Effect of drying process assisted by high-pressure impregnation on protein quality and digestibility in red abalone (*Haliotis rufescens*)

Yamira Cepero-Betancourt¹ · Patricio Oliva-Moresco² · Alexis Pasten-Contreras¹ · Gipsy Tabilo-Munizaga¹ · Mario Pérez-Won³ · Luis Moreno-Osorio⁴ · Roberto Lemus-Mondaca⁵

Revised: 8 August 2017 / Accepted: 23 August 2017 / Published online: 12 September 2017
© Association of Food Scientists & Technologists (India) 2017

Abstract Abalone (*Haliotis* spp.) is an exotic seafood product recognized as a protein source of high biological value. Traditional methods used to preserve foods such as drying technology can affect their nutritional quality (protein quality and digestibility). A 28-day rat feeding study was conducted to evaluate the effects of the drying process assisted by high-pressure impregnation (HPI) (350, 450, and 500 MPa × 5 min) on chemical proximate and amino acid compositions and nutritional parameters, such as protein efficiency ratio (PER), true digestibility (TD), net protein ratio, and protein digestibility corrected amino acid score (PDCAAS) of dried abalone. The HPI-assisted drying process ensured excellent protein quality based on PER values, regardless of the pressure level. At 350 and 500 MPa, the HPI-assisted drying process had no negative effect on TD and PDCAAS then, based on nutritional parameters analysed, we recommend HPI-assisted drying process at 350 MPa × 5 min as the best process condition to dry abalone. Variations in nutritional parameters compared to casein protein were observed; nevertheless, the

high protein quality and digestibility of HPI-assisted dried abalones were maintained to satisfy the metabolic demands of human beings.

Keywords Dried abalone · Protein quality · High pressure · Biological evaluation · Digestibility

Introduction

Abalone is one of the most valuable seafood species in the world. Abalone (*Haliotidae* family) belongs to the gastropod molluscs, which are distributed along rocky shores and reefs of coastal temperate and tropical waters (Ghaneshree et al. 2014). Abalone provides high quality protein with all the essential dietary amino acids for the maintenance and growth of the human body; it is also low in fat and a good source of polyunsaturated fatty acids, vitamins, and minerals (Padula et al. 2016). However, common processing methods can negatively affect its nutritional value, digestibility, and protein quality (Deng et al. 2015; Zhang et al. 2017).

Dried abalone is a recommended product for the gastronomy industry due to its reduced weight and volume, and lower transport and storage costs (Pérez-Won et al. 2016). This commercial form of abalone can be produced by the convective drying method, in which dehydration process causes micro and macrostructure changes producing important negative effects on food functionality. Nevertheless, Pérez-Won et al. (2016) proposed a combination of high hydrostatic pressure (HHP) and osmotic dehydration (OD) (high-pressure impregnation, HPI) to assist the convective drying process to enhance uniformity, drying rates, and changes in final product quality. Changes in digestibility and protein quality of seafood proteins, such as

✉ Patricio Oliva-Moresco
poliva@ubiobio.cl

¹ Departamento de Ingeniería en Alimentos, Universidad del Bío-Bío, Av. Andrés Bello 720, Chillán, Chile

² Departamento de Nutrición y Salud Pública, Universidad del Bío-Bío, Av. Andrés Bello 720, Chillán, Chile

³ Departamento de Ingeniería en Alimentos, Universidad de La Serena, Av. Raúl Bitrán Nachary 1305, La Serena, Chile

⁴ Departamento de Ciencias Básicas, Universidad del Bío-Bío, Av. Andrés Bello 720, Chillán, Chile

⁵ Departamento de Ciencias de los Alimentos y Tecnología Química, Universidad de Chile, Av. Santos Dumont 964, Santiago, Chile

squid (Deng et al. 2015) and shrimp (Toomer et al. 2015), are mainly attributable to modified protein structures. It is known that the HHP treatment has a disruptive effect on the protein structure and promotes folding/unfolding and dissociation (Achouri and Boye 2013). Osmotic pressure also causes conformational fluctuations that contribute to the collapse of internal cavities and protein compression (Boonyaratanakornkit et al. 2002). Therefore, HPI could positively modify the digestibility and protein quality of this seafood. The effect of the drying process assisted by HPI on abalone quality protein is not yet known.

Proteins are considered as the most important macronutrients for humans and their nutritional value differs substantially depending on their capacity to satisfy metabolic demands for amino acids and nitrogen (N). Therefore, protein quality in food is usually determined by the essential amino acid (EAA) composition. Also, measurements of protein digestibility corrected amino acid score (PDCAAS) are suggested for evaluating protein quality because this nutritional index considers the effectiveness with which absorbed dietary N can meet human amino acid requirements at the safe protein intake level (FAO/WHO/UNU 2007).

