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Molecular Characterization of Animal Glues for the Purpose of Restoration Treatments

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Abstract

Several thousand years ago, mankind perceived the gap and the need for an adhesive in order to make complicated objects consisting of two or more different or even same materials. Among all the materials that have been used throughout history, animal glue has been employed as an adhesive for several various tasks and still has kept its application. Animal glue has been used in book binding, painting binders, furniture manufacturing, to name but a few. Today, in cultural heritage field, it is being used as adhesive in restoration treatments. It is of great importance for conservators to be confident in their knowledge of the original materials they are using and introducing to the 'matrix' of cultural heritage objects in order to make proper decision in the process of restoration. Animal glues, however, are intrinsically challenging materials due to the fact that different animals' collagen proteins exhibit different behaviors or performances due to their different origin or preparation processes. Accordingly, different animal glues are used for different tasks. Therefore, it is critical for conservators to know the composing materials of animal glues they employ.

In this thesis, collagen proteins of several samples of animal glue which have been provided by restoration laboratories of both S. Orsola Benincasa, Naples, Italy and Museo del Prado, Madrid, Spain were identified by MALDI-TOF and LC-MS/MS coupled with Mascot bioinformatic tool with the goal of protein identification. Samples of S. Orsola Benincasa were analyzed by LC-MS/MS and Mascot in order to identify the specie(s) and tissue(s) they have been made of. Out of 8 samples, only one of them was made of what the label claimed to be. These results showed the importance of these experiments for the art conservation community as using known and standard materials forms a great part of their actions toward restoration of cultural heritage objects. Additionally, one of the samples, rabbit glue totten sixties, were chosen to demonstrate, in a preliminary manner, the capabilities of proteomics in the evaluation of degradation phenomenon.

Samples of Museo del Prado were analyzed by MALDI-TOF in order to demonstrate differences between the amount of information one can gain from MALDI-TOF in comparison with LC-MS/MS. Finally, one of the samples of Museo del Prado was used to improve the sample preparation protocol, by examining the hypothesis of whether or not combining two protocols could yield higher sequence coverage and better-quality spectra. Results of the series of experiments showed that adding ZipTip clean-up step after StageTip protocol increases the number of peptides in respect to what can be obtained by using a single chromatographic step.

Stay close to anything that makes you glad

you are alive.

Hafiz

To My Family

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1 Proteomics—Principles and Applications in Cultural Heritage

This chapter serves as a short introduction to principles of proteomics and its applications in cultural heritage area of study as well as to the case study of this project—animal glue. The chapter, therefore, have been divided to three main sections; the first part is the basics of proteomics as well as techniques and instrumentations which is chiefly about proteomic-based mass spectrometric strategies, the second part is about how proteomics have assisted researchers to address issues related to analyzing cultural heritage materials and several examples of it, and eventually, the last section includes a brief description of animal glue and its main component, collagen; different types; the preparation methods as well as chemical and mechanical properties of animal glues.

1.1 Proteomics

Proteomics is the discipline dedicated to the study of proteins, including their identification, quantification, and their chemical and post-translational modification [1]. The word "proteome" was proposed for the first time in 1995 to designate a protein set expressed by the genome of a cell, a tissue, and an organism at a precise moment of its development and in a precise environment [2]. While, the genome of an organism is essentially invariant and static in all its cells, proteomes vary from cell to cell, with time and as a function under developmental, physiological, pathological, pharmacological and aging conditions. Therefore, proteomics is a challenging discipline owing to proteomes' change quantitatively and qualitatively to reflect changes in the physiological state of cells, organs and organisms. As a consequence of post-transcriptional events and post-translational changes, the number of proteins that comprise a proteome is vastly larger than the number of genes that constitute a genome [3]. Today, not only does proteomics rely on the powerful analytical protein-separation technologies and the developments in mass spectrometers, but it takes advantage of the wide and growing range of genome and protein data stored in databases as well [4] so as to identify proteins accurately.

1.2 Mass Spectrometry

1.2.1 Basic Principles and Instrumentation

Mass spectrometry (MS) is one of the most powerful methodologies for identifying, structurally characterizing, and quantitating wide classes of molecules. At the end of the 1970s, wide classes of volatile organic molecules with low to medium molecular weight

could be characterized by this methodology. Later in 1990s, the introduction of soft ionization techniques, however, allowed MS to extend its applications to classes of nonvolatile, polar, thermally unstable and high molecular weight analytes. Parallel to the development of mass spectrometric instrumentation and methodologies, the improvements of separation techniques, such as gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), and of their coupling with MS made the study of complex mixtures, that are major issues in most studies, possible [5]. Basically, Mass spectrometers have three principal components: a source of ions, a mass analyzer and an ion detector. The function of the ionization source is to convert the analyte into gas phase ions in a vacuum. The ions are then accelerated in an electric field towards the analyzer, which separates them according to their mass-to-charge (*m/z*) ratios on their way to the detector (Figure 1.1). The function of the detector is to record the impact of individual ions [6]. Hereunder, these components with a special attention to the instruments that have been employed in this thesis are briefly described. More information on other types of mass spectrometers' instrument configurations can be found in [6,7].

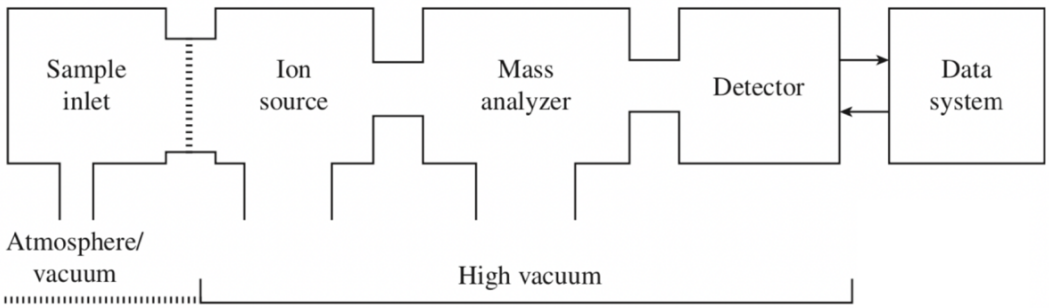


Figure 1.1 Schematic view of mass spectrometer components [7]

1.2.2 Ionization Source; Soft Ionization Techniques

Through a direct inlet or a separation technique, the sample is introduced into the ion source. The choice of ionization technique depends on different factors such as the chemico-physical properties of the analyte under investigation, its molecular weight, polarity, to mention but a few.

The term 'soft' is opposite to 'hard' and both of these terms are used to refer to the amount of energy deposited onto the molecule during the ionization process. In hard ionization techniques, among which electron ionization (EI) is the most common, a large amount of energy is deposited onto the molecular ion which results in abundant fragmentation and structural information. In soft ionization methods, however, the excess

energy deposited onto the ionized molecule is very small and stable even-electron ions are formed. This leads to easy determination of the molecular weight of the analyte. Soft ionization methods can be classified into three different groups: (a) gas phase soft ionization; (b) spray ionization techniques; (c) desorption ionization techniques [5].

Table 1.1 A technical comparison between ESI and MALDI [8]

Ionization technique	Analyte properties	Working phase	Ionizing agent	General features
Electrospray ionization (ESI)	Polar, low to very high MW molecules	Liquid	Electric field (kV)	Spray soft technique, even-electron ions, multicharged ions, no fragments, adducts
Matrix assisted laser desorption ionization (MALDI)	Polar, low to very high MW molecules	Solid	Photons (laser beam)	Desorption soft technique, protonated/ deprotonated molecules, even-electron ions, no fragments, adducts.

1.2.2.1 Electrospray Ionization (ESI)

In ESI, ions are formed from peptides and proteins by spraying a dilute solution of these analytes at atmospheric pressure from the tip of a fine capillary where a potential difference between the capillary and the inlet to the mass spectrometer results in the generation of a fine mist of charged droplets [9]. As the droplets evaporate, the peptide and protein molecules in the droplets pick up one, two, or more protons from the solvent to form singly or, more frequently, multiply charged (e.g., $[M+H]^+$, $[M+2H]^{2+}$) ions. The number of charges acquired by a molecule is roughly equivalent to the number of possible sites of proton attachment. As the droplets continue to shrink, the charge density on the surface of each droplet increases to the point where charge repulsion overcomes the forces holding the droplet and the solvated ions, contained within it, together. Ions are then 'emitted' or 'evaporated' from the droplet surface via coulombic repulsion. The ions are sampled into the high-vacuum region of the mass spectrometer for mass analysis and detection (Figure 1.2).

The typical solvent for peptides and proteins is a mixture of water and an organic modifier such as CH_3CN, and up to a few percent by volume of acetic, formic, or other volatile acid to enhance ionization of sample constituents [10].

A significant improvement in ESI technology occurred with the development of nanospray ionization. The low flow rates possible with nanospray ionization reduce the amount of sample consumed and increase the time available for analysis [2].

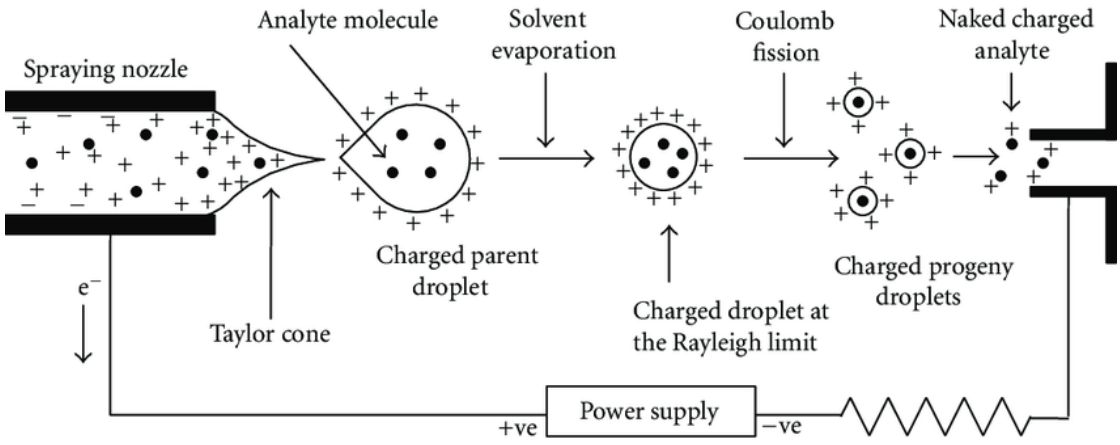


Figure 1.2 A scheme of ESI ionization technique [11]

1.2.2.2 Desorption Ionization Techniques

Matrix-Assisted Laser Desorption Ionization (MALDI)

In this method, the analyte should be mixed with a large excess of an aromatic 'matrix compound' that have strong absorption at the wavelength of the laser used with the mass spectrometer. For instance, the matrix compound a-cyano-4-hydroxycinnamic acid can absorb the energy from a nitrogen UV laser (337 nm). These two factors, matrix excess and its strong absorption, ensure that the energy from the laser pulse is absorbed by the matrix and not by the analyte, thus avoiding its decomposition [6].

The analyte and matrix are dissolved in an organic solvent and then spotted on a metallic target. Later, the solvent evaporates leaving matrix and analyte co-crystallized. The target is placed in the vacuum chamber of the mass spectrometer and a high voltage is applied. At the same time, the crystals are bombarded with a short laser pulse (1–20 ns) which causes the sample and matrix to be volatilized. The formation of ions occurs by acid–base reaction between the ionized matrix molecules and the analyte molecules. The product of the desorption process is the formation of singly charged (protonated or deprotonated) molecules of the analyte, with dimensions ranging from a few hundred daltons to several hundred thousand daltons [12]. This process is simply illustrated in Figure 1.3

1.2.3 The Analyzer

Once the ions are formed in the ion source, they are accelerated towards the mass analyzer where separation according to their *m/z* ratio occurs. The analyzer in mass spectrometer plays a key role as it essentially defines the performance of the instrument including mass accuracy, resolution, sensitivity, and the MS/MS capability [10].

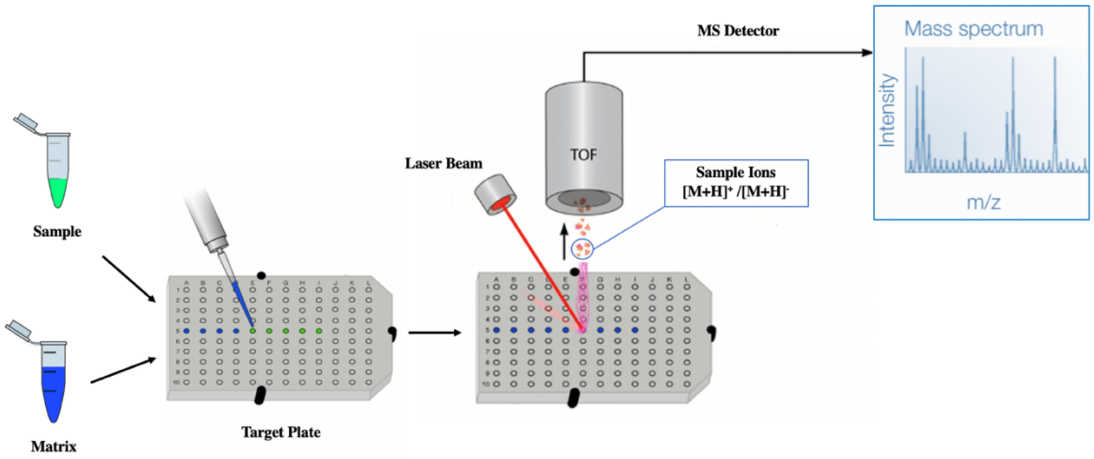


Figure 1.3 A schematic view of MALDI-TOF analysis from spotting samples on target plate to obtaining mass spectra

Analyzers are classified on the basis of separating ions according to either distance over which ions travel or time it takes for them to reach detector. Magnetic and electrostatic sectors, quadrupole, and time of flight analyzers (TOF) belong to the first group, while ion trap, Orbitrap and Fourier transform ion cyclotron resonance (FTICR) analyzers separate ions in time [5].

1.2.3.1 Time of Flight Analyzer

The TOF analyzer separates ions according to the time they spend travelling inside the flight tube. The ion beam formed in the source is accelerated towards the flight tube. Since the kinetic energy (E_κ) is related to the mass (m) by Equation 1.1 a charged species with high molecular weight will take more time than a low molecular weight compound to reach the detector [5,13]. Therefore, from the flight time it is possible to determine the *m/z* ratios of the ions as follows:

$$E_\kappa = \frac{1}{2}mv^2 \qquad \textbf{Equation 1.1}$$

Where v is velocity and (L) is the flight path divided by the time (t) it takes the ion to travel over that distance:

$$v = \frac{L}{t} \qquad \textbf{Equation 1.2}$$

Replacing this expression for v in Equation 1.1 gives:

$$E_\kappa = \frac{mL^2}{2t^2} \qquad \textbf{Equation 1.3}$$

Thanks to many improvements through years, due to the introduction of "reflector" in the instrumentation of TOF analyzer, all ions with the same amount of *m/z* values but different kinetic energy arrive at the same time to the detector, resulting in narrow peaks and higher resolution (Figure 1.4).

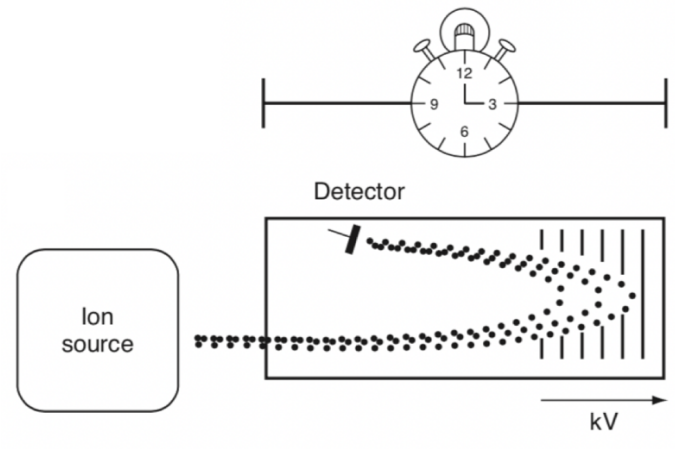


Figure 1.4 A scheme of TOF analyzer with reflector [5]

One of the significant advantages of the TOF analyzer is that it has no restriction on the *m/z* range which enables it to analyze ions with *m/z* values ranging from a few hundred to 500000 and higher. This is an important feature for its coupling with MALDI in the study of large molecules. In fact, as ions produced by MALDI are generally singly charged species, their *m/z* values can be extremely high and an analyzer able to explore such high *m/z* ranges is of great importance. Owing to its scan speed, high resolution and mass accuracy capabilities, TOF is currently coupled with all ionization techniques [5].

1.2.3.2 Orbitrap Analyzer

In orbitrap, ions are trapped in an electrostatic field produced by two electrodes: a central spindle-shaped and an outer barrel-like electrode (Figure 1.5). Ions are moving in harmonic, complex spiral-like movements around the central electrode while shuttling back and forth over its long axis in harmonic motion with frequencies dependent only on their *m/z* values [5]. The oscillating ions create electric current in the outer electrode, and the mass spectrum is obtained by Fourier transform of the recorded current. The longer the current recording period the better is the resolution [14].

1.2.4 Tandem Mass Spectrometry and MS_n

In tandem MS experiments two (MS/MS or MS_2) or more (MS_n) consecutive stages of mass analysis are used in order to examine selectively the decomposition of given ions, occurring out of the ion source, in a mixture of ions.

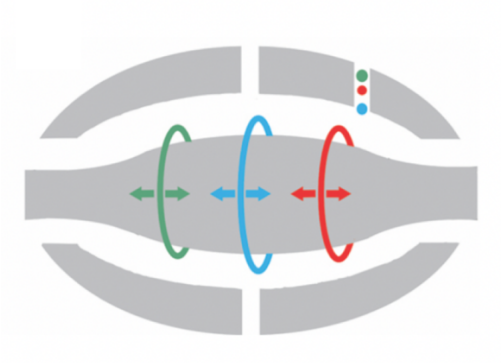


Figure 1.5 Ions enter orbitrap through the opening in outer electrode. Under the influence of an electric field they start to move around and along the central electrode creating concentric rings. Motion of ions induce image current in the outer electrode which is recorded and used for determination of *m/z* ratios of ions by Fourier transform [14].

The most common reactions that cause ion decomposition at this step are called unspontaneous decompositions, namely collision-induced dissociation (CID) and collision-activated decomposition (CAD) reactions. Accordingly, different kinds of experiments can be carried out by tandem MS; the most common, however, is the product ion scan. This is carried out by selecting ions with a given *m/z* value among all those formed in the source and detecting selectively their product ions formed due to CIDs. In this regard, the first analyzer is fixed in such a way that only ions with a specific *m/z* value can pass through it, while all the others are eliminated. The second analyzer scans over a *m/z* range in order to detect all the product ions formed owing to CIDs in a collision cell located in between the two analyzers. To increase the efficiency of collisions, a voltage (collision energy) is applied to the collision cell [2]. Triple-quadrupole (QqQ) mass spectrometers are the most common of this kind. The two, or more, analyzers constituting the tandem may be the same, for example quadrupoles, but their coupling can be also hybrid, among which are double focusing mass spectrometers (BE), Quadrupole ion trap (QIT) and Q-TOF. These processes occur for analyzers separating ions in space. In the case of analyzer separating ions in time, however, the three main processes—ion separation, CID and analysis of product ions—occur inside the same analyzer by just changing the forces acting on the ions over time [5].

Peptide precursor ions, dissociated by the most usual low-energy collision conditions, fragment along the backbone at the amide bonds, forming structurally informative sequence ions and less useful non-sequence ions by losing small neutrals like water, ammonia, etc. The amino acid backbone has three different types of bonds (NH-C_{α}H, C_{α}H-CO and CO-NH) and each of them can be fragmented originating different fragment ions. Each bond breakage gives rise to two species, one neutral and the other

one charged, and only the charged species is monitored by the mass spectrometer. Hence there are six possible fragment ions for each amino acid residues and these are labeled with the a, b, and c ions having the charge retained on the N-terminal fragment, and the x, y and z ions having the charge on the C-terminal fragment (Figure 1.6). The most common cleavage sites are at the CONH bonds which give rise to the b and/or the y ions [2,15].

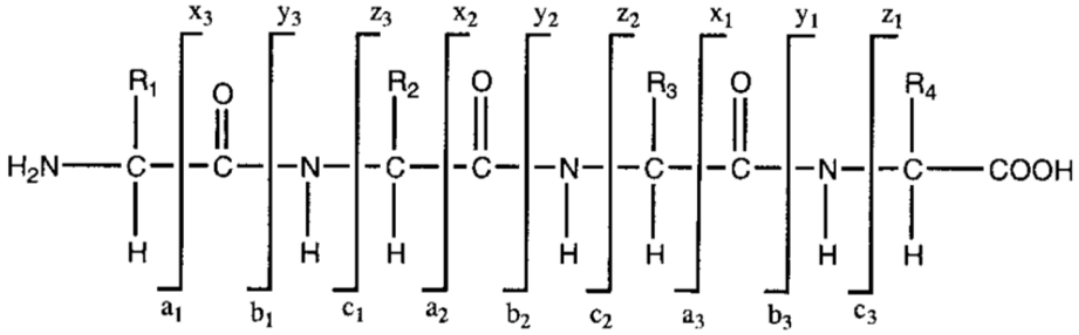


Figure 1.6 Peptide ion fragmentation nomenclature. Low-energy collisions promote fragmentation of a peptide primarily along the peptide backbone. Peptide fragmentation which maintains the charge on the C-terminus is designated a y-ion, whereas fragmentation which maintains the charge on the N-terminus is designated a b-ion. The other additional types of fragmentation are also indicated [2].

1.2.5 The Mass Spectrum

The mass spectrum is a two-dimensional graph that reports the *m/z* ratio of ions and their relative intensity. The most abundant ions are assigned as 100%. The appearance of the mass spectrum is greatly determined by the amount of energy deposited on the molecule during the ionization. If it is high, the mass spectrum is characterized by the molecular and fragment ions. However, if the amount of energy deposited on the molecule during the ionization is small, as occurs in soft ionizations, the mass spectrum shows only the presence of the protonated/deprotonated molecule, and eventually a few adduct ions but not fragment ions [5].

1.2.6 Database Searching and Interpretations

The goal of database searching is to be able to quickly and accurately identify large numbers of proteins. The success of database searching depends on the quality of the data obtained in the mass spectrometer, the quality of the database searched, and the method used to search the database [2]. Generally, the information produced by the mass spectrometer, such as lists of peak intensities and mass-to-charge (*m/z*) values, can be manipulated, compared and matched with lists generated from 'theoretical' digestion of a protein or 'theoretical' fragmentation of a peptide to identify the unknown sample.

Basically, sequences in the database are digested in silico, masses of peptides calculated and compared to experimentally obtained precursor masses. When a match is found, a theoretical fragmentation spectrum is created and compared to the measured MS/MS spectrum. Moreover, the number of hits which means the number of experimentally obtained peptide masses matching theoretical masses in the database, is reflected by the so-called score; a higher score means a higher reliability of protein identification. Bearing in mind that the MS/MS spectra only identify peptides, the last step, therefore, is to use identified peptides to produce a list of possible proteins present in the sample. At least two independent peptides are required for a reliable identification of a protein. When an identified peptide is unique, specific protein recognition is straightforward; however, some peptides might be present in multiple proteins due to the fact that homologous proteins from the same organism (or closely related organisms) have similar sequences.

