards creating functional foods .

The specific target group for antihypertensive functional food products is the middle-aged people, as they have an increased risk of developing hypertension.

3.1 Approach to collect information about the average Danish consumer

A quantitative design in form of a questionnaire has been selected. The purpose of using a questionnaire is to gain an overview of the consumers in Denmark in respect to acceptance and attitude towards meat products containing hydrolysates processed proteins, and willingness to compromise on taste. This approach is chosen to identify a number of similar quantifiable variables. Further it is wished to reveal knowledge about personal health perception influence awareness of functional meat products and awareness linked with own benefit among consumers with hypertension.

A questionnaire approach is useful because the construction of questions can be made so they indirectly lead to what issues influences the Danish consumer acceptance of hydrolysates in meat products (Riis, 2005). However, through a questionnaire it may be difficult to inquire into the factors behind the phenomenon, as people often do not know why they choose as they do (Jensen K.O., 2008; Riis, 2005). But it ensures an independent position to answer the questions, which is important since it is desired to examine the consumer's own attitude. Furthermore it is a good tool because it is possible to reach many respondents with relatively few resources. As the studied population becomes larger and more representatives it is easier to generalize compared to qualitative methods such as interviews. A disadvantage of a quantitative approach is that it is not possible to ask clarifying questions while answering, which could lead to the respondent not answering as intended. Another disadvantage of the quantitative approach is that questionnaires often use closed response categories where it can be difficult for respondents to answer properly, if the desired response is missing (Riis, 2005). These issues have been taken into account in the selection of questions and response categories and most of the questions had a "Do not know" opportunity. Using an online questionnaire could be an advantage since the respondent would feel more anonymous and therefore answer more honestly than in a questionnaire filled out on location.

4 Materials and Methods

This study consists of two main parts: An investigation of four different hydrolysates in four different traditionally Danish meat products and consumer attitudes towards functional meat products. The following chapter describes the experimental work where 12 traditional meat products of four types have been developed. Inhibition of ACE activity have been measured for each product. A conventional descriptive analysis are performed using a trained panel. Consumer tests of the products are performed using the Holistic by DMRI™ method. Two questionnaire surveys were completed.

4.1 Overview of the experimental work

An overview of the experimental work conducted is exemplified in Figure 7.

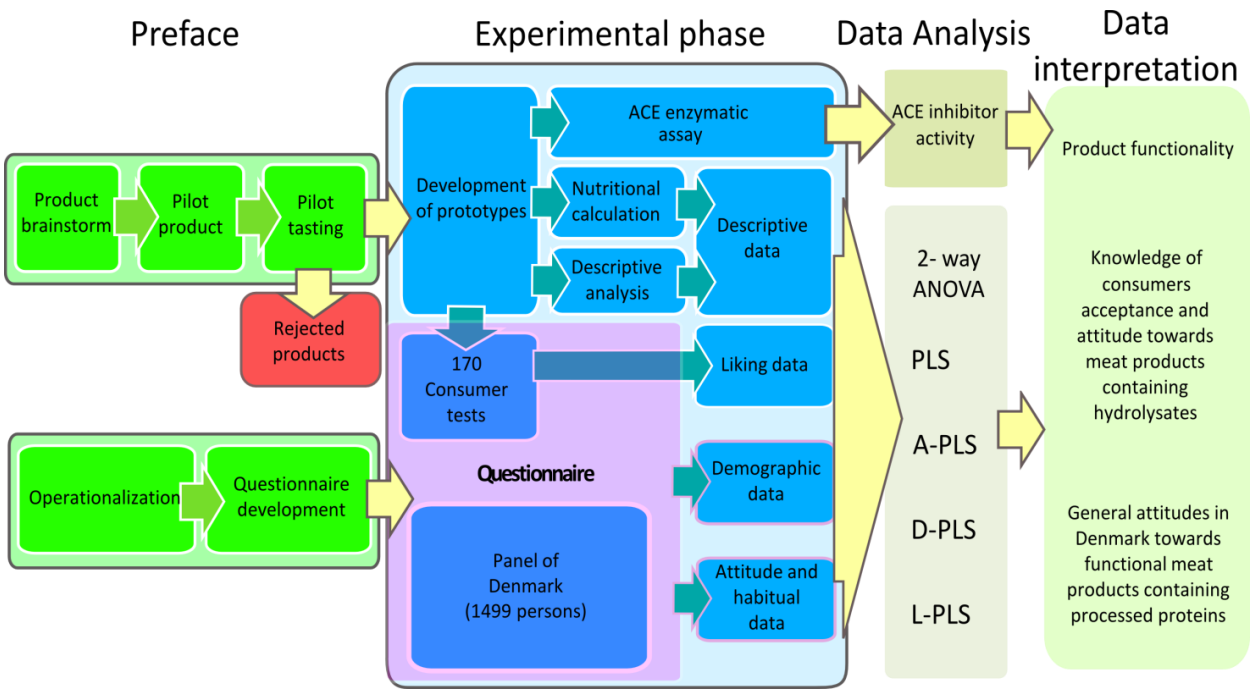

Figure 7: Overview of the work performed in the study. From development of products over experimental work to data analysis and interpretation.

4.2 Hydrolysates and inhibition of ACE activity

4.2.1 Hydrolysate preparation (performed by Dat-Schaub)

HBG, HPP11, HPG1.1 and HPL are produced from bovine and pork low-value products. The products are homogenized and heated to 80 °C for 12-24 hours followed by fat removal. All are food grade approved by the veterinary staff at the slaughterhouse (personal communication (Hansen,)).

HPP11, HBG, HPG1.1 and HPL are produced with the food grade enzymes Alcalase®, Protamex® and Flavourzyme®, which is prepared by Novozymes A/S. The enzymatic treatment of the remains are performed at 53-56 °C for one hour, prior to enzyme inactivation through heating to 90 °C. The samples are concentrated by vacuum and either submitted directly to spray drying or purified by an ultrafiltration step before spray drying.

4.2.2 ACE inhibitory Assays

The hydrolysates are assayed both from pure form and after incorporated in different products to make sure that they could resist the product processing in order to maintain the ACE-inhibitory effect.

ACE inhibitory assays were performed according to the method of Ahhmed and Muguruma (2010). The hydrolysate was solubilized in 0.1 M borate buffer (0.1 M H_3BO_3, pH 8.3) to a final concentration between 20 and 30 mg/ml. This solution was used to make dilution series of 6 and a blank sample without hydrolysate. 100 µl substrate solution (0.1 M H_3BO_3, 7.6 mM HHL (Hyppuryl-Histidyl-Leucine-OH), 0.61 M NaCl, pH 8.3 was added to 12 µl each of the hydrolysate solutions. The reaction was started (t=0) with addition of 40 µl enzyme solution (60 mU/ml rabbit-ACE enzyme (6778, Sigma-Aldrich), 0.25 M H_3BO_3, pH8.3) and performed at 37 °C water bath. After t = 30 minutes the reaction was stopped by adding 1100 µl of 0.1 M HCl. A sample with added HCl prior to t=0 was used prepared as a reference for no reaction. Carnenosine was used as a positive control for the assay using the same dilution series.

Hippuric acid released from the HHL was extracted from the reaction solution using 3.0 ml ethyl acetate added while stirring vigorously. The samples were centrifuged for 10 minutes at 2500 rpm and 2 ml of the top layer containing ethyl acetate. Then the hippuric acid was extracted and evaporated to dryness. Hippuric acid was re-dissolved in 2 ml 1 M NaCl and transferred to plastic cuvettes (plastibrand UV cuvette), and the absorbance at 228 nm was measured using a spectrophotometer (Perkin Elmer).

The inhibitory effect was then calculated as:

Inhibition, % = [(B-A)/(B-C)] x 100, Where A is the optical density of the sample, B is the optical density of the sample, C is the optical density of the blank sample for no reaction.

Screenings results from *in vitro* assays of HPP11, HBG, HPG1.1 and HPL is measured in IC_{50} value. With information from analysis of the clean hydrolysates done by Eurofins Steins Laboratorium A/S the IC_{50} value in protein content is calculated with the form:

IC_{50} (hydrolysate) x dry weight (%) = IC_{50}(dry weight)

IC_{50} (hydrolysate) x protein content (%) = IC_{50}(protein)

4.3 Questionnaire Survey (n=1499)

A questionnaire was developed to gain an overview of the consumers in Denmark concerning acceptance and attitude towards functional meat products and the consumers' willingness to compromise on taste. The questions were designed to provide background information on the individual or to be categorized within one of the three key concept groups described by (Bornkessel et al., 2011). Those were *Consumer characteristics, Purchasing situation and Product characteristics* and inspire the questionnaire. Operationalization of the three key concepts was completed and presented in Table 3. The operationalization is a utility to concretize the underlying concepts of consumer attitudes towards functional meat products ((Riis, 2005) and intends to define the key concepts and their dimensions so specific and understandable questions can be formulated to identify attitudes of the Danish consumers towards functional meat products. The key concepts are presented in the first column in the operationalization table (Table 3) and was defined in the conceptual framework in chapter 2.6 Acceptance of functional foods. The second column defines the associated dimensions. In column three the questions was formulated from each key concept and followed by a column, which presents response options for each question. The questions were of three types: i) Attitude questions to elicit norms, attitudes or values. ii) Factual questions to clarify behavior, habits and beliefs. iii) Background questions. These are emphasized in the last column of category.

Table 3 (page1): Questionnaire overview. The key concepts Consumer characteristics, Purchasing situation and Product characteristics are presented in the first column and are identified dimensions of consumer acceptance towards functional food products. The second column defines the associated dimensions. In column three the questions are formulated from each key concept. The fourth column presents response options for each question. The abbreviations are labels used for the analysis and fulgurate in the following figures and the Category indicates type of data.

Concept	Dimension	Question wording (English)	Response categories	Abbreviation	Catagory
Key concepts	*The key concepts dimensions*	*Questions for the questionnaire*	*Answer possibilities*	Label in the plot	Data
Background	Age, gender, education and demographic				
Consumer characteristics	Hypertension	Do you suffer from high blood pressure?	yes/no/don't know	HighBP, NotHBP, Don'tKBP	F
		Does someone from your family suffer from high blood pressure?	yes/no/don't know	RelativesHBP NA	
	Consumer intake of selected traditional Danish meat products	How often do you eat salami/ Paté/Meat balls/ Wiener sausage (e.g. hotdog sausage)	2 or more times per week/ weekly/ monthly/ Less than monthly/Never/ Don't know	EatSalami, EatPate, EatMeatBall, EatSausage	H
	Personal health status VS product attributes and self-relevant consequences	To what extent do you agree with this statement:			
	Knowledge linked with own benefit	You consider yourself a healthy person?	1-7 Scale	I'mHealthy	A
		You consider yourself as not sick?	1-7 Scale	I'mNotSick	A
		You eat healthy	1-7 Scale	EatHealthy	A
		You are interested in new food products with special health promoting attributes.	1-7 Scale	InterestNewHealthProduct	A
		You prioritize products, that you believe, have special health promoting attributes.	1-7 Scale	PriorityHealthProduct	A
		You take an interest in that what you eat originates from a natural source	1-7 Scale	NatSourceFood	A
		You take interested in that what you eat is sustainable	1-7 Scale	SustainableFood	A
		You accept to eat foods with added vitamins	1-7 Scale	AddVitamin	A
		You accept to eat foods with added proteins	1-7 Scale	AddExtraProtein	A
		You accept to eat meatpoducts with added processed proteins	1-7 Scale	AddProcesdProtein	A
		You believe that functional foods (e.g. Becel butter or probiotic yoghurt) are beneficial for your health.	1-7 Scale	FunctFoodHealthier	A
	Awareness of functional ingredients	Probiotic yoghurts strengthens gut healthiness and boost the immune defense.	True/false/ don't know	ProYogHealthy	F
		Becel butter have a lowering effect on the blood cholesterol	True/false/ don't know	BcelLowCho	F
		Products enriched with omega-3 fatty acids are healthy?	True/false/ don't know	Ω3Healthier	F

Table 3 (page2): Questionnaire overview. The key concepts *Consumer characteristics, Purchasing situation and Product characteristics* are presented in the first column and are identified dimensions of consumer acceptance towards functional food products. The second column defines the associated dimensions. In column three the questions are formulated from each key concept. The fourth column presents response options for each question. The abbreviations are labels used for the analysis and fulgurate in the following figures and the Category indicates type of data.

Concept	Dimension	Question wording (in Danish)	Response categories (in Danish)	Code	Catagory
Purchasing situation	Recommendations (nutritionist, by media, by a doctor)	To what extent do you agree with this statement? If an advertisement recommends a health-promoting product, would you be more inclined to purchase it?	 1-7 Scale	 AdvertsImpact NA	 A
		If a dietitian in a magazine recommends a health promoting product, would you be more inclined to purchase it?	1-7 Scale	MagazinImpact NA	A
		If a friend recommends a health-promoting product, would you be more inclined to purchase it?	1-7 Scale	FriendImpact NA	A
		If your doctor recommends a health-promoting product, would you be more inclined to purchase it?	1-7 Scale	DoctorImpact NA	A
	Familiarity (known product, known brand)	You are interested in the development towards a health beneficial effect of products you usually purchase?	1-7 Scale	IncrsHealthierProduct	A
		You would purchase new products you normally do not eat if you have the impression that they benefit your health?	1-7 Scale	BuyNewImprovHealth	A
		A well known company introduce a paté, which helps maintain a healthy blood pressure. This product will have a beneficial effect on you?	1-7 Scale	PurRelavance	A
		It increases your interest in a product if Hjerteforningen's logo is visible on the packaging? (Hjerteforeningen is one of Denmarks largest disease-fighting organizations, this include working with research within cardiovascular disease and prevention of heart disease)	1-7 Scale	HeartLogoInterest	A

Table 3 (page 3). Questionnaire overview. The key concepts *Consumer characteristics, Purchasing situation and Product characteristics* are presented in the first column and are identified dimensions of consumer acceptance towards functional food products. The second column defines the associated dimensions. In column three the questions are formulated from each key concept. The fourth column presents response options for each question. The abbreviations are labels used for the analysis and fulgurate in the following figures and the Category indicates type of data.

Concept	Dimension	Question wording (in Danish)	Response categories (in Danish)	Code	Catagory
Product characteristics	Quality attributes	If the Hjerteforeningens logo is visible on the product package, would you be more inclined to purchase the product?	yes/no/don't know	HeartLogoBuy	
		To what extent do you agree with this statement?			
	Taste (compromise on taste in terms of health value)	Are you willing to compromise on taste, if you know what you eat is healthy?	1-7 Scale	ComproTaste	A
		You would purchase meat products with added processed proteins, if it helps you maintain a healthy blood pressure.	1-7 Scale	ProductRelevance	A
	Source of ingredients (processed proteins)	Would you buy a product with natural health promoting ingredients added if it tastes good?	yes/no/don't know	BuyTasteGood	F
		Would you buy a product with natural health promoting ingredients added if it tastes less good?	yes/no/don't know	BuyTasteLessGood	F
	Quality attributes	Would you buy a product, which claims to help you maintain a healthy blood pressure?	yes/no/don't know	BuyClaimBP	F
		Do you see yourself in target group for such a product?	yes/no/don't know	I'mTarget	F
	Health claim (Knowledge linked with own benefit)	If you feel a positive and beneficial effect from a product, would you then purchase it again although the taste does not appeal to you?	yes/no/don't know	BuyLessGoodTasteBeneficial	F
		Would you purchase meat products with processed proteins added?	yes/no/don't know	BuyProcessMeat	F

NA= not analysed in this study. 1-7 scale where; 1=Strongly disagree, 4= indifferent, 7= Strongly agree, Don't know. Category: F = Factual data, A= attitude data, H=Habitual data.

Finally the questionnaire was generated online in the online software program Defgo.net (www.defgo.com, Copyright 1997-2012, defgo.net). The respondents were shown a brief introduction, which clarified the purpose and how the data would be used. The questionnaire was containing 19 questions with sub-questions, which had to be answered by checking one answer choice, unless other options were indicated. It was not possible to click forward without answering the previous question. Completion of the questionnaire was estimated to take approximately 10 minutes. A print view of the original questionnaire in Danish can be seen in appendix B. An overview of the questions translated to English is listed in Table 3.

4.3.1 Recruitment of respondents

By using the electronic questionnaire rather than mail delivery, it was assumed that the response rate would increase, as the answers were relatively easy and quick to fill out (Riis, 2005). Electronic questionnaires will also make the data analysis easier. The questionnaire was sent to the Panel of Denmark through Interresearch A/S. It is assumed that this is the easiest way to reach the Danish population. The Panel of Denmark is recruited through invitations on a sample of the largest Danish websites including Krak.dk. Krak.dk has been identified as one of the most visited website in Denmark with the widest combination of visitors. The total recruitment base is two million Danish internet users (*Interresearch.dk. question your knowledge.*). All panelists were invited randomly. They had received the invitation via a pop-up window where they could accept or reject. No participants were self-recruited to this questionnaire.

4.4 Production of meat products

This chapter contains the description of both the meat product development through a pilot study and the final production. The pilot study is performed to clarify to what extent addition of hydrolysates would affect traditional Danish meat products with regards to flavor and cooking attributes.

Following hydrolysates (Dat-Schaub A/S, Denmark) were examined in this study:

- DATPRO HPP11 (HPP11) derived from shield bloody meat (Batch 201108-21)
- DATPRO HBG (HBG) derived from bovine greaves (Batch 201109-22S)
- DATPRO HPG 1.1 (HPG 1.1) derived from pork greaves (Batch 201110-22S)
- DATPRO HPL-1 (HPL) derived from pork liver (batch 200901-24)

HPP11, HBG, HPG1.1 and HPL are produced with food grade enzymes Alcalase®, Protamex® and Flavourzyme®, all prepared by Novozymes A/S and is food grade approved by veterinary staff at the slaughterhouse (Dat-Schaub). All ingredients for the meatballs and liver pates are common conventional foods in Denmark and are available in ordinary supermarkets.

4.4.1 Procedure pilot study

4.4.1.1 Meatball - Pilot

Table 4: Ingredients and quantities from Frøken Jensens Kogebog

Ingredients	Quantity
Minced pork (maximum 6 % fat)	500 gram
Salt	1,5 tsp = 9 gram
Pepper	0.5 tsp = 1.5 gram
Egg	1 (approx. 50 g)
Onions	1 (approx. 100 g)
Low-fat milk (0,5 % fat)	2.5 dl
Flour	1 dl
Oil	1 tsp

The meatballs were prepared using the standard recipe from Frøken Jensens kogebog (Table 4). Ingredients from the recipe were mixed to a homogenous basic forcemeat through stirring. The mass was divided into 9 and the hydrolysates HPP11 and HBG were added either directly or with 1 dl of milk in two different concentrations (Table 5).

Table 5: Overview of meatballs prepared in the pilot study

#	Hydrolysate	(%)	Carrier
1	HBG	3.8	-
2	HBG	7.4	-
3	HPP11	3.8	-
4	HPP11	7.4	-
5	HBG	3.1	1 dl milk
6	HBG	6	1 dl milk
7	HPP11	3.1	1 dl milk
8	HPP11	6	1 dl milk
9	Reference	-	-

The mixtures were chilled for 1 hour at 5°C and browned in 50 g portions on an industrial frying table (Friberg Panocopter FBLB40/6 -5775, Fribergs Verkstäder AB, Sweden) at 220°C for 2 minutes followed by 10 minutes of steaming at 100 % moisture in an industrial oven (Electrolux AOS10EA).

4.4.1.2 Liver pate - pilot

Table 6: Ingredients and quantities for the basic mass, from Irma.dk.

Ingredients	Quantity
Minced pig liver and lard[a]	500 gram
Salt	1 tsp = 6 gram
Pepper	1.5 tsp = 4.5 gram
Thyme	0.5 tsp
Egg	1 (approx. 50 g)
Onions	1 (approx. 100 g)
Low-fat milk (0,5 % fat)	2 dl
Wheat flour	1 dl

[a]Minced pig liver and lard from Top Food A/S

The ingredients (Table 6) were mixed to create a basic forcemeat. Seven aliquots of the basic mass was prepared to cover two concentrations, 4 % and 8%, of each of the hydrolysates HBG, HPP11 and HPG1.1

and a reference sample (Table 7). The mixtures was baked at 180°C for 32 minutes in an industrial oven (Electrolux AOS10EA) in 500 g portions in foil salvers.

Table 7: Meat product overview of pilot study for liver paté.

#	Hydrolysate	(%)	Mixture
1	HBG	4	16 g HBG and 384 g basic forcemeat
2	HBG	8	38 g HBG and 368 g basic forcemeat
3	HPP11	4	16 g HPP11 and 384 g basic forcemeat
4	HPP11	8	38 g HPP11 and 368 g basic forcemeat
5	HPG1.1	4	16 g HPG1.1 and 384 g basic forcemeat
6	HPG1.1	8	38 g HPG1.1 and 368 g basic forcemeat
7	Reference	-	400 g Basic mass

4.4.2 Considerations and results from pilot study

The meat products from the pilot study were evaluated to get clarification on how they affected the product during and after processing. Important parameters, which received special attention, were liquid absorption during preparation, the interplay with other ingredients, tin size and sensory comparison. Furthermore inhibition of ACE activity was measured in the final products after processing. An earlier study by DMRI concludes that HPP11 could be added to a commercial bacon liver pate from Tulip in concentrations up to 7.5 % and in meatballs up to 5% without sensory distinction from the reference. Additionally HPL contributes with a strong liver taste and was therefore not suitable for meatballs (DMRI).

The key results from the pilot study was that meatballs had the strongest bi-taste from the hydrolysates. The bi-taste increased with increased amount of hydrolysates. Supplementary, the bi-taste was not as prominent in chilled cold products, which must be taken into account when considering which products the hydrolysates should be incorporated into. Added milk received a negative feedback on the consistence of meatballs. HBG received negative feedback in meatball and liver pates. It was noted, when adding 8 % HPG1.1, there was no sensory distinction from the reference. HPL was clearly distinguishable by color compared to the other liver pates; resulting in a very dark brown and almost black crust when 8 % HPL was added. In addition the HPL emphasized the flavor of liver in the pate as reported in a previous study (DMRI), however this taste attribute is not necessarily a bad attribute in a liver pate and the hydrolysate was therefore not rejected for this product.

4.5 Development of main products

Based on the observations obtained from the pilot study four different products, which could be considered as traditionally Danish, were chosen for investigation of incorporating hydrolysates. The four products included, in addition to meatballs and liver pates, also salami and wiener sausage. The salami was chosen as its inherent fermentative flavor were expected to disguise the flavor from the hydrolysates, similarly the smoked and spicy flavor from the wiener sausage were also expected to give good results. In total 12 products was developed, Table 8. Not all hydrolysates were tested in all products. HBG was only produced in salamis with the hypotheses that the fermentation could disguise the less good taste. Since HPL contributed with a flavor of liver it was in the nature only to add it to the liver paté. HPG1.1 was tested in all products because of the positive results from the pilot study. The production took place at DMRI. The liver pates and meatballs were generated in two batches, as close to the consumer tests as possible to ensure they were freshly made.

Table 8: Overview of meat products tested in the main experiment.

Sensory code	Product	Type	Hydrolysates
325	Salami	HPG 1.1	8 %
347	Salami	Reference	0 %
379	Salami	HBG	8 %
402	Meat Ball	HPP11	8 %
439	Meat Ball	HPG 1.1	8 %
491	Meat Ball	Reference	0 %
543	Wienersausages	Reference	0 %
562	Wienersausages	HPG 1.1	8 %
604	Liver pate	HPP11	8 %
621	Liver pate	HPG 1.1	8 %
657	Liver pate	HPL	8 %
689	Liver pate	Reference	0 %

4.5.1 Procedure for production of meat products used in the main study

4.5.1.1 Meatballs – Main study

The basic recipe used in the pilot study (Table 4) was used to prepare the meatballs for the main study. The forcemeat was divided into three and one of the hydrolysates HPP11 or HPG 1.1 was added directly to

reach a final concentration of 8 % in the hydrolysate product (Table 8). The mixtures were chilled for 1 hour at 5°C, followed by browning in portions of 50 g on an industrial frying table (Friberg Panocopter FBLB40/6-5775, Fribergs Verkstäder AB, Sweden) at 220°C for 2 minutes followed by 10 minutes of steaming at 100 % moisture in an industrial oven (Electrolux AOS10EA).

4.5.1.2 Liver pate – Main study

As in the pilot study the ingredients (Table 6) were mixed to create a basic forcemeat. The basic forcemeat was divided into four aliquots and hydrolysate corresponding to 8% of the final product was added creating products either with HBG, HPP11 and HPG1.1 or without hydrolysate as a reference sample (Table 8). The mixtures were baked at 180°C for 32 minutes in an industrial oven (Electrolux AOS10EA) in 500 g portions in foil salvers.

4.5.1.3 Wiener sausages – Main study

A low fat DMRI recipe (Table 9) was used and shrinkage was estimated to be approximately 7-8 %, based on previous productions. The added hydrolysates were calculated to reach a concentration of 8 % in the final product after shrinkage.

Table 9: List of ingredients for the wiener sausages. Quantity percent and amount (kg or gram) specified for reference (Ref) and hydrolysate recipes respectively.

Ingredients	Quantity % (Ref)	Quantity (ref)	Quantity % (Hydrolysate)	Quantity (Hydrolysate)
Pork shoulder	65	6.5 kg	65	6.5 kg
Water	32.17	3.22 kg	32.17	3.217 kg
Hydrolysate	-	-	8	800 g
Vacuum salt[a]	1.0	100 g	0.862	86.2 g
Nitrite salt 0,6 %[a]	1.0	100 g	1.0	107.7 g
Wiener Sausage spice[b]	0.8	80 g	0.8	86 g
Total	99.97	9.98 kg	107.832	10.777 kg

[a]From GC Rieber, [b]Kryta A/S

All ingredients were mixed to a homogenous forcemeat using an industrial blending machine (Kila F051019). HPG1.1 was dissolved in the water before addition. The forcemeat were filled into lamb casings (Dat-Schaub) using a sausage filling machine (Handtmann VF50 (ct)) and hanged on an industrial drying bracket and processed in an industrial oven (Doleschal Austria SC6001) adjusted according to the program in Table 10. After production all sausages were packaged in modified atmosphere (MAP) in batches of ten and stored at 5°C until use.

Table 10: Process program for wiener sausages in an industrial oven (Doleshal Austria SC6001).

Program	Time
Pre-heating	5 min
Drying	15 min
smoke 1	13 min
Boil	20 min
Drying	2 min
Proces stop	3 min
Sprinkling	10 min

4.5.1.4 Salami - Main study

A fat reduced recipe from DMRI with added potato fibers were used (Table 11). Based on DMRI specialist knowledge and experience shrinkage was expected to be around 30 %, correspondingly the amount of hydrolysate added accounted for 8 % of the salami post shrinkage. Each hydrolysate were dissolved in water and added directly to the other ingredients and mixed to a homogenous mass in an industrial blending machine (Kila F051019) running with adjusted speed between 1 and 2 on rotational speeds between 1920-3840 rpm. An equivalent portion without hydrolysate were prepared as a reference.

