



## Variation in the proximate composition and fatty acid profile recovered from Argentine hake (*Merluccius hubbsi*) waste from Patagonia

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**SUMMARY:** The fish processing operations in Patagonia produce large amounts of waste. The main fishery resource in Argentina is the Argentine hake (*Merluccius hubbsi*). The ports of the province of Chubut (the most important of which are Puerto Madryn, Rawson and Comodoro Rivadavia), together with Caleta Paula Port (province of Santa Cruz), in the Argentine Patagonia, capture more than 82,000 tons of hake annually, 80% of which are of *M. hubbsi*, which is mostly converted into fillets. From this capture, about 2,296 tons of liver would be available for the extraction of oil. To promote the recovery and industrial use of fish oil, in the present study, we determined the variation in the proximate composition and fatty acid profile of Argentine hake waste from the ports mentioned above at different catch times. Proximate composition was determined according of the Official Methods of Analysis (AOAC). Fatty acid profile was analyzed by gas chromatography of the fatty acid methyl esters (FAMES). A standard mixture of FAMES was run under identical conditions to identify the compounds on the basis of their retention times. Fatty acids were quantified using heptadecanoic acid (C17:0) as internal standard. The highest lipid recovery (27.0 to 41.8% of total lipids) was obtained from the liver fraction. Palmitic acid (C16:0), oleic acid (18:1 n9), docosahexaenoic acid (22:6 n3), eicosapentaenoic acid (20:5 n3) and palmitoleic acid (16:1) were the main constituents. Protein levels in viscera without livers (V-L) were higher than those in the liver. The extraction of marine fish oil and the production of fish offal meal from waste from fish factories would contribute to the sustainability of the regional industry, because it would also decrease the volume of waste, with benefits to the environment.

**KEYWORDS:** *Liver oil; Merluccius hubbsi; Proximate composition*

**RESUMEN:** *Variación en la composición proximal y perfil de ácidos grasos recuperados a partir de residuos del procesamiento de merluza argentina (Merluccius hubbsi) en Patagonia.* El procesamiento de pescados en Patagonia produce gran cantidad de residuos. El recurso de pesca más importante en la Argentina es la merluza argentina (*Merluccius hubbsi*). En Patagonia, los puertos de la provincia del Chubut (de los cuales los más importantes son Puerto Madryn, Rawson y Comodoro Rivadavia), y Caleta Paula (provincia de Santa Cruz), capturan más de 82.000 t anuales de merluza, de las cuales el 80% corresponden a *M. hubbsi*. La misma es en su mayoría convertida en filetes. De esta captura, aproximadamente 2.296 toneladas de hígados estarían disponibles para la extracción de aceites. Para promover la recuperación y el uso industrial del aceite de pescado, se ha determinado la variación en la composición proximal de los residuos y el perfil de ácidos grasos de hígados de merluza argentina capturada en diferentes épocas en los puertos mencionados arriba. La variación en

composición proximal fue determinada según la metodología propuesta por los Official Methods of Analysis (AOAC). El perfil de ácidos grasos fue analizado por cromatografía gaseosa de los ésteres metílicos de los ácidos grasos (FAMES). Una mezcla estándar de FAMES fue corrida en idénticas condiciones para la identificación de los compuestos en base a la comparación de sus tiempos de retención. Para la cuantificación de los ácidos grasos se utilizó ácido heptadecanoico como estándar interno (C17:0). El hígado presentó el mayor contenido en lípidos (27.0 a 41.8% de lípidos totales), siendo los ácidos palmítico (C16:0), oleico (C18:0), docosahexaenoico (C22:6 n3), eicosapentaenoico (20:5n3) y palmitoleico (C16:1) los más abundantes. Los niveles de proteína en el resto de las vísceras sin incluir el hígado (V-L) fueron superiores que en el hígado. La extracción de aceite de pescado marino y la producción de harinas de pescado a partir de los desechos industriales contribuirían a la sustentabilidad de la industria regional, pues esto disminuiría el volumen de residuos, lo cual beneficiaría al medio ambiente.

**PALABRAS CLAVE:** *Aceite de hígado; Composición proximal; Merluccius hubbsi*

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## 1. INTRODUCTION

In the last decade, the fishing activities in Argentina, where the Patagonian coast plays a major role, have shown an important increase. The Argentine hake (*Merluccius hubbsi*) is the resource with the greatest production in the Argentine Sea. *M. hubbsi*, and has been historically captured by vessels landing in the Province of Buenos Aires, but currently, it is the most abundant resource at the ports located below 41° S, in particular, at the ports of the Patagonian province of Chubut (the most important of which are Puerto Madryn, Rawson and Comodoro Rivadavia) and the port Caleta Paula (in the province of Santa Cruz, within the boundaries of Golfo San Jorge and near Comodoro Rivadavia).