1.3 Proteomics' Mass-Spectroscopic Based Approaches

Strategies for mass spectrometric peptide analysis are common to modern and archaeological samples. In general, there are two main approaches which have been employed on cultural heritage objects; peptide mass fingerprinting (PMF) and amino acid sequencing of the peptide using tandem mass spectrometry (MS/MS). The most common proteomic approach, so-called bottom-up approach, is the same for both of these methods and composed of several steps, including the hydrolysis of the proteins, the MS-based analysis of the resulting peptides, and data handling using bioinformatics tools. The schematic process of protein identification by these two methods is illustrated in Figure 1.7. Briefly, in PMF analysis, an enzyme is used to cut polypeptides at specific sites under defined conditions. This results in a mixture of peptides that are characteristic for each protein which serve as fingerprint for the protein. Almost always, trypsin is the protease of the choice, owing to its being highly specific and stable. Moreover, tryptic peptides are generally of proper length for MS analysis [10]. Trypsin is a serine endopeptidase with high specificity for peptide bonds behind positively charged amino acid residues of lysine and arginine—if the next amino acid to them is not proline. It has an appropriate pH-optimum and is commercially available in high purity and quality [12]. Finally, the accurate determination of peptide *m/z* ratio, typically using a MALDI-TOF mass spectrometer, permits the identification of the unknown protein by matching the resulting peptide masses with the theoretical peptide masses of proteins in a database (such as NCBI and Swiss-Prot).

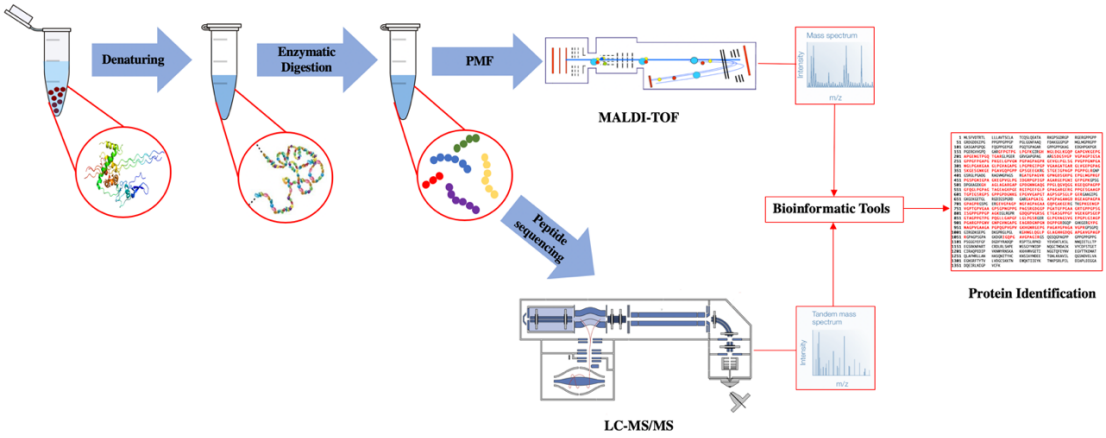


Figure 1.7 A schematic process of protein identification from the sample pre-treatment to bioinformatic data analysis step

For peptide sequence analysis, peptides obtained by proteolytic cleavage are subjected to MS/MS to be fragmented along the amide backbone. The amino acid sequence of the peptide is then obtained from the differences in *m/z* ratios for a series of daughter ions. Subsequently, the sequence ions and the intact peptide masses are matched against protein databases to identify the unknown protein. Since Protein identification by PMF is based on the peptide masses solely, this method may fail when analyzing highly modified proteins or complex protein mixtures (it increases the complexity of the peptide mass fingerprint) [16]. This issue, however, could be overcome by employing LC-MS/MS since it offers the possibility to determine peptide backbone sequence and its modifications.

LC-MS/MS is considered as a great tool in proteomics with respect to its ability to offer information about degradation phenomenon in the cultural heritage objects since peptide fragmentation facilitates the detection of post-translational modifications (PTM) sites. PTM of proteins are chemical or enzymatic modifications of amino acids after protein synthesis that change their molecular weights which can be then measured by mass spectrometry techniques. An example of PTMs that can be observed is oxidation of methionine (mass shift of +16 Da) that can occur both *in vivo* and *in vitro* as well as during sample manipulation. A high number of hydroxyproline and hydroxylysine modifications that occur in fibrillar collagens are reliable indicators of the collagen presence, and therefore animal glue when dealing with cultural heritage objects. Amongst all, deamidation in particular has gained growing attention since it is being considered as an aging marker. Deamidation (mass shift of +0.984 Da) is a non-enzymatically occurring

post-translational modification by which asparagine (Asn or N) and glutamine (Gln or Q) residues transform to aspartic acid (Asp or D) and glutamic acid (Glu or E) respectively by losing an amino group. Since this process continues postmortem, it could indicate the amount of damage proteinaceous substances have undergone. Since Gln deamidation happens at a slower rate in comparison with Asn deamidation, Gln might be considered to be a more useful indicator of again when it comes to cultural heritage objects [17].

In Table 1.2, MALDI-TOF and LC-MS/MS techniques have been compared to each other. It goes without saying that the choice of employing one over the other relies on the purpose of the research.

Table 1.2 A comparison between two common methods of proteomics in cultural heritage; PMF and LC-MS/MS

MALDI-TOF	LC-MS/MS
Protein identification on the basis of peptides' masses	Protein identification on the basis of peptide sequencing
Not reliable for complex mixtures or when proteins are highly modified	Compatible when complex mixture of proteins
The fastest and cheapest method of protein identification	Costly and time-consuming
Information on simple samples with large databases	- Information on taxonomy and tissue - Information on degradation rate (deamidation)
The technique is completely automated and so is for large-scale proteomics work	The technique is more complex and less scalable

1.4 The Application of Proteomics in Cultural Heritage

Characterization and identification of cultural heritage objects' [1] consisting materials—organic and inorganic parts—is of significant importance in the field of archaeological science and art conservation. Generally speaking, taking into account a unique object solely, information regarding the composing material of an artefact not only improves ones' knowledge about the technique, level of technology of past and the material artisans have had at their disposal, but also helps understand the conservation state of the object in order to make a proper decision for the purpose of restoration treatments. Moreover, it aids with attributing objects to a particular artist and even in differentiating fakes from originals. In terms of archaeological artefacts, however, fragmentary objects such as bones and pot sherds that do not pose an aesthetic value could provide information about the diet, domestications and trades. Nonetheless, studying

[1] The objects that are of importance in terms of their historical and cultural values .This definition includes archaeological finds that even though may not be unique but can provide information regarding past's traditions, lifestyle, to name a few.

cultural heritage materials is not a straightforward task due to the fact that cultural heritage objects, and in particular organic materials, have undergone deterioration process—the amount of which however depends on the age and environmental conditions—and are mostly presented in complex matrices of organic and inorganic materials, and as a consequence imposes great challenges. Despite this, developments in analytical techniques have helped researchers to a great extent to encounter issues related to these matters. New developments, for instance, in the technologies and methodologies of separation science, such as new stationary phases enabling more sensitive and selective chromatographic performance, as well as nanoscale chromatography combined with mass spectrometers with higher sensitivity and resolution have led to much better sequence coverage and the ability to measure small amounts of protein [18].

The first paper to analyze proteins applying proteomics in archaeology have been published in 2000 [19]. As it can be inferred, the application of proteomics in cultural heritage is quite a recent trend. Nevertheless, its potentiality in this field has been examined by different research groups who seek various purposes such as degradation rates as well as identification and characterization of proteins to the level of taxonomy and the kind of tissue in various artefacts which goes from the range of textile, leather, parchment as well as bone and ivory to adhesives and binders and even the organic residues which have been left in pottery sherds and ceramics.

Thanks to the high-throughput and high-resolution of mass spectrometers, proteomics approaches came into archaeology and anthropology to aid identifying ancient proteins to the taxonomic and tissue level by the name of palaeoproteomics [20]. Generally, palaeoproteomics is used to describe a discipline in which mass spectrometric approaches are employed to study ancient proteomes [21]. One of the very interesting approaches in archaeology is a method called ‘ZooMS’ which have been coined by Michael Buckley and Matthew Collins and is short for ZooArchaeology[2] by Mass Spectrometry [22]. This method is based on analyzing proteins from fragments of bone, ivory, or skin to identify animal species that might otherwise be undistinguishable due to these fragments being very small, and therefore there is no way for zooarchaeologist to differentiate these fragments on the basis of their shapes. Moreover, proteomics coupled with gas chromatography mass spectrometry (GCMS) analysis has proved to be a promising approach to obtain the contents of cooking pots and storage or simply food

[2] Zooarchaeology is a broad and interdisciplinary field which studies animal remains from archaeological sites, aiming to learn about the interactions of people and animals and the consequences of these relationships for people and their environments [53].

vessels which have been left behind as residues, as the combination of these two allows for analysis of lipids, polysaccharides as well as animal and vegetable proteins [23].

More recently, beside the identification of proteinaceous materials, particular attention has been placed on deamidation as a potential aging marker to study deterioration process in archaeological objects. Since this process continues postmortem, it could indicate the amount of damage proteinaceous substances have undergone and thereby, could shed light onto the conservation state of an object, deterioration degree or aging and the possible factors that could play a role in this respect. A number of applications of proteomics on cultural heritage can be seen in Table 1.3.

Table 1.3 Applications of proteomics in cultural heritage

Object	Technique	Objective	Highlights	
Fossil Bones	ZooMS (MALDI-TOF)	Taxonomy/ tissue characterization of fossil bones [24–26]	Species identification of collagenous tissues/ Deterioration	*Identification/ Deterioration*
	LC-MS/MS	Determining the extent of deamidation in collagen as an aging marker [27–29]		
Paintings/ others	MALDI-TOF / LC-MS/MS	Characterization of proteinaceous binding medium (animal glue [30,31] and egg-based [32–34] or others [17,35–42])	Painting technique/ Restoration treatments	
Pot sherds	LC-MS/MS	Identification of the biological source of protein remains (taxonomy and tissue level) [23,43,44]	Past diets/ Culinary practices/ Animal domestications	

1.5 Animal Glue—A Brief Introduction

Animal glues are natural polymers derived from mammalian or fish collagen and that is where they have taken their adhesion property and their tendency to aggregate into complex supra-molecular structures [12]. Beside collagen, which is the main constituent of animal glues, other materials could have been added to glues to enhance their properties. Therefore, due to various glues' treatments and recipes as well as different animal sources to prepare them, animal glues may differ from one another on the basis of their appearance, chemical composition and physical properties [45,46]. Typically, animal glues have been employed throughout history as adhesives, consolidants, and binders. Today, in the field of cultural heritage, animal glues are still being used for conservation and restoration of artefacts. Showing various empirical performances at the time of binding and drying, over the years, different kinds of animal glues have been used to best fit specific purposes. Since a wide variety of animal glues exist on the market, it is of critical importance for restorer who deal with unique objects to know best the

consisting components of materials they use regularly. Nevertheless, the information on the animal glues' source, treatments, and additives (such as preservatives of some kind (e.g. sulphur dioxide) [46]) with which restorers are provided may not as accurate and reliable as it should be and cause undesired results, and hence, emphasizing on the necessity of employing analytical techniques to assess information about their compositions.

1.5.1 Classification of Animal glues

Basically, Animal glues are classified according to their source of extraction. Generally, hide glues are prepared from bovine skins and those of smaller mammals, for the most part, but sometimes connective tissue as well. Bone glues, however, are made of from cattle and pig fresh bones or sometimes extracted bones to a great degree. Hide and bone glues are produced and sold as coarse powders, pearls, cubes, and cakes or plates. Additionally, regarding the degree of purification of animal glues, they could be divided from lesser purified ones known as strong glues (used mainly as adhesives) to the pure ones, that almost entirely consist of collagen, so called gelatin. Commercial gelatins may be obtained from either skin or bone sources and are supplied in the form of thin sheets, plates or powder [47].In contrast, since strong glues are made from boiling animal bones coming from slaughterhouses, the prime source and their compositions are not clear. However, their elasticity and reversibility have made them an ideal type of glue for wooden objects' restorations [45]

The purest form of fish glue is known as isinglass and is made almost entirely from the swim bladders of fish. The glue extracted from the swim bladders of sturgeon (*Acipenseridae* sp.) is known as sturgeon glue. Other type of fish glue is much less expensive product and is extracted from heads, skins and skeletal waste from cod (*Gadus* sp.), haddock (*Melanogrammus aeglefinus* sp.) and mackerel (*Scomber scombrus* sp.) and other fish as a by-product of the fresh and frozen food markets [45]. In medieval Europe fish glues were used as a size for parchment and as a medium or binder for paintings or polychrome wooden objects. The property of fish glue to adhere well to the porous surface of parchment made it a useful material for the illumination of manuscripts with gold leaf and painting. In China it was used as a binding medium by calligraphers. It has been used by conservators as an adhesive and consolidant [45,48].

1.5.2 Animal Glues' Preparation Methods

In order to make glue out of collagen, it should be transformed from insoluble

collagen to soluble gelatin which can be carried out by hot water extraction, during the process of denaturing. Usually either acidic or basic pre-treatment is prerequisite for most kinds of collagens. Extraction process breaks H-bonds in the triple-helix structures of the collagen which results in disordered 'random' coils of single protein chains. The temperature (T_d) at which denaturing occurs depends on the chemical structure of proteins particularly on the amount of proline (Pro) and hydroxyproline (Hyp) due to their being responsible for the stabilizing H-bonded water bridges in the triple helix. These two amino acids are present in mammalian collagen to a greater extent than in marine collagens. As a result, adult mammalian collagen denatures at 40–41°C, while lower temperature for isinglass and other fish collagens is adequate.

Generally speaking, the harsher the conditions of extractions (higher T_d, longer process, more extreme the pH), the more bonds cleave that makes proteins with lower molecular weights. As it is pointed out earlier, the severity of each of these factors is linked to the prime source of collagen. For instance, mild extraction is suitable for the hides of young mammals, as well as all fish skin and swim bladders, because they are rich in collagen and the collagen is not so strongly stabilized by the additional chemical bonds that develop in older mammals. Furthermore, glues that are derived from fish cleave more easily on extensive heating than those of mammalian origin owing to their chemical structure [46]. A summary of a comparison between different animal glues in terms of their chemical and mechanical properties is reported in Table 1.4.

1.5.3 Collagen in Cultural Heritage

On the grounds that one of the factors that makes analyzing cultural heritage objects challenging is related to the small quantity of materials while this situation could be exacerbated when analyzing organic materials which by nature are more prone to degradation, collagen could be a suitable candidate to be investigated by proteomic approaches; considering that collagen is the most ubiquitous protein in the animal kingdom. Moreover, collagen is known to persist for a long time (it offers studying archaeological fossil bones) and could even endure thermal degradation (it aids in studying past diet and food). Among the wide range of proteinaceous materials that proteomics approaches can be applied, in the present thesis, the focus is on collagen protein to study animal glues' source and tissue of origin.

Table 1.4 Comparison of the properties of different glue types. The glues are qualitatively ranked relative to one another for each property [46]

Molecular Weight	*Gel / Bloom Strength*	*Degree of Helicity*	*Viscosity*	*pH*	*Mechanical Strength*	*Elasticity*	*Stress Development in RH*	*Stability in Fluctuating Environment*
Bone Glue								
L-M	L-M	L-M	L-M	5-7	L-M	> hide glue but also more brittle	M	< hide / rabbit skin glue
Hide glue								
H	H	M-vH	M-H	6.5-7.4	H	< bone glue	H	> bone glue
Rabbit Skin Glue								
H	H	H-vH	H	5-7.5	H	> *hide glue*	H	less sensitive to moisture than hide glue
Isinglass								
c.150000 and higher up to 300000	M-H	M-H	Highest	6-7.5	H	> *hide glue*	vH	> mammalian gelatin
Fish Gelatin (from fish skin, bone and cartilage)								
96000–196000	L-M	M	M-H	3.5-5	M	> mammalian gelatin	M	n.a.

L: low, M: medium, H: high, vH: very high, and n.a.: data not available

1.5.4 Collagen Structure

Collagen consists of three polypeptide chains, each of which composed of naturally occurring amino acids that are linked by covalent peptide bonds in a repeating pattern in their sequences, that wound very tightly around each other to form a triple helix structure (Figure 1.8). Generally speaking, collagen chains are made of the triplet of Gly-X-Y where X and Y could be any amino acid. However, X is often proline and Y hydroxyproline (post-translational modification). Hydroxyproline is only present in some few other animal proteins and could be served as a marker for animal glue. The spatial conformation of these three amino acids, however, plays a key role in the structure of collagen. The large side chains of proline and hydroxyproline force the chain in sharp twists and forms kinks. Whereas glycine, which is the smallest amino acid because it does not have side chain, is the only amino acid that can fit to this space and hence is required at every third position [49].

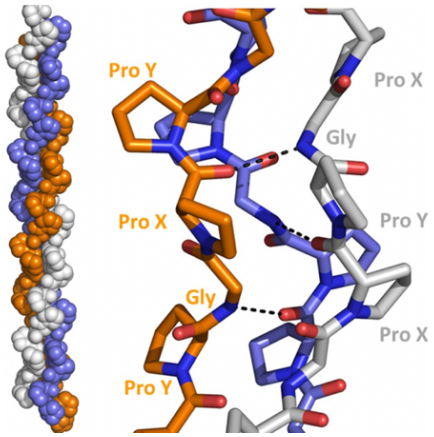


Figure 1.8 Collagen triple helix structural and the sequence of amino acids

Collagen occurs in all multicellular animals and is the most abundant protein of vertebrates, comprising almost 30% of their protein mass. It is an extracellular protein that is organized into insoluble fibers of great tensile strength. Vertebrates have 46 genetically distinct polypeptide chains making up 28 collagen types that occur in different tissues, where some are comprised of three identical chains (homotrimers) while others are made up of genetically distinct chains (heterotrimers). Among all, only types I to III are found in the connective tissues used for making animal glues where collagen type I is the most abundant collagen [50]. Table 1.5 shows the composition and the distribution of the most abundant collagens in tissues.

Table 1.5 A brief highlight on most abundant collagens [50]

Type	Chain Composition	Distribution
I	$[\alpha1(I)]_2\alpha2(I)$	Skin, bone, tendon, blood vessels, cornea
II	$[\alpha1(II)]_3$	Cartilage, intervertebral disk
III	$[\alpha1(III)]_3$	Blood vessels, fetal skin

2 Experimental

The case studies of the present thesis are several animal glues which have been given to the laboratory of the department of chemical science of Università degli Studi di Napoli Federico II by the restoration laboratories of both S. Orsola Benincasa, Naples, Italy and Museo del Prado, Madrid, Spain. In spite of what the labels of these animal glues declare, the restorers experienced diverse behavior of animal glues that was supposed to be from same source from which the glues have been manufactured. For this reason, the laboratory was asked to provide a report on the protein content of these samples to examine this hypothesis that whether or not the different behavior of the animal glues has something to do with their animal source and tissue.

2.1 Materials

2.1.1 Chemicals and Reagents

The reagents that have been used in the experiments are as follows: Ammonium hydrogen carbonate (AMBIC) (Fluka), Urea (Fluka) and trypsin were from Sigma; Formic acid (FA) and Acetonitrile (ACN) were purchased from Baker. Deionized water was obtained from Millipore cartridge equipment.

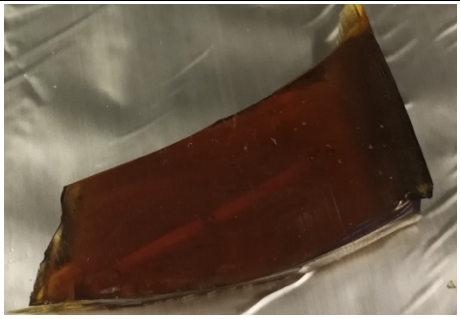
2.1.2 Samples

Totally 8 animal glue samples in different shapes and from various sources have been provided by the restoration laboratory of S. Orsola Benincasa, Naples, Italy for protein identifications. The name of the samples along with their reported source and their image can be found in Table 2.1. In addition, images of 3 animal glue samples provided by Museo del Prado, Madrid, Spain can be found in Figure 2.1.

Strong Glue Sample (8) Fish glue Sample (4) Rabbit Glue Sample (7)

Figure 2.1 The list of animal glue samples provided by Museo del Prado restoration laboratory

Table 2.1 The list of animal glue samples provided by S. Orsola Benincasa restoration laboratory

Types	Name of the Samples	
Rabbit Glue	 Rabbit Glue Toten (sixties)	 Rabbit Glue (of the first postwar)
	 Rabbit Glue (CTS)	 Rabbit Glue (Giosi)
Fish Glue	 Fish Glue (flakes)	 Sturgeon Fish Glue (pearls)
Strong Glue	 Strong Glue (pearls)	 Strong Glue (old manufacturing)

2.2 Methods

2.2.1 Sample Preparation

In brief, sample preparing for mass analysis consists of pre-treatment, protein digestion as well as clean-up and purification steps which will be explained in detail below respectively.

2.2.1.1 Sample Pre-treatment

Since it is important for protease to have access to the polypeptide chains in order to cleave them at specific sites, denaturing step have been carried out before protein digestion step in order to alter the shape of protein in some way to increase the probability of tryptic cleavages. Denaturing can be considered as a sort of opening of the three-dimensional structure to facilitate protease access to cleave sites. To this end, 10 µL of a solution of 6M Urea were added to the samples (≈1mg) and incubated for 10 min at RT, followed by sonication for 20 min. Urea was then 6-fold diluted with 50 µL of AMBIC 10 mM. This dilution step is required to make trypsin work, since trypsin itself is a protease and does not work on the presence of high denaturant concentration.

2.2.1.2 Protein Digestion

After Urea pre-treatment of the samples, enzymatic digestion was accomplished by adding 25 µL of trypsin (following the ratio of 50:1 sample/trypsin (w/w)) to the solution. Afterwards, the solution is incubated at 37°C for 16 hours. The reaction was stopped by adding 10 µL of FA 10% in Milli-Q water in order to lower the pH to around 3 and cooling down. Finally, peptides were extracted and vacuum dried.

2.2.1.3 The Concentration and Purification of Peptides on the Reverse Phase

After protein digestion step, peptide mixture usually needs to be cleaned to remove salts, which otherwise will damage the LC switching valves or clog the columns. Most importantly, however, salts impair mass spectrometric detection since they are, on their own, charged species, thus competing with peptides, resulting in peptide ions suppression.

In this step, peptides are concentrated and cleaned through their binding to a small quantity of reversed-phase material and eluted in organic solution, prepared for MS analysis. This step is of crucial importance for the overall quality and sensitivity of the analysis and robustness of the experiments. For this purpose, the stop-and-go-extraction tips (StageTips) or ZipTips (Millipore) could be used. The latter is rather costly and have limited capacity in comparison to the former. ZipTips are 10 µL pipette tips with a bed of

chromatographic support fixed at the end for concentrating, desalting and fractionating samples. Although ZipTips are commercially available, StageTips can be self-made by placing a small portion of Empore material in an ordinary pipette tip. Empore disks have chromatographic beads immobilized in a Teflon meshwork. From the commercially available large sheet, a small disk is stamped out using a blunt-ended syringe needle. The portion size is determined by the inner diameter of the needle and can thus be adapted to the size needed. In the present thesis, C18 materials have been used to pack the StageTips. Nonetheless, other materials for different purposes of desalting and fractionation can be packed [51,52].

