The Stability of Natural History Specimens In Fluid-preserved Collections

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Abstract

Natural history museum collections are incomparable storehouses of geological, biological, and genetic resources throughout the world. Some of the materials composing the specimens in these collections are vulnerable to deterioration when stored in fluids. Samples were taken from liquid storage media surrounding mammal specimens in the National Museum of Natural History (NMNH), Smithsonian Institution and analyzed for amino acids, lipids, and fatty acids using gas chromatography-mass spectrometry and high- performance ion-exchange chromatography. The results show the presence of the C12, C14, C16, C18, C20, C22, and C24 fatty acids in the fluids. The percent of each fatty acid in the mixture is typical for the lipids found in the sampled specimens. Amino acid profiles indicated that peptides and amino acids also were being leached into the storage fluids. These profiles indicated a general protein loss, but the high glycine and alanine content (about 30%) also indicates some structural protein loss. In addition, heating experiments using the proteins collagen and keratin in different preservative fluids show that 70% ethanol is a better preservative than 50% iso-propanol, and that, in general, hair (keratin) is more stable than skin (collagen). These projects form the baseline for an understanding of the deterioration process of proteinaceous materials in storage fluids and provide information from which new preservation policies can be developed. Reconsideration of present methods is especially important because of developments that allow examination of specimens at the molecular level. Fluids that preserve morphology may not adequately preserve sources of chemical information, such as DNA.

Introduction

Natural History Collections have been formed through the systematic input of invaluable specimens through the centuries (beginning of the sixteenth century). The actual number of specimens stored in institutions around the world is about 1.5 billion (the NMNH collection alone includes about 124 million specimens) and this number is increasing at a rate of about 50 million per year (Peake, 1989). In part, this high number is due to the need to collect modern examples because of the rapid decrease in biological diversity in the world.

The significance of these collections is remarkable, and they have been the most important source of information in studies of biodiversity. Technological development during the last decades has allowed research to go far beyond taxonomic studies to include the chemical information content of these collections, such as the sequencing of DNA. However, it should be noted that preservation techniques used for these specimens, for example the use of ethyl alcohol as preservative introduced by Boyle in the seventeenth century, as Down (1989) cites, has persisted until today without any major changes. Ensuring the appropriateness of storage conditions, as well as any conservation treatments that are used, requires an understanding of any deterioration process occurring in these collections.

This paper presents studies that correlate the type and amount of proteinaceous material and lipids released by the specimens into their storage fluids with the deterioration processes that take place in the specimen-fluid system. Heating experiments were

performed on the structural proteins collagen and keratin, the two most common proteins of fluid-preserved specimens, in different preservative fluids. Chromatography (HPLC amino acid analyzer, and GC) was used to determine amino acid profiles present in storage fluids. GC and GC-MS were used to elucidate other compound presents in the fluids (such as lipids).

Materials and Methods

50 ml samples from storage fluids surrounding mammal specimens at the National Museum of Natural History were taken. Each was split into two 25ml aliquots. These type specimens stored in 70% ethyl alcohol and water solution were collected more than 100 years ago. They are inspected every six months and any losses are "topped".

The first aliquot was dried under vacuum in the presence of silica gel and extracted twice with 0.1 molar HCl and used directly for amino acid analysis. Samples prepared in this way were analyzed on a high-performance liquid chromatograph (HPLC) specially designed for ion exchange separation of amino acids, similar to the one described by Hare et al. (1985) and in its current modified form described by von Endt (1994). Analysis was performed on a St. John Model 2000 Amino Acid Analyzer with Alcott 708AL Autosampler. Post column derivatization with ortho-phthalaldehyde (OPA) and 2-mercaptoethanol (MCE) as detecting reagents was used. This technique enables the separation and quantification of most of the amino acids found in proteinaceous materials. However, it does not detect secondary amines and thus two common imino acids, proline and hydroxyproline, are not reported (Benson and Hare, 1975).

Lipids from the second 25ml aliquot were extracted three times with 2ml methylene chloride. The extracts were combined and reduced under a nitrogen gas stream. The extracts were used for gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), either directly or as methyl esters. Derivatization was accomplished by treating the extracts with boron trichloride (BCl_3) in methyl alcohol, and extracting the mixture with hexane. The hexane was then dried over anhydrous Na_2SO_4 (Anon., 1991).

