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# Functional Ingredients in Food

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# Preface

This thesis constitutes the completion of a master's degree at the Department of Biotechnology (IBT) at the Norwegian University of Science and Technology (NTNU). The project was conducted in cooperation with SINTEF Ocean in connection with their project "Helhetlig bioØkonomisk utnyttelse av verpehøNE (HØNE).

First of all, I would like to thank my supervisor, professor Turid Rustad, for all her knowledge, help and guidance provided during my lab work and throughout the work on my thesis. I would also thank my co-supervisor at SINTEF Ocean, Kirsti Greif, for the regular monitoring and the help in the product development. I want to thank the remaining personal at the "næringsmiddellabben", and especially Siri Stavrum and Veronica Hjellnes, for helping me with my work, and for keeping a good mood during the lab days.

Finally, I would like to thank my mother Agnete Segalstad and the rest of my family. Your encouragement and support have been important for me during my time in Trondheim.

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Mickel Segalstad-Slaatto

# Abstract

In Norway there are more than 4.3 million laying hens that produce an average of 6 eggs every week between the age of five to twelve months. After twelve months the egg production starts to decrease, and when the hens are about 18 months old, the egg production is too low to be economically beneficial. With no suitable slaughter line for laying hens, more than 95% of them are destroyed, and some used in concrete. The UN has estimated a demand for a 70% increase in food production by 2050. Higher utilization of today's rest raw material can cover some of this demand without increasing the use of resources.

In this study, the objective was to characterize protein hydrolysate recovered by enzymatic hydrolysis of spent laying hens (HPH), and to explore new possibilities for use of the hydrolysates. Enzymatic hydrolysis is a process that can be used to extract valuable fractions such as proteins and lipids from rest raw materials. Functional properties like solubility, water holding capacity and emulsifying capacity was analysed. Total amino acid composition and free amino acids to investigate the nutritional value of HPH. The results were compared to the properties of two commercial protein powders, ScanPro T-95 and ScanPro FCP 75 to investigate the possibility of replacing existing food supplements.

With the exception of high solubility, HPH had poor functional properties compared to the commercial protein powders. The water holding capacity and emulsifying properties of HPH were very low, and HPH is not suitable for increasing the functional properties of food. Despite the poor functional properties, HPH might have an application as dietary supplement. With a high protein content, consisting of 41,6±0,6% essential amino acids, the nutritional properties are better compared to the commercial protein powders. HPH has a $PER^c$ value more than 1,4 times higher than the commercial protein powders, and it could therefore be used in foods where an increased protein content is more important than the addition of functional properties. With the good nutritional value, the sensory and functional properties of meatloaf with varying amounts of HPH added was analysed. The sensory analysis gave promising results for further use of HPH as a dietary supplement for human consumption.

# Sammendrag

I Norge har vi mer enn 4.3 millioner verpehøns. I gjennomsnitt legger de 6 egg i uken fra de fyller 5 måneder til de blir 12 måneder gamle. Etter 12 måneder går eggproduksjonen ned, og ved fylte 18 måneder er det ikke økonomist forsvarlig for bøndene å beholde dem. Det finnes per i dag ingen funksjonelle slaktelinjer for verpehøns. 95% av verpehønsene blir kastet, hvorav noen blir brukt som bindemiddel i betong. FN har estimert et behov for 70% økning av dagens matproduksjon innen 2050. En høyere utnyttelse av dagens restråstoff vil bidra til å nå øke matproduksjonen uten et større bruk av ressurser.

Målet med denne oppgaven var å karakterisere et proteinhydrolysat gjenvunnet ved enzymatisk hydrolyse av verpehøns (HPH), samt utforske nye bruksområder for det nevnte hydrolysatet. Enzymatisk hydrolyse brukes for å ekstrahere proteiner og fett fra restråråstoff. De funksjonelle egenskapene løselighet, vannbindingsegenskap og emulsjonsdannelse ble analysert. Total aminosyresammensetning og andel frie aminosyrer ble også bestemt for å karakterisere den næringsmessige nytteverdien av HPH. Resultatene ble sammenliknet med de kommersielle proteinpulverne ScanPro T-95 and ScanPro FCP 75 for å utforske muligheten av å erstatte kommersielle tilsetningsprodukter med HPH.

HPH hadde høy løselighet, men de resterende funksjonelle egenskapene var dårlige sammenliknet med de kommersielle proteinpulverne. De dårlige vannbindingsegenskapene og emulsjonsdannelsen for HPH gjør hydrolysatet uegnet til bruk for å øke de funksjonelle egenskapene i mat. HPH har et høyt innhold av proteiner, og da spesielt essensielle aminosyrer. Med en $PER^c$-verdi 1,4 ganger høyere enn de kommersielle produktene, har HPH en høy enæringsmessig verdi, og har derfor et mulig bruksområde som kosttilskudd i mat. Dette gjør at hydrolysatet har en høyere ernæringsmessig verdi enn de kommersielle produktene, og kan ha et bruksområde som kosttilskudd. Med grunnlag i de ernæringsmessige verdiene av HPH ble det lagt kjøttpudding tilsatt HPH. Det ble gjennomført sensoriske og funksjonelle analyser av kjøttpuddingen. Det var positive resultater både på smak og utseende av kjøttpuddingen, og resultatene indikerer at HPH har et bruksområde som kosttilskudd for å øke proteininnholdet i matvarer.

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# 1. Introduction

## 1.1 Spent Laying Hens

Laying hens have the highest egg production within their first year of living. Between the age of five and twelve months they lay an average of six eggs a week (Stranden 2016). After one and a half year the egg production is so inefficient compared to younger hens that killing them is more economically beneficial for the farmers. According to the Norwegian Directorate of Health, the yearly average consumption of egg has stabilized on 12.5 kg of eggs per citizen (Helsedirektoratet 2016). In 2015 there were 4.3 million laying hens in Norway, and according to SINTEF, 95% were treated as high risk waste and destroyed (Carvajal 2015).

The increased cost of slaughtering spent hens, both for farmers and producers, is one of the reasons why most spent hens are destroyed without any further utilization (Stranden 2016). Laying hens are bred to produce more eggs, and there will therefore be differences in physiology and anatomy compared to chickens, which in turn are bred to produce the highest yield of meat. The differences in anatomy, primarily the smaller size of hens, make the hens unavailable for the already existing slaughter line used by today's producers (Stranden 2016). Increased cost of production together with smaller size of the filets make it economically unprofitable to produce food from spent hens. To increase the utilization of spent hens, one possibility is to use the residual raw material to produce hydrolysate with functional and nutritional benefits. According to Food and Agriculture Organization of the United Nations food production must increase by 70 percent from 2005 to 2050. The use of rest raw material from animals will therefore be a way to cover some of this demand without requiring increased resources.

## 1.2 Objective of This Study

The overall objective of this project was to study the utilization of spent laying hens, and find new applications for spent laying hens in food products. This study was divided into two subunits. First of all, functional and nutritional properties of hen hydrolysates recovered by enzymatic hydrolysis (HPH) performed by Veronica Hammer Hjellnes in her master project was analysed (Hjellnes 2016). The results will be compared to the results of commercial protein powders to determine whether HPH can replace commercial products and improve the quality of the foods, both nutritional and functionality. Secondly, HPH was added to a food

product to determine how it behaves in a food system, and whether it gives the food undesirable taste.

## 1.3 Proteins

### 1.3.1 Essential amino acids

Proteins are chains of amino acids with different lengths. In total there are 20 single amino acids. The different sequence of amino acids gives each protein a different structure and function. Most of the amino acids can be synthesised by humans, however, eight of them are essential amino acids (Table 1.1); amino acids humans are not able to synthesise, and must therefore be obtained through the diet. There are several methods to determine the nutritional value of proteins, e.g. net protein utilization (NPU), biological value (BV) or protein efficiency ratio (PER) (Satterlee, Marshall et al. 1979). Both NPU and BV use human subjects to compare the protein quality. This makes the methods both time consuming and expensive.

*Table 1.1: Overview of the essential- and non-essential amino acids. The conditionally essential amino acids are synthesised in humans, but the pathway require the presence of an essential amino acid. Arginine and histidine (marked with *) are synthesised at a lower rate than the other amino acids, and are recommended to be supplemented through diet.*

| **Essential amino acids** | **Conditionally essential amino acids** | **Non-essential amino acids** |
|---|---|---|
| Phenylalanine | Cysteine | Alanine |
| Isoleucine | Tyrosine | Arginine* |
| Leucine | | Asparagine |
| Lysine | | Aspartate |
| Methionine | | Glutamine |
| Threonine | | Glycine |
| Tryptophan | | Histidine* |
| Valine | | Proline |
| | | Serine |

The PER values can be calculated using three different equations (Appendix E), all developed by Alsmeyer et al. and Lee et al. (Šližytė, Daukšas et al. 2005). The PER equations are calculated from the essential amino acid content of the hydrolysate (Appendix E). With the total amino acid composition being the only requirement for the determination of a hydrolysate quality, the PER values are a fast and inexpensive method compared to BV and NPU. The PER values were therefore used to determine the quality of the protein powders in this study. An increased amount of essential amino acids in hydrolysates will therefore make the hydrolysate more suitable as a nutritional booster in foods. Cysteine and Tyrosine are conditionally essential amino acids (Damodaran, Parkin et al. 2007). They can be synthesised in the body, but the synthesis pathway requires one of the eight essential amino acids.

Histidine and arginine are amino acids synthesised in the body, but they have a lower synthesis rate than the other amino acids. It is recommended to supplement the uptake of histidine through the diet (Snyderman 1971, not seen, cited after, Kopple and Swendseid 1975, Fürst and Stehle 2004). Amino acids that are conditionally synthesised in the body, or synthesised at a lower rate than needed, are categorised as dietary essential (Mitchell 1962, not seen, cited after Fürst and Stehle 2004).

### 1.3.2 Functional properties

The functional properties of proteins can be defined as "those physicochemical properties which affect the behaviour or proteins in food systems during preparation, processing, storage and consumption" (Kinsella 1979). The functional properties of a proteins are an indication of how they will behave in a food matrix. For a hydrolysate it is important to possess good functional properties to have a wider application in food than just nutritional value. A protein hydrolysate will have functional properties according to the amino acids found in the protein source. However, changing the conditions of the enzymatic hydrolysis like pH, temperature and degree of hydrolysis will influence the functional properties (Kristinsson and Rasco 2000). A proteins ability to interact with water is reflected in its solubility, and is one of the most important factors affecting its functional properties.

Water holding capacity and emulsification are both functional properties improved with increased solubility (Kristinsson and Rasco 2000). Conditions that affect the protein-water interaction, and hence affect the functional properties of a hydrolysate, are pH, hydrophilicity, ionic strength and protein conformation. A hydrolysate will have a higher hydrophilicity than the original proteins du to smaller peptides. The increased number of peptides will also have a higher number of exposed amino- and carboxyl groups that can bind water directly (Barrow and Shahidi 2007).

The ability to bind and retain water is an important functional property of proteins in food, and it is defined as the proteins water holding capacity; "a quantitative indication of the amount of water retained within a protein matrix under certain conditions" (Chou and Morr 1979). Proteins can increase the water holding capacity of food products by binding directly to water, a protein-water interaction, by the physical entrapment of water. They can also react with proteins in the food and increase their stability and ability to bind water molecules (Kristinsson 2007). Water holding capacity is positively correlated with water binding capacity (Damodaran, Parkin et al. 2007). For a peptide, the water binding capacity will change based on the amino acid composition. Charged polar amino acids binds 6 moles of

water per residue while uncharged- and nonpolar amino acids respectively bind only 2 and 1 moles of water per residue (Damodaran 1997).

As well as contributing to the water holding capacity, proteins play an important role in the formation and stabilization of an emulsion. The homogenisation of two immiscible liquids will form a emulsion by dispersing one of the liquids in the other liquid (McClements 2015). Proteins are amphiphilic; they have both hydrophobic and hydrophilic properties, and they will therefore tend to place themselves between a water- and an oil phase and act as an emulsifier (Santiago, Maldonado-Valderrama et al. 2008). An emulsion can either be water-in-oil emulsion or oil-in-water emulsion, however, a hydrolysate will promote oil-in-water emulsions (Wilding, Lillford et al. 1984, not seen, cited after Kristinsson and Rasco 2000). In order to work as an emulsifier, proteins must expose their hydrophobic parts. Once the proteins are absorbed to the interface they start to unfold and rearrange their hydrophobic parts, and their flexibility, enhanced by partial denaturation of proteins, is therefore one of the most important characteristics for proteins in emulsification (Damodaran, Parkin et al. 2007). Proteins with higher solubility will therefore have increased emulsifying properties (Kristo and Corredig 2014). Proteins promote formation and stabilization of emulsions by reducing the interfacial tension between the two phases, and formation of a viscoelastic film around the droplets (Walstra 2002). The protective protein layer will reduce the chance of coalescence, hence increasing the stability during storage (Damodaran, Parkin et al. 2007). The emulsification properties of proteins are affected by the pH, and food proteins are generally good emulsion stabilizers at pH around their isoelectric point (pI). However, at this pH proteins will have a lower ability to form emulsions, in addition to lower solubility (Barrow and Shahidi 2007). At a pH outside the pI-range of proteins, protein-protein interactions are favoured over protein-water interactions, leading to the formation of a protective film around the droplets (McClements 2004). Proteins should therefore be used as emulsifiers at a pH outside their pI.

In addition to contribute to the functional properties of food, proteins can also affect the taste and appearance of a food product. When exposed to heat, browning occur as the result of a reaction between the amino group of a free amino acid, peptide or protein and the carbonyl group of a reducing sugar (Damodaran, Parkin et al. 2007). In the late stage of this Maillard reaction melanoidins, insoluble, colouring compounds, are produced. Depending on the reactive amino acid and sugar, aldehydes produced alongside melanoidins give flavour to the food (Van Boekel 2006).

While the Maillard reaction might give the food desirable flavour, bitterness is a undesirable property that is common for protein hydrolysates (Damodaran, Parkin et al. 2007). The bitter taste was first investigated by Murray and Baker. Their findings indicated a correlation between bitter taste and hydrophobic peptides (Murray and Baker 1952, not seen, cited after, Maehashi and Huang 2009). This correlation was later shown for peptides ranging from 1000-6000 Daltons (Ney 1971, not seen, cited after Damodaran 1997). Bitterness is not likely to occur in larger peptides (Damodaran, Parkin et al. 2007), nor in free amino acids broken down from the bitter peptides (Kristinsson and Rasco 2000).

## 1.4 Enzymatic hydrolysis

Enzymatic hydrolysis is a biotechnological processing method used to utilise animal rest raw materials. One of its applications is to recover proteins (Šližytė, Rustad et al. 2005). Proteins are recovered as hydrolysate; defined as proteins broken down to peptides by the cleavage of peptide bonds between amino acids (Kristinsson and Rasco 2000). The recovery of protein as a hydrolysate from rest raw material can either be done by enzymatic hydrolysis or chemical hydrolysis. However, chemical hydrolysis has been shown to damage the end product and have a lower protein recovery rate than by the use of enzymes (Šližyte, Daukšas et al. 2005). In addition to have a higher protein recovery rate, enzymatic hydrolysis produces an end product with increased functional and nutritional properties. Partly degraded proteins often have increased functional properties compared to the original larger proteins (Clemente 2000, Šližytė, Rustad et al. 2005). Enzymatic hydrolysis will increase the water-protein interactions of the hydrolysate compared to the original proteins, thus increasing the solubility (Opheim, Šližytė et al. 2015). Enzymes used to break the peptide bonds can either be endogenous enzymes in the raw material or added enzymes (exogenous) (Adler-Nissen 1986).

### 1.4.1 Process

Before the enzymatic hydrolysis starts, rest raw material is ground to improve the availability of the proteins as substrate, and increase the efficiency of proteases. Water is added to the rest raw material to homogenize the mixture (Kristinsson and Rasco 2000), and to improve the protein recovery at the expense of lipids (Šližyte, Daukšas et al. 2005). To have full control over the hydrolysis, the use of commercial enzymes and the inactivation of endogenous enzymes might be necessary (Šližytė, Rustad et al. 2005). Heat exposure of the rest raw material prior to the hydrolysis results in inactivation of endogenous enzymes but it

could also result in a lower degree of hydrolysis (Cui, Zhou et al. 2009). Proteins will have increased solubility up to 40-50°C, but above this they will start to denature (Pelegrine and Gasparetto 2005).

