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Potential of innovative pre-treatment technologies for the revalorisation of residual materials from the chicken industry through enzymatic hydrolysis

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ABSTRACT

The effect of microwave, ultrasound and high-pressure pre-treatment prior to enzymatic hydrolysis of mechanically deboned chicken residuals was evaluated. Increased protein extraction yield was observed for high-pressure pre-treatment at ≥400 MPa. Enhanced antioxidant activity was found in hydrolysates pre-treated with high-pressure (200 MPa) and ultrasound (900 W). Hydrolysates with higher protein solubility over pH were found after pre-treatments at 200 and 600 MPa, and 1 and 10 min microwave at 40 °C, with the most efficient option being the shortest microwave treatment. Furthermore, pre-treatments with ultrasound at 600 W, microwave for 1 min at 40 °C, and high-pressure at 200 MPa seem to have the potential to induce formation of peptides with higher lipid associating properties.

Industrial relevance: The increase in meat consumption resulting from the rise in human population, has led to increased demand for relevant nutritional (protein) sources together with the need for proper utilisation of residual materials and reduction of food waste footprint. Thus, assessment and validation of novel technologies that allow unexploited materials, earlier regarded as waste, to be transformed into high-value products is of great interest for the industry within the current global framework. Enhanced extraction yield and bio- and techno-functionality will lead to high-value markets, while minimising environmental footprint. This article focuses on the potential of microwave, ultrasound and high-pressure technologies as pre-treatment strategies prior to enzymatic hydrolysis of mechanically deboned chicken residuals towards production of high-value peptides. The results show that the applied technologies are able to enhance the properties of the resulting peptides in terms of overall protein extraction and bio- and techno-functional properties (such as protein solubility over pH, antioxidant activity). Microwave pre-treatment is shown as a rapid method for increased protein solubility over pH; ultrasound pre-treatment increases the antioxidant activity of the peptides in a power-dependent manner; high-pressure pre-treatment has the potential to increase protein solubility, antioxidant properties and protein extraction, depending on the applied pressure. To achieve certain bio- or techno-functional peptide properties, choosing specific technologies and operational conditions is needed. Thus, relevant industry sectors will benefit from these results in light of their strategy towards valorisation of residual materials similar to mechanically deboned chicken, through enzymatic hydrolysis.

1. Introduction

Although meat consumption per capita in Europe has remained relatively constant since the early 90s, the production and consumption of chicken meat, beef and eggs are on the rise in many countries (Landbruksdirektoratet, 2018). Indeed, a rising demand is expected due to the increasing global population (about 10 billion by 2050) with a shift towards higher poultry and pig meat-consumption (Kanerva, 2013). In Europe (2016) approximately 62 million tonnes of meat were produced (primary production/carcass weight) (FAO, 2017). During meat production, 40–60% of the animal weight does not comprise the main product (Aspevik et al., 2017), thus large amounts of residuals are generated. Traditional low-value applications of such residuals are incineration, composting and animal feed (Rustad, Storrø, & Slizyte, 2011). Projects and legislations launched by the European Union intend to change the current perception and utilisation of these residuals as waste into resources applicable for value-added products. Enhancing the efficiency of food production systems, generating less food-waste

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through redesigning food supply chains and introducing innovative and sustainable technologies, which might both reduce food losses and waste and allow for recycling resources, are believed to be important. Since these residuals contain valuable proteins and lipids, they are a well-suited raw material for valorisation. For instance, several methods have been developed for the extraction of proteins from food residuals, with isopropanol extraction and both acidic and alkaline hydrolysis being reported (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013). However, disadvantages such as extraction losses (amino acid degradation) and toxicity are avoided and the nutritional value is maintained when using proteases to release value-added peptides in protein-rich residual materials. Bioactive peptides are protein fragments which upon human consumption may affect body function or condition positively (Kitts & Weiler, 2003). These peptides are inactive when in the sequence of the parental protein but may be released through enzymatic hydrolysis. Protein hydrolysis can also release peptides that improve techno-functional properties in food, for instance protein solubility over a wide pH range, water-holding capacity, gelation and coagulation, emulsification, foaming and surface hydrophobicity (Panyam & Kilara, 1996). On the other hand, extensive peptide bond cleavage may reduce not only the molecular weight but also the functional properties of the peptides (Halim, Yusof, & Sarbon, 2016). The properties of the peptides released and therefore the hydrolysate properties may vary depending on factors such as enzyme and proteinaceous substrate, protein cleavage sites exposed and treatment time.

The implementation of innovative pre-treatment technologies as chemical/structural modifiers prior to enzymatic hydrolysis could increase extraction yields and enhance the hydrolysate properties (Konno, Yamamoto, Takahashi, & Kato, 2000; Martinez-Monteagudo & Saldaña, 2014; Vlaisavljevich et al., 2015). For instance, microwave (MW) pre-treatment causes a change in protein structure through thermal fluctuations, and potentially, a non-thermal mechanism for protein denaturation, thus exposing new cleavage sites for the enzyme (Murphy & Marks, 2000). Innovative non-thermal technologies, e.g. high-pressure (HP) and ultrasound (US), have the potential to affect protein structures without changing the temperature. While HP is reported to affect protein structures through volume reduction (Martinez-Monteagudo & Saldaña, 2014), and thus exert an opposite effect as compared to the thermal effect of MW, there might be several mechanisms of action associated with US. In liquid systems with high gas/vapor-bubble content, inertial cavitation (water radical formation, shockwave, high temperature regions) is believed to be the driving mechanism (Dong et al., 2019; Zou et al., 2019, 2020), but in biological tissues with high content of solid structures, gradual fatigue and disruption/denaturation of protein structures could prevail (Miller, 1985).

The objective of the present work is to investigate the potential of innovative technologies (HP; MW; US) as a pre-treatment prior to enzymatic hydrolysis of mechanically deboned chicken residuals (MDCR) with regards to their ability to increase the protein yield, and enhance the bioactive (DPPH radical scavenging activity) and functional properties (protein solubility over pH) of the hydrolysates.

2. Materials and methods

2.1. Materials

Residuals from mechanically deboned chicken carcasses were kindly supplied by a local producer. An industrially relevant enzyme (Promod™ 950L, Biocatalysts Limited, UK) was used for the trials. The protein content of the raw material was 16.29 ± 1.25 g/100 g as determined by the Kjeldahl method (N x 6.25). The raw material was minced (Garant type MTK662, MADO GmbH, Germany, Dornhan) for 10 s at low speed followed by 10 s at high speed. Thereafter, 500 g of the minced residual was vacuum packed (99%) in double 29.5 × 20.0 cm sous-vide bags (FH-S Supermax C V3, Webomatic, Germany, Bochum) and stored at −40 °C. Prior to experimental trials (conducted in triplicate), the minced samples were thawed at 4 °C overnight. All reagents for further analysis were of analytical grade.