A Hewlett Packard 5790 series gas chromatograph with a flame ionization detector was used. A J&W DB-1 0.32 mm i.d × 30 m long silica column was used for the separation with hydrogen as carrier gas. Gas chromatography-mass spectrometry was carried out on a Finnigan MAT model 8230 combined GC-MS. This is a magnetic sector instrument which is connected as the detector for a Carlo-Erba Mega series gas chromatograph. A DB-1 column was used as before with hydrogen as the carrier gas. The source was operated in the electron impact mode at 70 kV.

For heating experiments, keratin (human, horse, guinea pig and cat hair) and collagen (rawhide) samples were used. One-milligram (keratin) and three-milligram (collagen) samples were placed in 12 × 35 mm screw-capped vials containing one milliliter of "storage solution" (70% ethanol, 70% ethanol + 1% formalin, and 50% 2-propanol). The vials were heated for different periods of time at 120, 130 and 140C. The samples were then dried under vacuum using a liquid nitrogen trap. 200 L of 6N HCl was added to each. The vials were flushed three times with dry nitrogen to prevent oxidation and then they were capped using Teflon® cap liners. The samples were hydrolyzed during 24 h at 100°C. After hydrolysis they were dried under vacuum using a liquid nitrogen trap. The dried samples were then dissolved in 1 ml of dilute HCl (2 ml of 12 M HCl in 1 L de-ionized water). A St. John Model 2000 Amino Acid Analyzer was used for amino acid analysis as before.

Results and Discussion

Previous work (von Endt, 1994) reported that mammal specimens were leaching amino acids and lipids into their respective storage fluids. Analyses indicate that saturated and unsaturated fatty acids of even number of carbon atoms were leached into the preservative fluids, with C16 and C18 accounting for most of the content of the lipids found. The respective percent of each fatty acid is typical for these fats (Hilditch and Williams, 1964). Amino acid analyses showed that general, as well as structural, proteins had been released into the media by the specimens. Figure 1 plots some of the representative amino acids from collagen against those from a field mouse *Peromyscus leucopus texanus* showing the similarity between this common structural protein in natural collection specimens and the pattern from the specimen in question. But it should also be noted there are differences in the concentration of lysine. Lysyl residues contain one the reactive groups in the specimen that are involved in the formation of crosslinks that tie together the molecules in the tissue during the fixative process, especially when using aldehyde fixatives. Fixation is a preparation step in the preservation of the biological specimens that attempts to avoid any changes in the tissue, especially morphology, after death (Stoddart, 1989) and any leaching or rearrangement process will be affected by the degree of fixation. This could also account for a possible explanation of the data collected in heating experiments of structural proteins in different storage fluids (von Endt, 2000). Here samples of keratin and collagen were heated in 70% ethanol, 70% ethanol + 1% formalin (to simulate residual fixative), and 50% 2-propanol. The results showed that in general collagen leached into the solvents tested faster than keratin. Both proteins lost weight faster in 50% 2-propanol than in 70% ethanol and 70% ethanol + 1% formalin. In the case of collagen the rate of weight loss was faster in 70% ethanol than in 70% ethanol + 1% formalin. With keratin it was the opposite, losing weight more quickly in 70% ethanol. The differences in the slopes are not so marked as in collagen. As von Endt explained, one of the reasons could be the formation of additional crosslinking in collagen. As we can see in Table 1 the content of lysine in collagen is approximately 4.5% while in keratin it is 1.1%. Also note that ethanol is, aside from being a preservative, a pseudo-fixative. This type of fixative removes water from the proteins causing unwinding and disordering (Stoddart, 1989). Because of this a slower rate weight loss in 70% ethanol than in 50% 2-propanol might be expected.

To provide a few examples Figure 2 shows amino acid profiles from different specimens stored in 70% ethyl alcohol and water solution at the NMNH (von Endt, in press). The data reported in that paper cover a wide range of taxa, and it shows different deterioration patterns. Note that in all the specimens presented in the Figure the levels of ammonia found in the fluids is very high compared to the control. Ammonia is one of the final deterioration products of amino acids and proteins. It should also be pointed out that in all the chromatograms the ratio of lysine in the specimens to the lysine content in collagen (used as control) is lower. This is expected since part of the lysine content reacts during the fixation of the specimens. Nucleic acids also undergo a similar process. Figure 3 shows the lysine content for the specimens analyzed and the lysine content in collagen and keratin, the two most common structural proteins in natural history specimens.

