Schricker, B. R.; Miller, D. D.; Stouffer, J. R. Measurement and
Content of Non-Heme and Total Iron in Muscle. *J. Food.
Sci.* 1982, 47, 740–743.

Scott, M. L.; Nesheim, M. C.; Young, R. J. *Nutrition of the
Chicken*; ML Scott and Associates: Ithaca, NY, 1976.

Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 6th ed.;
Iowa State University Press: Ames, IA, 1967.

Wilson, B. R.; Pearson, A. M.; Shorland, F. B. Effect of Total
Lipids and Phospholipids on Warmed-Over Flavor in Red
and White Muscle from Several Species as Measured by the
Thiobarbituric Acid Analysis. *J. Agric. Food Chem.* 1976,
24, 7–11.

Witte, V. C.; Krause, G. F.; Bailey, M. E. A New Extraction
Method for Determining 2-Thiobarbituric Acid Value of Pork
and Beef during Storage. *J. Food Sci.* 1970, 35, 582–586.

Characterization of Hake (*Merluccius merluccius* L.) and Trout (*Salmo irideus* Gibb) Collagen

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An analysis of the different types of collagen present in various body structures, the amino acid composition of the collagen, and the degree of collagen aggregation for hake and trout is presented. Type I collagen was found at all body sites, and its amino acid profile and degree of hydroxylation are considered. Hydroxyproline values, the ratio of hydroxyproline to the other amino acids, and the proportions of alanine, tyrosine, and methionine are also discussed. Values for collagen solubility in salt and acid indicated a lower insolubility rate for skin collagen than for muscle collagen. The proportions of the α, β, and γ components in the acid-soluble fractions of skin and muscle collagen are also examined; the proportion of γ components is lower in hake muscle collagen than in hake skin collagen, whereas the converse holds true in trout.

The study of such biochemical characteristics of fish collagen as amino acid composition, collagen types, cross-linking, and the like is an essential basis for further investigation into technical aspects of more widespread industrial applications for collagen.

The characteristics of collagen at different body sites in food animals and other higher vertebrates have been studied in some detail (Bailey and Sims, 1977; Sims and Bailey, 1981; Bailey et al., 1984). However, except for gaping, which has received considerable attention (Love, 1970; Love and Haq, 1970; Love and Lavety, 1972), only a few studies have dealt with collagen in fish and other marine organisms (Yamaguchi et al., 1976; Sikorski et al., 1984). Published results concerning collagen types in fish are limited (Bogason, 1984; Almas, 1986), and we were unable to discover in the literature any references to collagen types according to the anatomical location of the connective tissue.

The object of the present study was to ascertain the biochemical characteristics of fish collagen in order to increase our understanding of this protein and thus lay the groundwork for future research into industrial technology and uses for the functional properties of fish collagen. To this end, collagen types, amino acid composition, and degree of aggregation in the skin and in the connective tissue in various muscle structures were determined for two different fish species.

MATERIALS AND METHODS

Hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb) were the species used. The hake were caught by long-lining on the continental shelf off Galicia, Spain, in March; total sample weight was 20.1 kg. The trout were reared on a fish farm and hence were all similar in size, and total sample weight was 17.7 kg. Mean individual weight was 2.1 kg for the hake and 1.7 kg for the trout; mean length was 65 cm for the hake and 45 cm for the trout. Specimens were kept refrigerated from the time of capture until use at the laboratory some 24 h later.

Extraction of Connective Tissue. Specimens were headed, gutted, and filleted, and the fillets were skinned.

Before the collagen or connective tissue was separated from the dermis, any remnants of muscle tissue or fat adhering to the dermis or epidermis were eliminated by hand.