On the other hand, the assessment of protein digestibility is based on chemical analysis (in vitro) or biological methods (in vivo). The latter can be performed by studies in small animals, such as rats or rabbits, because results are directly applicable to solve human nutritional problems (Murlin et al. 1941; Owusu-Apenten 2002). Animal studies focus on determining nutritional parameters, such as food intake (FI), body weight, protein efficiency ratio (PER), true digestibility (TD), net protein utilization (NPU), and biological value (BV) (Sung-Wook et al. 2015). The usual method involves feeding an N-free or low N ration during the experiment to measure endogenous N losses in urine and metabolic N losses in faeces (Ochuko et al. 2011). These chemical and biological parameters reflect the efficient use (metabolic, structural, and physiological) of the macromolecule to benefit human nutrition. Therefore, the aim of this study was to evaluate the effect of the drying process assisted by HPI on protein quality and in vivo digestibility of dried abalone (*Haliotis rufescens*).

Materials and methods

Raw materials

Red abalone (*Haliotis rufescens*) specimens were obtained from a local aquaculture company (Live Seafood Chile S.A, Coquimbo, Chile). Alive abalone ($n = 48$) with a body weight of 80 ± 12 g were delivered to the university laboratory under refrigeration conditions (below 7 °C).

Abalones were slaughtered, shucked, eviscerated, and the blue pigment (hemocyanin) was removed with fresh water to extract the abalone muscle. They were then stored at 4 °C for no more than 48 h until further HPI and drying processes.

High-pressure impregnation (HPI) and drying process

Pieces of abalone muscle (5.0 ± 0.2 cm each side and 1.1 ± 0.1 cm thickness) were packed in polyethylene bags (6 abalones pieces per bag) in an osmotic solution (15% NaCl; 1:3 abalone:solution ratio), heat-sealed, and exposed to a high-pressure treatment. The HHP treatments were performed in an isostatic pressing system (Avure Inc., Kent, WA, USA) with a cylindrical pressure chamber (700 mm length, 60 mm diameter). Water was the pressurizing medium; it was applied at 17 MPa/s ramp rate and decompression time was less than 5 s. The HHP treatment was applied at 350, 450, and 500 MPa pressure levels with a holding time of 5 min at 20 °C. Samples were removed from the osmotic solution after the high-pressure treatment. Samples (102.4 ± 1.2 g) were then arranged as a thin layer in a stainless steel basket and the drying process was carried out on a convective tray dryer (Gallenkamp, OVA-031, Leicester, UK). Air velocity was kept constant at 2.0 ± 0.2 m/s in each test. The drying process air inlet temperature was 60 °C. Sample weight and drying process time were recorded until constant weight was reached (i.e., equilibrium condition) with a final moisture contents of $11.4 \pm 0.8\%$. An unpressurized dried sample (dried abalone control) was also prepared from abalones immersed in an osmotic solution (15% NaCl) for the same length of time prior to the drying process. Dried samples were vacuum-packed into polyethylene bags until further analysis.

Proximate analysis and essential amino acid contents

Chemical compositions, such as protein, lipid, ash, and crude fibre, were analysed according to methods established by the Association of Official Analytical Chemists (AOAC 2005). The N-free extract was calculated by difference. Amino acid composition was determined by protein acid hydrolysis pre-column derivatization with PITC and identified by HPLC at 254 nm (HPLC/UV-PITC) (Kwanyuen and Burton 2010). Sample analysis was performed in triplicate.

Diet preparation and composition

Casein (“Vitamin-Free” Test), vitamin mix (CA.40060, Teklad), and mineral mix (Rogers-Harper 170760) were

purchased from Harlan Laboratories, Inc., USA. Wheat fibre (Winkler 850223) and cornstarch (Winkler 101570) were purchased from Winkler Ltda, Chile. Corn oil was purchased from a local market.

Five diets were prepared, one basal casein-based, one dried abalone control, and three HPI-assisted dried abalone as the only source of dietary protein. The protein level was adjusted to approximately 10% (NPU standard) in all groups (Miller & Payne 1961). An N-free diet was also included to obtain an estimate of endogenous metabolic protein. The composition of experimental diets was based on recommended by Reeves et al. (1993) to satisfy fat (10 g/100 g), mineral (4 g/100 g), vitamin (1 g/100 g) and energy (73.6–85 g/100 g) requirements.

Animal testing

Thirty-six Wistar rats with a mean age of 23 day and initial body weight of 60 ± 5 g were used to measure PER, NPR (net protein ratio), TD, and PDCAAS. Animals were divided into six groups ($n = 6$), and the differences in mean group weight was not greater than 10 g as recommended by the AOAC (2005). They were housed individually in cages with adequate facilities for separate faecal collection in rooms lit for 12 h per day at a constant temperature of 23 ± 1 °C. All experiments and animal procedures complied with protocols for the use and care of living animals in scientific research approved by the University of Bio-Bio Animal Ethics Committee.