Between 2009 and 2013, the annual Argentine average of wild fish harvest was approximately 750,000 tons, 46% of which was of hake species, mostly *M. hubbsi*, and the capture volume was relatively constant (Ministerio de Agricultura, Ganadería y Pesca de la Nación, 2014). In the same period of time, the ports of the province of Chubut together with Caleta Paula Port recorded more than 82,000 annual tons of hake, 80% of which were of *M. hubbsi*. The hake is mostly converted into fillets at the industry. This filleting process generates approximately 40% of weight in fish waste, i.e. approximately 26,000 tons, which is mostly discarded within or close to urban waste tips. The high amounts of waste produced due to the inefficient use of this natural resource generate sites of local pollution, thus affecting the environment. Furthermore, the kelp gull (*Larus dominicanus*) consumes fish waste, and is thus expanding in Patagonia. This causes derived effects on other coastal species through predation and competition for breeding space and kleptoparasitism. In addition, the kelp gull is a source of bacterial contamination for the human population (Yorio and Caille, 2004).

The hake liver is part of the fish waste considered interesting because some of its constituents have a high nutritional value. The constituents can be extracted for the preparation of products with added commercial value. Fish liver oil is in general rich in omega-3 fatty acids, including alpha linolenic acid (ALA) 18:3, eicosapentaenoic acid (EPA) 20:5, and docosahexaenoic acid (DHA) 22:6. These fatty acids are considered essential for mammals, because they cannot be biosynthesized and their consumption has been associated with significant benefits for cardiovascular and brain health (Simopoulos, 2011a and 2011b; Bourre, 2007).

The overall composition of fish lipids and fatty acids is highly variable and depends on the season of capture, geographical location, temperature of the water, size and sex of the fish and time of the reproductive cycle at the moment of capture (Guil Guerrero *et al.*, 2011). Although some data on the composition of viscera and livers from hake waste from other regions of the Atlantic have been reported (Grompone *et al.*, 2004; Njinkoué *et al.*, 2002; Mendez, 1997), we have found no data on that from hake waste from the Patagonian region. Both aquaculture and the demand for fish oil as an ingredient of a balanced diet are booming (Montero *et al.*, 2005; Food and Agriculture Organization of the United Nations, 2014), and the global production of fish oil is not enough to cover this demand (Montero *et al.*, 2005; Izquierdo *et al.*, 2005). For this reason, most of the commercial formulations have changed fish oil for plant oil as an ingredient. However, plant oils are poor in essential fatty acids (EFAs), such as EPA and DHA. EFAs are necessary for the growth and development of the nervous system, pigmentation and behavior of fish (Medina *et al.*, 2014). In aquaculture, it is known that, feed accounts for over 50% of the production cost (Rana *et al.*, 2009). In Patagonia, these costs are higher due to the higher transportation costs

because of the long distances to the feed production plants. At present, the feed purchased in Patagonia is based on plant sources. However, it would be possible to make high-quality feed from regional resources that are currently discarded.

In an effort to find an activity with social and economic returns for the Argentine Patagonia and to improve the sustainability of the current fishing industry, the data obtained will hopefully be useful to evaluate the feasibility of implementing a process of hake oil production and or fish offal meal for commercial purposes.

## 2. MATERIALS AND METHODS

### 2.1. Samples

Fresh fish samples of *M. hubbsi* were provided by the fishery Mar del Chubut, Comodoro Rivadavia, in October 2011, March 2012, June 2012 and September 2012. Fifteen fish were randomly selected for each sampling, measured, weighed, eviscerated and then hand filleted. These procedures were carried out in a cold room (+4 °C). The different body parts were separated and stored on ice for 1–3 h before mincing. The raw material mixtures were separated into two groups: livers (L) and viscera without livers (V-L). The skin, head and backbone were not included in the experiments. Following Stansby (1986), the raw material from each group was pooled in a single sample and minced with a manual mincer with the addition of butylated hydroxytoluene as an antioxidant (Guil Guerrero *et al.*, 2011). Both L and V-L samples were stored under nitrogen at –20 °C until analysis.

### 2.2. Proximate composition

Moisture, ash and protein were analyzed according to the AOAC methodology (Official Methods of Analysis, 1990), total lipids were determined by Soxhlet (Hamilton, 1992), and carbohydrates were calculated by difference.

