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# Seasonal changes in the chemical and lipid composition of fillets of the Southwest Atlantic hake (*Merluccius hubbsi*)

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The pH, chemical and lipid composition of fillets of the Southwest Atlantic hake (*Merluccius hubbsi*) was studied over the course of a year. Mean values for the chemical composition were (wet basis): total non-volatile nitrogen, 2·87%; water, 79·5%; lipids, 1·5%; ash, 1·20%. Water and lipid content were linearly related ($P < 0.05$). Seasonal changes in the chemical composition were related to the energetic status of the hake, as assessed through the condition factor values. Lipid classes showed seasonal changes. Sterol content varied between 65 and 275 mg% (wet basis). Mean values for eicosapentaenoic and docosahexaenoic acids were 0·09 and 0·35 g% (wet basis). Results are compared to those reported for other hake species. © 1997 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The genus *Merluccius* is composed of a great number of species of economic importance. Hake species have a widespread distribution and occur predominantly in colder waters. The Southwest Atlantic hake (*Merluccius hubbsi*) abounds in the Southwest Atlantic Ocean and along the Río de la Plata.

Several papers have dealt with the chemical composition of hake species. Angel & Baker (1977) studied the chemical composition of muscle of *Merluccius merluccius*. Seasonal changes in the chemical composition were reported for *M. merluccius* (Pérez-Villareal & Howgate, 1987) and *M. hubbsi* (Chiodi, 1963). Data on chemical composition of *Merluccius productus*, *Merluccius capensis*, *M. hubbsi* and *Merluccius senegalensis* were reviewed by Pérez-Villareal & Howgate (1987).

Muscle fatty acid composition has been reported for *Merluccius australis* (Vlieg & Body, 1988), *M. capensis* and *Merluccius paradoxus* (Wessels & Spark, 1973). Kinsella (1987) reviewed literature data on chemical composition for *M. productus* and *M. bilinearis*. To our knowledge, there is no report on the seasonal changes in the fatty acid composition of hake species.

The Southwest Atlantic hake is the most important demersal resource for Uruguay, representing 60% of the annual catch (Instituto Nacional de Pesca, 1993). Most of the catch is used by the filleting industries. Hake fillets are locally consumed and are also exported to Europe, USA and Israel, frozen or processed. Due to the popularity of this species in Uruguay and the importance of this resource for the fishing industry, there is a requirement to establish the composition of *M. hubbsi* and its seasonal variation as background information for the fishing industry in Uruguay.

## MATERIALS AND METHODS

### Sample preparation

Six batches of Southwest Atlantic hakes, each comprising ten fish, were obtained from the Argentinean–Uruguayan common fishing zone in February, March, April, July, September and December 1992. All fish specimens were female, as assessed by macroscopic examination of the gonads (Christiansen & Cousseau, 1971).

Fish samples were maintained in ice until they arrived at the laboratory (2–3 days). The mean length of the specimens was 48 cm (range: 42–54 cm) and the mean weight was 800 g (range: 588–1075 g), corresponding in every case to mature specimens (Simonazzi & Otero, 1986).

From each specimen, a portion of the dorsal muscle was excised, taking out the skin and subdermal fat. The fillets were homogenized in a food processor and stored under a nitrogen atmosphere at −20°C prior to sampling for chemical analysis. The experimental design used followed that of Deng *et al.* (1976), in which the chemical analyses are performed over the composite sample.

## Methods

All the chemical analyses were performed in duplicate (unless otherwise specified) over the medium sample of the ten specimens in each sampling month.

