Racemization Kinetics of Aspartic Acid in Fish Material under Different Conditions of Moisture, pH, and Oxygen Pressure

Umberto Luzzana,*,† Tiziana Mentasti,† Johannes Opstvedt,‡ Einar Nygård,‡ Vittorio M. Moretti,† and Franco Valfrè†

Università degli Studi di Milano, Facoltà di Medicina Veterinaria, Istituto di Zootecnica, via Trentacoste, 2-20134 Milano, Italy, and Norwegian Herring Oil and Meal Industry Research Institute, Kjerreidviken 16, N-5033 Fyllingsdalen, Norway

The racemization kinetics of aspartic acid in heat-treated whole herring have been studied under conditions of treatment comparable to those that may occur in processing of fish meal. D-Aspartic acid content in the samples has been measured by RP-HPLC with precolumn automatic derivatization. The major parameters affecting the rate of racemization of aspartic acid k_{Asp} have been demonstrated to be temperature (elevation of temperature from 95 to 120 °C resulted in an increase of k_{Asp} from 0.46 to 3.39 $\times$ 10^{-3} min^{-1}), moisture of the raw material (reduction of the moisture content of the raw material from 80 to 15% lowered k_{Asp} measured at 95 °C from 0.46 to 0.06 $\times$ 10^{-3} min^{-1}), and to a lesser extent, pH (k_{Asp} at 95 °C was lowered from 0.46 to 0.37 $\times$ 10^{-3} min^{-1} following a decrease of pH from 7.0 to 4.0). No significant effects on the racemization rate of aspartic acid was observed for reducing the oxygen pressure to 0.8 %. The results from the present study show that the content of D-aspartic acid in fish material is a function of heat exposure and may be used to predict the thermal history of fish meal.

Keywords: *Fish meal; Clupea harengus; D-aspartic acid; racemization kinetics; quality assessment*

INTRODUCTION

Aquaculture is reported to presently consume as a protein source 17% of the global fish meal production, but in 2010 this amount is predicted to increase to 40% (Barlow and Pike, 1998), following the expansion of semi-intensive and intensive farming systems. This is expected to result in further increases of fish meal prices due to increased competition for the use of this limited resource (Rumsey, 1993; Hardy, 1996). This trend for scarcity and consequently higher prices for fish meal will make it increasingly important to efficently process the available resources to products of highest possible quality. Therefore, knowledge of the chemical reactions deteriorating protein quality as a basis to develop improved equipments and processes is needed. Also required are reliable, rapid, and cheap methods to measure protein quality. Fish meal quality is presently evaluated by means of a range of chemical and/or biological methods (FAO, 1986; Hardy, 1989; Pike et al., 1990; NRC, 1993). Since biological tests are time-consuming and expensive, much attention is being paid by the industry to chemical methods, among which the most commonly used are probably the measurement of levels of available lysine or of pepsin digestibility using porcine enzymes. Unfortunately, these methods do not have satisfactory reliability to be used for quality evaluation of commercial fish meal (IFOMA, 1993) being often incapable of distinguishing between a good quality fish meal and a superior quality fish meal (Hardy, 1995).

* To whom correspondence should be addressed (telephone, +39-0226415802; fax, +39-022154671; e-mail, Umberto.Luzzana@unimi.it).

† Università degli Studi di Milano.

‡ Norwegian Herring Oil and Meal Industry Research Institute.

In addition to the above-mentioned parameters, several other chemical methods have also been proposed to evaluate fish meal quality, such as the measurement of the changes in the ratio between disulfide bonds and sulfydryl groups (Opstvedt et al., 1984) and the evaluation of in vitro digestibility using fish pyloric caeca enzymes (Dimes and Haard, 1994; Bassompierre et al., 1997). However, the problem of the availability of rapid, cost-effective, and reliable chemical methods to evaluate fish meal quality is still far from being satisfactorily solved (Hardy, 1995, 1996).