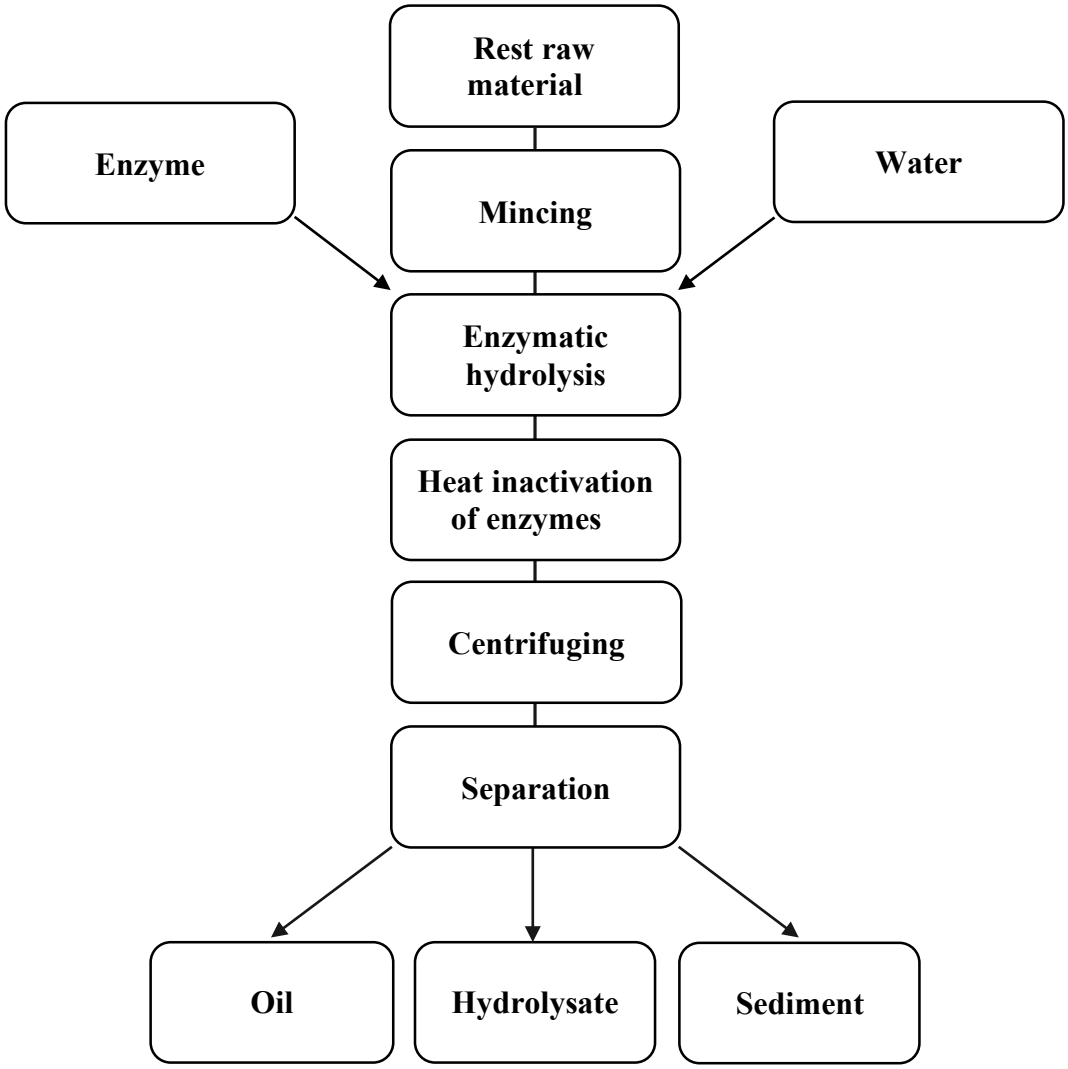


*Figure 1.1: Flow diagram for the enzymatic hydrolysis process of rest raw material. Rest raw material from spent laying hens were used to produce two hen hydrolysates (HPH1 and HPH2) in a former master project by Veronica H. Hjellnes. The diagram is freely edited after (Kristinsson and Rasco 2000).*

To initiate enzymatic hydrolysis, enzyme is added as soon as the mixture of water and rest raw material is heated to the temperature optimal for enzyme activity and protein recovery. When the wanted degree of hydrolysis is reached, the enzyme is inactivated by heat exposure for a short period of time (Opheim, Šližytė et al. 2015). The three phases produced by enzymatic hydrolysis; oil, water soluble proteins and sediment are separated by

centrifugation. The water soluble phase is dried and protein hydrolysate is produced (Šližytė, Mozuraitytė et al. 2009).

### 1.4.2 Protein Hydrolysates

The main product from enzymatic hydrolysis is the water soluble phase, termed protein hydrolysate. The hydrolysate consists of proteins, fat and minerals. The composition of the hydrolysate, and thereby its functional and nutritional characteristics, is influenced by the composition of the rest raw material, endo- and exogenous enzyme activity and the conditions of the hydrolysis process (Opheim, Šližytė et al. 2015). The quality of the hydrolysate is positively correlated with the protein content (Šližytė, Rustad et al. 2005), while the opposite is true for lipid content. A higher protein content at the expense of lipids make the hydrolysate less exposed to oxidation. A high lipid concentration can potentially impair the sensory qualities (Opheim, Šližytė et al. 2015).

The degree of hydrolysis has a direct impact on the functionality of the hydrolysates. Higher degree of hydrolysis results in more broken peptide bonds, and the number of smaller peptides increases. Smaller peptides consist of more ionised amino acids and carboxyl groups, thus increasing the solubility of the hydrolysate (Panyam and Kilara 1996). Smaller peptides will also give the hydrolysate a higher nutritional value as they are more bioavailable than larger proteins. Increased solubility and nutritional value are both good qualities for a product being implemented into human diet.

However, a higher degree of hydrolysis also has some drawbacks for the hydrolysate. The water holding capacity will decrease (Šližytė, Mozuraitytė et al. 2009), and this will make the hydrolysate lose some of its functional properties. A severe negative quality that comes with higher degree of hydrolysis is the formation of bitter taste (Dauksas, Slizyte et al. 2004). The bitter taste is a result of the exposure of hydrophobic amino acids during hydrolysis, with the likes of leucine, isoleucine and valine (Adler-Nissen 1976, Pedersen 1994, not seen, cited after, Nilsang, Lertsiri et al. 2005). To reduce the bitter taste of protein hydrolysates, the degree of hydrolysis can be lowered to between 3-5% (Adler-Nissen 1984), or exopeptides can be used during the enzymatic hydrolysis (Adler-Nissen 1976).

Protein hydrolysate has a wide field of application in the food industry, but it all comes down to the functional and nutritional characteristics. Changing the enzymatic hydrolysis conditions will produce different hydrolysates with different use. A hydrolysate produces with a long hydrolysis time, high degree of hydrolysis, will be more suited to increase the protein content of foods and dietary supplements rather than provide functional characteristics.

Higher degree of hydrolysis reduce water holding capacity and emulsifying capacity by the production of smaller peptides. The reduction in size will, however, make the peptides more bioavailable for humans, thus increasing the nutritional value. Hydrolysates with high water binding capacity can be added to minced meat products, increasing its ability to retain water during cooking. This will result in a final product with increased juiciness and tender texture.

During enzymatic hydrolysis of proteins recognition sites for the immunoglobulin E can be cleaved, which results in a lower allergenicity of the hydrolysate. An allergic reaction is due to the amino acid sequence, not the amino acid itself, and with the use of specific enzymes the peptide bond of the relevant epitope will be hydrolysed (Damodaran, Parkin et al. 2007). Enzymatic hydrolysis alone will only reduce allergenicity, however, with additional treatment enzymatic hydrolysis has been shown to produce hypoallergenic protein hydrolysates (Clemente 2000).

# 2. Materials and Methods

## 2.1 Materials

During this study, four different protein powders were characterised for functional and nutritional properties (Table 2.1). The two commercial products, ScanPro T-95 and Scanpro FCP 75, are categorised as protein powders. The values from the data sheets were used for these two protein powders (Appendix A). ScanPro T-95 is a protein powder manufactured from natural food grade pork raw material, while ScanPro FCP 75 is produced from natural food grade chicken raw material. The two remaining products, HPH1 and HPH2, are hydrolysates recovered by enzymatic hydrolysis of spent laying hens. HPH1 and HPH2 are both recovered from the same rest raw material. HPH2 was more coarsely ground prior to the enzymatic hydrolysis, and this is the only difference between the two hydrolysates. In further discussion, "protein powder" will be used whenever one or two of the commercial protein powders are included. When the commercial ScanPro products are not featured, HPH1 and HPH2 will be categorized as "hydrolysates". The enzymatic hydrolysis was performed by Veronica Hammer Hjellnes in her master project, and the detailed hydrolysis process can be found in her thesis (Hjellnes 2016).

*Table 2.1: Overview of which analyses included hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75).*

| Experiment | HPH1 | HPH2 | ScanPro T-95 | ScanPro FCP 75 |
|---|---|---|---|---|
| **Water holding capacity** | X | X | X | X |
| **Emulsifying Property** | X | X | X | X |
| **pH-Measurements** | X | X | | |
| **Dry Weight and Ash** | X | X | | |
| **Complete Amino Acid Composition** | X | X | | |
| **Hydroxyproline** | X | X | | |
| **Cook Loss** | X | X | X | X |
| **Molecular Weight Distribution** | X | X | X | X |
| **Pilot Product development** | X | X | | |
| **Product development** | X | X | | |

## 2.2 Dry Weight and Ash

Approximately 2 g fish was added to a pre-weighed beaker and 0,5 g HPH was added to a pre-weighed porcelain crucible and placed in a heat cabinet at 105°C for 24 hours. The

samples were cooled for 2 hours in an exicator before weighing. The porcelain crucibles were placed in a Nabertherm muffle furnace at 550°C for 17 hours. After cooling for 1,5 hours in an exicator, the porcelain crucibles were weighed again. Sample dry weight and ash was determined gravimetrically.

## 2.3 Water Holding Capacity

The water holding capacity (WHC) was analysed as described by (Børresen 1980). Frozen cod filet was thawed and minced with a food processor. Approximately 2 g fish was weighed out in pre-weighed test tubes. The tubes had a polyester membrane at the bottom surface allowing water to escape. The sample holders were placed in centrifuge tubes filled with glass beads and centrifuged for 5 minutes at 210 x g (Sigma 202 centrifuge). The sample holders were then weighed a second time after centrifugation. The influence of HPH and Scanpro on the WHC was determined by adding protein powder of different concentrations to the fish samples before centrifugation. The same procedure was carried out for 1, 2, 3, 5 and 10% protein powder concentration. The WHC was measured as water lost during centrifugation against the water lost during 24 hours in heat cabinet. All measurements were performed in quadruplicates

## 2.4 Emulsifying Properties

Emulsification capacity was analysed as described by (Šližytė, Mozuraitytė et al. 2009). Five different protein powder concentrations were tested, namely 0,5, 1, 2, 3 and 4%. The different protein concentrations, dissolved in distilled water (5 mL), were mixed with 5 mL of rapeseed oil in a 15 mL centrifuge tube. The mixture was homogenized with an IKA T10 basic Ultra-Turrax for 90 seconds at level 6. The test tubes were centrifuged for 3 minutes at 2400 x g in an eppendorf centrifuge 5804R. After centrifugation the amount (mL) of the three separated phases (water, emulsion and oil) was determined by directly reading off the centrifuge tube. The test tubes were left at room temperature for 24 hours, then centrifuged for 3 minutes at 2400 x g, to investigate the emulsion stability. The emulsification capacity was expressed as mL of emulsified oil per 1 g protein powder, while emulsion stability was expressed as percentage of initial emulsion remaining after 24 hours. All measurements were performed in duplicates.

## 2.5 pH-Measurements

Thawed cod filet was used to measure pH changes as a result of addition of HPH. The fish samples (2 g) were mixed with 2 mL 0.15 M KCL and the pH was measured using the pH-meter MP220 Basic pH/mV/°C Meter. The pH was then measured upon addition of HPH in two different concentrations: 1 and 3% of fish weight. The pH of the HPH itself was also measured by mixing 0,2 g powder with 10 mL distilled water.

## 2.6 Solubility

Protein powder (0,02 g) was mixed with 5.0 mL distilled water to determine the solubility using the Lowry method (Lowry, Rosebrough et al. 1951). A standard curve was prepared from the solution of bovine serum albumin (BSA). Sample solutions, standard solutions and blank (0,5 mL) were pipetted into test tubes. Alkaline copper reagent (2,5 mL), made from 1 mL 1% $CuSO_4$, 1 mL 2% potassium sodium tartate and 100 mL 2% $Na_2CO_3$ in 0.1M $N_aOH$, was added and each tube was mixed using a whirlmixer. The test tubes were left at room temperature for precisely 10 minutes. Folin-Ciocalteu reagens (0,25 mL), made from 1 part Folin reagent and 2 parts doubly distilled water, was added to the tubes and mixed. The tubes were left to stand for 30 minutes at room temperature, then mixed. A Genesys 10S UV-Vis spectrophotometer was used to read absorbance at 750 nm.

## 2.7 Total Amino Acid Composition

Total amino acid composition was determined after acid hydrolysis using high-performance liquid chromatography (HPLC), as described by (Blackburn 1968). HPH (0,1 g) was dissolved in 2 mL 6M HCL in a glass tube with screw cap. Hydrolysis was performed in a heat cabinet at 105°C for 22 hours. The glass tubes were cooled to room temperature and the solutions were flushed into a 10 mL volumetric flask. pH was adjusted to 7,0 using NaOH. The samples were filtrated through a Whatman glass microfiber filter GF/F using a vacuum pump. The filtrated solutions were made up to 10 mL volume. The samples were filtrated through a 0,22 mL filter using a syringe and stored in eppendorf tubes. Sample solution was diluted 1:1000 for HPLC, while the rest was stored in the freezer for later experiments. The diluted samples (0,205 mL) were pipetted to a glass test tube with a screw cap and analysed on high performance liquid chromatography (HPLC) by Siri Stavrum. During HPLC the samples will be pumped through a column by applied pressure. Amino acids are being separated based on polarity. Depending on the use of column, either polar or non-polar amino

acids will stick to the column, and the amino acids can be determined by the time they use to travel through the column. The measurements were performed in duplicates, and the amount of each amino acid was measured in g/100 g product.

## 2.8 Hydroxyproline

Hydroxyproline content was measured as described by (Leach 1960). Standard L-hydroxyproline solutions were made by dissolving 0,0125 g hydroxyproline in 100 mL distilled water. Concentrated HCL (10 mL) was added, followed by 25 mL distilled water. The standard solution, with a concentration of 100 μg/ml, was diluted to three different concentrations, namely 5, 10 and 15 μg/ml using distilled water. Frozen sample after acid hydrolysis was used and diluted 1:20. Sample (0,5 mL) was added to each tube, followed by 0,5 mL 0,05M $CuSO_4$ and 0,5 mL 2,5 M NaOH. The samples were mixed using a whirlmixer and covered with marbles. The samples were incubated in a water bath at 40°C for 5 minutes. $H_2O_2$ (0,5 mL, 6%), made fresh from 30%-solution, was added before mixing again. The samples were incubated in water bath at 40°C for 10 minutes and cooled to room temperature. $H_2SO_4$ (2 mL, 1,5M) and 1 mL 5% p-dimethylaminobenzaldehyd in 1-propanol were added in fume hood. The samples were mixed fore incubation in a water bath at 70°C for 16 minutes. The samples were cooled to room temperature. Absorbance was measured at 555 nm using a Genesys 10S UV-Vis spectrophotometer.

## 2.9 Cook Loss

The cook loss of fish added protein powder was determined as described by (Børresen 1980). Measurements were taken for 2 different protein powder concentrations, namely 1 and 3%. Approximately 2 g thawed and minced cod filet, was mixed with protein powder and added to a test tube containing a polyester membrane at the bottom. The test tubes were placed in a heat cabinet at 80°C for 15 minutes. After cooling to room temperature, the tubes were weighed and centrifuged for 5 minutes at 210 x g in a Sigma 202 centrifuge as for determination of WHC. The weight loss of dry sample was used as a reference to determine the relative amount of cook loss after adding protein powder. All measurements were performed in quadruplicates.

## 2.10 Molecular Weight Distribution

Gel filtration using a FPLC system was used to determine the molecular weight distribution for HPH and the commercial Scanpro products. The samples were prepared by dissolving protein powder, respectively 0,1 g HPH and 0,01 g Scanpro, in 4 mL 0,05 M sodium acetate buffer with pH 5 filtrated through a syringe with 0,2 μm filter. The solutions were then filtrated into a eppendorf tube through another 0,2 μm filter using a syringe. The computer program UNICORN was used to run the FPLC machine. Sodium acetate buffer (0,05M, pH 5) was used as the eluent and Superdex peptide 10/300 GL was the column. The sample (0,8 mL) was added to the FPLC machine.