Connective tissue was removed from the following sites: the fasciae surrounding the fillet; the myocommata separating the myotomes; the interior of the myotomes.
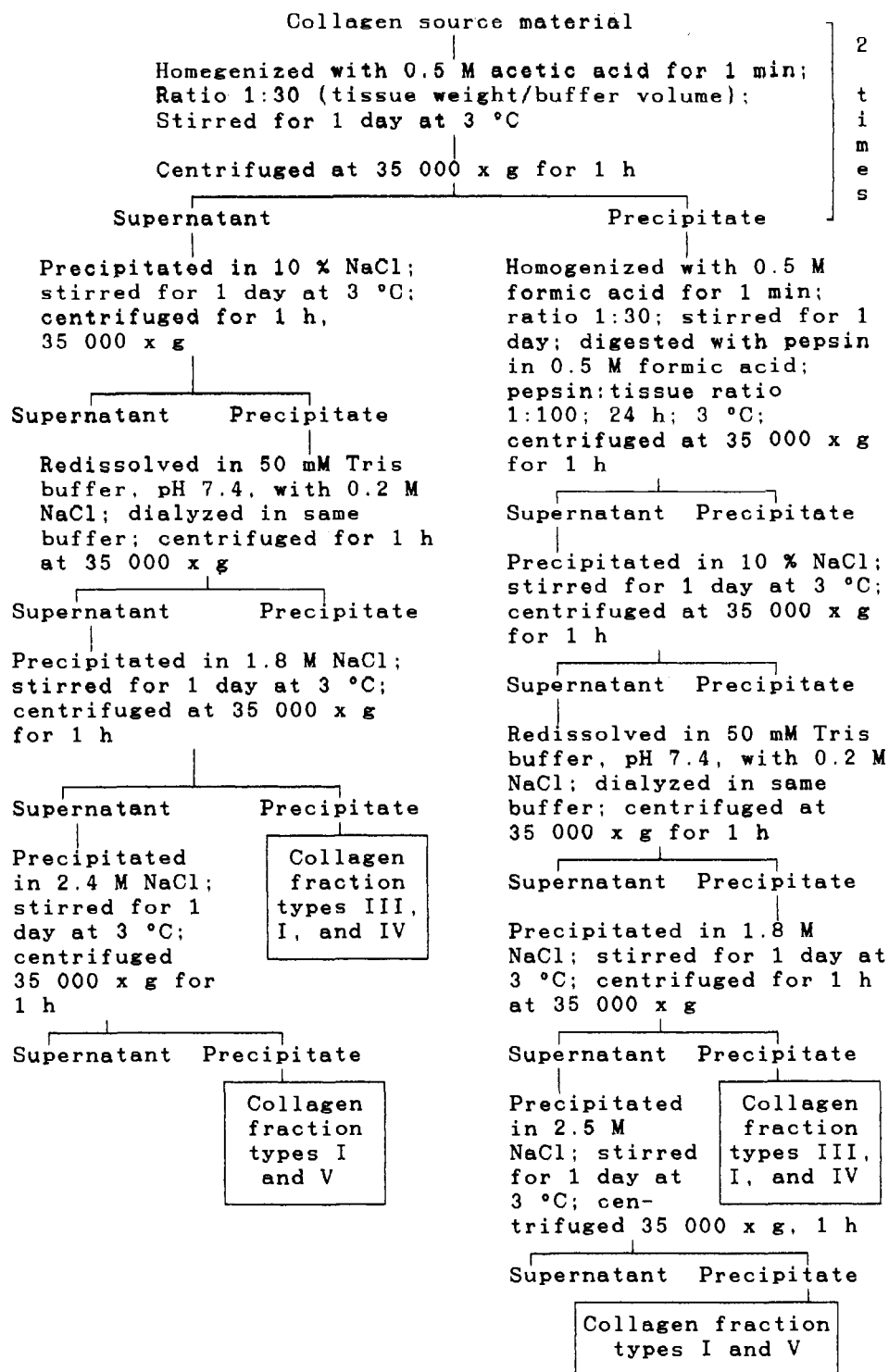
The connective tissue was separated out by dissection with a surgical knife. The types of collagen present in each body structure were then determined.

Separation and Purification of the Connective Tissue. The connective tissue was separated and purified by the method described by Borderías and Montero (1985).

Purification and Fractionation of the Different Collagen Types. A modified version of the fractionation method of Timpl et al. (1975) was used, as illustrated in Figure 1.

Electrophoresis on Polyacrylamide Gel Containing Sodium Dodecyl Sulfate. Electrophoresis on polyacryla-