### 2.3. Fatty acid composition

For the analysis of fatty acids, total lipids were transesterified according to Lepage and Roy (1984) with acetyl chloride in methanol 1:20 v/v. After the reaction, the tubes were cooled in water and the reaction mixture was diluted with 1 mL water and extracted three times with 1 mL hexane. The hexanic phase was dried under a nitrogen stream at atmospheric pressure and at room temperature. Fatty acids as fatty acid methyl esters (FAME) were re-suspended with 100 µL hexane for injection into a Hewlett-Packard 589 gas chromatograph with a flame ionization detector. A capillary column Varian Factor Four VF-23 ms (30 m×0.25 mm×0.25 µm; Varian INC Lake Forest,

CA, USA) was used. Hydrogen was used as carrier gas. The injection split was set at 25/1. The temperature in the column was held at 120 °C for 1 min, then increased at 10 °C·min<sup>-1</sup> until 160 °C (ramp 1), and finally increased to 3 °C·min<sup>-1</sup> until 200 °C (ramp 2). The injector and detector temperatures were 240 °C and 260 °C, respectively. Determinations were carried out in triplicate. A standard of FAME (Supelco Inc., Supelco Park, Bellefonte, USA) was run under identical conditions to identify compounds on the basis of their retention times. Fatty acids were quantified using heptadecanoic acid (C17:0) as internal standard. Thus, an aliquot of 125 µg heptadecanoic acid dissolved in 5 µL toluene was added to the biological samples before transesterification. Quantitative data for fatty acid content represent the mean of three independent experiments.

### 2.4. Statistical analysis

The experiments were performed in triplicate and conventional statistical methods were used to calculate the mean and standard deviations. The results are presented as mean ± standard deviation of determinations for triplicate samples.

## 3. RESULTS AND DISCUSSION

### 3.1. Biomass distribution

Biomass distribution in the samples, i.e. percent of weight for L and V-L, are shown in Table 1. Liver comprised 3.5% of the weight of the fish. Taking into account that the annual volume of capture in Chubut is on average 65,600 tons (Ministerio de Agricultura, Ganadería y Pesca de la Nación, 2014), nearly 2,296 tons of liver would be currently available for the extraction of oils.

### 3.2. Proximate composition

A highest lipid recovery (27.0 to 41.8% of total lipids) was obtained from the liver fraction in all the months analyzed, although the highest was in June, which is autumn/winter in South America (Table 2). Guil Guerrero *et al.* (2011) reported a lipid recovery of 36% from *M. hubbsi* liver from European coasts in

TABLE 1. Biomass distribution in samples of *Merluccius hubbsi* waste. Percentage expressed in g·100 g<sup>-1</sup> biomass

Month of capture	Unprocessed fish (g)	Livers (%)	Rest of viscera (%)
October	8006	3.16	7.08
March	6300	3.92	5.52
June	7955	3.71	2.58
September	7250	3.81	3.08

TABLE 2. Proximate composition of liver and rests of viscera from *M. hubbsi* in different times of capture. g·100 g<sup>-1</sup> biomass, mean ± standard deviation, n=3

	Livers				Viscera without liver			
	October	March	June	September	October	March	June	September
Lipids	36.13±2.20	27.05±1.36	41.78±1.37	39.55±0.88	3.84±0.47	5.75±1.13	0.39±0.01	5.36±2.66
Proteins	9.28±2.16	9.67±0.55	10.86±0.01	8.82±0.59	12.38±3.00	14.09±0.84	13.56±0.01	11.95±1.91
Ashes	0.93±0.20	0.95±0.03	1.21±0.07	0.74±0.03	1.33±0.01	1.68±0.09	1.88±0.01	1.30±0.02
Moisture	49.06±0.52	57.37±0.27	45.52±0.43	47.04±1.25	77.48±0.05	76.47±0.44	81.64±0.50	71.42±1.57

June, which is spring/summer in Europe. This value is similar to that obtained in this work in October (36%). Taking into account the high availability of lipids measured and the volume of capture reported for Chubut, it would be possible to obtain between 620–960 annual tons of liver oil. Protein levels in the viscera without livers (V-L) (11.9–14.1%) were higher than those in the liver (L) (8.8–10.9%) in all the months analyzed. The protein content in the rest of the viscera on dry basis was between 42 and 76%, whereas minerals were between 4.6 and 10%. This makes the rest of the viscera a very interesting material, because it can provide high-quality offal meal (Guillaume *et al.*, 2001). Offal meal is very simple to obtain and has the advantage that it does not generate new residues (Yano *et al.*, 2008).