Total lipids were extracted from a 10 g sample, purified by the Folch *et al.* (1957) method and quantified gravimetrically. They were then redissolved in $CHCl_3$/MeOH (2:1, v/v) and stored under a nitrogen atmosphere at −20°C for further analysis. The total non-volatile nitrogen (TN) was determined using a 1 g sample by the Kjeldahl procedure in a Kjelthec System (Tecator, Sweden) (González *et al.*, 1982). Ash was determined as the remnant weight after calcination of a 2 g sample in a muffle furnace at 550°C during 4 h (González *et al.*, 1982). The water content was determined using a 10 g sample by heating at 103°C to constant weight (González *et al.*, 1982). The coefficient of variation for the proximate analysis was less than 1%.

pH measurements were performed by homogenizing a 5 g portion of the muscle in 45 ml of deionized water. Measurements were replicated six times, with a coefficient of variation less than 0·3%.

Lipids were fractionated on silica gel G plates, 0·25 mm thickness (Machery-Nagel, Germany). The solvent mixture, petroleum ether/diethyl ether/acetic acid (80:20:1, v/v/v), was used for thin-layer chromatographic (TLC) development (Christie, 1989). Quantitation was carried out by means of direct densitometric measurements over the plate stained with 10% (w/v) $CuSO_4$/8% (v/v) $H_3PO_4$ (Bitman & Wood, 1982) and heated at 140°C for 20 min. Pure standards (Sigma, USA) and hake liver oil previously characterized (Méndez, 1993) were used for identification. A TLC scanner (Shimadzu CS-9000, Japan) was employed and measurements were made at 500 nm in the reflectance mode. Coefficients of variation for the different lipid classes were less than 2%.

Lipids were saponified with 0·5 N NaOH/MeOH and methylated with 14% $BF_3$/MeOH (AOCS, 1988). Fatty acid methyl esters were recovered in iso-octane and analyzed in a Hewlett-Packard 5840A gas chromatograph equipped with flame-ionization detector and an electronic integrator. A stainless steel column (3 m×3 mm i.d.) packed with 10% 2330 in Supelcoport 100/200 mesh AW (Supelco, USA) was used. Fatty acid methyl esters were identified in isothermic runs at 200°C, with the aid of pure standards (Sigma, USA), hake liver oil previously characterized (Méndez, 1993) and semilogarithmic graphs of retention time relative to stearic acid versus carbon chain length. For the quantitative analysis, a programmed temperature was used: 12 min at 185°C, heated to 230°C at a rate of 2°C $min^{-1}$, and then constant temperature (230°C). Coefficients of variation for this analysis were less than 5% for those fatty acids in percentages greater than 2%.

The condition factor (CF) for each fish was calculated as: CF=1000 (weight of eviscerated fish)/(total length)$^3$.

Correlation matrices and linear regression analyses were performed using MICROSTAT software.

## RESULTS AND DISCUSSION

### Seasonal changes in the chemical composition

The seasonal values of the chemical composition of the Southwest Atlantic hake fillets are summarized in Table 1. Mean values were coincident with those reported for other species of the same genus (*M. productus*, *M. merluccius*, *M. capensis* and *M. senegalensis*), as reviewed by Pérez-Villareal & Howgate (1987).

March and September are hake spawning months (Méndez, 1993). In these periods, TN and lipid contents as well as CF values decreased, due to the use of muscle lipids and proteins as energy reserves. In particular, lipids were almost completely depleted from muscle in September. In Autumn (April), the CF decreased, but the TN and lipid contents increased. During Winter (July), when the hake is not feeding, the TN content and the CF remained at constant values, while lipid content decreased, indicating the use of lipids as fuel during food shortage.

