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Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*)

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Abstract

Acid soluble collagen (ASC) was extracted from the skins of young and adult Nile perch (*Lates niloticus*) using 0.5 M acetic acid and precipitation with 0.9 M NaCl. The ASC yields, on a dry weight basis, were 63.1 and 58.7%, respectively for young and adult fish skins. SDS-PAGE showed that the collagens contained two alpha components (α1 and α2). ASC from Nile perch was found to contain more imino acids (19.3 and 20.0%, respectively, for young and adult fish) than most fish species. The denaturation temperature for the collagens from the skins of young and adult Nile perch was determined to be 36 °C, which is also higher than that for most other fish species. Fourier transform infrared spectroscopy showed a higher degree of molecular order in ASC from adult than from young Nile perch. The results indicate that age-related changes in Nile perch skin collagen are not very pronounced, probably because there is minimal development of mature cross-links.

Keywords: Nile perch; Fish collagen; Imino acids; Fish waste; Denaturation temperature

1. Introduction

Collagen is the most abundant protein of animal origin, comprising approximately 30% of total animal protein. There are at least 19 variants of collagen, named type I–XIX (Bailey, Paul & Knott, 1998). Types I, II, III and V are the fibrous collagens. Type I collagen is found in all connective tissue, including bones and skins. It is a heteropolymer of two α1 chains and one α2 chain. It consists of one-third glycine, contains no tryptophan or cysteine and is very low in tyrosine and histidine.

Several studies have focussed on the characterisation of different fish collagens (Kimura & Ohno, 1987; Montero, Alvarez, Marti & Borderias 1995; Montero, Gòmez-Guillèn, & Borderias, 1999; Nagai & Suzuki, 2000; Piez, 1965; Rigby, 1968; Sato, Yoshinaka, Yoshiaki & Sato, 1989; Sivakumar, Arichandran, Suguna, Mariappan & Chandrakasan, 2000). Most fish collagens have been found to consist of two α-chain variants, which are normally designated as α1 and α2 (Gòmez-Guillèn et al., 2002; Nagai, Yamashita, Taniguchi, Kanamori, & Suzuki, 2001). These α-chain variants, though having approximately the same molecular weight (~95,000 Da), can be separated by SDS PAGE due to their different affinity for SDS. Alpha 2 has a higher affinity for SDS and consequently exhibits a higher mobility than α1 (Kubo & Takagi, 1984). Piez (1965) isolated three variants of α-chains (α1, α2 and α3) from cod skin collagen and found that these variants differed in their amino acid composition. Alpha 3 has also been isolated from rainbow trout (Saito, Takenouchi, Kunisaki & Kimura, 2001), common horse mackerel (Kimura, Zhu, Matsui, Shijoh, & Takamizawa, 1988; Yoshida, Fujisawa, Mizuta, & Yoshinaka, 2001) and eel (Kimura et al., 1988).

In addition to differences in molecular species, fish collagens have been shown to vary widely in their amino acid composition. In particular, the levels of imino acids (proline and hydroxyproline) vary significantly among fish species (Balian & Bowes, 1977; Gudmundsson & Hafsteinsson, 1997; Poppe, 1992). The amount of imino acids, especially hydroxyproline, depends on the environmental temperature in which the fish lives and it

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affects the thermal stability of the collagens (Balian & Bowes, 1977; Kimura et al., 1988; Rigby, 1968). Collagens derived from fish species living in cold environments have lower contents of hydroxyproline and they exhibit lower thermal stability than those from fish living in warm environments. This is because hydroxyproline is involved in inter-chain hydrogen bonding, which stabilises the triple helical structure of collagen (Darby & Creighton, 1993). Cold water fish species are also reported to contain higher levels of hydroxyamino acids, serine and threonine (Balian & Bowes, 1977). Grossman and Bergman (1992) showed that gelatin from tilapia, a warm water fish species, contains higher levels of imino acids than cold water fish collagens.

Nile perch (*Lates niloticus*), like tilapia, is a warm water fish species. It is the most important commercial fish species in East Africa. Approximately 100,000 t of Nile perch are processed in Uganda alone, annually. The fish landed vary greatly in size. Young (<80 days old) fish measure as little as 6.4 cm and the largest adult (13+ years old) fish measure up to 160 cm long for males and 190 cm for females (Acere, 1993). The smallest length for sexually mature fish is 53.5 cm and 67.5 cm for males and females, respectively (Acere, 1993; Ogutu-Ohwayo, 2000). This occurs between the ages of 1 and 2 years.