The ZipTips C18 Pipette Tip

In this step, digested samples were resuspended in 10-20 µL of Milli-Q H_2O (FA 0.1%) to be prepared for being refined and desalted using a reverse-phase C18 ZipTip pipette tip (Millipore). By so doing, the peptides that are rather hydrophobic are retained on the reverse phase, while the inorganic salts stay in solution and can be easily washed out from the microcolumn.

To this end, the ZipTip C18 pipette tip was pre-conditioned with a wetting solution (ACN-Milli-Q H_2O solution (50/50 (v/v))), in order to activate the column, followed by an equilibration solution (0.1% FA). After conditioning, samples were carefully loaded on the C18 pipette tip. The organic salts and other unretained compounds passed through the tip, and thus were removed and discarded. The residual contaminants and salts that might be still on the column were washed from the tip with a slightly acidic water using water with 0.1% FA. Peptides were then eluted in a mixture composed of (ACN– Milli-Q H_2O solution (50/50 (v/v))) acidified with 0.1% FA. Finally, the solvent was evaporated by vacuum centrifuge and the samples were prepared for the next step.

StageTips—the Standard Protocol

To begin with, digested samples were resuspended in 50 µL of Milli-Q H_2O (FA 0.1%). Afterwards, StageTips were pre-conditioned with 150 µL (ACN-Milli-Q H_2O solution (50/50 (v/v))), followed by an equilibration solution (0.1% FA). Samples were subsequently loaded on the columns. What did not bind to the columns was kept as 'unbound'. Next, the columns were washed by slightly acidic water using 150 µL water with 0.1% FA and again what was left in the Eppendorf tubes was kept as 'wash' for further analysis of the present peptides. Finally, the peptides bound on the C18 resins were eluted by 150 µL (ACN– Milli-Q H2O solution (50/50 (v/v))) acidified with 0.1%

FA. The ‘elute’ sample were kept and vacuum dried. This protocol has been employed for the purification of reference samples (rabbit glue (of first post war) and strong glue (old manufacturing)) in the next chapter.

StageTips C18—the Current Lab’s Protocol

The extracted peptides were dissolved in 50 µL of Milli-Q H2O (FA 0.5%). Afterwards, StageTips were pre-conditioned with 100 µL MeOH, then ((ACN-Milli-Q H2O (0.5% FA) (80/20 (v/v))), followed by 100 µL of an equilibration solution (0.5% FA). Samples were subsequently loaded on the columns. What did not bind to the columns was kept as ‘unbound’ to be analyzed for any present peptides. Next, columns were washed by slightly acidic water using water with 0.5% FA and again what was kept as ‘wash’ for further analysis of any present peptides. Finally, the peptides bound on the C18 resins were eluted by different mixtures of firstly 50 µL (ACN-Milli-Q H_2O (0.5% FA) (50/50 (v/v))) and then 50 µL of ACN-Milli-Q H_2O (0.5% FA) 80/20 (v/v). The ‘elute’ sample was kept and vacuum dried. This protocol has been employed for the purification of samples from Museo del Prado in the next chapter.

2.2.2 Sample Measurement by MALDI-TOF Mass Spectrometry

MALDI-TOF analysis were performed to obtain a qualitative idea of the samples. Quite frequently, MALDI-TOF analysis is enough to identify protein collagen present in animal glues, by comparing with standard glues.

Accordingly, samples were resuspended in 10 µL of FA 0.1% in order to be analyzed by MALDI-TOF. Afterwards, 0.5 µL of each of alpha-Cyano-4-hydroxycinnamic acid (10 mg/mL in ACN-Citric acid 50 mM (70/30(v/v))) as matrix and samples were spotted on the target plate and left to be crystalized. Positive Reflector MALDI spectra were recorded on a 5800 MALDI TOF/TOF instrument (Sciex, Framingham, MA) equipped with a nitrogen laser (337 nm).

Mass spectra were externally calibrated against some mixture of standard peptides which have been purchased from Sciex. Mass spectra were acquired over the *m/z* range of 400–5000 for 10000 laser shots total from randomly chosen spots per sample position; Laser intensity, however, remained fixed for all the analyses.

MS/MS analyses were performed using 1 kV collision energy with air as CID gas. Raw data were analyzed using data explorer software provided by the manufacturer. Mass spectra were analyzed by manual inspection against the theoretical list of peptides, providing protein identification.

2.2.3 Sample Measurement by LC-MS/MS Mass Spectrometry

Samples were analyzed by LC-MSMS on LTQ- Orbitrap-XL (ThermoScientific, Bremen, Germany). Samples were injected onto a capillary chromatographic system consisting of a 2 cm length trapping column (C18, ID 100 µm, 5 µm) and a 20 cm C18 reverse phase silica capillary column (ID 75 µm, 5 µm) (Nanoseparations). Peptides were fractionated with acetonitrile-based eluents (solvent A: 0.2% formic acid, 2% acetonitrile in water; solvent B: 0.2% formic acid, 5% water in acetonitrile) with a gradient from 10 to 60% of B in 70 min, and then from 60 to 100% in 5 min, at 0.25 µL/min flow rate. MS analysis was performed with a resolution set to 30,000, and mass range from *m/z* 400 to 1,800 Da. The five most intense doubly, triply and fourthly charged ions were selected and fragmented in the ion trap by using CID fragmentation. Number of precursors selected for tandem-MS in each scan cycle: top 5 peaks; Mass window for precursor ion selection: 2 (*m/z*); Normalised collision energy: 35.0; Dynamic exclusion settings: 120 sec.

2.2.4 Data Handling

In order to analyze the data obtained from mass spectrometers, a licensed version of Mascot software (www.matrixscience.com) version 2.4.0. was used. Firstly, data obtained from mass spectrometer were transformed in Mascot Generic files (.mgf) format and used to query the database COLLE which have been created by the research group of the department of chemical science of Università degli Studi di Napoli Federico II and includes data only from animals that are most likely to be a source for animal glue. The Mascot search parameters that have been inserted for PMF and LC-MS/MS analysis are showed in Table 2.2. Accordingly, no fixed chemical modification was inserted, but possible oxidation of methionine residues, and deamidation at asparagines and glutamines were considered as variable modifications, and possible hydroxylation at lysines and proline. Only proteins presenting two or more peptides were considered as positively identified. Individual ion score threshold provided by Mascot software to evaluate the quality of matches in MS/MS data was 25.

Table 2.2 Mascot parameters for PMF and LC-MS/MS spectra

Mascot parameters for PMF	
Enzyme	Trypsin
Allowed number of missed cleavages	2
Fixed chemical modification	-
Variable modifications	Oxidation of met (M) Deamidation at asn and glu (NQ) Hydroxylation of lys and pro (Hydro KK-Hydro PP)
MS tolerance	10 ppm
MS/MS tolerance	0.6 Da
Mass Values	MH^+ Monoisotopic
Mascot parameters for LC-MS/MS spectra	
Enzyme	Trypsin
Allowed number of missed cleavages	1
Fixed chemical modification	-
Variable modifications	Oxidation of met (M) Deamidation at asn and glu (NQ) Hydroxylation of lys and pro (Hydro KK-Hydro PP)
MS tolerance	10 ppm
MS/MS tolerance	0.6 Da
Peptide charge	+2 to +3
Mass Values	Monoisotopic
Data format	Mascot generic

3 Results and Discussion

This chapter includes the discussions on the results of experiments on samples from restoration laboratories of both S. Orsola Benincasa, Naples, Italy and Museo del Prado, Madrid, Spain. The first section is dedicated to protein identification of S. Orsola Benincasa samples, including spectra interpretation of LC/MS-MS coupled with Mascot bioinformatic tool. The second section discusses the preliminary strategy for degradation evaluation. The third section is about protein identification of three animal glue samples from Museo del Prado by MALDI-TOF. The last section is about the StageTips/ZipTips protocol development in order to gain more peptide sequences, employing samples from Museo del Prado.

3.1 Samples of S. Orsola Benincasa, Naples, Italy

Samples of S. Orsola Benincasa have been enzymatically digested, cleaned and concentrated following *the standard protocol of StageTip* in order to be analyzed by LC-MS/MS as it was explained in detail in previous chapter.

3.1.1 Protein Identification by LC-MS/MS

Since Mascot compares the observed spectra to a database of known proteins and determines the most likely matches, there is, therefore, possibilities of reporting unlikely proteins in the sample. This issue can be particularly relevant in collagen protein due to the high sequence homology existing in the proteins among various species. Thus, a meticulous examination of the results is always required in order to confidently attribute a protein in a sample. It is, indeed, quite straightforward to identify collagen in samples but it is rather difficult to confidently identify the organisms from which collagen was extracted.

3.1.1.1 Rabbit glue (First Postwar)

Results of MS/MS spectra, employing Mascot bioinformatic tool in order to identify proteins in rabbit glue of the first postwar sample are shown in Table 3.1. As it can be inferred, results are promising to conclude that the sample according to what its label says ‘rabbit glue’ has been purely and solely prepared by rabbit glue bone and cartilage (due to presence of collagen α1(I) and α2(I)) and skin (due to presence of Collagen α1(III)). Although Mascot also showed several other proteins belonging to

diverse species matched with the experimental results of 'rabbit glue of the first postwar' sample, the proteins gathered in Table 3.1 which belong to *Oryctolagus cuniculus* had the highest scoring match with a considerable number of unique peptides, confirming the attribution of this animal glue to pure rabbit glue. In another words, in the other proteins, Mascot could only find peptides which are similar among these species where *Oryctolagus cuniculus* proteins had the statistically strongest assignments. Furthermore, in the match list of these species, there are no reports of unique peptides, thereby reasonably ruling out the possibility of their presence in the sample. A detailed list of the identified peptides with clear identification of unique ones and their ion scores of the sample examined by Mascot can be found in Table I-1, appendix I.

Table 3.1 Protein identification of *rabbit glue of the first postwar* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset and considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Protein Name	Taxonomy	Protein Identifier	Protein Score	Sequence Coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Oryctolagus cuniculus*	P02456	2887	57	47	6
Collagen α2(I)	*Oryctolagus cuniculus*	Q28668	2847	55	47	17
Collagen α1(III)	*Oryctolagus cuniculus*	G1T8J0	1689	33	32	15

3.1.1.2 Rabbit Glue (Giosi)

From Mascot protein identification results, the presence of both bovine and porcine collagen was observed (Table 3.2).The high sequence coverage and presence of unique peptides of each species are indicators of this conclusion. Additionally, the identification of Collagen α1(III) confirms the use of connective tissues and skin. It is worth emphasizing that the label on the animal glue sample was 'rabbit glue' and no collagen from *Oryctolagus cuniculus* was identified. Details on the identified peptides and score scan be found in Table I-2, appendix I.

Table 3.2 Protein identification of rabbit glue (Giosi) MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Bos taurus*	P02453	2698	48	48	2
Collagen α1(I)	*Sus scrofa*	A0A287A1S6	2492	48	12	5
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	2140	45	36	12
Collagen α2(I)	*Bos taurus*	P02459	1802	39	18	7
Collagen α1(III)	*Bos taurus*	P02458	1119	28	22	7
Collagen α1(III)	*Sus scrofa*	F1RYI8	865	23	6	4

3.1.1.3 Rabbit Glue Toten (sixties), Rabbit Glue (CTS)

As it can be seen from the results showed in Table 3.3, bovine bone, cartilage and possibly skin have been used in order to prepare the rabbit glue (CTS) and rabbit glue totten (sixties). The high protein score, sequence coverage and presence of prototypic peptides are all in line with this claim. Collagen α1(I) is more conserved during evolution in comparison with collagen α2(I) and collagen α1(III). Consequently, despite higher sequence coverage, only one unique peptide has been found. The higher number of unique peptides in collagen α2(I) and collagen α1(III), however, allowed a confident assignment of the bovine protein in the samples. The full list of identified peptide sequences and their relative score can be found in Table I-3 and Table I-4, appendix I.

Table 3.3 Protein identification of *rabbit glue Toten (sixties)* and *rabbit glue (CTS)*MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Rabbit Glue Toten (sixties)						
Protein name	**Taxonomy**	**Protein identifier**	**Protein Score**	**Sequence coverage (%)**	**No. of peptides**	**No. of Unique Peptides**
Collagen α1(I)	*Bos Taurus*	P02453	2704	53	47	1
Collagen α2(I)	*Bos Taurus*	P02459	2568	54	42	7
Collagen α1(III)	*Bos Taurus*	P04258	1669	34	27	10
Rabbit Glue (CTS)						
Protein Name	**Taxonomy**	**Protein Identifier**	**Protein Score**	**Sequence Coverage (%)**	**No. of Peptides**	**No. of Unique Peptides**
Collagen α1(I)	*Bos Taurus*	P02453	2345	44	43	1
Collagen α2(I)	*Bos Taurus*	P02459	1781	36	29	6
Collagen α1(III)	*Bos Taurus*	P04258	622	16	13	10

3.1.1.4 Strong Glue (Pearls)

Results of protein identification for strong glue (pearls) sample which are shown in Table 3.4 demonstrate the use of bovine and porcine bones and cartilage as sources for preparation of this sample. High sequence coverage, number of identified and unique peptides have led to an unambiguous assignment. Details on the identified peptides and score scan be found in Table I-5, appendix I.

3.1.1.5 Strong Glue (Old Manufacturing)

The protein identification results obtained by Mascot suggests that a combination of bovine, porcine, and donkey have been used to prepare the strong glue (old manufacturing). High sequence coverage, number of independent peptides and unique

peptides are all in agreement with the hypothesis that bone and cartilage of these animals have been employed (Table 3.5).

Table 3.4 Protein identification of *strong glue (pearls)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α2(I)	*Bos Taurus*	P02459	2555	51	44	9
Collagen α1(I)	*Bos Taurus*	P02453	2507	44	41	1
Collagen α1(I)	Sus scrofa	A0A287A1S6	2367	42	16	5
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	2292	43	11	9

The absence of any collagen type (III) confirms that no skin was used in the preparation of this glue which is totally in agreement with the fact that strong glue is made of bones. Details on identified peptides and scores can be found in Table I-6, appendix I. A BLAST [3] alignment analysis which has been performed on a unique peptide (K.SGDRGEAGPAGPAGPIGPVGAR.G) of *Equus asinus* shows 100% similarities of this sequence with a sequence of horse and 96% with bovine. In order to see whether the proteins belong to donkey or horse, a sequence alignment has been created on the whole collagen α1(I) sequence of *Equus asinus* versus *Equus sp.* putting uniport/swissprot as database and narrow it down by choosing mammals (taxid:40674) as the organism. As it can be seen in Figure 3.1 there are three peptides out of six peptides that are shared within the family Equidae belonging to donkey which further confirms the results of protein report by Mascot. On a side note, it is worth noting that Mascot, on the other hand, matches only exactly identical peptides and even a single substitution (i.e. a highly homologous sequence) fails to match the peptide.

Table 3.5 Protein identification of *strong glue (old manufacturing)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α2(I)	*Bos Taurus*	P02459	2660	55	45	7
Collagen α1(I)	*Bos Taurus*	P02453	932	45	42	_
Collagen α1(I)	*Sus Scorfa*	A0A287A1S6	2250	42	7	4
Collagen α2(I)	*Sus Scorfa*	A0A286ZWS8	2064	40	14	8
Collagen α1(I)	*Equus asinus*	B9VR88	2372	43	4	3
Collagen α2(I)	*Equus asinus*	B9VR89	2027	45	12	8

1 [3]BLAST which stands for Basic Local Alignment Search Tool is available online at: (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)

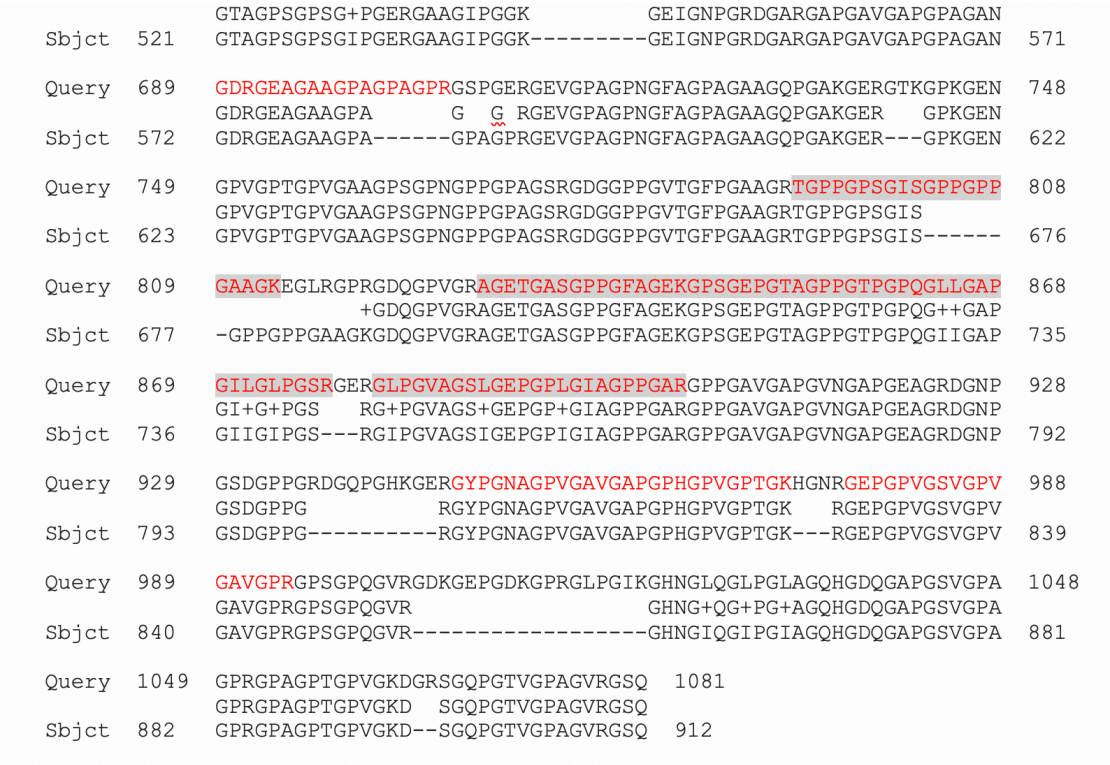


Figure 3.1 A piece of sequence alignment of collagen α1(I) sequence of *Equus asinus* (Sbjct) versus *Equus sp.* (Query) created by BLAST where red sequences are unique peptides of *Equus asinus* that have been found in Mascot report and highlighted sequences are the peptides specific to only *Equus asinus* compared with *Equus sp.*

3.1.1.6 Fish Glue (Flakes)

According to Mascot, the three proteins reported in the Table 3.6 were confidently identified in the sample. A considerable number of unique peptides in each protein led to conclude that the sample has been manufactured solely from porcine bone, cartilage and possibly skin and to rule out the possibility that any fish collagen was exist. Details on identified peptides and scores can be found in Table I-7, appendix I.

Table 3.6 Protein identification of *fish glue (flakes)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Protein Name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Sus scrofa*	A0A287A1S6	3107	54	52	8
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	3026	55	47	19
Collagen α1(III)	*Sus scrofa*	F1RYI8	1345	33	29	15

It is worth mentioning that other proteins belonging to other species were identified by their homologous peptides which were shared by porcine. A summary of the protein identification results is demonstrated in Table 3.7 to show further the high level of

confidence toward the protein assignment in this sample. Although proteins that belong to other species rather than porcine have high score and sequence coverage, all the peptides they cover are peptides that are shared with porcine and are not unique.

Amongst all the proteins matched for the protein content of sample, excluding porcine, there is a unique peptide of collagen α1(I) *Oryctolagus cuniculus*. Since the ion has a roughly low score (27) and the fragmentation spectrum is not of good quality, the hypothesis of the presence of rabbit was rejected.

Table 3.7 A comparison of data of 15 top protein matches reported by Mascot of fish flake sample

No.	Protein name	Protein Score	Sequence coverage (%)	Unique Peptide
1	Collagen α1(I) *Sus scrofa*	3107	54	8
2	Collagen α2(I) *Sus scrofa*	3026	55	19
3	Collagen α1(I) *Capra hircus*	2110	38	_
4	Collagen α1(I) *Equus asinus*	2101	35	_
5	Collagen α1(I) *Bos taurus*	2063	37	_
6	Collagen α1(I) *Canis lupus familiaris*	2019	37	_
7	Collagen α1(I) *Oryctolagus cuniculus*	2019	44	1
8	Collagen α1(I) *Felis catus*	1986	37	_
9	Collagen α1(I) *Rattus norvegicus*	1350	27	_
10	Collagen α1(III) *Sus scrofa*	345	33	15
11	Collagen α1(I) *Macaca mulatta*	1233	25	_
12	Collagen α2(I) *Equus asinus*	1233	25	_
13	Collagen α2(I) *Canis lupus familiaris*	1193	21	_
14	Collagen α1(I) *Mus musculus*	1186	23	_
15	Collagen α2(I) *Ovis aries*	1150	21	_

3.1.1.7 Sturgeon Fish Glue (Pearls)

Mascot results for this sample show unique peptides as identified peptides of two species, cat shark and porcine. There are no other protein matches in Mascot list of confident assignments, confirming that the sample has been prepared by combining animal bone and cartilage waste of cat shark and porcine.

Table 3.8 Protein identification of *sturgeon fish glue (pearls)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of peptides	No. of Unique Peptides
Collagen α1(I)/ Partial	*Scyliorhinus canicula*	D0PQF7	586	13	11	8
Collagen α1(I)	*Sus scrofa*	A0A287A1S6	425	11	6	3
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	205	8	5	4

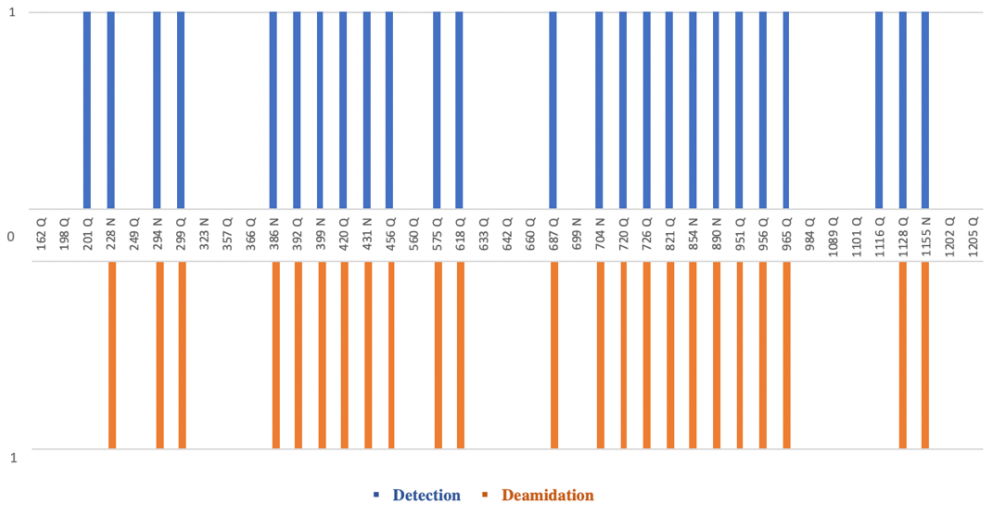
Although the sample goes by the name of sturgeon fish which is known to be used as collagen source for preparing the glue, no peptide of this specie has been reported by Mascot. Details on identified peptides and scores can be found in Table I-8, appendix I.