2.2. Sample treatments prior to enzymatic hydrolysis

2.2.1. MW volumetric heating

MW pre-treatment was performed in a MW-heated batch autoclave (Gigatherm AG, Flawil, Switzerland) operating at 2.45 GHz and 1000 W. Samples of mechanically deboned chicken residuals were adapted to a flat, circular shape with a diameter of about 16.5 cm and rounded edges to avoid overheating in sharp areas (1 cm thickness). Thermal sensors were placed at 1, 8 and 12 cm from the sample edge along a constant trajectory, with a depth of approx. 0.5 cm. Treatment times of 1, 5 and 10 min (MW1, MW5 and MW10) were recorded from the point at which an average temperature of 40 °C was reached (after about 154–190 s, depending on the trial). The maximum temperature values achieved during MW heating for 1, 5 and 10 min (average of 3 replicates per time condition) were 50.6, 51.7 and 52.6 °C, respectively. Such maximum values were recorded with the thermal sensor placed at 1 cm from the sample edge.

2.2.2. US pre-treatment

US treatment was conducted using a BT 130H bench top system (UPCORP, Illinois, USA). The US transducers were configured into two banks, each of 500 W, operating at 67 and 170 kHz, respectively. A constant frequency sweep rate of 1000 Hz was used, as it is recommended for viscous media. MDCR samples packed in a single vacuum bag were placed approximately 2.5 cm above the tank bottom. US powers of 300, 600 and 900 W (US300, US600 and US900) were investigated for 30 min exposure time. The initial temperature of the water bath was set up such that 29–30 °C (Anritsu HD-1250K Thermometer, Atsugi, Japan) were reached at the end of the pre-treatment.

2.2.3. HP pre-treatment

Double-packed MDCR samples were pre-treated at 100, 200, 400 and 600 MPa (HP100, HP200, HP400 and HP600) (QFP 2L-700, Avure Technologies Inc., Columbus, USA), with a holding time of 15 min. The pressurisation rate ranged between 47 and 59 bar/s and depressurisation was achieved within 15 s. Adiabatic heating/cooling was observed during pressurisation/depressurisation. HP at 600 MPa increased/decreased the water reservoir temperature (21 °C) with 13 °C/ 8 °C. HP at 400 MPa increased/decreased the water reservoir temperature (21 °C) with 10 °C/ 5 °C. HP at 200 MPa increased/decreased the water reservoir temperature (21 °C) with 4 °C/ 2 °C. HP at 100 MPa increased and decreased the water reservoir temperature (21 °C) with 1 °C.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the control (no pre-treatment) and pre-treated samples was conducted in a 2 L Radleys ReactorReady (Radleys, Saffron Walden, UK) coupled with a Hei-Torque400 (Heidolph, Germany, Schwabach) with stirring speed of 360 rpm. 990 mL distilled water was initially added to the jacketed vessel and allowed to reach 50 °C (Anritsu HD-1250K Thermometer, Atsugi, Japan) before the pre-treated or control sample was added. 10 mL enzyme solution (1 g/100 g sample) was added when the reactor content stabilized again at 50 °C (approx. 15 min). 15 mL samples were taken at 0.5, 5, 15, 30 and 60 min reaction time (time-samples), and the enzyme inactivated in a regular MW (1800 W) for 0.5 min following 15 min in a water bath at 95 °C. The remaining hydrolysis volume was inactivated by MW (1800 W) for 4 min prior to the 15 min water bath at 95 °C. The pH was not controlled during the hydrolysis, however, the pH in the resulting hydrolysis liquid was measured to 6.8–7.0 (Mettler Toledo SevenGo Pro pH/ion meter, Mettler Toledo, Norway).

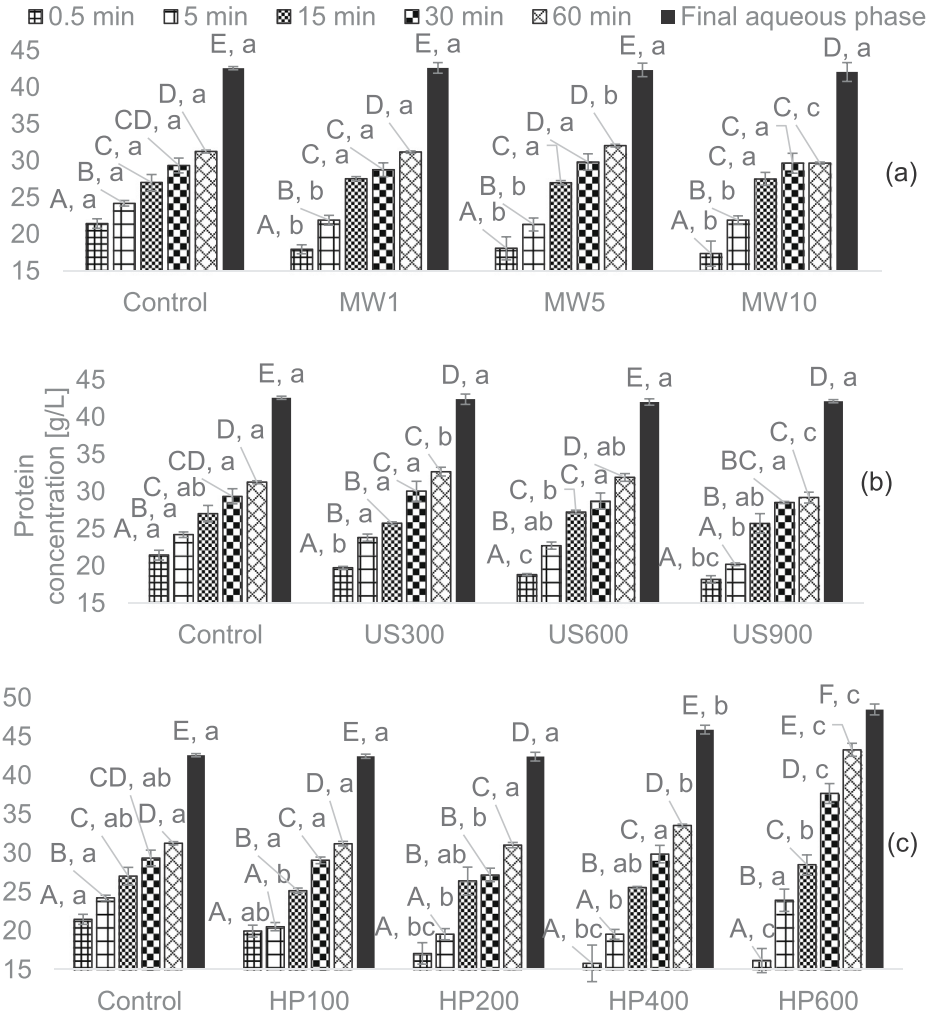

Fig. 1. Protein concentration [g/L] for time-samples (15 mL aliquots; at 0.5, 5, 15, 30 and 60 min) and for the remaining reactor volume (Final aqueous phase; approx. 1 L of liquid) after 60 min of hydrolysis, for (**a**) MW (1, 5 and 10 min), (**b**) US (300, 600 and 900 W) and (**c**) HPP (100, 200, 400 and 600 MPa) pre-treatments, as compared to the control samples. Within each treatment, bars bearing different uppercase letters are significantly different ($P \leq 0.05$). Among different treatments, bars bearing different lowercase letters are significantly different ($P \leq 0.05$).