About 30% of the total fish weight remains as waste in the form of skins and bones during preparation of fish fillets (Shahidi, 1994). This waste, if utilised in the manufacture of value added products, such as collagen, could contribute significantly to the economic value of the fish. The characteristics of collagen, the main component of these waste materials influences their potential for utilisation. The aim of this study was to characterise collagen from skins of Nile perch and to investigate the effect of fish age on collagen properties. It has been demonstrated for mammals that the solubility of their collagen reduces as they age, due to changes in the amount and type of cross-links (Bailey et al., 1998).

2. Materials and methods

2.1. Raw materials

Skins of Nile perch were procured from Nge-ge Ltd, Kampala, Uganda. These were by-products of fillet processing. The very small skins from young fish (skin thickness <0.4 mm) and the large ones from adult fish (skin thickness >1.5 mm) were selected and used in this study. Portions were taken for the determination of chemical composition. These were immediately refrigerated (~7 °C) and analysed within 48 h. The rest of the selected skins were frozen until required for extraction of collagen.

2.2. Proximate analysis

Portions were taken from different parts of the skins, blended together and used for proximate analysis. Moisture, lipid, ash and protein contents of skins from young and adult Nile perch were determined by AOAC (1995) methods 950.46, 960.39, 900.2A and 928.08, respectively. Protein digestion was done as described by Eastoe and Eastoe (1952) to ensure complete hydrolysis of collagen. A conversion factor of 5.4 was used in calculating the protein content from the Kjeldahl nitrogen content since collagen, the main protein in skin, contains approximately 18.7% nitrogen (Eastoe & Eastoe, 1952).

2.3. Extraction of collagen

The method described by Gòmez-Guillèn and Montero (2001) was used to obtain collagen from skins of young and adult Nile perch. The method involves washing of the skins with chilled (~5 °C) water for a period of 10 min. During this time, the skins were pressed intermittently by hand. The skins were then washed with 0.8 M NaCl for three periods of 10 min each, followed by rinsing in running water. The volumes and solids contents of the wash liquors (water and NaCl solution) were determined and used to calculate the amounts of solids lost in the wash liquors. Collagen was then extracted using 0.5 M acetic acid solution (1 g of skin per 20 ml of 0.5 M acetic acid). The extraction was conducted for 16 h, with intermittent stirring. The viscous collagenous material was separated from the insoluble components by sieving through cheesecloth. The volume and solids content of the filtrate were determined and used to determine the total acid-soluble solids from the skins. The collagen solution was then centrifuged and salt solution (0.9 M NaCl) was added to the supernatant to precipitate the collagen. The precipitated collagen (acid soluble collagen) was separated by centrifugation at 2500 × *g* for 30 min. To further purify the collagen, it was re-dissolved in acetic acid and re-precipitated as described above. The collagen extraction, precipitation and separation were conducted at room temperature (approximately 15 °C). The acid-soluble collagens from young and adult Nile perch were separately freeze-dried and used for analysis.

2.4. Determination of collagen denaturation temperature

Determination of denaturation temperature was based on the method described by Kimura et al. (1988). An Ostwald's viscometer was filled with 0.1% (m/v) collagen solution in acetic acid. The viscometer was then immersed in a water bath held at 30 °C and left to stand for 30 min, to allow the collagen solution to equilibrate to the water bath temperature. The

temperature was raised stepwise up to 50 °C and maintained at each temperature for 10 min. Collagen solution viscosities were measured at temperature intervals of about 2 °C from 30 °C up to 50 °C. Fractional viscosities were computed for each temperature as follows:

Fractional viscosity = (maximum viscosity − measured viscosity)/maximum viscosity-minimum viscosity.

Thermal denaturation curves were then obtained by plotting the fractional viscosities against temperature for young skin and adult fish skin collagen. The denaturation temperature was taken to be the temperature at which fractional viscosity was 0.5.