Table 11: List of ingredients for salami. Quantity percent and amount (kg or gram) specified for reference (Ref) and hydrolysate recipes respectively.

Commodity	Quantity % (Ref)	Quantity (Ref)	Quantity % (Hydrolysate)	Quantity (Hydrolysate)
Pork shoulder[a]	86.87	8.7 kg	88.34	8.8 kg
Lard pork meat	2.00	200 g	2.00	200 g
Vacuum salt	1.33	133 g	1.33	133 g
Nitrite salt	1.67	167 g	1.67	167 g
F-SC.111[b]	0.03	3 g	0.03	3 g
Dextrose	0.10	10 g	0.30	30 g
Sodium ascorbate	0.03	3 g	0.03	3 g
Potato Flakes[c]	8.00	800 g	-	-
Dried onion	0.60	60 g	0.60	60 g
White pepper	0.10	10 g	0.10	10 g
Water	6.00	6 dl	6.00	6 dl
Hydrolysate	00	0	5.6	560 g

[a]Pork shoulder (Tican Foods, Denmark) [b]F-SC.111 Bactoferm ® from Chr. Hansen, [c]200 g potato flakes soaked in 600 g water, (Kryta A/S, Denmark).

In total four batches were manufactured. The mix of ingredients were filled into 60 mm water soaked fiber sheaths (60/60, Faser) with a sausage filling machine (Handtmann VF50), hanged on an industrial drying bracket and processed in an industrial oven (Scaniro with a friction smoke generator(NESS,UK) or Doleschal Austria SC6001). Two salamis from each batch were weighed to follow the shrinkage, see in appendix C. Similarly, the pH was measured with a calibrated pH-meter (Knick Portamess, Mettler Toledo) periodically until the pH remained stable, shown in appendix D. In case visible mold growth was observed on the surface the salamis were washed by instruction from professional staff at DMRI with a soft cloth soaked in 4 % vacuum salt solution.

Table 12: Process program used in salami production in two industrial ovens Scaniro (S) with a friction smoke generator(NESS, UK) and Doleschal Austria SC6001 (D)

Step #	Oven	Temperatuer	% Relative humidity	Time	Smoke
1. Fermenting	S	24 °C	86 %	3 days	-
2. drying	S	18 °C	90 %	3 days	2x 30 minutes
3. drying	D	18 °C	88 %	20 hours	1x 30 minutes
4. drying	S	16°C	94 %	8-9 days	-

4.5.2 Measurement of nutritional value

Nutrient calculation of all products have been performed in WinFood® 4.0 (Kosttabelforlaget, Denmark) and are enclosed in appendix E

4.6 Descriptive Analysis

The descriptive analysis was conducted on the 12 developed products. A trained panel of nine members carried out the descriptive analysis according to the regulations and requirements listed standard in ISO 13299 and ASTM-MNL 14. It took place at the sensory laboratory at DMRI in Roskilde, approved and accredited for sensory analysis by DS/EN – ISO/IEC 17025.

4.6.1 Training and development of attributes

Trained panelists familiar with the sensory descriptive profile were used for the descriptive assesment. Training sessions were carried out on each type of products on a 15 cm unstructured line scale the day before the final profiling. The panelist wrote attributes to describe and characterize the products. The panel trained on the words and products to reach consensus. Throughout the training the understanding and usage of attributes were discussed and determined. A common set of attributes and the evaluation order was defined between the panelist and panel leader. Those are listed in Table 13 in order as judged.

Table 13: Attributes used in profile for meatball, salami, liver pate and wiener sausages.

Descriptors	Meatball	Salami	Liver paté	Wiener Sausages
Odor	Meatball Fried Onion Off	Salami Smoked Sour Spicy Off	Liver Spicy Burnt Soy	Smoked Spicy Bouillon Off
Appearance	External color Internal color	Red Yellow	Brown Rosa	Wrinkly Rosa Brown
Flavor	Meatball Onion Spicy Salt Sweet Sour Bitter Off	Salami Smoked Sour Salt Bitter Spicy Chemical Off	Liver Thyme Spicy Salt Sour Bitter Sweet Soy Off	Smoked Spicy Salt Floury Off Sour Bouillon Bitter
Texture	Firmness Juiciness Sticky Creamy	Dry Firmness	Firmness Roughly Creamy	Crisp Juiciness Firmness
After Flavor	Off Spicy Onion	Sour Smoked Bitter Off	Bitter	Smoked Spicy
Hand touch				Crispy Firmness

4.6.2 Profile

The samples were served in a bloc design and evaluated by all panelists during 3 or 4 sessions, depending on product type. The samples were served in random orders with 5 minutes intervals between the samples. One sample was served in each session to avoid a directly comparison. The samples were served in dosed petri dish at chill temperature 5 °C. Cold regular tap water was available in a pitcher. The panelists scored the intensity of each attribute on a 15 cm unstructured line scale with anchor points, established through the program FIZZ Acquisition 2.46B (Biosystems, France). All the intensities were collected in FIZZ Calculation 2.46B (Biosystems, France) and exported to Excel.

4.7 Consumer test

The aim was to investigate Danish consumers immediately perception of the developed product. Hence the test was performed as a blind test without any introduction to the attributes of the products, meaning that consumers did not have any preconceived attitudes and prejudices beyond normal towards the products. The selection of consumers was based on the age 18 and above. The recruitment and test was performed on the central location in the canteens at 3 different companies located in Roskilde, Sorø and Høje Taastrup. A poster attracted the consumers, which were posted in advance, (poster in appendix F) or they were directly addressed. In total 170 consumers were recruited and asked to choose a set of 6 products. Two different sets were available i) two series of wiener sausages together with four series of liver patés (Figure 8, A) and ii) three series of salami together with three series of meatballs (Figure 8, B).

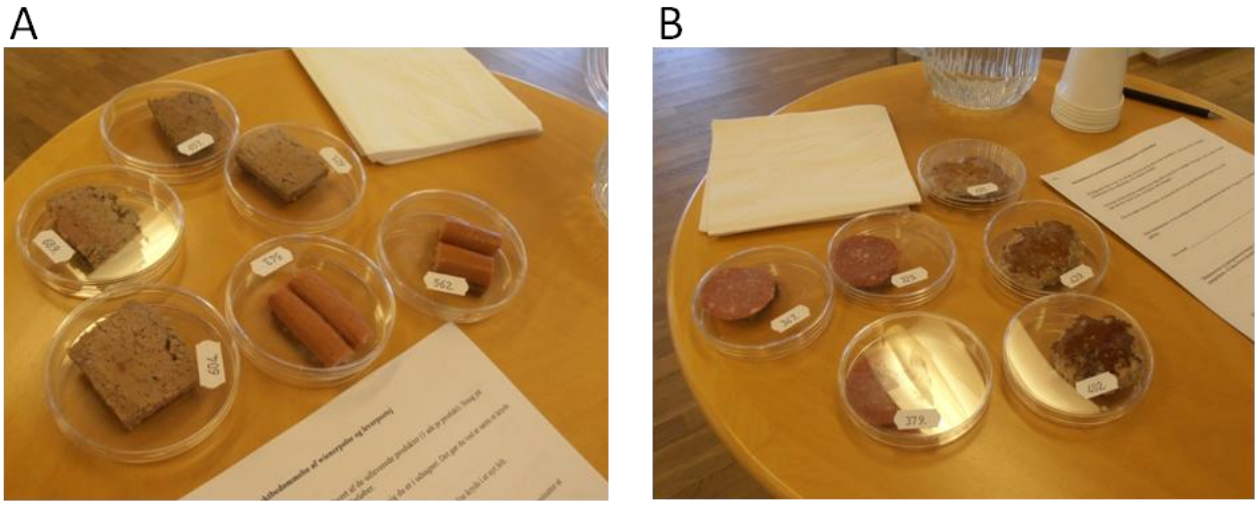

Figure 8: Setup with liver paté and wienersausages (A). Setup with salami and meatballs (B)

Each product sample was served on a little piece of rye bread in a small Petri-box and coded with a three-digit code. All products were evaluated cold at approximately 5°C. The consumers received the set of product samples in a small plastic bag. They were asked to taste and evaluate their perception of the products related to 10 abstract words. The words were graduated on a structured 15 point scale going from strongly disagree to strongly agree for each product in a randomized order to avoid first product biasness (Exampel of evaluation lists in appendix G). The 10 abstract words, shown in Table 14, were chosen in advance. As ”appetizing” can be linked to liking, it was decided not to ask directly to liking of the products. Quantification of abstract words is a part of the Holistic by DMRI™ method, see analysis directions in appendix H. The consumers received a short introduction on how to use the scale but formal training was not performed. They were also asked not to communicate with each

other during the evaluation. All consumers agreed to answer an online questionnaire that was consequently sent to their email. All consumers received a box of chocolate as thank for participating.

Table 14: The 10 selected abstract words.

Original words in Danish	Word translated to English
Traditionelt	Traditional
Anderledes	Different
Velkendt	Familiar
Mærkelig	Strange
Kedelig	Boring
Eksklusiv	Exclusive
Discount	Discount
Indbydende	Appetizing
Naturligt	Natural
Kemisk	Chemical

4.7.1 Online questionnaire

The consumers were asked to answer the same online questionnaire as the Panel of Denmark (Table 3, and Appendix B). All the ratings of the products were typed in a developed pre-questionnaire in Defgo.net and used as a tool to register data. All the consumers were registered by email in a panel in Defgo.net. Emails were encoded with an instruction code during the registration in Defgo.net. A personally link to the online questionnaire was sent to each consumer's personal email and the registration of their answers were connected to their ratings of the products. The online registration of the questionnaire ended the 14th of March 2012. 157 of the 170 consumers had completed all questions.

4.8 Data Analysis

Sensory and consumer data is 'real world data' having many aspects that influences the structure between samples and variables. This type of data is multi-factorial, hence it need a multivariate analysis to discover, extract, compress and exploit the underlying structure and latent variables. These could otherwise be difficult to gather from the data. An interpretation of relation between variables and samples can be expressed in simple models and give an overview of complex data (www.camo.com).

It is possible by Partial least square regression (PLS) to find the main systematic variation patterns from two different data tables, X and Y, of same samples. E.g. demographic and background data as X,

and health attitudes, Y, from the same persons. With PLSR it is possible to interpret the structure between and within X- and Y-variables. This provides a good overview and a more detailed insight of complicated multivariate data (H. Martens & Martens, 2001; M. Martens, Tenenhaus, Esposito Vinzi, Martens, & MacFie, 2007).

Deciding what to be either X or Y of the data matrixes, depends on the intention of the analysis and the available data. When analysing the effect of designed variables on the response variables it is called ANOVA-PLSR (APLSR) having the known classification is used as X. APLSR is useful when analyzing design effects. It is called a Discriminant- PLS (DPLS) when the known classification is used as Y, resulting in a regression of Y on X. DPLS is useful to discriminate or classify data variables. ((H. Martens & Martens, 2001; M. Martens et al., 2007).

4.8.1 Questionaire

4.8.1.1 Classification

The respondents were divided into four age group classifications: youth, young adults, middle adults and seniors. The classifications are based on cognitive development according to Eriksons stage of psychosocial development. Youth referring to Erikson's Adolescent stage were as young adults and middle adults are adopted from Erikson's model and seniors correspond to Erikson's late adulthood stage (Erikson, 1980).

4.8.1.2 Responses

All the attitude response in "Don't Know" was given the value 4 corresponding to the answer"*either or*" in the middle of the 7 point scale. This was chosen to avoid biasing the model. If the value 0 or 8 was chosen it would have weighted as either strongly agree or strongly disagree on the model.

4.8.1.3 Partial least square regression

1499 Danes and 157 Danish consumers answered the questionnaire (Table 3, page 18). The two groups of respondent were analyzed separately according to survey data studied by PLSR method (M. Martens, Frøst, & Martens, 2005) using The Unscrambler X10.1 (CAMO AS, Trondheim, Norway). The data was separated in background, factual and attitude variables. The questions were labeled as shown in column 4 in Table 3).

APLS was completed on the background and factual variables in a binary system (0/1) as X and the attitude scores (1-7) as Y respectively. Both were unweighted. DPLS was used to find characteristic attitudes describing the variation in the background and factual variables. From the APLSR and DPLSR the significant variables and samples were selected and a APLS was completed with full cross

validation, unweighted, mean centered and maximum five components and was requested. A validation table with the p-vaues of beta-coefficients was made to provide an overview of the results and the significance between different variables and samples of matter. Since the questionnaire provide a large amount of data, and a great deal of the removed background not was significant, this study is focused on the three chosen variables, gender, age and state of health with respect to blood pressure.

Removed from background data: Education level, Employ circumstances, Income, Geographical position

Removed from factual data: Relatives blood pressure

Removed from Attitude data: Impact from Advertising, Magazines, Doctor and Friends.

Eating frequency of liver pate, meatball, salami and wiener sausages was removed and analysed separately in excel.

4.8.2 Cross tabulation

A cross tabulation of all the questions is made by Interresearch A/S in SPSS and delivered in a excel report.

4.9 Sensory evaluation

4.9.1 ANOVA

2-way ANOVA was preformed with Bonferroni Least Significance Difference (LSD) in PanelCheck software, (ver. 1.4.0, Nofima, Norway) on sample means of descriptive attributes and the mean of consumer perception of abstract words. Analysis of variance (ANOVA) was advantageous to use in significance testing as it can break down the variation in responses into comparable parts. By using sample means and Bonfferoni LSD experiment sensible error is prevented from growing as the number of comparison increase.

4.9.2 Overall interpretation of the products

APLS was performed using The Unscrambler X10.1 (CAMO AS, Trondheim, Norway) to provide a compact representation of the common average descriptive attributes of the two set of products as X related to the perception of the 10 abstract words from Holistic in Y. It was chosen to analyses meatball and salami as well as liver pate and wiener sausages together as they were judged together in the consumer test.

4.10 **Preference Mapping**

For investigation of the main drivers of liking a PLS was performed separately on both groups of 85 consumers who judged either liver pate and wiener sausages (A) or meatball and salami (B) respectively, using The Unscrambler X10.1 (CAMO AS, Trondheim, Norway). All consumers individual perceptions of familiar, traditional, boring, discount, chemical, strange, different, exclusive and natural for all 6 product within a set were used as X, and related to all individual perceptions of appetizing for all products in Y. The data was organized similar to method 1 described by (Næs, Lengard, Bølling Johansen, & Hersleth, 2010)

Consumer preferences were investigated by a PLS performed using The Unscrambler X10.1 (CAMO AS, Trondheim, Norway). The average of common sensory descriptors and the average consumer perception of the holistic words (X) was related to all the individual consumer perceptions of appetizing (Y) for all 6 products. The analysis was performed separately on consumers belonging to the two groups of 85 who judged either liver pate and wiener sausages or meatball and salami respectively. Data is organized similar to method 3 described by (Næs et al., 2010), without the consumer attributes.

4.11 **L-PLS**

L-PLS is an analysis with data matrices consisting of three variables, X = product descriptors, Y= Consumers product liking and Z = consumer descriptors. L-PLS was performed in The Unscrambler X10.1 (CAMO AS, Trondheim, Norway). The L-shape of such X, Y and Z matrices is a result of X and Z shares a dimension consisting of same numbers of columns with Y. L-PLSR builds on the same principles of PLSR just with an extra dimension Z having regression of Y on X and Z with Y weighted by bi-linear interactions of underlying variables from X and Z. With the L-PLSR model it is possible to explain and link patterns from consumer's attitude and their demographic background directly to their liking of products (H. Martens et al., 2005).

In total four LPLS models were made, two for wiener sausages and liver pate data and two for meatball and salami data, having either background or attitude variables included, respectively. The consumers individual perception of appetizing in Y was explained by the products mean common sensory descriptive data together with the holistic words in (X) and background variables or attitude variables (Za and Zb respectively).

All analysis was performed using full cross validation. The consumers who did not enter Defgo.net and completed the questionnaire is removed from both Y and Z data. In total 13 consumers' were removed.

This corresponded to 9 from the wiener sausages and liver pate consumer group and 4 from the meatball and salami consumer group.

5 Results and discussion

In this chapter the inhibition of ACE activity will be investigated, for the pure hydrolysates and the various meat products. Furthermore, substantiation of a EFSA claim and results of the two consumer studies will be evaluated. As the collected data are substantial and highly diverse, the results will be discussed as a continuation of their presentation. This is chosen to enhance readability and consistency between results and discussion. The first section will evaluate the inhibition of ACE activity of HPP11, HBG, HPG1.1 and HPL and maintained activity after processing in four types of developed products. In that respect, 12 developed products will be characterized in respect to hydrolysate and taste. Subsequent, accept of the developed products will be discussed in the light of completed consumer test and a questionnaire survey and the Danish consumer attitudes towards functional meat products are evaluated briefly. Then an evaluation of the health benefits and substantiation in respect to achieve a health claim will be examined according to the EFSA's scientific requirements.

5.1 Inhibition of ACE activity

Results from *in vitro* screening assays on HPP11, HBG, HPG1.1 and HPL. The calculated IC_{50}-values based on dry weight and pure protein content is shown in Table 15.

Table 15: Screening results from ACE inhibitory Assays by DMRI on pue hydrolysates. The IC50 values are calculated on protein content from analysis information of the clean hydrolysates done by Eurofins Steins Laboratorium A/S.

Hydrolysate	IC_{50} value (hydrolysate)	IC_{50} value (protein)
HPP11	16 mg/ml	12 mg/ml
HPG 1.1	10 mg/ml	9 mg/ml
HBG	9 mg/ml	8 mg/ml
HPL	11 mg/ml	8 mg/ml

When considering the inhibition of ACE activity from peptides, it is important to distinguish the IC50 values derived from heterologous peptide mixtures containing multiple different peptides, from IC50 values derived from purified peptides with known sequences. The purified peptides are usually isolated from a mixture of proteins using chromatography. The hydrolysates in this study is comparable to those found in meat hydrolysate from porcine by (Ahhmed & Muguruma, 2010) having an IC_{50} value of 3.69 mg/ml and the IC50 value of 14 mg/ml in Carnosin used as a control in ACE inhibitory assay by DMRI. The assay screenings of HPP11, HBG, HPG1.1 and HPL was in the same

range showing IC_{50} values of 16 mg/ml, 10 mg/ml, 9 mg/ml and 11 mg/ml respectively. As the inhibition of ACE activity is related to peptides the IC50 values are calculated in respect to their pure protein content, which resulted in a slight stronger inhibiting effect. Calculations on pure protein content reveals that HPL and HBG are the most powerful ACE inhibitors both with an IC50 values of 8 mg/ml followed by HPG1.1 and HPP11 with IC50 values of 9 and 12 mg/ml respectively. HPP11, HBG, HPG1.1 and HPL can be assumed to be competent ACE inhibitors compared to other ACE inhibiting meat peptides derived from pork and bovine, when considering that they are not purified, hence, they are potential antihypertensive agents for a natural functional food compound on condition that the inhibition of ACE activity is maintained after processing.

The IC_{50}-values obtained from *in vitro* screenings assays of processed meat products containing HPP11, HBG, HPG1.1 and HPL is shown in Table 16.

Table 16: Screening results from ACE inhibitory Assays by DMRI on meat products. IC50 values of HPP11, HBG, HPG1.1 and HPL and maintained activity after processing of Meatball, Liverpaté, Salami and Wiener sausages (NM= No Measurement).

Product / Hydrolysat %	IC_{50} values in product	IC_{50} values of hydrolysates (%)	Maintained activity
Meatball			
HPP11 7,4 %	250 mg/ml	19 mg/ml	86 %
HBG 3,6 %	607 mg/ml	22 mg/ml	41 %
HBG 3.6 % *	408 mg/ml	15 mg/ml	61 %
HBG 7,4 %	200 mg/ml	15 mg/ml	61 %
HPG1.1 8 %	255 mg/ml	20 mg/ml	49 %
Liverpaté			
HPG1.1 8 %	383 mg/ml	31 mg/ml	33 %
HPL 8 %	301 mg/ml	24 mg/ml	46 %
HPL 4 %	355 mg/ml	14 mg/ml	77 %
HPP11 8 %	499 mg/ml	40 mg/ml	40 %
salami			
Reference	364 mg/ml	+	+
HBG 8%	495 mg/ml	40 mg/ml	23%
HPG 1.1 8%	277 mg/ml	22 mg/ml	45%
Wiener sausage			
DATPRO HPG1.1 8 %	189 mg/ml	15.12 mg/ml	66%

*Double sample measurement in the ACE inhibitory Assays.

The screening assays shows that there is a large variation in maintained inhibition of ACE activity for HPP11, HBG, HPG1.1 and HPL in Meatball, Liverpaté, Salami and Wiener sausages. HBG in salami had

the lowest maintained activity of 23 % whereas HPP11 in meatball had the highest maintained activity of 86 %. Variation between products was expected, as the preparation processing is different between products. The preparation processing of the four different products include fermentation, steaming, smoking, browning and baking.

There was not found any of inhibition of ACE activity in the references of liver pate, meatball and wiener sausages. The salami reference had an IC_{50} value of 364 mg/ml, which is a stronger inhibiting effect than the salamis with 8 % HBG and 8 % HPG1.1, having IC_{50} values of 495 mg/ml and 277 mg/ml respectively. This inhibition of ACE activity could be contributed to chemical changes of the proteins and peptides from the meat during the fermentation process with lactic acid bacteria. The proteins in the salami reference could be degraded to peptides inhibiting the ACE activity whereas the peptides within the hydrolysates HBG and HPG1.1 may be degraded to amino acids and aromatic compounds by microorganisms (Zhang et al., 2010)). The fermentation process could therefore be responsible for the difference in the inhibition of ACE activity between the reference and HBG and HPG1.1 salamis, as reported previously (Zhang et al., 2010).

The IC_{50}-values of the meatball containing 3.6 % HBG is showed from both the screening and the double sample measurements (Table 16). The results with double sample measurements show stronger inhibiting results (Figure 9) compared to the screening results (Figure 10), 408 mg/ml and 607 mg/ml respectively.

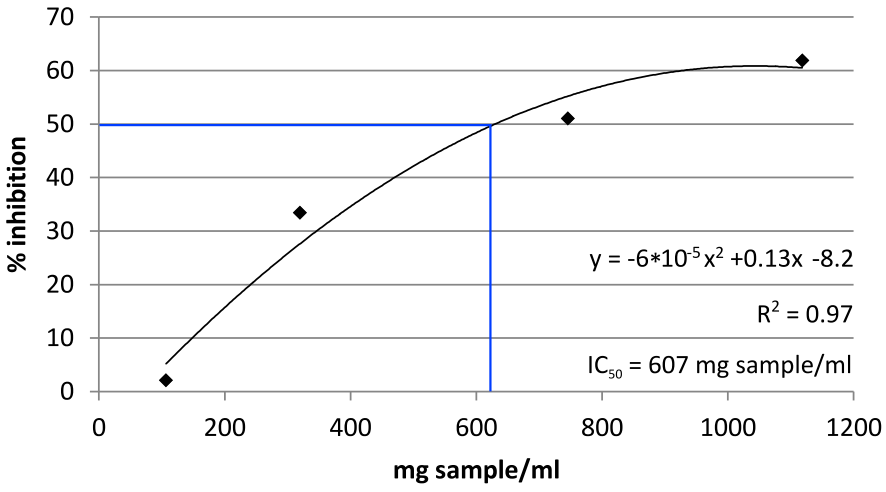

Figure 9: Average inhibition % and concentration in mg/ml from screening meatball containing 3,6 % HBG

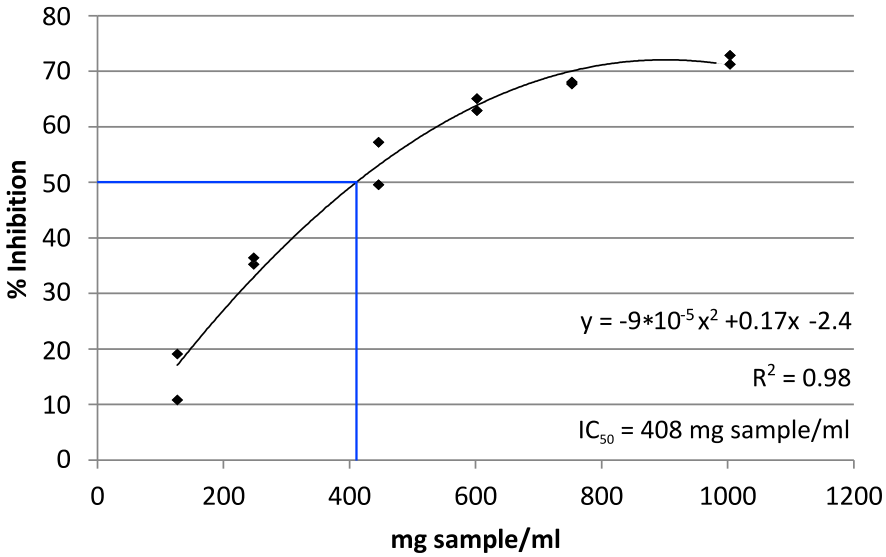

Figure 10: Average inhibition % and concentration in mg/ml in meatball containing 3,6 % HBG from double sample measurements.

The inhibition of ACE activity for liver paté products containing HPL in the concentrations of 4 % and 8 % seems strange, as stronger inhibition is found for the product containing 4 % compared to product containing 8 %. However, these measurements is performed as screenings and the IC_{50}-value can be a resultant of a single outlier in the measurement. This can illustrated by comparing the screening (Figure 9) and the double measurements (Figure 10) of the meatball containing 3.6 % HBG showing that, double measurements with several data points is necessary to give an accurate result. Hence, the results derived from screenings must be seen as indicators of ACE inhibition rather than comparable values. Additional measurements are not a part of this study. Prior to further investigations in inhibition of ACE activity, a reasonable estimate of consumer preferences and attitude towards functional meat products is needed, to give an indication as to how far the Danish market have foundation for innovating functional meat products.

5.2 The Danish attitude towards functional meat products

This chapter will accentuate results from the large questionnaire survey answered by 1499 respondent distributed from all parts of Denmark. As the questionnaire has contributed with large amounts of data and results, it is chosen to focus on gender and age as the main background variables. First an evaluation of interdisciplinary relations between selected variables will give an overview of the results. Hereafter the study population will be examined for hypertension prevalence, within age and gender distribution of Danish consumers with respect to intake frequency of the liver pate, salami, meatball and wiener sausages and willingness to compromise on taste.

As mentioned, Danish consumers are among the most skeptical towards functional foods in Europe (Lähteenmäki, 2012; Zhang et al., 2010). Acceptance and preferences can change over time and are depended on various factors and type of product. Following part will review the survey data analysis of the Panel of Denmark (n=1499). The questionnaire aims to elicit whether the general Dane will accept products containing hydrolysates referred to as processed proteins, their willingness to purchase functional meat products claimed to help maintaining a healthy blood pressure, and their willingness to compromise on taste and perception of own health is investigated.

The study population (18+) is a representative selection of the Danish population (*n*=1499). Frequency is represented in Table 17. Both gender and demographic areas are represented corresponding to statistical numbers of the population (*Interresearch.dk. question your knowledge.*). The younger age groups in the survey are under-represented compared to the actually Danish population, where the older part of the population is represented in a higher percentage. This can only be seen as an advantage in this study as the general middle-aged population is assumed to be the target population of interest, since the frequency and risk of developing hypertension increases with age.