Production of fish meal is done by a wet rendering process during which most of the water and, for fatty fish, also part of the fat found in the fish raw material are removed (Schmidtsdorff, 1995). The process involves the following main steps: (1) heating, which coagulates the protein and facilitate; (2) pressing, which separates the heated fish material into press cake and press liquor; (3) separation of the press liquor into a water phase (stick water) and an oil phase; (4) evaporation of moisture from the stick water; and (5) drying of the press cake and concentrated stick water mixture. Heat is added, and the temperature of the fish is increased in steps 1, 4, and 5. The heat exposure of the fish material depends on temperature level and time of exposure. When the process is properly run, the temperature in the fish material may not exceed 90 °C. However, the temperature of the equipment surfaces and the hot air used in some dryers are usually well above 100 °C. Thus, when the process is not properly run, the fish material may have been subjected to temperatures well above 100 °C for 1 h or more. Recently low-temperature fish meal processing has become common for the production of high-quality fish meals. In this process, the temperature of the fish

10.1021/jf9813438 CCC: $18.00
Published on Web 06/26/1999

material may not exceed 70 °C. To avoid overheating of the fish material, the removal of water is done to various extents under vacuum in fish meal processing. Previous studies have shown that temperature exposure during processing is the single-most important factor determining protein digestibility in fish meal (FAO, 1986; Pike et al., 1990; Schmidtsdorff, 1995; Hardy, 1995, 1996; Goddard, 1996). To avoid deterioration of the fish raw material before processing, the fish may be ensiled by the addition of an organic acid, lowering the pH to 4.0−5.0.

The content of protein-bound D-aspartic acid of fish meal has recently been proven to give indications about the thermal history of the product (Luzzana et al., 1996) and therefore has a potential to be used in evaluating the quality of fish meal. Indeed, the formation of D-amino acids has repeatedly been reported to be correlated with a reduction in protein digestibility (Maruyama et al., 1972; Hayashi and Kameda, 1980a,b; Friedman et al., 1981, 1985; Bunjapamai et al., 1982; Jenkins et al., 1984; Man and Bada, 1987). It has been suggested that the decrease in protein digestibility following racemization of bound amino acids could be explained by the fact that the extended range of the peptide chain around the racemized amino acid residues cannot be used by the substrate binding site of proteases (Hayashi and Kameda, 1980a,b; Chung et al., 1986) or by nonspecifical binding of D-amino acid residues to the active site of peptidases (Friedman et al., 1985), although growth depression in chicks fed free D-amino acids has also been reported (Maruyama et al., 1972; de Moraes et al., 1987) and absorption of intact oligopeptides synthesized from D-amino acids has been observed in rats (Pappenheimer et al., 1994). Whether aspartic acid racemization directly affects protein bioavailability or simply reflects other damages caused by heating such as cross-linking formation, knowledge of racemization kinetics is important. Kinetics of aspartic acid racemization obtained as a function of time, temperature, and acid or alkaline treatment are described for the free amino acid and for several purified and natural proteins (Masters Helfman and Bada, 1976; Masters et al., 1977; Liardon and Jost, 1981; Liardon and Hurrel, 1983; Bada, 1984; Friedman and Liardon, 1985; Liardon and Ledermann, 1986; Liardon et al., 1991), but to our knowledge, no data are available as regards aspartic acid racemization kinetics in fish protein. On the other hand Liardon et al. (1981) reported that the degree of racemization of protein-bound amino acids depends not only on the amino acid but also on its neighbors in the protein chain, as also demonstrated by the fact that different proteins show different rates of racemization of residues (Hayase et al., 1975; Liardon and Hurrel, 1983; Chung et al., 1986; Liardon and Ledermann, 1986). It has also been reported that the presence of lipids may influence amino acid racemization rates (Liardon and Hurrel, 1983). Moreover, the treatments in the above-mentioned kinetics studies are not always comparable with the conditions likely to be observed in fish meal production plants. Therefore, to evaluate the reliability of D-aspartic acid content as an indicator of the severity of thermal treatments during fish meal manufacturing and, consequently, its value as a tool to evaluate fish meal quality, kinetic data must be obtained on whole fish subjected to physical conditions similar to those that may occur in fish meal production.

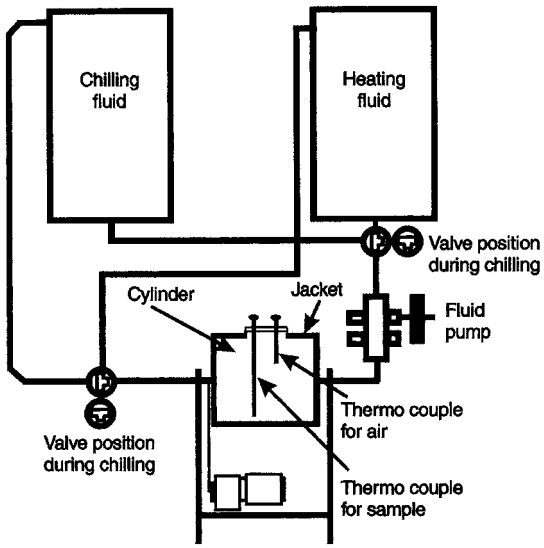

Figure 1. Sketch of the instrument designed for samples treatment.