2.5. Amino acid analysis

Amino acid analysis was conducted by the Pico.Tag method (Bidlingmeyer, Cohen, & Tarvin, 1984). This method involves derivatisation of amino acids using phenylisothiocyanate (PITC) and determination of the phenylthiocarbamyl derivative of amino acids (PTC amino acids) using reversed phase HPLC. Dry collagen (10–20 mg) from skins of young and adult Nile perch was mixed with 6 M HCl (1 ml) containing 1% phenol (v/v). The mixture was evacuated, blown with N_2 and vacuum-sealed before hydrolysis at 110 °C for 24 h. After hydrolysis, the samples were cooled and diluted to 5 ml with de-ionised water. A portion (25 μl) was then dried and derivatised. Derivatisation involved addition of 10 μl of a mixture of methanol, water and trimethylamine (2:2:1), mixing and then drying for 5 min. This was followed by addition of 20 μl of a mixture of methanol, water, trimethylamine and phenylisothiocyanate (7:1:1:1). The sample was left to stand for 20 min at room temperature (20–25 °C), dried under vacuum and then dissolved in 200 μl of pH 7.4 phosphate buffer and filtered with a 0.45 μm filter. Portions (20 μl) of the filtered samples were injected using an automatic loader (WISP™) (Millipore Corp, Milford, MA, USA) into the Pico.Tag column (part no 88131, 3.9 mm × 13 cm) (Millipore Corp, Milford, MA, USA) for amino acid analysis.

2.6. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis (SDS-PAGE) was conducted using the discontinuous Tris–HCl/glycine buffer system (Laemmli, 1970), with 7.5% resolving gel and 4% stacking gel. Samples containing approximately 5 μg of solids per μl were prepared by dissolving 10 mg of collagen in 2 ml sample buffer prepared with and without 2-mercaptoethanol. A portion (20 μl) of sample was loaded per well. Calf skin acid-soluble collagen (Sigma Chemical Co, St Louis, MO, USA) and molecular weight markers were loaded alongside the Nile perch collagen samples. The molecular weight markers (ICN Biomedicals Inc., Aurora, OH, USA) contained cytochrome C (horse heart), myoglobin (horse heart), chymotrypsinogen A, ovalbumin, bovine serum albumin, gamma globulin (human) and apoferritin. These were mixed in sample buffer to a make a solution containing 2 μg of each protein per μl and 10 μl were loaded per well.

Electrophoresis was conducted using the Protean II xi vertical cell and the 1000 powerpac (Bio-Rad laboratories, Hercules, CA, USA) at a constant current of 30 mA and a temperature of 10C. Gels were stained using 0.1% Coomassie Brilliant Blue R250 dissolved in water, methanol and trichloroacetic acid (5:4:1) and de-stained using a solution containing methanol, distilled water and acetic acid in a ratio of 5:4:1.

2.7. Fourier transform infrared spectroscopy

FTIR spectra were obtained from discs containing 2 mg collagen in approximately 100 mg potassium bromide (KBr). All spectra were obtained using a Bruker infrared spectrophotometer (Bruker Instruments, Billerica, MA) from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} per point. Background was subtracted using the Opus software (Bruker Instruments, Billerica, MA). Triplicate samples of collagen from young and adult Nile perch skins were analysed and spectra for the triplicate runs averaged. Fourier self deconvolution was conducted on the average spectra for the amide I band, using a resolution enhancement factor of 1.8 and full height band width of 13 cm^{-1}. The self deconvolution provided information on the number and location of sub-bands. Curve fitting was then performed using peakfit software (SPSS Inc., Chicago, IL, USA).

2.8. Statistical analysis

Means for the properties for the adult and young fish skin collagens were compared using *t*-test and *P*-values are presented wherever applicable.

3. Results and discussion

3.1. Proximate composition of Nile perch skins

The skins from young and adult Nile perch were found to contain similar amounts of protein (20–22%) (Table 1). The lipid content was however, higher for the skins of adult fish than for the skins of young fish ($P = 0.02$). It seems that the fish accumulate subcutaneous fat as they age. The ash content was also considerably higher for skins of adult fish probably because of increased mineralisation with age.

Table 1
Proximate composition of skins from young and adult Nile perch[a]

	Young fish	Adult fish	*P*-value
Moisture	72.7 (1.3)	68.4 (0.6)	0.54
Protein	20.3 (2.0)	21.6 (1.3)	0.16
Lipid	5.0 (0.7)	6.8 (0.3)	0.02
Ash	3.7 (0.5)	6.0 (0.2)	0.16

[a] Values in parentheses are standard deviations for triplicate samples.