3.1.2 Degradation Evaluation, Sample Rabbit Glue Totten (Sixties)

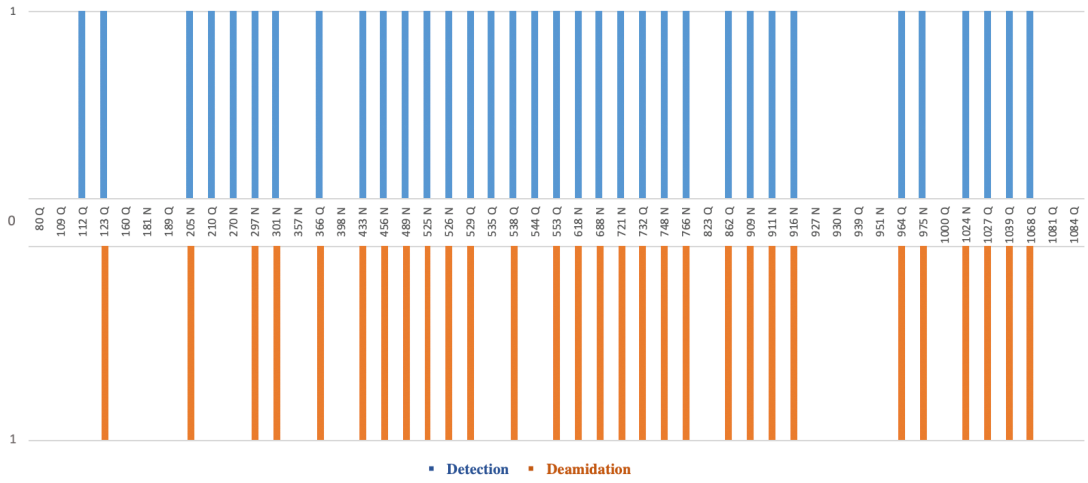
In this section, in order to demonstrate degradation evaluation by means of LC-MS/MS, 'rabbit glue totten (sixties)' sample has been chosen. As it was addressed earlier in this chapter, this sample has been prepared solely by bovine bone, cartilage, and connective tissues. The procedure to evaluate degradation is simply to check on Mascot report that whether or not deamidation for asparagine (N) and glutamine (Q) and oxidation for methionine (Met or M) have occurred. Thus, the amino acid positions were checked for deamidation and oxidation for three *Bos taurus* collagen chains—collagen α1(I), collagen α2(I), and collagen α1(III). In this regard, Q and N were checked for four different states—not detected, being only detected and not deamidated, partially deamidated, as well as only deamidated. In the graphs (Figure 3.2), however, to simplify the degradation pattern, each graph is illustrated in two series against each other, one series shows the detection (where the amino acids which have not been detected at all by the instrument were given the number 0 and the rest number 1), while the second series shows the deamidated amino acids (where the ones that were deamidated, partially or full, were given the number 1 and the rest number 0).

As it can be seen, all the amino acids that have been detected were also deamidated but a few. To evaluate oxidation, M positions were checked and where the instrument have detected M oxidized, it was given the number 1, otherwise 0. Similar to deamidation situation, wherever the instrument has detected M, it has been oxidized (Figure 3.3).

This procedure is a preliminary evaluation on the extent of degradation. This procedure can be extended in order to compare different types of samples with various degree of degradation. From a more general view, as far as animal glues are considered, since deamidation rate is affected by the degree or severity of temperature and pH which is related to preparation method and aging of the material itself, it is important to take all these factors into account and analyze the pattern of deamidation.



(a)



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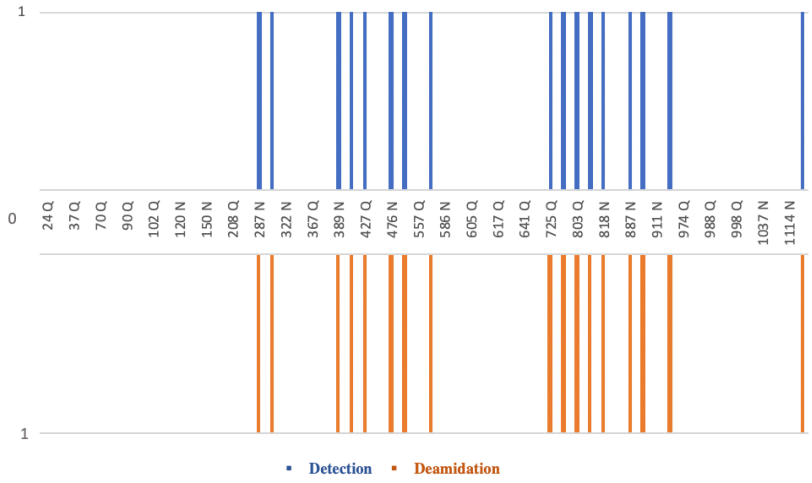
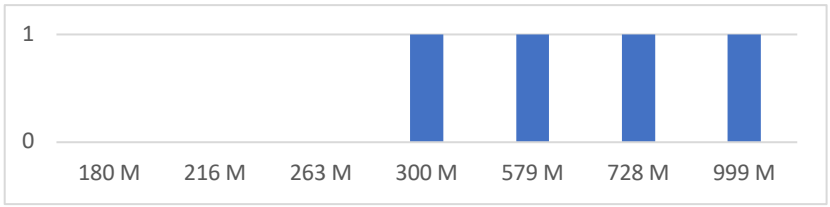
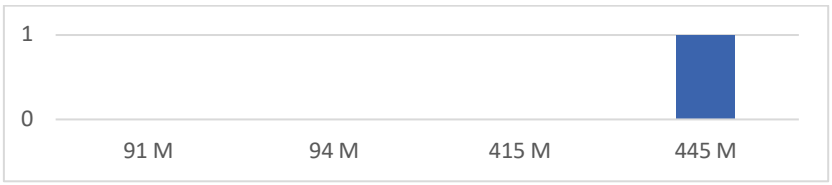


(c)

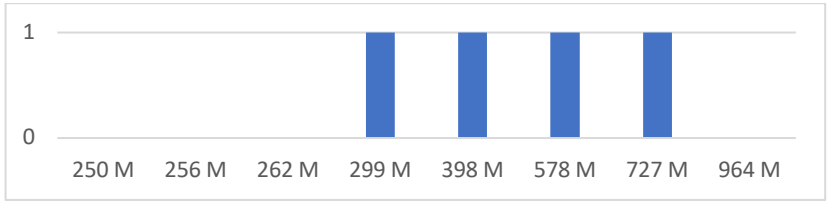
Figure 3.2 Deamidation evaluation of rabbit glue totten (sixties) for each identified protein, *Bos taurus* collagen α1(I), collagen α2(I), and collagen α1(III) as shown in a, b , and c graphs respectively.



(a)



(b)



(c)

Figure 3.3 Oxidation evaluation of rabbit glue totten (sixties) for each identified protein, *Bos taurus* collagen chains—collagen α1(I), collagen α2(I), and collagen α1(III) as shown in a, b , and c graphs respectively.

3.2 Protein Identification; Samples of Museo del Prado, Madrid, Spain

Three animal glues, namely fish glue (4), strong glue (8), and rabbit glue (7) were analyzed by MALDI-TOF (the whole procedure from sample preparation to data handling is thoroughly explained in previous chapter). Afterwards, the mass results of each were compared with mass results of two samples that have been analyzed before and their source of organism was confidently identified (the MS/MS detailed results of which are available in appendix I). Therefore, these two samples—rabbit glue (of the first post war) and strong glue (old manufacturing) were used as references for animal glue source. In the results of protein identification of these two glues by Mascot tool, it was deduced that rabbit glue (of the first post war) has been prepared from pure rabbit bones, cartilage, and skin wastes. As far as the strong glue (old manufacturing) sample is concerned, it is concluded that the sample has been prepared from bone and cartilage of bovine, porcine and donkey. As a result, comparing samples with these two 'references' (which somewhat is a collagen protein dataset of most common animal's with respect to animal glue preparation instructions) can help giving clues as to which animals' waste bone and connective tissues are more likely to be used in the samples. The procedure here is overlapping mass spectra of each of these samples with the 'reference' samples and by only taking into account the masses in common (considering the mass error of ±0.5),

identifying proteins according to the data of protein identification of MS/MS of the reference samples provided by Mascot bioinformatic tool.

Since collagen is a class of protein that has been conserved during the evolution of species, collagen α1(I) and collagen α2(I) (more than collagen α1(III)) of most of species) have a great number of peptides in common. For example, bovine collagen α1(I) chain presents more than 99% sequence homology with collagen from goat (*Capra hircus*) and sheep (*Ovis aries*); more than 97% sequence homology with collagen from porcine (Sus scrofa), domestic dog (*Canis lupus familiaris*) and cat (*Felis catus*); as well as more than 95% with collagen from rhesus macaque (*Macaca mulatta)* and donkey (*Equus asinus*). As it can be inferred, the possibility to detect species-specific peptides for this protein is therefore greatly reduced due to this considerable similarity. In another words, the lower the sequence homology with other species, the higher the probability of identifying specific peptides. Thus, despite the fact that some peptides that have been matched with the 'references' were shared in collagen proteins of some odd species—rhesus macaque *(Macaca mulatta),* red junglefowl *(Gallus gallus),* cat *(Felis catus),* domestic dog (*Canis lupus familiaris*)*,* brown rat *(Rattus norvegicus)* and house mouse *(Mus musculus)*—along with more common species, such as rabbit (*Oryctolagus cuniculus),* donkey *(Equus asinus),* goat *(Capra hircus)*, sheep (*Ovis aries*), bovine *(Bos taurus)* and porcine *(Sus scrofa),* for the sake of more confident assignments, in the process of assigning masses to peptides, only the most common animals that their bones and wastes were more likely to be used in animal glue preparation were taken into account in this preliminary approach for the protein identification.

3.2.1 Fish Glue (4)

Mass spectra of fish glue (4) versus mass spectra of 'references' rabbit glue (of first postwar) and strong glue (old manufacturing) are shown in Figure 3.4 and Figure 3.5. According to the results gathered in Table 3.9 and Table 3.10, rabbit (*Oryctolagus cuniculus)*, bovine *(Bos taurus)*, and grass carp *(Ctenopharyngodon Idella)* are species that are more likely to be existed in the fish glue (4) since masses of unique peptides of which have been matched with masses of sample.

Detection of peptides belonging to some other fish species, apart from grass carp, such as R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G that could also belong to Japanese rice fish (*Oryzias latipes),* as well as R.GPPGERGETGAPGPAGFAGPPGADGLPGAK.G that belongs to either grass carp *(Ctenopharyngodon Idella)* or Zebrafish *(Danio rerio)* and Crucian carps *(Carassius)*

could imply that in the preparation of this animal glue along with bones and cartilage of mostly farm animals, waste parts of different fish have also been used. Owing to that mass 2705.11 can belong to two different peptides (see Table 3.10), further analyses by LC-MS/MS is needed in order to assign it to the correct peptide, and thus species.

Table 3.9 Matched masses of fish glue (4) with 'reference' rabbit glue (of first post war) and identified protein and peptides according to MS/MS results and Mascot tool

Fish Glue (4) vs Rabbit Glue (of first post war)				
***m/z* MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
898.38	898.48	CO1A1	_	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1105.44	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1532.57	1532.66	CO1A2	_	R.GDGGPPGMTGFPGAAGR.T + 2 Hydroxylation (P) (P)[2]
1832.60	1832.88	CO1A2	_	R.GPPGPQGLPGLAGTAGEPGR.D + 3 Hydroxylation (P) (P)[3]
2704.86	2704.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)[4]

[1]this peptide is common/shared between several species, namely *Oryctolagus cuniculus, Equus asinus, Capra hircus, Bos taurus* and *Sus scrofa*. [2]within the group of (*Oryctolagus cuniculus, Equus asinus, Capra hircus, Bos taurus* and *Sus scrofa*) this peptide has only been detected in protein Collagen α1(I) of *Oryctolagus cuniculus*. [3]within the group of (*Oryctolagus cuniculus, Equus asinus, Capra hircus, Bos taurus* and *Sus scrofa*) this peptide has been detected in protein Collagen α1(I) of *Oryctolagus cuniculus* and *Bos taurus*. [4]this peptide is common/shared between several species, namely *Oryctolagus cuniculus, Equus asinus, Capra hircus, Bos Taurus, Sus scrofa* and *Oryzias latipes*.

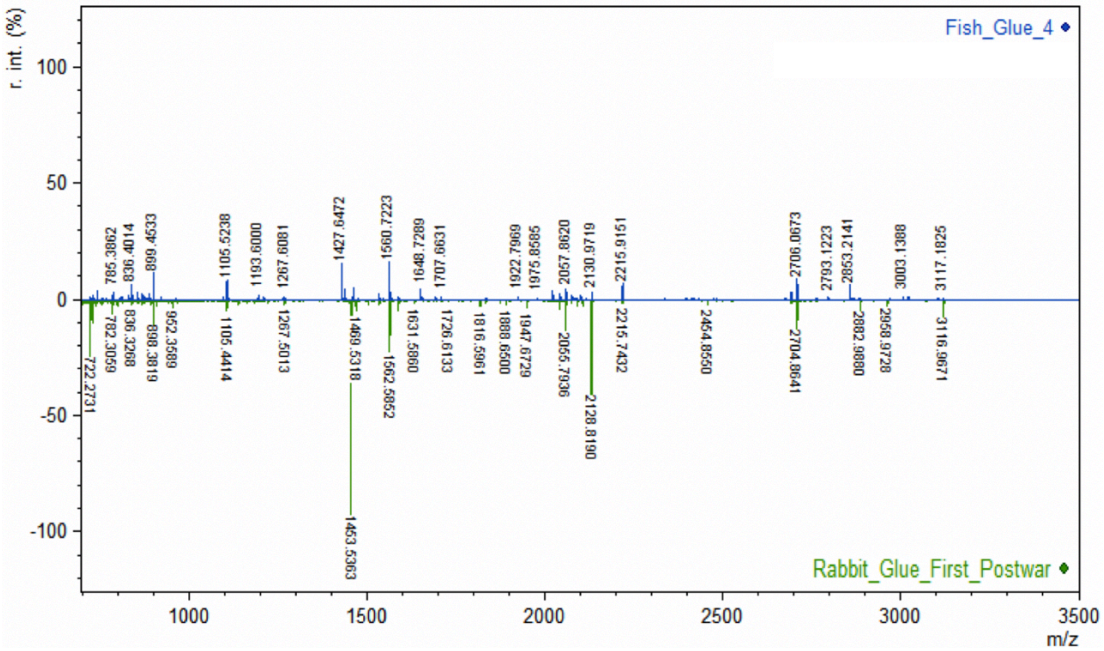


Figure 3.4 Mass spectra of fish glue (4) versus mass spectra of rabbit glue (of first postwar) obtained by MALDI-TOF

Identification of proteins from fish species is complicated by the actual fact that not many genomes or protein sequences from fish are available. Nevertheless, in identifying a fish origin, researchers rely on the fact that sequences are well-conserved (this is even more relevant in the case of collagen), and therefore peptides could be matched with

closely related species.

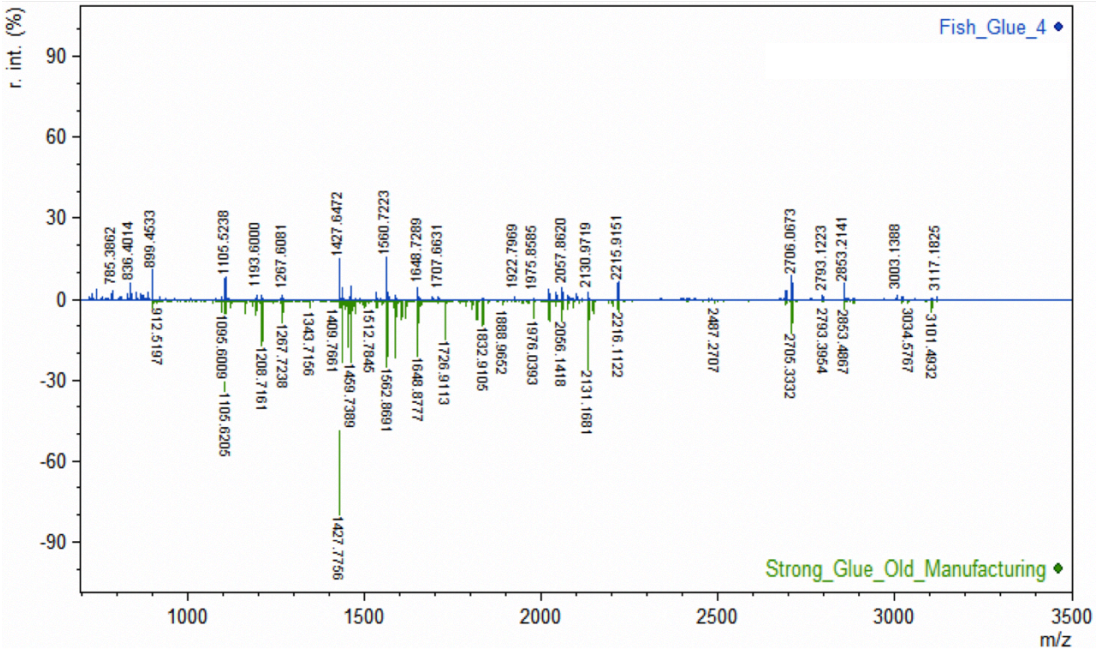


Figure 3.5 Mass spectra of fish glue (4) versus mass spectra of strong glue (old manufacturing) obtained by MALDI-TOF

Table 3.10 Matched masses of fish glue (4) with 'reference' strong glue (old manufacturing) and identified protein and peptides according to MS/MS results and Mascot tool

Fish Glue (4) vs Strong Glue (old manufacturing)				
***m/z* MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
1078.51	1078.56	CO1A1	_	R.GRPGAPGPAGAR.G + Hydroxylation (P) (P)[1]
1105.48	1105.54	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1192.62	1192.54	CO1A1	_	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P); Oxidation (M); Hydroxylation (K) (K)[1]
1208.61	1208.64	CO1A2-*Bos taurus*	U	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
1500.64	1500.69	CO1A1-*Oryctolagus cuniculus*	U	R.GSPGPPGATGFPGAAGR.V + 3 Hydroxylation (P) (P)
1655.72	1655.79	CO1A1 Ctenopharyngodon idella	U	K.GSPGTPGIAGAPGFPGPR.G + 4 Hydroxylation (P) (P)
1706.69	1706.75	CO1A1	_	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)[2]
2019.87	2019.95	CO1A1	_	K.GEPGPTGIQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[3]
2072.91	2072.98	CO1A1	_	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
2199.93	2199.93	CO1A1	_	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)[4]
2215.94	2215.93	CO1A1	_	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)[4]

Table 3.10 ***Continued.***

Fish Glue (4) vs Strong Glue (old manufacturing)				
m/z **MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
2689.10	2689.24	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[2]
2705.11	2705.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)[2]
	2705.27	CO1A1	_	R.GPPGERGETGAPGPAGFAGPPGADGLPGAK.G + 4 Hydroxylation (P) (P)[5]

[1]this peptide is common/shared between several species, namely *Bos taurus, Sus scrofa, Capra hircus, Ovis aries, Equus asinus, Oryctolagus cuniculus.* [2]within the group of (*Bos taurus, Sus scrofa, Capra hircus, Ovis aries, Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected in all but protein Collagen α1(I) of *Ovis aries*. [3]within the group of (*Bos taurus, Sus scrofa, Capra hircus, Ovis aries, Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected only in the protein Collagen α1(I) of *Bos taurus* and *Equus asinus.* [4]this peptide is shared in the protein Collagen α1(I) of the above-mentioned group and *Scyliorhinus canicular*. [5]this peptide is common/shared between several species, namely *Ctenopharyngodon Idella, Danio rerio* and *Carassius auratus*.

3.2.2 Rabbit Glue (7)

Mass spectra of rabbit glue (7) versus mass spectra of 'references' rabbit glue (of first postwar) and strong glue (old manufacturing) are shown in Figure 3.6 and Figure 3.7. According to the results gathered in Table 3.11 and Table 3.12 , it is more likely that the sample is a mixture of bone and cartilage wastes of rabbit and bovine since unique peptides of these two species were matched with masses of 'references'. Moreover, a unique peptide of grass carp, along with another peptide has been matched which could suggest also the presence of fish species in the sample. Although, presence of fish in a sample going by the name of rabbit glue is odd, LC-MS/MS analysis will be instrumental in providing more confident identifications.

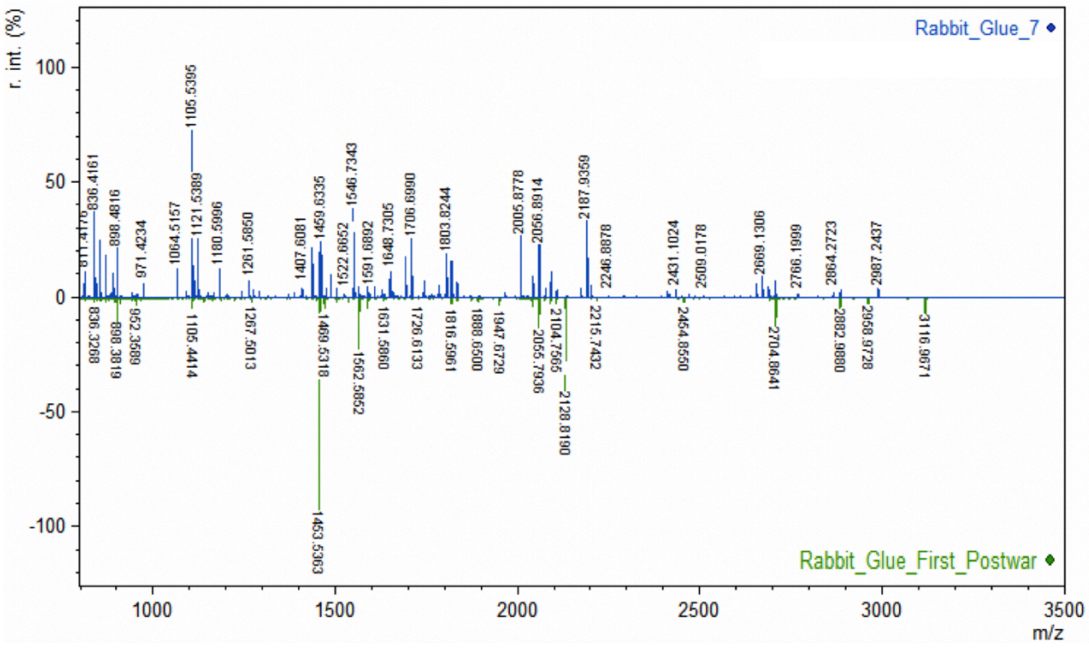


Figure 3.6 Mass spectra of rabbit glue (7) versus mass spectra of rabbit glue (of first postwar) obtained by MALDI-TOF

Table 3.11 Matched masses of rabbit glue (7) with rabbit glue (of first post war) 'reference' sample and identified protein and peptides according to MS/MS results and Mascot tool

Rabbit Glue (7) vs Rabbit Glue (of first post war)				
m/z **MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
868.34	868.43	CO1A2	_	R.GPSGPQGIR.G + Deamidated (NQ)[1]
898.38	898.48	CO1A1	_	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)[2]
1105.44	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[2]
1545.58	1545.78	CO1A2	U	R.GEPGPAGSIGPVGAAGPR.G
1561.56	1561.78	CO1A2	U	R.GEPGPAGSIGPVGAAGPR.G + Hydroxylation (P) (P)
1585.55	1585.74	CO1A1	_	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1631.58	1631.79	CO1A2	U	K.GEIGPVGNPGPSGPAGPR.G + Hydroxylation (P) (P)
1816.6	1816.89	CO1A2	_	R.TGPPGPSGITGPPGPPGAAGK.E + 3 Hydroxylation (P) (P)[3]
1832.6	1832.89	CO1A2	_	R.GPPGPQGLPGLAGTAGEPGR.D + 3 Hydroxylation (P) (P)[4]
2104.76	2104.99	CO1A1	_	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[2]
2704.86	2704.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)[2]

[1]within the group of (*Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*) this peptide has only been detected in protein Collagen α1(I) of *Bos taurus*. [2]this peptide is common/shared between several species, namely *Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*. [3]within the group of (*Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*) this peptide has only been detected in protein Collagen α1(I) of *Oryctolagus cuniculus*. [4]within the group of (*Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*) this peptide has been detected in protein Collagen α1(I) of *Oryctolagus cuniculus* and *Bos taurus*.