The control (basal casein-based) and experimental (dried abalone control and HPI-assisted dried abalone) diets were fed ad libitum to the respective animal groups along with water for 28 day. The food consumption level and body weight of the experimental animals were recorded weekly until the end of the experimental period (day 28). Growth performance of the experimental animals, such as FI and body weight gain (BWG), were recorded, and PER and NPR were then calculated.

Nitrogen balance studies were conducted for 14 day to calculate TD and PDCAAS (Vadivel and Pugalenthil 2010). One animal group was also fed a protein-free basal diet, and faeces were collected. After the collection period, faeces of each animal group were collected, dried, pooled, chilled, and the N content was determined by the Kjeldhal method (AOAC 2005).

Protein quality measurements

The PER was determined by relating protein intake and empty BWG (Sung-Wook et al. 2015):

$$\text{PER} = \frac{\text{BWG}}{\text{Protein intake}}$$

The NPR was calculated from the body weight loss of the N-free diet group according to the equation:

$$\text{NPR} = \frac{\text{BWG on protein diet} - \text{Body weight loss on N-free diet}}{\text{Protein intake}}$$

TD of each food protein was determined by measuring the amount of N ingested in the diet, amount excreted in the faeces (faecal nitrogen, FN), and the metabolic loss in the faeces (endogenous faecal nitrogen, EFN): this corresponds to faecal N of the protein-free diet group and is expressed by the following equation:

$$\text{TD}(\%) = \frac{\text{Nitrogen intake} - (\text{FN} - \text{EFN})}{\text{Nitrogen intake}} \times 100$$

Amino acid scores (AASs) were used to calculate PDCAAS based on the method described by FAO/WHO/UNU (2007).

$$\text{AAs} = \frac{\text{mg of limiting amino acid in 1 g of protein}}{\text{mg of limiting amino acid in 1 g of reference protein}}$$

The PDCAAS was calculated by multiplying the AASs by protein TD.

Statistical analysis

Results were expressed as mean values $\pm$ standard deviations. Linear regression analysis was carried out for the body weight against time, and the growth rate was represented by the slope values ($n = 6$) for each treatment. Correlations between nutritional parameters (PER, NPR, TD, and PDCAAS) and pressure levels were assessed by Pearson's correlation test. Data were subjected to a one-way analysis of variance and the significance of difference between means at 5% was determined by Duncan's multiple range test (DMRT) using the Statgraphics centurion XVI version 16.1.11 software (Statistical Graphics Corp., USA).

Results and discussion

Proximate analysis and amino acid contents

Proximate composition (Table 1) showed that dried abalone (*Haliotis rufescens*) has high protein (43.9–31.5% dry base) and low lipid (2.4–3.7%) contents. HPI-assisted dried abalones showed protein content lower ($P < 0.05$) than control. This could be related to the solubilization of the muscle proteins (myofibrillar proteins) in salts solution due to the HPI treatment (HHP and OD combination). According to Niwa (1992), the partial release and

Table 1 Chemical and essential amino acid (EAA) composition of dried abalone

		Abalone control ¹	Abalone 350 MPa ²	Abalone 450 MPa ²	Abalone 500 MPa ²
Chemical composition (g/100 g)					
Protein		43.90 ± 0.30 ^a	33.70 ± 0.60 ^b	33.50 ± 0.40 ^b	31.50 ± 0.20 ^b
Lipid		3.72 ± 0.06 ^a	2.40 ± 0.10 ^b	3.04 ± 0.02 ^b	2.90 ± 0.10 ^b
Ash		4.80 ± 0.05 ^c	25.90 ± 0.60 ^a	22.90 ± 0.20 ^b	22.80 ± 0.20 ^b
Crude fibre		0.56 ± 0.03 ^a	0.09 ± 0.04 ^b	0.07 ± 0.01 ^b	0.40 ± 0.20 ^{ab}
Nitrogen-free extract		47.02 ± 0.48 ^a	37.91 ± 0.12 ^c	40.49 ± 0.14 ^{bc}	42.40 ± 0.34 ^b
Essential amino acid (mg/g protein)					
Histidine	18 ³	21.43 ± 0.08 ^b	22.36 ± 0.07 ^a	23.40 ± 0.20 ^a	23.43 ± 0.10 ^a
Isoleucine	25 ³	24.87 ± 0.20	26.60 ± 0.05	26.73 ± 0.04	25.33 ± 0.12
Leucine	55 ³	60.20 ± 0.58	60.93 ± 0.55	62.60 ± 0.46	58.67 ± 0.40
Lysine	51 ³	70.53 ± 0.18	73.53 ± 0.51	69.07 ± 0.50	75.83 ± 0.48
Methionine + Cysteine	25 ³	43.80 ± 0.65 ^a	38.07 ± 0.12 ^b	46.13 ± 0.33 ^a	38.90 ± 0.76 ^b
Phenylalanine + Tyrosine	47 ³	155.45 ± 1.89 ^b	191.25 ± 0.25 ^a	164.00 ± 0.32 ^b	187.05 ± 1.31 ^{ab}
Threonine	27 ³	37.23 ± 0.42	39.43 ± 0.22	34.77 ± 0.11	37.23 ± 0.37
Valine	32 ³	43.07 ± 0.57	43.03 ± 0.01	44.57 ± 0.21	42.10 ± 0.11