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                        Collagen source material
                                   |
                  Homogenized with 0.5 M acetic acid for 1 min;     2
                  Ratio 1:30 (tissue weight/buffer volume);         t
                  Stirred for 1 day at 3 °C                         i
                                   |                                 m
                  Centrifuged at 35 000 x g for 1 h                 e
                    _____|_____             s
                   |                                   |
               Supernatant                         Precipitate
                   |                                   |
         Precipitated in 10 % NaCl;        Homogenized with 0.5 M
         stirred for 1 day at 3 °C;        formic acid for 1 min;
         centrifuged for 1 h,              ratio 1:30; stirred for 1
         35 000 x g                        day; digested with pepsin
                   |                        in 0.5 M formic acid;
          _____|_____                pepsin:tissue ratio
         |                  |               1:100; 24 h; 3 °C;
     Supernatant       Precipitate          centrifuged at 35 000 x g
                           |                 for 1 h
              Redissolved in 50 mM Tris          ____|_____
              buffer, pH 7.4, with 0.2 M        |          |
              NaCl; dialyzed in same        Supernatant  Precipitate
              buffer; centrifuged for 1 h       |
              at 35 000 x g                  Precipitated in 10 % NaCl;
          _____|_____                  stirred for 1 day at 3 °C;
         |                 |                  centrifuged at 35 000 x g
     Supernatant      Precipitate            for 1 h
         |                                        ____|_____
   Precipitated in 1.8 M NaCl;               |          |
   stirred for 1 day at 3 °C;            Supernatant  Precipitate
   centrifuged at 35 000 x g                 |
   for 1 h                               Redissolved in 50 mM Tris
         |                                buffer, pH 7.4, with 0.2 M
    _____|_____                     NaCl; dialyzed in same
   |                 |                     buffer; centrifuged at
 Supernatant     Precipitate              35 000 x g for 1 h
   |            _____                    ____|_____
 Precipitated  | Collagen  |             |          |
 in 2.4 M NaCl;| fraction  |         Supernatant  Precipitate
 stirred for 1 | types III,|             |
 day at 3 °C;  | I, and IV |         Precipitated in 1.8 M
 centrifuged   |_____|         NaCl; stirred for 1 day at
 35 000 x g for                      3 °C; centrifuged for 1 h
 1 h                                 at 35 000 x g
    ____|_____                         ____|_____
   |           |                     |          |
 Supernatant Precipitate         Supernatant  Precipitate
            _____              |        _____
           | Collagen  |        Precipitated | Collagen  |
           | fraction  |        in 2.5 M     | fraction  |
           | types I   |        NaCl; stirred| types III,|
           | and V     |        for 1 day at | I, and IV |
           |_____|        3 °C; cen-   |_____|
                                trifuged 35 000 x g, 1 h
                                    ____|_____
                                   |          |
                               Supernatant  Precipitate
                                          _____
                                         | Collagen fraction |
                                         | types I and V     |
                                         |_____|
```

Figure 1. Diagram of the fractionation and purification procedures for the different collagen types.

mide gel containing sodium dodecyl sulfate (SDS) was based on the method of Laemmli (1970).

The gels were prepared as 1-mm-thick slabs with an acrylamide concentration of 7%, an acrylamide to bisacrylamide ratio of 38:1, and 3% SDS (w/v) at pH 8.8 in 0.4 M Tris buffer. The upper or stacking gel layer contained 4% acrylamide and was made at pH 6.8 in 0.12 M Tris buffer.

A total of 40 μg of sample was dissolved in 100 μg of 0.065 M Tris buffer containing 3% SDS (w/v) and 0.8 M urea at pH 6.8. Samples were also reduced by adding 5% β-mercaptoethanol.

Electrophoresis was carried out in 0.025 M Tris buffer, 0.192 M glycine, and 0.1% SDS at pH 8.3. Staining with 0.02% bromophenol blue (w/v) was carried out to visualize migration.

During electrophoresis a current of 30 mA was applied until the front was 5 mm from the edge of the gel.