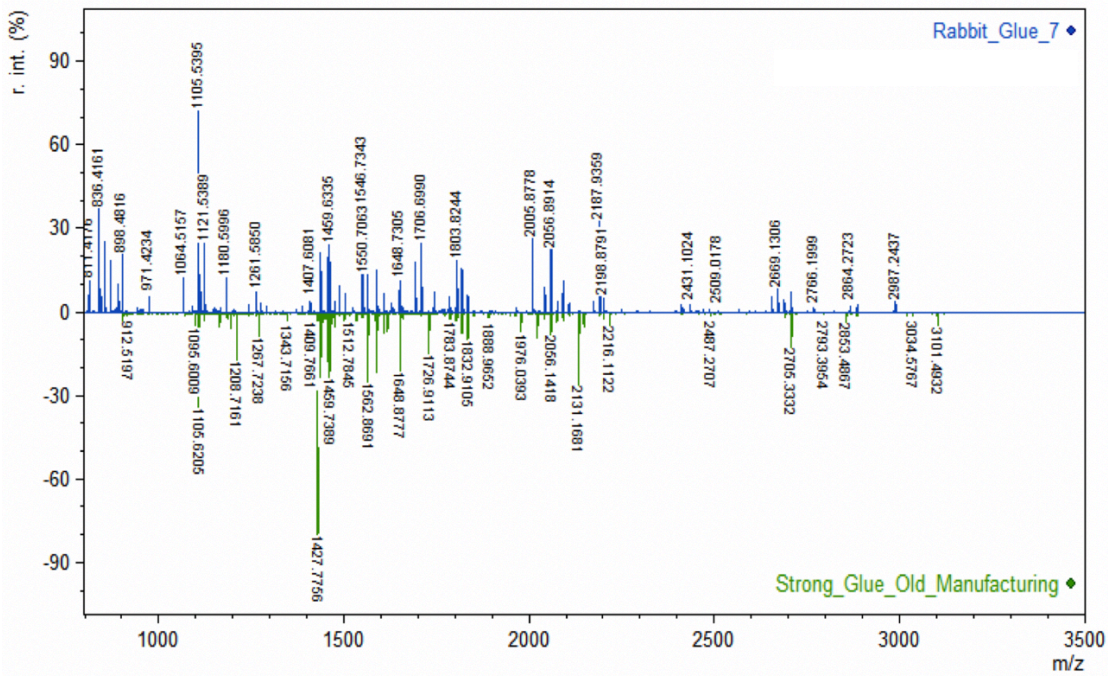


Figure 3.7 Mass spectra of rabbit glue (7) versus mass spectra of strong glue (old manufacturing) obtained by MALDI-TOF

Table 3.12 Matched masses of sample rabbit glue (7) with strong glue (old manufacturing) 'reference' sample and identified protein and peptides according to MS/MS results and Mascot tool

Rabbit Glue (7) vs Strong Glue (old manufacturing)				
***m/z* MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
1105.54	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1161.52	1161.5	CO1A1	_	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)[1]
1201.52	1201.55	CO1A2	_	R.GEPGNIGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)[1]
1239.60	1239.66	CO1A1	_	R.GVVGLPGQRGER.G + Hydroxylation (P) (P)[2]
1435.63	1435.67	CO3A1-*Bos taurus*	U	R.GPPGPPGTNGVPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1500.66	1500.69	CO1A1-*Oryctolagus cuniculus*	U	R.GSPGPPGATGFPGAAGR.V + 3 Hydroxylation (P) (P)
1655.73	1655.79	CO1A1-Ctenopharyngodon idella	U	K.GSPGTPGIAGAPGFPGPR.G + 4 Hydroxylation (P) (P)
1706.70	1706.75	CO1A1	_	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)[3]
1726.70	1726.72	CO1A1	_	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Hydroxylation (P) (P); Oxidation (M)[1]
1765.77	1765.86	CO1A2	_	R.GHNGLDGLKGQPGAPGVK.G + Deamidated (NQ); 2 Hydroxylation (P) (P); 2 Hydroxylation (K) (K)[1]
1961.89	1961.91	CO1A1	_	R.GEPGPAGLPGPPGERGGPGSR.G + 4 Hydroxylation (P) (P)[4]
2072.92	2072.98	CO1A1	_	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
2198.88	2198.94	CO1A1	_	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)[2]
	2198.96	CO1A1		R.NGEPGMPGSKGMTGSPGSPGPDGK.T + Deamidated (NQ)[4]
2689.14	2689.24	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[3]
2705.13	2705.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)[3]

[1]this peptide is common/shared between several species, namely *Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus, Oryctolagus cuniculus.* [2]within the group of (*Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected in all but protein Collagen α1(I) of *Capra hircus*. [3]within the group of (*Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected in all but protein Collagen α1(I) of *Ovis aries*. [4]this peptide is common/shared between several species, namely *Ctenopharyngodon Idella*, *Danio rerio* and *Carassius auratus*.

3.2.3 Strong Glue (8)

As far as the sample strong glue (8) is concerned, it is very likely that the sample is made of bone and cartilage wastes of bovine and donkey since unique peptides of them have been matched. Furthermore, unique peptides of rabbit and mouse has also been matched which suggests their presence in the sample. The exitance of these two species is somewhat odd for a strong glue but may imply an attempt of altering properties of the sample.

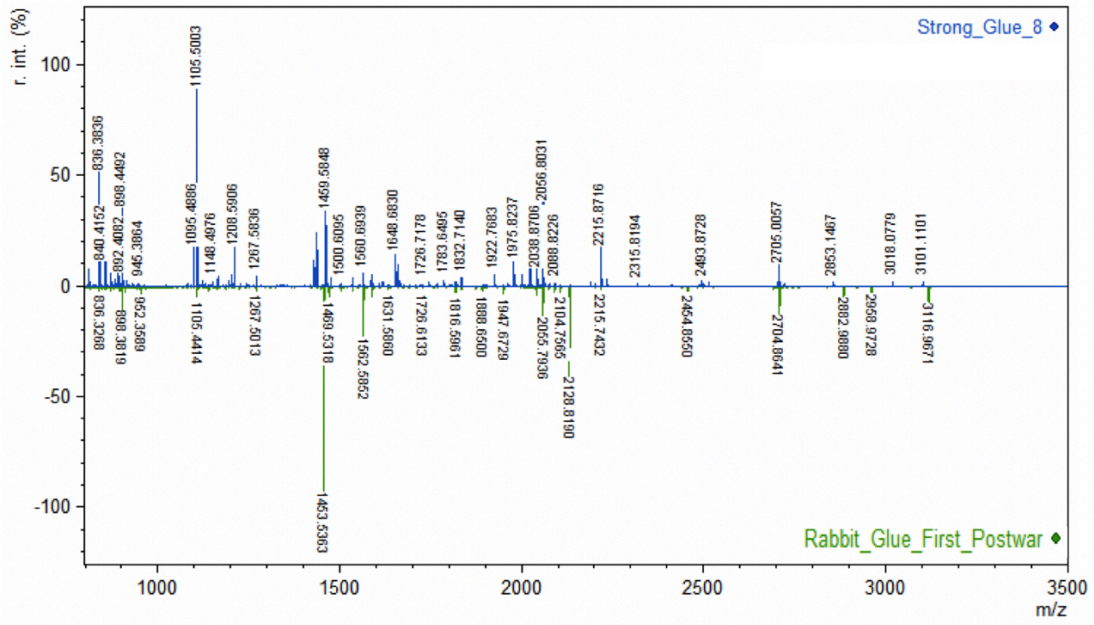


Figure 3.8 Mass spectra of strong glue (8) versus mass spectra of rabbit glue (of first postwar) obtained by MALDI-TOF

Table 3.13 Matched masses of strong glue (8) with rabbit glue (of first post war) 'reference' sample and identified protein and peptides according to MS/MS results and Mascot tool

Strong Glue (8) vs Rabbit Glue (of first post war)				
***m/z* MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
898.38	898.48	CO1A1	_	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1105.44	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1532.57	1532.66	CO1A2	_	R.GDGGPPGMTGFPGAAGR.T + 2 Hydroxylation (P) (P)[2]
1631.59	1631.79	CO1A2	U	K.GEIGPVGNPGPSGPAGPR.G + Hydroxylation (P) (P)
1816.60	1816.89	CO1A2	_	R.GPNGDSGRPGEPGLMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)[2]
1832.60	1832.88	CO1A2	_	R.GPPGPQGLPGLAGTAGEPGR.D + 3 Hydroxylation (P) (P)[3]
2005.68	2005.93	CO1A1	_	K.GEPGPTGVQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[4]
2055.79	2055.96	CO1A1	_	K.TGPPGPAGQDGRPGPPGPPGAR.G + 4 Hydroxylation (P) (P)[1]
2071.78	2071.96	CO1A1	_	K.TGPPGPAGQDGRPGPPGPPGAR.G + 5 Hydroxylation (P) (P)[1]
2104.76	2104.99	CO1A1	_	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[1]
2704.86	2704.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)[1]

[1]this peptide is common/shared between several species, namely *Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*. [2]within the group of (*Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*) this peptide has only been detected in protein Collagen α1(I) of *Oryctolagus cuniculus*. [3]within the group of (*Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*) this peptide has been detected in protein Collagen α1(I) of *Oryctolagus cuniculus* and *Bos taurus*. [4]within the group of (*Oryctolagus cuniculus*, *Equus asinus*, *Capra hircus*, *Bos taurus* and *Sus scrofa*) this peptide has been detected in protein Collagen α1(I) of *Oryctolagus cuniculus* and *Sus scrofa*.

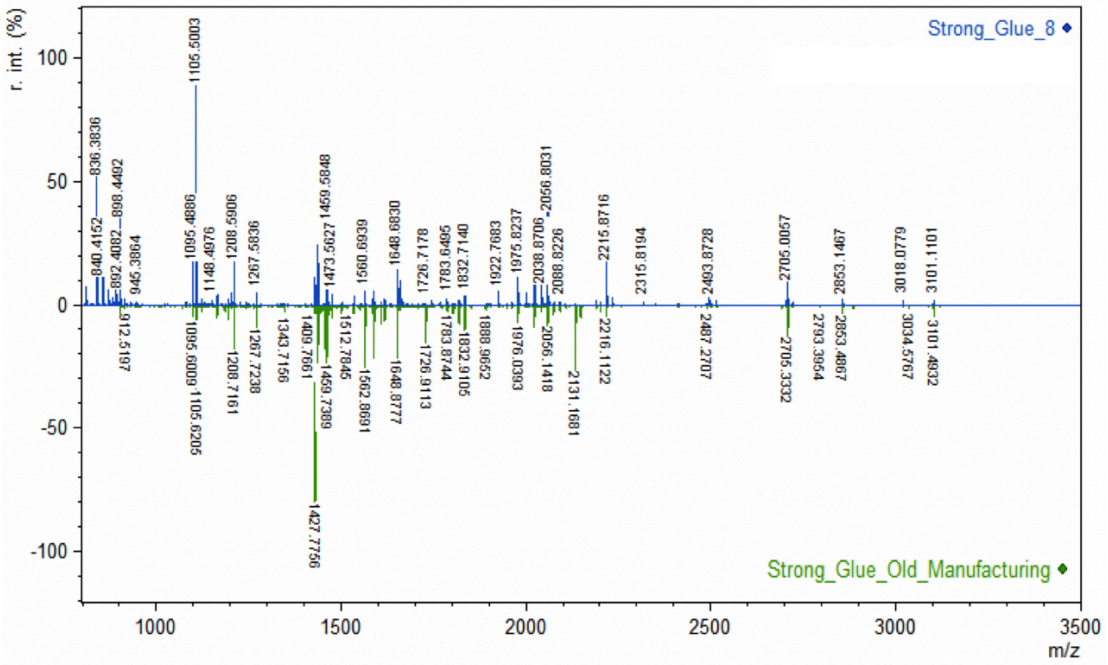


Figure 3.9 Mass spectra of strong glue (8) versus mass spectra of strong glue (old manufacturing) obtained by MALDI-TOF

Table 3.14 Matched masses of strong glue (8) with strong glue (old manufacturing) 'reference' sample and identified protein and peptides according to MS/MS results and Mascot tool

Strong Glue (8) vs Strong Glue (old manufacturing)				
***m/z* MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
1078.59	1078.56	CO1A1	_	R.GRPGAPGPAGAR.G + Hydroxylation (P) (P)[1]
1105.62	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1161.62	1161.55	CO1A1	_	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)[1]
1177.62	1177.54	CO1A1	_	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Oxidation (M)[1]
1192.72	1192.55	CO1A1	_	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P); Oxidation (M); Hydroxylation (K) (K)[1]
1201.62	1201.57	CO1A2	_	R.GEPGNIGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)[1]
1208.72	1208.65	CO1A1	_	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1332.69	1332.63	CO1A1	_	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1343.72	1343.63	CO1A1	_	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)[1]
1435.73	1435.67	CO3A1- *Bos taurus*	U	R.GPPGPPGTNGVPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1500.78	1500.69	CO1A1- *Oryctolagus cuniculus*	U	R.GSPGPPGATGFPGAAGR.V + 3 Hydroxylation (P) (P)
1615.86	1615.80	CO1A2	_	K.GELGPVGNPGPAGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1726.91	1726.72	CO1A1	_	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Hydroxylation (P) (P); Oxidation (M)[1]
1743.84	1743.71	CO1A1	_	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)[1]
1765.91	1765.86	CO1A1	_	R.GHNGLDGLKGQPGAPGVK.G + Deamidated (NQ); 2 Hydroxylation (P) (P); 2 Hydroxylation (K) (K)[1]

Table 3.14 ***Continued.***

Strong Glue (8) vs Strong Glue (old manufacturing)				
m/z **MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
1783.88	1783.88	CO1A1- *Equus asinus*	U	R.GPPGPVGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
2199.03	2199.06	CO1A1	_	R.GETGPAGRPGEVGPPGPPGPAGEK.G + 2 Hydroxylation (P) (P)[2]
2703.32	2703.21	CO1A1	_	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Hydroxylation (K) (K)[1]
2705.33	2.705.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)[1]
3101.49	3101.39	CO1A1- *Mus Musculus*	U	R.GLPGPPGAPGPQGFQGPPGEPGEPGGSGPMGPR.G + 7 Hydroxylation (P) (P)

[1]this peptide is common/shared between several species, namely *Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus, Oryctolagus cuniculus.* [2]within the group of (*Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected in all but protein Collagen α1(I) of *Capra hircus*.

3.3 Protocol Development; Samples of Museo del Prado, Madrid, Spain

In this section, it was attempted to improve sequence coverage by combining two purification protocols (C18 column) in sequence. The observation that by using two different C18 columns each purification protocol yields different sequences which were not covered by the other one, triggered to combine the two protocol in a series of experiments to examine this hypothesis.

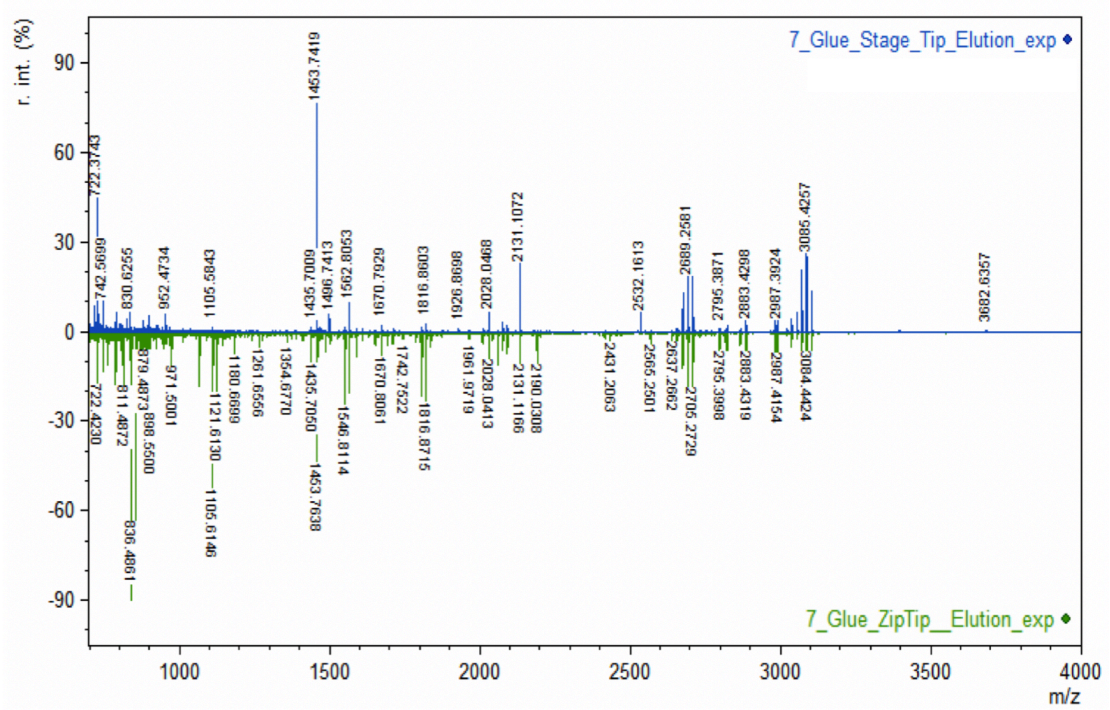


Figure 3.10 A comparison between two spectra obtained by MALDI-TOF after two different clean-up steps StageTip and ZipTip

Figure 3.10 shows a comparison between the masses of animal glue (7) analyzed by MALDI-TOF yielded after two different purification steps separately; employing StageTip and ZipTip. As it can be seen, there are some peptides present in spectra after StageTip that are not exist in ZipTip and vice versa[4].

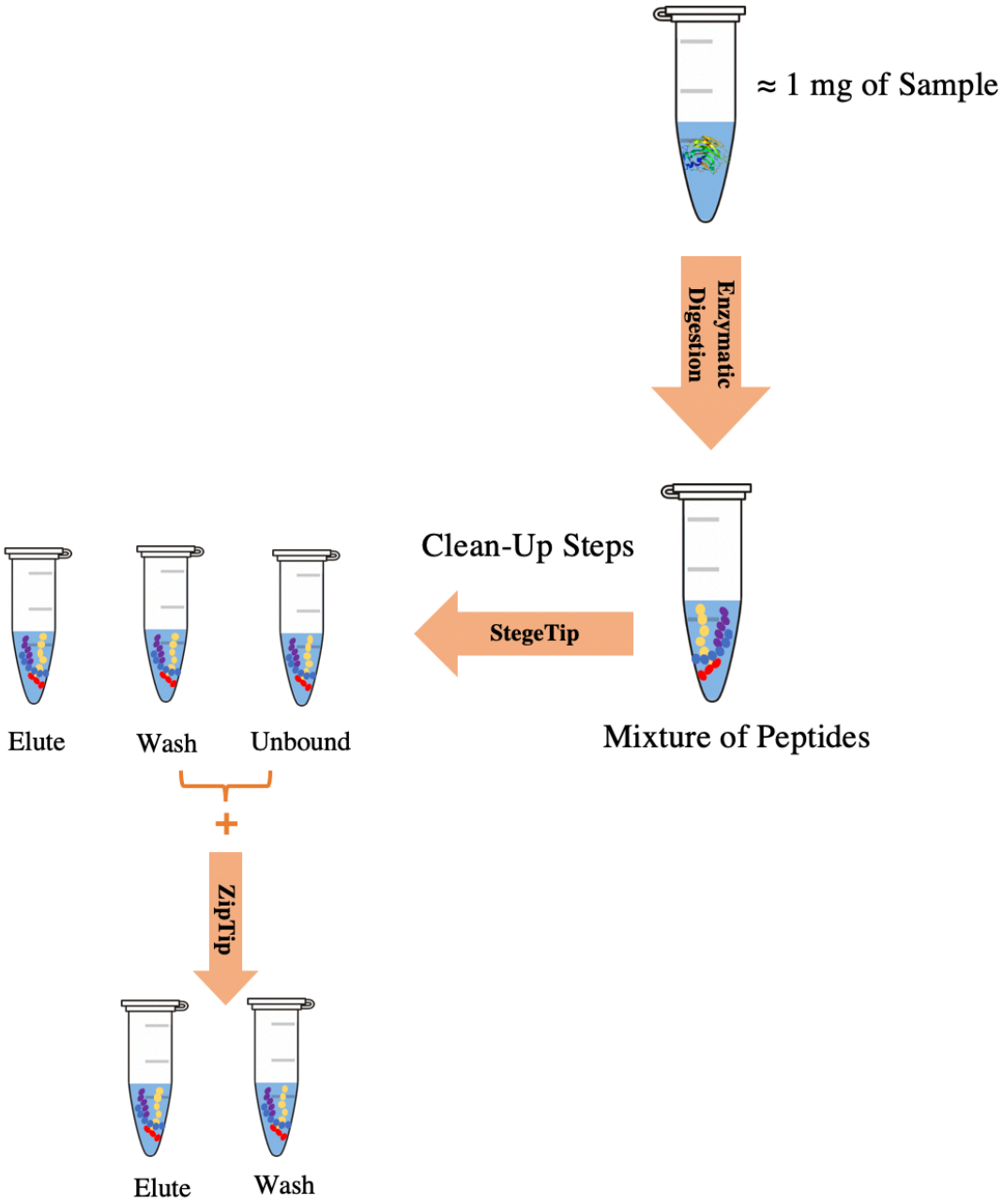


Figure 3.11 Simple schematic process of cleaning up peptides in two steps of purification by StageTip (C18) followed by a ZipTip (C18)

[4] Spectra obtained after ZipTip purification step has been compared with two reference spectra (see previous section) to show in a qualitative way that neither of two purification steps are superior in terms of sequence coverage than the other one (Appendix II).

To this end, the two protocols were combined in order to test that if this idea can aid to obtain a higher sequence coverage and better-quality spectra. Accordingly, animal glue (7) were purified in StageTip step, each of 'elute', 'unbound' and 'wash' were kept. Afterwards, 'wash' and 'unbound' of StageTip were cleaned-up in an additional step of ZipTip purification with the idea that whatever has not been bound with column in StageTip in the first step (and therefore exist in wash and unbound of StageTip) will bind in the second step and will be collected in 'elute' of ZipTip (Figure 3.11 illustrates this process). It is worth noting that although it was possible to split the sample after digestion step into two and to carry out each purification protocol on each of the halves, discarding a part of sample (in wash and unbound) was attempted to be avoided as much as possible in order to present a strategy for cultural heritage objects due to the little quantity of organic materials left in them.