Chemical composition expressed as dry matter. Values are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$)

Nitrogen-free extract = 100 – (protein + lipid + fibre + ash)

¹ Dried abalone (60 °C)

² HPI-assisted dried abalone for 5 min at 20 °C

³ Reference standard of the EAA content (Institute of Medicine 2005)

solubilisation of myofibrillar proteins from filaments is conditioned by the diffusion of salt ions that interact with basic and acidic amino acid residues that are exposed on the molecular surface; this causes the rupture of ionic bonds between proteins and increases their affinity for water. In addition, salt diffusion from the aqueous medium promotes protein dehydration through the osmotic effect (Martínez-Alvarez and Gómez-Guillén 2006). The HP treatment also promotes protein solubilisation since cell wall permeability and protein hydration increase because of myofibril disintegration and protein unfolding (Grossi et al. 2016; Iwasaki et al. 2006). After pressure is released, unfolded proteins tend to recover their conformation and stability, although there are no new covalent interactions with salts leading to myofibril contraction and water loss (Grossi et al. 2016; Martínez-Alvarez and Gómez-Guillén 2006). The solubilised myofibrillar proteins then move towards the aqueous medium and the salt concentration increases on the muscle surface. Finally, the drying process removes the solubilised protein and decreases total protein content.

Zhang et al. (2017) and Barrios-Peralta et al. (2012) reported that the protein structure after HP treatment became loose and destabilised because of the non-covalent protein bond rupture, which allowed water to enter into the interior of the protein. Moreover, it is known that during

OD treatment, the flux of water occurs simultaneously with a counter influx of solutes through the semi-permeable cell membranes present in biological materials, resulting in dehydration and increase on solute concentration in the muscle (Nguyen et al. 2014).

The lipid contents of HPI-assisted dried abalones were lower ($P < 0.05$) than control. The HPI treatment prior to drying process could be promoted conditions which could accelerate lipolysis and lipid oxidation processes (Nguyen et al. 2014). Moreover, sodium chloride in the aqueous medium can act as a pro-oxidant agent because of its ability to inhibit enzyme antioxidant activity and disrupt cell membrane integrity by facilitating the access of oxidising agents to lipid substrates (Mariutti and Bragagnolo 2017). On the other hand, the drying process could increase enzyme activity by increasing free fatty acids that are later oxidised in low molecular weight compounds (Nguyen et al. 2014). In addition, low water activity also accelerates lipid oxidation during the drying process (Nguyen et al. 2014).

Furthermore, the crude fibre content of HPI-assisted dried abalones at 500 MPa was higher ($P < 0.05$) than those at 350 MPa and 450 MPa; no significant differences were observed compared to the control. No clear trends were perceived when using HHP. According to Vega-Gálvez et al. (2014) and Briones-Labarca et al. (2011), this

result is attributable to an increase in moisture, which probably has a dilution effect on other constituents of the HP-treated samples. However, abalone samples had low fibre contents (<1%). Significant increases in ash contents in HPI-assisted dried abalone also suggest that salts present in the osmotic solution could penetrate into the abalone muscle because pressure treatments enhance protein hydration and increase water absorption (Zhang et al. 2017).

The essential amino acid (EAA) profile of dried abalone protein consisted of histidine, isoleucine, leucine, lysine, methionine + cysteine, phenylalanine + tyrosine, threonine, and valine (Table 1). Phenylalanine + tyrosine and lysine were the highest EAA found in the control and HPI-assisted dried abalone (Table 1); these contents were higher than values recommended for 1- to 2-year-old children and all other age groups (Institute of Medicine 2005). In addition, histidine and phenylalanine + tyrosine contents on HPI-assisted dried abalone were higher compared to control samples; this behaviour probably is due to protective effect of high pressure process. According to Zhang et al. (2017) when HP is applied protein molecules stretched and unfolded, and conformation structure became loose and destabilized, leading to new molecular interaction where some amino acids became part of the core of the protein structure, being less exposed to environment; then drying process conditions does not affect the amino acid composition. However, in HPI-assisted dried abalones treated at 350 and 500 MPa, methionine + cysteine content was lower than control, thus it might be possible that these amino acids could be more exposed to environment during drying process. In all cases, control and HPI-assisted dried abalone proteins had EAA compositions that were higher than quantities recommended by the Institute of Medicine (2005). According to AAs values (all > 1), none of the dried abalone samples exhibited limiting amino acids. This means that no amino acid from the target food was found at the lowest concentration when compared to results found in a reference protein (FAO/WHO/UNU 2007); this ensures sufficient quantities in HPI-assisted dried abalone proteins. The proximate composition and the presence of eight EAAs in sufficient amounts to meet 100% of the nutritional requirements of human adults (FAO/WHO/UNU 2007) indicate that HPI-assisted dried abalone has a high quality protein for human consumption.