The gels were then stained by applying a solution of 1.0% Coomassie blue, 45% methanol, 9% acetic acid, and 45% water for 15 min at ambient temperature. They were destained in a solution of 45.5% methanol, 4.5% acetic acid, and 50% water. Two replications of all determinations were performed.

Amino Acid Analysis. A Durrum D500 autoanalyzer was used according to the method of Gavilanes et al. (1984), except for the hydroxyproline determinations, which were carried out by the method of Leach (1960).

Collagen Solubility. The method of fractionation in different solutions was a modified version of the technique of Timpl et al. (1975), as described by Borderías and Montero (1985). Two replications of each extraction were performed.

Molecular Sieve Chromatography. The chromatographic method used was based on the technique of Chandrakasan et al. (1976) and Krieg et al. (1981), as modified by

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Table I. Yield of Connective Tissue (%, w/w) from Skin and Muscle in Hake and Trout[a]

connective tissue	hake	trout
skin	34.2 ± 2.0	48.8 ± 2.3
muscle	1.7 ± 0.1	1.4 ± 0.1

[a] Determinations were performed on 10 individuals.

Table II. Yield of Connective Tissue at Various Sites in the Muscle of Hake and Trout (%, w/w; Connective Tissue/Muscle)[a]

connective tissue	hake	trout
fasciae	37 ± 3.4	30 ± 3.1
myocommata	32 ● 5.0	33 ± 2.8
myotomes	31 ± 2.9	37 ± 4.0

[a] Determinations were performed on 10 individuals.

Borderías and Montero (1985). The results presented herein are the means of two determinations.

Statistical Analysis. Standard deviation was calculated for yield of connective tissue for skin and muscle, amount of hydroxyproline in the collagen, collagen solubility, and the proportions of the α, β, and γ components in the acid-soluble collagen fraction. Two-way analysis of variance using Tukey's test was applied to determine the degree of significance for the differences between the means for collagen solubility and for the proportions of the α, β, and γ components in the acid-soluble collagen fraction.

RESULTS AND DISCUSSION

Table I presents connective tissue yields from skin and muscle in the hake and the trout. The amount of connective tissue in the skin was 34.2% in the hake and 48.8% in the trout; these values were much higher than the amounts of connective tissue in the muscle, which were comparable for the two species. The data agreed with those reported by Jacquot (1961).

The values of Table II show the proportion of connective tissue in the muscle to be similar in the fasciae, myocommata, and myotomes.

Fractionated precipitation was carried out employing differing salt concentrations at pH 7.4. Complete precipitation of type III collagen was achieved at a sodium chloride concentration of 1.8 M, while type I collagen remained mostly soluble at that same salt concentration, finally precipitating at an NaCl concentration of 2.5 M. In the main, only a single collagen type was detectable in the electrophoretic analyses of the collagen from the various salt fractionation stages. The fraction precipitating at the NaCl concentration of 1.8 M yielded a small amount of precipitate that consisted of type I collagen and was devoid of type III collagen, inasmuch as the electrophoretic diagram for the samples reduced with β-mercaptoethanol was similar to that for the unreduced samples, indicating the absence of the disulfide bonds characteristic of type III collagen (Figures 2 and 3). Similar results were obtained for all the samples tested, indicating that the collagen in the skin, fasciae, myocommata, and myotomes of both the hake and the trout was type I. Other workers, e.g., Bogason (1984) and Almas (1986), also recorded type I collagen exclusively in connective tissue from rockfish (*Sebastes spp.*) and cod (*Gadus morhua*) muscle analyzed without regard to location in the muscle tissue. Type I collagen exclusively was also reported from lobster (*Palinurus spp.*) muscle (Kimura and Tanaka, 1986) and grouper (*Epinephelus spp.*) muscle (Kimura et al., 1983) and from dogshark (*Squalus spp.*) skin (Lewis and Piez, 1964) and octopus (*Octopus spp.*) skin (Takema and Kimura, 1982). In octopus mantle a majority of type I collagen, along with smaller
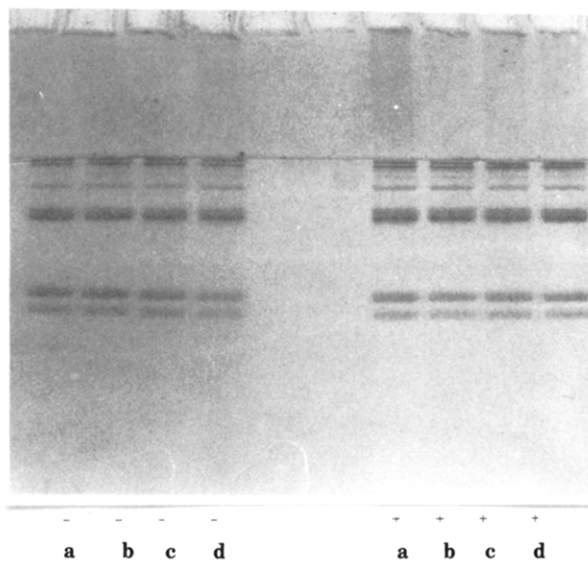
Figure 2. Electrophoresis of hake collagens from skin (a), fasciae (b), myocommata (c), and myotomes (d) on 7% polyacrylamide gel containing sodium dodecyl sulfate and with (+) or without (−) β-mercaptoethanol.
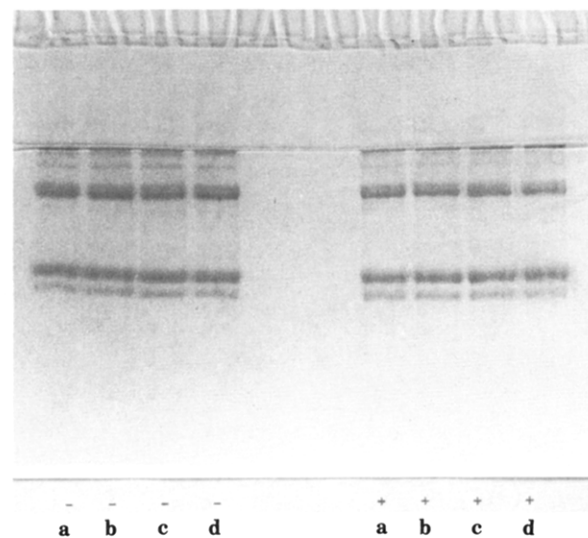
Figure 3. Electrophoresis of trout collagens from skin (a), fasciae (b), myocommata (c), and myotomes (d) on 7% polyacrylamide gel containing sodium dodecyl sulfate and with (+) or without (−) β-mercaptoethanol.