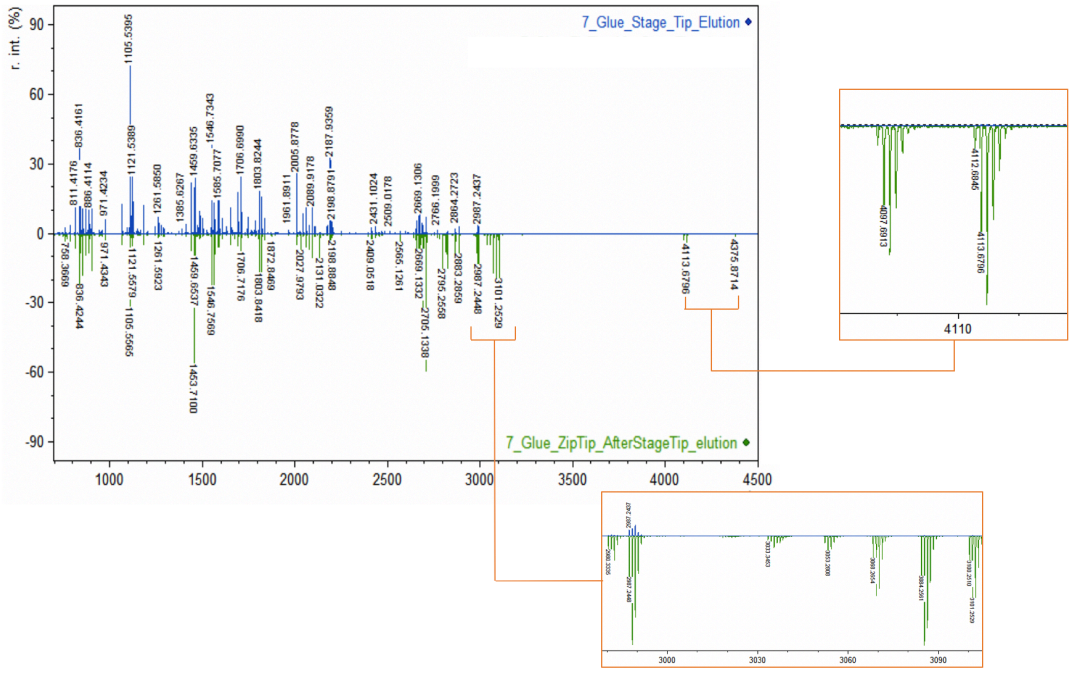


Figure 3.12 Spectra obtained by MALDI-TOF after the combined clean-up step; the 'elute' spectra of StageTip and ZipTip shows a number of not similar masses yielded by each protocol

The differences between the masses acquired from each purification step is demonstrated in Figure 3.12. In order to investigate if these additional masses yielded by ZipTip can represent useful data, a peptide mass fingerprint (PMF) was carried out on these masses with a licensed version of Mascot software (www.matrixscience.com) version 2.4.0, using the Colle database and considering peptide mass tolerance at 100 ppm, allowed trypsin missed cleavages up to 2. No fixed chemical modification was inserted, but possible hydroxylation of prolines, oxidation of methionine residues, and

deamidation at asparagines and glutamines were considered as variable modifications. According to the results represented by PMF, the additional masses yielded by two chromatographic steps belong to protein collagen (I) alpha 2 of *Oryctolagus cuniculus*, *Capra hircus* and *Ovis aries.* From previous analysis we know that it is most likely that rabbit glue (7) is made of collagen protein of rabbit, bovine, donkey and maybe mouse (previous section).

```
Query  421   RGATGPAGVRGPNGDSGRPGEPGLMGPRGFPGSPGNIGPAGKEGPAGLPGIDGRPGPIGP  480
             RGATGPAGVRGPNGDSGRPGEPGLMGPRGFPGSPGNIGPAGKEGP GLPGIDGRPGPIGP
Sbjct  421   RGATGPAGVRGPNGDSGRPGEPGLMGPRGFPGSPGNIGPAGKEGPVGLPGIDGRPGPIGP  480

Query  481   AGARGEPGNIGFPGPKGPTGDPGKAGEKGHAGLAGPRGAPGPDGNNGAQGPPGLQGVQGG  540
             AGARGEPGNIGFPGPKGP+GDPGKAGEKGHAGLAG RGAPGPDGNNGAQGPPGLQGVQGG
Sbjct  481   AGARGEPGNIGFPGPKGPSGDPGKAGEKGHAGLAGARGAPGPDGNNGAQGPPGLQGVQGG  540

Query  961   PGPQGPVGPTGKHGSRGEPGPVGAVGPAGAVGPRGPSGPQGIRGDKGEPGDKGPRGLPGL  1020
             PGPQGPVGP GKHG+RGEPGP GAVGPAGAVGPRGPSGPQGIRGDKGEPGDKGPRGLPGL
Sbjct  961   PGPQGPVGPVGKHGNRGEPGPAGAVGPAGAVGPRGPSGPQGIRGDKGEPGDKGPRGLPGL  1020
```

(a)

```
Query  275   MGPAGSRGATGPAGVRGPNGDSGRPGEPGLMGPRGFPGSPGNIGPAGKEGPAGLPGIDGR  334
             MGPAGSRGATGPAGVRGPNGDSGRPGEPGLMGPRGFPGSPGNIGPAGKEGP GLPGIDGR
Sbjct  415   MGPAGSRGATGPAGVRGPNGDSGRPGEPGLMGPRGFPGSPGNIGPAGKEGPVGLPGIDGR  474

Query  335   PGPIGPAGARGEPGNIGFPGPKGPTVR----------------------ITTTSSPSAL  371
             PGPIGPAGARGEPGNIGFPGPKGP+                        P  L
Sbjct  475   PGPIGPAGARGEPGNIGFPGPKGPSGDPGKAGEKGHAGLAGARGAPGPDGNNGAQGPPGL  534

Query  717   GPVGAAGAPGPQGPVGPTGKHGSRGEPGPVGAVGPAGAVGPRGPSGPQGIRGDKGEPGDK  776
             GPVGAAGAPGPQGPVGP GKHG+RGEPGP GAVGPAGAVGPRGPSGPQGIRGDKGEPGDK
Sbjct  953   GPVGAAGAPGPQGPVGPVGKHGNRGEPGPAGAVGPAGAVGPRGPSGPQGIRGDKGEPGDK  1012
```

(b)

Figure 3.13 Comparing collagen α2(I) sequence of both *Ovis aries* (a) and *Capra hircus* (b) as query with *Bos taurus* as subject by BLAST sequence alignment tool; the two red peptides are the unique peptides shared with *Capra hircus* and *Ovis aries* (only pieces of these sequence alignments are shown here).

The assignment of some peptides by PMF to collagen (I) alpha 2 of *Oryctolagus cuniculus* is not something new. Nevertheless, PMF assignment of some peptides to sheep or goat is a suggestion to examine more precisely. The fact that PMF could not differentiate between *Capra hircus* and *Ovis aries* comes as no surprise since the protein sequence of both of them present homology to a high degree. However, both of these species share great part of their protein collagen sequence with *Bos taurus*.

Therefore, in order to prove this hypothesis, the peptides—according to which, PMF technique claims to belong to *Capra hircus* or *Ovis aries* and not *Bos Taurus*—were checked with BLAST sequence alignment tool, comparing collagen α2(I) sequence of

both *Capra hircus* and *Ovis aries* with *Bos taurus* separately. Among the peptides that PMF technique have assigned to *Capra hircus* and *Ovis aries,* two peptides were found to be unique to *Capra hircus* and *Ovis aries*, confirming that there is a high possibility that animal glue (7) is made of sources from one or both of these species (Figure 3.13).

These results are of importance, since as it was pointed out before, high homology between collagen protein of *Capra hircus, Ovis aries* and *Bos taurus* to differentiate between these species is a difficult task and if not a unique peptide to be found, the data may be considered to be related to both species.

4 Conclusion

Animal glue has been used through the history of mankind for a long time as an adhesive. In general, it still has kept its application in painting binding medium, bookbinding, sizing, wood manufacturing, to mention but a few, and in particular in the field of cultural heritage in the restoration treatments. From this point of view, characterization of animal glue is crucial for a restorer to know in detail the composition of the material they introduce into the "matrix" of cultural heritage objects. In this thesis, samples of animal glue from restoration laboratories of both S. Orsola Benincasa, Naples, Italy and Museo del Prado, Madrid, Spain with the aim of protein characterization have been analyzed.

As for the first part of the experiments which aimed to identify sources of animal glues employed in samples of S. Orsola Benincasa, Naples, Italy to the level of species and tissues, following conclusions can be drawn:

- According to the results that a summary of which is illustrated in Table 4.1, only 1 sample out of 8 samples, the rabbit glue (of the first postwar), in fact, was made of what it is claimed on the label to be the source. In most cases, the samples were made of a combination of two or more animal sources.
- Since in all rabbit animal glues collagen α1 (III) has been found, it is highly probable that not only bone and cartilage but also skin waste and connective tissues of animals have been used. Preparing animal glues from a mixture of tissues in the so-called 'rabbit glue' samples may imply attempts toward enhancing glue properties; for example, altering the degree of viscosity. Hide glues are made of skin waste of animals and have higher viscosity in comparison with bone glues and possibly for this reason, manufacturers mixed all these parts to achieve a higher viscosity as supposedly 'skin rabbit glue' should have. The same theory can be applied to the fish flake sample where collagen α1 (III) along with collagen α1 (I) and collagen α2 (I) have been detected.
- In the case of sturgeon fish glue, although collagen of a fish species (*Scyliorhinus canicula*) has been detected, it is no surprise that no collagen of sturgeon has been detected since some species of sturgeon are extinct and

several are on the verge of extinction. In addition, many species are classified as endangered.

- In the case of strong glues, no peptide of collagen α1(III) have been found. This is in total agreement with the instructions of making strong glues (otherwise known as bone glue) in which animal bones should solely be used.
- As for the strong glue (old manufacturing), since the presence of donkey waste parts and bones in the sample was not a common case, the presence of a mixture of these three sources (bovine, porcine, and donkey) may relates to providing bones from mostly farm animals coming from slaughterhouses and not paying much attention as to which kind of animal to use as source for glue preparation.

Additionally, preliminarily evaluation of degradation rate by the information acquired by LC-MS/MS has showed potential applications of this technique toward this area of study.

Table 4.1 A summary of the results of animal glues' protein identification by LC-MS/MS and Mascot.

Name of the sample	Taxonomy	Collagen α1(I)	Collagen α2(I)	Collagen α1(III)
Rabbit glue (of the first postwar)	*Oryctolagus cuniculus*	✓	✓	✓
Rabbit Glue Toten (sixties)	*Bos Taurus*	✓	✓	✓
Rabbit glue (CTS)	*Bos Taurus*	✓	✓	✓
Rabbit glue (Giosi)	*Bos Taurus*	✓	✓	✓
	Sus scrofa	✓	✓	✓
Fish glue (flakes)	*Sus scrofa*	✓	✓	✓
Sturgeon fish glue (pearls)	*Scyliorhinus canicula*	✓		
	Sus scrofa	✓	✓	
Strong glue (pearls)	*Bos Taurus*	✓	✓	
	Sus scrofa	✓	✓	
Strong glue (old manufacturing)	*Bos Taurus*	✓	✓	
	Equus asinus	✓	✓	
	Sus scrofa	✓	✓	

Identifying collagen proteins according to the procedure which have been taken in the third part of the project provides a general strategy to give preliminary analysis of the glues and a first glimpse to which animal sources have been employed in the preparation of animal glue samples. This strategy has been adopted to analyze three samples from Museo del Prado. This is somewhat a quick way to have an idea of what samples are possibly made of and if required, to take next step and analyze samples with LC-MS/MS techniques.

Moreover, in the last part of the project, one of the samples of Museo del Prado (rabbit glue (7)) was used to ameliorate the existing protocol, by testing the hypothesis of whether combining two C18 chromatographic could yield higher sequence coverage and better-quality spectra. Results of the series of experiments showed that adding ZipTip clean-up step after StageTip protocol on the unbound and wash fractions could improve the number of recovered peptides.

From a general point of view, unique applications of proteomics in cultural heritage have opened up new perspectives toward analyzing cultural heritage materials, making it possible to go even deeper in characterizing materials. In the case of identification of proteinaceous materials in cultural heritage area of study, it is possible with this method to gain information about the taxonomy and tissue of the composing materials of the sample. Since, LC-MS/MS provides information on peptide sequences and measures masses of peptides, this data also can be used for measuring degradation phenomenon.

Appendix I

Rabbit glue of the first postwar

Table I-1 Protein identification of *rabbit glue of the first postwar* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset and considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein Name	Taxonomy	Protein Identifier	Protein Score	Sequence Coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Oryctolagus cuniculus*	P02456	2887	57	47	6
Collagen α2(I)	*Oryctolagus cuniculus*	Q28668	2847	55	47	17
Collagen α1(III)	*Oryctolagus cuniculus*	G1T8J0	1689	33	32	15

Collagen alpha-1(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
350.69	29	K.EGGKGPR.G
426.21	45	R.GFSGLDGAK.G
449.76	39	R.GVVGLPGQR.G + Hydroxylation (P) (P)
450.25	26	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
536.77	33	R.GFPGADGVAGPK.G
544.77	56	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
545.78	39	R.GVQGPPGPAGPR.G + Deamidated (NQ)
553.29	26	R.GVQGPPGPAGPR.G + Hydroxylation (P) (P)
553.78	36	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
573.78	44	R.GQAGVMGFPGPK.G + Deamidated (NQ)
581.29	60	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P)
581.29	55	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K)
581.78	56	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
588.82	53	R.GVPGPPGAVGPAGK.D + Hydroxylation (P) (P)
589.28	47	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P); Oxidation (M)
589.77	34	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
589.78	45	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Oxidation (M)
596.82	50	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
621.80	67	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.80	88	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
656.32	94	K.GETGPSGPAGPTGAR.G
656.83	50	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.82	74	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
664.83	38	R.GFPGLPGPSGEPGK.Q + Hydroxylation (K) (K); Hydroxylation (P) (P)
666.83	66	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
667.32	58	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)
672.82	66	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
674.83	55	R.GPSGPQGPSGPPGPK.G + 2 Hydroxylation (P) (P)
675.32	48	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
733.35	65	R.GEPGPTGLPGPPGER.G + 3 Hydroxylation (P) (P)
743.35	73	R.GSPGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-1(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
751.35	98	R.GSPGPPGATGFPGAAGR.V + 3 Hydroxylation (P) (P)
766.89	70	R.GETGPAGPAGPIGPAGAR.G
785.88	51	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
793.39	41	K.GANGAPGIAGAPGFPGAR.G + 3 Hydroxylation (P) (P)
793.88	70	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
795.91	59	R.GLTGPIGPPGPAGAPGDK.G + 2 Hydroxylation (P) (P)
828.40	35	K.GSPGEAGRPGEAGLPGAK.G + 3 Hydroxylation (P) (P)
845.89	85	K.DGEAGAQGPPGPAGPAGER.G
853.88	80	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
854.38	72	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)
871.87	38	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P)
872.36	81	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
872.86	80	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
879.87	52	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P); Oxidation (M)
880.36	83	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
590.93	36	R.EGSPGAEGSPGRDGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
885.89	46	R.EGSPGAEGSPGRDGAPGPK.G + 3 Hydroxylation (P) (P)
908.93	66	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
912.96	25	R.VGPPGPSGNAGPPGPPGPVGK.E + 2 Hydroxylation (P) (P)
916.93	78	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P); Oxidation (M)
614.31	25	R.VGPPGPSGNAGPPGPPGPVGK.E + 3 Hydroxylation (P) (P)
920.96	41	R.VGPPGPSGNAGPPGPPGPVGK.E + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
921.45	38	R.VGPPGPSGNAGPPGPPGPVGK.E + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
924.93	39	R.GPPGPMGPPGLAGPPGESGR.E + 3 Hydroxylation (P) (P); Oxidation (M)
925.43	87	K.GEPGPTGVQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
925.92	59	K.GEPGPTGVQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
928.96	49	R.VGPPGPSGNAGPPGPPGPVGK.E + 4 Hydroxylation (P) (P)
929.45	28	R.VGPPGPSGNAGPPGPPGPVGK.E + Deamidated (NQ); 4 Hydroxylation (P) (P)
974.48	61	K.SGDRGETGPAGPAGPIGPAGAR.G
649.99	48	K.SGDRGETGPAGPAGPIGPAGAR.G
655.32	26	K.SGDRGETGPAGPAGPIGPAGAR.G + Hydroxylation (P) (P)
663.99	29	K.GEPGPTGVQGPPGPAGEEGKR.G + Hydroxylation (P) (P)
1003.48	71	K.GEPGPTGVQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1003.97	66	K.GEPGPTGVQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1037.01	73	K.GSPGADGPAGAPGTPGPQGIAGQR.G
1045.00	77	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Hydroxylation (P) (P)
1045.50	70	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
1045.99	87	K.GSPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1053.00	85	K.GSPGADGPAGAPGTPGPQGIAGQR.G + 2 Hydroxylation (P) (P)
1053.50	26	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1059.49	53	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-1(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1060.99	74	K.GSPGADGPAGAPGTPGPQGIAGQR.G + 3 Hydroxylation (P) (P)
1061.49	39	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1067.49	28	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1067.98	27	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1069.04	72	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
1075.49	52	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1093.03	63	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
733.66	28	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1099.98	43	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 3 Hydroxylation (P) (P); Oxidation (M)
1100.48	65	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1108.47	67	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1108.47	79	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1108.53	29	R.GETGPAGRPGEVGPPGPPGPAGEK.G + 3 Hydroxylation (P) (P)
739.37	29	R.GETGPAGRPGEVGPPGPPGPAGEK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1108.97	62	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1117.07	96	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G
1125.06	76	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (K) (K)
1125.06	68	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (P) (P)
1125.56	53	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
1133.07	103	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1136.03	51	R.GFPGLPGPSGEPGKQGPSGASGER.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1141.06	57	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1141.56	41	K.GDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
762.02	52	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Hydroxylation (P) (P)
1142.53	57	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Hydroxylation (P) (P)
767.35	37	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1151.02	35	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
767.68	30	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1158.52	60	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1159.02	38	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
774.69	28	R.GPPGAPGKNGDDGEAGKPGRPGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
822.41	28	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
1241.12	61	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P)
1247.54	34	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
832.03	44	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-1(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
832.36	55	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
1249.10	48	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
837.03	36	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 4 Hydroxylation (P) (P)
837.03	45	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P); Oxidation (M)
837.36	31	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
837.36	46	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
1257.10	72	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1258.60	51	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (P) (P)
1266.61	68	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 2 Hydroxylation (P) (P)
1267.10	46	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1274.60	71	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 3 Hydroxylation (P) (P)
1275.09	54	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1282.60	78	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
896.42	30	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
896.75	26	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1345.63	89	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
901.75	33	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1352.12	44	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1353.13	103	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)
902.75	86	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
915.09	43	K.QGPSGASGERGPPGPMGPPGLAGPPGESGR.E + Hydroxylation (P) (P); Oxidation (M)
920.43	38	K.QGPSGASGERGPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P); Oxidation (M)
920.76	35	K.QGPSGASGERGPPGPMGPPGLAGPPGESGR.E + Deamidated (NQ); 3 Hydroxylation (P) (P)
925.76	28	K.QGPSGASGERGPPGPMGPPGLAGPPGESGR.E + 3 Hydroxylation (P) (P); Oxidation (M)
1440.69	44	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 3 Hydroxylation (P) (P)
1441.19	27	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1442.21	92	R.GLTGPIGPPGPAGAPGDKGETGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
1016.50	44	K.GEPGDAGAKGDAGPAGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1021.82	37	K.GEPGDAGAKGDAGPAGPAGPAGPPGPIGNVGAPGPK.G + 2 Hydroxylation (K) (K); Hydroxylation (P) (P)
1051.49	58	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1056.82	42	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1057.15	44	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1057.15	54	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-1(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1061.82	25	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1062.15	30	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1062.15	44	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1062.48	32	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
1081.13	42	R.GSNGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1081.14	26	R.GSNGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1086.14	58	R.GSNGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 5 Hydroxylation (P) (P); Oxidation (M)
1086.47	46	R.GSNGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1086.79	43	R.GSNGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1087.13	41	R.GSNGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1124.54	35	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGAR.G + 3 Hydroxylation (P) (P)
1130.19	43	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGAR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1300.92	37	R.GEPGPPGPAGAAGPAGNPGADGQPGAKGANGAPGIAGAPGFPGAR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 7 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
437.2	31	R.GVMGPQGAR.G + Deamidated (NQ)
456.23	31	R.GHNGLDGLK.G + Deamidated (NQ)
549.79	46	R.GLVGEPGPAGTK.G + Hydroxylation (P) (P)
592.28	37	K.GGGPGPMGLMGPR.G
601.29	44	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
601.79	41	R.GEPGNIGFPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
402.55	43	R.SGHPGTVGPAGLR.G
603.81	54	R.GFPGTPGLPGFK.G + 2 Hydroxylation (P) (P)
618.31	62	R.GEAGAAGPAGPAGPR.G
620.32	67	R.GLPGSPGNVGPAGK.E + 2 Hydroxylation (P) (P)
620.81	26	R.GLPGSPGNVGPAGK.E + Deamidated (NQ); 2 Hydroxylation (P) (P)
634.34	85	R.GIPGPVGAAGATGAR.G + Hydroxylation (P) (P)
476.91	40	K.GETGLRGEIGNPGR.D + Hydroxylation (P) (P)
719.37	28	R.GLPGEFGLPGPAGPR.G + Hydroxylation (P) (P)
727.37	81	R.GLPGEFGLPGPAGPR.G + 2 Hydroxylation (P) (P)
735.37	71	R.GLPGEFGLPGPAGPR.G + 3 Hydroxylation (P) (P)
755.86	42	R.GAPGAVGAPGPAGATGDR.G + 2 Hydroxylation (P) (P)
767.34	59	R.GDGGPPGMTGFPGAAGR.T + 2 Hydroxylation (P) (P)
773.89	43	R.GEPGPAGSIGPVGAAGPR.G
775.36	74	R.GDGGPPGMTGFPGAAGR.T + 2 Hydroxylation (P) (P); Oxidation (M)
781.89	106	R.GEPGPAGSIGPVGAAGPR.G + Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-2(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
781.91	71	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
783.87	97	R.GAPGESGAAGPPGPIGSR.G + 2 Hydroxylation (P) (P)
804.88	98	R.GSPGEPGSAGPAGPPGLR.G + 3 Hydroxylation (P) (P)
808.41	56	K.GEIGPVGNPGPSGPAGPR.G
812.88	39	R.GSPGEPGSAGPAGPPGLR.G + 4 Hydroxylation (P) (P)
816.90	82	K.GEIGPVGNPGPSGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
868.42	33	R.GPPGAVGSPGVNGAPGEAGR.D + 2 Hydroxylation (P) (P)
868.91	69	R.GPPGAVGSPGVNGAPGEAGR.D + Deamidated (NQ); 2 Hydroxylation (P) (P)
583.96	27	R.GHNGLDGLKGQPGAPGVK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
876.41	101	R.GPPGAVGSPGVNGAPGEAGR.D + 3 Hydroxylation (P) (P)
876.91	102	R.GPPGAVGSPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
882.93	43	K.RGSPGEPGSAGPAGPPGLR.G + 3 Hydroxylation (P) (P)
588.96	36	K.RGSPGEPGSAGPAGPPGLR.G + 3 Hydroxylation (P) (P)
891.91	30	R.GPNGDSGRPGEPGLMGPR.G + 2 Hydroxylation (P) (P)
595.27	31	R.GPNGDSGRPGEPGLMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
909.45	78	R.TGPPGPSGITGPPGPPGAAGK.E + 3 Hydroxylation (P) (P)
917.45	29	R.TGPPGPSGITGPPGPPGAAGK.E + 4 Hydroxylation (P) (P)
676.34	51	K.HGNRGEPGPAGSIGPVGAAGPR.G + Hydroxylation (P) (P)
676.67	54	K.HGNRGEPGPAGSIGPVGAAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1018.00	44	R.GEVGPAGPNGFAGPAGAAGQPGAK.G
1018.49	92	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ)
1025.99	62	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Hydroxylation (P) (P)
1026.49	91	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (P) (P)
1034.49	72	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1065.07	77	R.GLPGVAGALGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1073.49	73	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + 2 Hydroxylation (P) (P)
716.32	67	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1074.48	50	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1127.04	76	R.GYPGNAGPVGAAGAPGPQGSVGPTGK.H + 2 Hydroxylation (P) (P)
1127.54	49	R.GYPGNAGPVGAAGAPGPQGSVGPTGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
1127.54	56	R.GYPGNAGPVGAAGAPGPQGSVGPTGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
1128.03	70	R.GYPGNAGPVGAAGAPGPQGSVGPTGK.H + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
789.05	38	R.GEVGPAGPNGFAGPAGAAGQPGAKGEK.G + Hydroxylation (K) (K)
789.38	29	R.GEVGPAGPNGFAGPAGAAGQPGAKGEK.G + Deamidated (NQ); Hydroxylation (K) (K)
794.02	26	K.GESGNKGEPGSAGPQGPPGPSGEEGK.R + Hydroxylation (P) (P)
1191.56	66	R.GEVGPAGPNGFAGPAGAAGQPGAKGEK.G + Deamidated (NQ); 2 Hydroxylation (K) (K)
1194.60	51	R.GEVGLPGVSGPVGPPGNPGANGLTGAK.G + 2 Hydroxylation (P) (P)
1195.10	62	R.GEVGLPGVSGPVGPPGNPGANGLTGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
799.35	38	K.GESGNKGEPGSAGPQGPPGPSGEEGK.R + 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-2(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
800.04	32	R.GEVGPAGPNGFAGPAGAAGQPGAKGEK.G + Deamidated (NQ); 2 Hydroxylation (K) (K); Hydroxylation (P) (P)
1202.61	64	R.GEVGLPGVSGPVGPPGNPGANGLTGAK.G + 3 Hydroxylation (P) (P)
1203.09	57	R.GEVGLPGVSGPVGPPGNPGANGLTGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1210.61	53	R.GEVGLPGVSGPVGPPGNPGANGLTGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1211.09	53	R.GEVGLPGVSGPVGPPGNPGANGLTGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1262.12	109	K.GENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + Hydroxylation (P) (P)
842.07	46	K.GENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + Deamidated (NQ); Hydroxylation (P) (P)
1262.61	96	K.GENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + Deamidated (NQ); Hydroxylation (P) (P)
1263.10	99	K.GENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1287.13	96	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1295.13	76	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
884.09	37	R.GSPGERGEVGPAGPNGFAGPAGAAGQPGAK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
884.42	31	R.GSPGERGEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
884.75	39	R.GSPGERGEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1364.16	33	R.GAPGAVGAPGPAGATGDRGEAGAAGPAGPAGPR.G + 2 Hydroxylation (P) (P)
915.46	39	K.GHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + Deamidated (NQ)
925.79	30	K.GHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + 2 Hydroxylation (P) (P)
936.13	31	K.GPKGENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + Deamidated (NQ); Hydroxylation (P) (P)
936.46	85	K.GPKGENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
941.13	97	K.GPKGENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
941.46	96	K.GPKGENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
941.79	100	K.GPKGENGVVGPAGPVGAAGPSGPNGPPGPAGGR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1418.67	44	K.GEQGPAGPPGFQGLPGPSGTAGEVGKPGER.G + 3 Hydroxylation (P) (P)
1471.25	68	K.GPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + 3 Hydroxylation (P) (P)
1479.25	65	K.GPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + 4 Hydroxylation (P) (P)
1052.50	36	R.GPSGPPGPDGNKGEPGVVGAPGTAGASGPGGLPGER.G + 3 Hydroxylation (P) (P)
1052.83	28	R.GPSGPPGPDGNKGEPGVVGAPGTAGASGPGGLPGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1586.25	50	R.GPSGPPGPDGNKGEPGVVGAPGTAGASGPGGLPGER.G + 4 Hydroxylation (P) (P)
1063.16	64	R.GPSGPPGPDGNKGEPGVVGAPGTAGASGPGGLPGER.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
836.43	25	R.GLPGIKGHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
839.93	29	R.GLPGIKGHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-2(I) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1119.90	67	R.GLPGIKGHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1120.24	41	R.GLPGIKGHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1214.52	37	R.GSQGSQGPAGPPGPPGPPGPPGASGGGYDFGYDGDFYR.A + 4 Hydro (P) (P)
1322.34	39	R.GEVGLPGVSGPVGPPGNPGANGLTGAKGAAGLPGVAGAPGLPGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 6 Hydroxylation (P) (P)
1346.97	36	K.GENGVVGPAGPVGAAGPSGPNGPPGPAGGRGDGGPPGMTGFPGAAGR.T + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
1347.29	44	K.GENGVVGPAGPVGAAGPSGPNGPPGPAGGRGDGGPPGMTGFPGAAGR.T + 2 Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
1401.69	38	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPKGEIGPVGNPGPSGPAGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1406.68	27	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPKGEIGPVGNPGPSGPAGPR.G + 5 Hydro (P) (P)
1407.02	42	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPKGEIGPVGNPGPSGPAGPR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1476.39	41	R.TGETGASGPPGFPGEKGPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1482.06	37	R.TGETGASGPPGFPGEKGPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1482.06	32	R.TGETGASGPPGFPGEKGPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1487.05	38	R.TGETGASGPPGFPGEKGPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
1487.39	46	R.TGETGASGPPGFPGEKGPSGEAGTAGPPGTPGPQGLLGAPGILGLPGSR.G + Deamidated (NQ); Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
Collagen alpha-1(III) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
536.76	27	R.GEAGSPGIPGAK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
562.80	47	R.GPPGLAGTPGLR.G + 2 Hydroxylation (P) (P)
569.78	60	R.GLAGPPGMPGPR.G + 2 Hydroxylation (P) (P)
588.80	29	R.GGPGGPGLPGPPGK.N + 2 Hydroxylation (P) (P)
602.29	38	R.GQPGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
602.78	43	R.GQPGVMGFPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
604.79	50	R.GGPGGPGLPGPPGK.N + 4 Hydroxylation (P) (P)
652.30	51	R.GSPGGPGAAGFPGAR.G + 3 Hydroxylation (P) (P)
707.85	52	R.GERGEAGSPGIPGAK.G + 2 Hydroxylation (P) (P)
725.85	40	R.GPPGPPGTNGIPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
725.85	40	R.GPPGPPGTNGIPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
726.34	53	R.GPPGPPGTNGIPGQR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
505.58	37	K.DGTSGHPGPIGPPGPR.G + Hydroxylation (P) (P)
761.86	56	R.GETGPAGPSGAPGPAGAR.G + Hydroxylation (P) (P)
510.91	46	K.DGTSGHPGPIGPPGPR.G + 2 Hydroxylation (P) (P)
819.39	89	R.GPAGPSGTPGEKGPAGER.G + Hydroxylation (P) (P)
821.87	61	K.GEVGPAGSPGSNGSPGQR.G + 2 Hydroxylation (P) (P)
822.36	69	K.GEVGPAGSPGSNGSPGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
835.90	61	K.GEMGPAGIPGAPGLMGAR.G + 2 Hydroxylation (P) (P)
843.89	80	K.GEMGPAGIPGAPGLMGAR.G + 2 Hydroxylation (P) (P); Oxidation (M)