3.2. Solubility of Nile perch skin solids

The solubilities of solids in water, salt solution and in acetic acid were not significantly ($P > 0.05$) different for the skins of young and adult fish (Table 2). Working with pig skin, Reich, Walther, and Stather (1962) found that the component soluble in water consisted only of non-collagenous matter but that the salt-soluble component contained both non-collagenous matter and collagen. The amounts of stable cross-links in collagen have been reported to increase with age in mammals (Sims, Avery, & Bailey, 2000). As a result, the solubility of mammalian collagen in salt solution and cold acid solutions decreases with age (Reich et al., 1962). The consistently and slightly lower solubilities for adult compared to young fish skin collagen may be indicative of some slight increase in the amounts or extents of stable cross-links.

Fish skin collagens have been reported to develop minimal amounts of mature cross-links (Hickman et al., 2000). Cohen-Salal, Le Lous, Allain, and Meunier (1981) also demonstrated, by measuring hydrothermal isometric tensions that fish skin collagen cross-links do not mature to thermally-stable bonds. As a result of its low content of stable cross-links, fish skin collagen can easily be solubilised, even from adult fish.

3.3. Denaturation temperature of Nile perch skin collagens

Fig. 1 shows the changes in fractional viscosity, with increasing temperature, for young and adult Nile perch skin collagens. Both the young and adult skin collagens exhibited a rapid loss of viscosity with heating. This can be attributed to denaturation of collagen. The thermal denaturation temperature (Td) was determined to be about 36.0 °C for collagen from the skin of young fish and about 36.5 °C for collagen from the skin of adult fish. The minimal difference between denaturation temperatures of collagens from young and adult Nile perch is also indicative of minimal differences in the extents of stable cross-links.

The denaturation temperature recorded in this study, for collagen from the skin of Nile perch, is higher than the values reported for those from temperate fish species. Collagen denaturation temperatures have been reported for cod (15 °C) (Rigby, 1968), Alaska pollack (16.8 °C) (Kimura & Ohno, 1987), muscle of carp (32.5 °C), eel (29.3 °C), common mackerel (26.1 °C), chum salmon (19.4 °C) (Kimura et al., 1988), Japanese seabass (30 °C), skip jack tuna (29.7 °C) and ayu (29.7 °C) (Nagai & Suzuki, 2000). The higher denaturation temperature for collagen of Nile perch may be attributed to the higher imino acid content than that of cold-water fish collagens.

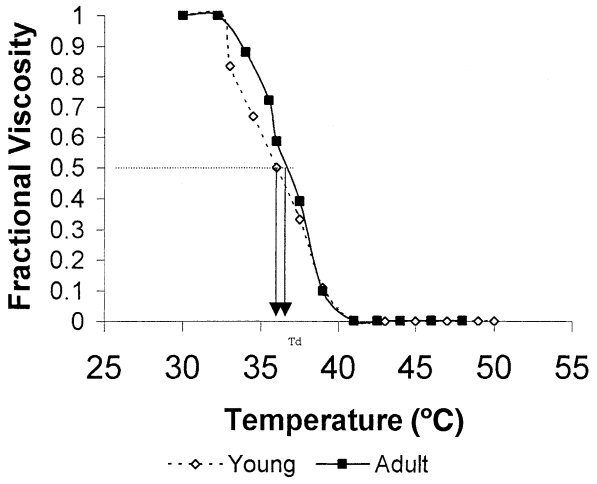

Fig. 1. Denaturation curves of collagens from skins of young and adult Nile perch as shown by change in fractional viscosity with temperature for 0.1% (m/v) solutions of collagen in acetic acid. T_d is the denaturation temperature.

Table 2
Solubility of solids from skins of young and adult Nile perch in solutions used in collagen preparation[a]

Component	% of total solids solubilised		*P*-value
	Young fish	Adult fish	
Water-soluble (%)	3.5 (0.2)	2.5 (0.1)	0.52
Salt-soluble (%)	3.4 (0.3)	2.4 (0.3)	0.29
Acid-soluble (%)	63.1 (3.3)	58.7 (3.4)	0.13
Insoluble (%)[b]	30.0 (1.7)	36.4 (3.3)	0.18

[a] Values in parentheses are standard deviations for triplicate experiments.
[b] Obtained by difference.