The objective of the present study was therefore to study the kinetics of aspartic acid racemization in heat-treated whole herring (*Clupea harengus*), evaluating the effects of temperature and time of treatment, oxygen pressure, moisture, and pH of the raw material under processing conditions of practical significance.

MATERIALS AND METHODS

Apparatus and Sample Preparation. The study of the racemization kinetics required the availability of fish samples treated for exact time periods under fixed physical conditions, i.e., temperature, moisture, oxygen pressure, and pH. Thus, special equipment had to be designed that allowed rapid temperature rise and fall within the samples. Moreover, since the water content of the fish material may also affect the formation of D-amino acids, it has been necessary to prevent changes in the moisture content of the samples following heat treatment. Figure 1 shows a sketch of the designed autoclave, which was built by Fish and Powder Technology (Bergen, Norway). Absolutely fresh (total volatile nitrogen less than 15 mg of N/100 g of fish) whole Norwegian spring spawning herring (moisture content about 80%) were coarsely ground and heat-treated under different physical conditions. A control sample not heated in the autoclave was homogenized, added to 300 mg of ethoxyquin/kg of dry matter, and freeze-dried (plate temperature 30 °C). The temperatures chosen for heat treatment (i.e., 70, 95, and 120 °C) were within the range of operational temperatures that may be used in fish meal production plants (FAO, 1986; Schmidtsdorff, 1995). Samples with different moisture contents were prepared by freeze-drying the raw material with plate temperature of 30 °C until the desired moisture content of 15% was achieved. After a time period when the intended moisture content was expected to be achieved based on previous experience, samples were removed from the freeze-drier and weighed, and the moisture content was calculated. When higher than intended moisture content was found, the samples were replaced in the freeze-drier, and the procedure was repeated. To evaluate the effects of reduced oxygen pressure, the autoclave containing the raw material was sealed, the hollow was evacuated for air and flushed with nitrogen in turns for 10 times until oxygen pressure was less than 0.8%, at which point heating started. The oxygen pressure did not change during the heat treatment. To study the effect of pH, the raw material was added to a 30% dilution of formic acid in a meat mincer until the desired pH of 4.0 was reached. The pH of the mixture was monitored in samples with 0.15 M KCl added. Following heat treatment, the samples were then homogenized; ethoxyquin was added

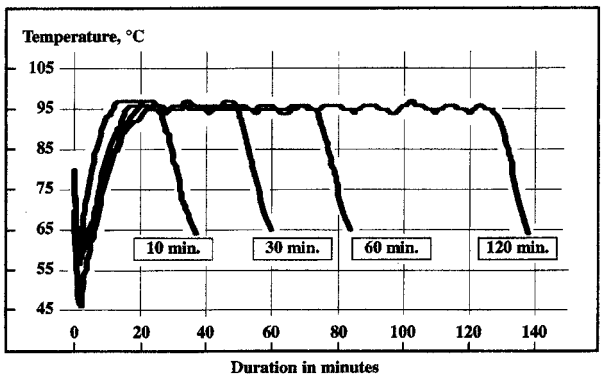

Figure 2. Development of the temperature in a sample of fish material heated under reduced oxygen pressure at 95 °C for 120 min.

as an antioxidant (300 mg/kg dry matter); the samples were placed in stainless steel beakers and frozen at −20 °C pending freeze-drying, which lowered their moisture content to less than 10%. The dried fish material was homogenized and stored under nitrogen in plastic bags, each holding 100 g, until analyzed.