The highlighted peptides are unique peptides.

Table I-1 ***Continued.***

Collagen alpha-1(III) [*Oryctolagus cuniculus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
573.25	36	R.GATGEPGRDGAPGSPGMR.G + 3 Hydroxylation (P) (P)
870.38	29	K.GENGLPGENGAPGPMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
878.88	48	K.DGSPGEPGANGLPGAAGER.G + 3 Hydroxylation (P) (P)
969.45	56	K.SGDRGETGPAGPSGAPGPAGAR.G + Hydroxylation (P) (P)
981.44	51	K.DGPPGPPGSNGAPGNPGVAGPK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1022.47	53	R.GLPGPPGNNGNPGPPGPSGGPGK.D + Deamidated (NQ); 4 Hydroxylation (P) (P)
1037.04	74	K.GSPGAQGPPGAPGPLGLAGITGAR.G + Deamidated (NQ); Hydroxylation (P) (P)
1044.54	72	K.GSPGAQGPPGAPGPLGLAGITGAR.G + 2 Hydroxylation (P) (P)
1045.04	95	K.GSPGAQGPPGAPGPLGLAGITGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1052.54	91	K.GSPGAQGPPGAPGPLGLAGITGAR.G + 3 Hydroxylation (P) (P)
1053.03	105	K.GSPGAQGPPGAPGPLGLAGITGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1095.51	80	K.GPAGPPGPPGASGTPGLQGMPGER.G + 2 Hydroxylation (P) (P); Oxidation (M)
1096.01	30	K.GPAGPPGPPGASGTPGLQGMPGER.G + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
1103.51	64	K.GPAGPPGPPGASGTPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1104.00	78	K.GPAGPPGPPGASGTPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1111.50	55	K.GPAGPPGPPGASGTPGLQGMPGER.G + 4 Hydroxylation (P) (P); Oxidation (M)
743.32	33	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
748.33	29	K.GEDGKDGSPGEPGANGLPGAAGER.G + 3 Hydroxylation (P) (P)
748.66	34	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1122.48	71	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1124.00	83	R.GEHGPPGPAGFPGAPGQNGEPGAK.G + 4 Hydroxylation (P) (P)
749.99	40	R.GEHGPPGPAGFPGAPGQNGEPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1124.49	63	R.GEHGPPGPAGFPGAPGQNGEPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
766.35	26	R.GENGSPGAPGAPGHPGPPGPVGPAGK.S + Deamidated (NQ); 5 Hydroxylation (P) (P)
1149.06	75	K.GEGGPPGIAGPPGGSGPAGPPGPQGVK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
826.41	27	R.GPVGPSGPPGKDGTSGHPGPIGPPGPR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
648.29	27	R.GEHGPPGPAGFPGAPGQNGEPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
648.29	31	R.GEHGPPGPAGFPGAPGQNGEPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
880.76	28	K.GEGGPPGIAGPPGGSGPAGPPGPQGVKGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1426.69	57	R.GAPGEKGEGGPPGIAGPPGGSGPAGPPGPQGVK.G + 2 Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1460.73	53	R.GPAGPIGPPGPAGQPGDKGEGGAPGLPGIAGPR.G + 4 Hydroxylation (P) (P)
981.82	33	K.GDAGQPGEKGSPGAQGPPGAPGPLGLAGITGAR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
987.15	61	K.GDAGQPGEKGSPGAQGPPGAPGPLGLAGITGAR.G + 4 Hydroxylation (P) (P)
1056.49	29	R.GGAGPPGPEGGKGPAGPPGPPGASGTPGLQGMPGER.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P); Oxidation (M)

The highlighted peptides are unique peptides.

Rabbit glue (Giosi)

Table I-2 Protein identification of *rabbit glue (Giosi)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Bos taurus*	P02453	2698	48	48	2
Collagen α1(I)	*Sus scrofa*	A0A287A1S6	2492	48	12	5
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	2140	45	36	12
Collagen α2(I)	*Bos taurus*	P02459	1802	39	18	7
Collagen α1(III)	*Bos taurus*	P02458	1119	28	22	7
Collagen α1(III)	*Sus scrofa*	F1RYI8	865	23	6	4

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
426.21	45	R.GFSGLDGAK.G
449.76	34	R.GVVGLPGQR.G + Hydroxylation (P) (P)
542.75	69	R.EGAPGAEGSPGR.D
544.77	64	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
545.29	41	R.GVQGPPGPAGPR.G
545.78	58	R.GVQGPPGPAGPR.G + Deamidated (NQ)
553.78	49	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
573.29	41	R.GQAGVMGFPGPK.G
573.78	43	R.GQAGVMGFPGPK.G + Deamidated (NQ)
581.28	56	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K)
581.28	49	R.GQAGVMGFPGPK.G + Oxidation (M)
581.28	62	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P)
581.78	56	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
588.82	38	R.GVPGPPGAVGPAGK.D + Hydroxylation (P) (P)
589.28	41	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
589.28	56	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P); Oxidation (M)
589.77	74	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Oxidation (M)
589.77	56	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
596.82	51	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
597.28	31	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P); Oxidation (M)
597.77	42	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P); Oxidation (M)
621.80	87	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.78	71	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
641.31	60	K.GEAGPSGPAGPTGAR.G
648.83	38	R.GFPGLPGPSGEPGK.Q
656.83	48	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.82	41	R.GFPGLPGPSGEPGK.Q + Hydroxylation (K) (K); Hydroxylation (P) (P)
664.8	69	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
666.83	61	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)

Table I-2 ***Continued.***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
667.32	63	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)
672.82	54	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
675.32	33	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
718.34	61	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)
722.35	48	R.GSAGPPGATGFPGAAGR.V + Hydroxylation (P) (P)
730.35	120	R.GSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)
780.91	68	R.GETGPAGPAGPIGPVGAR.G
781.39	31	K.DGLNGLPGPIGPPGPR.G + 3 Hydroxylation (P) (P)
781.89	38	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
785.88	32	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
793.38	36	K.GANGAPGIAGAPGFPGAR.G + 3 Hydroxylation (P) (P)
793.87	58	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
795.91	54	R.GLTGPIGPPGPAGAPGDK.G + 2 Hydroxylation (P) (P)
539.93	48	R.GFSGLDGAKGDAGPAGPK.G + Hydroxylation (K) (K)
828.39	45	K.GSPGEAGRPGEAGLPGAK.G + 3 Hydroxylation (P) (P)
845.89	104	K.DGEAGAQGPPGPAGPAGER.G
846.38	47	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ)
853.88	107	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
854.38	88	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)
576.93	30	R.EGAPGAEGSPGRDGSPGAK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
871.87	52	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P)
872.36	92	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
872.85	55	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
879.87	52	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P); Oxidation (M)
880.36	92	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
880.85	67	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
906.94	30	R.VGPPGPSGNAGPPGPPGPAGK.E + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
605.29	27	R.VGPPGPSGNAGPPGPPGPAGK.E + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
606.29	26	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
908.93	91	R.GPPGPMGPPGLAGPPGESGR.E + Hydroxylation (P) (P); Oxidation (M)
916.93	73	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P); Oxidation (M)
932.44	34	K.GEPGPTGIQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
932.93	73	K.GEPGPTGIQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
659.33	49	K.SGDRGETGPAGPAGPIGPVGAR.G
673.99	31	K.GEPGPTGIQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
674.32	53	K.GEPGPTGIQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1029.50	65	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ)
1029.99	85	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ)
1037.50	31	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
1037.99	51	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-2 ***Continued.***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1045.99	75	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1053.98	64	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
712.32	29	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1091.98	32	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 3 Hydroxylation (P) (P)
1093.02	38	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1099.98	45	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1100.48	88	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1100.97	67	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
1107.98	34	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P); Oxidation (M)
1108.47	61	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1108.97	43	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
762.67	30	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1146.56	55	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
768.01	44	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1154.56	31	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
773.34	56	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
775.37	31	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
807.69	38	R.GPSGPQGPSGPPGPKGNSGEPGAPGSK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
819.08	29	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
824.41	31	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
827.74	26	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P)
829.74	43	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
832.03	52	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
832.36	44	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
833.07	37	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
833.07	28	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
837.03	49	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P); Oxidation (M)
837.36	43	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
837.36	30	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
837.69	33	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
838.40	53	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
842.69	33	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P); Oxidation (M)
843.02	29	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1266.60	25	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)

Table I-2 ***Continued.***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1267.10	40	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1275.09	42	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
891.76	48	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Hydroxylation (P) (P)
1345.13	70	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Hydroxylation (P) (P)
1345.63	50	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1353.13	73	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)
902.75	93	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydro(P) (P)
1354.11	79	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
907.41	26	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
915.09	47	K.QGPSGASGERGPPGPMGPPGLAGPPGESGR.E + Hydro (P) (P); Oxidation (M)
945.13	37	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ)
946.47	28	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (P) (P)
951.80	31	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
951.80	28	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
960.79	33	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 3 Hydroxylation (P) (P)
961.12	27	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1036.15	26	K.GEPGDAGAKGDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 2 Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1041.49	49	K.GEPGDAGAKGDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1057.47	29	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 3 Deamidated (NQ); 3 Hydroxylation (P) (P)
1062.81	26	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 3 Deamidated (NQ); 4 Hydroxylation (P) (P)
1075.47	46	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 5 Hydro (P) (P)
1075.80	57	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1076.13	44	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1076.46	50	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); 5 Hydroxylation (P) (P)
1076.79	35	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 4 Deamidated (NQ); 5 Hydroxylation (P) (P)
1081.14	26	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1081.14	26	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
1081.46	26	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1081.79	41	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1082.12	35	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 4 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1134.19	29	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEGGPQGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1295.92	36	R.GEPGPPGPAGAAGPAGNPGADGQPGAKGANGAPGIAGAPGFPGAR.G + 3 Deamidated (NQ); 7 Hydroxylation (P) (P)
1371.97	26	R.GEQGPAGSPGFQGLPGPAGPPGEAGKPGEQGVPGDLGAPGPSGAR.G + Deamidated (NQ); 6 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-2 ***Continued.***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
596.84	61	R.IGQPGAVGPAGIR.G
597.33	50	R.IGQPGAVGPAGIR.G + Deamidated (NQ)
604.84	47	R.IGQPGAVGPAGIR.G + Hydroxylation (P) (P)
605.33	49	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
634.34	94	R.GIPGPVGAAGATGAR.G + Hydroxylation (P) (P)
644.81	34	R.GFPGSPGNIGPAGK.E + Deamidated (NQ); 2 Hydroxylation (P) (P)
714.36	81	R.GIPGEFGLPGPAGAR.G + 2 Hydroxylation (P) (P)
746.85	50	R.SGETGASGPPGFVGEK.G + Hydroxylation (P) (P)
766.89	47	R.GEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
769.86	46	R.GAPGAIGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
790.88	83	R.GPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
824.92	28	R.GSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
602.31	27	K.RGSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
904.90	40	R.GPPGNVGNPGVNGAPGEAGR.D + 3 Deamidated (NQ); 2 Hydroxylation (P) (P)
912.90	59	R.GPPGNVGNPGVNGAPGEAGR.D + 3 Deamidated (NQ); 3 Hydroxylation (P) (P)
641.64	46	R.GERGPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
666.66	48	K.HGNRGEPGPAGAVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1131.56	51	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
1132.05	65	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1216.60	54	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); 4 Hydro (P) (P)
1224.59	71	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
696.08	38	R.GAPGAIGAPGPAGANGDRGEAGPAGPAGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
931.77	48	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
932.10	48	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1066.17	44	R.GPSGPPGPDGNKGEPGVVGAPGTAGPSGPSGLPGER.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1071.50	56	R.GPSGPPGPDGNKGEPGVVGAPGTAGPSGPSGLPGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
Collagen alpha-1(III) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
528.77	36	R.GEAGSPGIAGPK.G + Hydroxylation (P) (P)
542.76	39	R.GPAGANGLPGEK.G + Deamidated (NQ); Hydroxylation (K) (K)
556.77	44	R.GLAGPPGMPGAR.G + 2 Hydroxylation (P) (P)
594.78	37	R.GQPGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
602.29	38	R.GQPGVMGFPGPK.G + 2 Hydroxylation (P) (P)
602.78	48	R.GQPGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydro(P) (P)
610.78	33	R.GQPGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P); Oxidation (M)
637.29	62	R.GSPGGPGAAGFPGGR.G + 2 Hydroxylation (P) (P)
645.29	70	R.GSPGGPGAAGFPGGR.G + 3 Hydroxylation (P) (P)
711.33	59	R.GPPGPPGTNGVPGQR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-2 ***Continued.***

Collagen alpha-1(III) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
719.33	60	R.GPPGPPGTNGVPGQR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
495.57	37	K.DGASGHPGPIGPPGPR.G + Hydroxylation (P) (P)
769.86	64	R.GETGPAGPSGAPGPAGSR.G + Hydroxylation (P) (P)
800.86	68	K.GEVGPAGSPGSSGAPGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
818.93	33	K.GEMGPAGIPGAPGLIGAR.G + Hydroxylation (P) (P)
826.93	35	K.GEMGPAGIPGAPGLIGAR.G + 2 Hydroxylation (P) (P)
834.92	41	K.GEMGPAGIPGAPGLIGAR.G + 2 Hydroxylation (P) (P); Oxidation (M)
565.29	26	R.GVAGEPGRDGLPGGPGLR.G + 2 Hydroxylation (P) (P)
570.62	37	R.GVAGEPGRDGLPGGPGLR.G + 3 Hydroxylation (P) (P)
651.97	41	K.SGDRGETGPAGPSGAPGPAGSR.G + Hydroxylation (P) (P)
982.94	30	K.DGPPGPPGSNGAPGSPGISGPK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1041.56	67	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Hydroxylation (P) (P)
1042.05	55	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); Hydroxylation (P) (P)
700.04	66	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + 2 Hydroxylation (P) (P)
1050.04	80	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1058.04	55	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1085.98	85	R.GSDGQPGPPGPPGTAGFPGSPGAK.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1090.50	58	K.GAAGPPGPPGSAGTPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1090.99	70	K.GAAGPPGPPGSAGTPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1098.49	28	K.GAAGPPGPPGSAGTPGLQGMPGER.G + 4 Hydroxylation (P) (P); Oxidation (M)
1098.99	45	K.GAAGPPGPPGSAGTPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1120.49	36	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1120.98	26	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 3 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
748.66	50	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
748.66	57	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
861.71	30	R.GEQGPPGPAGFPGAPGQNGEPGAKGER.G + 3 Deamidated (NQ); 4 Hydro(P) (P)
870.40	26	K.GDRGENGSPGAPGAPGHPGPPGPVGPAGK.S + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
918.11	30	R.GAPGEKGEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
918.44	46	R.GAPGEKGEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
923.44	61	R.GAPGEKGEGGPPGAAGPAGGSGPAGPPGPQGVK.G + 3 Hydroxylation (P) (P)
923.77	41	R.GAPGEKGEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
937.41	42	R.GGPGERGEQGPPGPAGFPGAPGQNGEPGAK.G + 3 Deamidated (NQ); 5 Hydroxylation (P) (P)
989.81	32	R.GPTGPIGPPGPAGQPGDKGESGAPGVPGIAGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
733.87	42	K.SAGISVPGPMGPSGPR.G
741.87	25	K.SAGISVPGPMGPSGPR.G + Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-2 ***Continued.***