## 2.11 Free Amino Acids

The amount of free amino acids was analysed as described by (Osnes and Mohr 1985). Protein powder (0,025 g) was weighed out and dissolved in 5 ml distilled water. Water soluble protein extract (1 mL) was added to an eppendorf tube and added 0,25 mL 10% sulphosalisylic acid. The tube was shaken vigorously and left in a cold room at 4°C for 30 minutes. The eppendorf tube was centrifuged at 6150 x g for 10 minutes in a eppendorf centrifuge 5415 R. The supernatant (1 mL) was tested for any unprecipitated proteins by adding 0,25 mL 10% sulphosalisylic acid. The supernatant was analysed on HPLC as previously described. The measurements were performed in duplicates.

## 2.12 Acid Soluble Peptides

The amount of acid soluble peptides was analysed as described by (HOYLE and MERRITT 1994). Water soluble extract (2 mL) as described in 2.10 was added to a test tube. Trichloroacetic acid (TCA) (2,0 mL, 20%) was added to the tube. The tube was mixed and left at room temperature for 30 minutes. The sample was filtrated through a Whatman glass microfiber filter GF/F using a vacuum pump. Amount of acid soluble peptides was determined by analysing the amount of acid soluble peptide by the Lowry method

## 2.13 Pilot Project - Product Development

Meatloaf was made on the following recipe:

- 400 g chicken minced meat
- 22 g potato flour
- 200 g milk (1,2% fat)
- 7 g salt
- 0,75 g pepper
- 0/5/10 g HPH

Salt, pepper and potato flour was stirred into the minced cheaken meat. The minced meat was continuously stirred while milk was added. The finished minced meat was divided into 6 portions of 100 g. Two of the portions were added HPH, respectively 5 and 10 g, and stirred. The meatloaf was cooked in small aluminium beakers in a baking tray filled with water one third up the beaker walls at 180°C for 40 minutes.

## 2.14 Product Development

Seven different versions of meatloaf were produced, with changes in HPH concentration, with water replacing milk and where potato flour was excluded (table 2.2). Minced chicken meat and salt was mixed using a food processor for 30 seconds. Pepper, potato flour and HPH was added to the mixture and mixed for another 30 seconds. Milk, or water when milk was replaced, was slowly and continuously mixed in for 1 minute, followed by 2 minutes with stirring. The temperature of the mixture was measured and divided into five portions of approximately 95 g in an aluminium beaker (Havbris). The meat loafs were cooked in a water bath one third up the side wall of the beaker at 180°C for 36 minutes. They were left in cold room overnight before analysed.

*Table 2.2: Recipe for chicken mince meatloaf produced during product development. A mixture of the two hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material, coarsely ground spent hen raw material (HPH) was added in different concentrations (5% and 10%). In two samples milk was replaced with water (WM), and two samples were made without potato flour (WPF).*

| Ingredient | Recipe (g) | Recipe (%) | Reference | 5 % | 10 % | 5 % WM | 10 % WM | 5 % WPF | 10 % WPF |
|---|---|---|---|---|---|---|---|---|---|
| **Chicken mince** | 400,0 | 62,4 | 312,1 | 296,5 | 280,9 | 296,5 | 280,9 | 312,6 | 296,2 |
| **Potato flour** | 33,0 | 5,2 | 25,8 | 24,5 | 23,2 | 24,5 | 23,2 | 0,0 | 0,0 |
| **Milk (1,2% fat)** | 200,0 | 31,2 | 156,1 | 148,3 | 140,5 | 148,3 | 140,5 | 156,3 | 148,1 |
| **Salt** | 7,0 | 1,1 | 5,5 | 5,2 | 4,9 | 5,2 | 4,9 | 5,5 | 5,2 |
| **Pepper** | 0,8 | 0,1 | 0,6 | 0,6 | 0,5 | 0,6 | 0,5 | 0,6 | 0,6 |
| **HPH** | 0,0 | 0,0 | 0,0 | 25,0 | 50,0 | 25,0 | 50,0 | 25,0 | 50,0 |
| **Total** | 640,8 | 100 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| **Protein (g/100g)** | | | 12,3 | 15,5 | 18,6 | 15,5 | 18,6 | 16,1 | 19,2 |
| **Dry weight (%)** | | | 24,1 | 27,6 | 31,1 | 27,6 | 31,1 | 24,7 | 28,4 |

## 2.15 Sensory Analysis

A panel of 10 people were given a bite of each of the seven different meat loafs together with a question to grade the samples from 1-6 (Appendix A). They were asked to give each product two grades; one for taste and texture, the other one for smell and the aesthetic.

## 2.16 Colour Measurement

The colour of the surface of each pudding were measured using a Konica Minolta chroma meter CR-400 on three different spots. A cross section of each meatloaf was made, and the colour of the inside was tested at three spots. The measurements were performed on three different meat loafs made with the same conditions.

## 2.17 Water loss of meatloaf – Centrifugation

Water loss of the meatloaf was analysed as described by (Børresen 1980). Approximately 2 g meatloaf was added to a test tube with a polyester membrane. The tubes were placed in centrifuge tubes containing glass beads and centrifuged for 5 minutes at 210 x g (Sigma 202 centrifuge). The test tubes were then weighed. The tubes were cooled to room temperature and measured. Measured water loss was compared and calculated against the water content of meatloaf. The measurements were performed in triplicates.

## 2.18 Water loss of meatloaf – Mechanical Pressure

A slice of approximately 1 cm was cut from the middle of the meatloaf and placed between 10 pre-weighed filter papers. A beaker filled with water weighing 1 kg was placed on top of the meatloaf for 2 minutes. The meatloaf was removed and the filter papers were weighed. The filter papers were placed in a heat cabinet at 105°C for 24 hours. They were cooled to room temperature for one hour and weighed. The measurements were performed as triplicates.

# 3. Results and Discussion

## 3.1 Dry Weight and Ash

The dry weight of HPH1 and HPH2 was measured to 94,33±0,78 and 93,98±0,05, respectively, with an ash content of 11,73±0,10 and 11,55±0,07 (Table 3.1). For the commercial ScanPro products, the values are taken from their respective data sheets (Appendix A). The dry weight of HPH is close to the values for the commercial products; >96% for ScanPro T-95 and 95-99% for ScanPro FCP 75 according to their datasheets. The dry weight of protein powders includes proteins, fat and minerals. In foods, ash refers to the inorganic content such as minerals (Pomeranz and Meloan 1994). In animals, minerals are primarily found in bones (Siri 1956), thus giving hydrolysates recovered from animals containing bones a higher ash content. This is most likely the explanation of the elevated ash values for HPH. HPH is recovered from spent laying hens including bones, unlike the commercial protein powders which is recovered from natural food grade (Appendix A). The composition of the ash was not further investigated.

*Table 3.1: Dry weight (%) and ash (%) of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) (mean ± SD, n = 4). The values for two commercial products ScanPro T-95 and ScanPro FCP 75 were taken from their datasheet (Appendix X).*

| Sample | Dry Weight (%) | Ash (%) |
|---|---|---|
| **HPH1** | 94,33 ± 0,78 | 11,73 ± 0,10 |
| **HPH2** | 93,98 ± 0,05 | 11,55 ± 0,07 |
| **ScanPro T-95** | >96 | 1-2 |
| **ScanPro FCP 75** | 95-99 | 3-5 |

According to the data sheets T-95 has the highest protein content of 94-98% while the protein content of FCP 75 and HPH is approximately 75% (Table3.4). HPH will therefore have a higher fat concentration than T-95. The indicated results from ash, total amino acid composition (Table 3.4) and hydroxyproline (Table 3.5) adds up to 88% of HPH components, leaving 6% of the dry weight unaccounted for. There are some amino acids left out during total amino acid composition, but it is reasonable to estimate the fat content of HPH to somewhere between 4-6%. The fat content was not determined.

## 3.2 pH-Measurements

The pH of fish was measured, and HPH was added in two different concentrations to observe any changes in pH. pH of pure fish mince was measured to 6.39±0,02, and a small reduction in pH was observed in samples with higher level of addition of HPH (Table 3.2).

The results indicate that HPH dissolved in distilled water has a pH in the middle of the pH range of the commercial ScanPro products; ranging from pH 5-8 (Table 3.3). The pH value of a hydrolysate influences the functional properties (Kristinsson and Rasco 2000). This is further discussed under water holding capacity (chapter 3.9) and emulsifying properties (chapter 3.10).

*Table 3.2: pH of fish mince was measured with increasing level of addition of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) (mean ± SD, n = 4). pH of HPH was also measured in distilled water (mean ± SD, n=2). Values for the commercial protein powders (*) were found in their datasheets (Appendix A).*

| Sample | pH |
|---|---|
| **Pure fish** | 6,39±0,02 |
| **Fish + 1% HPH1** | 6,36±0,01 |
| **Fish + 1% HPH2** | 6,37±0,01 |
| **Fish + 3% HPH1** | 6,33±0,02 |
| **Fish + 3% HPH2** | 6,33±0,01 |
| **Water + HPH1** | 6,27±0,00 |
| **Water + HPH2** | 6,27±0,01 |
| **ScanPro T-95*** | 5-7 |
| **ScanPro FCP 75*** | 6-8 |

## 3.3 Solubility

Solubility of the protein powders was measured spectrophotometrically after dissolving protein powder in distilled water. Solubility is an important functional property of proteins, and is it usually a correlation between solubility and other functional properties (Wilding, Lillford et al. 1984). Increased solubility of protein powders is therefore important to provide foods with increased functional properties like water holding capacity and emulsifying capacity. The solubility of HPH1 and HPH2 was found to be 58,1%±0,7 and 63,6%±0,3 g/100 g, respectively (Figure 3.1). The commercial ScanPro products were found to have lower solubility, with ScanPro T-95 having a solubility of 47,7%±1,2 and 15,9%±1,6 g/100 g for ScanPro FCP 75 .

Higher solubility promotes higher emulsifying properties by increasing the flexibility and the unfolding of proteins at the oil-water interface (Kristo and Corredig 2014). The results indicate that HPH will have better emulsifying properties than the ScanPro products. This was, however, not the case in this study. In contradiction, the commercial protein powders were found to have greater emulsifying capacity (Table 3.9). The solubility of hydrolysates increases with increased degree of hydrolysis (Chobert, Sitohy et al. 1988).

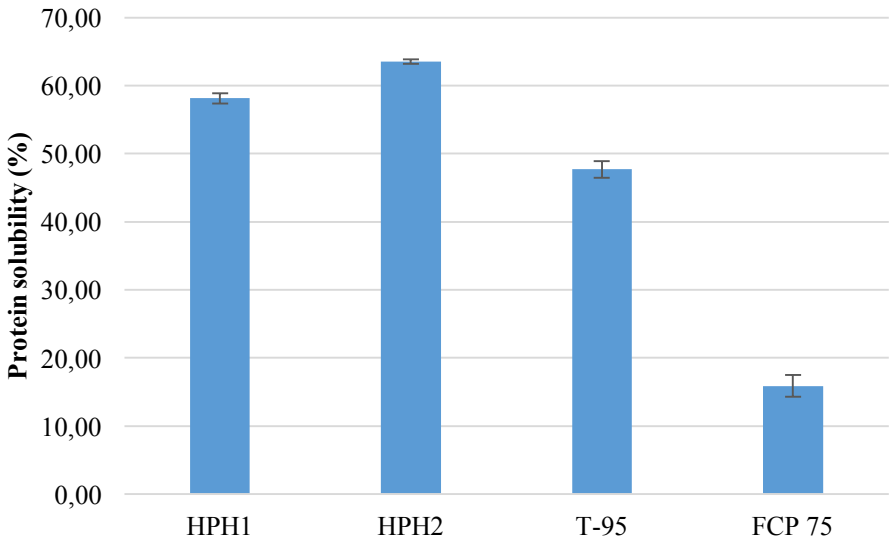


*Figure 3.1: Solubility of protein powders (%) in distilled water for hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75) (mean ± SD, n = 2).*

The contradictory results for the degree of solubility, emulsifying properties and water holding capacity of the protein powders could be explained with HPH being too degraded. To possess good emulsifying properties, the peptide size of a hydrolysate should be higher than 20 amino acid residues (Lee, Shimizu et al. 1987). With the given results, it is reasonable to think that the degree of hydrolysis of HPH has exceeded the balance between solubility and other functional properties. These results point towards the importance of peptide size and pH of the hydrolysate when it comes to functional properties, which is further discussed in chapter 3.9 and 3.10.

## 3.4 Molecular Weight Distribution

Molecular weight distribution of the protein powders was determined using FPLC with Superdex peptide 10/300 GL column. All four protein powders were analysed, together with a vitamin B12 standard. However, no peptides were detected in the sample of T-95, and this is therefore left out from the results. Separation range of the column used is 100-7000 Daltons (Da). The lack of results for T-95 could therefore be explained with the protein powder containing too large peptides for this method. The HPH samples have two major peaks at 13 and 21 mL (Figure 3.2). Vitamin B12, the standard used in this study, has a size of 1 357 Da and peaks after 18,5 mL. The results of the HPH analysis indicates therefore that the majority of the peptide content of HPH are in the region of 1-2 kDa. With smaller peaks ranging from

6 mL to 30 mL, HPH contain a limited amount of peptides with both higher and smaller molecular weight.

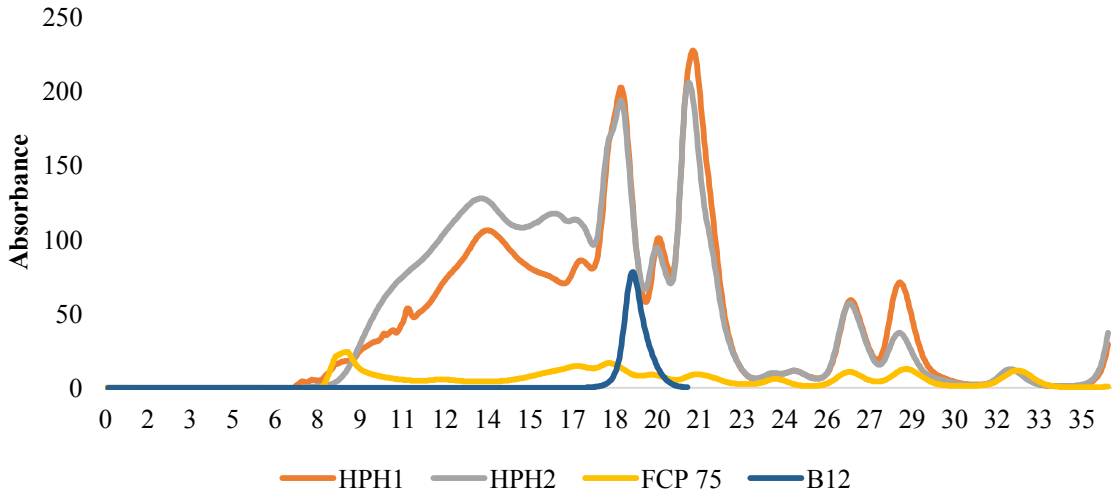


*Figure 3.2: Molecular weight distribution of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2), two commercial ScanPro protein powders (T-95 and FCP 75) and a standard of vitamin B12.*

The results show that HPH1 and HPH2 have the same peaks, consisting of peptides with the same molecular weight. The peptide concentration of ScanPro FCP 75 is very low, however, the results indicate peaks at the same molecular weight as for HPH. The low concentration is probably explained by the low solubility of the protein powder (Figure 3.1). This would reduce the amount of protein powder applied to the FPLC machine, resulting in a lower detection level. The similarities in the peptide size between the HPH1 and HPH2 are easily explained by source of proteins. Both are recovered from spent hen, and with FCP 75 recovered from chicken meat, it is expected to see similarities. With the low concentration of FCP 75 it is difficult to say which of the given peaks that are more dominant. However, the results from the water holding capacity and emulsifying properties, indicating better functional properties for ScanPro FCP 75 compared to HPH, it is reasonable to believe that ScanPro FCP 75 has a larger concentration of peptides with a higher molecular weight.

A previous study done by Hjellnes showed a majority of peptides with a molecular weight less than 6 kDa (Hjellnes 2016). The molecular weight of HPH changed due to different enzymatic hydrolysis conditions, with some enzymes resulting in a lower molecular weight. The observer results of HPH1 and HPH2 are in compliance with the previous research done on this

## 3.5 Total Amino Acid Composition

Total amino acid composition of HPH1 and HPH2 was measured after acid hydrolysis using HPLC. The results indicate that the two hen hydrolysates are closely linked in their amino acid composition, while the commercial ScanPro T-95 and ScanPro FCP 75 differ in their amino composition compared to HPH1 and HPH2 (Figure 3.3). For HPH1 and HPH2 the most prominent amino acids are glutamic acid and lysine. In ScanPro FCP 75 glycine and glutamic acid is the most prominent amino acids, while glycine, followed by proline are the most prominent amino acids in ScanPro T-95.