Chemical Analyses. Reagents were of analytical and HPLC grade (Merck, Darmstadt, Germany). TVN (total volatile nitrogen) of raw fish was determined by distillation (AOAC, 1996). For D-aspartic acid analyses, after a 2-h Soxhlet preliminary extraction using 40−60 boiling range light petroleum ether as the solvent, the samples were subjected to a further methanol−chloroform extraction as described in Luzzana et al. (1996). Of each sample, 100 mg was hydrolyzed in 20 mL of 6 N HCl under vacuum at 100 °C for 6 h to reduce the degree of hydrolysis-induced racemization as compared to standard hydrolysis conditions (Masters Helfman and Bada, 1976). The hydrolysate was then filtered on single-use filters (0.45 μm), and the hydrolysis tube was washed with 3 × 1 mL of 0.1 N HCl, which was added to the sample. A 1-mL aliquot was then taken and evaporated to dryness under nitrogen at 40 °C, and the dry residue was redissolved in 5 mL of distilled water. D-Aspartic acid content was determined by RP-HPLC with fluorescence detection (λ_{Ex} = 340 nm, λ_{Em} = 445 nm) after a precolumn automatic derivatization with *o*-phthaldialdehyde (OPA) together with *N*-isobutyryl-D-cysteine (IBDC) according to the method of Brückner et al. (1991). The HPLC system consisted of two Jasco model 980-PU pumps (Ishikawa-cho, Japan), a Jasco model AS-950 autosampler, and a Jasco model 821-FP fluorescence detector. Chromatograms were recorded and integrated on a HP 3365 ChemStation (Hewlett-Packard, Atlanta, GA). The OPA/IBDC reagent was dissolved in potassium borate buffer, pH 10.4 (170 mM OPA and 260 mM IBDC) (Pierce, Rockford, IL). A fresh solution was prepared daily. The reaction buffer was 0.4 M borate buffer, pH 10.4, made by mixing boric acid and sodium hydroxide. The separation was carried out by gradient elution. The eluent was varied linearly from methanol−sodium acetate buffer (40 mM, pH 6.0) (5:95) to methanol−sodium acetate buffer (19:81) over 17.5 min. A 25 cm × 4.6 mm i.d. column packed with Hypersil ODS 5 μm (Shandon HPLC, Astmoor Runcorn, U.K.) was used. The flow rate was 1 mL/min. Fifty microliters of borate buffer, 10 μL of OPA/IBDC reagent, and 20 μL of sample or standard solution were drawn up by the syringe of the autosampler and mixed in a reaction vial. After 2 min, 20 μL of the reaction mixture was injected onto the HPLC system.

Statistical Analysis. The kinetics equations were obtained by linear regression analysis, using the Prism version 2.01 statistical package (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

The designed equipment was proven to allow exact temperature control within the samples, as evident from Figure 2, which shows the continuously recorded temperature in a sample of fish material heated under reduced oxygen pressure at 95 °C for 120 min. Furthermore, the moisture content of the samples remained unchanged during the heat treatment.

Table 1. Inversion Rate Constants (k_{Asp}) and Half-Life Racemization Periods ($t_{1/2}$) for Fish Material Treated under Different Conditions

temp (°C)	moisture (%)	O_2 pressure	pH	k_{Asp} (10^{-3} min^{-1})	$t_{1/2}$ (min)
95	80	normal	7.0	0.46	1510
120	80	normal	7.0	3.39	204
95	15	normal	7.0	0.06	11159
95	80	reduced	7.0	0.43	1613
95	80	normal	4.0	0.37	1848

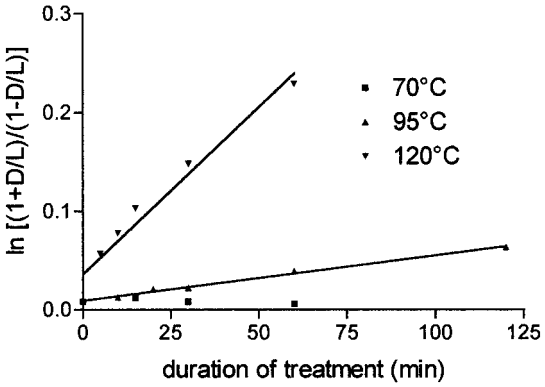

Figure 3. First-order rate plots for the racemization of aspartic acid in fish material at 80% moisture, normal oxygen pressure, and pH 7.0 under different temperatures. The equations for the lines are $Y = 0.00046 + 0.00939X$ ($R^2 = 0.9929$) and $Y = 0.00339 + 0.03578X$ ($R^2 = 0.9557$) for 95 and 120 °C, respectively.