amounts of type III collagen, was found (Takema and Kimura, 1982). This is also a characteristic of food animals and other higher vertebrates, in which, although both type I and type III collagen are present in the skin and type I, type III, and type V collagen are present in the muscle, type I collagen predominates in both tissues (Sims and Bailey, 1981; Light and Champion, 1984).

The amino acid composition of the collagens from the skin, fasciae, myocommata, and myotomes is given in Table III for the hake and Table IV for the trout. Glycine accounted for approximately one-third of the amino acid residues, in line with the structural characteristics of collagen, in which glycine frequencies of one of every three residues have been reported (Ramachandran and Kartha, 1955; Rich and Crick, 1955; Ramachandran and Ramakrishnan, 1976). In certain instances, e.g., the collagen from the skin and myocommata in the hake, the values were somewhat lower.

The content of the amino acids proline and hydroxyproline was 16–21% in the hake and 15–18% in the trout.

Hake and Trout Collagen

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Table III. Amino Acid Composition (Number of Residues/1000 Amino Acid Residues) of the Collagen Fractions Extracted from the Skin, Fasciae, Myocommata, and Myotomes in Hake (Estimated Error 5%)

amino acid	skin	fasciae	myocommata	myotomes
Hyp	69	62	103	61
Asx	56	58	54	49
Thr	30	33	29	28
Ser	50	51	46	41
Glx	74	77	69	67
Pro	122	114	108	98
Gly	255	314	265	334
Ala	147	100	136	139
Val	23	26	23	23
Met	16	17	16	14
Ile	11	12	11	12
Leu	20	22	20	23
Tyr	4	4	4	5
Phe	16	16	15	14
His	9	11	9	9
Hyl	4	5	4	4
Lys	31	28	30	29
Arg	63	50	58	50

Table IV. Amino Acid Composition (Number of Residues/1000 Amino Acid Residues) of the Collagen Fractions Extracted from the Skin, Fasciae, Myocommata, and Myotomes in Trout (Estimated Error 5%)

amino acid	skin	fasciae	myocommata	myotomes
Hyp	70	49	61	59
Asx	75	61	64	26
Thr	25	28	29	46
Ser	43	52	53	75
Glx	71	79	83	74
Pro	110	117	95	105
Gly	313	309	290	313
Ala	113	104	118	111
Val	20	22	24	18
Met	14	16	15	15
Ile	12	13	15	11
Leu	22	24	26	22
Tyr	3	3	6	3
Phe	13	15	16	13
His	9	10	10	10
Hyl	7	9	11	9
Lys	26	27	23	24
Arg	54	62	59	65

It should be noted that the hydroxyproline content in both skin and muscle collagen is considerably lower in fish than in food animals and other vertebrates, around 40–80 residues/100 amino acid residues in fish as compared to 100–130 residues/100 amino acid residues in meat (McClain et al., 1970; Sikorski et al., 1984).

The values for hydroxyproline in both the hake and the trout were similar to those in some other fish species, e.g., cod, hake, dogsharks, and rockfish (Lewis and Piez, 1964; Yamaguchi et al., 1976; Bogason, 1984). Somewhat higher values have been reported for other aquatic species like carp and octopus (Kubota and Kimura, 1975; Takema and Kimura, 1982; Kimura et al., 1981).

The ratio between hydroxyproline and the other amino acids yielded hydroxyproline-based conversion factors for both muscle and skin collagen. Factor values were 14.4 for skin collagen and 13.3 for muscle collagen in the hake and 14.2 for skin collagen and 17.8 for muscle collagen in the trout.

The proportion of alanine in trout collagen, approximately 11% (Table IV), agreed with the values reported by other investigators (Eastoe and Leach, 1958; Piez and Gross, 1960; Lewis and Piez, 1964; Yamaguchi et al., 1976; Sikorski et al., 1984; Bogason, 1984). The values for alanine were, however, somewhat higher in the hake collagen.

Table V. Degree of Hydroxylation of Proline and Lysine [Percent Hydroxylated Amino Acid to Total (Hydroxylated = Unhydroxylated) Amino Acids] in Hake and Trout Collagen

species	site	% Hyp	% Hyl
hake	skin	36.7	10.9
	fasciae	35.2	14.3
	myocommata	48.6	12.9
	myotomes	38.4	12.0
trout	skin	38.8	20.4
	fasciae	29.6	25.8
	myocommata	39.3	32.4
	myotomes	36.1	28.6

The tyrosine content ranged between 3.9 and 5.0% in the hake and 2.8 and 5.5% in the trout. These values, though low compared to those for nearly all the other amino acids, were normal for this amino acid, given its location in the telopeptide area.

The amount of methionine ranged from 14 to 17% in both the trout and the hake. As in the above case, these values were similar to those obtained for other fish species (Lewis and Piez, 1964; Yamaguchi et al., 1976; Bogason, 1984; Sikorski et al., 1984). All the values were higher than those obtained for mammals and other higher vertebrates (Harrington and von Hippel, 1961; McClain et al., 1970). The values for the remaining amino acids were also similar to the proportions reported in the literature (Yamaguchi et al., 1976; Bogason, 1984; Sikorski et al., 1984).