### 3.3. Fatty acid composition

The chemical composition of the FAMES from the liver is shown in Table 3. All the months analyzed showed similar profiles, with palmitic acid (C16:0), oleic acid (18:1 n9), DHA (22:6 n3), EPA (20:5 n3) and palmitoleic acid (16:1) as the main constituents. Throughout the year, saturated fatty acids (SFAs) accounted for 29–33%, polyunsaturated fatty acids (PUFAs) accounted for 29–33%, and monounsaturated fatty acids (MUFAs) accounted for 36–39% of the total fatty acid composition (Table 4). Although the value of SFA content was higher than that reported for Uruguay and Spain (Mendez, 1997; Guil Guerrero *et al.*, 2011), the PUFAs/SFAs ratio (0.9 to 1.1%) indicates that hake liver oil is a good supplement of PUFAs. Table 4 also shows that the total amount of n3 PUFAs (26–30%) was higher than that of total n6 PUFAs, with the latter being only 2–3% of the total lipid profile of hake liver. It is known that the n3/n6 ratio can be used as an index to compare the relative nutritional values of fish oils from different species (Huynh and Kitts, 2009). The n3/n6 ratio for hakes from San Jorge Gulf is very high (10.1–16.0), unlike that found for Mediterranean (4.3) and European hakes (9.4) (Guil Guerrero *et al.*, 2011). The values reported for European, Mediterranean and Uruguayan hake species (Guil Guerrero *et al.*, 2011; Mendez, 1997) indicate that the DHA content is higher than the

EPA content. In this work, both EPA and DHA were found in similar proportions, at all catch times. The fatty acid content in the tissues comes from two different sources: *de novo* biosynthesis and diet. The latter varies with the habitat. EPA + DHA values reported for hakes from other parts of the world

TABLE 3. Chemical composition of FAMES from liver oil of *M. hubbsi* from different capture times. Total Fatty acids (TFAs) (g·100 g<sup>-1</sup> fresh weight), FA (area % on TFA area). Mean ± standard deviation, n=3

Peak ID	October	March	June	September
<b>TFAs</b>	9.07±0.45	7.13±0.36	9.96±0.81	10.26±1.69
<b>SFAs</b>				
C14:0	n.d.	2.67±1.54	3.69±0.91	3.38±0.72
C15:0	0.43±0.02	0.41±0.03	0.59±0.05	0.60±0.08
C16:0	24.51±1.34	22.88±0.37	25.06±2.41	26.49±2.24
C18:0	2.82±0.05	3.23±0.09	2.75±0.17	1.98±1.72
C20:0	0.11±0.02	0.12±0.01	0.12±0.03	0.18±0.08
<b>MUFAs</b>				
C14:1c	0.16±0.05	0.20±0.05	0.36±0.03	0.32±0.02
C16:1c	10.64±0.34	10.88±0.30	10.36±1.83	10.71±1.22
C17:1	0.43±0.18	0.37±0.01	0.65±0.24	0.67±0.09
C18:1n9c	23.65±0.73	22.09±0.16	17.78±5.73	21.10±1.91
C20:1n9	2.55±1.63	5.78±0.14	6.18±0.36	5.75±0.06
C22:1n9	1.09±0.03	0.07±0.01	0.10±0.02	0.01±0.03
C24:1n9	0.54±0.09	0.03±0.01	0.12±0.16	0.06±0.06
<b>PUFAs</b>				
C18:2n6c	1.67±0.04	1.5±0.03	1.95±0.11	2.05±0.06
C18:3n6	0.16±0.01	0.20±0.01	0.17±0.05	0.18±0.05
C18:3n3	1.20±0.01	1.35±0.03	1.56±0.04	1.62±0.03
C20:2	0.26±0.15	0.35±0.01	0.48±0.08	0.46±0.06
C20:3n6	0.08±0.01	0.09±0.01	0.09±0.01	0.04±0.04
C20:4n6	1.02±0.04	n.d.	n.d.	n.d.
C20:3n3	0.25±0.03	0.25±0.01	0.44±0.33	0.51±0.42
C20:5n3	11.80±0.35	14.09±0.30	11.95±0.52	11.51±0.84
C22:3	n.d.	0.02±0.02	0.03±0.03	0.03±0.02
C22:5n3	n.d.	0.69±0.04	0.60±0.11	0.66±0.11
C22:6n3	15.34±1.26	13.46±0.26	14.87±2.38	11.73±2.28

n.d.: not detected.

TABLE 4. Content of different fatty acid groups from *M. hubbsi* at different catch times (values are expressed as mean  $\pm$  s.d.)