Amino acid inversion follows first-order kinetics and can be considered an irreversible first-order reaction when the extent of racemization in the system is small, i.e., D/L < 0.15 (as is the case in this study); therefore, being described by the equation $\ln[1 + D/L] - \ln[1 - D/L] = kt$ (Bada, 1984; Liardon and Ledermann, 1986; Man and Bada, 1987). First-order rate plots are reported in Figures 3 and 5−7, while the values obtained for inversion rate constants (k_{Asp}) and half-life racemization periods ($t_{1/2}$) are given in Table 1. The samples treated at 70 °C show limited extent of aspartic acid racemization that is not appreciably different from the D-aspartic acid content measured in freeze-dried samples (Figure 3). These figures for D-aspartic acid content (in the range 0.3−0.6% expressed as D/D + L%) therefore have to be considered as a measure of the degree of acid-catalyzed racemization that occurrs during hydrolysis (Masters Helfman and Bada, 1976; Liardon et al., 1981). Interestingly, Pike et al. (1990) reported that small changes in the feeding value of fish meal for Atlantic salmon (*Salmo salar*) occurred with processing temperatures in the range between 70 and 90 °C, but that the value fell more markedly as temperatures rose above 90 °C. The fact that, in this study, no appreciable aspartic acid racemization occurred at 70 °C seems to support the reliability of D-aspartic acid content of fish meal as a quality parameter to evaluate the nutritional value of the product as affected by thermal treatment. It is noticeable that at 120 °C the line interpolating the observed values does not go through the origin of the plot (which is at 0.008364 due to the hydrolysis-induced racemization) (Figure 3). A similar observation about some bound amino acids has also been reported by

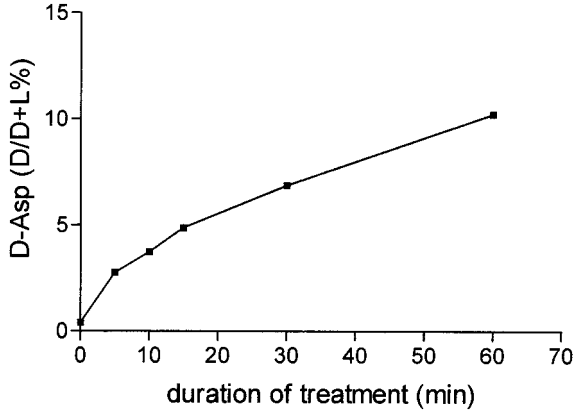

Figure 4. Racemization time course for aspartic acid in fish material treated at 120 °C.

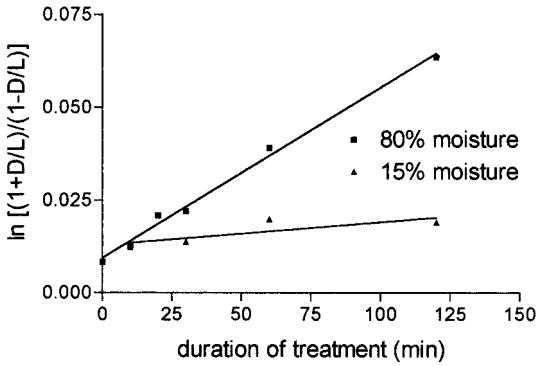

Figure 5. First-order rate plots for the racemization of aspartic acid in fish material treated at 95 °C, normal oxygen pressure, and pH 7.0 under different moisture conditions. The equations for the lines are $Y = 0.000062 + 0.000033X$ ($R^2 = 0.6413$) and $Y = 0.00046 + 0.00939X$ ($R^2 = 0.9929$) for 15% and 80% moisture, respectively.

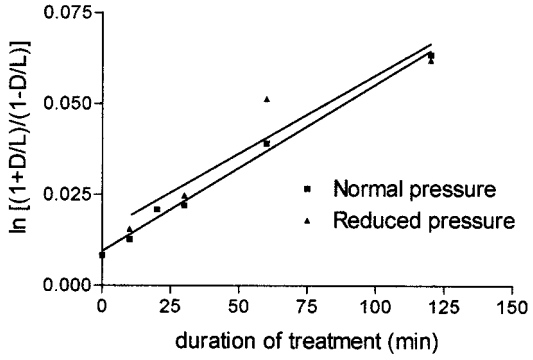

Figure 6. First-order rate plots for the racemization of aspartic acid in fish material treated at 95 °C, 80% moisture, and pH 7.0 under different oxygen pressure conditions. The equations for the lines are $Y = 0.00043 + 0.01479X$ ($R^2 = 0.8915$) and $Y = 0.00046 + 0.00939X$ ($R^2 = 0.9929$) for reduced (<0.8%) and normal oxygen pressure, respectively.