2.4. Phase separation

Time-samples (0.5–60 min) and the reactor content after 60 min hydrolysis were centrifuged after cooling down to room temperature at 2250 ×g (Thermo Scientific Heraeus multifuge x3 FR, Osterode, Germany) and 25 °C for 20 min. Approximately 2 mL of the aqueous phase of centrifuged time-samples was filtered with an acetylated cellulose syringe filter (w/0.45 µm, VWR international, Art. Nr. 514-0063) and stored at −40 °C for further analysis ("0.5–60 min" in Fig. 1). After centrifugation, the reactor content after 60 min hydrolysis was divided into solid, aqueous ("final aqueous phase" in Fig. 1), lipid and multi-component phases by gently pouring the liquid phase through a sieve into a phase-separation funnel. The multicomponent phase was entrapped in the sieve while the lipid-water mixture was allowed to separate in the funnel for 15 min. The aqueous phase was vacuum filtered (Pall filter systems, Depth Filters, Material nr. 1,426,045) using a Büchner filtration system to remove the remaining solid and lipid particulates and stored at −40 °C for further analysis.

2.5. Protein solubility

The protein concentration in the aqueous phase was determined by the method of Lowry (Lowry, Rosebrough, Farr, & Randall, 1951) with bovine serum albumin as standard. The absorbance was measured at 750 nm (Shimadzu UV-mini 1240, Japan).

2.6. The DPPH radical scavenging activity

The DPPH radical scavenging activity was determined as described by Thiansilakul, Benjakul, and Shahidi (2007) with slight modifications. One day in advance, a solution of 0.15 mM DPPH in 96% EtOH was prepared and stirred at 4 °C overnight in the dark. 1.5 mL of the suitably diluted hydrolysate sample was added to 1.5 mL of 0.15 mM DPPH and vortexed vigorously for 10 s. The samples were left at room temperature in the dark for 60 min. The DPPH radical scavenging activity was calculated according to Eq. (1), where A and B are the absorbance at 517 nm of the hydrolysate and blank (distilled water), respectively.

$$\text{Radical scavenging activity}\ (\%) = (B–A)/B * 100\% \quad (1)$$

2.7. Protein solubility over pH

Samples of the final aqueous phase (pH 6.8–7.0) were mixed with distilled water and HCl (0.01, 0.1 or 1 M) to obtain a pH of 2 and 4, while distilled water and NaOH (0.1 or 1 M) were added in order to obtain a pH of 11. After addition of acid or base, samples were vortexed for 5 s. The total volume and the protein concentration after pH

adjustment was 25 mL and 8.2–8.6 g/L, respectively. The samples were then left at room temperature for 30–60 min and centrifuged at 11625 ×*g* for 15 min at 20 °C. The protein concentration was determined with the Lowry method. The protein solubility at different pH values was expressed as gram of soluble protein per gram of protein in the sample.

2.8. Statistical analysis

The double-sided Student *t*-test assuming different samples variances was applied using the Excel function T.TEST to determine whether there are any significant differences between means at a 95% confidence level ($\alpha = 0.05$). Test statistics were regarded as significant when P was ≤ 0.05.

3. Results and discussion

3.1. MW pre-treatment

Applying MW prior to enzymatic hydrolysis reduced the protein concentration after 0.5 and 5 min of hydrolysis as compared to the control (Fig. 1a). However, after 15 min of hydrolysis, there was no significant difference in the protein concentration between the MW treated samples and the control. The protein concentrations following 15 min of hydrolysis only varied significantly at 60 min of hydrolysis, where MW5 samples showed a significant increase as compared to MW10 and control samples; MW10 samples exhibited a significant decrease as compared to the remaining conditions, likely due to protein aggregation, which results in less substrate being available during the hydrolysis. MDCR is reported to contain 16–20% total protein where 18% corresponds to muscle proteins, such as myofibrillar and sarcoplasmic proteins, and the remaining content is mainly collagen (McCurdy, Jelen, Fedec, & Wood, 1986). In general, muscle protein solubility is negatively affected by MW treatment due to aggregation of originally soluble proteins. During thermal treatments, the initial protein unfolding is often associated with no apparent loss in solubility; this is often followed by aggregation and loss of solubility (Li-Chan, Nakai, & Wood, 1984). Protein aggregates are found to be resistant towards enzymatic degradation (Šližyte, Daukšas, Falch, Storrø, & Rustad, 2005). For components such as actomyosin in MDCR, the mechanism is attributed to hydrophobic interactions (Feng, Xue, Li, Wang, & Xue, 2017). Increasing the pre-treatment time could therefore promote aggregation, which explains the reduction in protein concentration after 60 min of hydrolysis for MW10 samples. Alternatively, since the protein concentration is determined by the Lowry method, which relies on peptide bonds upon complex formation (Olson & Markwell, 2007) to confirm presence of peptides, small peptides and free amino-acids might be neglected and explain the drop in protein concentration. Konno et al. (2000) suggested that the myosin S-1 fragment could be one of the components inducing aggregation upon prolonged treatment. This could be accompanied by denaturation of sarcoplasmic proteins. On the other hand, the significant increase in protein concentration for MW5 samples after 60 min of hydrolysis could be attributed to the release of low molecular weight proteins reportedly released for certain thermal treatments (Murphy & Marks, 2000). The protein content in the final aqueous phase was significantly higher than that in the 60 min time-samples, which is attributed to varying solid contents. MDCR hydrolysates are reported to contain significant amounts of collagen which, if present in the solid phase, could solubilise during enzyme inactivation (Yannas, 1972).

According to Table 1 listing phase contents, no significant differences were observed in the amount of solid, particulate, aqueous, multicomponent or lipid phases between the MW-treated and control samples. However, differences were observed in the multicomponent phase content among MW treatments. MW1 samples showed a significantly higher multicomponent phase content as compared to the MW5 and MW10 samples, which could be attributed to the presence of lipid associating peptides. Myofibrillar proteins are often considered rich sources of peptides with antioxidant properties (Borawska, Darewicz, Vegarud, & Minkiewicz, 2016) as compared to sarcoplasmic proteins, while both protein types are related to functional properties such as stabilization of oil-water interphases and producing emulsion structures (Hegarty, Bratzler, & Pearson, 1963). Although prolonged treatment times and short peptide chain lengths are often associated with reduction in the latter property, peptide adsorption to the oil surface may still occur (Pacheco-Aguilar, Mazorra-Manzano, & Ramírez-Suárez, 2008) and this would explain the reduction in the multicomponent phase while the lipid content increases when MW pre-treatment increases from 1 to 5 min. Long MW pre-treatment times have been reported to reduce the oil extraction yield (Bruno, Kudre, & Bhaskar, 2019), similarly to the observations in this work, which was attributed to reduced release of lipids due to a lower protein hydrolysis caused by heat-induced aggregation.