3.4. Amino acid composition of Nile perch skin collagens

Table 3 shows the amino acid compositions of the acid-soluble collagen extracted from young and adult fish skins of Nile perch. The amino acid contents of collagens from the skins of young and adult fish were not significantly different from each other, suggesting that amino acid composition of collagen is independent of age. The collagens were found to contain no tryptophan or cysteine. They were also very low in methionine, tyrosine and histidine, like other collagens (Balian & Bowes, 1977; Grossman & Bergman, 1992;

Gudmundsson & Hafsteinsson, 1997; Yoshida, Fujisawa, Mizuta, & Yoshinaka, 2002). A significant observation was the high total imino acid content (20.0 and 19.3%, respectively, for young and adult fish skin collagen) of acid-soluble Nile perch skin collagen in comparison to other fish collagens. The total imino acid content of Nile perch skin collagens, though lower than the 25.4% for tilapia (Grossman & Bergman, 1992) is among the highest reported for fish collagens. Collagen from cold-water fish species contains 16–18% imino acids (Gilsenan & Ross-Murphy, 2000; Gudmundsson & Hafsteinsson, 1997; Norland, 1990). The higher imino acid content and higher denaturation temperature of collagen of Nile perch, in comparison with cold-water fish species are in agreement with observations by Rigby (1968) that thermal stability of collagen increases with imino acid content.

The high imino acid content, especially the hydroxyproline content is also significant because it affects the functional properties of gelatin that can be derived from collagen (Gilsenan & Ross-Murphy, 2000; Gòmez-Guillèn et al., 2002). Fish gelatin has potential for use in several applications (Norland, 1990; Osborne, Voight, & Hall, 1990), however, low gel strength is a major problem, hindering increased production and use of fish gelatins. The low gel strength of fish gelatins has been attributed to the low imino acid content of fish collagens (Gilsenan & Ross-Murphy, 2000; Gòmez-Guillèn et al., 2002). Nile perch collagen contains more imino acids and therefore has potential for use in the manufacture of gelatins with good gelling properties.

Table 3
Amino acid composition of acid soluble collagen from skins of young and adult Nile perch[a]

	Amino acid content g/100 g protein		*P*-value
	Young fish	Adult fish	
Asp	6.14 (0.04)	5.91 (0.02)	0.24
Gln	10.0 (0.01)	9.85 (0.01)	0.05
Hyp	7.88 (0.01)	8.05 (0.03)	0.83
Ser	3.47 (0.01)	3.34 (0.03)	0.58
Gly	21.1 (0.11)	22.1 (0.11)	0.81
His	1.16 (0.05)	1.10 (0.02)	0.74
Arg	8.10 (0.01)	8.15 (0.02)	0.24
Thr	3.24 (0.01)	3.04 (0.01)	0.05
Ala	9.77 (0.02)	10.1 (0.02)	0.64
Pro	11.4 (0.11)	12.0 (0.14)	0.11
Tyr	0.96 (0.03)	0.86 (0.02)	0.26
Val	2.47 (0.02)	2.35 (0.02)	0.56
Met	1.72 (0.01)	1.58 (0.04)	0.56
Ile	1.38 (0.01)	1.26 (0.02)	0.72
Leu	3.19 (0.01)	2.83 (0.03)	0.85
Phe	2.48 (0.02)	2.31 (0.05)	0.74
Lys	4.07 (0.01)	3.77 (0.15)	0.55
Hyl	1.44 (0.01)	1.43 (0.05)	0.39

[a] Values in parentheses are standard deviations for duplicate samples.

The degree of hydroxylation of proline and lysine, influences the thermal stability of collagen (Kimura et al., 1988). A higher degree of hydroxylation is associated with higher denaturation temperature, for collagens with similar amino acid profiles. The total degree of hydroxylation of proline and lysine for Nile perch collagen (Table 4) was found to be similar to that reported for pike (34%) and cod (32%) skin collagens (Piez & Gross, 1960) but higher than that reported by Gòmez-Guillèn et al., (2002) for sole (25.3%), megrim (25%), and hake (24.6%). The denaturation temperature for cod has been reported to be 15 °C (Rigby, 1968). It appears that it is the higher imino acid content, rather than the extent of hydroxylation that seems to be the reason for the higher denaturation temperature observed for Nile perch skin collagen.