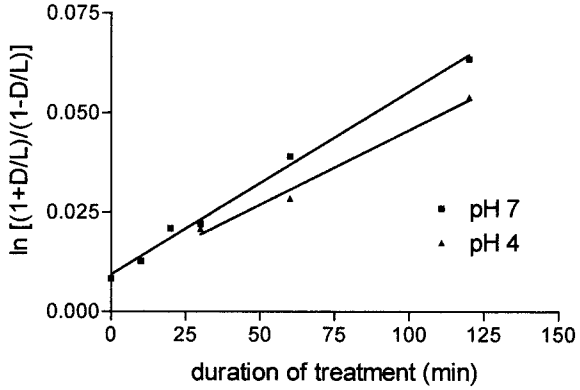

Figure 7. First-order rate plots for the racemization of aspartic acid in fish material treated at 95 °C, 80% moisture, and normal oxygen pressure under different pH conditions. The equations for the lines are $Y = 0.00037 + 0.00814X$ ($R^2 = 0.9878$) and $Y = 0.00046 + 0.00939X$ ($R^2 = 0.9929$) for pH 4.0 and pH 7.0, respectively.

Liardon and Jost (1981). Following prolonged heat treatments, a decrease of the racemization rate is usually evident, presumably due to protein denaturation (Friedman and Liardon, 1985; Liardon and Ledermann, 1986), and this may also be the case for severe heating, as seems to be indicated by the results shown in Figure 4. However, since the change in racemization rate seems to occurr already after a 5-min treatment at 120 °C and no data points were available between 0 and 5 min, it has been considered prudent to calculate the k_{Asp} by linear regression on the complete data set, especially when taking into account the high R^2 value (0.9557). Among the different chemical and physical parameters evaluated as affecting D-aspartic acid formation, temperature of treatment, and moisture content of the raw material appear to have the strongest effects on the extent of aspartic acid racemization (Table 1, Figures 3 and 5). Elevation of temperature from 95 to 120 °C resulted in an increase of k_{Asp} from 0.46 to 3.39 $\times$ 10^{-3} min^{-1} (Table 1), supporting our previous observations that D-aspartic acid content of fish meal can give indications about the thermal history of the product (Luzzana et al., 1996). Reduction of the moisture content of the raw material from 80 to 15% greatly reduced the extent of aspartic acid racemization following thermal treatment at 95 °C, as expressed by the lowering of k_{Asp} from 0.46 to 0.06 $\times$ 10^{-3} min^{-1} (Table 1). The extent of racemization in the samples with reduced moisture content did not exceed 1% (expressed as D/D + L%), quite close to the values measured in freeze-dried samples, confirming that water activity in the raw material is critical in allowing racemization to occurr (Liardon and Ledermann, 1986). A less dramatic reduction of racemization rate followed pH decrease in the raw material, lowering of k_{Asp} from 0.46 to 0.37 $\times$ 10^{-3} min^{-1} following a decrease of pH from 7.0 to 4.0 (Table 1), which was expected since it is well-known that amino acid racemization proceeds most rapidly at basic pH where it can occurr even at moderate temperatures of less than 80 °C (Bunjamapai et al., 1982; Liardon and Ledermann, 1986). From a practical point of view, it would be interesting to evaluate if acidification of the raw material before thermal treatment during fish meal production also leads, parallel to a reduction in D-aspartic acid content, to a product with higher protein quality, which could be expected from the reported decreased digestibility of alkali-treated proteins (Friedman et al., 1981, 1985; Jenkins et al., 1984; Chung et al., 1986). Reduced oxygen pressure did not appear to induce major changes in aspartic acid inversion rate constants, which was only reduced from 0.46 to 0.43 $\times$ 10^{-3} min^{-1} following a reduction of oxygen pressure in the autoclave to less than 0.8% (Table 1). This is accordance with the results of Hayase et al. (1972), who reported that atmospheric oxygen takes no part in the dissociation of an α-hydrogen atom attached to the peptide linkage during amino acid racemization.

In conclusion, the results from the present study support the reliability of D-aspartic acid content of fish

meal as a tool to predict the heat exposure to which a fish meal has been subjected in processing (within the limits studied in the present experiment). Processing temperature and moisture content of the raw material are the major factors affecting the rate of racemization of aspartic acid in fish meal, followed by pH. No significant effects of oxygen pressure on the inversion rate has to be expected on the other hand. If a correlation between the D-aspartic acid content of fish meals and their feeding value for farmed fish is confirmed by ongoing studies, the results from the present study may be utilized as an aid in the process control of fish meal production. Thus, the combined effect of temperature and time would focus the attention on processing units that operate at low temperatures or at higher temperatures only for limited periods of time. Furthermore, the effect of moisture indicates that the focus on heat exposure should be directed to stages in the process where the fish material has higher moisture contents and the heat damage appears to be more evident. The positive effect of reduced pH should create interest in using fish silage as a raw material for fish meal production. Finally, the modest effect of a considerable reduction of oxygen pressure reduction is not encouraging with regard to protein deterioration from D-aspartic acid for processes using reduced oxygen pressure.