Regarding the DPPH radical scavenging activity (Fig. 2a) for protein concentrations of 0.5, 1.0 and 2.0 mg/mL, no significant variation was observed among the MW-treated or control samples. However, significant differences were found among MW treatments and/or the control for protein concentration of 4.0 mg/mL. MW1 samples displayed a significantly lower DPPH activity relative to both MW10 and control samples, although the activity of MW10 samples was still lower than that of the control samples. The apparent drop in antioxidant properties when increasing the protein concentration from 2.0 to 4.0 mg/mL could be attributed to a high content of charged residues within the peptides interacting unfavourably with the relatively non-polar solvent (ethanol) used for the analysis. Such groups are reported to present low DPPH activity and might exaggerate this effect by precipitating and absorbing light at the corresponding wavelength (Cumby, Zhong, Naczk, & Shahidi, 2008). This effect could increase as the protein concentration increases (Dissanayake, Ramchandran, Donkor, & Vasiljevic, 2013) and is found to depend on the enzyme and peptide properties (Centenaro et al., 2014). Also, peptides in the MW1 hydrolysate with antioxidant properties could contribute to the high multicomponent phase content and thereby explain the low DPPH activity for the 4 mg/mL protein concentration. In general, high antioxidant properties are correlated with peptides with high hydrophobic group content (Saiga, Tanabe, & Nishimura, 2003). As the MW treatment time was increased to 10 min, the multicomponent phase content was reduced significantly while the DPPH radical scavenging activity increased significantly. This could indicate that groups capable of scavenging the DPPH radical were released and a higher content was available in the hydrolysate. However, the scavenging activity of MW10 samples was still lower than that of the control, which could be due to protein aggregation through hydrophobic interactions possibly occurring in the late stages of hydrolysis.

Fig. 3a shows that MW1 and MW10 samples displayed significantly higher protein solubility at all pH values as compared to the control (except MW10 at pH 4). The highest increase in the protein solubility was achieved at pH 6 and 11 as compared to pH 2 and 4. Enhanced solubility is often associated with increased degree of hydrolysis (DH%) or peptides with reduced molecular weight, which increases the charges present per chain length at both acidic and alkaline conditions as additional carboxylic and amide groups are introduced upon hydrolysis of the peptide bond. Indeed, increasing content of low molecular weight peptides has been reported upon exposure of MDCR constituents to 40 °C (Murphy & Marks, 2000). Higher solubility over pH due to increased number of charged/polar groups would also confirm the reduced antioxidant activity, as such charged groups would interact unfavourably with ethanol. Bruno et al. (2019) reported that 5 and 10 min MW pre-treatment of fish head slurry resulted in higher DH% (55 °C, 2 h) and thus, exposure of more cleavage sites, while a decrease in DH% was observed for the 15 min pre-treatment. In this work, protein aggregation in MW10 samples would be in agreement with the

Table 1
Phase content for pre-treated and control samples. For each technology, different lowercase letters indicate significant differences for a certain phase content among treatments and the control ($P \leq 0.05$).

Treatment		Solid [g]	Particulate [g]	Multi-component[g]	Lipid [g]	Filtered aqueous [g]
Control		45.1 ± 2.1^{a}	18.3 ± 1.9^{a}	1.3 ± 0.3^{ab}	9.1 ± 1.0^{a}	999.3 ± 8.5^{a}
MW [min]	1	43.1 ± 1.8^{a}	17.8 ± 0.2^{a}	2.4 ± 0.3^{a}	8.3 ± 2.2^{a}	989.7 ± 10.8^{a}
	5	43.1 ± 2.1^{a}	18.9 ± 1.2^{a}	0.8 ± 0.7^{b}	11.1 ± 2.4^{a}	990.8 ± 10.3^{a}
	10	43.9 ± 2.7^{a}	18.4 ± 1.3^{a}	0.5 ± 0.3^{b}	11.0 ± 1.9^{a}	996.1 ± 9.0^{a}
US [W]	300	44.5 ± 1.3^{a}	19.6 ± 1.2^{a}	2.7 ± 0.4^{a}	7.7 ± 0.5^{a}	1009.7 ± 3.7^{a}
	600	45.0 ± 1.6^{a}	18.8 ± 1.5^{a}	3.3 ± 1.1^{a}	8.5 ± 0.2^{ab}	1000.2 ± 8.9^{a}
	900	44.1 ± 1.6^{a}	19.7 ± 0.6^{a}	1.8 ± 0.5^{a}	9.0 ± 0.3^{b}	1004.9 ± 11.8^{a}
HP [MPa]	100	45.4 ± 1.1^{a}	16.7 ± 2.0^{a}	3.0 ± 1.9^{ac}	8.6 ± 1.1^{a}	996.3 ± 21.8^{ac}
	200	45.9 ± 1.1^{a}	15.4 ± 1.2^{a}	2.7 ± 1.4^{ac}	7.7 ± 0.3^{a}	1014.1 ± 13.5^{a}
	400	46.4 ± 1.3^{a}	17.8 ± 2.5^{a}	1.8 ± 0.1^{a}	11.5 ± 0.4^{b}	975.6 ± 3.9^{bc}
	600	45.9 ± 2.1^{a}	17.0 ± 0.6^{a}	0.4 ± 0.1^{bc}	12.4 ± 0.6^{b}	980.6 ± 3.9^{bc}

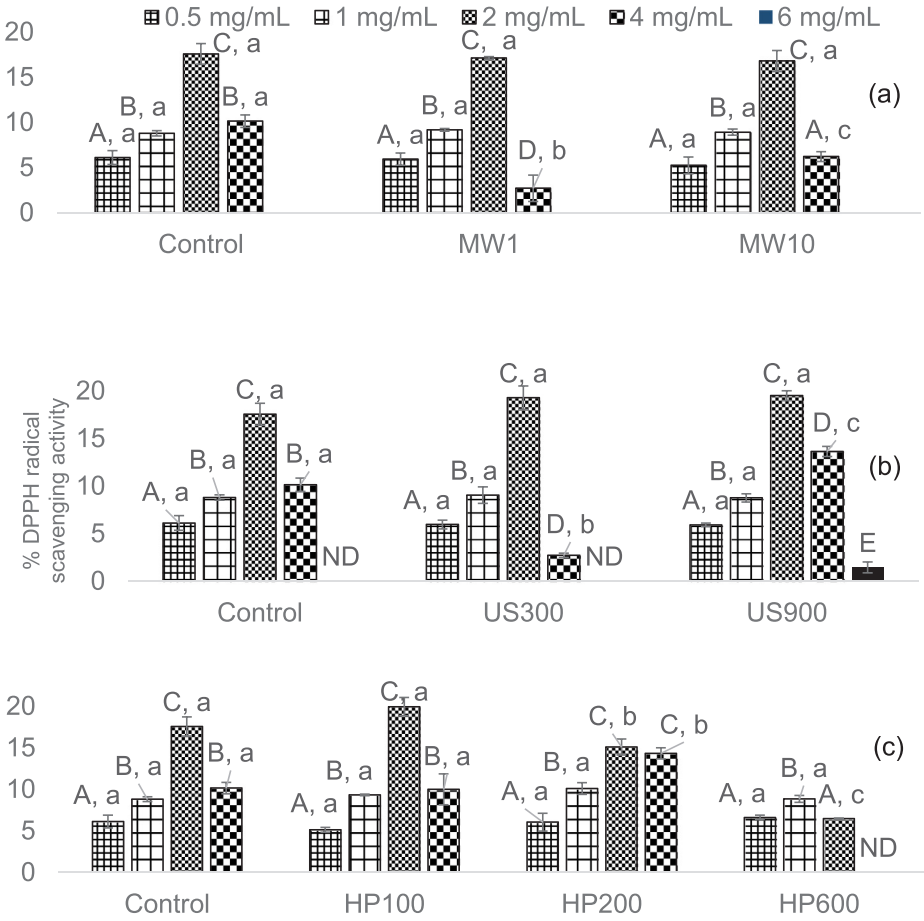

Fig. 2. DPPH radical scavenging activity (%) for (**a**) MW (1 and 10 min), (**b**) US (300 and 900 W) and (**c**) HP (100, 200, 600 MPa) pre-treatments, as compared to control samples, at protein concentrations of 0.5, 1.0, 2.0, 4.0 and 6.0 mg/mL. Within each treatment, bars bearing different uppercase letters are significantly different ($P \leq 0.05$). Among different treatment, bars bearing different lowercase letters are significantly different ($P \leq 0.05$).

reduction in DH% observed by Bruno et al. (2019).