**Table 1. Mean, standard deviation and confidence interval ($P<0·05$) for the proximate analysis, pH and condition factor of hake muscle tissue**

| Month | % TN | % Water | % Lipid | % Ash | pH | CF |
|---|---|---|---|---|---|---|
| February | 2·96 | 76·7 | 3·4 | 1·40 | 6·90 | 7·4 |
| March | 2·51 | 81·2 | 1·1 | 1·40 | nd | 6·9 |
| April | 3·09 | 78·3 | 1·7 | 1·08 | 7·07 | 6·3 |
| July | 2·98 | 78·2 | 1·3 | 1·07 | 7·00 | 6·3 |
| September | 2·78 | 81·6 | Trace | 1·14 | 6·91 | 6·1 |
| December | 2·91 | 81·0 | 1·4 | 1·10 | 6·94 | 7·3 |
| Overall mean | 2·87 | 79·5 | 1·5 | 1·20 | 6·96 | 6·7 |
| Standard deviation | 0·20 | 2·0 | 1·1 | 0·16 | 0·07 | 0·56 |
| Confidence interval[a] | 0·16 | 1·6 | 0·9 | 0·13 | 0·06 | 0·45 |

[a] $P<0·05$.

TN, total non-volatile nitrogen; CF, condition factor; nd, = not determined.

**Table 2. Correlation coefficients for the chemical composition and condition factor of hake muscle tissue**

| | TN | Lipid | Water | Ash |
|---|---|---|---|---|
| Lipid | 0·41546 | | | |
| Water | −0·67784 | −0·82889[a] | | |
| Ash | −0·57895 | 0·46008 | −0·11432 | |
| CF | −0·11063 | 0·67579 | 0·57883 | 0·57883 |

[a]Significant at $P < 0·05$ (critical value: 0·81165).
TN, total non-volatile nitrogen; CF, condition factor.

The highest (absolute) correlation coefficient (Table 2) was that between lipid content and water ($P < 0·05$). The linear regression fitted by the least squares procedure was:

$$\% \text{ lipid} = 37·44 - 0·452(\% \text{ water})$$

This equation allows the estimation of the lipid content from the water content analysis, with a simpler analytical procedure. The standard error of the estimation using this equation is 0·7 %units, less than 1·1, which is the standard deviation for the annual values for lipid content.

Ash content also showed seasonal variations. From March to April (Autumn), the ash content decreased 44% (Table 1), coincidentally with an important drop of the water content. An increase in water content can be observed in March (Table 1) and is in accordance with Love's suggestion (Love, 1980) that the water content must increase to a critical value before minerals can be mobilized and excreted.

The factor to convert TN values into protein content was calculated from the ratio of the overall mean residual solid (calculated as: 100 − % lipid − % ash) to the overall mean TN (Pérez-Villareal & Howgate, 1987). The present data gave a value of 6·20, less than the suggested value of 6·25 (World Health Organization, 1973). The sums of water, lipid, ash and protein content ranged from 99·2% to 101·5%, and the differences between 100 and those sums did not differ significantly from 0 ($P < 0·05$).

### Seasonal changes in muscle pH

Muscle pH showed seasonal changes (Table 1) that can be related to the migratory movement of the hake. The increase in muscle pH is due to the lactate production resulting from liver glycogen mobilization (Love, 1980). After the two spawns (autumn and spring), hake makes important migratory movements, from the Argentinean Patagonian waters to the Río de la Plata (Podestá, 1990). During these periods, food availability becomes reduced, and the hake has to use its glycogen reserves to produce the necessary energy for the muscular effort. This is reflected in the increase of muscular pH in these periods.

Muscular pH increase has technological consequences. High values of pH have been related to the 'gapping' of the muscle (Love, 1980). This phenomenon has not been observed in the samples processed.

### Seasonal changes in the lipid class composition

In Table 3, the seasonal values of lipid class composition are summarized. To our knowledge, no equivalent data have been reported for other hake species. All lipid classes showed seasonal changes that, as expected, followed those of the lipid content. Neutral lipid (triacylglycerols and wax esters) was the predominant lipid class in all the sampling months.

From a nutritional point of view, sterol content is of importance, and ranged from 65 to 275 mg per 100 g muscle (Table 3). An important decrease in the phospholipid content was produced after the autumn spawn, with a quick recovery in the following month. Among the neutral lipids, the main variations were due to the wax ester content of the muscle (Table 3).