Table 17: Frequency of gender, demographic, age, status of blood pressure represented in the study population compared to the Danish population.

	n	Percent	*n* DK(weighted)[a]	Percent DK(weighted)[a]
Gender				
Male	787	52,5	754,4	50,3
Female	712	47,5	744,3	49,7
Demographic				
Capital	513	34,2	467,0	31,2
Zealand	241	16,1	217,8	14,5
South Denmark	330	22	318,0	21,2
Central Denmark	301	20,1	337,4	22,5
NorthDenmark	114	7,6	158,6	10,6
Age group (years)				
18-29	154	10,3	296,7	19,8
30-39	188	12,5	272,1	18,2
40-49	291	19,4	302,0	20,2
50-59	424	28,3	268,1	17,9
60+	442	29,5	359,8	24,0
Status of blood pressure				
Hypertension	359	23,9	293,2	19,6
Normal blood pressure	1017	67,8	1066,5	71,2
Do not know	123	8,2	138,9	9,3

[a] The weights calculated manually in SPSS by Interresearch A/S (Appendix I), where the selected background variables are multiplied by a weight in order to adapt the distributions in the population from Danish Statistics (Danmarks Statistik)

Figure 11 summarizes the results from PLSR in terms of X (background and factual data) and Y (attitude) loadings and gives an overview of interdisciplinary relations between the variables. The two first PLSR components describe a total of 20 % of the variance in the attitude data. It is important to stress that 80 % of the variance is unexplained and hence the results are used to interpret connection rather than directly predictions. The plot spans two main components (factor 1 and 2). The factual and background X-variables are all found to be significant at the 5% level and are marked with circles in the loadings plot.

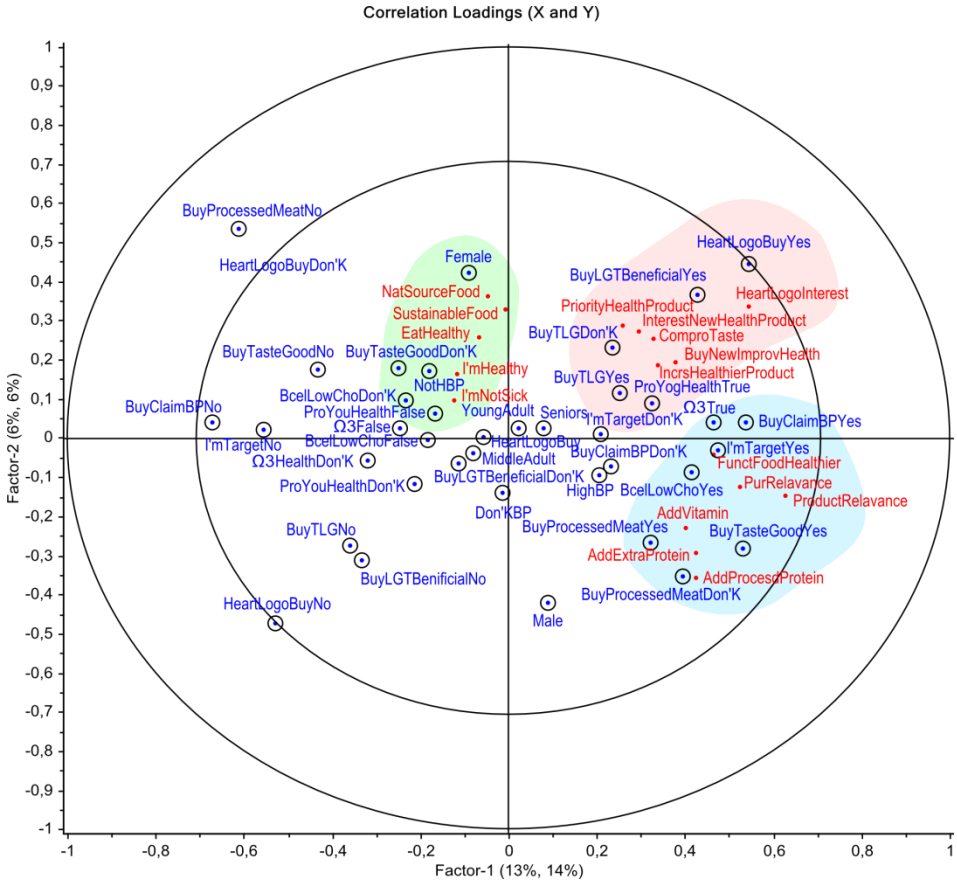

Figure 11: Partial Least Squares (Mean centered). X = background and factual data and Y = attitude loading. Jack-knifing by full cross validation with 1499 segments. Optimal number of factors used for uncertainty test: 3. The inner and outer circles indicate 50 and 100 % explained variance, respectively. Three identified consumer groups are illustrated with green, red and blue background colors. Labels explanation in table X, chapter x.

The horizontal dimension (Figure 11) tends to describe self-perceptions of health related to buy meat products, which claims to benefit a healthy blood pressure. People who see themselves as a part of the target group are negatively correlated to those who do not. The vertical component seems to be mostly segmented by gender. Male and female respond in the opposite directions.

In general a notable tendency seems to divide consumers into three groups: i) The health and environmental concerned, which see themselves as healthy. They are in the upper left quadrant where

attitudes are orientated against healthiness and consumers who see themselves as not sick (in Danish "rask") where concerns address natural source products and sustainable values (I'mHealthy, I'mNotSick, EatHealthy, NatSourceFood, SustainableFood). Those attitudes are associated with females with normal blood pressure, who do not believe that current functional foods such as becel butter, probiotic yoghurts and products enriched with omega-3 fatty acid benefit health, express they are not likely to buy products with bad taste and processed proteins. ii) Consumers who to some extend accept additives and show interest in functional foods. Consumer attitudes about: functional food are healthy (FunctFoodHealthy), familiar product which helps maintaining a healthy blood pressure are of relevance (PurRelevance), purchase meat products with added processed proteins, if it helps maintain a healthy blood pressure (ProductRelevance) and accept of additives in form of vitamins, extra proteins and processed proteins in meat products (AddVitamins, AddExtraprotein, AddProcesdProtein) are correlated in the lower right quadrant. Those attitudes are associated with males who suffer from hypertension, who express they are prone to buy a product which claims to benefit a healthy blood pressure, are likely to buy healthier products with less good taste and will buy products with processed proteins. These results can be connected to (Urala & Lähteenmäki, 2004) "reward from using functional foods" which had most influence on consumer readiness towards functional foods. iii) Consumers who compromise on taste for health and believe functional foods are healthy. Those are assosiated with interest in products with the Danish Heart association (Hjerteforeningen) logo, interest in and purchase of unfamiliar products if they benefit health, (InterestNewHealthProduct, BuyNewImprovHealth), interested in the development towards a health beneficial effect of familiar products (IncrsHealthierProduct), willingness to trade-off taste in respect to health (ComproTaste) and those who prioritize products with special health promoting attributes (PriorityHealthProduct) correlated in the upper right quadrant and associated to expressions accept to buy products with less good taste and processed proteins, probiotic yoghurts and products enriched with omega-3 fatty acid benefit health.

Although the variable pattern can be interpreted in the correlation loadings it is difficult to interpret the variables impact on the model in a plot. Figure 12 shows the P-values of the beta-coefficients. The significance level shows the respondents perception of own health as well as attitudes to functional foods, taste and processed proteins and how these variables are related to the factual and background data. The darker the blue the higher the impact, the variables have on the model.

It is clear that both female and male have high significant impact on several attitude variables. As well as the taste variables, healthiness related to functional meat products and perceived purchase relevance. The plot in Figure 11 confirms the difference as men seems to be mostly correlated with the

group ii) and women with group i) which are concordant with the gender differences emphasized in section >2.6.2 Gender< on page 31.

It seems that there exist a difference between young adults, middle-aged adults and seniors, which could be influenced of various factors and strategies. The accept of additives in form of vitamins, extra proteins and processed proteins in meat products has a higher impact at the young adults corresponding to the findings by (Krystallis et al., 2008) where motivations and cognitive structures of consumers were investigated and the results showed that young adults had higher attention to increased attributes such as vitamin and minerals while the middle age adults attention was more on attributes preventing diseases. To some extent the same can be extracted from the Danish population as significance level increase with age on the interest in improvement or development of new healthy products. Compromise on taste seems to have higher impact on seniors.

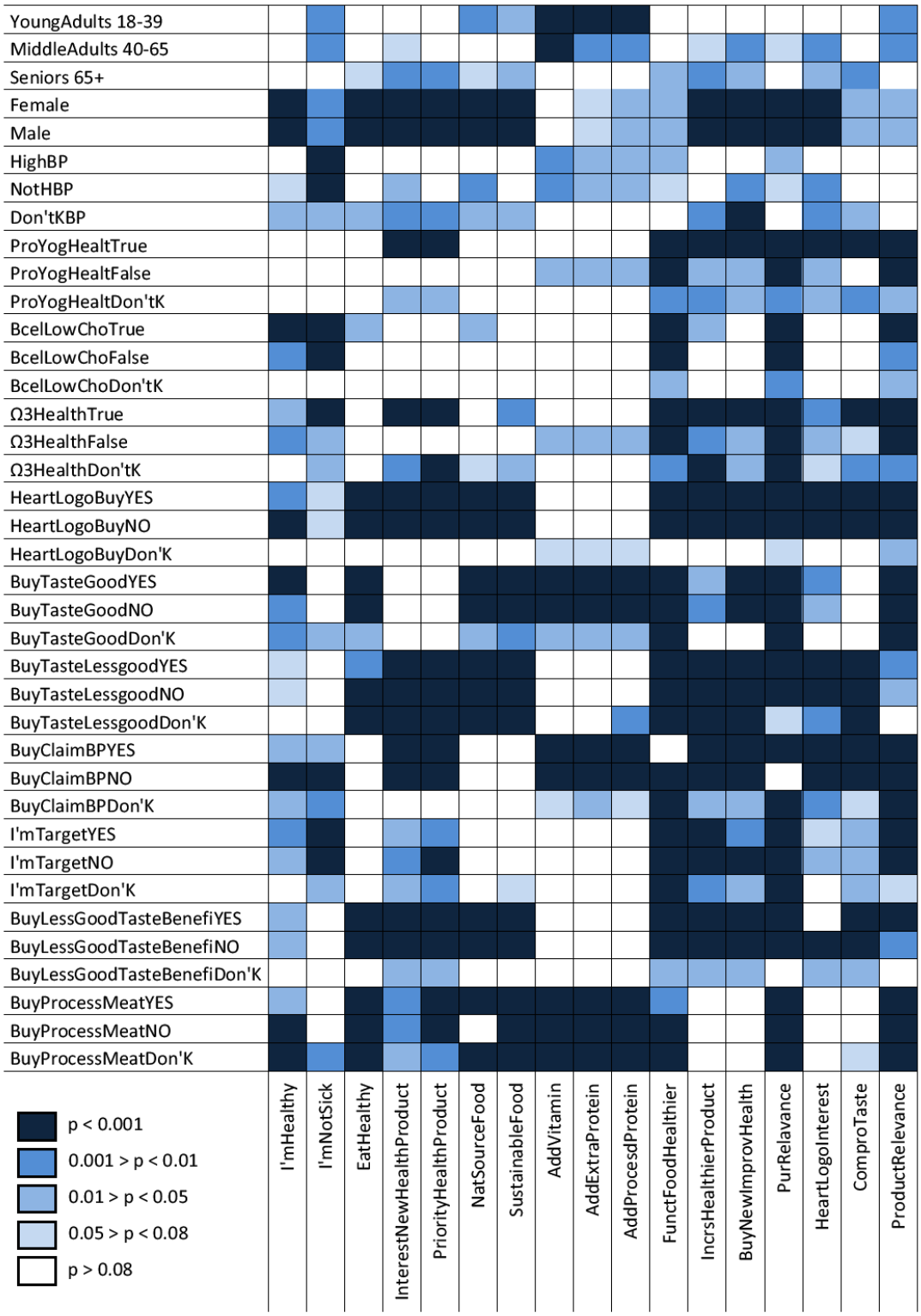

Figure 12: P-values of Beta-coefficients determined by jackknifing at optimal factors (3 factors). Graphical summary of significance level are quantified to five levels according to indications in the left corner. Question labels are shown in Table 3, on page 42.

5.2.1 Status on blood pressure in Denmark

Results show that 23,9 % of the study population (n=1499) suffer from hypertension. Weighted in order to adapt the distributions in the population Denmark it corresponds to 19.6 % (Table 17, on page 64). These results confirm the recently index from the Danish Heart Association which indicate that every fifth Dane have hypertension. The weighted result of age and gender distribution in Danish population asked: *Do you suffer from high blood pressure?* is shown in Figure 13.

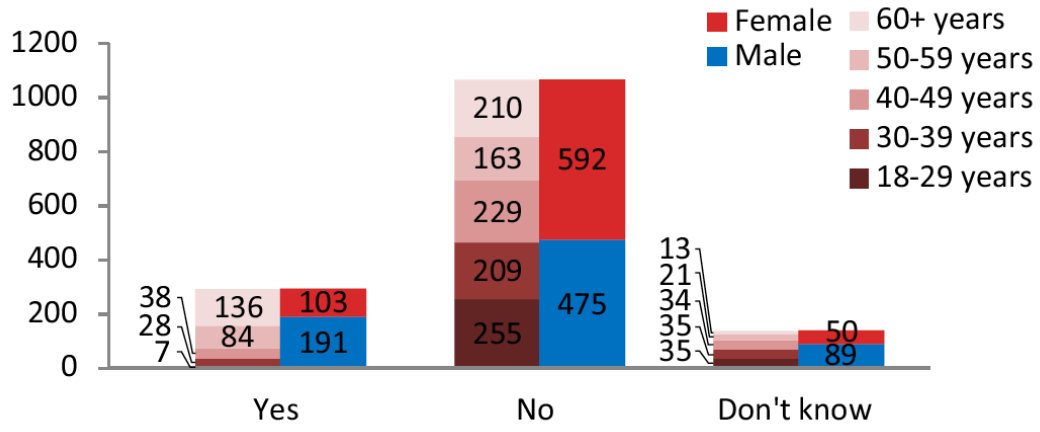

Figure 13: Age and gender distribution of Danish population with respect to blood pressure (n= 1499) is multiplied by a weight in order to adapt the distributions in the Danish population from Danish Statistics). Question asked: Do you suffer from high blood pressure? (The selected background variables are weighted in order to adapt the distributions in the population from Danish Statistics)

The results shows that men account for approximately 65 % of the incidences and furthermore 75 % are in the age group 50 +. This can be explained by physiological changes related to age, which cause the heart to work harder to pump the blood around in the body. But also revealed to be influenced by environmental and lifestyle factors such as diet with high content of salt and saturated fats (Ahhmed & Muguruma, 2010).

The hypertension problem and these results pinpoint the specific target group as middle-age due to increased risk of developing hypertension for that age group. Moreover men have increased risk compared to women.

5.2.2 Intake frequency of traditional Danish meat products

As the majority of hypertension cases are among men, and it is revealed that men eat more meat compared with women ((Fagt et al., 2008), it can be assumed that traditionally Danish meat products are good potential functional products to reach the target group. Age and gender distribution of Danish consumers with respect to intake frequency of liver paté (A), meatballs (B), salami (C) and wiener sausages D) are shown in Figure 14 and Figure 15.

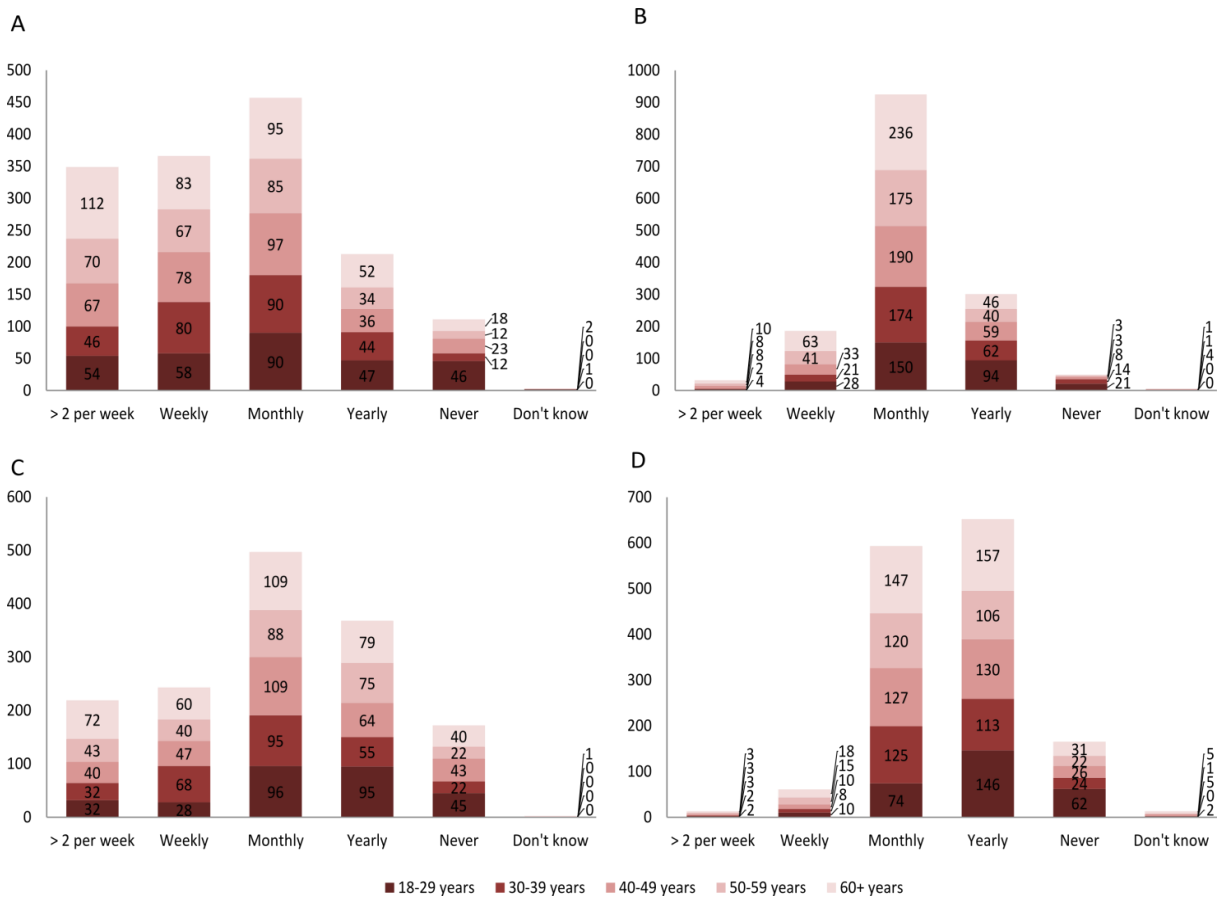

Figure 14: Age distribution of Danish consumers with respect to intake frequency of traditional Danish meat products A) Intake of liver paté, B) Intake of meatballs, C) Salami and D) Intake of wienersausage. (Age are weighted in order to adapt the distributions in the population from Danish Statistics)

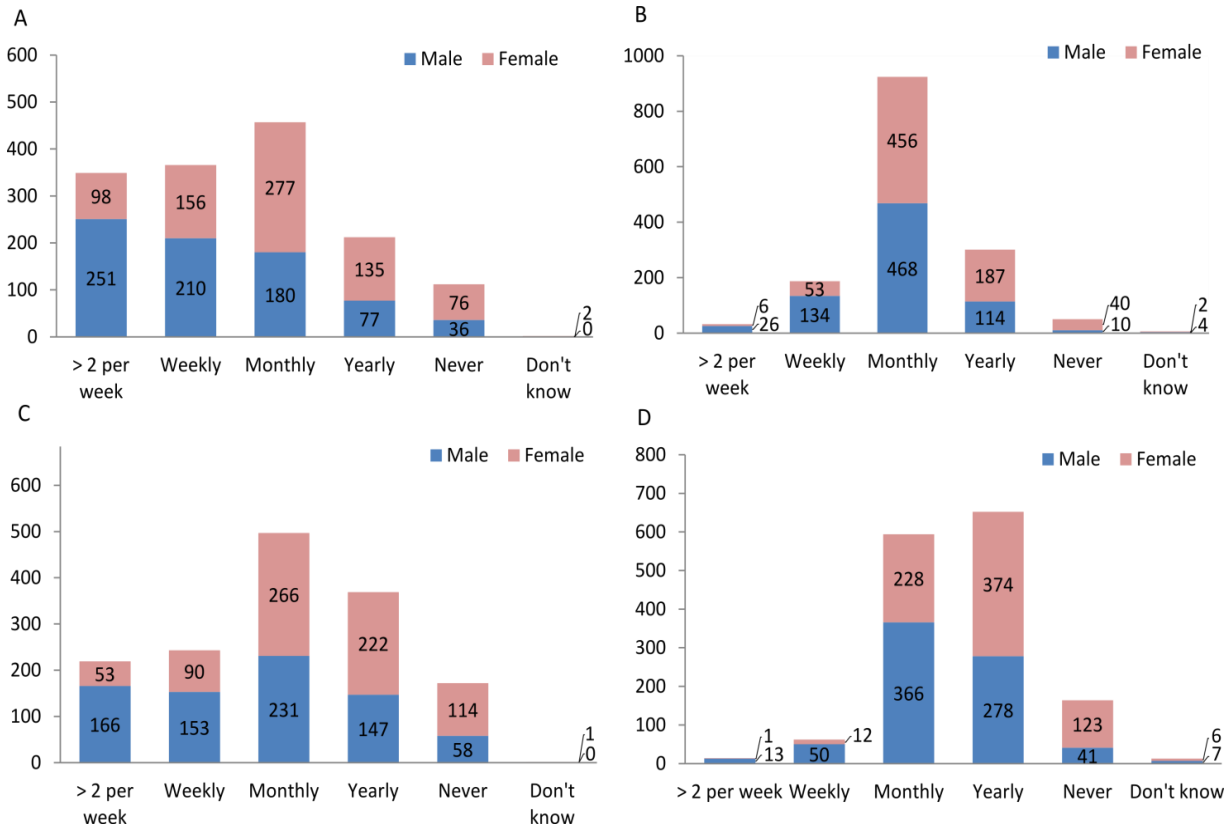

Figure 15: Gender distribution of Danish consumers with respect to intake frequency of traditional Danish meat products. A) Intake of liver paté, B) Intake of meatballs, C) Intake of Salami and D) Intake of wiener sausage. (Gender are weighted in order to adapt the distributions in the population from Danish Statistics)

In general males have are a more frequent intake than females and the intake of the products increases with age. It can be seen that liver pate is the most frequently consumed among the four types of products, which indicate that liver pate is an everyday product followed by salami. However meatballs are consumed more rarely with most frequent intake on monthly basis along with wiener sausages, which are most frequent intake on monthly and yearly basis. This is in accordance with the findings by (Holm, 1997) that lunch meal is characteristically consisting of Danish open sandwiches with typically Danish spreads such as salami or liver pate. The evening dishes have larger variations and explain that meatballs and sausages are consumed less frequent than liver pate and salami (Holm, 1997). These patterns are culture dependent and can be explained by the role of appropriateness of the four different products in different meals.

5.2.3 Taste attitudes

Taste and health have been investigated to be among important determinants when associating the consumption of different food groups and expected outcomes (Shepherd & Sparks, 1994). The Danish consumers attitude towards purchase of functional foods with respect to taste are shown in Figure 16 with age and gender as background variables. A) Consumers willingness to buy functional foods, which taste good. B) Consumers willingness to purchase functional foods, which taste less good. C) Consumers willingness to purchase functional foods, which taste less good but have a health benefit.

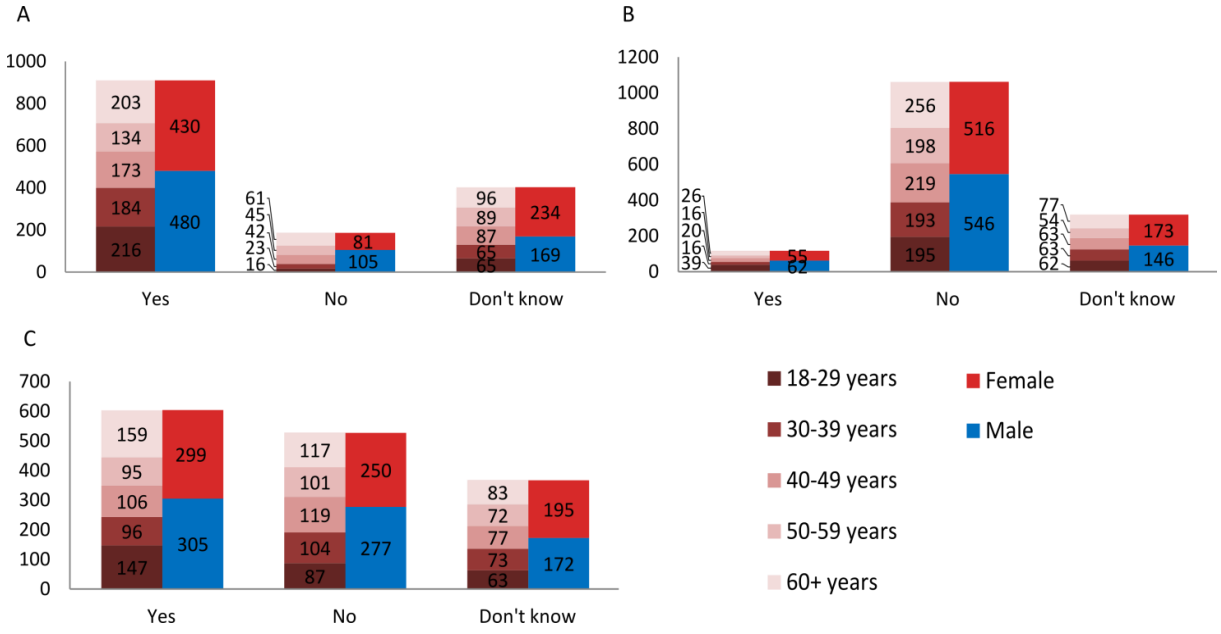

Figure 16: Attitude of Danish consumers towards purchase of functional foods with respect to flavor, age and gender. A) Consumers willingness to buy functional foods, which taste good. B) Consumers willingness to purchase functional foods, which taste less good. C) Consumers willingness to buy functional foods with an evident health benefit, but it tastes less good. (The selected background variables are weighted in order to adapt the distributions in the population from Danish Statistics).

Results from the panel of Danish consumers (Figure 16) shows that 910 out of 1499, which corresponds to 61 %, are willing to buy functional meat products, which taste good whereas only 8 % of the population are willing to buy functional foods, which taste less good. The same tendency has been found among Belgian consumers by (Verbeke, 2005). However, 40 % of Danish consumers are willing to purchase functional foods, which taste less good when an appreciable health benefit is evident. This is in accordance with the significance level in Figure 12 on page 68, that shows that compromise on taste related to intensions of buying functional products with less good taste variables have high significant impact on the model. These results conflicts with studies that shows low willingness to compromise on taste for health (K. G. Grunert, 2010; Verbeke, 2006). However the result can be interpreted as the negative prejudice of functional foods may have decreased, like it has

been found to be the case among consumers in Belgium (Verbeke, 2006). The distribution of age and gender are not significant different.