ACKNOWLEDGMENT

The assistance of Prof. Armando Negri is gratefully acknowledged.

LITERATURE CITED

AOAC. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC International: Gaithersburg, MD, 1996.

Bada, J. L. *In Vivo* Racemization in Mammalian Proteins. *Methods Enzymol.* **1984**, *106*, 97−115.

Barlow, S. M.; Pike, I. H. Aquaculture Feed Ingredients in Year 2010: Fish Meal and Fish Oil. Presented at Aquavision 98−Second Nutreco Aquaculture Business Conference, Stavanger, Norway; May 1998.

Bassompierre, M.; Børresen, T.; Sandfeld, P.; Rønsholdt, B.; Zimmermann, W.; McLean, E. An evaluation of open and closed systems for *in vitro* protein digestion of fish meal. *Aquacult. Nutr.* **1997**, *3*, 153−159.

Brückner, H.; Wittner, R.; Godel, H. Fully Automated High-Performance Liquid Chromatographic Deparation of DL-Amino Acids Derivatized with *o*-Phthaldialdehyde together with *N*-Isobutyryl-Cysteine. Application to Food Samples. *Chromatographia* **1991**, *32*, 383−388.

Bunjapamai, S.; Mahoney, R. R.; Fagerson, I. S. Determination of D-Amino Acids in Some Processed Foods and Effect of Racemization on *In Vitro* Digestibility of Casein. *J. Food Sci.* **1982**, *47*, 1229−1234.

Chung, S.-Y.; Swaisgood, H. E.; Catignani, G. L. Effects of Alkali Treatment and Heat Treatment in the Presence of Fructose on Digestibility of Food Proteins as Determined by an Immobilized Digestive Enzyme Assay (IDEA). *J. Agric. Food Chem.* **1986**, *34*, 579−584.

De Moraes, G. H. K.; Rogler, J. C.; Featherston, W. R. Effects of D-Amino Acids on Growth Rate and Kidney D-Amino Acid Oxidase in Chicks. *Poult. Sci.* **1987**, *66*, 98−102.

Dimes, L. E.; Haard, N. F. Estimation of protein digestibility−I. Development of an *in vitro* method of estimating protein digestibility of salmonids (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **1994**, *108A*, 349−362.

FAO. *The production of fish meal and oil;* FAO Fisheries Technical Paper No. 142, Rev. 1; Fishery Industries Division of the FAO: Rome, Italy, 1986.

Friedman, M.; Liardon, R. Racemization Kinetics of Amino Acid Residues in Alkali-Treated Soybean Proteins. *J. Agric. Food Chem.* **1985**, *33*, 666−672.

Friedman, M.; Zahnley, J. C.; Masters, P. M. Relationship between In Vitro Digestibility of Casein and its Content of Lysinoalanine and D-Amino Acids. *J. Food Sci.* **1981**, *46*, 127−131, 134.

Friedman, M.; Grosjean, O.-K.; Zahnley, J. C. Carboxypeptidase Inhibition by Alkali-Treated Food Proteins. *J. Agric. Food Chem.* **1985**, *33*, 208−213.

Goddard, S. *Feed Management in Intensive Aquaculture;* Chapman & Hall: New York, 1996.

Hardy, R. W. Diet Preparation. In *Fish Nutrition*, 2nd ed.; Halver, J. E., Ed.; Academic Press: San Diego, CA, 1989; pp 475−548.

Hardy, R. W. Current Issues in Salmonid Nutrition. In *Nutrition and Utilization Technology in Aquaculture*; Lim, C. E., Sessa, D. J., Eds.; AOCS Press: Champaign, IL, 1995; pp 26−35.

Hardy, R. W. Alternate protein sources for salmon and trout diets. *Anim. Feed Sci. Technol.* **1996**, *59*, 71−80.

Hayase, F.; Kato, H.; Fujimaki, M. Racemization of Amino Acid Residues in Proteins during Roasting. *Agric. Biol. Chem.* **1972**, *37*, 191−192.

Hayase, F.; Kato, H.; Fujimaki, M. Racemization of Amino Acid Residues in Proteins and Poly(L-amino acids) during Roasting. *J. Agric. Food Chem.* **1975**, *23*, 491−494.