### Fatty acid composition

In Table 4, the mean values and annual range of the nutritionally important fatty acids are summarized, along with the fatty acid composition reported for other hake species. Palmitic acid (16:0) and oleic acid (18:1) were the main saturated and monounsaturated fatty acids, respectively. The sum of the eicosapentaenoic fatty acid (EPA, 20:5 $n-3$) and docosahexaenoic fatty acid (DHA, 22:6 $n-3$) percentages accounted for one-third of the total fatty acids. These figures are similar to those reported for *M. productus* (Kinsella, 1987), *M. paradoxus* and *M. capensis* (Wessels & Spark, 1973). In the case of *M. bilinearis* (Kinsella, 1987), although the percentage of polyunsaturated fatty acids (PUFAs) is high, the EPA + DHA content is lower than for the other hake species because of the relatively high percentage of 20:4 $n-3$ in comparison to the other hake species. There is a notably low percentage of PUFAs reported for *M. australis* muscle (Vlieg & Body, 1988),

**Table 3. Seasonal changes in the lipid class composition of hake muscle**

| | February | March | April | July | September | December |
|---|---|---|---|---|---|---|
| Phospholipids (g per 100 g muscle) | 0·75 | 0·10 | 0·35 | 0·20 | nd | 0·18 |
| Sterols (mg per 100 g muscle) | 275 | 65 | 139 | 70 | nd | 83 |
| Neutral lipids[a] (g per 100 g muscle) | 2·38 | 0·93 | 1·18 | 1·03 | nd | 1·05 |

[a]Includes triacylglycerols, wax esters, diacylglycerols and free fatty acids.
nd, no data.

**Table 4. Comparison between the fatty acid composition (wt %) of the Southwest Atlantic hake fillet (mean values and annual ranges in parentheses) and other hake species reported in the literature**

| Fatty acids | *M. hubbsi* | *M. australis*[a] | *M. productus*[b] | *M. bilinearis*[b] | *M. paradoxus*[c] | *M. capensis*[c] |
|---|---|---|---|---|---|---|
| 14:0 | 2·8 (2·3–4·1) | 2·1 | nr | nr | 1·1 | 1·7 |
| 16:0 | 18·0 (16·2–18·9) | 26·6 | nr | nr | 23·3 | 23·6 |
| 18:0 | 3·2 (2·4–3·5) | 5·7 | nr | nr | 3·8 | 4·4 |
| 16:1 | 5·2 (3·9–6·5) | 6·3 | 8·0 | 9·3 | 4·7 | 5·0 |
| 18:1 | 14·9 (12·6–17·3) | 30·0 | 14·0 | 18·2 | 20·6 | 20·5 |
| 20:1 | 4·8 (3·4–6·4) | 9·2 | 2·2 | 3·7 | 4·3 | 4·4 |
| 22:1 | 5·2 (3·9–7·6) | 4·0 | nd | 0·5 | nd | nd |
| 18:2 *n*–6 | 2·0 (1·5–2·2) | 1·1 | 2·4 | 2·3 | 0·6 | 0·7 |
| 18:4 *n*–3 | 2·6 (2·3–2·9) | nr | 1·8 | 2·6 | nr | nr |
| 20:4 *n*–3 | 1·2 (0·8–1·5) | nr | 3·8 | 8·4 | 2·0 | nd |
| 20:5 *n*–3 (EPA) | 6·3 (5·2–7·6) | 2·0 | 18·9 | 10·5 | 6·9 | 6·6 |
| 22:6 *n*–3 (DHA) | 25·7 (15·9–32·3) | 7·0 | 19·6 | 13·5 | 27·8 | 24·0 |

[a]Vlieg & Body (1988).
[b]Kinsella (1987).
[c]Wessels & Spark (1973).
nr, not reported; nd, not detected. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