3.5. Electrophoretic pattern of Nile perch skin collagens

SDS PAGE showed that both young and adult fish skin acid-soluble collagen consisted of α chains and their dimers (β chains) (Fig. 2). The α components showed two distinct species varying in their mobility, for both reducing and non-reducing conditions. It may be concluded, therefore, that Nile perch acid-soluble collagen is made up of at least two α species (α1 and α2). This is similar to the pattern observed for several other fish species (Gomez-Guillen et al., 2002; Nagai et al., 2001) and is typical of type I collagen (Bailey & Light,

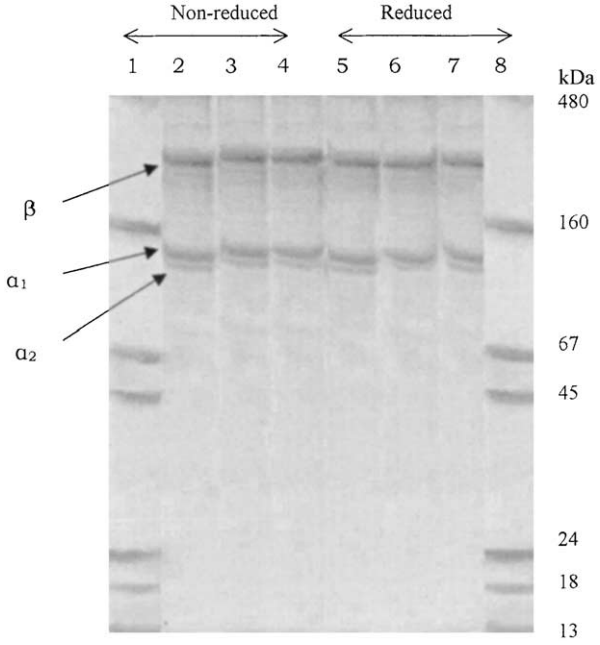

Fig. 2. SDS polyacrylamide gel (7.5%) electrophoretic pattern for acid soluble collagen under non-reducing and reducing conditions. 1 and 8—Molecular weight markers, 2 and 5—calf skin collagen, 3 and 6—collagen from skin of adult Nile perch, 4 and 7—collagen from skin of young Nile perch.

Table 4
Proportion of total imino acids and percent hydroxylation of lysine and proline in collagens from skins of young and adult Nile perch

	Young fish	Adult fish
Total imino acids (%)	19.3	20.0
% Hydroxylation		
Lysine	26.1	27.5
Proline	40.9	40.2
Total	37.6	37.6

1989). The electrophoretic pattern of Nile perch skin collagen was similar to that of calf skin collagen (Fig. 2).

The α2 was the minor component of the two species and it seems that Nile perch collagen exists as trimers consisting of two α1 and one α2 chains. This is typical of type I collagen (Bailey & Light, 1989), which is the major collagen in dermal tissue (Bailey & Light, 1989; Bailey et al., 1998).

There was no clear difference between the electrophoretic patterns under reducing and non-reducing conditions, suggesting absence of disulphide bonds. This is consistent with the observation that the collagen was almost devoid of sulphur-containing amino acids. No consistent difference was observed between the electrophoretic pattern of collagen from young and adult fish skins.

As observed by Hayashi and Nagai (1979), the mobility of alpha chains was lower than would be expected for globular proteins of similar molecular weights (ca. 95 kDa) and, when globular proteins are used as molecular weight markers, the molecular weight of collagen could be overestimated. This is because of the unique amino acid profile of collagen. The differences observed in mobility, between collagenous proteins and globular protein, have been attributed to the high content of the relatively small amino acid residues, glycine, proline and alanine of the former (Noelken, Wisdom, & Hudson, 1981). The estimated molecular weight for α-chain, using globular protein standards was approximately 120 kDa (Fig. 2).

3.6. Fourier transform infrared spectra for acid soluble collagens

The Nile perch acid soluble collagens exhibited FTIR spectra (Fig. 3) similar to that exhibited by other collagens (Jackson, Choo, Watson, Halliday, & Mantsch, 1995; Liu, Dixon, & Mantsch, 1998; Sai & Babu, 2001).