3.2. US pre-treatment

Independently of the applied power (300, 600 and 900 W), US pre-treatment significantly reduced the protein concentration after 0.5 min of hydrolysis as compared to the control and a similar but weaker trend was observed after 5 min of hydrolysis (Fig. 1b). After 30 min of hydrolysis, no significant difference in dissolved peptide content among the different treatments and the control samples was observed. After 60 min of hydrolysis, US300 samples displayed significantly higher protein concentration than that of the control; US900 samples presented significantly lower protein concentration as compared to other treatments/control; US600 pre-treatment was not statistically different from the US300 pre-treatment nor the control. Soluble protein content in the final aqueous phase was similar among all the treatments and significantly higher than that of the 60 min time-samples. Overall, the observed behaviour is attributed to a combination of effects from the US, the enzyme and the thermal conditions in the reactor. In general, the US effect on raw materials similar to MDCR is often attributed to inertial cavitation and thus, to hot-spot formation, sonochemical effects and/or shock-wave formation upon collapsing bubbles (Leighton, 2007). However, in the present work such effects would depend on the close association between the collapsing bubble and protein or cells (Duerkop, Berger, Dürauer, & Jungbauer, 2018; M. W. Miller, Miller, & Brayman, 1996). The threshold for inertial cavitation has been demonstrated to be close to that of de-gassed water in biological tissues (Vlaisavljevich et al., 2015), which bear resemblance to the material used in this work. Indeed, it is suggested that effects observed on biological tissue treated with US neither is of thermal origin nor from

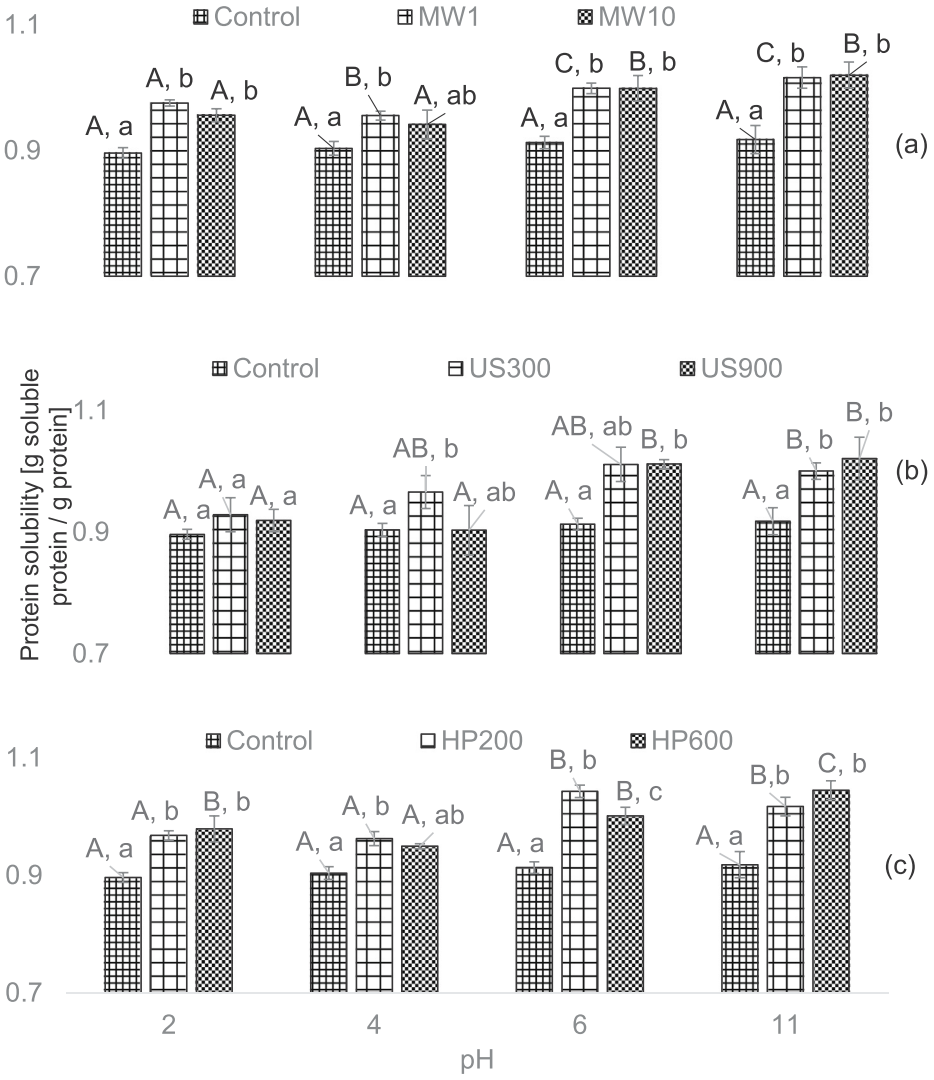

Fig. 3. Protein solubility [g soluble protein/g protein] over pH (2, 4, 6 and 11) for (**a**) MW (1 and 10 min), (**b**) US (300 and 900 W) and (**c**) HP (200 and 600 MPa) pre-treatments, as compared to control samples. Within each treatment, bars bearing different uppercase letters are significantly different ($P \leq .05$). Among different treatments, bars bearing different lowercase letters are significantly different ($P \leq .05$).

inertial cavitation but due to the formation of transverse shear waves denaturing specific protein components in the raw material through strain accumulation (Frenkel, Kimmel, & Iger, 2000). Such an effect could cause entanglement and prevent/delay dissolution as seen when vacuum packed carrots were treated with US (Ricce, Rojas, Miano, Siche, & Augusto, 2016), but also the low protein concentrations during early stages of hydrolysis in the present work. A potential explanation is that certain susceptible molecular structures are disrupted by the oscillating positive/negative pressure phases (push/pull effect) and form entangled structures when not flushed out by the surrounding water (due to vacuum-packing). Shrunken sarcomeres, intracellular cavities and protein aggregates within the extracellular space have been reported in vacuum-packed beef treated with US (Chang, Wang, Tang, & Zhou, 2015), which indicate that the extracellular space may display inertial cavitation while other regions in the raw-material are exposed to the milder push/pull effect. Degradation of the protein desmin at a treatment time of 30 min is found to be heavily dependent on the US power applied (Kang, Gao, Ge, Zhou, & Zhang, 2017), and could be one of the components responsible for the protein solubilisation results observed in the present work.

Regarding the protein solubility over pH (Fig. 3b), at pH 2 there were no significant differences in protein solubility among the various US treatments and the control; at pH 4, US300 samples displayed higher protein solubility as compared to US900 and the control, which were statistically similar; at pH 6 and 11 the protein solubility was significantly higher for US300 and US900 as compared to the control, but there was no statistically significant difference between the US treatments. The highest increase in protein solubility as compared to the control was observed at pH 6 and 11, although it did not vary among US treatments. These results suggest that specific protein structures were affected differently by the US treatment and a push/pull mechanism may thus have occurred during the treatment. Wang, Yang, Tang, Ni, and Zhou (2017) observed this type of specificity when treating actomyosin with US. Above a certain treatment period myosin exhibited slight denaturation while actin completely disappeared.