Measuring protein quality by animal experiments

The chemical compositions of diets (Fig. 1) were according to standard recommendations (Reeves et al. 1993). Diets promoted the good growth of the rats because all the animal groups showed a positive trend of body weight evolution over time. The growth rates, represented by the

slope mean value of each curve (Fig. 1), were higher ($P < 0.05$) in abalone-fed rats groups (control: 3.7 ± 0.3 g/day; 350 MPa: 3.9 ± 0.7 g/day, 450 MPa: 4.0 ± 0.9 g/day and 500 MPa: 4.3 ± 0.6 g/day) rather than in casein-fed rats group (2.6 ± 0.3 g/day) (Fig. 1). Moreover, abalone-fed rats had higher BWG than casein-fed rats ($P < 0.05$, Table 2); this is probably caused by their higher FI (Table 2). Boakes et al. (2016) reported that rats exhibit reduced acceptance of a low palatability diet, which causes lower protein intake, a negative effect on muscle protein synthesis, and BWG. It is possible that a difference in palatability between the casein and abalone diets can contribute to the low FI of casein-fed rats. On the other hand, rats fed with casein and dried abalone control diets had similar protein intake, while the HPI-assisted dried abalone diets indicated higher protein intake values (Table 2). All diets contained the protein requirements necessary to satisfy a good growth of the rats ($10 \pm 1\%$) (Reeves et al. 1993), thus the differences in protein intake among diet groups were basically related to the differences in animal FI.

A PER value greater than 2.5 (standard value of casein protein) is considered to be an excellent protein source (Hoffman and Falvo 2004). The PER values of abalone-based diets were greater than 2.5 (Table 2), thus confirming that dried abalone is a good source of high quality proteins. The PER values of HPI-assisted dried abalone at 350 and 450 MPa were not significantly different ($P < 0.05$) than those of the casein diet, while PER values of the dried abalone control and HPI-assisted dried abalone at 500 MPa were higher than in the casein diet. The NPR is similar in value to PER, except that an additional factor (mean weight loss of rats fed a non-protein diet) is considered (Boye et al. 2012); it is useful to determine body N expenditure with respect to the minimum necessary for maintenance. Therefore, the NPR method credits protein used for both growth and maintenance. PER and NPR variabilities can be attributed to different FI and BWG, and the differences how a biological organism can use efficiently a protein (FAO/WHO/UNU 2007). The NPR results were similar to those of PER and PER/NPR index was approximately 1 (Table 2); thus confirming the high quality of dried abalone proteins to promote animal growth and maintenance. Furthermore, abalone-fed rats had a lower PER/NPR index than casein-fed rats. This could be associated with the lower body N expenditure necessary for maintenance and higher body N expenditure for growth of abalone-fed rats, which could also contribute to their greater BWG compared to casein-fed rats.

Digestibility indicates protein bioavailability, meaning the amount of ingested protein that is hydrolysed by digestive enzymes (Reis and Oliveira 2008). Thus, TD was calculated by measuring the amount of nitrogen ingested in