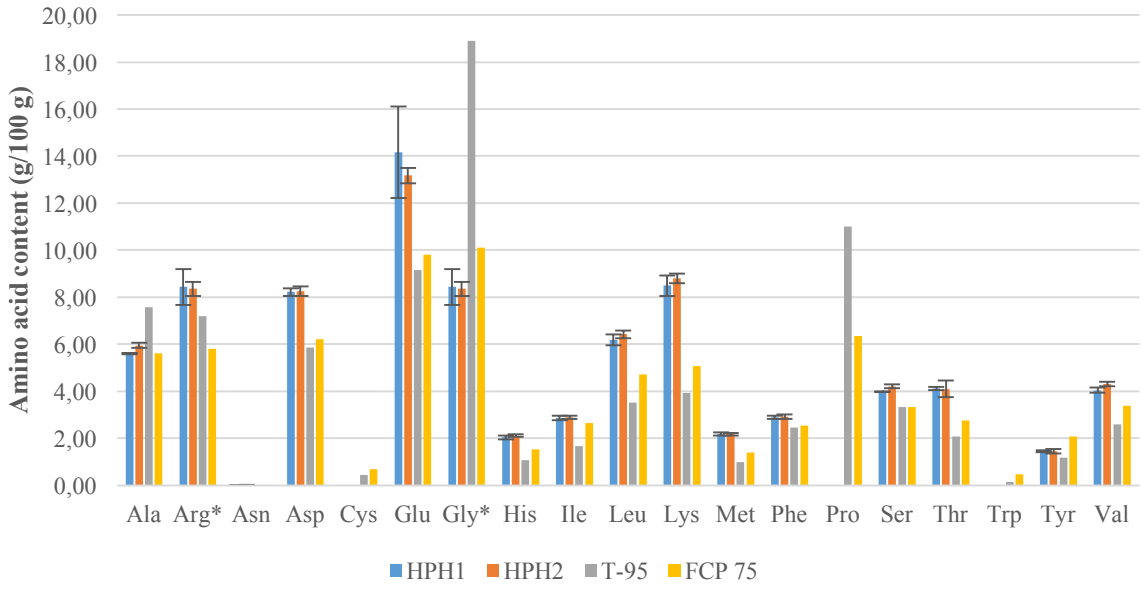


*Figure 3.3: Total amino acid composition of HPH1 and HPH2 (g/100g) was analysed for hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) (mean ± SD, n = 2). Values for the commercial protein powders ScanPro T-95 and ScanPro FCP 75 are taken from their datasheets (Appendix X). Glycine and arginine (*) are returned as one combined value after HPLC. For HPH1 and HPH2 the total combined value is presented for both amino acids.*

The similarities in the amino acid composition of HPH1 and HPH2 are to be expected, as they are recovered from the same rest raw material. The total amino acid content of HPH is measured to approximately 75 g/100g (Table 3.3), however, the actual amino acid concentration of HPH is higher. Tryptophan is destroyed during acid hydrolysis (Kristinsson and Rasco 2000), while hydroxyproline is not detected with the use of OPA derivatisation. The amount of hydroxyproline in the hydrolysates was, however, measured in a separate analysis (Table 3.5). The quality of hydrolysates is often determined by their amount of proteins. With a protein content >75 g/100g, HPH must therefore be considered a hydrolysate of high quality, suitable for use in foods to increase protein concentration.

*Table 3.3: Protein content of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) based on the total amino acid composition (mean ± SD, n = 2). Values for the commercial protein powders ScanPro T-95 and ScanPro FCP 75 are taken from their datasheets (Appendix A).*

| HPH1 | HPH2 | ScanPro T-95 | ScanPro FCP 75 |
|---|---|---|---|
| 74,7±1,7 | 75,2±2,2 | 94-98 | 72-78 |

The quality of a protein powder can be determined using the protein efficiency ratio (PER) developed by Alsmeyer et al. and Lee et al. $PER^c$ is the most comprehensive equation, including the following seven amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine. $PER^a$ includes the amino acids leucine and tyrosine, while $PER^b$ includes methionine, leucine, histidine and tyrosine (Alsmeyer, Cunningham et al. 1974, Lee, ELLIOTT et al. 1978, both not seen, cited after Šližytė, Daukšas et al. 2005).

The calculations give a $PER^c$ value for HPH1 of 2,38 and 2,45 for HPH2 (Table 3.4). This is more than 1,4 times greater than for ScanPro FCP 75, the best of the two commercial protein powders. The PER values are based on the essential amino acid concentration of the protein powders. The obtained values show that HPH has a higher nutritional value than the commercial protein powders.

*Table 3.4: The protein efficient ratio (PER) values for hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75).*

| | HPH1 | HPH2 | ScanPro T-95 | ScanPro FCP 75 |
|---|---|---|---|---|
| **$PER^a$** | 2,16 | 2,27 | 1,00 | 1,44 |
| **$PER^b$** | 3,01 | 3,22 | 0,49 | 0,85 |
| **$PER^c$** | 2,38 | 2,45 | 1,29 | 1,71 |

Higher PER values for HPH makes these hydrolysates more suitable than the commercial protein powders analysed in foods where increased nutritional properties are more important than the addition of functional properties. With the high protein content, and an essential amino acids content of 41,6±0,6% in particular, HPH can be used in foods for people who have a reduced intake of food, e.g., elderly people, to maintain important biological functions.

## 3.6 Hydroxyproline

Hydroxyproline, an amino acid not determined by the HPLC analysis, of HPH was determined using the Leach method. The results show that HPH1 and HPH2, with a hydroxyproline concentration of 1,4±0,0 and 1,6±0,0 g/100g, respectively (Table 3.5). This is a significantly lower amount of hydroxyproline than the commercial ScanPro products T-95

and FCP 75. The values for the commercial protein powders were found in their datasheets (Appendix A). ScanPro T-95 had the highest hydroxyproline concentration, with a value of 9,18 g/100g.

*Table 3.5: Hydroxyproline was measured for hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) (mean ± SD, n = 3). The values for the commercial ScanPro products (T-95 and FCP 75) were found in their datasheets (Appendix X).*

| Sample | Hydroxyproline (g/100g) |
|---|---|
| **HPH1** | 1,4±0,0 |
| **HPH2** | 1,5±0,0 |
| **T-95** | 9,2 |
| **FCP 75** | 3,8 |

Hydroxyproline is almost exclusively found in collagen, and the collagen content of a protein powder can therefore be determined by measuring the amount of hydroxyproline. Hydroxyproline constitutes about one third of the amino acids in collagen (Ramshaw, Shah et al. 1998, not seen, cited after Shoulders and Raines 2009), and it also plays an important role in stabilising the collagen structure (Berg and Prockop 1973). Collagen is a key factor when it comes to increasing the water holding capacity of a food product (Ranganayaki, Asghar et al. 1982). The observed high value of hydroxyproline in T-95, and the low concentration in HPH, corresponds well with the observed results from the water holding capacity analysis for the respective protein powders (Figure 3.5).

## 3.7 Free Amino Acids

The concentration of free amino acids in the protein powders was measured by the use of HPLC after precipitation of proteins and peptides with sulphosalisylic acid. Both the hen hydrolysates and the commercial ScanPro products were analysed. HPH1 was found to have the highest concentration of free amino acids, with a concentration of 7,4±1,7 g/100 g (Table 3.6). HPH2 followed with a free amino acid concentration of 5,5±0,6 g/100 g, while ScanPro FCP 75 were found to only have 0,5±1,2 g/100 g free amino acids. The amount of free amino acids constitutes less than 10% of the total protein concentration for the protein powders. Scanpro T-95 showed no content of free amino acids, and is therefore not shown the results.

*Table 3.6: Total concentration of free amino acids of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and one commercial ScanPro protein powder (FCP 75) (mean ± SD, n =2).*

| Sample | Free amino acids (g/100g) |
|---|---|
| **HPH1** | 7,4±1,7 |
| **HPH2** | 5,5±0,6 |
| **FCP 75** | 0,5±1,2 |

With a few exceptions, the pattern in the concentration of the individual free amino acids (Figure 3.4) follows the concentration of each amino acid from the total amino acid composition (Figure 3.3). As for total amino acid composition, the essential amino acids are the prominent part of the free amino acid concentration.

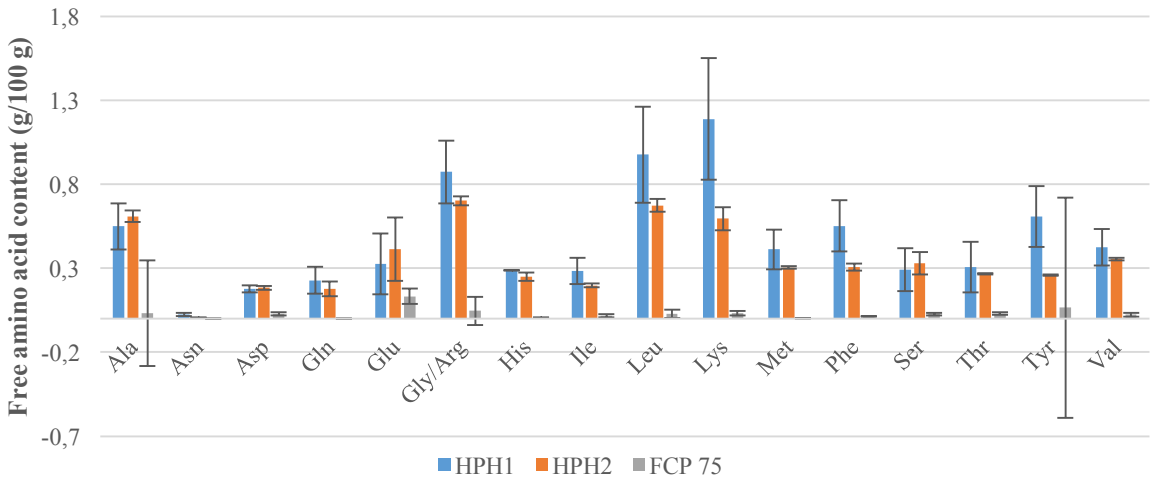


*Figure 3.4: Concentration of free amino acids distribution of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and one commercial ScanPro protein powder (FCP 75) (mean ± SD, n =2).*

HPH1 has a higher content of free amino acids than HPH2. The high stander deviation will, however, show the uncertainty of the method running this few parallels (n=2), and is it reasonable to believe that the concentration of free amino acids would be higher for HPH2 following the more coarsely grinding of the rest raw material prior to the enzymatic hydrolysis.

## 3.8 Acid Soluble Peptides

The amount of acid soluble peptides was determined by the use of trichloroacetic acid (TCA) and Lowry method. The results indicate that HPH1 and HPH2 had an acid soluble peptide concentration of 26,5±2,2 and 29,8±2,3 g/100 g , respectively (Table 3.7). This is

higher than what was found for both the commercial ScanPro products, with a concentration of 7,2±0,1 for T-95 and 24,0±1,6 g/100 g for FCP 75.

*Table 3.7: The concentration of acid soluble peptides of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75) (mean ± SD, n = 4).*

| Sample | Acid soluble peptides (g/100g) |
|---|---|
| **HPH1** | 26,5±2,2 |
| **HPH2** | 29,8±2,3 |
| **T-95** | 7,2±0,1 |
| **FCP 75** | 24,0±1,6 |

A 10% TCA solution will have an average peptide size of 3-4 amino acid residues (Greenberg and Shipe 1979). The content of acid soluble peptides will therefore reflect the peptides with a chain length of only a few amino acids, including free amino acids. For ScanPro T-95, the low concentration of acid soluble peptides can therefore explain the absent of peptide detection during the molecular weight distribution analysis (Chapter 3.4). For HPH1, HPH2 and ScanPro FCP 75 the observed high amount of acid soluble peptides is in accordance with the observed high amount of low molecular weight of the protein powder (Figure 3.2).

## 3.9 Water Holding Capacity and Cook Loss

The ability to retain water is an important function for foods in to stay juicy and be appealing to the customers. The ability for protein powders to increase water holding capacity of a product is an important functional property. The ability for the protein powders to increase the water holding capacity of fish minces was therefore measured after centrifugation. In general, the addition of the commercial ScanPro products had an improved effect on the water holding capacity of fish mince compared to the addition of the two hen hydrolysates (Figure 3.5). ScanPro T-95 showed the best water holding capacity, and was the only product that showed a considerable decrease in water loss as a result of increased level of protein powder added to fish mince. A halving in water loss of the fish mince was observed as a result of 3% addition of ScanPro T-95 compared to the control.

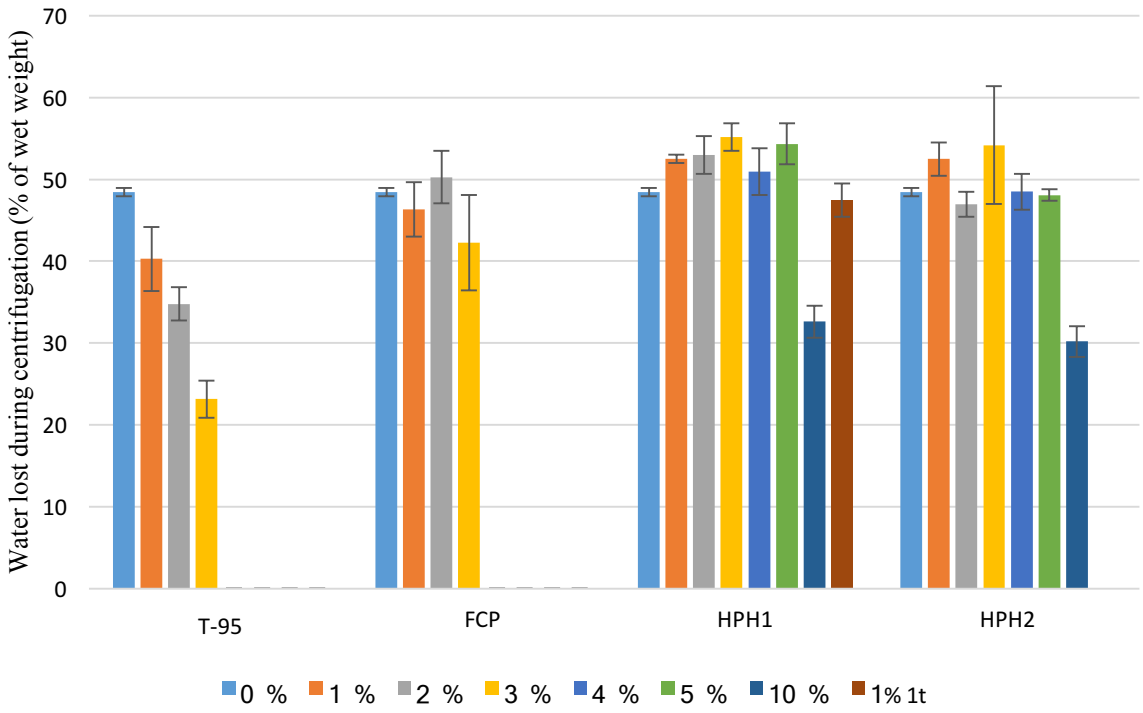


*Figure 3.5: Water loss during centrifugation (% of water content in fish mince) of fish mince added hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75) (mean ± SD, n = 4). One fish mince sample added 1% HPH1 (n=4) was left at room temperature for 1 hour before centrifuged (1% 1t).*

The results of adding HPH was increased water loss at the lower levels of addition. However, with the addition of 10% of fish mince weight, HPH was found to reduce the water loss of fish mince. For HPH1 the addition of 1% hydrolysate was performed with the fish and HPH1 mixture left for one hour at room temperature before centrifuging. This resulted in a slight reduction in water loss was shown compared to water loss of the original sample added 1% HPH. However, compared to the pure mince, there is no indication reduced water loss. With the high standard deviation between the parallels, the observed increased water loss when HPH was added to fish mince might be a result of uncertainty in the parallels.

These results indicate that HPH does not increase water holding capacity of fish mince when less than 10% of fish weight is added. The intended role to increase the functional properties of foods will therefore require a hydrolysate concentration of 10% or above. This is significantly higher than what is required for ScanPro T-95, which improved the water holding capacity even at the addition of 1% of fish mince weight. According to the datasheet, ScanPro T-95 acts as a commercial water binder, and have good functional properties (Appendix A). With reduced water loss at the addition of 10% of fish weight, HPH can be

used to increase the functional properties of food. However, this will increase the concentration of hydrophobic peptides in the food. Hydrophobic peptides have been shown to give the food a bitter taste (Adler-Nissen 1976), and a balance between taste and water holding capacity will therefore be important when using HPH. However, despite the poor functional property as a water holder reagent, HPH can be used to increase the nutritional value of foods by increasing the protein content, and raise the amount of essential amino acids. As long as the food does not require increased functional properties, HPH can therefore be added in lower concentrations to increase the nutritional value of foods, or by replacing other commercial protein powders. This can be important for people that might have a reduced food intake like the elderly.