Table V presents the degree of hydroxylation of lysine and proline in collagen from the skin, fasciae, myocommata, and myotomes in the hake and the trout.

The proportion of hydroxylated proline was similar in both species and higher than that of lysine. Hydroxylation of lysine in the trout was appreciably higher than in the hake. This is important in view of the fact that hydroxylated proline, i.e., hydroxyproline, plays a role in stabilizing the triple helix (Ramachandran, 1988) and that hydroxylysine contributes to the formation and stabilization of cross-links, giving rise to complex, nonhydrolyzable bonds (Stimler and Tanzer, 1977; Asghar and Henrickson, 1982). In contrast, the degree of hydroxylation of both lysine and proline is much higher in food animals and other higher vertebrates (Bailey and Etherington, 1980). The degree of hydroxylation is very useful in applied technical studies, since it indicates the maximum cross-linking capacity, which in turn determines functional capacity.

Since the collagen from the different muscle structures was all the same type (type I), type III collagen containing sulfhydryl (SH) groups, which would confer differing characteristics on the connective tissue from the different structures, was absent, and there were no sizable differences in collagen solubility or in the proportion of α, β, and γ components. The experiments described below were carried out using muscle collagen from all three muscle structures combined.

The solubility values for the muscle and skin collagen appear in Table VI. The acid-soluble fraction was most abundant in all cases, which agreed with the results of earlier work performed at our laboratory (Borderías and Rudzki, 1985) and with data reported by Yamaguchi et al. (1976), although the proportion of acid-soluble collagen in the hake obtained in the present experiment (69.0%) was higher than the level (55%) reported by Yamaguchi et al. (1976).

The values for the acid-soluble fraction in the trout muscle collagen showed close agreement with those

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Table VI. Solubility (%) of Muscle and Skin Collagen in Hake and Trout[a]

| fraction | hake | | trout | |
	muscle	skin	muscle	skin
SS	$4.8^a \pm 0.1$	$2.5^b \pm 0.1$	$2.6^a \pm 0.1$	$2.6^a \pm 0.3$
AS	$69.6^a \pm 2.1$	$90.6^b \pm 1.9$	$70.4^a \pm 0.5$	$93.9^b \pm 2.0$
I	$25.6^a \pm 0.2$	$6.9^b \pm 0.0$	$27.0^a \pm 0.9$	$3.5^b \pm 0.0$

[a] (a, b) Different superscripts for the values in each row, within each species separately, indicate significant differences ($P \leq 0.05$).

Table VII. Proportion of α, β, and γ Components (Percent Total Components) in Acid-Soluble Muscle and Skin Collagen in Hake and Trout[a]

| component | hake | | trout | |
	muscle	skin	muscle	skin
α	$51.0^a \pm 0.8$	$52.3^a \pm 1.2$	$48.7^a \pm 1.3$	$65.8^b \pm 1.4$
β	$36.2^a \pm 0.4$	$26.5^b \pm 0.2$	$36.0^a \pm 1.0$	$34.2^a \pm 0.9$
γ	$11.6^a \pm 1.2$	$21.2^b \pm 0.1$	$15.4^b \pm 0.4$	$0.0^a \pm 0.0$

[a] (a, b) Different superscripts for the values in each column indicate significant differences ($P \leq 0.05$).

reported in a previous paper for similar specimens (Borderías and Montero, 1985).

The collagen extracted in neutral salt solutions consisted mainly of α-chain monomers. In addition to the α monomers, the acid-soluble fraction contained two distinct aggregates, a high proportion of β dimers and a small quantity of γ trimers. The insoluble collagen fraction contained highly reduced γ components with stable bonds and other highly aggregated components (Asghar and Henrickson, 1982).

The data in Table VI indicate a lower insolubility rate in skin collagen than in muscle collagen in both the hake and the trout, coinciding with the results obtained by Yamaguchi et al. (1976) for hake, cod, and dogfish (*Anarhichas spp.*). The higher solubility rates for the skin collagen could be associated with lower hydroxylysine levels, suggesting that there are fewer cross-links in the skin collagen in both species. This is extremely important in the development of applications for the functional properties of collagen in food products.

Table VII sets out the properties of the α, β, and γ components in the acid-soluble fraction of the skin and muscle collagen. Generally speaking, the proportion of α monomers was higher than that of the other components. Similar data were reported by Yamaguchi et al. (1976), Piez (1967), and Mohr (1971).