In the past most of the specimens in natural history collections were used for taxonomic studies and the principal goal was to preserve their morphology. The main concern was dealing with deterioration process such as distortion, shrinkage, and discoloration. Today researchers have access to techniques that allow them to go far beyond taxonomic studies to examine the chemical information content of these collections. For this reason the kind of information to be preserved is different, including for instance protein composition and structure, and DNA sequences. The expansion of types of information that should be preserved requires a re-examination of the various preservation techniques.

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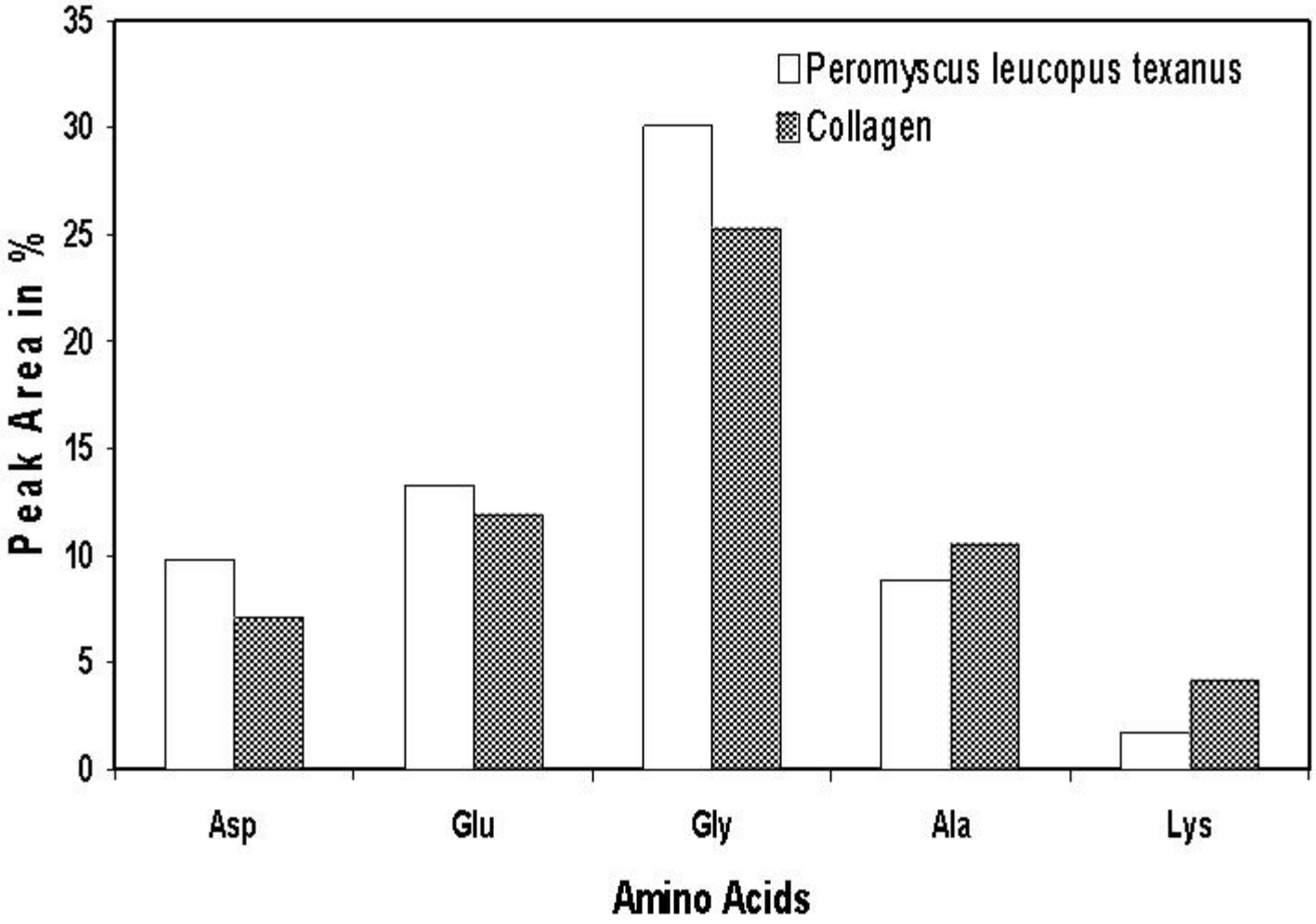
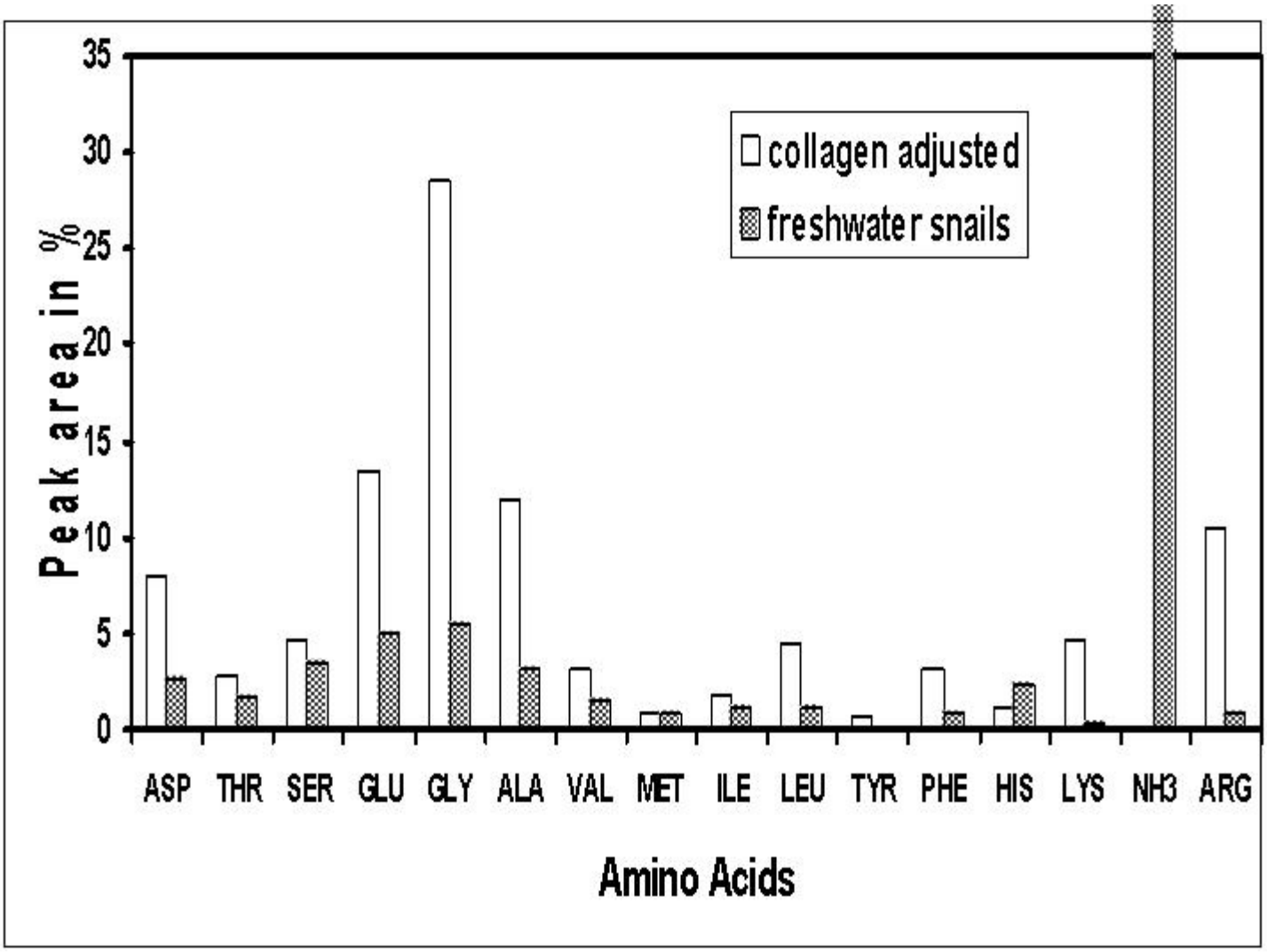