There has not been done much work on hen hydrolysate before but there have been a lot of work on water holding capacity of hydrolysate from other sources, e.g. fish. Fish hydrolysate has been shown to improve water holding capacity of food products (Kristinsson 1998, not seen, cited after Kristinsson and Rasco 2000). The enzymatic hydrolysis performed by Kristinsson was performed under different conditions than what was the case for HPH1 and HPH2. It is therefore reasonable to believe that HPH can possess better water holding capacity than shown in this study with altered enzymatic hydrolysis conditions.

One explanation for the poor water holding capacity of HPH could be the low molecular weight of the peptides. HPH consists primarily of peptides below 3 kDa (Figure 3.2). Almost 30% of the hydrolysate is acid soluble peptides (Table 3.7), dissolved in 10% TCA with an average peptide size of 3-4 (Greenberg and Shipe 1979). With increased amount of small peptides, the hydrolysate will be capable of binding more water. Free amino- and carboxyl groups of peptides will bind to water (Barrow and Shahidi 2007), and the increased number of peptides will therefore increase the amount of water bound to the hydrolysate. Peptides with smaller molecular weight will, however, reduce the water holding capacity of a hydrolysate. Water retained in a food system is mainly entrapped water (Huff-Lonergan and Lonergan 2005). Smaller peptides have a lower capacity to physically entrap water, and despite the increased number of amino- and carboxyl groups binding water, the total water holding capacity will therefore decrease as a function of reduces peptide size.

The pH of fish mince was measured both with and without HPH. A lowering in pH of the fish mince will release entrapped water in the mince (Offer, Knight et al. 1989), and result in a reduced water holding capacity. This could be an explanation for the poor water holding capacity of HPH. However, no changes in pH of fish mince after addition of HPH was observed (Table 3.2). A pH value close to the proteins pI will result in equal numbers of

positive and negative charges, and protein-protein interactions will be promoted over protein-water interactions (Huff-Lonergan and Lonergan 2005). This results in a decreased water holding capacity compared to a higher pH value duo to less water bound to the charged groups of the proteins. The observed pH of HPH dissolved in distilled water of 6,27±0,01, which is close to the pH of raw hen meat at 6,2 (Wang, Wu et al. 2013), is therefore contributing negatively to the water holding capacity of HPH.

Another factor that might explain the poor water holding capacity of HPH compared to commercial protein powders is the lower protein concentration of HPH. ScanPro T-95, a commercial protein powder with a high water binding capacity consist of 94-98 g/100g protein while HPH1 has a protein content 74,7±1,7 g/100 g and HPH2 has a protein content of 75,2±2,2 g/100 g (Table 3.3). At a given level of addition, the total protein concentration will therefore be lower in the sample added HPH.

Cook loss was measured to investigate whether heat exposure of fish mince with added protein powder before centrifugation would enhance water holding capacity compared to non-heated samples. The results from measuring cook loss will also give an indication of how HPH will perform in a cooked food product. Fish mince added HPH showed an improved water holding capacity after heat exposure compared to non-heated samples with up to 6% less water loss during centrifugation (Figure 3.6). Compared to the water loss without heat exposure, fish mince added commercial protein powders showed increased water loss. Pure fish mince showed no changes in water holding capacity as a result of preheating. The increased water holding capacity of samples added HPH did, however, not make up for the poor initial water holding capacity, and the net result of cook loss was higher than for the control.

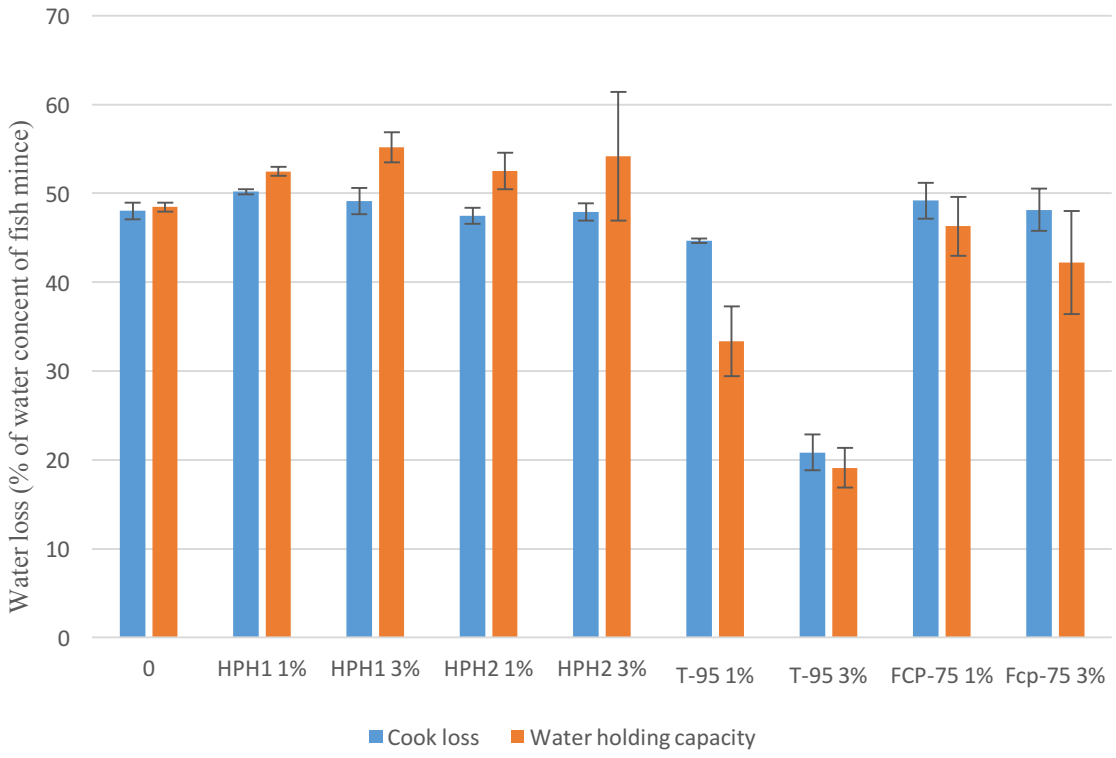


*Figure 3.6: Cook loss of fish mince compared to water holding capacity (% of water content in fish mince) of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75) (mean ± SD, n = 4).*

A possible explanation for the reduced water loss of fish mince with added HPH samples and the elevated water loss of fish mince with added ScanPro is the cleavage of peptide bonds between amino acids as a result of heat exposure. This will result in a smaller peptide size in the protein powder, with a lower capacity to physically entrap water. As already mentioned, the water holding capacity of a product is mainly the result of the entrapped water within the product (Huff-Lonergan and Lonergan 2005). According to the molecular weight distribution (Figure 3.2) and amount of acid soluble peptides (Table 3.7), HPH have a high content of small peptides. The amount of entrapped water will therefore be lower than for commercial products, in particular ScanPro T-95; a protein powder consisting of peptides with higher molecular weight, and higher observed water holding capacity. For HPH, which already showed low water holding capacity in fish mince, amount of entrapped water will be low. The cleavage of peptides, and exposure of additional amino- and carboxyl groups following heat exposure will therefore increase the amount of bound water, thus increasing the water holding capacity of fish mince. The high water holding capacity of ScanPro T-95 is likely to be a result of high amount of entrapped water. The result of heat exposure, and cleavage of the

large peptides, will therefore lower the ability to physically entrap water. For ScanPro T-95 the increased exposure of amino- and carboxyl groups binding water will not be able to cover for this loss of functionality, and the water holding capacity will therefore be reduced compared to non-heated samples.

## 3.10 Emulsifying Properties

Since proteins contain both hydrophilic and hydrophobic properties they can act as emulsifiers (Santiago, Maldonado-Valderrama et al. 2008). They have the ability to absorb and unfold at the oil-water interface, and stabilize the formation of emulsion (Damodaran, Parkin et al. 2007). The emulsifying properties of the commercial products ScanPro T-95 and ScanPro FCP 75, and hen hydrolysates from enzymatic hydrolysis of spent laying hens, HPH1 and HPH2, were tested to investigate the use of HPH as a food stabilizer. Each protein powder was tested up to a protein powder concentration of 4% of the liquid. An increased formation of emulsion as a result of higher protein powder concentration was observed (Table 3.8).

Proteins will form a protective membrane around the oil droplets to prevent coalescence (McClements 2004), and the observer results of higher emulsion with increased level of addition of protein powders are therefore as expected. It would be reasonable to believe that higher protein level of addition of protein powder also would lead to a more stable film surrounding the oil droplets, thus stabilising the emulsion. However, this is not the case in this analysis, with the lowest stability at the highest protein concentration. The results indicate no correlation between protein powder concentration and the emulsion stability, with the lowest stability at the highest protein powder concentration.

The unexpected results may be due to an unsuitable method to test for emulsifying properties. The standard deviations are high, and for some of the samples there are increased emulsion after 24 hours at room temperature. With the given method used for this experiment, it was difficult to reproduce the exact same conditions for each parallel. The use of a handheld Ultra-Turrax might have resulted in different level of homogenisation of the oil and water phase, thus resulting in variation in the formation of emulsion both within the parallels, but also between the different samples. A lower homogenization will not allow for the formation of oil droplets, thus reducing the formation of emulsion. The volume of the three phases was read with the naked eye. It was difficult to read the smaller emulsion phases correctly without any uncertainty. Despite the difficulties using this method, it was chosen because it is an easy

method to perform, and it gives an indication of emulsifying properties between the different protein powders.

*Table 3.8: Emulsifying properties of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75) (mean ± SD, n = 2).*

| Protein powder concentration (%) | Sample | Emulsion formed (mL) | Emulsion capacity (mL/g protein powder) | Emulsion stability (%) |
|---|---|---|---|---|
| **0,5** | **T-95** | 3,15±0,07 | 3,45±0,21 | 109,52±0,28 |
| | **FCP 75** | 2,50±0,71 | 1,50±0,71 | 60,00±0,00 |
| | **HPH1** | 1,00±0,14 | 0,75±0,35 | 75,00±0,21 |
| | **HPH2** | 0,10±0,00 | 0,10±0,00 | 100,00±0,00 |
| **1** | **T-95** | 4,65±0,49 | 4,65±0,49 | 100,00±0,00 |
| | **FCP 75** | 2,95±1,06 | 2,90±0,99 | 98,31±0,07 |
| | **HPH1** | 0,85±0,49 | 0,80±0,57 | 94,12±0,07 |
| | **HPH2** | 0,20±0,00 | 0,20±0,00 | 100,00±0,00 |
| **2** | **T-95** | 2,25±1,06 | 2,25±1,06 | 100,00±0,00 |
| | **FCP 75** | 3,95±0,07 | 3,70±0,14 | 93,67±0,07 |
| | **HPH1** | 1,45±0,07 | 1,25±0,35 | 86,21±0,42 |
| | **HPH2** | 0,10±0,00 | 0,10±0,00 | 100,00±0,00 |
| **3** | **T-95** | 5,15±0,49 | 5,15±0,49 | 100,00±0,00 |
| | **FCP 75** | 4,55±0,07 | 4,55±0,07 | 100,00±0,00 |
| | **HPH1** | 3,65±2,05 | 3,75±1,91 | 102,74±0,14 |
| | **HPH2** | 0,20±0,00 | 0,20±0,00 | 100,00±0,00 |
| **4** | **T-95** | 5,70±1,27 | 5,25±0,64 | 92,11±0,64 |
| | **FCP 75** | 4,65±0,21 | 0,50±0,14 | 10,75±0,07 |
| | **HPH1** | 2,50±0,42 | 2,20±0,14 | 88,00±0,28 |
| | **HPH2** | 0,20±0,00 | 0,20±0,00 | 100,00±0,00 |

HPH showed lower emulsifying properties than the commercial protein powders. The emulsifying capacity of HPH2 was very low, while HPH1 gave increased formation of emulsion with increasing level of addition of hydrolysate. Formation of emulsion depend on several characteristics of the protein powder. Formation of emulsion depend on the exposure of hydrophobic regions of the peptides (Damodaran, Parkin et al. 2007). A good emulsifier should rapidly be absorbed to the formed oil droplets (Walstra 2002). The poor emulsifying properties of HPH compared to the ScanPro products could be a result of slower absorbance to the formed oil droplets for HPH. Another important factor in the emulsifying properties of a protein powder is degree of degradation, and the size of the peptides. An extended hydrolysis process will result in a loss of emulsifying properties for the hydrolysate (Mahmoud 1994). The low molecular weight of the peptides of HPH will increase the

solubility, which promotes emulsifying properties (Kristinsson and Rasco 2000). However, for a peptide containing good emulsifying properties, the chain length should exceed 20 amino acid residues (Lee, Shimizu et al. 1987). The low emulsifying properties of HPH can therefore be explained by the low molecular weight. Out of the four protein powder that have been tested, ScanPro T-95 showed the best emulsifying properties as well as peptides with the highest molecular weight. The extended cleavage of HPH could therefore indicate that the hydrophobic parts of the peptides have been cleaved during the enzymatic hydrolysis.

The cleavage of the hydrophobic parts during enzymatic hydrolysis will also explain the differences in the formation of emulsion between HPH1 and HPH2. HPH2 is more coarsely ground prior to the enzymatic hydrolysis. A more homogenous raw material might give the enzymes better access to their substrate, thus reducing the number of hydrophobic regions in the hydrolysate. As for water holding capacity, the emulsifying properties of a hydrolysate is affected by the pH. With the pH close to the pI of the proteins, proteins show poor emulsifying capacity (Kristo and Corredig 2014). However, proteins will be good emulsification stabilisers at pH close to the pI (Barrow and Shahidi 2007). This corresponds well with the observer results, with low emulsifying capacity, but high emulsifying stability of HPH.

## 3.11 Pilot Project

Despite the poor functional properties of HPH compared to the two commercial ScanPro protein powders, the observed nutritional value of HPH was so promising that it was decided to proceed to work on product development. It was decided to work on chicken mince meatloaf enriched with HPH. Because of the observed similarities between the two hen hydrolysate, it was decided to mix the two hydrolysates before they were added to the meatloaf. As the amount of hydrolysate was the limiting factor in the product development, this was done to be able to produce more meatloaf.

HPH was added to meatloaf in a small-scale production to investigate the sensory properties of the hydrolysate. The meatloaf was tested by three people that had knowledge of the experiment. The pilot project concluded that the meatloaf added HPH had a better texture than the ones without, but at the highest concentrations there could be a slight taste of bitterness, which is normal for hydrolysates recovered by enzymatic hydrolysis. The bitter taste will increase with higher concentration of hydrolysate as a result of increased amount of hydrophobic amino acids, and the experienced bitterness was only pointed out for the

meatloaf containing 10% HPH. According to the test panel the bitter taste was so marginal that it would be no problem to cover it by adding more spices, or simply using more flavour giving components in the meatloaf.

The pilot project was performed simply to investigate the possibility of using HPH as a nutritional booster in foods, and to determine whether it has a strong flavour or not. The improved texture of meatloaf with added HPH experienced by the panel is probably caused by the increased dry weight of the meatloaf. HPH was added after the meat loaf was made, simply increasing the dry weight of the samples by respectively 5 and 10%, and the improved texture should therefore not be emphasized highly. With the lack of additional flavour in samples added HPH, the pilot project showed promising results for further use, and indicates that HPH can be used in foods as a nutritional booster to increase the protein concentration.

## 3.12 Water Loss of Meatloaf

Water loss of the finished meatloaf product was tested using two different methods; applying gravitational force by centrifugation and mechanical pressure using filter paper to absorb lost water. The observed water loss of meatloaf during centrifugation is less than 10% of meatloaf weight (Table 3.9). A reduction in water loss of meatloaf compared to fish samples with added HPH indicates that HPH act together with the proteins of minced chicken meat to improve water holding capacity of the product. However, the water holding capacity is not consistently increased with increased level of HPH added to meatloaf. An increased water holding capacity of meatloaf would be expected if HPH provided the product with increased water holding capacity.

*Table 3.9: Water loss during centrifugation of chicken mince meatloaf added a mixture of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material, coarsely ground spent hen raw material (HPH). Two samples were made were water replacing milk (WM), and two samples were made without potato flour (WPF).*

| Sample | Water loss (% of theoretical maximum) |
|---|---|
| **Reference** | 8,15±2,01 |
| **5% HPH** | 5,26±0,40 |
| **10% HPH** | 8.35±1,08 |
| **5% HPH, WM** | 7,28±2,18 |
| **10% HPH, WM** | 7,06±1,91 |
| **5% HPH, WPF** | 7,21±1,17 |
| **10% HPH, WPF** | 7,68±2,72 |

The results of 5% added HPH points toward improved water holding capacity of meatloaf added HPH. However, with increased water loss of samples added 10% HPH compared to 5% HPH makes it difficult to believe that HPH provided the products with increased water holding capacity. In addition, there is only a slight reduction in water loss of samples added HPH compared to the control.