Based on these findings it is chosen to examine the developed products among Danish consumers. In principle it is decided to perform a blind-labeled test with focus on taste, where consumers taste without further information about the products. This is chosen to obtain the consumers immediate perceptions of the product and then afterwards they were asked to complete the questionnaire. In this way it is as far as possible avoided that consumers were biased towards the products.

5.3 Sensory evaluation of the products

In this chapter the sensory modalities of HPP11, HBG, HPG1.1 and HPL will be evaluated in four traditional Danish products with main focus on flavor. First results from the descriptive analysis will be reviewed followed by results from consumer tests of the products performed according to the method Holistic by DMRI™.

5.3.1 Descriptive analysis

The professional panel of DMRI has examined the sensory modalities odor, appearance, hand touch, flavor, texture and after-flavor in four types of liver pates (HPP11 8%, HPG1.1 8 %, HPL 8% and reference), three types of meatballs (HPP11 8 %, HPG1.1 8 % and reference), two types of wiener sausages (HPG1.1 8 % and reference) and three types of salamis (HBG 8 %, HPG1.1 8% and reference). Results of 2-way ANOVAs of mean attribute ratings and replicates across nine panelists are shown for the 12 products in Table 18, Table 19, Table 20 and Table 21. Almost all sensory modalities show significant differences.

Table 18: Liver Paté. 2-way ANOVA sample means of product effect are shown with significance from Bonferroni LSD in superscript.

Attribute	HPG1.1	HPL	HPP11	REF	P-values
Odor					
Liver	5.1^{abc}	8.0^{a}	3.1^{c}	6.2^{ab}	P<0.001
Spicy	5.1^{ab}	5.0^{ab}	3.5^{b}	5.6^{a}	P<0.05
Burnt	3.41^{b}	6.5^{c}	1.7^{ab}	0.6^{a}	P<0.001
Soy	2.5^{a}	7.3^{b}	2.0^{a}	0.8^{a}	P<0.001
Appearance					
Brown	6.2^{a}	11.2^{b}	5.8^{a}	5.0^{a}	P<0.001
Rose	2.1^{a}	0.4^{b}	2.3^{a}	2.7^{a}	P<0.001
Flavor					
Liver	5.9^{ab}	8.5^{b}	3.1^{a}	4.9^{a}	P<0.001
Thyme	5.1^{ab}	2.6^{bc}	3.0^{c}	6.1^{a}	P<0.01
Spicy	5.2^{ba}	5.5^{b}	4.1^{a}	5.2^{ab}	P<0.05
Salt	2.2^{a}	3.7^{b}	2.3^{a}	1.9^{a}	P<0.01
Sour	3.7^{a}	5.5^{b}	4.7^{ab}	3.8^{a}	P<0.01
Bitter	5.6^{a}	9.4^{b}	6.3^{a}	4.3^{a}	P<0.001
Sweet	3.2	3.2	4.0	3.7	NS
Soy	2.3^{a}	7.6^{b}	2.2^{a}	1.0^{a}	P<0.001
Off	2.7^{a}	3.8^{a}	8.1^{b}	1.8^{a}	P<0.01
Texture					
Firmness	7.3^{b}	6.6^{ab}	9.2^{c}	5.2^{a}	P<0.001
Roughly	6.4^{a}	6.6^{a}	4.7^{b}	6.7^{a}	P<0.001
Creamy	6.5^{a}	7.3^{ab}	8.4^{b}	6.1^{a}	P<0.01
After Flavor					
Bitter	5.2^{a}	8.7^{b}	5.4^{a}	3.9^{a}	P<0.001

Table 18 shows the mean attribute ratings in liver pate. HPL is significant different from HPG1.1, HPP11 and the reference in liver pate on burnt and soy odor, in the brown and rosa appearance, in the salty, bitter, soy flavor and the bitter after flavor. HPL's brown appearance are extremely high with an average intensity on 11.2 compared with HPG1.1, HPP11 and the reference with average intensities on

6.2, 5.8 and 5.0 respectively. HPP11 is significantly different from HPG1.1, HPL and the reference in liver pate in off flavor and roughly texture ($p<0.001$). Off flavor in HPP11 are rated quite high with average intensity 8.1 compared with HPG1.1, HPL and the reference with average intensities 2.7, 3.8 and 1.8 respectively. The sweet flavor is not significant in liver pates.

Table 19 shows the mean attribute ratings in salami. The reference is significant different from with HPG1.1 and HBG in salami on several attributes. Salami, smoked, sour and off odor, yellowness in appearance, salami, smoked, sour, salt, bitter and off flavors as well as dry and firmness in texture and sour, bitter and off in after flavor. All salamis are high significant different from each other on off odor, off flavor and off after-flavor ($p<0.001$), were HBG have the highest rated average intensities on 6.7, 8.8 and 6.6 respectively. Spicy and chemical flavor are not significant.

Table 19: Salami. 2-way ANOVA sample means of product effect are shown with significance from Bonferroni LSD in superscript.

Attribute	HBG	HPG1.1	REF	P-values
Odor				
Salami	3.9^{b}	5.8^{b}	8.4^{a}	P<0.001
Smoked	4.3^{b}	5.0^{b}	6.4^{a}	P<0.001
Sour	4.5^{b}	4.8^{b}	6.4^{a}	P<0.001
Spicy	3.5^{b}	4.1^{ab}	5.4^{a}	P<0.01
Off	6.7^{b}	3.7^{c}	0.5^{a}	P<0.001
Appearance				
Red	5.3^{b}	5.6^{ab}	7.7^{a}	P<0.05
Yellow	6.3^{b}	4.9^{b}	0.9^{a}	P<0.001
Flavor				
Salami	3.7^{b}	5.7^{b}	8.3^{a}	P<0.001
Smoked	4.8^{b}	5.4^{b}	6.5^{a}	P<0.001
Sour	5.4^{b}	5.6^{b}	7.0^{a}	P<0.001
Salt	5.9^{b}	5.8^{b}	7.7^{a}	P<0.001
Bitter	6.0^{b}	5.8^{b}	3.2^{a}	P<0.001
Spicy	3.9	4.5	5.6	NS
Chemical	5.3	4.4	3.2	NS
Off	8.8^{b}	5.2^{c}	0.5^{a}	P<0.001
Texture				
Dry	3.5^{b}	3.9^{b}	8.2^{a}	P<0.001
Firmness	4.1^{b}	4.4^{b}	8.6^{a}	P<0.001
After Flavor				
Sour	3.7^{b}	3.9^{b}	5.6^{a}	P<0.01
Smoked	3.8^{b}	4.7^{ab}	5.6^{a}	P<0.01
Bitter	4.4^{b}	4.7^{b}	2.4^{a}	P<0.01
Off	6.6^{b}	3.6^{c}	0.4^{a}	P<0.001

The fermentation of salami causes several physical, biochemical and microbial changes which can contribute with improved sensory characteristics. The formation of lactic acid and degradation of proteins and peptides to low molecular weight compounds is mainly due to the fermentation process.

These compounds are peptides, free amino acids, aldehydes, organic acids and amines and are important flavor contributors (Zhang et al., 2010).

Table 20: Wiener sausages. 2-way ANOVA sample means of product effect are shown with significance from LSD.

Attribute	HPG1.1	REF	P-values
Odor			
Smoked	6.3	9.0	P<0.001
Spicy	6.0	7.2	NS
Bouillon	3.2	2.3	P<0.05
Off	5.7	1.2	P<0.01
Appearance			
Wrinkly	2.9	8.9	P<0.001
Rose	2.0	7.3	P<0.001
Brown	7.0	1.4	P<0.01
Hand touch			
Crispy	3.7	2.9	NS
Firmness	6.5	3.5	P<0.01
Flavor			
Smoked	5.9	9.1	P<0.001
Spicy	7.1	8.3	NS
Salt	8.6	7.9	NS
Floury	3.8	1.7	P<0.01
Off	7.0	1.5	P<0.01
Sour	5.2	4.9	NS
Bouillon	4.2	3.1	P<0.05
Bitter	6.2	3.1	P<0.05
Texture			
Crisp	3.5	2.0	NS
Juiciness	4.4	5.6	P<0.05
Firmness	5.5	3.2	P<0.001
After			
Flavor			
Smoked	5.8	8.2	P<0.01
Spicy	7.1	8.1	NS

Table 20 shows the mean attribute ratings of HPG1.1 in wiener sausages and a reference. The two sausages are highly significant different ($p<0.001$) at smoked odor, rosa and wrinkly appearance, smoked flavor and at their firmness in texture. Further, the reference and HPG1.1 are significant different at off odor, brown appearance, firmness in hand touch, floury and off flavor at 1 % level ($p<0.01$). A bouillon odor, bitter and bouillon flavor as well as juiciness in texture were found at to be significant different in the two sausages at 5 % level ($p<0.05$). Off flavor in HPG1.1 are rated quite high with an average intensity on 7 compared to the reference with average intensity on 1.5. Spicy odor, crispy hand touch, spicy, salt and sour flavor, crisp texture and spicy after flavor are not significant different in the two wiener sausages.

As a result of the recipe the reference dropped a higher amount of water shown on the high intensity of wrinkly appearance compared to the reference. However it shows that HPG 1.1 binds the liquid, which can be advantageous in low-fat recipes as used in present study.

Table 21: Meatball. 2-way ANOVA sample means of product effect are shown with significance from Bonferroni LSD in superscript.

Attribute	HPG1.1	HPP11	REF	P-values
Odor				
Meatball	3.4[b]	1.5[b]	7.2[a]	P<0.001
Fried	2.8[a]	1.9[b]	3.5[a]	P<0.01
Onion	2.9[b]	2.3[b]	5.7[a]	P<0.001
Off	5.2[b]	9.5[c]	0.7[a]	P<0.001
Appearance				
External color	6.9[b]	7.8[b]	5.5[a]	P<0.001
Internal color	6.6[b]	7.5[b]	4.3[a]	P<0.001
Flavor				
Meatball	3.3[b]	2.0[b]	7.5[a]	P<0.001
Onion	3.4[b]	3.1[b]	5.9[a]	P<0.01
Spicy	5.8	5.36	5.5	NS
Salt	5.0	4.8	4.8	NS
Sweet	3.6[ab]	5.52[b]	3.33[a]	P<0.05
Sour	4.5	4.8	4.6	NS
Bitter	5.9[b]	5.4[b]	2.3[a]	P<0.01
Off	7.3[b]	10.3[c]	1.0[a]	P<0.001
Texture				
Firmness	3.8[b]	4.1[b]	5.0[a]	P<0.01
Juiciness	3.1[b]	3.0[b]	3.9[a]	P<0.01
Sticky	7.7[b]	7.93[b]	4.1[a]	P<0.001
Creamy	6.4[b]	6.2[b]	3.4[a]	P<0.001
After Flavor				
Off	6.2[b]	8.4[c]	0.9[a]	P<0.001
Spicy	6.2	5.8	5.9	NS
Onion	3.0[b]	3.1[b]	5.7[a]	P<0.001

Table 21 shows the mean attribute ratings in meatballs. HPG1.1, HPP11 and the reference are significant different from each other on off odor, off flavor and off after flavor ($p<0.001$). The reference is significant different from with HPG1.1 and HPP11 on meatball and onion odor, external and internal color in appearance, meatball, onion and bitter flavors as well as firmness, juiciness, sticky, and creamy in texture and onion in after flavor. There is no significance difference in the spicy and salt flavor and spicy after flavor in the meatballs.

Overall, the developed products differ from each other within the four types of hydrolysates. Bitter and off odor, off flavors and off after flavor is prominent in products with the hydrolysates compared to the references, which also are expressed as higher intensity values in the ratings means. This is not surprising as hydrolysates themselves are described as bitter. The hydrolysates contain short peptides since they are ACE inhibiting, and it is found that the intensity of bitterness increases as peptide length gets shorter dependent on the sequences (Nishimura & Kato, 1988).

5.3.2 Consumer perception of taste

This part will evaluate the sensory perception of the products by consumers. The 12 developed products have been examined according the Holistic by DMRI™ method. In total 170 consumers were assessing either meatball and salami or liver pate and wiener sausages on their perception of 10 abstract words in relation to taste. Results of 2-way ANOVAs of mean perception (1 replicate) are shown in Table 22 and Table 23. In total 85 consumers found highly significant differences at 0.1 % level ($p<0.001$) when tasting salami and meatball. The 85 consumers who tasted liver pate and wiener sausages found significant difference at a minimum of 5 % level ($p<0.05$). Such variation is assumed to be acceptable among consumers in a blind test.

Table 22 shows the mean perception of the 10 holistic words related to the taste of three types of salamis and three types of meatballs. HPG1.1, HPP11 in meatball were significant different from the meatball reference and all three salamis on the perception of traditional, different, familiar, strange, exclusive, appetizing and chemical. The reference meatball was perceived as the most traditional in the taste. HPG1.1 in meatball in average is perceived with a higher strange intensity in taste whereas HPP11 in meatball on average is rated with highest perceived intensity of chemical taste.

Table 22: 2-way ANOVA (1 Rep) sample means of product effect of salami (S) and meatballs (MB) from consumer tests (n=85) are shown with significance from Bonferroni LSD in superscript.

Attribute	S_HPG1.1	S_REF	S_HBG	MB_HPP11	MB_HPG1.1	MB_REF	P-values
Traditional	9.1^{a}	9.2^{a}	9.3^{a}	6.9^{b}	7.0^{b}	10.0^{a}	P<0.001
Different	7.1^{a}	6.7^{a}	7.0^{a}	8.9^{b}	8.9^{b}	5.8^{a}	P<0.001
Familiar	9.5^{a}	9.5^{a}	9.2^{a}	7.2^{b}	7.0^{b}	9.9^{a}	P<0.001
Strange	6.0^{a}	6.4^{a}	6.2^{a}	8.5^{b}	9.5^{b}	5.8^{a}	P<0.001
Boring	8.1^{a}	8.7^{ab}	8.5^{a}	9.9^{b}	9.9^{b}	8.7^{ab}	P<0.001
Exclusive	5.5^{a}	5.3^{a}	5.7^{a}	4.0^{b}	3.6^{b}	5.6^{a}	P<0.001
Discount	7.3^{a}	8.0^{ab}	7.8^{ab}	9.3^{bc}	9.6^{c}	8.5^{abc}	P<0.001
Appetizing	7.8^{a}	7.2^{a}	7.7^{a}	5.8^{b}	5.4^{b}	8.2^{a}	P<0.001
Natural	8.0^{a}	7.7^{a}	7.9^{a}	6.4^{b}	6.1^{b}	8.7^{a}	P<0.001
Chemical	6.3^{ab}	6.7^{ab}	6.5^{ab}	8.4^{c}	7.7^{bc}	5.6^{a}	P<0.001

Table 23: 2-way ANOVA (1 Rep) sample means of product effect of Liver pate (L) and Wiener sausages (W) from consumer test are shown with significance from Bonferroni LSD in superscript.

Attribute	L_HPP11	L_HPG1.1	L_HPL	L_REF	W_REF	W_HPG1.1	P-values
Traditional	7.2^{a}	8.4^{ab}	7.2^{a}	9.0^{b}	9.4^{b}	9.7^{b}	P<0.001
Different	9.4^{a}	7.3^{b}	9.5^{a}	7.7^{b}	7.4^{b}	7.1^{b}	P<0.001
Familiar	6.5^{a}	8.1^{bc}	6.8^{ab}	8.8^{c}	9.3^{c}	9.3^{c}	P<0.001
Strange	8.9^{a}	7.4^{ab}	8.7^{a}	6.1^{b}	6.0^{b}	6.4^{b}	P<0.001
Boring	9.6	9.6	8.6	8.7	8.2	8.3	P<0.05
Exclusive	4.7^{a}	5.1^{ab}	5.3^{ab}	6.3^{b}	4.8^{a}	4.8^{a}	P<0.01
Discount	9.9^{a}	8.3^{bc}	8.7^{abc}	7.8^{c}	8.5^{abc}	9.4^{ab}	P<0.01
Appetizing	5.9^{a}	6.8^{abc}	6.2^{ab}	7.8^{c}	6.5^{abc}	7.4^{bc}	P<0.001
Natural	6.6^{a}	7.5^{ab}	7.5^{ab}	8.6^{b}	7.7^{ab}	7.2^{ab}	P<0.01
Chemical	7.7^{a}	7.0^{ab}	7.2^{ab}	5.8^{b}	6.8^{ab}	7.8^{a}	P<0.001

As seen from the mean perception of 10 holistic words in taste of four different types of liver pates and two types of wiener sausages (Table 23), HPP11 and HPL in liver pates are significantly different from HPG1.1 in liver pate, the liver pate reference and the two types of wiener sausages on the perception of "different". The consumers perceived boring in the products with significance difference at a 5 % level ($p<0.05$) from the overall ANOVA but when corrected the p values to take into account Bonferroni multiple comparison, it was failed to reach significance. By inspection of the average intensities of boring it should be mentioned that the HPP11 and HPG1.1 were perceived with the highest intensity. HPP11 in liver pate are in average perceived with the highest intensity of strange in taste. In average HPG1.1 in wiener sausages are is perceived as more appetizing compared to the reference. Among the liver pates the reference is perceived most appetizing followed by the HPG1.1.

As consumers have different strategies for food choices and individually preferences, they perceive products differently. Hence it is important that consumers within the target group judge the products. A trained panel is not optimal for this type of rating as the target consumer is assumed to be in the middle age and in risk of developing hypertension. The Holistic by DMRI™ method requires no training because it is the individual consumer's own perception of the words that underlie the assessment. This form of assessment is a very effective way to evaluate how the products differ from each other. In the present study it is an advantage because the method allows consumer differences where perception and evaluations of foods reflect habitual conscious and unconscious decisions. According to (Furst et al., 1996) and (Connors et al., 2001) food choice and preferences are based on individual strategies where taste perception is an individually value. The Holistic by DMRI™ method comply with the complexity of the food choice process in a heuristic approach as it evaluates from the consumers perspective.

5.3.3 Overall interpretation of taste and perception

Holistic by DMRI™ method is applicable to describe products with sensory differences and by comparing the results with a sensory descriptive analysis, it is possible to see which sensory properties that may explain variations in the holistic assessment among the products. The average of common sensory descriptors (X) was related to the average consumer perception of holistic words (Y) by PLSR and describes how HBG, HPP11, HPG1.1 and HPL influence the taste in the four products. The analysis is performed on two consumer groups who tasted either liver pate and wiener sausages (Figure 17) or salami and meatball (Figure 18).

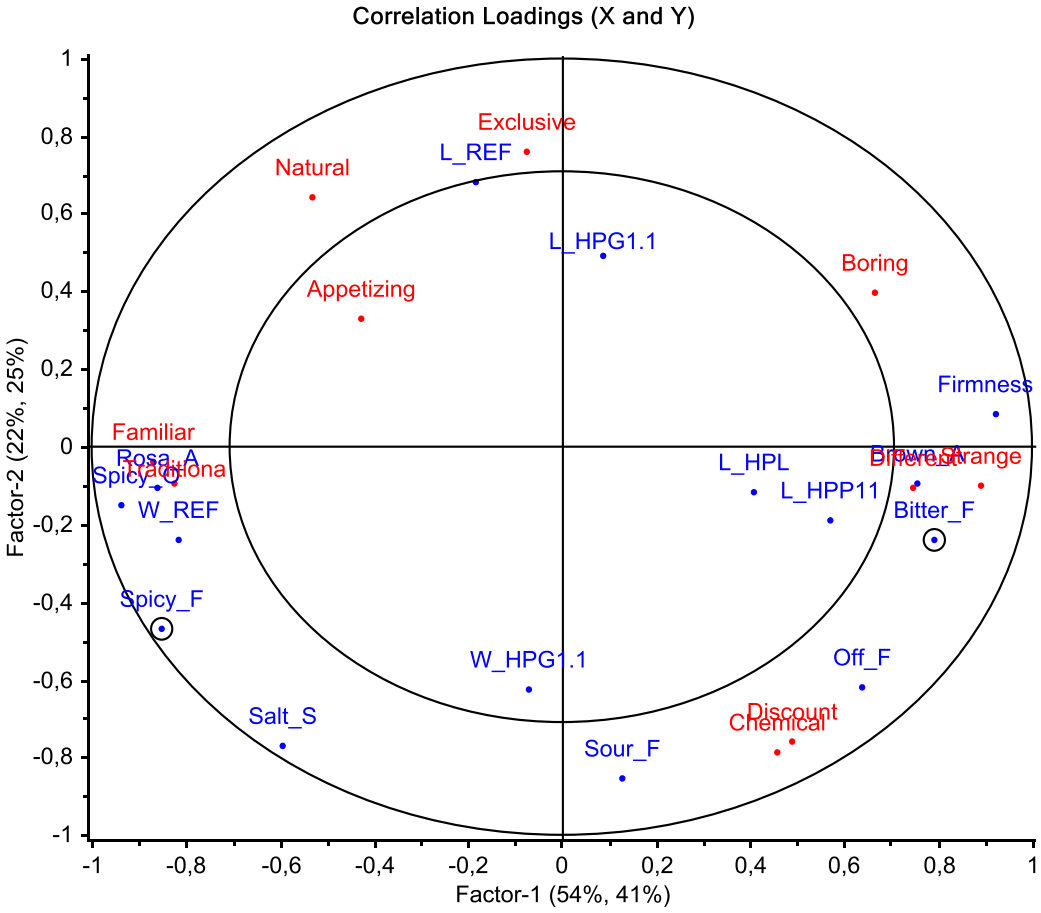

Figure 17: APLS correlation loading for average of sensory descriptors and product matrix in blue (X) and Holistic in red (Y). The two first dimensions showing the difference among HPL, HPP11, HPG1.1 and a reference (REF) in Liver pate (L) together with HPG1.1 and a reference (REF) in Wiener Sausages (W). Full cross validation with all six products. The inner and outer circles indicate 50 and 100 % explained variance, respectively.

The two first principal components describe 66 % of the variance in taste of wiener sausages and liver pates (Figure 17). The horizontal dimension describes novelty going from familiar and traditional to different and strange. Familiar and traditional is associated with rose appearance and spicy odor in the reference of wiener sausages while different and strange is associated with bitter flavor, firmness in

texture and brown appearance in HPL and HPP11 in Liver pate. The vertical dimension describes the quality parameters in which discount and chemical are associated to sour and off flavor, which are negatively correlated to the liver pate reference, which is perceived as exclusive and natural. Liver pate containing HPG1.1 and the perception of appetizing is described with 50 % variance but is mostly correlated to exclusive and natural as well. Salty and spicy flavors show a slight correlation to traditional and familiar and are anti-correlated with boring. The consumers did not perceive boring with significant differences and it could be interpreted that the consumers did not have a general conventional perception of boring and the word can be associated in different way. As the tested products are common consumed Danish meat products, it means that they are well known by the Danish consumers and therefore can be perceived as boring by default. However, if the tasted product is perceived as boring, it could be interpreted, as the product does not differ from the reference taste expected by the consumer. HPG1.1 in wiener sausage can slightly be associated to sour and off flavor, but must be interpreted with caution, as only 50 % of the variance is explained. The result agree with the descriptive results as HPL is significantly different from HPG1.1, HPP11 and the reference in the brown and rosa appearance along with bitter flavor and HPP11 is significantly different from HPG1.1, HPL and the reference in off flavor in liver pate. HPG1.1 in liver pate can be associated to the liver pate reference which is substantiated in the descriptive attributes whereas HPG1.1 did not differ from the reference attributes, except from burnt odor and firmness in texture (Table 18 page 74). The wiener sausages reference was significantly different in the rosa appearance (Table 20 on page 76, wiener).

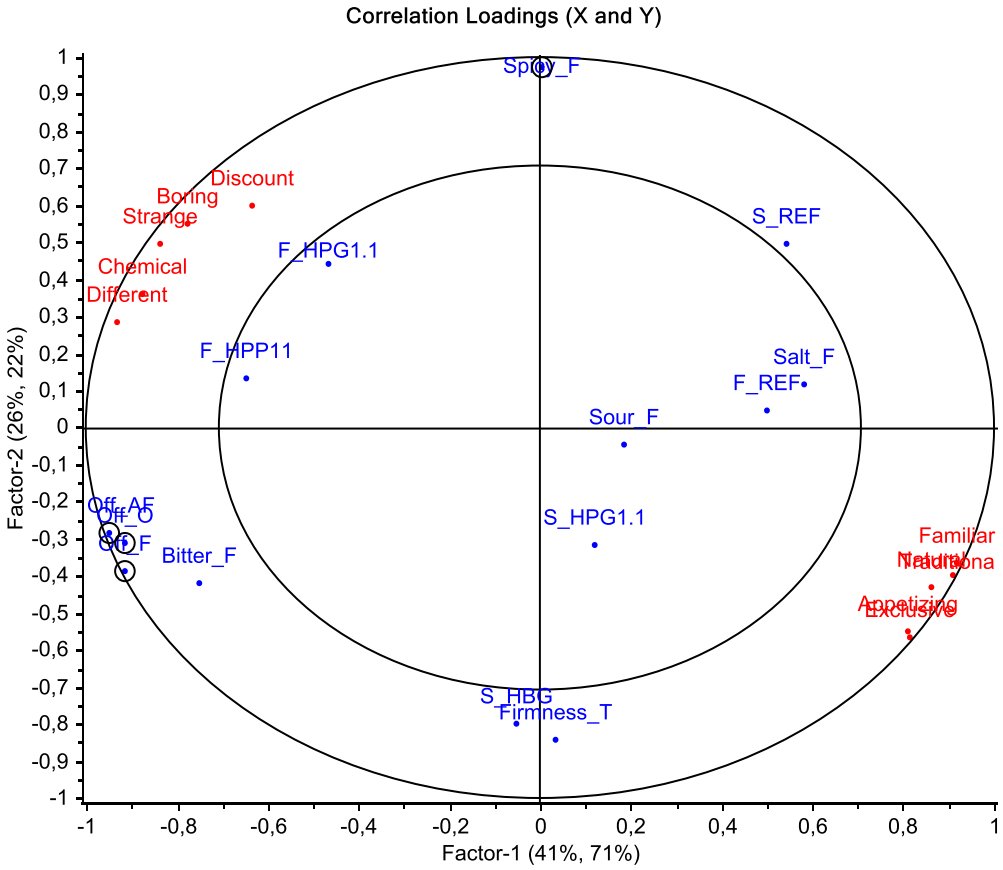

Figure 18: APLS correlation loading for average of sensory attributes and product matrix in blue (X) and holistic perception in red (Y). The two first dimensions showing the difference among HBG, HPG1.1 and a reference (REF) in Salami (S) together with HPP11, HPG1.1 and a reference (REF) in Meatball (F). Full cross validation with all six products. The inner and outer circles indicate 50 and 100 % explained variance, respectively.