**Table 5. Seasonal changes of the nutritionally important fatty acids**

| Fatty acids[a] (g per 100 g muscle) | February | March | April | July | September | December |
|---|---|---|---|---|---|---|
| 14:0 | 0·12 | 0·02 | 0·04 | 0·03 | nd | 0·03 |
| 16:0 | 0·52 | 0·18 | 0·25 | 0·18 | nd | 0·22 |
| 18:0 | 0·07 | 0·03 | 0·05 | 0·04 | nd | 0·04 |
| 16:1 | 0·16 | 0·04 | 0·08 | 0·04 | nd | 0·08 |
| 18:1 | 0·46 | 0·12 | 0·18 | 0·16 | nd | 0·21 |
| 20:1 | 0·18 | 0·03 | 0·06 | 0·06 | nd | 0·06 |
| 22:1 | 0·21 | 0·04 | 0·07 | 0·06 | nd | 0·05 |
| 18:2 *n*–6 | 0·06 | 0·01 | 0·03 | 0·02 | nd | 0·03 |
| 18:4 *n*–3 | 0·07 | 0·02 | 0·04 | 0·03 | nd | 0·03 |
| 20:4 *n*–3 | 0·04 | 0·01 | 0·02 | 0·02 | nd | 0·01 |
| 20:5 *n*–3 (EPA) | 0·17 | 0·07 | 0·08 | 0·06 | nd | 0·09 |
| 22:6 *n*–3 (DHA) | 0·45 | 0·31 | 0·38 | 0·30 | nd | 0·32 |

[a]Calculated according to Exler *et al.* (1975).
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; nd, no data.

which is clearly different from the other hake species. For all the hake species, the ratio EPA/DHA is less than 1.

### Seasonal changes in the fatty acid content

In Table 5, seasonal changes in the content of nutritionally important fatty acids are summarized. As expected, due to its highest lipid content, all figures for February showed the highest values. These values had recovered to approximately one-half in December. In the middle months, values were less than these two extremes, having decreased approx. one-third below the February values. The DHA content remained practically constant during the year, in contrast to the EPA content, which followed more or less the same pattern as the other fatty acids. This is in agreement with the fact that long-lived marine fishes tend to accumulate DHA for reserves (Ackman, 1989).

From a nutritional point of view, the EPA + DHA content is of interest because of the incidence of these fatty acids in the therapy and prevention of cardiovascular diseases (Uauy & Valenzuela, 1992). From Table 5, the mean values for EPA and DHA content were 0·09 and 0·35 g per 100 g muscle tissue (wet basis), respectively. This means that, for the recommended daily ingestion of 1 g of EPA + DHA (Ackman, 1989), 230 g of hake fillets are needed. This value is comparable with other fishes from the Río de la Plata and hake roes previously reported (Méndez *et al.*, 1992, 1993, 1996).

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## REFERENCES

Ackman, R. G. (1989). Nutritional composition of fats in seafoods. *Prog. Food Nutr. Sci.*, **13**, 161–241.

Angel, S. & Baker, R. C. (1977). A study of the composition of three popular varieties of fish in Israel, with a view towards further processing. *J. Food Technol.*, **12**, 27–35.

AOCS (1988). *Official Methods and Recommended Practices of the American Oil Chemists' Society*, ed. R. C. Walker. AOCS, Champaign, IL.

Bitman, J. & Wood, D. L. (1982). An improved copper reagent for the quantitative densitometric thin-layer chromatography of lipids. *J. Liquid Chromatogr.*, **5**, 1155–1162.

Chiodi, O. C. (1963). Variaciones estacionales de la composición química de la merluza del Atlántico Sudoccidental. Industrialización. CARPAS Doc. Tco. 4, 43 pp., Montevideo, Uruguay.

Christiansen, H. E. & Cousseau, M. B. (1971). La reproducción de la merluza (*Merluccius hubbsi*) en el Mar Argentino. 2. La reproducción de la merluza y su relación con otros aspectos biológicos de la especie. *Bol. Inst. Biol. Mar. Argent.*, **20**, 43–73.