	October	March	June	September
$\Sigma$ SFA	30.49 $\pm$ 1.34	29.31 $\pm$ 1.59	32.21 $\pm$ 2.59	32.64 $\pm$ 4.64
$\Sigma$ MUFAs	39.05 $\pm$ 1.83	39.41 $\pm$ 0.37	35.56 $\pm$ 6.04	38.64 $\pm$ 2.27
$\Sigma$ PUFAs	33.28 $\pm$ 1.32	32.09 $\pm$ 0.40	32.22 $\pm$ 2.47	28.79 $\pm$ 2.47
$\Sigma$ n3PUFAs	29.59 $\pm$ 1.31	29.85 $\pm$ 0.40	29.42 $\pm$ 2.46	26.03 $\pm$ 2.46
$\Sigma$ n6PUFAs	2.94 $\pm$ 0.06	1.87 $\pm$ 0.03	2.29 $\pm$ 0.12	2.27 $\pm$ 0.09
n3/n6 RATIO	10.06 $\pm$ 0.49	15.96 $\pm$ 0.33	12.85 $\pm$ 1.27	11.47 $\pm$ 1.75
PUFAs/SFAs RATIO	1.09 $\pm$ 0.06	1.09 $\pm$ 0.06	1.00 $\pm$ 0.11	0.88 $\pm$ 0.15
EPA+DHA	27.14 $\pm$ 1.30	27.56 $\pm$ 0.40	26.82 $\pm$ 2.44	23.24 $\pm$ 2.43

range between 21.3 and 24.9% (Mendez, 1997; Guil Guerrero *et al.*, 2011). Values found in hakes from Chubut are higher (23.2–27.6%). According to that explained by Guil Guerrero *et al.* (2011), this difference could be due to the differences in water temperature in their respective habitats.

In aquaculture, the n3/n6 ratio is a very important feature. However, most of the commercial fish feed is based on plant oils, because of reduced costs (Tacon and Metian, 2008; Guillou *et al.*, 1995). As a consequence, the n3/n6 ratio in the fish muscle is reversed, and a re-feeding period with fish oil-based feed before slaughter is necessary. The resulting filets in general have lower nutritional quality in EPA than fish fed animal oil-based feed (Montero *et al.*, 2005; Izquierdo *et al.*, 2005). This is true for marine fish because they do not have the necessary enzymes (elongases and desaturases) for the conversion of EFAs like EPA, DHA and arachidonic acid from n6 linoleic acid (Izquierdo *et al.*, 2005; Guillou *et al.*, 1995). The absence of EFAs can affect the normal development of fish. As fish is the main source of PUFAs for humans (Montero *et al.*, 2005), a deficiency in EFAs in fish can affect the health of consumers. Humans are also unable to synthesize EFAs (Guillou *et al.*, 1995). On the other hand, extracting the lipid content from fish viscera increases the quality of fish meal, because the meal is priced on the basis on the protein content (Yano *et al.*, 2008). Palmitic and oleic fatty acids are the main constituents of Patagonian hake oil. They are useful for formulations based on fish oil because fish use both acids as an energy source during the production of eggs and the spawning time, respectively (Medina *et al.*, 2014). Fish oil is used mainly in the aquaculture industry, but increasingly for human consumption as well, with a better-paying market, particularly for nutraceutical purposes (Italy; Food and Agriculture Organization of the United Nations 2014). Regarding the high n3 PUFA content found in *M. hubbsi* liver oil from Patagonia, it is important to emphasize how beneficial the consumption of foods rich in n3 PUFAs is for human health. The beneficial health effects of the omega-3 fatty acids EPA and DHA include

low rates of coronary heart disease, asthma, type 1 diabetes mellitus, multiple sclerosis, benefits related to cancer, inflammatory bowel disease, rheumatoid arthritis, psoriasis, and mental health. Moreover, the balance of omega-6/omega-3 fatty acids is an important determinant in maintaining homeostasis, normal development, and mental health throughout the human life cycle (Simopoulos 2011a).

#### 4. CONCLUSIONS

*Merluccius hubbsi* liver oil from Chubut has a high nutritional value. The rest of the viscera provide a good protein source. Both features, together with the high volume of capture that already exists at present in Chubut, would justify the development of a Patagonian economy of added-value products. The nutritional constituents present in the fish waste could serve as raw material for the preparation of balanced feed for aquaculture, currently under development in the Chubut province. The extraction of marine fish oil and the production of fish offal meal from the waste from fish factories would contribute to the sustainability of the regional industry because it would also decrease the total waste products, with benefits to the environment.

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