Meatloaf exposed to mechanical pressure (1kg for 2 minutes) lost between 1 and 4% of their water weight (Table 3.10). Filter papers used to measure weight loss of meatloaf were dried in an attempt to determine how much of the lost weight that was water, and how much was fat. This was unsuccessful as the filter papers weighed less after drying than before the experiment started. This is probably due to moisture in the filter papers, and the weight loss of the filters (Appendix K) were too inaccurate to be used. The fat loss of meatloaf was not measured otherwise in this experiment, and it is therefore disregarded in further discussions. The water loss of < 4% shows that not much water is lost. The reduced water loss compared to samples exposed to gravitational forces indicates that HPH can be used in foods cooked the same way as meatloaf without resulting in a dryer product. However, the best results were the reference product. Just like water holding capacity of fish mince (Figure 3.5), the water holding capacity of meatloaf was reduced with the addition of HPH compared to the reference.

*Table 3.10: Water loss during mechanical pressure (1 kg for 2 minutes) of chicken mince meatloaf added a mixture of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material, coarsely ground spent hen raw material (HPH). Two samples were made were water replacing milk (WM), and two samples were made without potato flour (WPF).*

| Sample | Water loss (% of theoretical maximum) |
|---|---|
| **Reference** | 1,45±0,01 |
| **5% HPH** | 1,90±0,00 |
| **10% HPH** | 1,93±0,00 |
| **5% HPH, WM** | 3,27±0,01 |
| **10% HPH, WM** | 2,61±0,01 |
| **5% HPH, WPF** | 2,49±0,01 |
| **10% HPH, WPF** | 2,22±0,00 |

The reduced water holding capacity could be explained by removing milk and potato flour from the recipe, as they are ingredients which will increase water holding capacity of foods. Potato flour contains starch which increases the water holding capacity, and previous studies show that potato flour reduce the water loss of food products (Yanez, Ballester et al. 1981, not

seen, cited after Kotoki and Deka 2010). Milk contain proteins, and it is reasonable to assume that the increased protein content with the use of milk instead of water will increase the water holding capacity of the meatloaf to some extent. Had the reduced water holding capacity solely been a result of the the omission of milk and potato flour, the same pattern should have been shown in the water loss during the centrifugation. However, that was not the case, and based on the results from the initial water holding capacity experiment (Figure 3.5), the likelihood is that addition of HPH provides no water holding capacity when added to a food product.

As mentioned in the chapter discussing water holding capacity of fish mince with added protein powders (Chapter 3.9), the pH can influence the water holding capacity of proteins. A study done by (Barbut 1997) showed that the pH of chickens range from 5,56 to 6,42. The measured pH of HPH (Table 3.2) is in this range, indicating a pH close to the pI of chickens. As a result of equal positive and negative charges of the peptides, protein-protein interactions will be favoured over protein-water interactions. This results in a lower water holding capacity compared to a product with a pH outside the pI of the proteins.

## 3.13 Colour Measurements

The colour of a food product affects how appetizing it looks. It is therefore important that addition of HPH does not change the colour of meatloaf to the worse. The colour was measured for lightness (L*), redness (a*) and yellowness (b*). Both the surface and a cross section of the seven meat loafs were measured. There was not observer any notable changes on the colour of the cross section when HPH was added (Table 3.11).

*Table 3.11: Colour measurements of cross section of meatloaf added a mixture of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material and coarsely ground spent hen raw material (HPH) (mean±SD, n=9). Two samples were made were water replacing milk (WM), and two samples were made without potato flour (WPF).*

| | **Mean±SD L*** | **Mean±SD a*** | **Mean±SD b*** |
|---|---|---|---|
| **Reference** | 71,12±2,42 | 1,18±0,35 | 12,45±0,52 |
| **5 % HPH** | 72,78±3,92 | -0,31±0,40 | 14,13±0,37 |
| **10 % HPH** | 72,85±1,20 | -1,15±0,28 | 16,33±0,56 |
| **5% HPH, WM** | 71,57±2,78 | -0,94±0,62 | 13,55±0,43 |
| **10% HPH, WM** | 72,13±0,70 | -1,50±0,25 | 15,53±1,01 |
| **5% HPH, WPF** | 74,72±0,41 | -0,55±0,24 | 15,02±0,32 |
| **10% HPH, WPF** | 69,52±1,21 | 0,67±0,22 | 14,10±0,44 |

There is a slight variation in the values between the different meat loafs. There is no correspondence between meat loafs 5% and 10% added HPH under the different recipes. It is therefore reasonable to believe that the small variations have nothing to do with the added

HPH. Without automated systems, there will naturally be some differences between two parallels of the same food product.

The surface measurements showed meatloaf added 10% HPH varied from the six other meat loafs (Table 3.12). The measurements, indicating a darker and more red colour corresponded well with the observed frying crust on the meat loafs. Frying crust was an important trait, and meatloaf added 10% HPH was ranked the most appealing meatloaf during the sensory analysis (Table 3.13). Meatloaf added 10% HPH showed the best foaming capacity during cooking, and this could be a possible explanation for the darker colour. With higher foaming capacity, the surface of the meatloaf will be thinner compared to the other meat loafs. Thus, the surface will dry out faster and hold a higher temperature.

*Table 3.12: Colour measurements of the surface of meatloaf added a mixture of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material and coarsely ground spent hen raw material (HPH) (mean±SD, n=9). Two samples were made were water replacing milk (WM), and two samples were made without potato flour (WPF).*

| | **Mean±SD L*** | **Mean±SD a*** | **Mean±SD b*** |
|---|---|---|---|
| **Reference** | 68,95±1,91 | 1,36±0,43 | 18,54±1,26 |
| **5 % HPH** | 68,84±2,52 | 0,77±0,88 | 21,40±2,49 |
| **10 % HPH** | 59,44±5,92 | 10,12±5,93 | 28,47±4,15 |
| **5% HPH, WM** | 67,79±3,93 | 0,48±0,71 | 18,85±1,54 |
| **10% HPH, WM** | 68,19±1,76 | 1,55±1,89 | 23,43±4,09 |
| **5% HPH, WPF** | 71,70±2,37 | 1,02±1,76 | 22,42±4,53 |
| **10% HPH, WPF** | 65,88±2,38 | 0,95±0,77 | 17,72±1,31 |

## 3.14 Sensory Analysis

The seven different meat loafs were given to a panel of 10, consisting of other students and employees at NTNU. They were asked to grade each and every meatloaf separately with a grade between 1 and 6. A more direct comparison would probably have been better to distinguish the meat loafs from each other, and to determine the effects on taste by changing the parameters in the recipe. However, by giving each sample grade based solely on their respective taste and look, it would be possible to conclude that the changes had no effect on the meatloaf. The panel was not trained to take part in a sensory analysis, and individual preferences might therefore occur, especially if they have either an especially good or bad relationship to meatloaf.

Besides two deviations, the appearance of the products is closely rated by the panel. The one meatloaf with 10% added HPH stood out as the most appealing of the products, meanwhile the meatloaf added 10% HPH without using potato flour came off worst (Table 3.13). The meatloaf with 10% HPH added was the only product with frying crust, so the results indicate the importance of frying crust for the product to look appealing. It is also

mentioned by the test panel as a positive thing. There were also a few comments on the rest of the products not looking cooked enough. As frying crust was the only factor which affected the appearance of the product in a positive way, future product developments should work towards achieving this. There were a few comments on the smell om the products but there was no agreement between the members of the panel, and the comments are probably due to personal preferences.

*Table 3.13: Sensory analysis with grades given on taste and appearance of chicken mince meatloaf added a mixture of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material, coarsely ground spent hen raw material (HPH). Two samples were made were water replacing milk (WM), and two samples were made without potato flour (WPF). Taste and appearance are presented as mean±SD and ranked from 1-7.*

| Sample | Taste | Appearance |
|---|---|---|
| **Reference** | 4,10±0,99 (4) | 4,00±0,71 (6) |
| **5% HPH** | 3,60±1,35 (6) | 4,11±0,93 (2) |
| **10%HPH** | 4,20±0,75 (2) | 4,50±1,00 (1) |
| **5% HPH, WM** | 4,35±1,00 (1) | 4,06±1,01 (5) |
| **10% HPH, WM** | 3,85±1,00 (5) | 4,11±1,17 (2) |
| **5% HPH, WPF** | 4,15±1,53 (3) | 4,11±1,05 (2) |
| **10% HPH, WPF** | 3,40±1,58 (7) | 3,33±1,32 (7) |

In comparison to the appearance, the results given for the taste of the products are more varied. There are only small variations in the grades given for the taste, and it can therefore, as mentioned, be difficult to see any clear differences between the products. Compared to the reference, half of the products came out better, while the other half came out worse. There is a gap between the reference and the three most negatively rated samples. With two out of three of the products with 10% HPH added rated lower than the reference, there is an indication of products with 10% HPH added have a less appealing flavour. The bitter taste was mentioned for products with 10% HPH added. This was expected given the results from the pilot project. For the same products there were also comments about the meat loafs having an undesirable aftertaste. There are strong indications of HPH providing the foods with undesirable taste. Without trying to mask the taste, addition of 5% HPH seems to be fine without providing the product with bitter and undesirable flavour. Every meatloaf was made with only a small amount of spices, and no other components to give a flavour to the product. Meatloaf that was added 10% HPH without excluding any of the other ingredient came out second best. This result indicate that the bitter taste is masked by the use of milk/potato flour. The addition of 10% to foods should therefore not be a problem.

# 4. Conclusion

With the exception of high solubility, HPH had poor functional properties compared to the commercial protein powders. The water holding capacity and emulsifying properties of HPH were very low, and HPH is not suitable for increasing the functional properties of food. Despite the poor functional properties, HPH might have an application as dietary supplement. With a high protein content, consisting of 41,6±0,6% essential amino acids, the nutritional properties are better compared to the commercial protein powders. HPH has a $PER^c$ value more than 1,4 times higher than the commercial protein powders, and it could therefore be used in foods where an increased protein content is more important than the addition of functional properties. With the good nutritional value, the sensory and functional properties of meatloaf with varying amounts of HPH added was analysed. The sensory analysis gave promising results for further use of HPH as a dietary supplement for human consumption.

# 5. Future Work

Despite the poor functional properties, the hen hydrolysate (HPH) showed promising results when added to meatloaf for human consumption. These results should be used to explore additional applications for HPH by adding the hydrolysate to new food products. The product development did not compare meatloaf with added HPH to meatloaf with added commercial protein powders. This should be done in future product developments to compare the taste and behaviour of the hydrolysate compared to approved dietary supplements. A potential test panel should also consist of trained member to avoid personal preferences, and to get a more professional opinion.

The use of HPH in foods with different pH values should also be investigated. A pH outside the pI of the hydrolysate could alter the functional properties of HPH, and open up for new applications. During the cooking of the meatloaf, HPH showed to provide the meatloaf with foaming properties. The foaming properties of HPH should be more firmly determined to investigate the possibility of HPH in products like cream and cakes.

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APPENDIX A – Datasheet for the Commercial ScanPro Protein Powders (ScanPro FCP75 and ScanPro T-95)

# Product specification

## SCANPRO FCP 75/SF
Item No.: 94180-000

BHJ

| | | | |
|---|---|---|---|
| **Description** | SCANPRO FCP 75/SF is a functional chicken protein, manufactured from natural food grade chicken raw material by mechanical and thermal treatment. | | |
| **Ingredients** | Chicken protein, rosemary extract | | |
| **Recommended dosage** | 0.1-3 % | | |
| **Application area** | Ground products, emulsified products, injected products, reformed products | | |
| **Functionality** | Water binding, fat binding | | |
| **Raw material origin** | EU - Chicken | | |
| **Manufactured in** | EU | | |
| **Appearance** | | | |
| | Particle size | Superfine powder | |
| | Colour | Creamy | |
| | Flavour | Mild chicken | |
| **Nutrition (typical values)** | | | **Method** |
| | Protein (N x 6,25) | 72-78 % | ISO 937 |
| | Fat (total) | 15-21 % | ISO 1443 |
| | - Saturated fat | 6 % | ISO 5508-09/661/15304 |
| | - Mono-unsaturated fat | 8 % | ISO 5508-09/661/15304 |
| | - Polyunsaturated fat | 3 % | ISO 5508-09/661/15304 |
| | Moisture | 1-5 % | ISO 1442 |
| | Carbohydrates | < 1 % | Calculated |
| | Ash | 3-5 % | ISO 936 |
| | NaCl | < 1 % | ISO 1841-1 |
| | pH | 6-8 | |
| | Dietary fibre | < 1 % | AOAC |
| | Phosphate (natural occurrence) | < 3 % | Calc. Lavetan |
| | Collagen | 27-33 % | ISO 3496 |
| **Energy** | 1950 kJ/470 kcal | | |
| **Microbiology** | | | **Method** |
| | TPC | < 10,000 /g | ISO 4833 |
| | Salmonella - negative in | 25 g | ISO 6579 |
| | Coliforms | < 100 /g | ISO 21528-2 |
| | Sulphite-red. clostridia | < 100 /g | ISO 15213 |
| | Mould & yeast | < 100 /g | Afnor NF V08-059 |
| | Bacillus cereus | < 100 /g | ISO 7932 |
| **Production and packaging** | | | |
| | Packaging | White polyethylene bag | |
| | Unit weight | 20 kg | |
| | Pallet type | One-way plastic pallet 100x120 cm | |
| | Storage condition | Ambient temperature and dry condition | |
| | Shelf life unopened | 12 months | |
| | Pallet weight | 1000 kg | |

# Product specification

## SCANPRO FCP 75/SF
Item No.: 94180-000

**Statements**

| | | |
|---|---|---|
| | Safety and handling | A material safety data sheet is available upon request |
| | GMO Statement | The product does not contain or consist of GMO's and is not produced from GMO according to the definition of Regulation (EC) No. 1829/2003. |
| | Legislation | The product has been manufactured in accordance with and meet all current relevant EU-legislation. |

| **Amino acid profile (typical values – g/100 g product)** | | |
|---|---|---|
| | Alanine | 5.63 |
| | Arginine | 5.82 |
| | Aspartic acid | 6.22 |
| | Cystine + cystein | 0.70 |
| | Glutamic acid | 9.82 |
| | Glycine | 10.1 |
| | Histidine | 1.54 |
| | Hydroxyproline | 3.80 |
| | Isoleucine | 2.64 |
| | Leucine | 4.73 |
| | Lysine | 5.08 |
| | Methionine | 1.4 |
| | Phenylalanine | 2.54 |
| | Proline | 6.35 |
| | Serine | 3.32 |
| | Threonine | 2.75 |
| | Tryptophane | 0.48 |
| | Tyrosine | 2.07 |
| | Valine | 3.39 |

| **Minerals (typical values)** | | |
|---|---|---|
| | Na | 0.51 g/100g |
| | K | 0.97 g/100g |
| | Fe | 52 mg/kg |
| | Ca | 0.07 g/100g |
| | P | 1.51 g/100g |
| | Mg | 0.06 g/100g |

| **Heavy metals (typical values)** | | |
|---|---|---|
| | Zn | 56 mg/kg |
| | Cd | < 0.05 mg/kg |
| | Pb | < 0.05 mg/kg |
| | As | < 0.10 mg/kg |
| | Hg | < 0.010 mg/kg |
| | Cu | < 10 mg/kg |
| | Cr | < 1.0 mg/kg |

# Product specification

## SCANPRO FCP 75/SF
Item No.: 94180-000

**Allergens**

| | Yes | No | | Yes | No |
|---|---|---|---|---|---|
| Peanuts and products thereof | | X | Eggs and products thereof | | X |
| Soybeans and products thereof | | X | Cereals containing gluten * and products thereof | | X |
| Fish and products thereof | | X | Shellfish and products thereof | | X |
| Crustaceans/molluscs and products thereof | | X | Nuts ** and products thereof | | X |
| Sulphur dioxide and sulphites | | X | Sesame and products thereof | | X |
| Celery and products thereof | | X | Lupin and products thereof | | X |
| Mustard and products thereof | | X | Cow's milk protein and products thereof (incl. of lactose) | | X |

| | |
|---|---|
| *** Cereals** | including wheat, rye, barley, oats, spelt, kumat |
| **** Nuts** | including almond, hazelnut, walnut, cashew, pecan, brazil, pistachio, macadamia |

**Intolerants data**

| | Yes | No | | Yes | No |
|---|---|---|---|---|---|
| Rye | | X | Beef | | X |
| Pork | | X | Chicken/turkey | X | |
| Maize and products thereof | | X | Cocoa and products thereof | | X |
| Yeast | | X | Carrot | | X |
| Benzoic acid/parabens (E210-E219) | | X | Legumes/pulses | | X |
| Glutamate added | | X | Azo dyes | | X |
| Taetrazine (E102) | | X | Cinnamon | | X |
| Vanillin | | X | Coriander | | X |
| Umbelliferae | | X | Suitable for ovo-lacto vegetarians | | X |
| Suitable for vegans | | X | Suitable for coeliacs | X | |
| Kosher certified | | X | Halal certified | X | |