Christie, W. W. (1989). *Gas Chromatography and Lipids: A Practical Guide*. The Oily Press, Glasgow, UK.

Deng, J. C., Orthoefer, F. T., Dennison, R. A. & Watson, M. (1976). Lipids and fatty acids in mullet (*Mugil cephalus*): seasonal and locational variations. *J. Food Sci.*, **41**, 1479–1483.

Exler, J., Kinsella, J. E. & Watt, B. K. (1975). Lipids and fatty acids of important finfish: new data for nutrient tables. *J. Am. Oil Chem. Soc.*, **52**, 154–159.

Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509.

González, R. M., Inocente, G. & Altamirano, J. (1982). *Métodos de Análisis*. Laboratorio de Análisis Químicos, Departamento de Control de Calidad, Instituto Nacional de Pesca, Ministerio de Ganadería, Agricultura y Pesca, Montevideo, Uruguay.

Instituto Nacional de Pesca (1993). *Boletín Comercial Enero—Diciembre 1992. Compendio Estadístico Pesquero 1975–1992*. Instituto Nacional de Pesca. Ministerio de Ganadería, Agricultura y Pesca, Montevideo, Uruguay, 61 pp.

Kinsella, J. E. (1987). *Seafoods and Fish Oils in Human Health and Disease*. Marcel Dekker, New York, pp. 246–247.

Love, R. M. (1980). *The Chemical Biology of Fishes*, Vol. 2. Academic Press, London, 943 pp.

Méndez, E. (1993). Estudio de los lípidos extraídos de pescados de interés nacional y de sus posibles aplicaciones. MSc Thesis, Universidad de la República, Uruguay, 143 pp.

Méndez, E., Fernández, M., Pazo, G. & Grompone, M. A. (1992). Hake roe lipids: composition and changes following cooking. *Food Chem.*, **45**, 179–181.

Méndez, E., Jachmanián, I. & Grompone, M. A. (1993). Lipid distribution in blackbelly rosefish (*Helicolenus dactylopterus lahillei*) in relation to its possible functions as hydrostatic agent and energy reserve. *Comp. Biochem. Physiol.*, **105B**, 193–198.

Méndez, E., González, R. M., Inocente, G., Giudice, H. & Grompone, M. A. (1996). Lipid content and fatty acid composition of fillets of six fishes from the Río de la Plata. *J. Food Comp. Anal.*, **9**, 163–170.

Pérez-Villareal, B. & Howgate, P. (1987). Composition of European hake, *Merluccius merluccius*. *J. Sci. Food Agric.*, **40**, 347–356.

Podestá, G. P. (1990). Migratory pattern of Argentine hake *Merluccius hubbsi* and oceanic processes in the Southwestern Atlantic Ocean. *Fish. Bull. U.S.*, **88**, 167–177.

Simonazzi, M. A. & Otero, H. O. (1986). Aspectos de la estructura de población de la merluza común (*Merluccius hubbsi*), I. Largo y edad de primer madurez, relación largo-peso. *Publ. Com. Téc. Mix. Fr. Mar.*, **1**, 135–146.

Uauy, R. & Valenzuela, A. (1992). Marine oils as a source of omega-3 fatty acids in the diet: how to optimize the health benefits. *Prog. Food Nutr. Sci.*, **16**, 199–243.

Vlieg, P. & Body, D. R. (1988). Lipid contents and fatty acid composition of some New Zealand freshwater finsfish and marine finfish, shellfish and roes. *N.Z. J. Mar. Freshwater Res.*, **22**, 151–162.

Wessels, J. P. H. & Spark, A. A. (1973). The fatty acid composition of the lipids from two species of hake. *J. Sci. Food Agric.*, **24**, 1359–1370.

World Health Organization (1973). Energy and protein requirements. WHO Technical Report No. 522, WHO, Rome.