The lower proportion of γ components detected in the hake muscle collagen, may, in light of the higher insolubility rate (Table VI), have been a result of the highly reduced and stable, and hence more complex, cross-links in such components. The γ components thus make up part of the insoluble collagen fraction rather than part of the acid-soluble collagen fraction (Sims and Bailey, 1981; Asghar and Henrickson, 1982). In contrast, in the trout the proportion of α components was lower and the proportion of γ components higher in the muscle collagen than in the skin collagen, which was consistent with the higher values for the acid-soluble fraction in Table VI.

The following conclusions can be drawn: Collagen from connective tissue taken from muscle (fasciae, myocommata, myotomes) and from skin consisted mainly of type I collagen in both hake and trout. The relative proportions of the various amino acids in the two fish species studied were different from those in the higher mammals. Collagen from connective tissue from muscle had

more cross-links than collagen from connective tissue from skin in both fish species considered.

NOMENCLATURE

AS, acid-soluble collagen fraction; Asx, asparagine + aspartic acid; Glx, glutamine + glutamic acid; HyL, hydroxylysine; HyP, hydroxyproline; I, insoluble collagen fraction; SDS, sodium dodecyl sulfate; SH, sulfhydryl; SS, salt-soluble collagen fraction.

LITERATURE CITED

Almas, A. L. The muscle cell envelope of cod; ultrastructure and chemical composition. Ph.D. Thesis, Department of Biochemistry, Norweign Institute of Technology, University of Trondheim, 1986.

Asghar, A.; Henrickson, R. L. Chemical, biochemical, functional and nutritional characteristics of collagen in food systems. In *Advances in food research*; Chichester, C. O., Mrata, E. M., Schweigert, B. S., Eds.; Academic Press: London, 1982; Vol. 28, pp 237–273.

Bailey, A. J.; Sims, T. J. Meat tenderness: distribution of molecular species of collagen in bovine muscles. *J. Sci. Food Agric.* 1977, 28, 565–570.

Bailey, A. J.; Etherington, D. J. Metabolism of collagen and elastin. In *Comprehensive Biochemistry*; Florkin, M., Neuberger, A., van Deenenll, M., Eds.; Elsevier: New York, 1980; pp 19B, 299–460.

Bailey, A. J.; Sims, T. J.; Light, N. Cross-linking in type IV collagen. *Biochem. J.* 1984, 218, 713–723.

Bogason, S. G. Characterization of the intramuscular connective tissue collagen in the three rockfish species (*Sebastes*). Ph.D. Thesis, Oregon State University, 1984 (Available from University Microfilms International, Ann Arbor, MI).

Borderías, A. J.; Montero, P. Changes in fish muscle collagen during frozen storage. In *Storage lives of chilled and frozen fish and fish products*; International Institute of Refrigeration: Aberdeen, U.K., 1985a, pp 85–91.

Borderías, A. J.; Rudzki, J. Estudios preliminares sobre el colágeno de merluza (*Merluccius merluccius* L.). *Rev. Agroquim. Aliment.* 1985b, 25 (1), 149–154.

Chandrakasan, G.; Torchia, D. A.; Piez, K. A. Preparation of intact monomeric collagen from rat tail tendon and skin and the structure of the nonhelical ends in solution. *J. Biol. Chem.* 1976, 251 (19), 6062–6067.

Eastoe, J. E.; Leach, A. A. *Recent advances in gelatin and glue research*; Stainsby, G., Ed.; Pergamon Press: New York, 1958.

Gavilanes, J. G.; Gonzáles de Buitrago, G.; Leyzarbe, M. A.; Municio, A. M.; Olmo, N. Stabilization of pericardial tissue by glutaraldehyde. *Connect. Tissue Res.* 1984, 13, 37–44.

Harrington, W. F.; von Hippel, P. H. The structure of collagen and gelatin. *Adv. Protein Chem.* 1961, 16, 1–139.

Jacquot, R. Organic constituents of fish and other aquatic animal food. In *Fish as food*; Borgstrom, G., Ed.; Academic Press: New York, 1961; Vol. 2.

Kimura, S.; Tanaka, H. Partial characterization of muscle collagens from prawns and lobster. *J. Food Sci.* 1986, 51 (2), 330–339.

Kimura, S.; Takema, Y.; Kubota, M. Octopus skin collagen. Isolation and characterization of collagen comprising two distinct α chains. *J. Biol. Chem.* 1981, 256 (24), 13230–13234.

Kimura, S.; Miura, S.; Park, Y. M. Collagen as the major edible component of jellyfish (*Stomolophus namural*). *J. Food Sci.* 1983, 48, 1758–1760.

Krieg, T.; Luderschmidt, C.; Weber, L.; Muller, P. K.; Braun-Falco, O. Scleroderma fibroblasts: some aspects of in vitro assessment of collagen synthesis. *Dermatol. Res.* 1981, 270, 263–277.

Kubota, M.; Kimura, S. The distribution of collagen and some properties of intramuscular collagen in fish. *Hikaku Kagaku (Leather Chem.)* 1975, 21, 80–83.

Laemmli, U. K. Cleavage and structural proteins during assembly of the head of the bacteriophage T$_4$. *Nature* 1970, 227, 680–685.

Leach, A. A. Notes on a modification of the Newman and Logan method for the determination of hydroxyproline. *Biochem. J.* **1960,** *74,* 62–70.