Figure 1. The amino acid profile for *Peromyscus leucopus texanus* plotted against the representative profile from collagen. The areas are reported as a fraction of the total area of the listed amino acids detected.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Peak area in %
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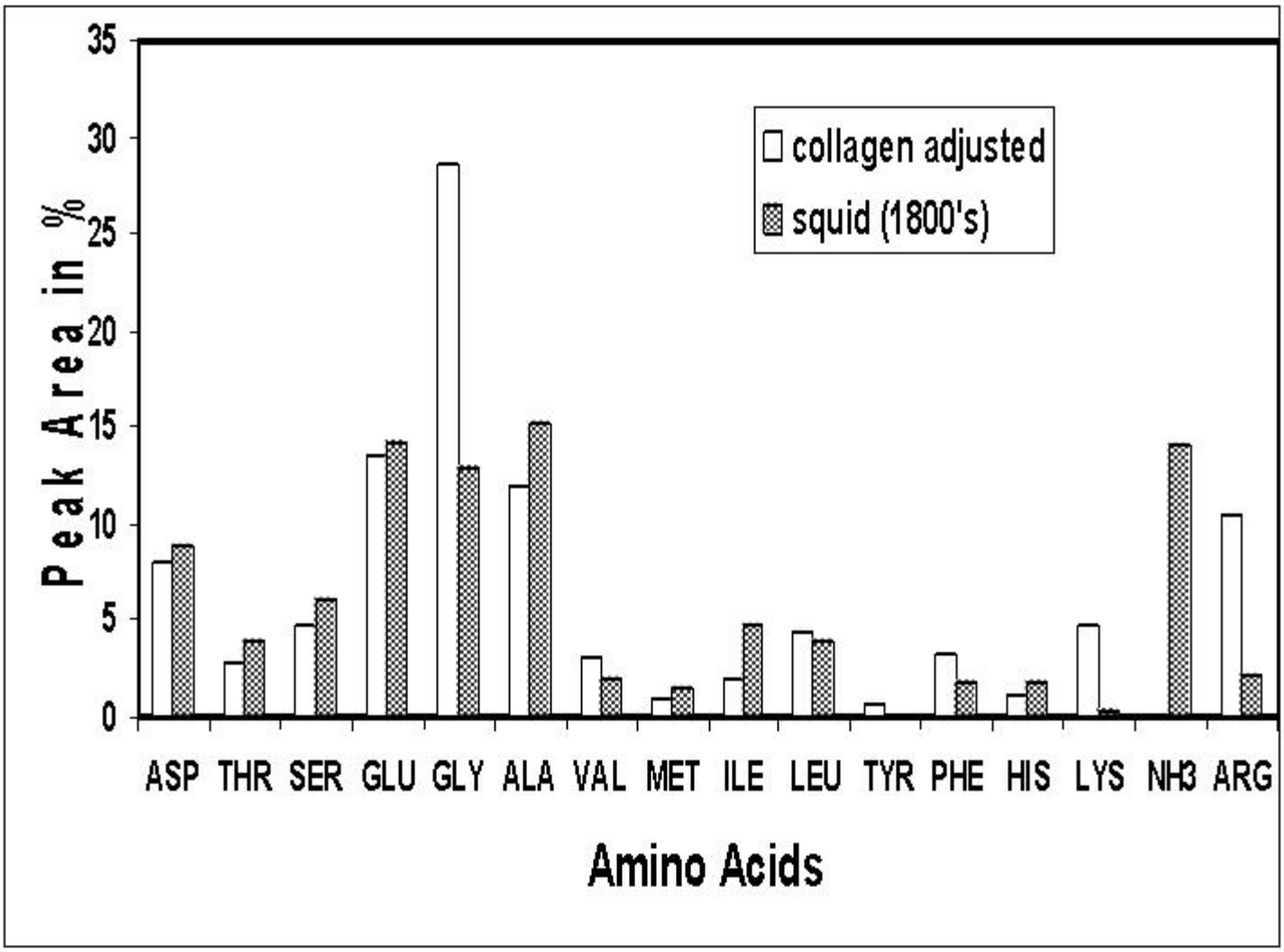
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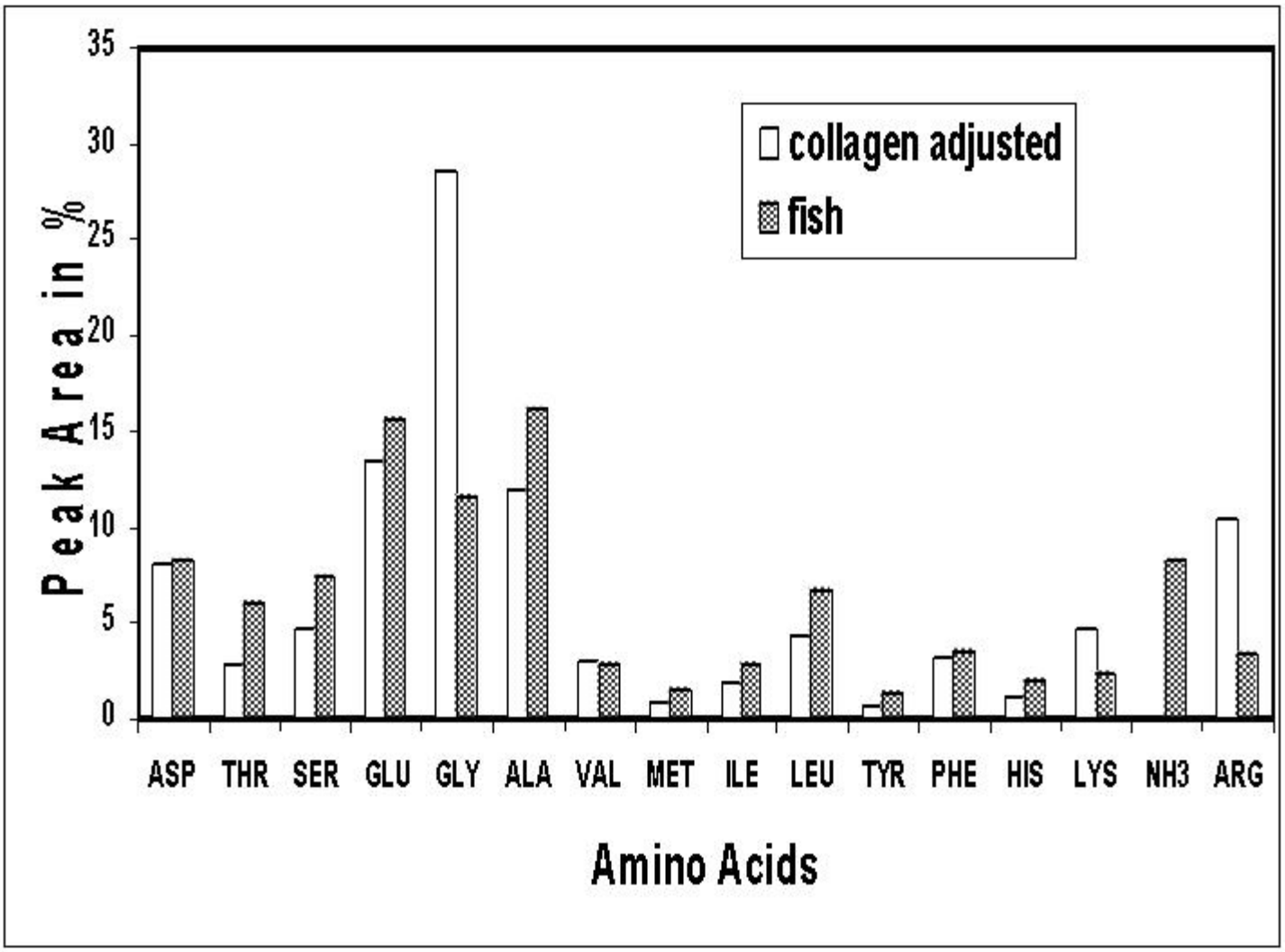