Regarding the phase contents (Table 1), the amount of lipid phase increased significantly when the US power was increased from 300 to 900 W. The US300 samples presented increased amounts of the multicomponent and aqueous phases, possibly due to the disruption of structures releasing phospholipids, which have been reported to associate to protein-rich phases, and thus potentially the aqueous and multicomponent phases (Slizyte, Nguyen, Rustad, & Storro, 2004). The multicomponent phase content further increased for US600 treatments but decreased for US900 samples, displaying levels slightly higher than those for the control samples. The aqueous phase content dropped to levels similar to that of the control when increasing the US power to

600 and 900 W, as compared to the higher contents in the US300 samples. This could indicate the presence of peptides redirecting the lipids from the aqueous to the multicomponent phase (for US600 samples) and lipid phase (for US900 samples) possibly through formation of protein-lipid structures through the introduction of peptides stabilising emulsion type structures or simply oil surface adsorption (Pacheco-Aguilar et al., 2008).

According to Fig. 2b, no differences in the DPPH radical scavenging activity were found among US pre-treatments for protein concentrations of 0.5, 1.0 or 2.0 mg/mL. However, a significant reduction in antioxidant activity was observed for US300 samples at a protein concentration of 4 mg/mL (the cause of this drop is discussed in Section 3.1). For the US300 treatment, the DPPH activity was significantly lower than that for the control and US900, the latter showing the highest value. At a protein concentration of 6.0 mg/mL, the DPPH activity was only detectable after the US900 treatment. As mentioned in Section 3.1, peptides with enhanced DPPH radical scavenging activity are often associated to certain non-polar/aromatic groups and also to short chain length. Both the surface charge content and hydrophobicity of actomyosin has been reported to increase with increasing US power before eventually experiencing a decrease to levels still higher than those for the control (Ye Zou et al., 2018). In the cited work, a similar trend was observed for the emulsifying activity, while emulsion stability declined with increasing US power, similarly to the observations for the multicomponent phase in the present work.

Kangsanant, Murkovic, and Thongraung (2014) treated a similar raw material with US prior to enzymatic hydrolysis and observed higher DPPH radical scavenging activity for the hydrolysate as compared to the control, likely due to thermally induced protein aggregation. These observations were in line with the results obtained for the US900 samples, where higher DPPH radical scavenging activity was observed alongside a decrease in protein concentration after 60 min of hydrolysis. Misir and Koral (2019) observed low structural alterations (small amino acid composition change in the hydrolysate) but increased matrix access (increased DH%) and oil binding capacity in samples hydrolysed during US treatment. These results would align with the observations for the US300 samples in the present work, where the increase in the multicomponent phase was likely due to peptide-lipid associations.

3.3. High-pressure pre-treatment

As shown in Fig. 1c, for a pre-treatment pressure of 100 MPa as compared to the control, the protein concentration was only different and reduced after 5 min of hydrolysis. Pre-treatment pressures of 200, 400 and 600 MPa caused the 0.5 min soluble peptide content to decrease as compared to the control. However, this initial reduction in protein concentration disappeared at later stages of hydrolysis, as observed for the protein concentration in the 60 min time-samples for HP400 and HP600 treatments, where the protein concentration is significantly higher than that of the control sample. HP is reported to reduce protein volume (Martinez-Monteagudo & Saldaña, 2014), on the other hand thermal fluctuations increase protein volume. While some MDCR proteins may become insoluble after the HP treatment, the thermal effect could gradually “unpack” the compressed protein state and increase the period of proteolytic susceptibility. Although such an effect should be confirmed through a reduction in the solid or particulate phases, there were no significant changes in the content of those phases as the pressure was increased (Table 1). However, the aqueous hydrolysate phase content slightly increased for HP200 samples. This was seen alongside a gradual decrease in the lipid and multicomponent phases. At pressures higher than 200 MPa, the multicomponent phase gradually decreased, while the lipid phase content increased significantly for both HP400 and HP600 as compared to the control and HP200. The increasing amount of lipid phase and final protein concentration for pressures above 200 MPa occurred with a reduction of the aqueous phase. Thus, it seems as if pressure-induced destabilisation of structures present in the aqueous phase is responsible for the increased protein concentration after 60 min of hydrolysis, but also the increased lipid phase content. Also, higher pressures reduced the protein concentration differences between 60 min time-samples and final aqueous phase samples and gradually less proteins were solubilised during the inactivation. Collagen has been reported as unaffected by high pressure (Gekko & Koga, 1983), thus the proteins released during enzymatic inactivation were not of a collagenous type as indicated in Section 3.1. Pressure-labile structures, potentially increasing the protein extraction yield in the present work, are reported in similar raw materials treated with high pressure (Chapleau, Mangavel, Compoint, & de Lamballerie-Anton, 2004). However, while certain structures are reported to dissolve upon pressure-treatment, other structures are reportedly formed, probably due to aggregation (Chapleau et al., 2004; Hsu, Hwang, Yu, & Jao, 2007; Ma & Ledward, 2004). The aggregates reported to occur when similar raw materials are treated with high pressure, are heat labile with a transition temperature close to 50 °C and seemingly formed by aggregated myosin and actin through S−S and H-bonds (Angsupanich, Edde, & Ledward, 1999; Hsu et al., 2007). Furthermore, these aggregates are reported as structures of increased surface area (Jung, de Lamballerie-Anton, & Ghoul, 2000), which potentially could benefit a subsequent hydrolysis but also for enhanced release of lipids. The increased multicomponent phase of HP100 and HP200 samples might be due to the formation of protein structures entrapping lipids (Chapleau et al., 2004), while at higher pressures such structures are disrupted.

HP100 presented no variations in DPPH radical scavenging activity as compared to the control, which suggests similar peptides within the hydrolysates (Fig. 2c). The scavenging activity measured for the HP200 hydrolysate with a peptide concentration of 2.0 mg/mL was significantly lower than that observed for HP100 and control samples. However, when increasing the protein concentration to 4.0 mg/mL, significantly higher scavenging activity was observed for the HP200 treatments. This indicates the presence of peptides relatively unstable in ethanol while simultaneously displaying high antioxidant properties and is observed alongside an increase in the aqueous phase and reduction in lipid phase content. Peptides presenting such antioxidant properties could potentially associate with lipids present in the aqueous phase (Slizyte et al., 2004) originating from disrupted membrane structures in the raw material containing, among other structures, phospholipids (Spector & Yorek, 1985). For HP600 treatments at protein concentrations of 0.5 and 2.0 mg/mL, statistically similar antioxidant activity was observed, which was the lowest value recorded among HP pre-treatments. At a protein concentration of 4 mg/mL, no scavenging activity was detected. The disassociation of protein-lipid structures seen at pressures above 200 MPa, could release proteins present in protein-lipid structures prior to enzymatic hydrolysis, as opposed to during heat inactivation. This could enhance the formation of short peptides with a high charge content per chain length thereby explaining the low antioxidant activity for the HP600 samples.