Fig. 1 Effect of diet composition on rat body weight gain

Table 2 Nutritional parameters of each protein source

Protein source	Casein control	Abalone control ¹	Abalone 350 MPa ²	Abalone 450 MPa ²	Abalone 500 MPa ²
BWG (g/animal)	75.43 ± 8.30 ^b	102.33 ± 8.37 ^a	106.19 ± 20.92 ^a	108.30 ± 25.18 ^a	118.27 ± 18.13 ^a
FI (g/animal)	304.55 ± 25.61 ^b	337.34 ± 16.24 ^b	385.49 ± 40.72 ^a	403.38 ± 45.44 ^a	406.30 ± 18.14 ^a
Food/gain ratio	4.05 ± 0.15 ^a	3.31 ± 0.22 ^b	3.68 ± 0.39 ^a	3.82 ± 0.53 ^{ab}	3.48 ± 0.40 ^b
Protein intake (g/animal)	30.61 ± 2.57 ^b	31.37 ± 1.51 ^b	37.82 ± 3.99 ^a	40.62 ± 4.57 ^a	37.30 ± 1.66 ^a
PER	2.5 ± 0.09 ^b	3.3 ± 0.21 ^a	2.8 ± 0.30 ^b	2.6 ± 0.39 ^b	3.2 ± 0.36 ^a
NPR	2.30 ± 0.10 ^b	3.1 ± 0.21 ^a	2.6 ± 0.31 ^b	2.5 ± 0.40 ^b	3.0 ± 0.37 ^a
PER/NPR	1.09 ± 0.01 ^a	1.06 ± 0.01 ^b	1.07 ± 0.01 ^b	1.04 ± 0.02 ^b	1.07 ± 0.01 ^b
Nitrogen intake (g/animal)	4.80 ± 0.40 ^b	5.02 ± 0.24 ^b	6.05 ± 0.64 ^a	6.36 ± 0.70 ^a	5.97 ± 0.27 ^a
FN-EFN (g/animal)	0.36 ± 0.09 ^c	0.46 ± 0.08 ^c	0.65 ± 0.11 ^b	1.07 ± 0.29 ^a	0.67 ± 0.13 ^b
TD (%)	92.50 ± 1.91 ^a	90.80 ± 1.48 ^{ab}	89.20 ± 1.38 ^b	83.30 ± 4.21 ^c	88.80 ± 2.21 ^b
rTD (%)	–	98.16 ± 0.02 ^a	96.43 ± 0.01 ^a	90.05 ± 0.04 ^b	96.00 ± 0.02 ^a
PDCAAS	0.92 ± 0.02	0.91 ± 0.01 ^b	0.95 ± 0.01 ^a	0.89 ± 0.04 ^b	0.90 ± 0.02 ^b

Values are given as mean ± standard deviation

Different letters in the same row indicate significant differences ($P < 0.05$)

BWG body weight gain; FI food intake; PER protein efficiency ratio; NPR net protein ratio; FN Faecal nitrogen; EFN Endogenous faecal nitrogen; TD true digestibility; rTD relative digestibility; PDCAAS protein digestibility corrected amino acid score

¹ Dried abalone (60 °C)

² HPI-assisted dried abalone for 5 min at 20 °C

the diet, the amount excreted in the faeces (FN), and metabolic loss in the faeces (EFN), which corresponds the faecal nitrogen of the protein-free diet group (Reis and Oliveira 2008). The FN-EFN (Table 2) value indicates the amount of protein not absorbed in the intestine, and it has a lineal correlation with TD ($r = 0.996$). TD of casein and dried abalone protein are shown in Table 2. The HPI-

assisted drying process at 350 and 500 MPa had no effect ($P < 0.05$) on TD compared to the dried abalone control (Table 2); however, TD was lower ($P < 0.05$) than in the casein diet (protein reference). On the contrary, the HPI-assisted drying process at 450 MPa had a lower TD value ($P < 0.05$) compared to the dried abalone control and casein diet. The HPI-assisted drying process at 450 MPa

could probably promote some modifications in protein structures and increase protein resistance to enzymatic hydrolysis. In contrary, the protein resistance to enzymatic hydrolysis decrease at 350 and 500 MPa. Studies to establish the relationship between protein molecular structures and digestive characteristics (Liu et al. 2013; Salazar-Villanea et al. 2016) have shown that the most commonly occurring protein secondary structures (α -helix and β -sheet) can influence protein quality, nutrient use, and digestive behaviour. Changes in protein conformation have an impact on the relative ease with which peptide bonds can be hydrolysed. There are no reports on protein digestibility of the abalone protein. According to Boye et al. (2012), fish digestibility and fish product protein is $94 \pm 3\%$ higher than the digestibility values found in the current study. Nevertheless, the relative digestibility (rTD) of dried abalone samples (Table 2), determined from values in Table 2, such as the ratio between TD of the experimental groups and TD of the casein diet group (Fontes et al. 2015), was greater than 90%. When associated with the good amino acid profile (Table 1) ensures the high protein quality of dried abalone.

The PDCAAS is the most relevant index to determine protein quality because it is a method based on both the amino acid requirements of humans and their ability to digest it, and it might eventually replace the need for assessing overall protein quality (Giménez et al. 2016). The HPI-assisted drying process at 350 MPa increased the PDCAAS of dried abalone protein compared to the casein and dried abalone control (Table 2). This indicates that the HPI-assisted drying process has not modified protein quality. Except for HPI-assisted dried abalone at 450 MPa, all the diets had PDCAAS values in accordance with those reported for fish and fish product protein (0.96 ± 0.04) (Boye et al. 2012). Based on the results obtained from the animal assays, reduced PDCAAS in HPI-assisted dried abalone at 450 MPa was a consequence of its lower digestibility because the amino acid composition showed no limiting amino acid in HPI-assisted dried abalone proteins (Table 1). This indicated that low digestibility could reduce proteins with high EAA composition (high quality protein) due to the lower availability of essential amino acids to be absorbed by the body (Murlin et al. 1941).