The spectra for acid soluble collagen from young and adult fish skins differed slightly, indicating some differences in the secondary structure of the two proteins. Table 5 is a summary of the major peaks identified in the FTIR spectra of ASC from young and adult Nile perch skins, and their assignments. Generally, the peaks for the young fish collagen appeared at a lower frequency than the corresponding peaks for the adult fish collagen.

The amide I and amide II peaks were at a lower frequency for the young fish skin (1650 and 1542 cm^{-1}, respectively) than the adult fish skin (1654 and 1555 cm^{-1}, respectively) collagen. Based on the location of the amide I and amide II peaks, it would seem that the acid-soluble collagen from the young skins had a lower

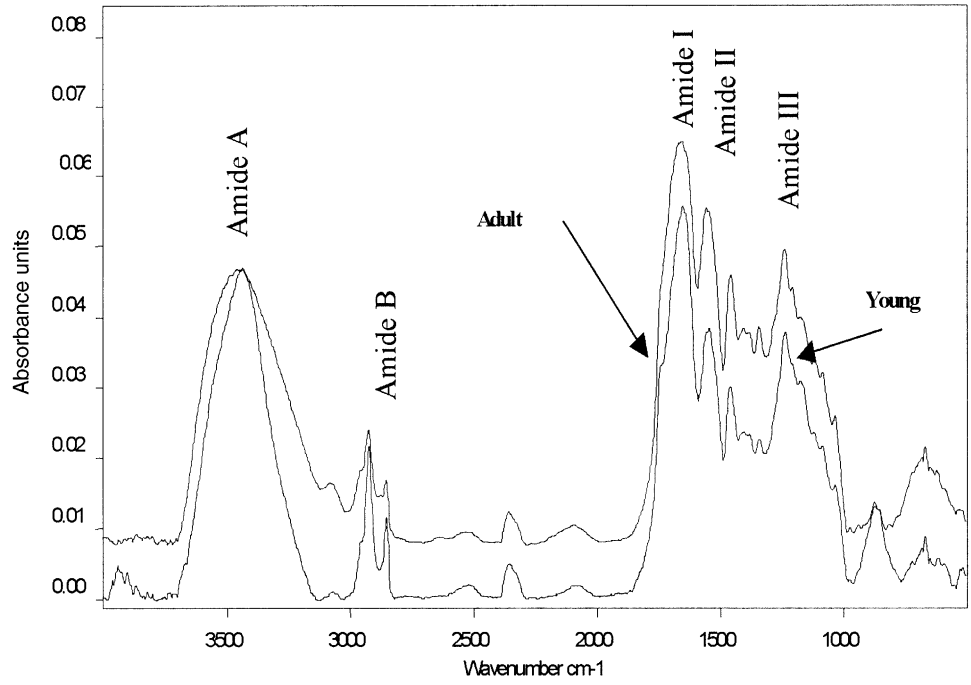

Fig. 3. Average FTIR spectra for triplicate samples of acid-soluble collagen derived from skins of young and adult Nile perch.

Table 5
FTIR spectra peak positions and assignments for acid soluble collagen from skins of young and adult Nile perch

Region	Peak wavenumber (cm^{-1})		Assignment	Reference
	Young	Adult		
Amide A	3434	3458	NH stretch, coupled with hydrogen bonding	Sai and Babu (2001)
Amide B	2924	2926	CH_2 asymmetrical stretch	Abe and Krimm (1972)
–	2853		CH_2 symmetrical stretch	Abe and Krimm (1972)
Amide I	1650	1654	C=O stretch/hydrogen bonding coupled with COO–	Jackson et al. (1995)
Amide II	1542	1555	NH bend coupled with CN stretch	Jackson et al. (1995)
–	1457	1455	CH_2 bend	Jackson et al. (1995)
–		1340	CH_2 wagging of proline	Jackson et al. (1995)
Amide III	1235	1238	NH bend	Jackson et al. (1995)
–	871	875	Skeletal stretch	Abe and Krimm (1972)
–	670	670	Skeletal stretch	Abe and Krimm (1972)

– No common name for the spectral region.