Overall, the protein solubility over pH for both HP200 and HP600 (Fig. 3c) samples significantly increased as compared to the control samples. However, for both treatments the highest increase in protein solubility was observed at pH 6 and 11 as compared to pH 2 and 4. Moreover, HP200 samples adjusted to pH 6 presented a significantly higher increase in protein solubility as compared to HP600 samples. The gradual pressure-induced alteration of major MDCR constituents could explain the differences observed among the HP200 and HP600 samples. High-pressure treated myofibrils have been reported to display significantly enhanced solubility, attributed to low molecular weight species (myosin light chain), when exposed to 300 MPa and higher (Chapleau et al., 2004; Jung et al., 2000). This together with the gradual increase of low molecular weight species generated from sarcoplasmic proteins upon increasing pressure (Villamonte, Pottier, & De Lamballerie, 2016), could explain the effective protein solubilisation in

HP400 and HP600 samples. Variations in the amount and type of such peptides would not necessarily present large variations in protein solubility over pH as short chain peptides in general present high solubility over a wide pH range.

Total soluble solids and total water soluble nitrogen have been reported to increase when conducting enzymatic hydrolysis of fish residuals at 300 MPa (Kim, Son, Maeng, Cho, & Kim, 2016), which are in the same range as the conditions exhibiting enhanced protein concentration in the present work. In addition, 12–21% increase in DH% was observed for various enzyme combinations at 300 MPa, which is attributed to enhanced peptide bond exposure. This, together with the high content of low molecular weight components, would have increased the charge content of the peptides in the hydrolysate, which would coincide with what seems to be an increasing content of hydrophilic peptides at the highest pressure levels investigated in the present work.

4. Conclusions

In this work the use of innovative technologies as a pre-treatment prior to enzymatic hydrolysis enhanced bio- and techno-functional properties of the resulting peptides (i.e. protein extraction and solubility over pH, antioxidant activity) in a technology and operational dependent manner. Microwave pre-treatment affected protein structures in the raw material leading to enhanced protein solubility over pH when applied at the shortest treatment time (1 min). While enhancement in protein solubility over pH was also observed with increasing treatment times, the concurrent detrimental effects, i.e. reduced protein extraction in the aqueous phase, occurred as a result of protein aggregation. Meanwhile, accurate temperature control and short treatment times could position microwave technology as an effective pre-hydrolysis treatment.

Ultrasound pre-treatment was found to enhance the antioxidant properties of the resulting peptides, although this effect was only observed for the highest ultrasonic power applied. This together with power-dependent trends on protein solubility over pH has been attributed to varying degrees of structural modification for different protein structures in the raw material. Thus, albeit ultrasound is effective towards enhanced peptide bioactivity, the applied ultrasonic power is crucial.

The application of high-pressure pre-treatment induced the peptides with enhanced antioxidant properties and protein solubility over pH when the treatment pressures of 100 and 200 MPa were applied. On the other hand, pressures higher than 200 MPa improved protein extraction, but reduced bio- and techno-functional properties. Such a wide range of effects by HPP alone could allow for production of peptides with different properties.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Angsupanich, K., Edde, M., & Ledward, D. (1999). Effects of high pressure on the myofibrillar proteins of cod and Turkey muscle. *Journal of Agricultural and Food Chemistry, 47*(1), 92–99.

Aspevik, T., Oterhals, Å., Rønning, S. B., Altintzoglou, T., Wubshet, S. G., Gildberg, A., ... Lindberg, D. (2017). Valorization of proteins from co-and by-products from the fish and meat industry. *Chemistry and Chemical Technologies in Waste Valorization* (pp. 123–150). Springer.

Borawska, J., Darewicz, M., Vegarud, G. E., & Minkiewicz, P. (2016). Antioxidant properties of carp (Cyprinus carpio L.) protein ex vivo and in vitro hydrolysates. *Food Chemistry, 194*, 770–779.

Bruno, S. F., Kudre, T. G., & Bhaskar, N. (2019). Impact of pretreatment-assisted enzymatic extraction on recovery, physicochemical and rheological properties of oil from Labeo rohita head. *Journal of Food Process. Eng. 42*(43), e12990.

Centenaro, G. S., Salas-Mellado, M., Pires, C., Batista, I., Nunes, M. L., & Prentice, C. (2014). Fractionation of protein hydrolysates of fish and chicken using membrane ultrafiltration: Investigation of antioxidant activity. *Applied Biochemistry and Biotechnology, 172*(6), 2877–2893.

Chang, H. J., Wang, Q., Tang, C. H., & Zhou, G. H. (2015). Effects of ultrasound treatment on connective tissue collagen and meat quality of beef semitendinosus muscle. *Journal of Food Quality, 38*(4), 256–267.

Chapleau, N., Mangavel, C., Compoint, J. P., & de Lamballerie-Anton, M. (2004). Effect of high-pressure processing on myofibrillar protein structure. *Journal of the Science of Food and Agriculture, 84*(1), 66–74.

Cumby, N., Zhong, Y., Naczk, M., & Shahidi, F. (2008). Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food Chemistry, 109*(1), 144–148.

Dissanayake, M., Ramchandran, L., Donkor, O. N., & Vasiljevic, T. (2013). Denaturation of whey proteins as a function of heat, pH and protein concentration. *International Dairy Journal, 31*(2), 93–99.

Dong, Z. Y., Li, M. Y., Tian, G., Zhang, T. H., Ren, H., & Quek, S. Y. (2019). Effects of ultrasonic pretreatment on the structure and functionality of chicken bone protein prepared by enzymatic method. *Food Chemistry, 299*, 125103.

Duerkop, M., Berger, E., Dürauer, A., & Jungbauer, A. (2018). Impact of cavitation, high shear stress and air/liquid interfaces on protein aggregation. *Biotechnology Journal, 13*(7), 1800062.

FAO. (2017). Food outlook June 2017, accessed 21.10.2019., www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Meat/Documents/Food_Outlook_June_2017_Meat_.pdf.

Feng, D., Xue, Y., Li, Z., Wang, Y., & Xue, C. (2017). Effects of microwave radiation and water bath heating on the physicochemical properties of actomyosin from silver carp (Hypophthalmichthys molitrix) during setting. *Journal of Food Processing and Preservation, 41*(4), e13031.

Frenkel, V., Kimmel, E., & Iger, Y. (2000). Ultrasound-induced intercellular space widening in fish epidermis. *Ultrasound in Medicine & Biology, 26*(3), 473–480.

Gekko, K., & Koga, S. (1983). The effect of pressure on thermal stability and in vitro fibril formation of collagen. *Agricultural and Biological Chemistry, 47*(5), 1027–1033.

Ghaly, A., Ramakrishnan, V., Brooks, M., Budge, S., & Dave, D. (2013). Fish processing wastes as a potential source of proteins. Amino acids and oils: A critical review. *J. Microb. Biochem. Technol, 5*(4), 107–129.

Halim, N., Yusof, H., & Sarbon, N. (2016). Functional and bioactive properties of fish protein hydolysates and peptides: A comprehensive review. *Trends in Food Science & Technology, 51*, 24–33.

Hegarty, G., Bratzler, L., & Pearson, A. (1963). Studies on the emulsifying properties of some intracellular beef muscle proteins ab. *Journal of Food Science, 28*(6), 663–668.