Hayashi, R.; Kameda, I. Racemization of Amino Acid Residues during Alkali-Treatment of Protein and Its Adverse Effect on Pepsin Digestibility. *Agric. Biol. Chem.* **1980a**, *44*, 891−895.

Hayashi, R.; Kameda, I. Decreased Proteolysis of Alkali-Treated Protein: Consequences of Racemization in Food Processing. *J. Food Sci.* **1980b**, *45*, 1430−1431.

IFOMA. *Tests to predict the quality of fish meals for special uses*; IFOMA Research Report 1993-1; IFOMA: St. Albans, Hertfordshire, U.K., 1993.

Jenkins, W. L.; Tovar, L. R.; Schwass, D. E.; Liardon, R.; Carpenter, K. J. Nutritional Characteristics of Alkali-Treated Zein. *J. Agric. Food Chem.* **1984**, *32*, 1035−1041.

Liardon, R.; Hurrell, R. F. Amino Acid Racemization in Heated and Alkali-Treated Proteins. *J. Agric. Food Chem.* **1983**, *31*, 432−437.

Liardon, R.; Jost, R. Racemization of free and protein-bound amino acids in strong mineral acid. *Int. J. Peptide Protein Res.* **1981**, *18*, 500−505.

Liardon, R.; Ledermann, S. Racemization Kinetics of Free and Protein-Bound Amino Acids under Moderate Alkaline Treatment. *J. Agric. Food Chem.* **1986**, *34*, 557−565.

Liardon, R.; Ledermann, S.; Ott, U. Determination of D-amino acids by deuterium labeling and selected ion monitoring. *J. Chromatogr.* **1981**, *203*, 385−395.

Liardon, R.; Friedman, M.; Philipposian, G. Racemization Kinetics of Free and Protein-Bound Lysinoalanine (LAL) in Strong Acid Media. Isomeric Composition of Bound LAL in Processed Proteins. *J. Agric. Food Chem.* **1991**, *39*, 531−537.

Luzzana, U.; Mentasti, T.; Moretti, V. M.; Albertini, A.; Valfrè, F. Aspartic acid racemization in fish meal as induced by thermal treatment. *Aquacult. Nutr.* **1996**, *2*, 95−99.

Man, E. H.; Bada, J. L. Dietary D-amino acids. *Annu. Rev. Nutr.* **1987**, *7*, 209−225.

Maruyama, K.; Sunde, M. L.; Harper, A. E. Effect of D-Alanine and D-Aspartic Acid on the Chick. *J. Nutr.* **1972**, *102*, 1441−1452.

Masters, P. M.; Bada, J. L.; Zigler, J. S., Jr. Aspartic acid racemisation in the human lens during ageing and in cataract formation. *Nature* **1977**, *268*, 71−73.

Masters Helfman, P.; Bada, J. L. Aspartic acid racemisation in dentine as a measure of ageing. *Nature* **1976**, *262*, 279−281.

National Research Council. *Nutrient Requirements of Fish;* National Academy Press: Washington, DC, 1993.

Opstvedt, J.; Miller, R.; Hardy, R. W.; Spinelli, J. Heat-induced changes in sulphydryl groups and disulfide bonds in fish

protein and their effect on protein and amino acid digestibilty in rainbow trout. *J. Agric. Food Chem.* **1984**, *32*, 929−935.

Pappenheimer, J. R.; Dahl, C. E.; Karnowski, M. L.; Maggio, J. E. Intestinal absorption and excretion of octapeptides composed of D amino acids. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1942−1945.

Pike, I. H.; Andorsdóttir, G.; Mundheim, H. *The role of fish meals in diets for salmonids;* IAFMM Technical Bulletin No. 24; International Association of Fish Meal Manufacturers: Potters Bar, U.K., 1990.

Rumsey, G. L. Fish meal and alternate sources of protein in fish feeds; update 1993. *Fisheries* **1993**, *18*, 14−19.

Schmidtsdorff, W. Fish Meal and Fish Oil−Not Only By-products. In *Fish and Fishery Products*; Ruiter, A., Ed.; CAB International: Wallingford, Oxon, U.K., 1995; pp 347−376.

Received for review December 14, 1998. Revised manuscript received May 3, 1999. Accepted May 10, 1999. The study has been carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD program CT96-1329, "Effect of processing technology on the quality of aquaculture feeds". The paper does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

JF9813438