The two first principal components describe 93 % of the variance in the taste of salami and meatball (Figure 18). The holistic words are clearly clustered in two anti-correlated groups. Left group encompass strange, different, chemical, boring and discount which can be associated with off odor, off flavor, off after flavor and bitter flavor in meatball with HPP11 and HPG1.1. These are reversely correlated with familiar, traditional, natural, appetizing and exclusive. The meatball and salami reference seems to be associated to salt flavor, but only explained with 50 % variance. In spite of this the salt flavor are not described with significant difference in the meatball attributes where as it is in the salami reference. The salami reference is anti-correlated with the off and bitter modalities. HBG in salami seems to be particular firm and low in spicy flavor.

The result from PLS shows the relationship between the variations between common sensory descriptors and consumer perception of the products and how different hydrolysates is connected. When comparing the different product categories, there is a clear difference between hydrolysate and product and how consumers perceive the taste of the products. The clusters of anti-correlated holistic

words is clear in meatball and salami which is perceived with higher significant differences among the consumers (Table 22, page 78) compared with liver pate and wiener sausages (Table 23, page 79). The result could express a tendency in the perception of liver pate and sausages as different among the consumers whereas meatball and salami is perceived more similar. Moreover it could be interpreted as if the hydrolysates are less prominent in the liver pate and wiener sausages as compared to the salami and meatball products. It seems that the liver pate and wiener sausages can be classified by novelty and quality parameters (Figure 17 on page 80). The off and bitter modalities is prominent in products with the hydrolysates compared to the references in the descriptive analysis (Table 18, Table 19, Table 20 & Table 21), whereas they by the consumers in general are associated with strange, different, chemical, boring and discount (Figure 17 and Figure 18).

5.4 Appetizing as a predictor for liking and preferences

The following chapter will evaluate results on consumer perception of appetizing to predict liking and preferences, which is important to understand in respect to development of functional meat products containing meat hydrolysates. As the descriptive and holistic evaluation do not reveal consumers accept and preferences of the product and hydrolysates, following section will evaluate appetizing used as predictor for liking of - and preferences for the four types of products. The aim is to reveal the consumer's drivers of liking and additionally the relationships between consumer liking and the sensory evaluation is investigated to identify the consumer's individual preferences.

5.4.1 Drivers of 'liking'

Drivers of liking is investigated and related to the four types of products. As the Holistic by DMRI™ data is a result of the individual consumer own perception that motivate their assessment, it is of interest to see how the different variables are related and if there is differences between the two consumer groups. The two plots in Figure 19 shows PLSR loading plots for consumer perception of the holistic words familiar, traditional, boring, discount, chemical, strange, different, exclusive and natural variables (X) related to their perception of appetizing (Y).

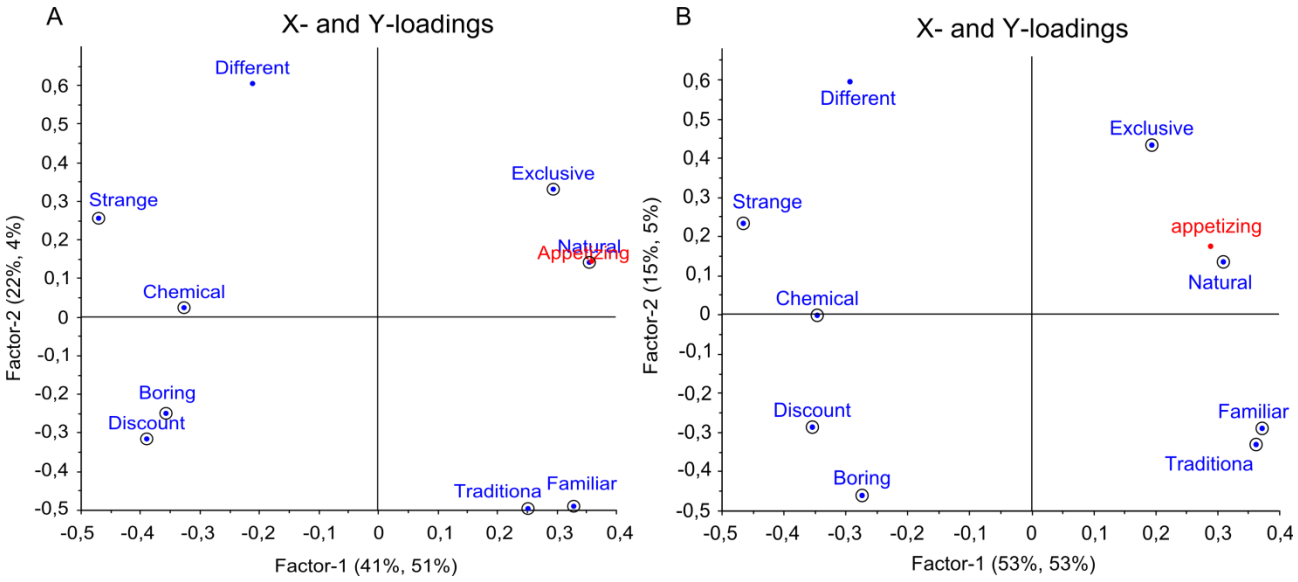

Figure 19: PLSR loading plot for consumer perception of familiar, traditional, boring, discount, chemical, strange, different, exclusive and natural related to perception of appetizing. Two groups of 85 consumers were judging either liver pate and wiener sausages (A) or meatball and salami (B) on their perception of 10 abstract words in relation to taste.

The two first principal components describe 55 % of the variance in perception of appetizing in wiener sausages and liver pate (Figure 19, A) and 58 % of the variance in perception of appetizing in

meatball and salami (Figure 19, B). The two plots are almost identical and confirm that the two groups of consumers are similar in their perception of appetizing related to the other variables. For both consumer groups natural are the big driver of appetizing with respect to the four different products. Chemical is anti-correlated and can be interpreted as the opposite of natural in the consumer perception. The perception of exclusive is correlated to appetizing and negatively correlated with boring and discount. Familiar and traditional are correlated reversely correlated with different and strange.

PLSR loading plots (Figure 19) clearly show how consumers' perceptions of the 10 holistic words are related in dimensions of novelty, quality and naturalness parameters, where naturalness are the big driver of liking in both consumer groups. It is in accordance with the fact that the more a product is perceived as natural and homemade the higher liked and more accepted among consumers (K. G. Grunert, 2010). These results underlines the tendency derived from the interpretation of the common descriptive attributes related to the perception of holistic words of liver pate and wiener sausages that were classified by novelty and quality parameters (figure x). As consumers seldom consume a food product they do not like whereas the consumption increases of those foods products with high liking (Graaf et al., 2007; Yeomans & MacFie, 2007), the perception of naturalness seems to be dominant for food choices. Nevertheless there is situations in which people choose the less liked food on the background of their state of hunger or other different motives.

5.4.2 Preference analysis

Consumer preferences are investigated and related to the four types of products. The consumers individual ratings for appetizing are used as predictors for preferences. The average of common sensory descriptors and the average consumer perception of holistic words (X) are related to all the individual consumer perceptions of appetizing by a PLSR preference mapping. The preference models were made on the two different consumer groups, which tasted either liver pate and wiener sausages (Figure 20) or salami and meatball (Figure 21), respectively.

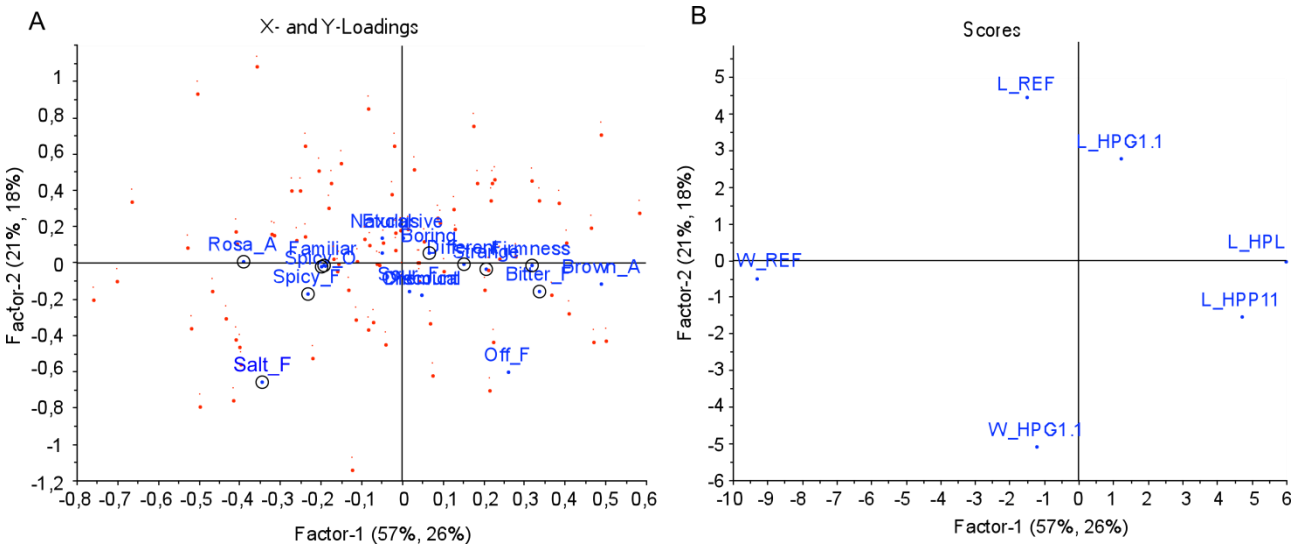

Figure 20: (A) PLSR loading plot for consumer perception of appetizing as a prediction of liking. Average of common sensory attributes and perception of holistic for wiener sausages and liver pate in blue are related to consumers preference (appetizing) represented by red dots. (B) Score plot for two wiener sausages (W) and four liver pates (L).

The two first principal components describe 44 % and of the variance of the consumer's preferences with respect to wiener sausages and liver pate (Figure 20, A). The score plot in Figure 20 (B) shows which products are responsible for the pattern seen in the X and Y - loading plot. Liver pate is located in the upper right side and the wiener sausages in the lower left side similar to Figure 17 in section 5.3.3 Overall interpretation of taste and perception. The horizontal dimension describes the hydrolysates in which the wiener sausages reference is anti-correlated with HPL and HPP11 in liver pate. The vertical dimension mainly describes the product preferences. The results of preference map show that the 85 consumers have clear differences in their 'preference' for the wiener sausages and liver pate products, as the consumers are spread in all four quadrants.

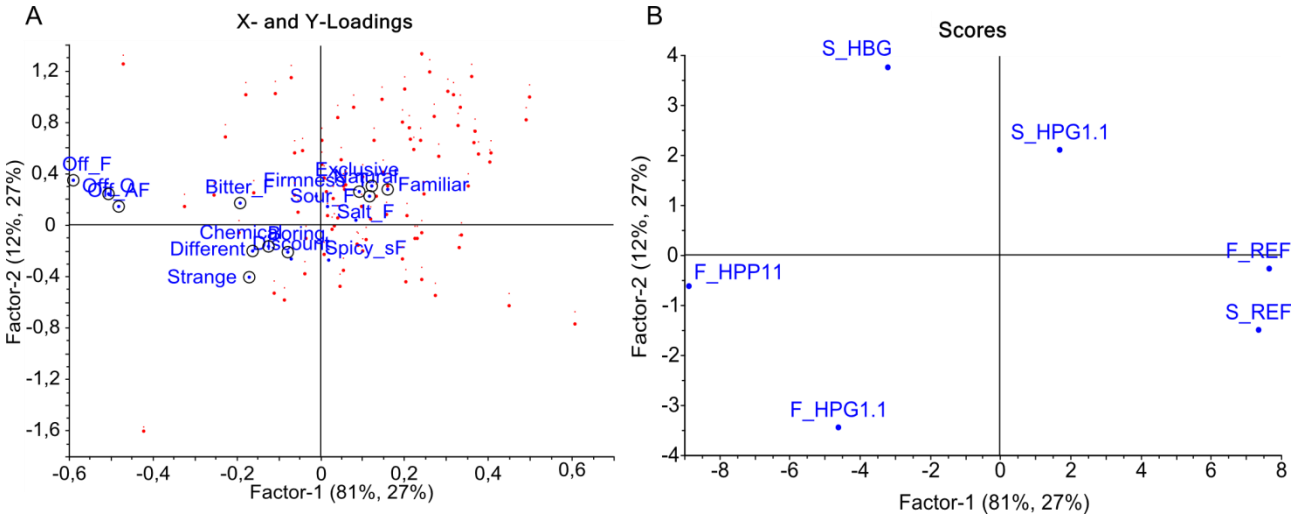

Figure 21: (A) PLSR loading plot for consumer perception of appetizing as a prediction of liking in salami and meatballs. Average of common sensory attributes and holistic in blue are related to consumers liking (appetizing) represented by red dots. (B) Score plot showing three meatballs (F) and three salamies (S).

The two first principal components describe 54 % of the variance in the consumer's preferences with respect to meatball and salami (Figure 21, A). The score plot in Figure 21, B show which products are responsible for the pattern seen in the X- and Y- loading plot. The horizontal dimension describes the hydrolysates having both the meatball and salami references negatively correlated with HPP11 in meatball. The vertical dimension mainly describes the product preferences. The results from preference map on salami and meatball shows that the 85 consumers mainly are clustered from the center to the upper right quadrant which is associated to HBG1.1 salami and the references in the score plot (Figure 21, A and B) and are negatively correlated to meatball with HPP11 and HPG1.1 in the lower left quadrant.

The plots in Figure 20 and Figure 21 illustrates how the average of common sensory attributes and perception of the holistic words can be related directly to the consumers in the loading plots (A) whereas the score plot is related to the product (B). The results show that the first consumer group has different perceptions of appetizing for wiener sausages and liver pate, in contrast to the second consumer group, which found salami more appetizing. As the preference is predicted from the consumer perception of appetizing (Danish "indbydende") the results from preference mapping must be seen more as how appealing the products are perceived rather than an exact consumer preference. The consumers did not have the opportunity to choose between the products, but as far as possible, they had the opportunity to choose between the two sets of products until only one set were left.

The off and bitter modalities, which is found prominent in meatball with HPP11 and associated with strange, different, chemical, boring and discount, is therefore perceived as unappetizing. The salami with HPG1.1 seems to be perceived as the most appetizing among the salamis and meatball products

in Figure 21. On the other hand the consumers perception of liver pate and wiener sausages are not clustered thus none of the products seem to be perceived as more appetizing than others. Again it indicates that the 85 consumers perceived the wiener sausages and liver pate products differently. Compared with the interpretations of the descriptive and holistic results in Figure 17 it seems that consumers have different preferences related to the sensory descriptors. Some find the brown appearance and bitter flavor of HPL in liver pate appetizing were as others find rose appearance and spicy odor in the reference of wiener sausages more appetizing. As liver pate and wiener sausages can be classified by novelty and quality parameters it seems that consumers perceive these parameters different in the two types of products. It can substantiate the interpretation that the off and bitter modalities related to the hydrolysates are not as prominent in the liver pate and wiener sausages as it is in the salami and meatballs. Moreover, the off and bitter modalities perceived as appetizing by some parts of the consumers can be related to different and strange in the novelty dimension.

6 Consumer questionnaire

The following chapter will evaluate consumer background and attitudes derived from the questionnaire (n=157). This information can be related to sensory modalities and the holistic perception to interpret and understand the reasons for the different perceptions of a product. The aim is to reveal relationships between liking (perception of appetizing) and sensory evaluation related to age, gender, state of health with respect to hypertension and attitudes.

6.1 Consumer background and attitudes

The online questionnaire completed by 1499 Danes at Defgo.net was completed by 157 of the 170 consumers after the test of meat products containing hydrolysates. These consumers must be representative minority of the Danish population in the target age group for the research to be valid. The background variables of 157 consumers are represented in Table 24. The middle age part of the consumers is represented in a higher percentage when compared to the large survey. This can only be seen as an advantage in this study as the general middle-aged population is assumed to be the target population of interest. The 60+ is represented in a low degree. 11.5 % of the consumers have hypertension which is a lower representation compared to the larger survey having 23.9 %.

Table 24: Overview of consumers from the tasting completing the questionnaire.

	n	Percent	Percent from Dk survey[b]
Gender			
Male	74	47.1	52.5
Female	83	52.9	47.5
Demographic			
Capital	33	21	34.2
Zealand	112	73.3	16.1
South Denmark	6	3.8	22
Central Denmark	5	3.2	20.1
NorthDenmark	-		7.6
Outside Denmark	1	0.6	-
Age group (years)			
18-29	13	8.3	10.3
30-39	32	20.4	12.5
40-49	56	35.7	19.4
50-65	45	28.6	28.3
60+	11[a]	7	29.5
Status of blood pressure			
Hypertension	18	11.5	23.9
Normal blood pressure	126	80.25	67.8
Do not know	13	8.3	8.2

[a]The 11 consumers in the 60+ group were between 60 and 64 and are included in the middle-aged category.

[b]To compare with the large questionnaire survey (n=1499)

To gain knowledge on the 157 consumers an overview of interdisciplinary relations between the variables are shown in Figure 22 as a results from PLSR of the background and factual data (X) related to attitudes data (Y). The exact same variables from the questionnaire answered by 1499 Danes are chosen for analysis.

The two first PLSR components described a total of 22 % of the variance in the attitude data. The plot spans two main components (factor 1 and 2) and is almost identical with Figure 11 (page 65) that shows results of the same variables from the large questionnaire survey in Denmark. The results shows that the consumers can be divided into three groups of consumers similar to respondents from the large questionnaire survey as revealed in section 5.2 page 63.

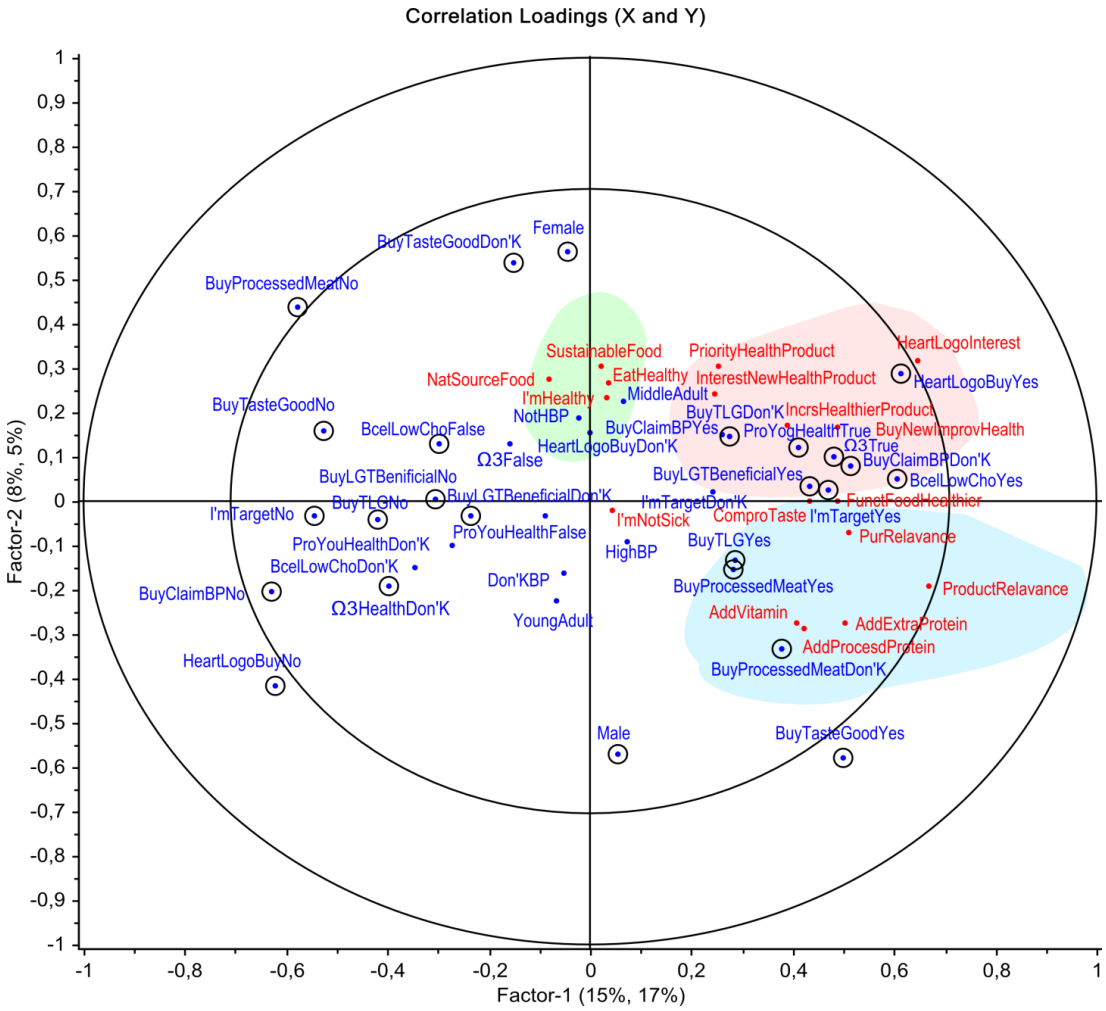

Figure 22: Partial Least Squares regression on X = background and factual data related to Y = attitude loading. Jack-knifing by full cross validation with 157 segments. Optimal number of factors used for uncertainty test: 2. The inner and outer circles indicate 50 and 100 % explained variance, respectively. Three identified consumer groups are illustrated with green, red and blue background colors. Labels are explained in Table 3, page 42.

To examine the variables impact on the model the P-values of the beta-coefficients are showed in Figure 23. The significance level shows consumers perception of own health, attitudes to functional

foods, taste and processed proteins and how these variables are related to the factual and background data. The darker the blue the higher the impact, the variables have on the model. When comparing the P-values from Figure 23 with of the beta-coefficients from the large questionnaire survey (Figure 12, page 68), the variables have a lower impact on the model. They are not significant seen by the high frequency of the lightest blue and white squares ($P>0.05$) However, both female and male have significant impact on health, nature and additives attitude variables at a 5 % level ($p<0.05$). In addition, added proteins, claims, taste, healthiness related to functional meat products and perceived purchase relevance is variables that interact with high significant impact ($P<0.001$).

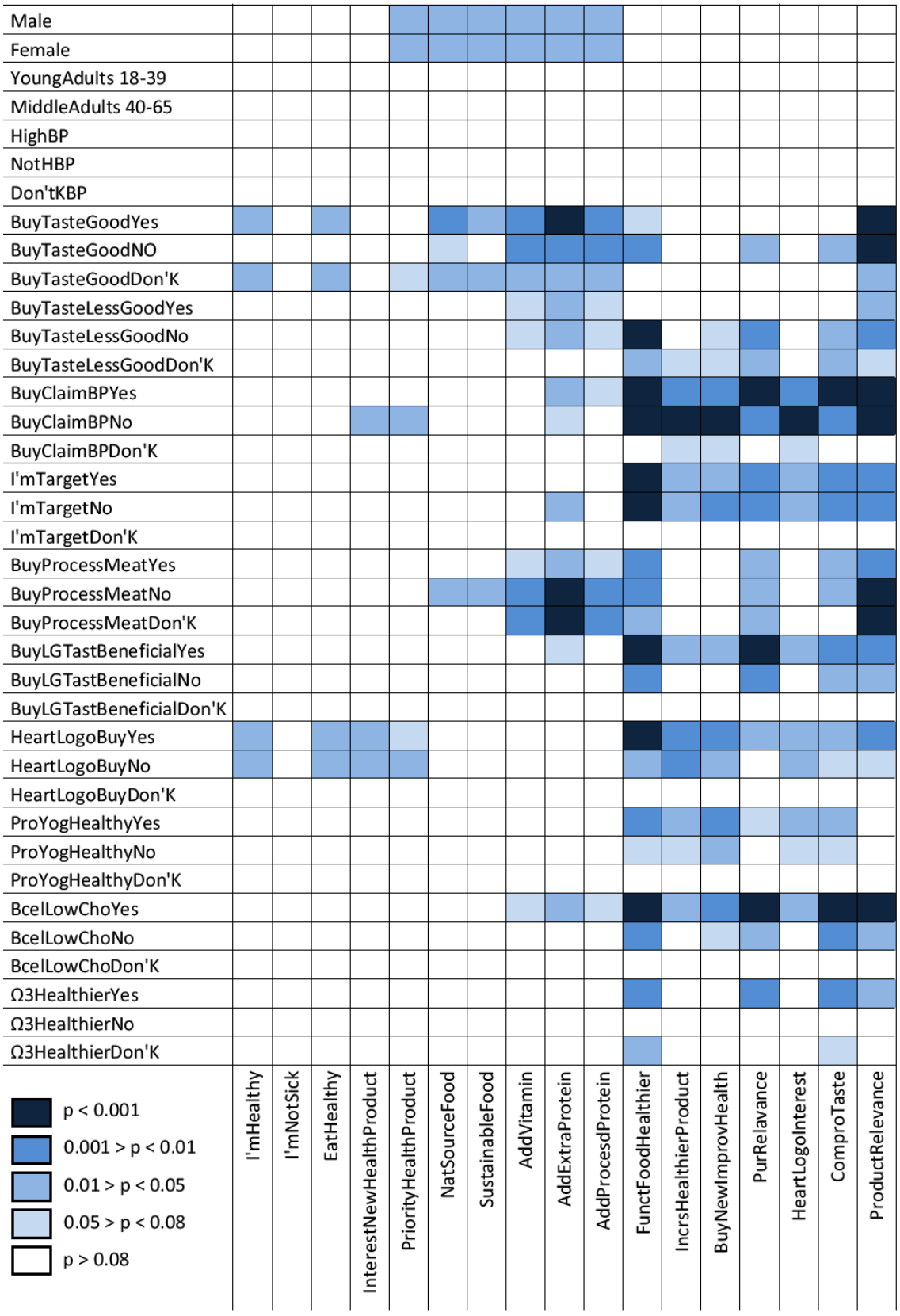

Figure 23: P-values of Beta-coefficients determined by jackknifing at optimal factors (2 factors). Graphical summary of significance level are quantified to five levels according to indications in the lower left corner.

The model in Figure 23 is build on the two first factors where as the model from the 1499 respondents in Figure 12 are build on the three first factors. As the impact of noise in the large survey data would be more equalized by the high number of respondent, the noise in the consumer survey data with 157 respondents would have a higher impact, hence, the significant differences not as clear in the small survey data when comparing the two models. Overall, the 157 consumers is a representative minority of the Danish population in the target age group, comparable to that of the large panel of Danish consumers.

6.1.1 LPLS Regression

Following results illustrates the consumers individual perception of appetizing (Y) explained by the sensory descriptive data together with the holistic words in (X) and background variables or attitude variables (Za and Zb respectively) analyzed by L-PLSR for wiener sausages and liver pate as well as meatball and salami. The plots will mainly be commented on how the background characteristics and attitudes are related to the two identified relations between sensory modalities and perceptions of the products.

6.1.1.1 Consumer perception of appetizing explained by product descriptors and background

The individual perception of appetizing explained by the variables gender, age group and state of health in respect to hypertension and the sensory descriptive data are investigated by L-PLSR. The covariation between common sensory descriptive attribute and holistic (X), consumers perception of appetizing (Y) and consumer background variables (Za) are visualized in correlation loadings plot in Figure 24 and Figure 25 for wiener sausages and liver pate as well as meat ball and salami respectively.