Hsu, K.-C., Hwang, J.-S., Yu, C.-C., & Jao, C.-L. (2007). Changes in conformation and in sulfhydryl groups of actomyosin of tilapia (Orechromis niloticus) on hydrostatic pressure treatment. *Food Chemistry, 103*(2), 560–564.

Jung, S., de Lamballerie-Anton, M., & Ghoul, M. (2000). Modifications of ultrastructure and myofibrillar proteins of post-rigor beef treated by high pressure. *LWT- Food Science and Technology, 33*(4), 313–319.

Kanerva, M. (2013). *Meat consumption in Europe: Issues, trends and debates.* Bremen: Universität., Bremen, Forschungszentrum Nachhaltigkeit (artec).

Kang, D.-c., Gao, X.-q., Ge, Q.-f., Zhou, G.-h., & Zhang, W.-g. (2017). Effects of ultrasound on the beef structure and water distribution during curing through protein degradation and modification. *Ultrasonics Sonochemistry, 38*, 317–325.

Kangsanant, S., Murkovic, M., & Thongraung, C. (2014). Antioxidant and nitric oxide inhibitory activities of tilapia (O reochromis niloticus) protein hydrolysate: Effect of ultrasonic pretreatment and ultrasonic-assisted enzymatic hydrolysis. *International Journal of Food Science & Technology, 49*(8), 1932–1938.

Kim, N., Son, S. H., Maeng, J. S., Cho, Y. J., & Kim, C. T. (2016). Enzymatic hydrolysis of anchovy fine powder at high and ambient pressure, and characterization of the hydrolyzates. *Journal of the Science of Food and Agriculture, 96*(3), 970–978.

Kitts, D. D., & Weiler, K. (2003). Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design, 9*(16), 1309–1323.

Konno, K., Yamamoto, T., Takahashi, M., & Kato, S. (2000). Early structural changes in

myosin rod upon heating of carp myofibrils. *Journal of Agricultural and Food Chemistry, 48*(10), 4905–4909.
Landbruksdirektoratet (2018). Vurdering av markedene for norske landbruksvarer. Accessed 31.10.2019 https://www.landbruksdirektoratet.no/no/produksjon-og-marked/egg/marknad-og-pris/_attachment/73784?_ts=1695282ed50.
Leighton, T. G. (2007). What is ultrasound? *Progress in Biophysics and Molecular Biology, 93*(1–3), 3–83.
Li-Chan, E., Nakai, S., & Wood, D. (1984). Hydrophobicity and solubility of meat proteins and their relationship to emulsifying properties. *Journal of Food Science, 49*(2), 345–350.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.
Ma, H.-J., & Ledward, D. (2004). High pressure/thermal treatment effects on the texture of beef muscle. *Meat Science, 68*(3), 347–355.
Martinez-Monteagudo, S. I., & Saldaña, M. D. (2014). Chemical reactions in food systems at high hydrostatic pressure. *Food Engineering Reviews, 6*(4), 105–127.
McCurdy, S. M., Jelen, P., Fedec, P., & Wood, D. F. (1986). Laboratory and pilot scale recovery of protein from mechanically separated chicken residue. *Journal of Food Science, 51*(3), 742–747.
Miller (1985). Microstreaming shear as a mechanism of cell death in Elodea leaves exposed to ultrasound. *Ultrasound in Medicine & Biology, 11*(2), 285–292.
Miller, M. W., Miller, D. L., & Brayman, A. A. (1996). A review of in vitro bioeffects of inertial ultrasonic cavitation from a mechanistic perspective. *Ultrasound in Medicine & Biology, 22*(9), 1131–1154.
Misir, G., & Koral, S. (2019). Effects of ultrasound treatment on structural, chemical and functional properties of protein hydrolysate of rainbow trout (ONCORHYNCHUS MYKISS) by-products. *Italian Journal of Food Science, 31*(2).
Murphy, R., & Marks, B. (2000). Effect of meat temperature on proteins, texture, and cook loss for ground chicken breast patties. *Poultry Science, 79*(1), 99–104.
Olson, B. J., & Markwell, J. (2007). Assays for determination of protein concentration. *Current Protocols in Protein Science, 48*(1) (3.4. 1-3.4. 29).
Pacheco-Aguilar, R., Mazorra-Manzano, M. A., & Ramírez-Suárez, J. C. (2008). Functional properties of fish protein hydrolysates from Pacific whiting (Merluccius productus) muscle produced by a commercial protease. *Food Chemistry, 109*(4), 782–789.
Panyam, D., & Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science & Technology, 7*(4), 120–125.
Ricce, C., Rojas, M. L., Miano, A. C., Siche, R., & Augusto, P. E. D. (2016). Ultrasound pretreatment enhances the carrot drying and rehydration. *Food Research International, 89*, 701–708.
Rustad, T., Storrø, I., & Slizyte, R. (2011). Possibilities for the utilisation of marine by-products. *International Journal of Food Science & Technology, 46*(10), 2001–2014.
Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry, 51*(12), 3661–3667.
Šližyte, R., Daukšas, E., Falch, E., Storrø, I., & Rustad, T. (2005). Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (Gadus morhua) by-products. *Process Biochemistry, 40*(3–4), 1415–1424.
Slizyte, R., Nguyen, J. V., Rustad, T., & Storro, I. (2004). Hydrolysis of cod (Gadus morhud) by-products: Influence of initial heat inactivation, concentration and separation conditions. *Journal of Aquatic Food Product Technology, 13*(2), 31–48.
Spector, A. A., & Yorek, M. A. (1985). Membrane lipid composition and cellular function. *Journal of Lipid Research, 26*(9), 1015–1035.
Thiansilakul, Y., Benjakul, S., & Shahidi, F. (2007). Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. *Journal of Food Biochemistry, 31*(2), 266–287.
Villamonte, G., Pottier, L., & De Lamballerie, M. (2016). Influence of high-pressure processing on the physicochemical and the emulsifying properties of sarcoplasmic proteins from hake (Merluccius merluccius). *European Food Research and Technology, 242*(5), 667–675.
Vlaisavljevich, E., Lin, K.-W., Maxwell, A., Warnez, M. T., Mancia, L., Singh, R., ... Cain, C. (2015). Effects of ultrasound frequency and tissue stiffness on the histotripsy intrinsic threshold for cavitation. *Ultrasound in Medicine & Biology, 41*(6), 1651–1667.
Wang, J.-y., Yang, Y.-l., Tang, X.-z., Ni, W.-x., & Zhou, L. (2017). Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. *Ultrasonics Sonochemistry, 38*, 225–233.
Yannas, I. (1972). Collagen and gelatin in the solid state. *Journal of Macromolecular Science—Reviews in Macromolecular Chemistry, 7*(1), 49–106.
Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., ... Xu, W. (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. *International Journal of Biological Macromolecules, 113*, 640–647.
Zou, Y., Yang, H., Li, P., Zhang, M., Zhang, X., Xu, W., & Wang, D. (2019). Effect of different time of ultrasound treatment on physicochemical, thermal, and antioxidant properties of chicken plasma protein. *Poultry Science, 98*(4), 1925–1933.
Zou, Y., Yang, H., Zhang, X., Xu, P., Jiang, D., Zhang, M., ... Wang, D. (2020). Effect of ultrasound power on extraction kinetic model, and physicochemical and structural characteristics of collagen from chicken lung. *Food Production, Processing and Nutrition, 2*(1), 3.