To relate nutritional parameters (PER, NPR, TD and PDCAAS) to AASs and pressure levels, a pairwise Pearson's correlation was conducted. PER and NPR values were found to be negatively correlated with the isoleucine score ($r = -0.98$, $P < 0.01$). For PDCAAS values no significant predictor was obtained. Thus, no correlation was found between the pressure level and nutritional parameters but higher pressure levels were associated with a greater histidine score ($r = 0.96$, $P < 0.05$) confirming that HP had a protective effect on this amino acid. Nevertheless, it was possible to

Fig. 2 Potential relation between growth rates, pressure level and protein digestibility corrected amino acid score (PDCAAS) of dried abalone

identify a range of pressure level and growth rate in which the highest values of PDCAAS were obtained (red zone in Fig. 2). This red zone, area of convergence between the growth rate (3.9–4.0 g/day) and HPI treatment (pressure levels: 300–370 MPa), is where high PDCAAS (0.94–0.95) values are reached; this is where HPI-assisted dried abalone at 350 MPa was found. Since the HPI-assisted dried abalone at 350 MPa had the highest PDCAAS value; PER, NPR, and TD values were also found within the standard values considered to be an excellent protein source. We propose that the HPI-assisted dried abalone at 350 MPa for 5 min is the most appropriate method to produce this type of commercial abalone. The evaluation of protein quality of dried abalone has not yet been determined; therefore, our findings contribute to public awareness of the health benefits of consuming abalone.

Conclusion

High-pressure impregnation (HPI) assisted dried abalones have a good amino acid profile. The HPI-assisted drying process ensured an excellent quality protein based on the protein efficiency ratio values, regardless of the pressure level. At 450 MPa, the HPI-assisted drying process reduced true digestibility and protein digestibility corrected amino acid score of dried abalone protein compared to the casein and dried abalone control. We recommend that the HPI-assisted drying process at 350 MPa is the best process condition to dry abalone based on its nutritional parameters. To sum up, some variations in nutritional parameters compared to casein protein were observed, but the high quality protein of the HPI-assisted dried abalone was maintained to satisfy the metabolic demands of human beings.

Acknowledgements The authors acknowledge the financial support by CONICYT/FONDECYT 1140067 and DIUBB Project 152920 2/R.