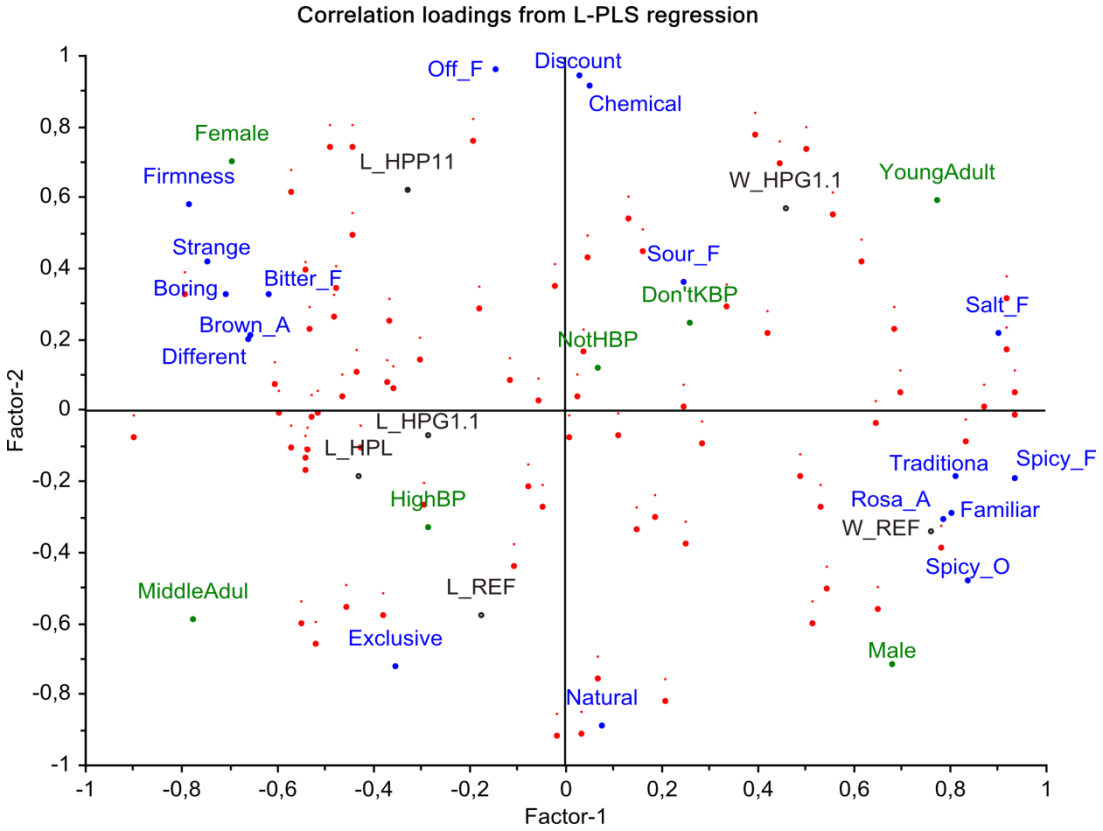

Figure 24: Liver paste and wiener sausages. Correlation loadings plot of the L-PLS model for the first two components (Factor -1 and -2). Average of sensory attributes and holistic perception of two wiener sausages (W) and four liver pates (L) are represented in blue (X) (O=odor, F=flavor, A=appearance). Consumers individually perception of appetizing are represented by red dots (Y); consumer background information issued from the questionnaire is shown in green (Z). Explained Variance of factor -1 and -2 are 49.2 % and 73.3 % in X, 1.9 % and 2.4 % in Y and 37.4 % and 69 % in Z respectively. HighBP = hypertension, Don'KBP = Do not know their blood pressure, NotHB = normal blood pressure

In the horizontal dimension (Figure 24) there is contrast between the meat products and novelty. As a result the wiener sausages reference is traditional and familiar, and opposite correlated to liver pate containing HPL and HPP11. The perceptions of appetizing are different between the six products shown by the scattered density of consumers (red dots) must be interpreted with caution, as explained variance of Y is very low of 1.9 % and 2.4 % in factor-1 and-2 respectively. The vertical dimension mainly are described by quality parameters were off flavor are associated with chemical and discount and as unnatural. The holistic perceptions and common sensory descriptors are related similar as described in Figure 17 (section 5.3.3 Overall interpretation of taste and perception) with no obvious differences. Gender and age groups describe the two diagonals. The first diagonal describes age parameters. Middle adults are associated with the liver pate reference, high blood pressure and exclusivity are reversely correlated with the young adults which are associated to with HPG1.1 in wiener sausages. Genders are opposite correlated in the other diagonal. Female are associated to HPP11 in liver pate and perceptions of strange, different, firmness, boring where male are opposite. The first principal component contrast the young males are in the right side associated with highest

perception of familiar and traditional and rose appearance, salty flavor and spicy odor in the two wiener sausages. Middle-aged females are in the right side and are related to bitter flavor, firmness in texture and brown appearance in liver pate.

The results in Figure 24 could be interpreted as male have higher preferences for wiener sausages and in general are associated with the more positive perceptions of the products and the females perceive the liver pate with more skepticism. This can be substantiated by the findings of (Verbeke, 2005) and (Turrell, 1997) who reveal that women is more thoughtful about food to a higher degree than men. The results can be related to the fact that males in general have a higher and more frequent intake of wiener sausages and liver pate compared with females as revealed in Figure 15 (page 71). This can explain the correlation of male with perceptions of familiar and traditional. This is in agreement with the finding by (Beardsworth et al., 2002) who showed that men consumed more meat than women as well as women and men have different perceptions and understanding of meat.

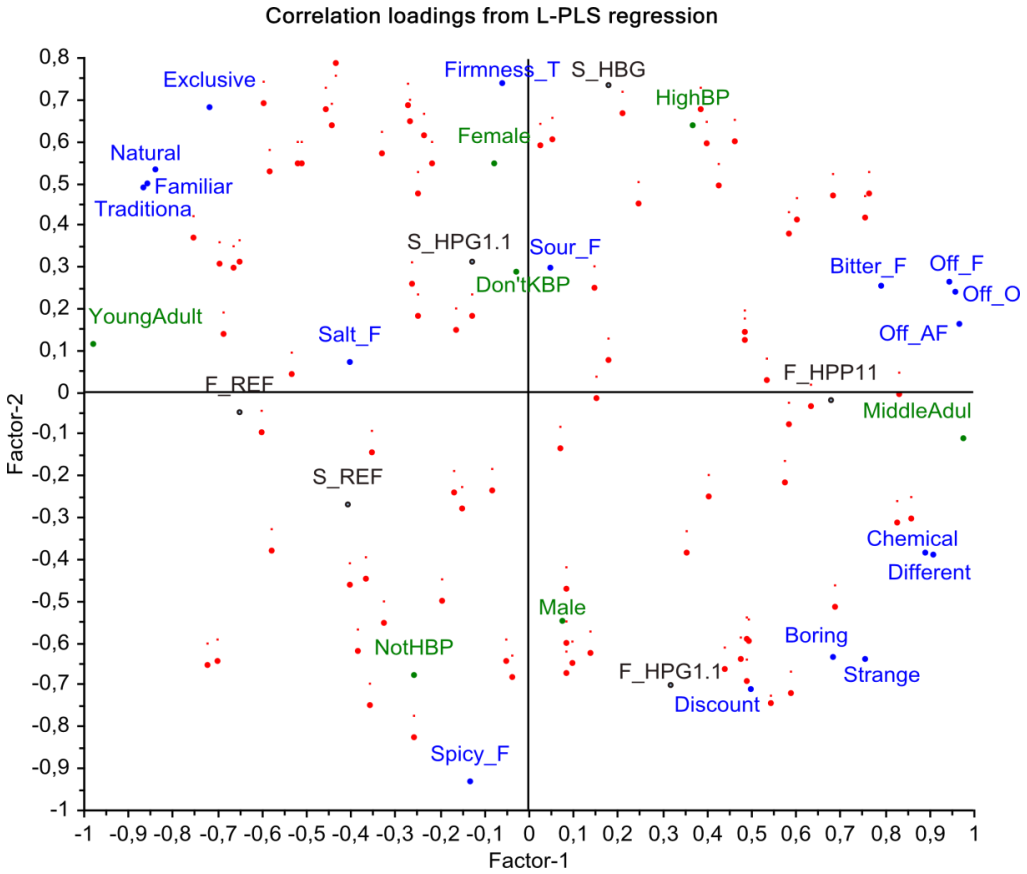

Figure 25: Salami (S) and meatball (F). Correlation loadings plot of the L-PLS model for the first two components (Factor -1 and -2). Average of sensory attributes and holistic are represented in blue (X) (O=odor, F=flavor, A=appearance, AF= after flavor). Consumers individually perception of appetizing are represented by red dots (Y); consumer background information issued from the questionnaire is shown in green (Z). Explained Variance of factor -1 and -2 are 78.4 % and 90.1 % in X, 0.7 % and 1.5 % in Y and 38.1 % and 58.3 % in Z respectively.HighBP = hypertension, Don'KBP = Do not know their blood pressure, NotHB = normal blood pressure.

The horizontal dimension in Figure 25 shows that the meatball and salami references are associated to young adults and are negatively correlated with HPP11 in meatball, which are characterized by bitter and off modalities and associated to the middle adults. The products are related identically to the score plot in Figure 21 (B). The vertical dimension associate female and hypertension to HBG in salami, which are low in spicy flavor. It is remarkable that the perceptions of appetizing among the 85 consumers differ, shown by the scattered pattern of consumers without any apparent density. That hold a different view from the loading plot (A) in Figure 21, presumably it is a result of the covariation from the background variables in the model.

Males seem to perceive HPG1.1 in meatball as discount, boring and strange Figure 25. In theory the perception of unfamiliar and untraditional are conflicting with boring. But as the consumers can interpret the holistic words very differently, the flavors may be perceived as boring in a way that not are connected to boredom. Additionally it is unexpected that HPP11 in meatball which are mostly characterized by the off and bitter modalities and yet correlated with the middle adults, but as mentioned the explained variance of perception of appetizing have to be interpreted with caution. This mean that the middle age not necessarily perceive HPP11 in meatball as appetizing.

Unexpectedly females are correlated with hypertension. Hypertension in general are more frequent among the middle age men (Figure 13 in 5.2.1 Status on blood pressure in Denmark). This can be explained by the low incidences of hypertension among the 157 consumers and that females are higher represented than the males.

6.1.1.2 Consumer perception of appetizing explained by product descriptors and attitudes

The individual perception of appetizing explained by attitude variables and the sensory descriptive data is investigated by L-PLSR. The co-variation between common sensory descriptive attributes and holistic words (X), consumers perception of appetizing (Y) and consumer attitude variables (Zb) are visualized in correlation loadings plots in Figure 26 and Figure 27 for wiener sausages and liver pate as well as meat ball and salami respectively.

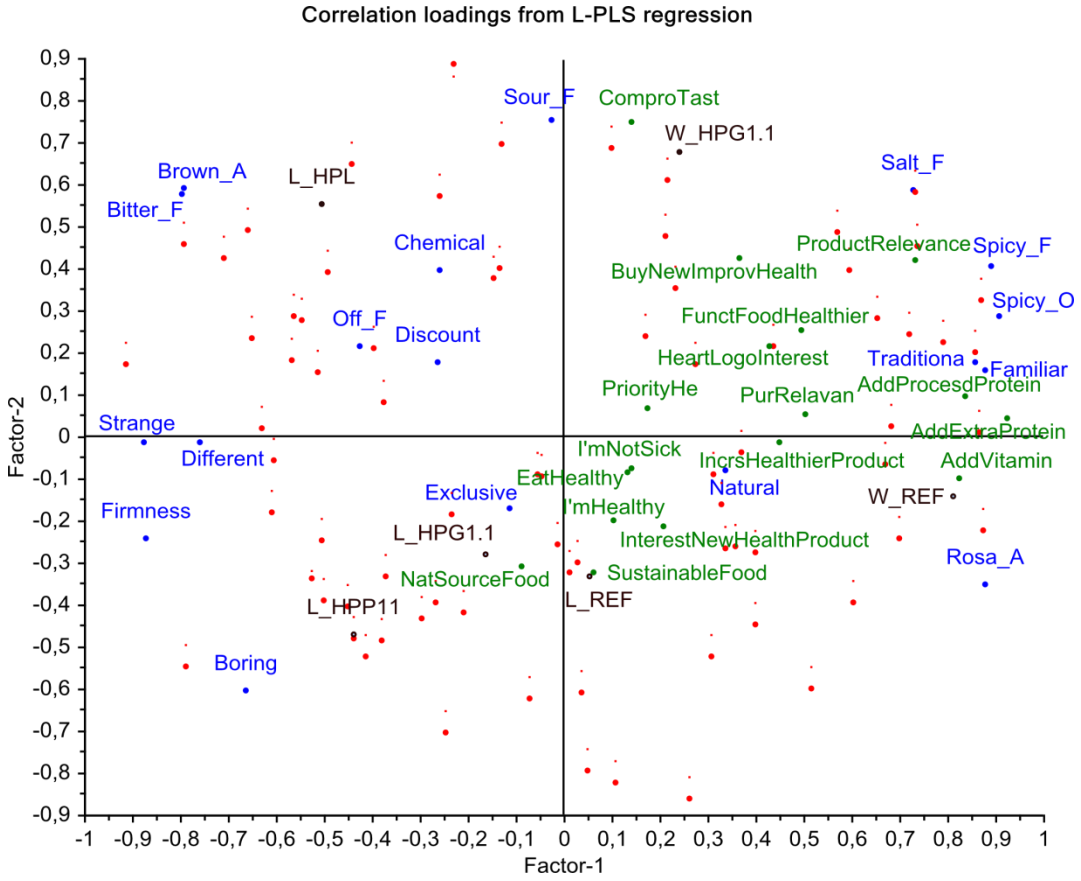

Figure 26: Liver paste and wiener sausages. Correlation loadings plot of the L-PLS model for the first two components (Factor -1 and -2). Average of sensory attributes and holistic perception of two wiener sausages (W) and four liver pates (L) are represented in blue (X). Consumers individually perception of appetizing are represented by red dots (Y); consumer attitude issued from questionnaire is shown in green (Z). Explained Variance of factor -1 and -2 are 56.8 % and 76.6 % in X, 2.7 % and 5.6 % in Y, and 25.1 % and 34.7% in Z respectively. Labels are explained in Table 3, page 42.

The horizontal dimension describes the wiener sausages reference as traditional, familiar, not strange and not different (Figure 26). It is characterized by rosa appearance, spicy odor and a slightly salty flavor which correspond to the findings in Figure 17. The general perceptions of appetizing is different between the six products as the density of consumers is not in a specific direction and as mentioned the explained variance of Y are slightly low of 2.7 % and 5.6 % in factor-1 and-2 respectively, and must be interpreted with caution. The vertical dimension mainly describes that HPG1.1 in wiener sausages are associated with sour flavor and is not perceived as exclusive however it is correlated with willingness to compromise on taste for health benefit (ComproTaste). The reference and HPG.1.1 in liver pate appeared opposite and are correlated to healthy self-concepts (I'mHealthy), interest in food originates from a natural source and is sustainable (NatSourceFood, SustainableFood) and exclusive perceptions. The attitudes are mainly in the right side of the plot and span mostly from the center to upper right quadrant. The three identified consumer groups (figure x) are visible though not as marked. Goup i) onsumers seems to be more correlated with HPG1.1 and the reference liver pate.

Group ii) Consumers are correlated with the wiener sausage reference and group iii) consumers mostly are correlated to wiener sausages with HPG1.1.

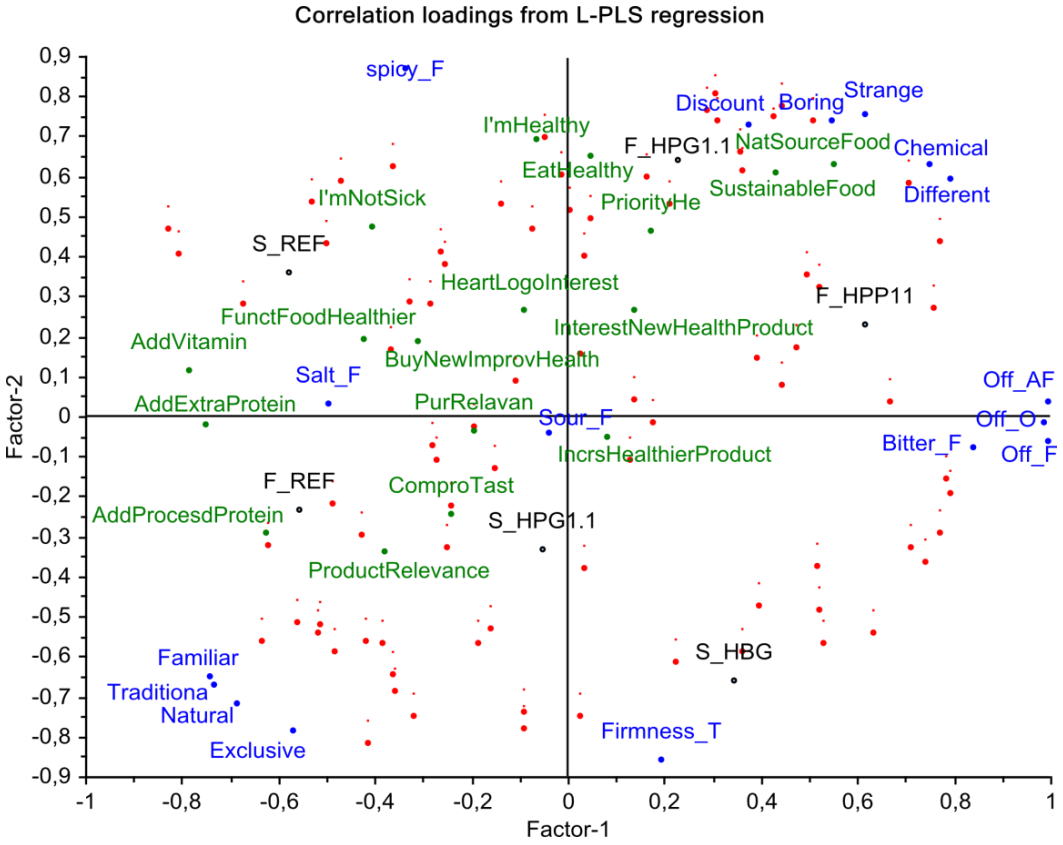

Figure 27: Salami and Meatball. Correlation loadings plot of the L-PLS model for the first two components (Factor -1 and -2). Average of sensory attributes and holistic are represented in blue (X). The developed meat product is denominated with (F) and (S) for meatball and salami, respectively. Consumers individually perception of appetizing are represented by red dots (Y); consumer background information issued from the questionnaire is shown in green (Z). Explained Variance of factor -1 and -2 are 80.3 % and 91.6 % in X, 1.6 % and 4.4 % in Y and 18.1 % and 32.3 % in Z respectively. Labels are explained in table X, page x

The consumer attitudes spans more vide in Figure 27, describing consumers who tasted meatball and salami. The two negatively correlated groups of holistic perceptions are independently associated to the identified consumer groups (Section 5.2). i) is correlated to meatballs with HPP11 and HPG1.1 which is associated with strange, different, chemical, boring and discount. Group ii) and iii) is mostly correlated with the meatball and salami reference as well as HPG1.1 in salami. The Meatball reference as well as HPG1.1 in salami is perceived as familiar, traditional, natural, appetizing and exclusive.

The perceptions of appetizing differs greatly between meatball and salami products (Figure 27) as the density of consumers is not in a specific direction and must be interpreted with caution as the explained variance of Y are slightly low of 1.6 % and 4.4 % in factor-1 and-2 respectively. This is different from the loading plot (A) in Figure 21, and presumably a result of the covariation from the attitude variables (Z) impact on the model. This means that the group i) consumers not necessarily

perceive HPP11 and HPG1.1 in meatball as appetizing but more associated them as strange, different, chemical, boring and discount.

The vertical dimension describes the acceptance of additives in form of vitamins, extra proteins and processed proteins in meat products (AddVitamins, AddExtraprotein, AddProcesdProtein) and are correlated with the references and salty flavor and are anti correlated with bitter and off modalities. As taste is a major factor for preferences and accept of foods it seem that consumers to some extend accept additives if the good taste is retained. In agreement with Verbeke (2006) who found that consumers not necessarily believe that good taste trade-off healthiness.

As it is described the explained variance of all the Y-loadings are rather weak for all four L-PLSR plots. The L-PLSR plot can be validated by examining both the sensory descriptive attributes and the holistic words (X) and the consumer background variables (Z) independently, by relating to the Y matrix (perception of appetizing). The average of common sensory descriptors and the average consumer perception of holistic words (X) is related to all the individual consumers perception of appetizing by a PLSR preference mapping in Figure 20and X for wiener sausages and liver pate as well as meatball and salami respectively. The variations between consumer background variables (Z) and perception of appetizing have not been inspected independently in present study.

7 Summary of analysis and sensory evaluations

In overall the developed products differ from each other within the four types of meat products. Bitter and off odor, off flavors and off after flavor is prominent in products with the hydrolysates as compared to the references. Hydrolysates are not perceived by the consumers as high significant different from the control in liver pate and sausages where as they are in meatball and salami. Hydrolysates are therefore less prominent in the liver pate and wiener sausages compared with the salami and meatball. Overall, the products containing the hydrolysates are in general associated with strange, different, chemical, boring and discount.

Consumers are similar in their perception of appetizing related to the other variables with respect to the four different types of products. Consumers perceptions of the 10 holistic words are related in dimensions of novelty, quality and naturalness parameters. Perceived naturalness seems to be the big driver of appetizing. In the dimension of quality the off modalities are associated with chemical, discount and as unnatural and are perceived as unappetizing

Consumers (n=157) and respondents from the large questionnaire (n=1499) survey highlight that Danish consumers can be divided into three groups with respect to attitudes towards functional meat

products: i) The health and environmental concerned, which see themselves as healthy. ii) Consumers who to some extend accept additives and shows interest in functional foods and iii) Consumers who compromise on taste for health and believe functional foods are healthy.

Male are in general associated with the more positive perceptions of the products whereas females perceive them more skeptical. Accepts of additives in form extra proteins and processed proteins in meat products are correlated with the references and salty flavor and are negatively correlated with bitter and off modalities.

7.1 Overall interpretation of hydrolysates in wiener sausages and liver pate

The results show that consumers have different preferences for wiener sausages and liver pate as none of the products seems to be perceived as more appetizing than others. HPP11 in liver pate is in average perceived with the highest intensity of strange taste.

Among the liver pates, the reference are perceived most appetizing followed by the HPG1.1 both are related to the health and environmental concerned consumers. Middle adults are associated with the liver pate reference, high blood pressure and exclusivity. Middle-aged females are related to bitter flavor, firmness in texture and brown appearance in liver pate. Females perceive HPP11 in liver pate as strange, different, firmness and boring.

On average HPG1.1 in wiener sausage is perceived as more appetizing compared with the reference, especially by the young male adults. The wiener sausages reference is opposite correlated to liver pate with HPL and HPP11. HPG1.1 in wiener sausages have a sour flavor and are not exclusive. It is correlated with willingness to compromise on taste for health benefit. Both the wiener sausages with HPG1.1 and the respective reference are related to consumers who want to improve their health, to some extend accept protein additives and shows interest in functional foods.

7.2 Overall interpretation of hydrolysates in meatball and salami

Consumers fund the salami more appetizing than meatball and the salami with HPG1.1 seems to be perceived as the most appetizing among the salamis and meatball products.

The health and environmental concerned, which have interest in food originates from a natural source and are sustainable and see themselves as healthy are correlated to meatballs with HPP11 and HPG1.1 which are associated with strange, different, chemical, boring and discount. The off and bitter modalities are found most prominent in meatball with HPP11, which is rated with highest perceived intensity of chemical taste and perceived as different by the middle adults. HPG1.1 in meatball is perceived with a higher strange intensity in taste and associated to discount and boring by males.

The consumers who want to improve their health, to some extend accept additives and shows interest in functional foods are correlated with the meatball references and HPG1.1 in salami as well as the salami reference. These are mainly perceived as familiar, traditional, natural, appetizing and exclusive. The reference meatball is perceived as the most traditional in the taste.

8 Reviewing the evidence for a claim

ACE inhibitory peptides have attracted much attention in the recent decades in the search for compounds that could comply with the growing hypertension problem. ACE inhibitory peptides had come into review as functional ingredients in food products that could be bearer of a health claim. An acute decrease in blood pressure has been discovered in studies of peptides and hydrolysates (section 2.2.3). Acute decrease in blood pressure is considered as a psychological effect and help to maintain a healthy blood pressure rather than a disease reduction. Hence the evaluation of HPP11, HBG, HPG1.1 and HPL will be in accordance with an article 13.1 health claim**.**

This section will review the substantiation of an article 13.1 health claims related to HPP11, HBG, HPG1.1 and HPL as ACE inhibitors used as food additives. The review will be approached according to EFSA's procedure described in section 8.1.1 . First characterization of the hydrolysates will be evaluated followed by their relevance of the claimed effect to human health and the beneficial physiological effect. A scientific substantiation of the claimed effect will be discussed according to PASSCLAIM requirements and an assumed EFSA opinion of that will be reviewed. Finally future aspects will be discussed.

Scientific Opinion on the substantiation of health claims related to the meat hydrolysates HPP11, HBG, HPG1.1 and HPL and maintenance of normal blood pressure pursuant to Article 13(1) of Regulation (EC) No 1924/2006.

8.1.1 Assessment

Characterization of HPP11, HBG, HPG1.1 and HPL and healthy blood pressure

Characterizations of HPP11, HBG, HPG1.1 and HPL are in progress as a part of the inSPIRe project nr. III-5: *Use of protein hydrolysates obtained from meat by-products as natural and healthy ingredients for food products***.** The project is collaboration between Dat-Schaub, DMRI and Copenhagen University, SCEINCE. However a characterization of the specific peptide sequences within the hydrolysates is not expected as a part of the project.

- HPP11 originate from shield bloody pork meat (Batch 201108-21)
- HBG originate from bovine greaves (Batch 201109-22S)
- HPG 1.1 originate from pork greaves (Batch 201110-22S)
- HPL originate from pork liver (batch 200901-24)

Process has been described in section 4.2.1. All raw meat material has been food grade by DVFA staff at the slaughter house (Personnel communication (Hansen,)). The peptides within HPP11, HBG, HPG1.1 and HPL all originate from meat and hence unspecified leading to a categorization as "*true inhibitor type*" peptides (Ryan et al., 2011). It could be expected that the hydrolysates will include peptides in the range of 2 to 12 amino acids according to other recognized ACE inhibitory peptides.

8.1.2 Relevance of the claimed effect to human health

ACE play an important role on RAS and consequently on the regulation of the blood pressure, as it catalyses the changes of the inactive form of Ang I to the active Ang II. Ang II act directly on vascular smooth cells. Further ACE deactivates the vasodilator peptide, Bradykinin, which among others functions are responsible for enlargement of the blood vessels and hence contribute to decrease in the blood pressure (Ahhmed & Muguruma, 2010; De Leo et al., 2009; Erdmann et al., 2008; Escudero et al., 2010; Ryan et al., 2011).