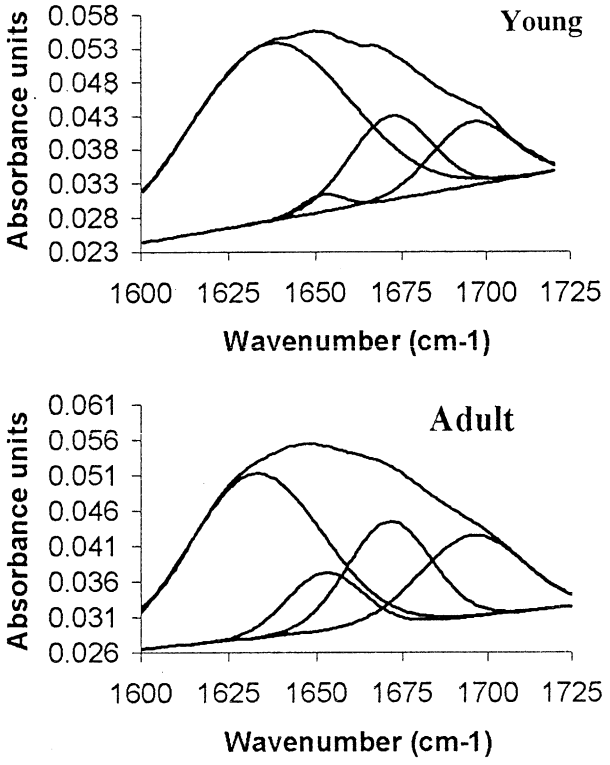

Fig. 4. Amide I band for collagens from the skins of young and adult Nile perch with fitted band components.

degree of molecular order, since a shift of these peaks to lower wave numbers is associated with a decrease in the molecular order (Payne & Veis, 1988). It would appear, therefore, that there were more intermolecular cross-links in the adult fish collagen. Amide I components (Fig. 4) showed adult Nile perch ASC amide I band to consist of a higher proportion of the component at 1695 cm^{-1} than the young fish ASC (Table 6). This band is linked to the extent of intermolecular interactions in collagen and collagen-like peptides (Doyle, Bendit, & Blout, 1975; Paschalis et al., 2001; Prystupa & Donald, 1996). The other considerable difference was the lower intensity of the component with a peak at 1652 cm^{-1} in young fish ASC (Table 6). This component has been attributed to random coils (Prystupa & Donald, 1996), suggesting a lower extent of unwinding of the triple helix in the young fish ASC. It seemed, therefore, that adult fish ASC retained more intermolecular cross-links during solubilisation with acetic acid but the triple helical structure, normally held together by intramolecular hydrogen bonds (Darby & Creighton, 1993) was extensively destroyed. The young fish ASC on the other hand, because of its lower content of stable intermolecular bonds, could be solubilised more easily and perhaps retained triple helices to a greater extent. The minimal differences in the extent of collagen cross-linking with age were therefore reflected in differences in the FTIR spectra of the collagens.

Table 6
Peak location (cm^{-1}) and percent area (in parentheses) of fitted components of amide I band for collagens from skins of young and adult Nile perch

	Young	Adult
1	1637 (69)	1634 (49.1)
2	1652 (1.8)	1653 (10.4)
3	1672 (16.7)	1671 (20.5)
4	1696 (12.6)	1695 (20)

4. Conclusions

Based on solubility and amino acid composition, it may be concluded that collagen from the skin of Nile perch differs considerably from mammalian and cold-water fish collagens. The collagen was easily solubilised from skins of both young and adult Nile perch using 0.5 M acetic acid, indicating that it has a low content of stable cross-links. The solubilities and denaturation temperatures of collagen from skins of young and adult Nile perch were similar, indicating that age-related changes in collagen are less pronounced in Nile perch skin than in mammalian collagen.

Based on the electrophoretic profile and amino acid composition, it may be concluded that collagen from Nile perch skins, like collagens from skins of most other fish species, is Type 1 collagen. Collagen from Nile perch skins, however, differs from collagens from skins of other fish species in some respects. The denaturation temperature and the imino acid content of collagen from the skin of Nile perch were found to be higher than those reported for most fish species and closer to those of mammalian collagens. Due to its high imino acid content, Nile perch collagen may be a source of gelatin with good gelling properties, since the gelling properties of gelatin are related to its imino acid content. The high acid solubility of Nile perch collagen has implications for gelatin manufacture from skins of Nile perch, since prolonged acid pre-treatment, before extraction of gelatin from the skins, would lead to high losses of collagen and low gelatin yield.

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