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# Product specification

## ScanPro™ T 95
Item No.: 95000-000

| | | | |
|---|---|---|---|
| **Description** | ScanPro™ T 95 is a high functional, cold setting animal protein manufactured from natural food grade pork raw material by mechanical and thermal treatment. | | |
| **Ingredients** | Pork protein, antioxidant (E306) | | |
| **Recommended dosage** | 1-3 % | | |
| **Application area** | Injected, reformed and emulsified products. | | |
| **Functionality** | Water binding / fat binding | | |
| **Raw material origin** | EU - Pork | | |
| **Manufactured in** | DK | | |
| **Appearance** | | | |
| | Particle size | Min. 99 % <106 µ | |
| | Colour | White | |
| | Flavour | Neutral | |
| **Nutrition (typical values)** | | | **Method** |
| | Protein (N x 6,25) | 94-98 % | NMKL 6 |
| | Fat (total) | 9-13 % | Bromnaphtalin |
| | - Saturated fat | 4 % | AOCS |
| | - Mono-unsaturated fat | 6 % | AOCS |
| | - Polyunsaturated fat | 1 % | AOCS |
| | Moisture | < 4 % | NMKL 23 |
| | Carbohydrates | < 1 % | Calculated |
| | Ash | 1-2 % | NMKL 173 |
| | NaCl | 1 % | NMKL 178 |
| | pH | 5-7 | |
| | Dietary fibre | < 1 % | AOAC 985.29 |
| | Phosphate (natural occurrence) | 0.39 % | Calc. DS13805 |
| | Collagen | 70-75 % | NMKL 127 |
| **Energy** | 2010 kJ / 480 kcal | | |
| **Microbiology** | | | **Method** |
| | TPC | < 10,000 /g | NMKL 86 |
| | Salmonella - negative in | 25 g | BRD RAPID |
| | Coliforms | < 100 /g | NMKL 44 |
| | Sulphite-red. clostridia | < 100 /g | NMKL 56 |
| | Mould & yeast | < 100 /g | NMKL 98 |
| | Bacillus cereus | < 100 /g | DS/ISO 7932 |
| **Production and packaging** | | | |
| | Packaging | White polyethylene bag | |
| | Unit weight | 20 kg | |
| | Pallet type | One-way wooden pallet 100x120 cm, heat-treated | |
| | Storage condition | Ambient temperature and dry condition | |
| | Shelf life unopened | 24 months | |
| | Pallet weight | 1000 kg | |

Essentia
Protein Solutions

# Product specification

## ScanPro™ T 95
Item No.: 95000-000

**Statements**

| | | |
|---|---|---|
| | Safety and handling | A material safety data sheet is available upon request |
| | GMO Statement | The product does not contain or consist of GMO's and is not produced from GMO according to the definition of Regulation (EC) No. 1829/2003 and 1830/2003. |
| | Legislation | The product has been manufactured in accordance with and meets all current relevant EU and Danish legislation. |

| **Amino acid profile (typical values – g/100 g product)** | | |
|---|---|---|
| | Alanine | 7.58 |
| | Arginine | 7.21 |
| | Aspartic acid | 5.86 |
| | Cystine + cystein | 0.435 |
| | Glutamic acid | 9.16 |
| | Glycine | 18.9 |
| | Histidine | 1.08 |
| | Hydroxyproline | 9.17 |
| | Isoleucine | 1.67 |
| | Leucine | 3.63 |
| | Lysine | 3.94 |
| | Methionine | 0.983 |
| | Phenylalanine | 2.45 |
| | Proline | 11.02 |
| | Serine | 3,32 |
| | Threonine | 2.07 |
| | Tryptophane | 0.16 |
| | Tyrosine | 1.17 |
| | Valine | 2.61 |

| **Minerals (typical values)** | | |
|---|---|---|
| | Na | 0.24 g/100g |
| | K | 0.12 g/100g |
| | Fe | 10 mg/kg |
| | Ca | 0.052 g/100g |
| | P | 0.17 g/100g |
| | Mg | 0.013 g/100g |

| **Heavy metals (typical values)** | | |
|---|---|---|
| | Zn | 13 mg/kg |
| | Cd | < 0.01 mg/kg |
| | Pb | < 0.05 mg/kg |
| | As | < 0.10 mg/kg |
| | Hg | < 0.005 mg/kg |
| | Cu | < 5 mg/kg |
| | Cr | < 1.0 mg/kg |



V

# Product specification

## ScanPro™ T 95
Item No.: 95000-000

**Allergens**

| | Yes | No | | Yes | No |
|---|---|---|---|---|---|
| Peanuts and products thereof | | X | Eggs and products thereof | | X |
| Soybeans and products thereof | | X | Cereals containing gluten * and products thereof | | X |
| Fish and products thereof | | X | Shellfish and products thereof | | X |
| Crustaceans/molluscs and products thereof | | X | Nuts ** and products thereof | | X |
| Sulphur dioxide and sulphites | | X | Sesame and products thereof | | X |
| Celery and products thereof | | X | Lupin and products thereof | | X |
| Mustard and products thereof | | X | Cow's milk protein and products thereof (incl. of lactose) | | X |

| | |
|---|---|
| *** Cereals** | including wheat, rye, barley, oats, spelt, kumat |
| **** Nuts** | including almond, hazelnut, walnut, cashew, pecan, brazil, pistachio, macadamia |

**Intolerants data**

| | Yes | No | | Yes | No |
|---|---|---|---|---|---|
| Rye | | X | Beef | | X |
| Pork | X | | Chicken/turkey | | X |
| Maize and products thereof | | X | Cocoa and products thereof | | X |
| Yeast | | X | Carrot | | X |
| Benzoic acid/parabens (E210-E219) | | X | Legumes/pulses | | X |
| Glutamate added | | X | Azo dyes | | X |
| Taetrazine (E102) | | X | Cinnamon | | X |
| Vanillin | | X | Coriander | | X |
| Umbelliferae | | X | Suitable for ovo-lacto vegetarians | | X |
| Suitable for vegans | | X | Suitable for coeliacs | X | |
| Kosher certified | | X | Halal certified | | X |



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# APPENDIX B - Dry Weight and Ash

The dry weight and ash of HPH was measured (Table 1B) using a muffle furnace.

*Table 1B: Ash and dry weight for hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2). pH of HPH was also measured in distilled water.*

| Sample | Protein powder (g) | After 105°C (g) | After 550°C (g) | Dry weigh t (%) | Ash (%) | Mean±SD Dry Weight | Ash |
|---|---|---|---|---|---|---|---|
| **HPH1** | 0,50 | 0,47 | 0,06 | 95,50 | 11,82 | 94,33± 0,78 | 11,73± 0,10 |
| | 0,50 | 0,47 | 0,06 | 93,95 | 11,58 | | |
| | 0,50 | 0,47 | 0,06 | 93,96 | 11,76 | | |
| | 0,51 | 0,48 | 0,06 | 93,92 | 11,77 | | |
| **HPH2** | 0,50 | 0,47 | 0,06 | 94,03 | 11,62 | 93,98±0,0 5 | 11,55± 0,07 |
| | 0,51 | 0,48 | 0,06 | 94,00 | 11,54 | | |
| | 0,51 | 0,48 | 0,06 | 93,92 | 11,58 | | |
| | 0,50 | 0,47 | 0,06 | 93,96 | 11,47 | | |

# APPENDIX C – pH

The pH of fish mince was measured to observe any changes in pH with HPH added in different concentrations (Table 1C). pH of HPH dissolved in distilled water was also measured.

*Table 1C: pH of fish mince with increasing level of addition of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2). pH of HPH was also measured in distilled water.*

| Sample | Parallel 1 | Parallel 2 | Parallel 3 | Mean±SD |
|---|---|---|---|---|
| **Pure fish** | 6,37 | 6,39 | 6,40 | 6,39±0,02 |
| **Fish + 1% HPH1** | 6,36 | 6,36 | 6,37 | 6,36±0,01 |
| **Fish + 1% HPH2** | 6,36 | 6,37 | 6,37 | 6,37±0,01 |
| **Fish + 3% HPH1** | 6,31 | 6,34 | 6,35 | 6,33±0,02 |
| **Fish + 3% HPH2** | 6,34 | 6,33 | 6,33 | 6,33±0,01 |
| **Water + HPH1** | 6,27 | 6,27 | x | 6,27±0,00 |
| **Water + HPH2** | 6,27 | 6,28 | x | 6,28±0,01 |

# APPENDIX D – Solubility

Absorbance of bovine serum albumin (BSA) (Table 1D) was measured at 750 nm and plotted against seven concentrations of BSA to create a standard curve (Figure 1D). The standard curve was used in the calculation of protein solubility for the protein powders HPH and ScanPro.

*Table 1D: Measured absorbance at 750 nm for bovine serum albumin (BSA) in seven known concentrations.*

| **Concentration (µg/ml)** | **12,5** | **25** | **50** | **100** | **150** | **200** | **300** |
|---|---|---|---|---|---|---|---|
| **1** | 0,045 | 0,076 | 0,135 | 0,244 | 0,348 | 0,428 | 0,593 |
| **2** | 0,045 | 0,074 | 0,136 | 0,246 | 0,350 | 0,424 | 0,591 |
| **3** | 0,047 | 0,072 | 0,137 | 0,241 | | 0,429 | 0,590 |
| **Average** | 0,046± | 0,074± | 0,136± | 0,244± | 0,349± | 0,427± | 0,591± |

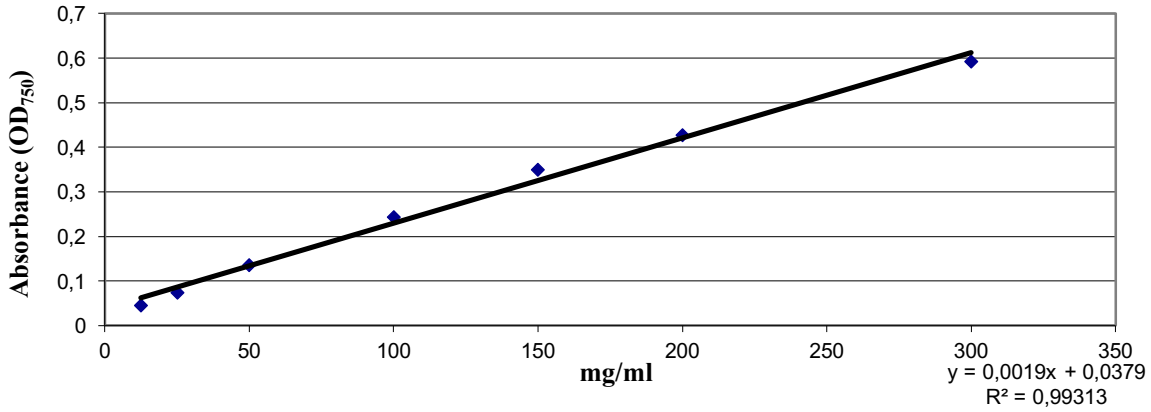


*Figure 1D: Standard curve generated from measured absorbance (750 nm) of seven bovine berum albumin (BSA) concentrations. The regression line and the associated equation are also presented.*

Linear regression (Microsoft Excel 2016) was used to calculate slope value (A), intercept value (B) and $R^2$. The solubility of the protein powders was calculated by the use of Equation A.

- A = 0,0019
- B = 0,0379
- $R^2$ = 0,99313

$$\frac{\left(\frac{1}{A} * (OD - B)\right) x\ dilution\ x\ volume\ extract\ (mL) x\ 100}{sample\ weight\ (g)\ x\ 1000\ x\ 1000} \qquad Equation\ A$$

Where A = the slope value, OD = measured absorbance and B = the intercept value.

# APPENDIX E – Total Amino Acid Composition

The amino acid content of HPH1 and HPH2 was calculated in g/100g from the measured HPLC values (µmol/l) (Table 1E) by the use of Equation B.

*Table 1E: Total amino acid composition of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2).*

| Amino Acid (µmol/l) | HPH1 (1) | HPH1 (2) | HPH2 (1) | HPH2 (2) |
|---|---|---|---|---|
| Asp | 5,81 | 5,64 | 5,65 | 5,85 |
| Glu | 9,65 | 7,94 | 8,04 | 8,31 |
| Asn | 0,00 | 0,00 | 0,02 | 0,00 |
| His | 1,17 | 1,23 | 1,22 | 1,26 |
| Ser | 3,67 | 3,68 | 3,83 | 3,93 |
| Gln | 0,00 | 0,00 | 0,00 | 0,00 |
| Gly/Arg | 7,33 | 6,45 | 6,66 | 7,00 |
| Thr | 3,30 | 3,23 | 3,06 | 3,45 |
| Ala | 6,31 | 6,34 | 6,63 | 6,79 |
| Tyr | 0,70 | 0,73 | 0,69 | 0,75 |
| Aba | 0,18 | 0,18 | 0,14 | 0,16 |
| Met | 1,31 | 1,37 | 1,31 | 1,35 |
| Val | 3,21 | 3,34 | 3,43 | 3,54 |
| Phe | 1,56 | 1,60 | 1,56 | 1,63 |
| Ile | 1,98 | 2,07 | 2,03 | 2,08 |
| Leu | 4,26 | 4,50 | 4,48 | 4,63 |
| Lys | 5,11 | 5,51 | 5,42 | 5,59 |
| Total | 55,55 | 53,80 | 54,16 | 56,31 |

$$\frac{[AA]\ x\ MM\ (AA)\ x\ 1{,}25\ x\ volume\ extract(mL)\ x\ dilution}{1\ x\ 1000\ x\ 1000\ x\ sample\ weight\ (g)} \qquad Equation\ B$$

Where [AA] = sample concentration of amino acid in µmol/l, MM = molecular mass of amino acids in proteins (without water).

The total amino acid composition for the four protein powders were used to to calculate the PER values of the respective protein powder with the use of Equation C-E.

$$PER^a = -0{,}468 + 0{,}45[LEU] - 0{,}105[TYR] \qquad Equation\ C$$

$$PER^b = -1{,}816 + 0{,}435[MET] + 0{,}780[LEU] + 0{,}211[HIS] - 0{,}944[TYR] \qquad Equation\ D$$

$$PER^c = 0{,}08084[\Sigma AA_7] - 0{,}1094 \qquad Equation\ E$$

Where $\Sigma AA_7$= threonine + valine + methionine + isoleucine + leucine + phenylalanine + lysine

# APPENDIX F – Hydroxyproline

Absorbance of L-hydroxyproline was measured at 555 nm (Table 1F). and plotted against the concentration was plotted against the concentration of L-hydroxyproline to create a standard curve (Figure 1F). The standard curve was used in the calculation of hydroxyproline content in the protein powders.

*Table F.1: Measured absorbance at 555nm for L-hydroxyproline in known concentrations.*

| L-hydroxyproline (µg/mL) | $OD_{555}$ | | | Mean | SD |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **5** | 0,08 | 0,08 | 0,09 | 0,08 | 0,01 |
| **10** | 0,15 | 0,16 | 0,14 | 0,15 | 0,01 |
| **15** | 0,26 | 0,25 | 0,24 | 0,25 | 0,01 |

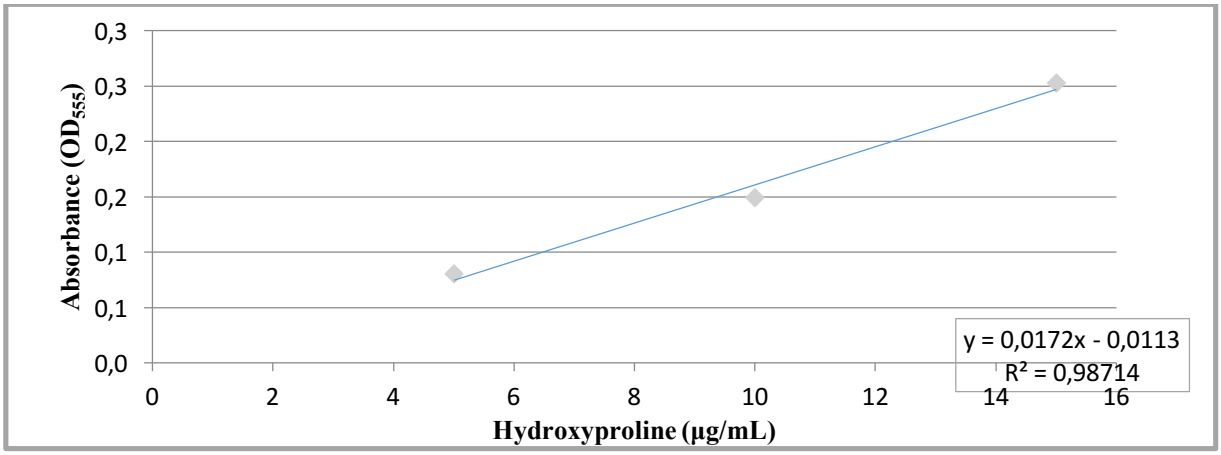


*Figure 1F: Standard curve generated from measured absorbance (555 nm) of three L-hydroxyproline concentrations. The regression line and the associated equation are also presented.*

Linear regression (Microsoft Excel 2016) was used to calculate slope value (A), intercept value (B) and $R^2$. The solubility of the protein powders was calculated by the use of Equation F.