By inhibition of ACE, a negative impact on the blood pressure could be prevented. Maintenance of healthy blood pressure would presumably be the wording of a claim. Based on *Guidance on the scientific requirement related to antioxidants, oxidative damage and cardiovascular health* maintenance of healthy blood pressure are considered as a beneficial physiological effect (EFSA on Dietetic Products, Nutrition and Allergies (NDA), 2011). According to the Danish Heart association the maximum systolic blood pressure is 140 mmHg and the maximum diastolic pressure is 90 mmHg for healthy people with normal blood pressure (Hjerteforeningen, 2012 (01.02.2012)).

The general middle-aged population is assumed to be the target population of interest, as risk of developing hypertension increases with age due to physiological changes. The elasticity of the wall of the arteries decrease and the smaller blood vessels become narrower with age (Ahhmed & Muguruma, 2010). As maintenance of healthy blood pressure would benefit the middle-aged population a claim can be considered as it is relevant for human health.

8.1.3 Scientific substantiation of the claimed effect

As revealed in section 0 (Inhibition of Ace activity, page 60) the screening results of HPP11, HBG, HPG1.1 and HPL *in vitro* have shown IC_{50}-values close to other ACE inhibitory hydrolysates derived from meat (Ahhmed & Muguruma, 2010). Liver paté, Meatballs, wiener sausages and salami containing HPP11, HBG, HPG1.1 and HPL in a 3,6 % - 8 % concentrations have maintained the ability to inhibit ACE activity after the meat product processing steps.

Scientific substantiation of a claim can be obtained from human intervention studies showing a short term reduction in the systolic blood pressure or a reduction in the diastolic blood pressure (EFSA on

Dietetic Products, Nutrition and Allergies (NDA), 2011). No human intervention studies have been carried out with HPP11, HBG, HPG1.1 and HPL.

8.2 A probable EFSA opinion

The food ingredients of interest, HPP11, HBG, HPG1.1 and HPL, is not sufficiently characterized in order to evaluate a cause and effect relationship in maintenance of healthy blood pressure. According to PASSCLAIM the food or food component to which the claimed effect is attributed should be characterized so it is possible to compare between studies and to ensure that levels of exposure can be linked quantitatively to the claimed outcome (Aggett et al., 2005).

The claim can be considered relevant with regard to human health as the general middle-aged populations are in risk of developing hypertension. So this population could benefit from ACE inhibitory hydrolysates derived from animal origin with respect to upholding a healthy blood pressure.

The findings in present study represent *in vitro* effects from screenings and a slightly optimistic opinion could be gathered from these. To substantiate a general health claim clinical trials and *in vivo* effects need to be proven. On the basis of this evidence, it is not adequate to establish a health claim that consumption of meat products with HPP11, HBG, HPG1.1 and HPL would benefit maintenance of a healthy blood pressure.

8.3 Future substantiation

The hydrolysates need to be sufficiently characterized with respect to their effects that not necessarily have to be connected to the sequences of peptide. The ACE inhibitory screenings have shown that the results must be seen as indicators for detection of the inhibition of ACE activity in HPP11, HBG, HPG1.1 and HPL and that their activity is maintained after food processing. HPP11, HBG, HPG1.1 and HPL need to be assayed for their exact IC_{50}-values before further estimations of their effect can be discussed and substantiated by human intervention studies. As the effects of ACE inhibitory peptides are able to result in an acute decrease in blood pressure (Table 1, page 18), HPP11, HBG, HPG1.1 and HPL will presumably be categorized in the diet related cardiovascular disease biomarker ((Aggett et al., 2005).

Time-course effect and dose response relationship have not yet been investigated. Based on the present gab in research it could be advantageous to relate the products to the market of functional foods in an early state and investigate whether consumers perceive and accept the products with HPP11, HBG, HPG1.1 and HPL. Consumer insight can indicate how far there is basis to proceed and can

turn the investigation in the right direction of finding the right product to apply HPP11, HBG, HPG1.1 and HPL and creating a success with functional meat products on the market.

9 Summary of Danish consumers acceptance of functional meat products

General considerations of applying hydrolysates to meat products will be evaluated in this section to provide a perspective of the findings in present study.

Danes attitudes towards functional meat products were investigated (*n*=1499). In overall a notable tendency seems to divide the Danish consumers in three groups: i) The health and environmental concerned, which see themselves as healthy. ii) Consumers who to some extend accept additives and shows interest in functional foods and iii) Consumers who compromise on taste for health and believe functional foods are healthy. Consumers who tasted the developed product could be divided into the same three groups as the respondents in the large questionnaire. This tendency confirms that consumers are different and seem to prioritize different from individual strategies and preferences. Acceptance of functional meat products is connected to food choice and preferences and is therefore among others dependent on situational factors such as time and place (Siegrist et al., 2007; Zhang et al., 2010). Furthermore, eating is more than just pleasing hunger. Satisfaction in eating is influenced by several individual values such as health, taste, convenience and naturalness (Furst et al., 1996; K. G. Grunert, 2010). However, taste satisfaction has been revealed as the main driver of the overall satisfaction with pork meat and pork meat products by (Resano et al., 2011).

Despite of taste being the main driver within satisfaction in eating 40 % of the Danish consumers answered they are willing compromise on taste for health if they can feel that the functional meat product has a beneficial effect. A high percentage compared to other studies, which shows that people in general do not consider trade-off of flavor to gain health as a possibility (K. G. Grunert, 2010; Verbeke, 2006), which is underlined by this study (Figure 16, page 72). Nevertheless, according to the study by (Wansink et al., 2005), described previously, consumers are willing to compromise taste if they are able to link the knowledge on health provided by the product to a self-relavant consequence of eating it. This could explain the high prevalence of the Danish consumers willingness to compromise taste if feeling a beneficial effect related to hypertension, as hypertension is a prevalent problem having many relate themselves as in a risk group. The tendency is related to using functional food as medicine or prevention, which has been highlighted by (Urala & Lähteenmäki, 2004) as an important factor for consumers' readiness for use functional foods. This can be associated to an old proverb:

"Let your food be your medicine and your medicine be your food" (Hippocrates, 460 B.C)

Hippocrates 2500-year-old observation can still be considered as current. However consumers acceptance of functional meat product seem to be dependent on state of health and act from different strategies to comply according their own conviction of what is best. In spite of this it must be considered that food as medicine or as prevention of diseases can be a misleading as it cannot be compared on effect. The literature and market analyses have pointed out that a new market for products, which can be can be categorized between food and drugs are increasingly in progress (Bornkessel et al., 2011). Although several functional foods can contribute to an overall state of health, it is important to remember that there still is a big difference between those and real medicine. In this perspective, functional products can be used for lowering some risks in respect to the state of health as a part of a normal diet whereas medicine only is prescribed for specific diseases in measured doses.

The result shows that 65 % of the males in the Danish population have problems with hypertension. As males both have higher consumption of meat and lower willingness to change diet compared to females (Beardsworth et al., 2002; Fagt et al., 2008). They can be considered as consumers who find it difficult to change their diet. For those functional food products can act as a compromise rather than changing their diets. Different eating patterns of men and women suggest that food preferences could be governed by norms, which means that expectations about how men and women should behave, is crucial for their eating habbits (Jensen K.O., 2008) which also comply with consumers identity. As mentioned food are defining identity. Hence traditional meat product from the Danish cuisine seem as a good option for application of meat derived hydrolysates. Results from present study found a tendency that males were associated with the more positive perceptions of the meat products whereas females are more skeptical in their perceptions, which are in agreement with earlier findings (Beardsworth et al., 2002).

Consumers (n=170) perceived naturalness as the main driver as how appetizing a product was perceived. In this relation it is demonstrated that the developed products with HPL, HPG1.1, HBG and Hpp11 is found to differ from each other in the sensory modalities within the four types of meat products. Bitter and off odor, off flavors and off after flavor is prominent in products with the hydrolysates when compared with their respective reference product. These are not exactly the most positive attributes for such food products. However the products containing hydrolysates is not perceived by the consumers (n=170) as being significantly different from the reference products in liver pate and wiener sausages whereas they are in meatball and salami. The results show that consumers have different preferences for wiener sausages and liver pate as none of the products seems to be perceived as more appetizing than others.

Information is an important part of acceptance and can be linked to consumer knowledge (Wansink et al., 2005). Informing consumers so they gain knowledge about bioactive peptides and their benefit, should be an important part of the marketing strategies when promoting these hydrolysates. A study demonstrated that the Nordic consumers mainly prefer familiar ingredients in functional foods and bioactive peptides were categorized as an unfamiliar ingredient (K. G. Grunert et al., 2009). As brands are associated with fact, thoughts, feelings, perceptions, images and experiences the consumers usually relate food brands with a certain value (Dam, Y.K. and Trijp, H.C.M., 2006), it seem obvious to add hydrolysates into products that the consumer already is familiar with. As familiar brands increases trust towards the beneficial effects in functional foods (Beardsworth et al., 2002) as well as consumers confidence with a brand and its value perception can surpass the actual quality of a product (Dam, Y.K. and Trijp, H.C.M., 2006) which is useful information to consider related to the unfavorable flavor of hydrolysates.

Consumer test of the product were performed in canteens, which can be considered as a familiar environment as it must be assumed that the tested subjects are eating there all weekdays when they are at work. Even though the environment was a natural setting; the product samples were served in petri dishes which could have an effect on the perceptions of the products as different from normal. It could be interesting to investigate the product as a part of consumer's private household, which could be assumed as the closest to natural settings as possible. The products are more suitable as a cold serving as unwanted flavors from the hydrolysates becomes more prominent when the product is warm. Since the Danish meal patterns are characterized with cold meals for breakfast and lunch, they would may be seen as most appropriate as a part of the lunch meal, which must be assumed to be consumed when the middle-aged target group are at work. Hence, the workplace environment might be the most important test-place.

10 Conclusion

Hydrolysates HPP11, HBG, HPG1.1 and HPL have ability to inhibit ACE shown by IC_{50} values from *in vitro* screening assays of 12 mg/ml, 8 mg/ml, 9 mg/ml and 8 mg/ml respectively. The hydrolysates maintained ACE inhibitory ability, when applied in concentrations of 8 % into four different traditional meat products from the Danish cuisine, for which the processing covers fermenting, smoking, steaming, baking and frying.

Danes attitudes towards functional meat products were investigated (*n*=1499). General a notable tendency seems to divide the Danish consumers into three groups: i) The health and environmental concerned, which see themselves as healthy. ii) Consumers who to some extend accept additives and show interest in functional foods and iii) Consumers who compromise on taste for health and believe functional foods are healthy.

Forty percent of the Danish consumers are willing to compromise on taste for health if they can feel that the functional product has a beneficial effect.

Generally the developed products differ from each other in the sensory modalities within the four types of meat products. Bitter and off odor, off flavors and off after flavor is prominent in products with the hydrolysates when compared with their respective reference product.

The hydrolysates are not perceived by the consumers (*n*=170) as being significantly different from the reference products in liver pate and wiener sausages whereas they are in meatball and salami. The results show that consumers have different preferences for wiener sausages and liver pate as none of the products seems to be perceived as more appetizing than others.

Consumers can be divided into the same three groups as the respondents from the large questionnaire survey. Males are in general associated with the more positive perceptions of the products whereas females are more skeptical in their perception.

HPP11 in liver pate are in average perceived with the highest intensity of strange taste. The reference liver pate are perceived as the most appetizing followed by HPG1.1 and are related to group i) consumers. Moreover the middle-aged females perceive bitter flavor, firmness in texture and brown appearance in liver pate more than males.

On average HPG1.1 in wiener sausages are perceived as more appetizing compared with the reference, especially by the young male adults. HPG1.1 in wiener sausages have a sour flavor and are not perceived as exclusive and is correlated with willingness to compromise on taste for health benefit. Both wiener sausage references are related to group ii) consumers.

Consumers found salamis more appetizing than meatballs and salami product with HPG1.1 seems to be perceived as the most appetizing among the salamis and meatball products. The off and bitter modalities are found more prominent in meatballs with HPP11, which are rated with highest perceived intensity of chemical taste and perceived as different by the middle adults

Group i) consumers perceive meatballs with HPP11 and HPG1.1 as strange, different, chemical, boring and discount. HPG1.1 in meatball is perceived with a higher strange intensity in taste and associated to discount and boring by males. Group ii) consumers perceive the meatball references and HPG1.1 in salami as well as the salami reference as familiar, traditional, natural, appetizing and exclusive. The reference meatball is perceived as the most traditional in the taste.

The risk of receiving a health claim based on the data available at present, has been evaluated according to the EFSA scientific procedure. On the basis of present evidence, it is not adequate to establish a health claim that consumption of meat products with HPP11, HBG, HPG1.1 and HPL would benefit maintenance of a healthy blood pressure. No human intervention studies have been carried out with HPP11, HBG, HPG1.1 and HPL and clinical trials and *in vivo* effects have to be proven.

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APPENDIX A

Amino acids and their molecular weight

Amino acids (aa)	M [g/mol]	Abreviation
Alanine	71.09	A
Cysteine	103.15	C
Aspartic Acid	115.09	D
Glutamic acid	129.12	E
Phenylalanine	147.18	F
Glycine	57.05	G
Isoleucine	113.16	I
Lysine	128.17	K
Leucine	113.16	L
Methionine	131.19	M
Asparagine	114.11	N
Proline	97.12	P
Glutamine	128.14	Q
Arginine	156.19	R
Threonine	101.11	T
Valine	99.14	V
Tyrosine	163.18	Y

Peptide sequences and

Peptide	**µg/ml**	**mg/ml**	**g/mol**	**Bonds**	**µM (µmol/L)**
RMLGQTP	388.29588	0.38829588	771.96	6	503
RMLGQTPTK	33.90616	0.03390616	997.24	8	34
ITTNP	490.345755	0.490345755	518.61	4	945.5
VKKVLGNP	23.42871	0.02342871	822.06	7	28.5
KRVITY	4.580795	0.004580795	750.95	5	6.1
VKAGF	10.040989	0.010040989	494.63	4	20.3
KRQKYDI	24.10662	0.02410662	920.1	6	26,2
Meat hydrolysate	3900	3.9			
Meat hydrolysate	3690	3.69			
RPR	154.901	0.154901	405.5	2	382
KAPVA	21.3528816	0.021352882	458.61	4	46.56
PTPVP	124.0024401	0.12400244	483.61	4	256.41
GFHI	64.3	0.0643		3	
DFHING	50.5	0.0505		5	
FHG	52.9	0.0529		2	
GLSDGEWQ	117	0.117		7	

$$M_{peptide}\left[{}^{g}/_{mol}\right] = \left(\sum M_{aa}\left[{}^{g}/_{mol}\right]\right) - 2 * Bonds$$

$$M_{peptide} = \left(\sum M_{aa}\right) - 2 * Bonds$$

$$M_{peptide}\left[{}^{g}/_{mol}\right] * c_{peptide}\left[{}^{mol}/_{l}\right] = \rho_{peptide}\left[{}^{g}/_{l}\right]$$

Example ITTNP:

M_{ITTNP} = (113.16+101.11+101.11+114.11+97.12)-2*4 = 518.61 g/mol

518.61 µg/µmol * 945.5 µmol/L = 490345.8 µg/L eller 490.3458 µg/ml

APPENDIX B

0% 0% 100%

Velkommen tilbage

TEKNOLOGISK INSTITUT

Danish Meat Research Institute

Velkommen tilbage til forbrugerundersøgelsen og tak fordi du vil besvare spørgeskemaet. Det indsamlede data vil blive brugt fortroligt og som baggrundsinformation til forskning på DMRI (*Danish Meat Research Institute* - Teknologisk institut).

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Køn?

TEKNOLOGISK INSTITUT

Danish Meat Research Institute

Kun ét svar

- Mand
- Kvinde

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0% 10% 100%

Alder?

TEKNOLOGISK INSTITUT

Danish Meat Research Institute

Kun ét svar

- Vælg fra listen -

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0% 15% 100%

I hvilken region er du bosat?

TEKNOLOGISK INSTITUT

Danish Meat Research Institute

Kun ét svar

- Region Hovedstaden
- Region Sjælland

- Region Syddanmark
- Region Midtjylland
- Region Nordjylland
- Jeg er bosat i udlandet

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0% 21% 100%

TEKNOLOGISK INSTITUT DMRI Danish Meat Research Institute

Hvad er din højeste, *gennemførte* uddannelse?

Kun ét svar

- Folkeskole, realeksamen (7.-10. klasse)
- Gymnasial uddannelse inkl. HF, studenterkurser, HHX, HTX
- Erhvervsuddannelse (f.eks. håndværker, HK'er og SOSU)
- Kort videregående uddannelse (1-2 år, f.eks. merkonom)
- Mellemlang videregående uddannelse (3-4,5 år f.eks. bachelor, lærer, sygeplejeske)
- Lang videregående uddannelse (5 år og mere inkl. phd og HD, f.eks. Cand.merc.)
- Ved ikke
- Anden uddannelse (angiv også gerne varighed):

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TEKNOLOGISK INSTITUT DMRI Danish Meat Research Institute

Lider du af forhøjet blodtryk? (Hvis du er rask skal dit blodtryk være 140 / 90 eller derunder)

Kun ét svar

- Ja
- Nej
- Ved ikke

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Kun ét svar

- Ja
- Nej
- Ved ikke

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0% 36% 100%

Hvor ofte spiser du..

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Kun ét svar i hver linje

	2 eller flere gange ugentligt	Ugentligt	Et par gange om måneden	Et par gange om året	Aldrig	Ved ikke
Spegepølse						
Leverpostej						
Frikadeller						
Wienerpølser (fx hotdog pølser)						

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0% 42% 100%

Hvor enig er du i følgende udsagn?

TEKNOLOGISK INSTITUT

DMRI Danish Meat Research Institute

Kun ét svar i hver linje

	Helt uenig 1.	2.	3.	Hverken/ eller 4.	5.	6.	Helt enig 7.	Ved ikke
Du ser dig selv som en sund person.								
Du ser dig selv som en rask person.								
Du spiser sundt.								
Du interesserer dig for nye produkter med særligt sundhedsfremmende egenskaber.								
Du prioriterer at købe produkter, du mener, har en speciel sundhedsfremmende effekt.								
Du interesserer dig for, at det du spiser stammer fra en naturlig kilde.								
Du interesserer dig for, at det du spiser er bæredygtigt.								

Du accepterer at spise fødevarer tilsat vitaminer.	○	○	○	○	○	○	○	○
Du accepterer at spise fødevarer tilsat ekstra protein.	○	○	○	○	○	○	○	○
Du accepterer at spise kødprodukter tilsat forarbejdede protein.	○	○	○	○	○	○	○	○
Du tror at funktionelle fødevarer (fx Becel smør eller probiotisk yoghurt) kan gavne din sundhed.	○	○	○	○	○	○	○	○

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0% 47% 100%

Sandt eller Falsk

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Kun ét svar i hver linje

	Sandt	Falsk	Ved ikke
Probiotiske yoghurter styrker tarmenes sundhed og immunforsvaret.	○	○	○
Becel smør sænker kolesterolindholdet i blodet.	○	○	○
Produkter beriget med omega-3 fedtsyrer er sunde.	○	○	○

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0% 52% 100%

Hvor enig er du i følgende udsagn?

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Kun ét svar i hver linje

	Helt uenig 1.	2.	3.	Hverken/ eller 4.	5.	6.	Helt enig 7.	Ved ikke
Hvis du ser en reklame der anbefaler et sundhedsfremmende produkt, vil det påvirke dig til at købe det.	○	○	○	○	○	○	○	○
Hvis en diætist i et magasin, fx I Form, anbefaler et sundhedsfremmende produkt, vil det påvirke dig til at købe det.	○	○	○	○	○	○	○	○
Hvis en ven anbefaler et sundhedsfremmende produkt, vil det påvirke dig til at købe det.	○	○	○	○	○	○	○	○
Hvis din læge anbefaler et sundhedsfremmende produkt, vil det påvirke dig til at købe det.	○	○	○	○	○	○	○	○
Du interesserer dig for udvikling af de produkter du normalt køber, så de også har en sundhedsgavnlig effekt.	○	○	○	○	○	○	○	○
Du vil købe nye produkter du normalt ikke spiser, hvis du har indtryk af at de gavner din sundhed.	○	○	○	○	○	○	○	○
En ny leverpostej med tilsat protein, hjælper med at opretholde et sundt blodtryk. Dette produkt vil have en gavnlig effekt for dig.	○	○	○	○	○	○	○	○

Det øger din interesse for et produkt hvis Hjerteforeningens logo er synligt på emballagen. (Hjerteforeningen er en af Danmarks største sygdomsbekæmpende organisationer, som bl.a. arbejder med forskning i hjertekarsygdomme og forebyggelse af hjertesygdomme)

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0% 57% 100%

TEKNOLOGISK INSTITUT DMRI Danish Meat Research Institute

Hvis Hjerteforeningens logo er synligt på emballagen, ville du så være mere tilbøjelig til at købe produktet?

Kun ét svar

- Ja
- Nej
- Ved ikke

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0% 63% 100%

TEKNOLOGISK INSTITUT DMRI Danish Meat Research Institute

Hvor enig er du i følgende udsagn?

Kun ét svar i hver linje

	Helt uenig 1.	2.	3.	Hverken/ eller 4.	5.	6.	Helt enig 7.	Ved ikke
Du vil gå på kompromis med smagen, hvis du ved at det du spiser er sundt.								
Du vil købe kødprodukter tilsat forarbejdede proteiner, hvis det kan hjælpe dig til at opretholde et sundt blodtryk.								

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0% 68% 100%

TEKNOLOGISK INSTITUT DMRI Danish Meat Research Institute

Vil du købe et produkt tilsat sundhedsfremmende naturlige ingredienser, hvis det smager godt?

Kun ét svar

- Ja
- Nej

Ved ikke

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0% 73% 100%

Vil du købe et produkt tilsat sundhedsfremmende naturlige ingredienser, hvis det smager mindre godt?

Kun ét svar

- Ja
- Nej
- Ved ikke

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0% 78% 100%

Vil du købe et produkt med påstand om at det kan hjælpe dig til at opretholde et sundt blodtryk?

Kun ét svar

- Ja
- Nej
- Ved ikke

Ser du dig selv i målgruppen for sådan et produkt?

Kun ét svar

- Ja
- Nej
- Ved ikke

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0% 89% 100%

Vil du købe et produktet igen hvis du ikke syntes så godt om smagen, men du kan mærke en positiv og gavnlig

effekt når du spiser det?

Kun ét svar

- Ja
- Nej
- Ved ikke

Vil du købe kødprodukter tilsat forarbejdede proteiner?

Kun ét svar

- Ja
- Nej
- Ved ikke

info Næste >

0% 100% 100%

Tak for din besvarelse

TEKNOLOGISK INSTITUT

DMRI Danish Meat Research Institute

Tusind tak for hjælpen! Dine svar er nu registreret.

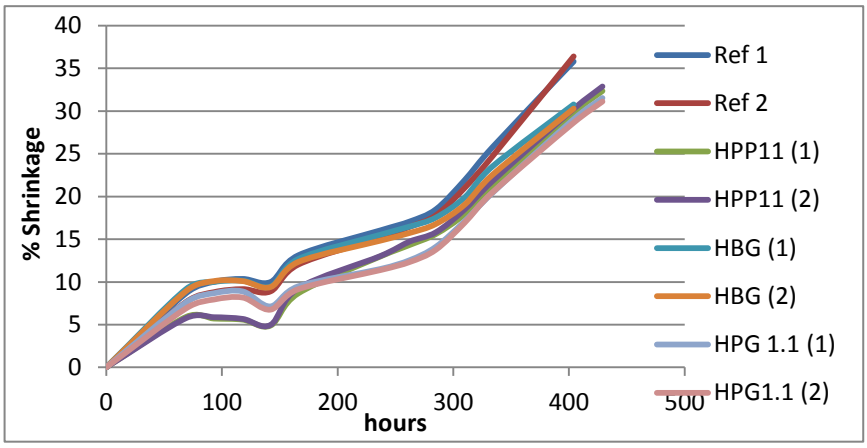
Jeg er specialestuderende ved Københavns Universitet. I forbindelse med mit afsluttende speciale på kandidatuddannelsen i Gastronomi og Sundhed og i samarbejde med DMRI på Teknologisk Institut, skal jeg undersøge danskernes holdning til funktionelle fødevarer. Mit fokus er på fødevarer tilsat naturlige bioaktive proteiner, som man regner med har en sundhedsfremmende effekt. Det er bl.a. påvist i forskellige studier at de positivt kan påvirke det system der regulerer vores blodtryk.

Mvh.
Marie Tøstesen

info Afslut >

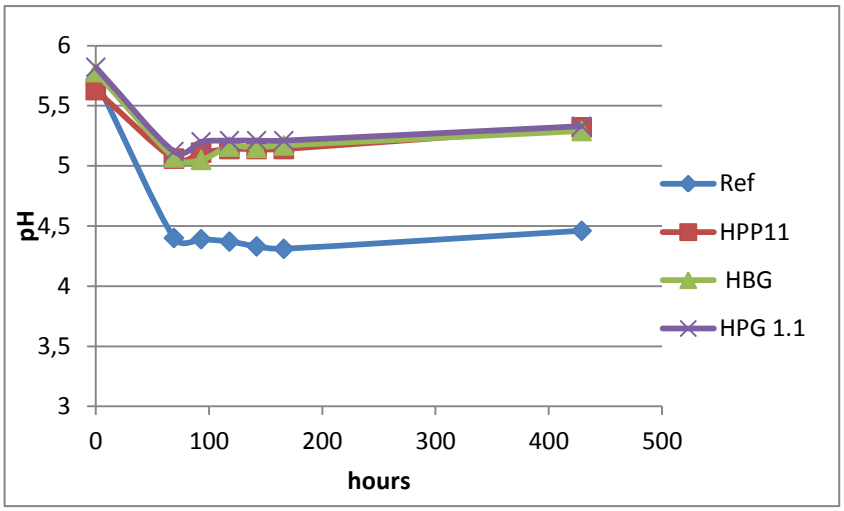
APPENDIX C

Two salamis from each batch were weighed to follow the shrinkage



APPENDIX D

The pH was measured with a calibrated pH-meter (Knick Portamess, Mettler Toledo) periodically until the pH remained stable.



APPENDIX E

Nutritional facts for meatball containing 8 % of the hydrolysate HPG1.1.

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	608.6	kJ		
	144.9	kcal.		
Protein:	19.5	g	54.5%	10 - 20%
Carbohydrate. total:	6.6	g	8.3%	50 - 60%
Fat. total:	4.6	g	27.8%	25 - 35%

Nutritional facts for meatball containing 8 % of the hydrolysate HPP11.

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	603.0	kJ		
	143.6	kcal.		
Protein:	18.4	g	51.8%	10 - 20%
Carbohydrate. total:	6.6	g	8.3%	50 - 60%
Fat. total:	4.9	g	30.3%	25 - 35%

Nutritional facts for the reference meatballs, without added hydrolysates.