References

- Achouri A, Boye JI (2013) Thermal processing, salt and high pressure treatment effects on molecular structure and antigenicity of sesame protein isolate. *Food Res Int* 53(1):240–251
- AOAC (2005) Official methods of analysis, 18th edn. AOAC International, Maryland
- Barrios-Peralta P, Pérez-Won M, Tabilo-Munizaga G, Briones-Labarca V (2012) Effect of high pressure on the interactions of myofibrillar proteins from abalone (*Haliotis rufescens*) containing several food additives. *LWT-Food Sci Technol* 49(1):28–33
- Boakes RA, Martire SI, Rooney KB, Kendig MD (2016) Individual differences in saccharin acceptance predict rats' food intake. *Phys Behav* 164(Part A):151–156
- Boonyaratankornkit BB, Park CB, Clark DS (2002) Pressure effects on intra- and intermolecular interactions within proteins. *Biochim Biophys Acta* 1595(1–2):235–249
- Boye J, Wijesinha-Bettoni R, Burlingame B (2012) Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *Brit J Nutr* 108:S183–S211
- Briones-Labarca V, Venegas-Cubillos G, Ortiz-Portilla S, Chacana-Ojeda M, Maureira H (2011) Effects of high hydrostatic pressure (HHP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple. *Food Chem* 128:520–529
- Deng Y, Luo Y, Wang Y, Zhao Y (2015) Effect of different drying methods on the myosin structure, amino acid composition, protein digestibility and volatile profile of squid fillets. *Food Chem* 171:168–176
- FAO/WHO/UNU (2007) Protein and amino acid requirements in human nutrition. Report of a joint FAO/WHO/UNU expert consultation (WHO Technical Report Series 935). World Health Organization, Geneva, Switzerland
- Fontes PR, Gomide LAM, Costa NMB, Peternelli LA, Fontes EAF, Ramos EM (2015) Chemical composition and protein quality of mortadella formulated with carbon monoxide-treated porcine blood. *LWT-Food Sci Technol* 64(2):926–931
- Ghaneshree M, Lethabo M, Rajesh L, Suren S (2014) Application of biological agents in abalone aquaculture: a South African perspective. In: Hernandez-Vergara M, Perez-Rostro C (eds) Sustainable aquaculture techniques. InTech, Rijeka, Croatia, pp 207–237
- Giménez MA, Drago SR, Bassett MN, Lobo MO, Samman NC (2016) Nutritional improvement of corn pasta-like product with broad bean (*Vicia faba*) and quinoa (*Chenopodium quinoa*). *Food Chem* 199:150–156
- Grossi A, Olsen K, Bolumar T, Rinnan Å, Øgandal L, Orlén V (2016) The effect of high pressure on the functional properties of pork myofibrillar proteins. *Food Chem* 196:1005–1015
- Hoffman JR, Falvo MJ (2004) Protein—Which is best? *J Sports Sci Med* 3(3):118–130
- Institute of Medicine (2005) Food and nutrition board. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids macronutrients. National Academy of Sciences. United States of America
- Iwasaki Noshiroya K, Saitoh N, Okano K, Yamamoto K (2006) Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty. *Food Chem* 95:474–483
- Kwanyuen P, Burton JW (2010) A modified amino acid analysis using pitc derivatization for soybeans with accurate determination of cysteine and half-cystine. *J Am Oil Chem Soc* 87(2):127–132
- Liu B, Thacker P, McKinnon J, Yu PQ (2013) In-depth study of the protein molecular structures of different types of dried distillers grains with solubles and their relationship to digestive characteristics. *J Sci Food Agric* 93(6):1438–1448
- Mariutti L, Bragagnolo N (2017) Influence of salt on lipid oxidation in meat and seafood products: a review. *Food Res Int* 94:90–100
- Martínez-Alvarez O, Gómez-Guillén M (2006) Effect of brine salting at different pHs on the functional properties of cod muscle proteins after subsequent dry salting. *Food Chem* 94:123–129
- Miller DS, Payne PR (1961) Problems in the prediction of protein values of diets. The influence of protein concentration. *Br J Nutr* 15:11–19
- Murlin JR, Marshall ME, Kochakian CD (1941) Digestibility and biological value of whole wheat breads as compared with white bread: one figure. *J Nutr* 15:11–19
- Nguyen MV, Arason S, Eikevik TM (2014) Drying of fish. In: Bozariis IS (ed) Seafood processing: technology, quality and safety, 1st edn. Wiley, Chichester, pp 161–175
- Niwa E (1992) Chemistry of surimi gelation. In: Lanier T, Lee C (eds) Surimi technology. Marcel Dekker Inc, New York, pp 389–428
- Ochuko LE, Oluwatoyin VO, Folasade A, Funmi OK, Olubanke A (2011) Nutritional evaluations of clerodendrum volubile, a tropical non-conventional vegetable as sole dietary protein source in rats. *Int J Biol Med Res* 2(1):374–377
- Owusu-Apenten RK (2002) Protein nutrient value. In: Owen R (ed) Food Protein Analysis. Quantitative effects on processing. Marcel Dekker Inc, New York, pp 341–374
- Padula D, Greenfield H, Cunningham J, Kiermeier A, McLeod C (2016) Australian seafood compositional profiles: a pilot study. Vitamin D and mercury content. *Food Chem* 15(193):106–111
- Pérez-Won M, Lemus-Mondaca R, Tabilo-Munizaga G, Pizarro S, Noma S, Igura N et al (2016) Modelling of red abalone (*Haliotis rufescens*) slices drying process: effect of osmotic dehydration under high pressure as a pretreatment. *Innov Food Sci Emerg Technol* 34:127–134
- Reeves PG, Nielsen FH, Fahey GCJ (1993) AIN-93 Purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing Committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 123(11):1939–1951
- Reis LS, Oliveira TC (2008) Ostrich (*Strutio camelus*) meat protein quality and digestibility. *Rev Bras Cienc Avic* 10(3):185–188
- Salazar-Villanea S, Bruininx E, Gruppen H, Hendriks WH, Carre P, Quinsac A, van der Poel AFB (2016) Physical and chemical changes of rapeseed meal proteins during toasting and their effects on in vitro digestibility. *J Anim Sci Biotechnol* 7:62–73
- Sung-Wook H, Kyu-Man C, Seong-Jun C (2015) Nutritional quality of rice bran protein in comparison to animal and vegetable protein. *Food Chem* 172:766–769
- Toomer OT, Do AB, Fu TJ, Williams KM (2015) Digestibility and immunoreactivity of shrimp extracts using an in vitro digestibility model with pepsin and pancreatin. *J Food Sci* 80(7):T1633–T1639
- Vadivel V, Pugalenth M (2010) Evaluation of nutritional value and protein quality of an under-utilized tribal food legume. *Indian J Tradit Knowl* 9(4):791–797
- Vega-Gálvez A, López J, Torres-Ossandón MJ, Galotto MJ, Puente-Díaz L, Quispe-Fuentes I, Di Scala K (2014) High hydrostatic pressure effect on chemical composition, color, phenolic acids and antioxidant capacity of Cape gooseberry pulp (*Physalis peruviana* L.). *LWT-Food Sci Technol* 58(2):519–526
- Zhang Z, Yang Y, Zhou P, Zhang X, Wang J (2017) Effects of high pressure modification on conformation and gelation properties of myofibrillar protein. *Food Chem* 217:678–686