- A = 0,0172
- B = 0,0113
- $R^2$ = 0,98714

$$\frac{\left(\frac{((OD\ x\ dilution) - B}{A}\right) x\ volume\ extract\ (mL)}{sample\ weight\ (g) x\ 1000\ x\ 1000\ x\ 100} \qquad Equation\ F$$

Where OD = measured absorbance, B = the intercept value and A = the slope value.

# APPENDIX G – Free Amino Acids

The free amino acid content of HPH1, HPH2, ScanPro T-95 and ScanPro FCP 75 was calculated in g/100g from the measured HPLC values (µmol/l) (Table 1G) by the use of Equation G.

*Table 1G: Free amino acid content of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and the commercial protein powder ScanPro FCP 75 (FCP 75).*

| Amino acid (µmol/l) | HPH1 (1) | HPH1 (2) | HPH2 (1) | HPH2 (2) | FCP 75 (1) | FCP 75 (2) |
|---|---|---|---|---|---|---|
| **Asp** | 1,07 | 1,05 | 1,13 | 1,12 | 0,16 | 0,17 |
| **Glu** | 1,70 | 1,84 | 2,19 | 2,34 | 0,71 | 0,75 |
| **Asn** | 0,16 | 0,15 | 0,05 | 0,04 | 0,00 | 0,00 |
| **His** | 1,48 | 1,48 | 1,30 | 1,28 | 0,04 | 0,04 |
| **Ser** | 2,28 | 2,15 | 2,56 | 2,49 | 0,21 | 0,22 |
| **Gln** | 1,28 | 1,22 | 0,99 | 0,96 | 0,00 | 0,00 |
| **Gly/Arg** | 5,53 | 5,70 | 4,54 | 4,56 | 0,26 | 0,34 |
| **Thr** | 2,00 | 2,14 | 1,81 | 1,81 | 0,21 | 0,20 |
| **Ala** | 4,85 | 5,02 | 5,49 | 5,54 | 0,09 | 0,49 |
| **Tyr** | 2,64 | 2,75 | 1,15 | 1,15 | 0,50 | 0,08 |
| **Met** | 2,17 | 2,26 | 1,65 | 1,65 | 0,02 | 0,02 |
| **Val** | 2,86 | 2,96 | 2,45 | 2,45 | 0,17 | 0,16 |
| **Phe** | 2,63 | 2,73 | 1,49 | 1,51 | 0,07 | 0,07 |
| **Ile** | 1,71 | 1,78 | 1,21 | 1,22 | 0,11 | 0,10 |
| **Leu** | 5,84 | 6,09 | 4,13 | 4,16 | 0,19 | 0,17 |
| **Lys** | 6,37 | 6,65 | 3,26 | 3,32 | 0,20 | 0,18 |

$$\frac{[AA]\ x\ MM\ (AA)\ x\ 1{,}25\ x\ volume\ extract(mL)\ x\ dilution}{1\ x\ 1000\ x\ 1000\ x\ sample\ weight\ (g)} \qquad Equation\ G$$

Where [AA] = sample concentration of amino acid in µmol/l and MM = molecular mass of amino acids in free form (with water).

# APPENDIX H – Acid Soluble Peptides

Absorbance of bovine serum albumin (BSA) (Table 1H) was measured at 750 nm and plotted against seven concentrations of BSA to create a standard curve (Figure 1H). The standard curve was used in the calculation of acid soluble peptide content for the protein powders HPH and ScanPro.

*Table 1H: Measured absorbance at 750 nm for bovine serum albumin (BSA) in known concentrations.*

| µg/ml | 12,5 | 25 | 50 | 100 | 150 | 200 | 300 |
|---|---|---|---|---|---|---|---|
| **OD 750** | 0,036 | 0,070 | 0,132 | 0,231 | 0,350 | 0,435 | 0,618 |
| | 0,038 | 0,072 | 0,133 | 0,231 | 0,367 | 0,445 | 0,637 |
| | 0,038 | 0,069 | 0,133 | 0,232 | 0,352 | 0,442 | 0,606 |

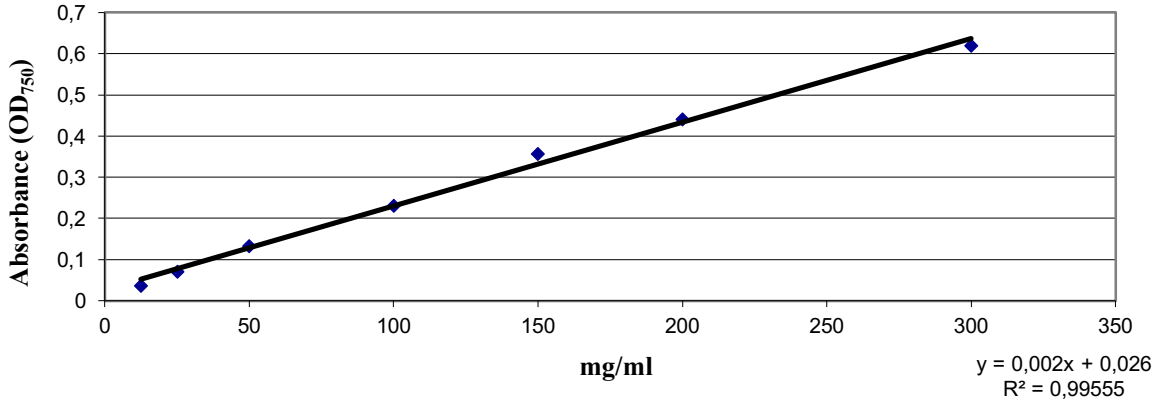


*Figure 1H: Standard curve generated from measured absorbance (750 nm) of seven bovine berum albumin (BSA) concentrations. The regression line and the associated equation are also presented.*

Linear regression (Microsoft Excel 2016) was used to calculate slope value (A), intercept value (B) and $R^2$. The solubility of the protein powders was calculated by the use of Equation H.

- A = 0,002
- B = 0,026
- $R^2$ = 0,99555

$$\frac{\left(\frac{1}{A} * (OD - B)\right) x\ dilution\ x\ volume\ extract\ (mL) x\ 100}{sample\ weight\ (g)\ x\ 1000\ x\ 1000} \qquad Equation\ H$$

Where A = the slope value, OD = measured absorbance and B = the intercept value.

# APPENDIX I – Water Holding Capacity and Cook Loss

Water holding capacity (Table 1I) and cook loss (2I) of fish mince was measured after centrifugation. Fish mince used to measure cook loss was exposed to heat (80°C for 15 min) prior to centrifugation. Increased level of protein powder was added to fish mince to observe any effects on the water holding capacity of fish mince.

*Table 1I: Water loss of fish mince before and after addition of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75). The water loss is presented as % of wet weight of fish mince.*

| Added protein powder (% of fish weight) | Parallel 1 | Parallel 2 | Parallel 3 | Parallel 4 | Mean±SD |
|---|---|---|---|---|---|
| **Pure fish** | 51,89 | 51,51 | 37,91 | 52,51 | 48,45±7,04 |
| **HPH1 1%** | 52,70 | 51,75 | 52,58 | 52,88 | 52,48±0,50 |
| **HPH1 2%** | 50,19 | 54,46 | 55,28 | 52,06 | 53,00±2,32 |
| **HPH1 3%** | 53,50 | 55,75 | 54,16 | 57,31 | 55,18±1,71 |
| **HPH1 4%** | 49,19 | 51,86 | 54,54 | 48,19 | 50,94±2,85 |
| **HPH1 5%** | 57,58 | 52,06 | 52,71 | 53,38 | 53,93±2,49 |
| **HPH1 10%** | 31,13 | 35,42 | 31,28 | 32,57 | 32,60±1,99 |
| **HPH1 1% + 1t** | 49,56 | 45,46 | 48,85 | 46,00 | 47,47±2,04 |
| **HPH2 1%** | 50,13 | 54,35 | 54,04 | 51,46 | 52,50±2,04 |
| **HPH2 2%** | 54,32 | 51,51 | 54,49 | 54,86 | 53,79±1,54 |
| **HPH2 3%** | 58,75 | 61,32 | 45,51 | 51,12 | 54,17±7,22 |
| **HPH2 4%** | 50,26 | 46,69 | 50,55 | 46,47 | 48,49±2,21 |
| **HPH2 5%** | 48,12 | 48,00 | 47,28 | 48,94 | 48,08±0,68 |
| **HPH2 10%** | 30,47 | 30,88 | 31,88 | 27,52 | 30,19±1,87 |
| **T-95 1%** | 35,91 | 41,15 | 45,22 | 38,83 | 40,28±3,93 |
| **T-95 2%** | 33,98 | 32,25 | 36,53 | 36,35 | 34,77±2,05 |
| **T-95 3%** | 21,08 | 22,88 | 22,25 | 26,31 | 23,13±2,24 |
| **FCP 75 1%** | 43,50 | 43,57 | 50,19 | 47,93 | 46,30±3,32 |
| **FCP 75 2%** | 46,67 | 49,61 | 50,31 | 54,51 | 50,27±3,23 |
| **FCP 75 3%** | 33,66 | 46,67 | 44,73 | 43,81 | 42,22±5,83 |

*Table 2I: Cook loss of fish mince exposed to 80°C for 15 minutes, before and after addition of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75). The water loss is presented as % of wet weight of fish mince.*

| Added protein powder (% of fish weight) | Parallel 1 | Parallel 2 | Parallel 3 | Parallel 4 | Mean±SD |
|---|---|---|---|---|---|
| **Pure fish** | 48,12 | 49,05 | 46,79 | 48,15 | 48,03±0,30 |
| **HPH1 1%** | 24,20 | 49,91 | 50,12 | 50,51 | 50,18±1,48 |
| **HPH1 3%** | 47,64 | 48,13 | 50,55 | 50,30 | 49,15±1,48 |
| **HPH2 1%** | 46,76 | 48,54 | 47,93 | 46,72 | 47,49±0,90 |
| **HPH2 3%** | 47,35 | 47,37 | 49,04 | 24,80 | 47,92±0,97 |
| **T-95 1%** | 44,60 | 44,57 | 44,46 | 44,99 | 44,66±0,23 |
| **T-95 3%** | 18,71 | 20,55 | 23,54 | 20,56 | 20,84±2,00 |
| **FCP 75 1%** | 49,04 | 50,33 | 46,39 | 50,94 | 49,18±2,02 |
| **FCP 75 3%** | 47,46 | 46,31 | 47,13 | 51,63 | 48,13±2,38 |

# APPENDIX J – Emulsifying Properties

Emulsion properties was measured in a mixture of 5,0 mL water and 5,0 mL oil when protein powder was added in increased levels (Table 1J).

*Table 1J: Emulsifying properties for hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material (HPH1), coarsely ground spent hen raw material (HPH2) and two commercial ScanPro protein powders (T-95 and FCP 75). The size of the three phases created during emulsion; water, emulsion and oil, was measured before and after 24 hours at room temperature.*

| Sample | Hydrolysate | Before | | | After | | |
|---|---|---|---|---|---|---|---|
| | | **Water** | **Emulsion** | **Oil** | **Water** | **Emulsion** | **Oil** |
| **0,5%** | **T-95** | 4,35 | 3,15 | 2,5 | 4,1 | 3,45 | 2,45 |
| | **FCP 75** | 5 | 2,5 | 3 | 5 | 1,5 | 3,5 |
| | **HPH1** | 4,9 | 1 | 4,1 | 5 | 0,75 | 4,25 |
| | **HPH2** | 5 | 0,1 | 4,9 | 5 | 0,1 | 4,9 |
| **1%** | **T-95** | 4,15 | 4,65 | 1,2 | 4,15 | 4,65 | 1,2 |
| | **FCP 75** | 4,9 | 2,95 | 2,15 | 4,9 | 2,9 | 2,2 |
| | **HPH1** | 5 | 0,85 | 4,15 | 5 | 0,8 | 4,2 |
| | **HPH2** | 4,95 | 0,2 | 4,85 | 4,95 | 0,2 | 4,85 |
| **2%** | **T-95** | 3,9 | 2,25 | 3,85 | 3,9 | 2,25 | 3,85 |
| | **FCP 75** | 5 | 3,95 | 1,05 | 5 | 3,7 | 1,3 |
| | **HPH1** | 5 | 1,45 | 3,3 | 5 | 1,25 | 3,75 |
| | **HPH2** | 5 | 0,1 | 4,9 | 5 | 0,1 | 4,9 |
| **3%** | **T-95** | 0,6 | 5,15 | 4,25 | 0,6 | 5,15 | 4,25 |
| | **FCP 75** | 4,9 | 4,55 | 0,55 | 4,9 | 4,55 | 0,55 |
| | **HPH1** | 4,65 | 3,65 | 1,7 | 4,75 | 3,75 | 1,5 |
| | **HPH2** | 5,1 | 0,2 | 4,7 | 5,1 | 0,2 | 4,7 |
| **4%** | **T-95** | 0,5 | 5,7 | 3,8 | 0,5 | 5,25 | 4,25 |
| | **FCP 75** | 4,55 | 4,65 | 0,8 | 4,7 | 4,5 | 0,8 |
| | **HPH1** | 5,05 | 2,5 | 2,45 | 5,05 | 2,2 | 2,75 |
| | **HPH2** | 5,15 | 0,2 | 4,65 | 5,2 | 0,2 | 4,6 |

# APPENDIX K – Water Loss of Meatloaf

Water loss of meatloaf was measured both by gravitational force (centrifugation) and mechanical pressure (2 kg for 2 minutes) (Table 1K).

*Table 1K: Water loss of meatloaf added a mixture of hen protein hydrolysate from enzymatic hydrolysis of finely ground spent hen raw material and coarsely ground spent hen raw material (HPH)*

| Meatloaf | CF (1) | CF (2) | CF (3) | Mean±SD | MP (1) | MP (2) | MP (3) | Mean±SD |
|---|---|---|---|---|---|---|---|---|
| **Ref** | 6,66 | 10,44 | 7,37 | 8,15±2,01 | 0,94 | 2,04 | 1,36 | 1,45±0,55 |
| **5% HPH** | 5,37 | 4,82 | 5,60 | 5,26±0,40 | 1,83 | 1,96 | 1,91 | 1,90±0,07 |
| **10% HPH** | 9,55 | 8,08 | 7,44 | 8,35±1,08 | 1,87 | 2,06 | 1,87 | 1,93±0,11 |
| **5% WM** | 9,54 | 7,12 | 5,19 | 7,28±2,18 | 4,91 | 2,23 | 2,66 | 3,27±1,44 |
| **10% WM** | 5,01 | 7,38 | 8,80 | 7,06±2,91 | 2,99 | 3,07 | 1,76 | 2,61±0,73 |
| **5% WPF** | 5,94 | 7,43 | 8,25 | 7,21±1,17 | 1,59 | 2,75 | 3,14 | 2,49±0,81 |
| **10%WFP** | 5,90 | 6,33 | 10,81 | 7,68±2,72 | 1,97 | 2,37 | 2,32 | 2,22±0,22 |

The weight loss of filter papers used to measure weight loss of meatloaf during mechanical pressure was measured after heat exposure (Table 2K). The high standard deviation made it difficult to measure water loss/weight loss of the meatloaf.

*Table 1K: Weight loss of filter paper used to measure water loss of meatloaf with mechanical pressure after after 24 hours in 105℃.*

| Parallel | Weight loss (%) | Mean±SD |
|---|---|---|
| **Filter 1** | 6,01 | |
| **Filter 2** | 4,10 | 3,90±2,21 |
| **Filter 3** | 1,59 | |

# APPENDIX L – Sensory Analysis

The sensory analysis was conducted by a group of 10, including students and employees at NTNU. All members of the panel were Norwegian, and the question sheet were therefore given out in Norwegian.

### 1. Smak og konsistens

Hvordan smakte produktet – gi karakter 1-6, der 6 er best. Gi gjerne en kommentar

| PRØVE | RESULTAT |
|---|---|
| **1** | |
| **2** | |
| **3** | |
| **4** | |
| **5** | |
| **6** | |
| **7** | |

## 2. Utseende og lukt

Ser produktet tiltalende ut – og hvordan lukter det?

| PRØVE | RESULTAT |
|---|---|
| **1** | |
| **2** | |
| **3** | |
| **4** | |
| **5** | |
| **6** | |
| **7** | |