Figure 2. The amino acid profiles for freshwater snails (Fig 2a), squid (1800's) (Fig 2b) and fish (Fig 2c) plotted against the representative profile from collagen. The areas are reported as a fraction of the total area of the listed amino acids detected.

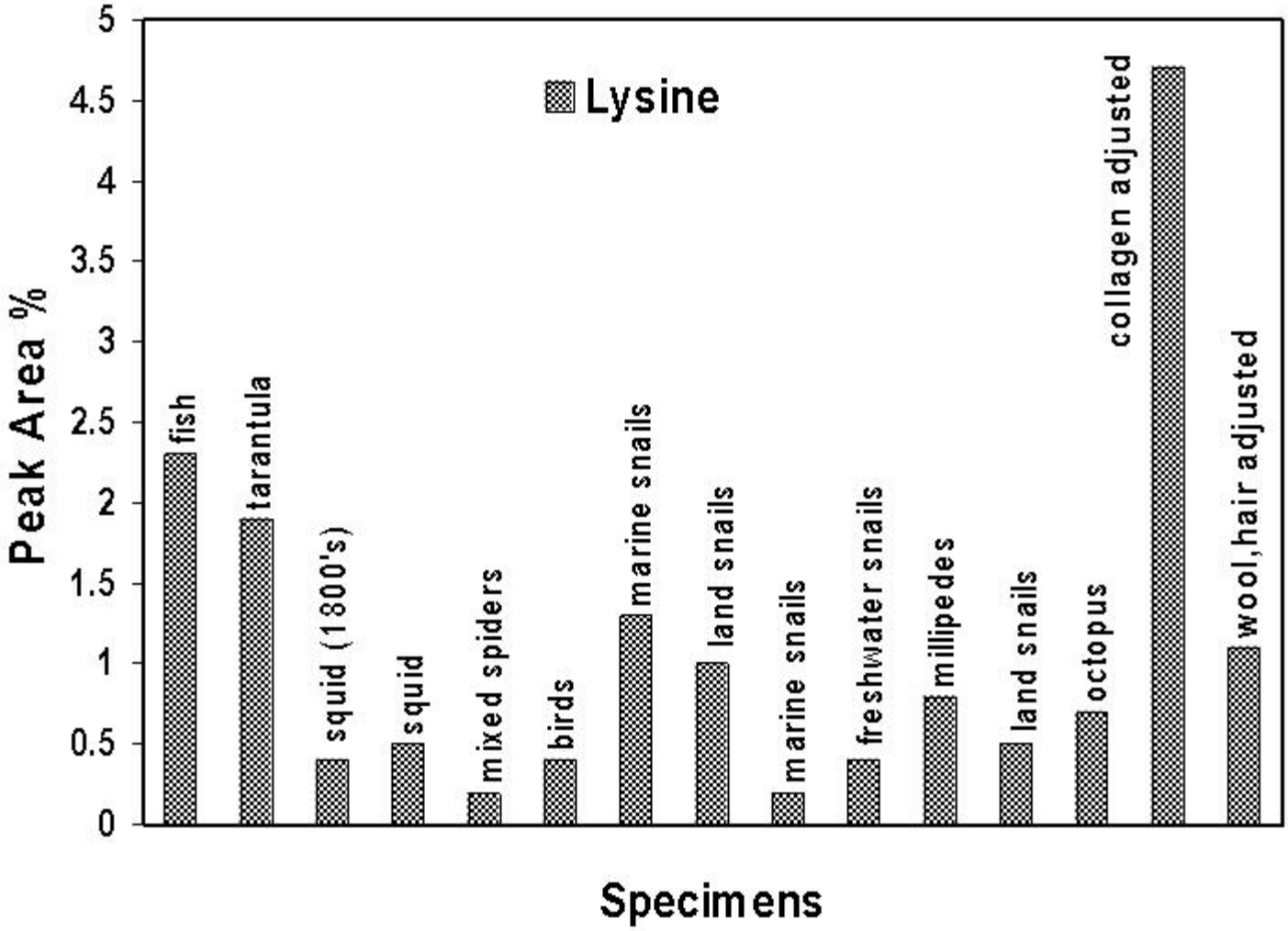

Figure 3. The lysine content from different specimens stored in 70% ethyl alcohol and water at the NMNH and from collagen and keratin. The areas are reported as a fraction of the total area of all the amino acids detected.

Amino Acids	Collagen adjusted	Wool,Hair adjusted	Horn,Feather adjusted
ASP	8	8	7.5
THR	2.8	7.4	5.7
SER	4.7	9.7	14.5
GLU	13.5	16.6	11.9
GLY	28.6	6.8	13.8
ALA	11.9	4.6	8.8
VAL	3.1	6.3	8.8
MET	0.9	0.6	0.6
ILE	1.9	4.6	4.4
LEU	4.4	9.1	8.8
TYR	0.7	6.3	3.8
PHE	3.2	4.6	3.8
HIS	1.1	3.5	1.3
LYS	4.7	1.1	0.6
ARG	10.4	10.8	5.7
Total	**99.9**	**100**	**100**

Table 1. The Parts Per Hundred of the amino acids in collagen, keratin in wool and hair, and keratin in horn and feather, adjusted to the amino acids detected.