Lewis, M. S.; Piez, K. A. The characterization of collagen from the skin of the dogfish shark, *Squalus acanthias. J. Biol. Chem.* **1964,** *239* (10), 3336–3340.

Light, N.; Champion, A. E. Characterization of muscle epimysium, perimysium, and endomysium collagens. *Biochem. J.* **1984,** *219,* 1017–1026.

Love, R. M. *The chemical biology of fishes*; Academic Press: London, New York, 1970.

Love, R. M.; Haq, M. The connective tissues of fish: III The effect of pH on gaping in cod entering rigor mortis at different temperatures. *J. Food Technol.* **1970,** *5,* 241–248.

Love, R. M.; Lavety, J. The connective tissues of fish: VII Postmortem hydration and ice crystal formation in myocommata and their influence on gaping. *J. Food Technol.* **1972,** *7,* 431–441.

McClain, P. E.; Creed, G. J.; Wiley, E. R.; Hornstein, I. Effect of post-mortem aging on isolation of intramuscular connective tissue. *J. Food Sci.* **1970,** *35,* 258–260.

Mohr, V. On the constitution and physico-chemical properties of the connective tissues and collagens of cod during starvation. Ph.D. Thesis, University of Aberdeen, 1971; *Comp. Biochem. Physiol.* **1971,** *55B,* 487–492.

Piez, K. A. Soluble collagen and the components resulting from its denaturation. In *Treatise on collagen*; Ramachandran, G. N., Ed.; Academic Press: New York, 1967; Vol. 1.

Piez, K. A.; Gross, J. The amino acid composition of some fish collagens: the relation between composition and structure. *J. Biol. Chem.* **1960,** *235,* 995–998.

Ramachandran, G. N. Stereochemistry of collagen. *J. Peptide Protein Res.* **1988,** *31,* 1–16.

Ramachandran, G. N.; Kartha, G. Structure of collagen. *Nature* **1955,** *176,* 593–595.

Ramachandran, G. N.; Ramakrishnan, C. Molecular structure. In *Biochemistry of collagen*; Ramachandran, G. N., Reddi, A. H., Eds.; Plenum Press: New York, 1976; pp 45–84.

Rich, A.; Crick, F. H. C. The structure of collagen. *Nature* **1955,** *176,* 915–917.

Sikorski, Z.; Scott, D.; Buisson, D. The role of collagen in the quality and processing of fish. *CRC Crit. Rev. Food Sci. Nutr.* **1984,** *20* (4), 301–338.

Sims, T.; Bailey, A. Connective tissue. In *Developments in meat science-2*; Ralston, L., Ed.; Applied Science Publishers: London, 1981; pp 28–59.

Stimler, N. P.; Tanzer, M. L. Location of the intermolecular crosslinking sites in collagen. In *Protein crosslinking. Nutritional and medical consequences*; Friedman, M., Ed.; Plenum Press: New York, 1977.

Takema, Y.; Kimura, S. Two genetically distinct molecular species of octopus muscle collagen. *Biochim. Biophys. Acta* **1982,** *706,* 123–128.

Timpl, R.; Ganville, R.; Nowack, H.; Wiedemann, H.; Fietzek, P.; Kühn, K. Isolation, chemical and electron microscopical characterization of neutral-salt soluble type III collagen and procollagen from fetal bovine skin. *Z. Physiol. Chem.* **1975,** *356,* 1783–1792.

Yamaguchi, K.; Lavety, J.; Love, R. M. The connective tissue of fish; VIII Comparative studies of hake, cod and catfish collagen. *Food Technol.* **1976,** *11,* 389–399.

Carotenoids and Provitamin A Activity of Carrot (*Daucus carota* L.) Cultivars

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The carotenoid and provitamin A content of 19 cultivars of orange carrot (*Daucus carota* L.) were analyzed by high-performance liquid chromatography. The amounts of the predominant carotenoids, α- and β-carotene, ranged from 2200 to 4900 and from 4600 to 10 300 μg/100 g of fresh weight, respectively. High α- and β-carotene contents were found especially in carrot cultivars Nantes Duke Notabene 370, Nantes Fancy Notabene 405, Narbonne F_1 BZ, Nelson F_1 BZ, Nantucket F_1 BZ, and Berlicum R. The tentatively identified 15-*cis*-β-carotene accounted for approximately 4.3% (range 1.3–12.9%) of the amount of *all-trans*-β-carotene. All carrot cultivars contained γ-carotene (range 630–2700 μg/100 g of fresh weight). Also, lutein (110–560 μg/100 g of fresh weight) was present. Expressed as retinol equivalents (RE, μg/100 g), the amount of provitamin A in carrot cultivars was between 1200 and 2300, an amount high enough to satisfy the human daily need of vitamin A.

Carrot is one of the most important vegetables grown and stored in Finland. Good orange color is essential for carrots sold on the fresh vegetable market and used for food processing. The main variation in the color of carrot is due to genotype (Bajaj et al., 1980; Gabelman, 1974), but the development stage of the plant (Banga et al., 1963; Bajaj et al., 1980; Evers, 1989; Hårdh et al., 1977) and temperatures during the growing season (Banga