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	505.8	kJ		
	120.4	kcal.		
Protein:	13.3	g	44.7%	10 - 20%
Carbohydrate. total:	7.0	g	10.8%	50 - 60%
Fat. total:	4.4	g	32.4%	25 - 35%

Nutritional facts for liverpaté containing 8 % of the hydrolysate HPG1.1

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	937.3	kJ		
	223.2	kcal.		
Protein:	17.0	g	30.9%	10 - 20%
Carbohydrate. total:	4.0	g	0.8%	50 - 60%
Fat. total:	15.7	g	62.1%	25 - 35%

Nutritional facts for liverpaté containing 8 % of the hydrolysate HPL

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	913.8	kJ		
	217.6	kcal.		
Protein:	15.5	g	28.9%	10 - 20%
Carbohydrate. total:	5.1	g	0.9%	50 - 60%
Fat. total:	15.2	g	61.8%	25 - 35%

Nutritional facts for liverpaté containing 8 % of the hydrolysate HPP11

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	931.7	kJ		
	221.8	kcal.		
Protein:	15.9	g	29%	10 - 20%
Carbohydrate. total:	4.0	g	0.8%	50 - 60%
Fat. total:	16.1	g	63.9%	25 - 35%

Nutritional facts for the reference liver paté, without added hydrolysates.

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	863.2	kJ		
	205.5	kcal.		
Protein:	10.6	g	20.9%	10 - 20%
Carbohydrate. total:	4.3	g	1%	50 - 60%
Fat. total:	16.5	g	71%	25 - 35%

Nutritional facts for salami containing 8 % of the hydrolysate HBG

Nutritional value pr. 100 g			After shrinkage per 100 g		Energy distribution	
					Percentage:	Recommended:
Energy:	829.6	kJ	1082.9	kJ		
	198.3	kcal.	258.8	Kcal		
Protein:	21.1	g	27.6	g	43.3%	10 - 20%
Carbohydrate. total:	0.5	g	0.6	g	0%	50 - 60%
Fat. total:	12.5	g	16.3	g	55.8%	25 - 35%

Nutritional facts for salami containing 8 % of the hydrolysate HPG1.1

Nutritional value pr. 100 g			After shrinkage per 100 g		Energy distribution	
					Percentage:	Recommended:
Energy:	830.6	kJ	1090.8	kJ		
	198.5	kcal.	260.7	kcal		
Protein:	21.1	g	27.7	g	43.2%	10 - 20%
Carbohydrate. total:	0.5	g	0.6	g	0%	50 - 60%
Fat. total:	12.5	g	16.4	g	55.8%	25 - 35%

Nutritional facts for the reference salami, without added hydrolysates.

Nutritional value pr. 100 g			After shrinkage per 100 g		Energy distribution	
					Percentage:	Recommended:
Energy:	740.2	kJ	1007.4	kJ		
	176.9	kcal.	240.8	kcal		
Protein:	15.7	g	21.3	g	36.1%	10 - 20%
Carbohydrate. total:	1.9	g	2.6	g	0.2%	50 - 60%
Fat. total:	11.9	g	16.2	g	59.6%	25 - 35%

Nutritional facts for wienersausage containing 8 % of the hydrolysate HPG1.1

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	596.8	kJ		
	142.6	kcal.		
Protein:	17.7	g	50.6%	10 - 20%
Carbohydrate. total:	0.5	g	0%	50 - 60%
Fat. total:	17.7	g	48.1%	25 - 35%

Nutritional facts for the reference wienersausage, without added hydrolysates.

Nutritional value pr. 100 g			Energy distribution	
			Percentage:	Recommended:
Energy:	499.2	kJ		
	119.3	kcal.		
Protein:	11.9	g	40.5%	10 - 20%
Carbohydrate. total:	0.4	g	0%	50 - 60%
Fat. total:	7.8	g	58.2%	25 - 35%

APPENDIX F

Kom og smag!

Deltag i forbruger-undersøgelse af fedtfattige kødprodukter

Teknologisk Institut Taastrup
Torsdag d. 9. februar 2012

Online spørgeskema sendes til din e-mail

-få lækker **chokolade** som tak

DMRI
Danish Meat Research Institute
En Division i Teknologisk Institut

Ved deltagelse skal du efterfølgende svare på et online spørgeskema, der sendes til din e-mail. Alle oplysninger behandles fortroligt!

APPENDIX G

Introduktion til produktbedømmelse af spegepølse og frikadeller

På følgende sider er der 10 udsagn om hvert af de udleverede produkter (1 ark pr produkt). Smag på produkterne i den rækkefølge som siderne forløber.

For hvert af dem skal du markere hvor enig eller uenig du er i udsagnet. Det gør du ved at sætte et kryds i det felt der svarer til din u/enighed med udsagnet.

Hvis du sætter krydset forkert, så streg det helt over og sæt det korrekte kryds i et nyt felt.

Efter bedømmelsen vil du modtage et anonymt spørgeskema pr mail som vil tage ca. 10 minutter at udfylde.

Din e-mail: _____

Bedømmelsen og spørgeskemaet hænger sammen, så derfor er det vigtigt at du besvare det tilsendte spørgeskema. Alt data bliver behandlet fortroligt.

På forhånd tusind tak for hjælpen

Med venlig hilsen

Marie Tøstesen
Specialestuderende i Gastronomi og Sundhed

Spegepølse 379

Jeg synes, at produktet er **traditionelt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **anderledes**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **velkendt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **mærkeligt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **kedeligt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **eksklusivt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **discount-agtigt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **indbydende**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **naturligt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **kemisk**

Helt uenig						**Neutral**								**Helt enig**

Spegepølse 347

Jeg synes, at produktet er **traditionelt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **anderledes**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **velkendt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **mærkeligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **kedeligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **eksklusivt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **discount-agtigt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **indbydende**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **naturligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **kemisk**

Helt uenig							**Neutral**							**Helt enig**

Spegepølse 325

Jeg synes, at produktet er **traditionelt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **anderledes**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **velkendt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **mærkeligt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **kedeligt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **eksklusivt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **discount-agtigt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **indbydende**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **naturligt**

Helt uenig						**Neutral**								**Helt enig**

Jeg synes, at produktet er **kemisk**

Helt uenig						**Neutral**								**Helt enig**

Frikadelle 402

Jeg synes, at produktet er **traditionelt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **anderledes**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **velkendt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **mærkeligt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **kedeligt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **eksklusivt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **discount-agtigt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **indbydende**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **naturligt**

Helt uenig						**Neutral**							**Helt enig**	

Jeg synes, at produktet er **kemisk**

Helt uenig						**Neutral**							**Helt enig**	

Frikadelle 439

Jeg synes, at produktet er **traditionelt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **anderledes**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **velkendt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **mærkeligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **kedeligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **eksklusivt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **discount-agtigt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **indbydende**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **naturligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **kemisk**

Helt uenig							**Neutral**							**Helt enig**

Frikadelle 491

Jeg synes, at produktet er **traditionelt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **anderledes**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **velkendt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **mærkeligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **kedeligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **eksklusivt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **discount-agtigt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **indbydende**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **naturligt**

Helt uenig							**Neutral**							**Helt enig**

Jeg synes, at produktet er **kemisk**

Helt uenig							**Neutral**							**Helt enig**

APPPENDIX H

Analyseforskrift

24.08.2011
Proj.nr.2000256
LME/CB/MDAG/MT

Sensorisk beskrivelse af produkter med Holistic by DMRI

1. Generelt

Holistic by DMRI er en hurtigmetode til beskrivelse af sensoriske indtryk af fødevarer. Den holistiske tilgang bygger på en helhedsopfattelse baseret på den enkelte dommers umiddelbare følelser omkring et givent produkt. Metoden består i princippet af to metoder, som også kan anvendes individuelt: 1) CATA, Check All That Apply og 2) kvantificering af udvalgte holistiske ord (helhedsord). Eksempler på helhedsord kan være ”traditionelt”, ”sommerligt”, ”spændende” og ”moderne”. Metodens resultater er unikke ved at kunne fungere som et direkte bindeled mellem sensorik og marketing, da ordene har en umiddelbar anvendelighed i begge sammenhænge.

2. Princip

Metoden bygger på, at utrænede dommere bedømmer givne produkter ud fra den enkelte dommers egen umiddelbare indskydelse/følelse/intuition/mavefornemmelse. Der gives således ikke en definition på ordene, det er helt op til dommeren selv. I den første del af metoden anvendes CATA til at udvælge de ord, der senere skal kvantificeres. Dette foregår ved, at dommerne krydser af på en bruttoliste, hvilke ord de kan associere med det givne produkt. Dommerne er også velkomne til at tilføje egne ord. Efterfølgende foregår der en udvælgelse af de ord, flest dommere har kunnet relatere til de givne produkter. Disse ord udgør resultatet af CATA. Sidste del af Holistic by DMRI indeholder en kvantificering af de udvalgte helhedsord, hvor et hold nye dommere bedømmer produkterne i forhold til de enkelte ord på en 15 cm linjeskala (gående fra lidt til meget), som det kendes fra klassisk sensorisk profilering.

3. Anvendelsesområde

Hvilke produkter?
Alle fødevarer, og mange andre produkter (f.eks. TV-skærme, mobiltelefoner mv.), kan beskrives med Holistic by DMRI.

Hvornår er metoden velegnet?
Holistic by DMRI kan benyttes i forskellige sammenhænge, men egner sig dog ikke til daglig

kvalitetskontrol. I den sidste del af en *produktudvikling* derimod, kan der med metoden gives et godt bud på, hvilket produkt der skal sættes i produktion og sendes videre ud i butikkerne. Metoden er desuden fundet særdeles velegnet til *forbrugerundersøgelser*.

4. Hvem kan være dommer?

I princippet kan alle personer deltage som dommere. Under forudsætning af at der skal lugtes/smages på et produkt, må dommerne ikke have nedsat lugte- og smagssans som f.eks. under en forkølelse.

Erfaringen har dog vist, at et trænet dommerpanel ikke egner sig til denne metode. De trænede dommere har svært ved at omstille sig fra at skulle være enige og bedømme veldefinerede egenskaber til at skulle bedømme ud fra egen intuition.

5. Antal dommere

Det er ikke veldefineret, hvor mange dommere, der skal indgå i en bedømmelse. Anvendes metoden under produktudvikling, bør antallet af dommere være på mindst 10. Anvendes metoden derimod til en forbrugerundersøgelse, bør min. 40 forbrugere pr. segment afgive bedømmelse.

6. Antal produkter

Antallet af produkter afhænger af, hvilken type produkt der er tale om. Hvis produkterne er meget krydrede, bliver dommerne hurtigere "sensorisk trætte", hvilket igen betyder, at det er svært at identificere forskelle. Erfaring med forskellige kødprodukter har vist, at mellem *6 og 10 produkt-prøver* kan håndteres. Der er ingen mindstegrænse for antallet af produkter.

7. Lokale

Det er væsentligt, at alle dommere har god bordplads og evt. også kan sidde ned, et mødelokale kan derfor være fint. Det er en klar fordel, hvis bedømmelsen kan foregå uforstyrret fra støj og lugte.

8. Klargøring af produkter

Produkterne bør forberedes, som det er tiltænkt hos slutbrugeren. Hvis det f.eks. er wienerpølser, der skal bedømmes, skal pølserne koges eller pandesteges inden bedømmelse.

9. Information og servering

Information til dommerne
Dommerne skal informeres om det praktiske ved selve bedømmelsen, så de forstår, hvad de skal gøre. Da dommerne jo også kan være forbrugere uden kendskab til sensorik, kan der være en del spørgsmål; besvar dem endelig. Men giv generelt ingen produktinformationer, så lader dommerne sig ikke begrænse eller anspore af denne information.

Det er meget væsentligt, at dommerne bliver informeret om, at de skal afgive deres bedømmelse efter egen umiddelbare indskydelse/følelse/intuition/mavefornemmelse, og at der ikke er nogen forkerte svar! Erfaringen har vist, at folk (især forbrugere) generelt er lidt tvivlende overfor anvendeligheden af deres bedømmelse, når nu ordene ikke er blevet defineret. Men erfaringen viser også, at langt de fleste har meget nemt ved at gennemføre bedømmelsen.

Servering
Det er som regel en god ide at randomisere rækkefølgen af prøverne, så ikke alle dommere bedømmer produkterne i den samme rækkefølge. Der foreligger dog ikke på nuværende tidspunkt undersøgelser af, hvor stor betydning randomisering vs. ikke-randomisering har for det endelige resultat.

Om dommerne skal have alle produkter på én gang eller enkeltvis må afhænge af praktikken omkring gennemførelsen, og om produkterne generelt er temperaturafhængige. I nogle situationer vil det være hensigtsmæssigt at servere alle prøver på en gang, f.eks. hvis mange personer bedømmer samtidig, og i andre situationer er det hensigtsmæssigt at servere produkterne et ad gangen f.eks. ved bedømmelse af is, der ikke må smelte.

10. Bedømmelse

Holistic by DMRI kan, som tidligere nævnt, opfattes som to selvstændige del-metoder, hvorfor metoderne kan gennemføres uafhængigt af hinanden.

CATA - udvælgelse af ord
Dommerne skal ud fra en bruttoliste af ord (se eksempel i bilag 1) krydse alle de ord af, som de kan associere med et givent produkt. Dommerne er ligeledes meget velkomne til at tilføje egne ord. Det er en god idé, at dommerne har en bruttoliste til hvert produkt. Dommerne må ikke tale sammen under bedømmelsen, og de skal placeres med afstand, så de ikke forstyrrer hinanden og heller ikke kan se hinandens bedømmelser.

Bruttolisten i bilag 1 er et eksempel og derfor ikke en endelig ordliste. Ved fremstilling af bruttolisten, kan det være en fordel at inddrage salgs- og marketingspersonale, da de ord, der bedømmes, måske kan anvendes i markedsføring af produkterne.

Erfaringen viser, at det ved forbrugerundersøgelser er vigtigt også at have mere negativt-

ladede ord på listen som f.eks. ”kedelig”, ”umoderne” eller ”mærkelig”, da det synes at øge undersøgelsens troværdighed i forbrugerens øjne.

Fra CATA til kvantificering - udvælgelse af ord
Med udgangspunkt i de markeringer, som dommerne har afgivet, kan der udvælges ord til kvantificering. Der kan være mange tilgange til at vælge ord ud, og der er ingen definition på, hvordan det skal gøres. En åbenlys tilgang er dog at udvælge de ord med flest markeringer, da antallet af markeringer indikerer, at flest personer kan relatere produktet til netop disse ord. Dette kan så med fordel kombineres med ønsker fra marketing.

Der er ligeledes ikke regler for, hvor mange ord der kan indgå i kvantificeringen. Valget af antal ord kan også afhænge af antallet af produkter, da det kan være overvældende at skulle kvantificere mange ord for mange produkter, især for forbrugere. Et forslag er at udvælge maks.10 ord, da de kan stå på en enkelt side (se eksempel i bilag 2). Det er overskueligt og har dermed en god psykologisk effekt. Der er ingen nedre grænsen for antallet af ord.

Kvantificering af ord
For hvert produkt markerer dommeren på linjeskalaen (15 cm) ud for hvert ord graden af ordets intensitet, f.eks. hvor ”sommerligt” synes dommeren produktet er på skalaen gående fra lidt til meget. Det er mest overskueligt, hvis hvert produkt bedømmes på hver sin side med sættet af ord svarende til bilag 2.

Dommerne må ikke tale sammen under bedømmelsen og skal placeres med afstand, så de ikke forstyrrer hinanden og heller ikke kan se og dermed påvirkes af hinandens resultater.

11. Opgørelse af resultater

CATA
Opgørelse af de afgivne bedømmelser fra CATA er blot en simpel optælling af markeringer. Optællingen kan opgøres på forskellige måder alt efter undersøgelsens formål som f.eks. A) et samlet resultat, B) en optælling pr. produkt eller C) en optælling pr. forbrugersegment.

Der kan opnås et godt overblik ved anvendelse af ”Pivot”-funktionen i Excel, hvor der nemt kan udarbejdes en opgørelse i forhold til undersøgelsens forskellige faktorer (se eksempel i bilag 3).

Kvantificering
Hver markering på samtlige linjeskalaer opmåles med lineal, og resultaterne (i cm) tastes ind i Excel. De indtastede data kan herefter analyseres i PanelCheck, se beskrivelse i bilag 4.

☐	Skøn	☐	Sund
☐	Harmonisk	☐	Traditionel
☐	Kompleks	☐	Overraskende
☐	Feminin	☐	Landlig
☐	Maskulin	☐	Nem
☐	Sexet	☐	Nyt
☐	Eksotisk	☐	Sofistikeret
☐	Eksklusiv	☐	Sommerlig
☐	Lækker	☐	Nordisk
☐	Ungdommelig	☐	Indbydende
☐	Intensivt	☐	Spændende
☐	Cool	☐	Mærkelig
☐	Umoderne	☐	Forvirrende
☐	Gør mig glad	☐	Hyggelig
☐	Velkendt	☐	
☐	Kedelig	☐	
☐	Flirtende	☐	
☐	Dansk	☐	

Lidt	**Sommerlig**	**Meget**
Lidt	**Kedelig**	**Meget**
Lidt	**Spændende**	**Meget**
Lidt	**Mærkelig**	**Meget**
Lidt	**Lækker**	**Meget**
Lidt	**Nem**	**Meget**
Lidt	**Traditionel**	**Meget**
Lidt	**Velkendt**	**Meget**
Lidt	**Indbydende**	**Meget**
Lidt	**Overraskende**	**Meget**

Opgørelsen er et konstrueret eksempel for optælling af ord fundet med CATA (udført med Pivot-funktion)
Dette kunne være bedømmelsen af 8 produkter afgivet af 7 medarbejdere, der er summeret over alle produkter og alle medarbejdere. De 10 mest markerede ord er angivet med rødt, og ord med blåt er medarbejdernes egne.

Helhedsord	Antal markeringer (Sum)
Antal af traditionel	21
Antal af sommerlig	16
Antal af overraskende	12
Antal af indbydende	12
Antal af nem	11
Antal af kedelig	11
Antal af velkendt	11
Antal af eksotisk	10
Antal af lækker	9
Antal af mærkelig	8
Antal af spændende	7
Antal af feminin	7
Antal af umoderne	6
Antal af maskulin	6
Antal af landlig	5
Antal af sund	5
Antal af ikke lækkert	4
Antal af kompleks	4
Antal af skøn	3
Antal af harmonisk	3
Antal af grim	2
Antal af praktisk	2
Antal af stærk	2
Antal af forvirrende	2
Antal af nordisk	2
Antal af nyt	2
Antal af cool	2
Antal af kedelig farve	1
Antal af uappetitlig	1
Antal af klam	1
Antal af klisterende	1
Antal af overvældene	1
Antal af slimet	1
Antal af eksklusiv	1
Antal af dansk	1
Antal af flirtende	1
Antal af sexet	1
Antal af tam farve	1
Antal af hyggelig	

Download program

PanelCheck downloades nemt og gratis fra hjemmesiden www.panelcheck.com

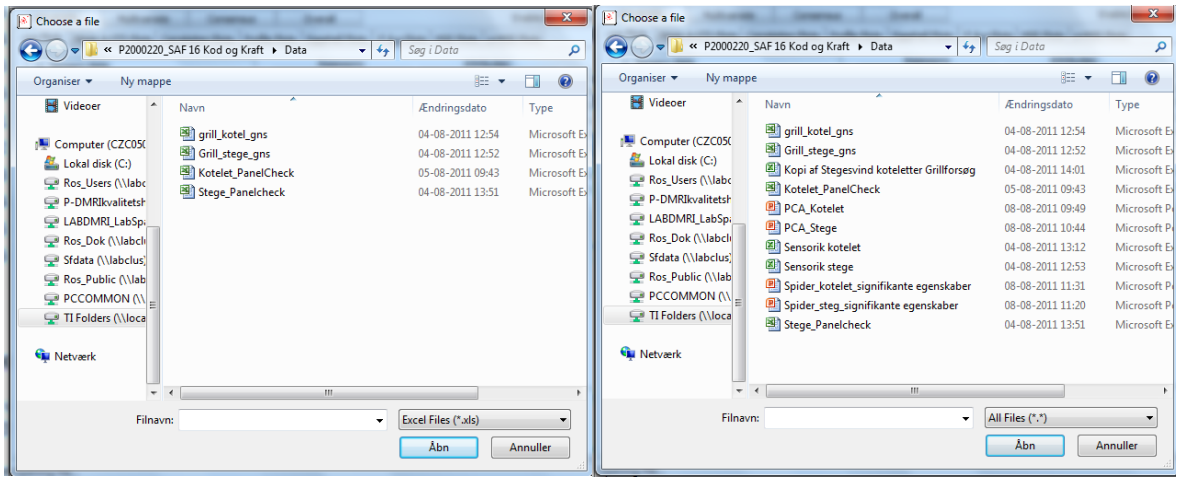
Opstilling af data

Data skal opstilles som vist her. PanelCheck kræver en kolonne med "Gentag", og da der ikke udføres gentag på dommerniveau, skrives blot 1. Anvend nedenstående kolonne-rækkefølge, så bliver data automatisk indlæst korrekt i programmet.

Dommer	Produkt	Gentag	Eksotisk	Mærkelig	Indbydende
1	Produkt 1	1	6,9	0,3	7,1
1	Produkt 2	1	10,7	0,3	11,3
1	Produkt 3	1	14,2	0,6	14,2
1	Produkt 4	1	2,9	6,4	0
1	Produkt 5	1	1	14,4	0,5
1	Produkt 6	1	8,5	2,5	8,9
2	Produkt 1	1	0,8	10,8	0,7
2	Produkt 2	1	7	0,3	14,6
2	Produkt 3	1	14,4	0,3	14,6
2	Produkt 4	1	6,8	6,8	6,7
2	Produkt 5	1	0,3	14,4	0,2
2	Produkt 6	1	0,2	0,4	7,1

Importer data til PanelCheck

Importer data fra Excel via "Filer", "Import", "Excel". Vær opmærksom på, at PanelCheck automatisk viser filer gemt i version "Excel 97-2003" (.xls) - men vælg blot "All files", så kommer også de nyere versioner frem i vinduet.



Kun Excel filer version 97-2003 Alle filer

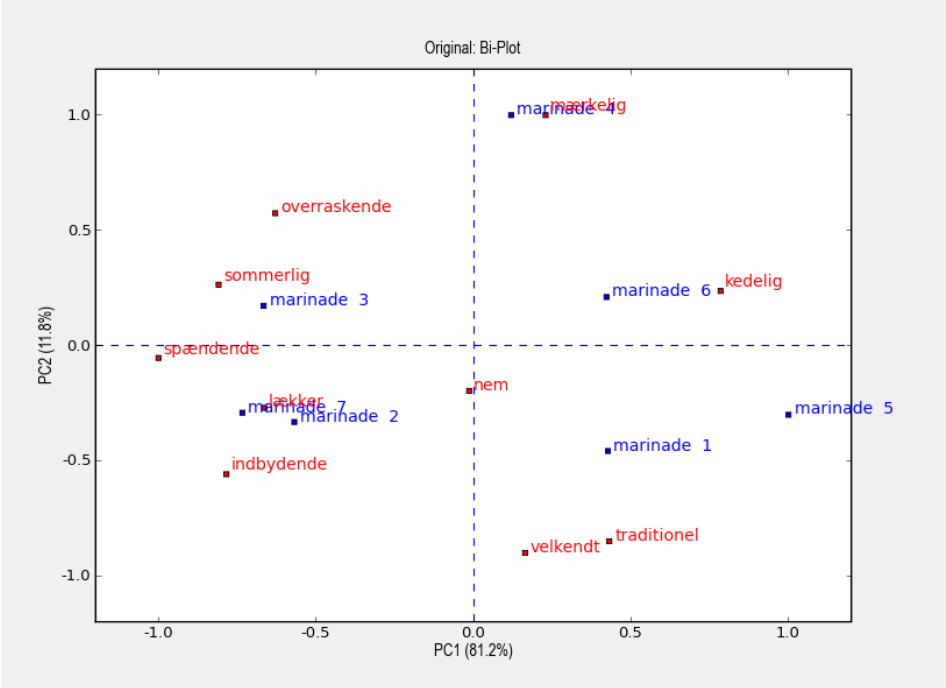
Når import af data er gennemført, fremkommer et kontrolvindue, hvori det angives, om der er manglende værdier. Derudover skal de to felter nederst med min- og max-værdier for de indtastede data kontrolleres. Programmet er sat til min = -1 og max = 16 (selv om disse værdier går fra 0 - 15). Hvis der står andre værdier, f.eks. max = 149, tyder det på, at der er glemt et komma - gå tilbage i Excel, ret fejl-værdien og importer igen.

Analyser data i PanelCheck

Vælg ”Consensus” og ”Original” i topbjælken. Tryk på ”+” ud for ”sensory data”, og dobbeltklik på biplot i den fremkomne række.

Fortolkning af resultatet

PCA-plottet viser det overordnede resultat, det vil sige, hvordan produkterne er placeret i forhold til de bedømte ord og i forhold til hinanden. I det viste biplot (venligst udlånt af Friland A/S) kan det ses, at marinade 2, 3 og 7 er grupperet sammen og er karakteriseret med ”lækker”, ”sommerlig”, ”spændende”, ”indbydende” og ”overraskende”.



APPENDIX I

Emne:RE: ** Teknologisk institut - Rapportering

Fra: Rasmus Toft, interresearch a|s (rat@interresearch.dk)

Til: mtoestesen@yahoo.dk;

Dato: 9:56 torsdag den 3. maj 2012

Hej Marie

Jeg beklager den sene tilbagemelding.

Datamaterialet er vejet op i mod Danmark Statistik ud fra køn, alder, region og uddannelse. Dvs. hvor det har været nødvendigt er disse baggrundsvariable blevet ganget med en faktor, så de følger fordelingen fra Danmarks Statistik – denne faktor er for den enkelte variable maksimalt 2 eller minimalt 0,5, hvis faktoren er 1 betyder det, at der er ikke er nogen forskel mellem det indsamlede materiale og fordelingen hos Danmarks Statistik. Når vejeprocessen er afsluttet kan der stadig være en forskel på +/- 0,3 mellem det vejede materiale og fordelingen i Danmarks Statistik – dvs. fx hvis Danmarks Statistiks fordeling på mænd er 50% kan den vejede fordeling for mænd ligge i intervallet 49,7%-50,3% (når vejeprocessen er afsluttet). Det skal påpeges, at når der vejes på en variable, påvirker dette selvfølgelig også de andre variable (ikke kun de vejede variable køn, alder, region og uddannelse) og da alt data hænger sammen. Derfor er det også en større udfordring, når der vejes på flere variable på en gang.

Jeg håber, at du kan bruge forklaringen. Jeg er på vej til møde nu og er tilbage efter kl. 15.

Held og lykke med specialet ☺

Hilsen Rasmus

Frederiksholms Kanal 4B - 1220 København K
Direkte nummer: +45 888 21 845 - Hovednummer: +45 888 21 888 - Fax: +45 888 21 889
E-mail: rat@interresearch.dk - Hjemmeside: http://www.interresearch.dk