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
773.90	50	R.GETGPAGPAGPVGPVGAR.G
925.43	48	K.GEPGPTGVQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
654.66	34	K.SGDRGETGPAGPAGPVGPVGAR.G
1053.49	31	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1062.03	26	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
757.35	49	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 2 Hydroxylation (P) (P)
760.05	33	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (P) (P)
1147.57	75	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); Hydro(P) (P)
1151.52	49	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 4 Hydroxylation (P) (P)
770.71	37	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 3 Hydroxylation (P) (P)
1155.57	40	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
776.04	29	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 4 Hydroxylation (P) (P)
794.04	30	K.GDTGAKGEPGPTGVQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
803.08	34	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
813.74	32	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
823.07	37	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P)
824.40	28	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
918.44	34	K.QGPSGPSGERGPPGPMGPPGLAGPPGESGR.E + Hydroxylation (P) (P)
1026.17	27	K.GEPGDAGAKGDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1031.49	46	K.GEPGDAGAKGDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1042.16	49	K.GEPGDAGAKGDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 5 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
379.69	37	R.GLPGADGR.A + Hydroxylation (P) (P)
435.22	49	R.GPSGPQGIR.G + Deamidated (NQ)
542.78	40	R.GLVGEPGPAGSK.G + Hydroxylation (P) (P)
550.78	29	R.GLVGEPGPAGSK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
598.82	40	R.TGQPGAVGPAGIR.G + Hydroxylation (P) (P)
601.29	47	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
601.78	38	R.GEPGNIGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
601.79	44	R.GEPGNIGFPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
603.81	26	R.GFPGTPGLPGFK.G + 2 Hydroxylation (P) (P)
620.32	90	R.GIPGPAGAAGATGAR.G + Hydroxylation (P) (P)
631.31	53	R.GEAGPAGPAGPAGPR.G
637.31	89	R.GFPGSPGNVGPAGK.E + 2 Hydroxylation (P) (P)
677.34	53	K.GVGAGPGPMGLMGPR.G
727.37	72	R.GIPGEFGLPGPAGPR.G + 2 Hydroxylation (P) (P)
729.34	83	R.GDGGPPGATGFPGAAGR.I + Hydroxylation (P) (P)
735.37	45	R.GIPGEFGLPGPAGPR.G + 3 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-2 ***Continued.***

Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
737.34	104	R.GDGGPPGATGFPGAAGR.I + 2 Hydroxylation (P) (P)
739.84	36	R.TGETGASGPPGFAGEK.G + Hydroxylation (P) (P)
762.36	29	R.GAPGAVGAPGPAGANGDR.G + 2 Hydroxylation (P) (P)
762.85	49	R.GAPGAVGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
774.88	71	R.GEPGPAGSVGPAGAVGPR.G + Hydroxylation (P) (P)
775.88	96	R.GPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
781.91	75	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
783.88	32	R.GPPGESGAAGPAGPIGSR.G + 2 Hydroxylation (P) (P)
808.41	60	K.GELGPVGNPGPAGPAGPR.G + Hydroxylation (P) (P)
808.90	67	K.GELGPVGNPGPAGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
824.40	50	R.GPNGEVGSAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
824.90	72	R.GPNGEVGSAGPPGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
881.92	76	R.GPPGAVGNPGVNGAPGEAGR.D + 2 Hydroxylation (P) (P)
882.42	98	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); 2 Hydroxylation (P) (P)
889.92	74	R.GPPGAVGNPGVNGAPGEAGR.D + 3 Hydroxylation (P) (P)
890.41	89	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
600.60	28	R.GPNGDSGRPGEPGLMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
602.30	45	K.RGPNGEVGSAGPPGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
631.64	42	R.GERGPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
671.67	55	K.HGNRGEPGPAGSVGPAGAVGPR.G + Hydroxylation (P) (P)
504.25	33	K.HGNRGEPGPAGSVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1034.48	72	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1034.98	52	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1066.06	97	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1073.49	41	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + 2 Hydroxylation (P) (P)
1073.99	44	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
716.33	43	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1075.03	67	K.GEPGVLGAPGTAGPSGPSGLPGER.G + 2 Hydroxylation (P) (P)
1103.04	74	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 2 Hydroxylation (P) (P)
1111.03	39	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 3 Hydroxylation (P) (P)
794.02	46	K.GESGNKGEPGAAGPQGPPGPSGEEGK.R + 2 Hydroxylation (P) (P)
798.72	39	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K)
1200.61	25	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1201.10	37	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
804.05	30	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
804.37	45	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1208.61	25	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + 4 Hydroxylation (P) (P)
1209.10	59	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-2 ***Continued.***

Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1217.10	59	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
935.45	37	K.GEQGPAGPPGFQGLPGPAGTAGEVGKPGER.G + 2 Hydroxylation (P) (P)
940.79	26	K.GEQGPAGPPGFQGLPGPAGTAGEVGKPGER.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
955.14	72	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
955.47	46	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
955.79	74	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
973.76	31	R.GPPGAVGNPGVNGAPGEAGRDGNPGSDGPPGR.D + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1070.51	29	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + 2 Hydroxylation (P) (P)
1075.85	52	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1124.04	26	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 6 Hydroxylation (P) (P)
Collagen alpha-1(III) [Sus scrofa]		
M (Obs.)	**Ion Score**	**Identified Peptide**
761.86	36	R.GETGPAGPAGAPGPAGSR.G + Hydroxylation (P) (P)
813.37	45	K.GEVGPAGSPGPSGSPGQR.G + 2 Hydroxylation (P) (P)
843.90	32	K.GEMGPAGIPGAPGLMGAR.G + 2 Hydroxylation (P) (P); Oxidation (M)
565.95	30	R.GVAGEPGRDGVPGGPGLR.G + 3 Hydroxylation (P) (P)
1037.52	50	K.GSPGPQGPPGAPGPGGISGITGAR.G + 2 Hydroxylation (P) (P)
697.34	30	K.GSPGPQGPPGAPGPGGISGITGAR.G + 3 Hydroxylation (P) (P)
1095.52	50	K.GPAGPPGPPGAAGTPGLQGMPGER.G + 4 Hydroxylation (P) (P)
750.00	26	R.GEHGPPGPAGFPGAPGQNGEPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
980.47	34	R.GAPGEKGEGGPPGIAGQPGGTGPPGPPGPQGVK.G + 2 Hydroxylation (K) (K); 3 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Rabbit glue (CTS)

Table I-3 Protein identification of *rabbit glue* (*CTS)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein Name	Taxonomy	Protein Identifier	Protein Score	Sequence Coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Bos Taurus*	P02453	2345	44	43	1
Collagen α2(I)	*Bos Taurus*	P02459	1781	36	29	6
Collagen α1(III)	*Bos Taurus*	P04258	622	16	13	10

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
426.21	42	R.GFSGLDGAK.G
449.76	26	R.GVVGLPGQR.G + Hydroxylation (P) (P)
450.25	32	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
544.77	63	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
545.29	30	R.GVQGPPGPAGPR.G
545.78	52	R.GVQGPPGPAGPR.G + Deamidated (NQ)
365.86	27	R.GRPGAPGPAGAR.G + 2 Hydroxylation (P) (P)
553.29	29	R.GVQGPPGPAGPR.G + Hydroxylation (P) (P)
553.78	57	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
581.28	47	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K)
581.28	42	R.GQAGVMGFPGPK.G + Oxidation (M)
581.78	68	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
581.78	48	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M)
581.79	30	R.GLPGTAGLPGMK.G + Oxidation (M); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
588.82	44	R.GVPGPPGAVGPAGK.D + Hydroxylation (P) (P)
589.2	46	R.GQAGVMGFPGPK.G + Oxidation (M); Hydroxylation (P) (P)
589.77	55	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
589.77	64	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (P) (P)
596.82	64	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
597.28	42	R.GQAGVMGFPGPK.G + Oxidation (M); Hydroxylation (K) (K); Hydroxylation (P) (P)
597.77	51	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); Hydroxylation (P) (P)
621.79	77	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.79	77	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
641.31	49	K.GEAGPSGPAGPTGAR.G
656.83	30	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.83	63	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
666.83	57	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
667.32	66	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)
672.82	60	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
672.82	32	R.GFPGLPGPSGEPGK.Q + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
675.32	68	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
718.34	72	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)

Table I-3 ***Continued.***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
730.35	124	R.GSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)
780.91	64	R.GETGPAGPAGPIGPVGAR.G
781.40	36	K.DGLNGLPGPIGPPGPR.G + 3 Hydroxylation (P) (P)
781.89	47	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
793.88	47	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
539.93	64	R.GFSGLDGAKGDAGPAGPK.G + Hydroxylation (K) (K)
828.40	55	K.GSPGEAGRPGEAGLPGAK.G + 3 Hydroxylation (P) (P)
845.89	58	K.DGEAGAQGPPGPAGPAGER.G
853.88	73	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
854.38	71	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)
872.85	56	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
880.36	94	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydro (P) (P)
880.85	65	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 3 Hydro(P) (P)
908.93	90	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); Hydroxylation (P) (P)
916.93	62	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); 2 Hydroxylation (P) (P)
924.93	51	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); 3 Hydroxylation (P) (P)
932.93	44	K.GEPGPTGIQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
659.33	40	K.SGDRGETGPAGPAGPIGPVGAR.G
673.99	36	K.GEPGPTGIQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
674.32	43	K.GEPGPTGIQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1029.50	42	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ)
1037.01	79	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Hydroxylation (P) (P)
1037.50	61	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
1037.99	72	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1045.00	39	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Hydroxylation (P) (P)
1045.49	52	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1045.99	55	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1053.98	54	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
712.33	27	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1067.98	27	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
717.66	31	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1085.03	27	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (K) (K); 2 Hydro(P) (P)
1093.02	46	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1100.48	54	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1100.97	60	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydro (P) (P)
1107.98	64	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
1108.47	53	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1108.97	65	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
764.71	25	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
767.68	33	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)

Table I-3 ***Continued.***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
768.01	42	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
770.04	44	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
773.34	50	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
1162.56	36	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
778.67	40	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1170.55	47	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
829.74	41	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
833.07	29	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
837.36	37	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
837.69	53	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
838.40	33	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
842.69	32	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
843.02	46	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
845.07	50	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1267.10	36	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1274.60	37	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 3 Hydroxylation (P) (P)
1275.09	80	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
902.75	73	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
903.08	79	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
951.81	32	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
955.46	30	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 2 Hydroxylation (P) (P)
955.79	31	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
957.14	33	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
961.13	35	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1036.16	27	K.GEPGDAGAKGDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1062.81	49	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 3 Deamidated (NQ); 4 Hydroxylation (P) (P)
1076.46	28	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); 5 Hydroxylation (P) (P)
1081.46	28	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1081.79	44	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1082.12	44	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 4 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1134.52	26	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEGGPQGPR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-3 ***Continued.***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
542.78	46	R.GLVGEPGPAGSK.G + Hydroxylation (P) (P)
596.84	59	R.IGQPGAVGPAGIR.G
597.33	51	R.IGQPGAVGPAGIR.G + Deamidated (NQ)
601.29	37	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
601.79	45	R.GEPGNIGFPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
604.84	61	R.IGQPGAVGPAGIR.G + Hydroxylation (P) (P)
605.33	62	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
619.80	30	R.GFPGTPGLPGFK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
631.31	45	R.GEAGPAGPAGPAGPR.G
634.34	95	R.GIPGPVGAAGATGAR.G + Hydroxylation (P) (P)
644.32	35	R.GFPGSPGNIGPAGK.E + 2 Hydroxylation (P) (P)
644.81	49	R.GFPGSPGNIGPAGK.E + Deamidated (NQ); 2 Hydroxylation (P) (P)
714.36	72	R.GIPGEFGLPGPAGAR.G + 2 Hydroxylation (P) (P)
729.34	90	R.GDGGPPGATGFPGAAGR.T + Hydroxylation (P) (P)
737.33	95	R.GDGGPPGATGFPGAAGR.T + 2 Hydroxylation (P) (P)
746.85	37	R.SGETGASGPPGFVGEK.G + Hydroxylation (P) (P)
766.89	73	R.GEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
769.86	44	R.GAPGAIGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
781.91	66	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
790.88	77	R.GPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
808.41	57	K.GELGPVGNPGPAGPAGPR.G + Hydroxylation (P) (P)
808.90	57	K.GELGPVGNPGPAGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
816.92	56	R.GSTGEIGPAGPPGPPGLR.G + Hydroxylation (P) (P)
824.91	28	R.GSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
851.86	86	K.GEPGAPGENGTPGQTGAR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
911.92	49	R.GPPGNVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
912.41	57	R.GPPGNVGNPGVNGAPGEAGR.D + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
961.97	39	R.GERGPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
500.00	35	K.HGNRGEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
666.66	41	K.HGNRGEPGPAGAVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1026.49	42	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (P) (P)
1026.98	83	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1034.98	39	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1058.06	29	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 2 Hydroxylation (P) (P)
1066.06	115	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1131.06	83	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Hydroxylation (P) (P)
1131.56	32	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
1132.05	65	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
798.71	26	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-3 ***Continued.***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
799.04	25	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
804.05	26	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
804.37	46	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1216.11	29	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydro (P) (P)
1216.59	67	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); 4 Hydro (P) (P)
1224.11	38	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1224.60	68	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
861.74	51	K.GENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydro (P) (P)
931.77	55	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
932.10	35	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
960.79	36	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
961.13	91	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1071.51	36	R.GPSGPPGPDGNKGEPGVVGAPGTAGPSGPSGLPGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
Collagen alpha-1(III) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
521.76	29	K.GEAGAPGIPGGK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
542.76	38	R.GPAGANGLPGEK.G + Deamidated (NQ); Hydroxylation (K) (K)
637.29	79	R.GSPGGPGAAGFPGGR.G + 2 Hydroxylation (P) (P)
719.33	76	R.GPPGPPGTNGVPGQR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
769.86	64	R.GETGPAGPSGAPGPAGSR.G + Hydroxylation (P) (P)
792.86	52	K.GEVGPAGSPGSSGAPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
800.86	70	K.GEVGPAGSPGSSGAPGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
570.62	32	R.GVAGEPGRDGLPGGPGLR.G + 3 Hydroxylation (P) (P)
651.97	37	K.SGDRGETGPAGPSGAPGPAGSR.G + Hydroxylation (P) (P)
694.71	26	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Hydroxylation (P) (P)
695.03	36	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); Hydroxylation (P) (P)
700.03	46	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + 2 Hydroxylation (P) (P)
700.37	49	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1098.50	57	K.GAAGPPGPPGSAGTPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
732.99	28	K.GAAGPPGPPGSAGTPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1120.48	26	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
748.66	30	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
984.48	25	R.GPTGPIGPPGPAGQPGDKGESGAPGVPGIAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
989.82	43	R.GPTGPIGPPGPAGQPGDKGESGAPGVPGIAGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Rabbit Glue Toten (sixties)

Table I-4 Protein identification of *rabbit glue Toten (sixties)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Bos Taurus*	P02453	2704	53	47	1
Collagen α2(I)	*Bos Taurus*	P02459	2568	54	42	7
Collagen α1(III)	*Bos Taurus*	P04258	1669	34	27	10

Collagen alpha-1(I) [Bos Taurus]		
M (Obs.)	**Ion Score**	**Identified Peptide**
426.21	44	R.GFSGLDGAK.G
449.76	32	R.GVVGLPGQR.G + Hydroxylation (P) (P)
450.25	32	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
473.22	38	K.QGPSGASGER.G
473.71	44	K.QGPSGASGER.G + Deamidated (NQ)
536.77	34	R.GFPGADGVAGPK.G
360.53	32	R.GRPGAPGPAGAR.G + Hydroxylation (P) (P)
544.77	58	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
548.28	37	R.GRPGAPGPAGAR.G + 2 Hydroxylation (P) (P)
367.18	34	K.GADGAPGKDGVR.G
553.78	60	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
581.78	29	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M)
588.82	39	R.GVPGPPGAVGPAGK.D + Hydroxylation (P) (P)
589.28	49	R.GQAGVMGFPGPK.G + Oxidation (M); Hydroxylation (P) (P)
589.77	58	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (P) (P)
589.78	50	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K)
596.82	31	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
597.77	51	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); Hydroxylation (P) (P)
621.80	53	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.79	38	K.GLTGSPGSPGPDGK.T + Hydroxylation (K) (K); Hydroxylation (P) (P)
629.79	29	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
422.18	28	R.GDKGETGEQGDR.G + Hydroxylation (K) (K)
648.83	49	R.GFPGLPGPSGEPGK.Q
656.83	51	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.83	56	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
664.83	51	R.GFPGLPGPSGEPGK.Q + Hydroxylation (K) (K); Hydroxylation (P) (P)
666.83	40	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
667.32	63	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)
667.32	48	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
672.82	45	R.GFPGLPGPSGEPGK.Q + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
672.82	60	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
675.32	64	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydro (P) (P)
718.34	68	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)
722.35	39	R.GSAGPPGATGFPGAAGR.V + Hydroxylation (P) (P)
730.34	99	R.GSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)
780.91	47	R.GETGPAGPAGPIGPVGAR.G
781.89	49	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
787.91	44	R.GLTGPIGPPGPAGAPGDK.G + Hydroxylation (P) (P)

Table I-4 ***Continued.***

Collagen alpha-1(I) [Bos Taurus]		
M (Obs.)	**Ion Score**	**Identified Peptide**
793.88	67	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
795.91	31	R.GLTGPIGPPGPAGAPGDK.G + 2 Hydroxylation (P) (P)
809.39	50	R.GFSGLDGAKGDAGPAGPK.G + Hydroxylation (K) (K)
828.40	39	K.GSPGEAGRPGEAGLPGAK.G + 3 Hydroxylation (P) (P)
845.89	69	K.DGEAGAQGPPGPAGPAGER.G
846.38	64	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ)
853.89	87	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
854.38	74	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)
872.36	28	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 2 Hydro(P) (P)
872.86	36	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 2 Hydro (P) (P)
880.36	70	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydro(P) (P)
880.85	106	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 3 Hydro (P) (P)
888.36	56	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 4 Hydro (P) (P)
888.85	41	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 4 Hydro (P) (P)
908.93	112	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); Hydroxylation (P) (P)
916.93	93	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); 2 Hydroxylation (P) (P)
924.93	51	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); 3 Hydroxylation (P) (P)
932.43	48	K.GEPGPTGIQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
932.93	88	K.GEPGPTGIQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
988.50	49	K.SGDRGETGPAGPAGPIGPVGAR.G
1010.48	71	K.GEPGPTGIQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1010.98	78	K.GEPGPTGIQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1029.51	58	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ)
1029.99	30	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ)
1037.50	66	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
1037.99	63	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1045.00	52	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Hydroxylation (P) (P)
1045.49	58	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1045.99	78	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1059.49	51	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 2 Hydroxylation (P) (P)
1059.99	43	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1061.04	42	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S
1067.49	44	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1067.98	53	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1069.03	63	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
1077.03	53	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P)
1085.03	64	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
1093.03	59	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1099.98	36	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 3 Hydroxylation (P) (P)
734.02	27	R.GETGPAGRPGEVGPPGPPGPAGEK.G + 2 Hydroxylation (P) (P)
1107.98	69	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
1108.48	54	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1108.48	43	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1108.97	64	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1116.53	47	R.GETGPAGRPGEVGPPGPPGPAGEK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1138.57	77	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)

Table I-4 ***Continued.***

Collagen alpha-1(I) [Bos Taurus]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1142.53	54	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Hydroxylation (P) (P)
1143.02	58	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 2 Hydro (P) (P)
1143.52	44	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 2 Hydro (P) (P)
1146.07	71	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (K) (K); Hydro (P) (P)
1146.57	68	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1150.53	37	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
767.68	39	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
768.01	44	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1154.56	76	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 3 Hydro (P) (P)
1154.56	35	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydro (K) (K); 2 Hydroxylation (P) (P)
770.04	27	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 3 Hydro (P) (P)
1158.53	40	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1159.02	39	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 4 Hydro (P) (P)
1159.51	53	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 4 Hydro (P) (P)
1162.06	51	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + 4 Hydroxylation (P) (P)
1162.56	79	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 4 Hydro (P) (P)
1166.53	30	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1167.02	29	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 5 Hydro (P) (P)
1167.02	43	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1167.51	68	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1167.51	28	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 2 Deamidated (NQ); 5 Hydro (P) (P)
783.36	26	R.GPPGPPGKNGDDGEAGKPGRPGER.G + Deamidated (NQ); 2 Hydroxylation (K) (K); Hydroxylation (P) (P)
807.37	30	R.GPSGPQGPSGPPGPKGNSGEPGAPGSK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1228.12	34	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydro (P) (P)
1233.11	56	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
1241.11	57	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P)
829.74	33	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydro (P) (P)
1249.10	66	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
837.36	39	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
837.69	41	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
838.40	40	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1257.11	62	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1259.11	74	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); Hydro (P) (P)
1263.54	33	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
843.02	29	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1264.04	31	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1266.61	46	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1267.10	72	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)

Table I-4 ***Continued.***

Collagen alpha-1(I) [Bos Taurus]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1267.10	41	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1274.61	33	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 3 Hydroxylation (P) (P)
1275.10	64	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1282.60	40	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1283.10	66	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1329.13	33	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G
1329.63	37	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ)
1337.13	48	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Hydroxylation (P) (P)
1337.63	107	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1345.13	77	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Hydroxylation (P) (P)
897.42	29	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1345.63	57	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1353.13	52	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)
1353.62	74	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
902.75	54	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
945.13	38	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ)
1419.22	56	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (P) (P)
1425.19	38	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)
1427.21	79	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
1427.21	49	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
1432.69	35	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 2 Hydroxylation (P) (P)
1433.19	25	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
957.14	28	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1441.18	38	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1566.71	37	R.GLPGPPGAPGPQGFQGPPGEPGEPGASGPMGPR.G + 8 Hydroxylation (P) (P)
1567.19	26	R.GLPGPPGAPGPQGFQGPPGEPGEPGASGPMGPR.G + Deamidated (NQ); 8 Hydroxylation (P) (P)
1081.46	42	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1081.79	27	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1081.79	72	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1082.12	36	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 4 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1128.87	29	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEGGPQGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1129.19	26	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEGGPQGPR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1371.97	28	R.GEQGPAGSPGFQGLPGPAGPPGEAGKPGEQGVPGDLGAPGPSGAR.G + Deamidated (NQ); 6 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-4 ***Continued.***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
405.22	43	K.GHAGLAGAR.G
542.78	55	R.GLVGEPGPAGSK.G + Hydroxylation (P) (P)
596.84	39	R.IGQPGAVGPAGIR.G
597.34	53	R.IGQPGAVGPAGIR.G + Deamidated (NQ)
601.29	57	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
601.78	39	R.GEPGNIGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
604.84	45	R.IGQPGAVGPAGIR.G + Hydroxylation (P) (P)
605.33	49	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
631.32	64	R.GEAGPAGPAGPAGPR.G
634.34	69	R.GIPGPVGAAGATGAR.G + Hydroxylation (P) (P)
644.81	57	R.GFPGSPGNIGPAGK.E + Deamidated (NQ); 2 Hydroxylation (P) (P)
714.36	68	R.GIPGEFGLPGPAGAR.G + 2 Hydroxylation (P) (P)
729.34	91	R.GDGGPPGATGFPGAAGR.T + Hydroxylation (P) (P)
737.34	107	R.GDGGPPGATGFPGAAGR.T + 2 Hydroxylation (P) (P)
746.84	74	R.SGETGASGPPGFVGEK.G + Hydroxylation (P) (P)
766.89	99	R.GEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
769.86	78	R.GAPGAIGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
781.91	45	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
790.88	97	R.GPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
808.42	51	K.GELGPVGNPGPAGPAGPR.G + Hydroxylation (P) (P)
816.92	30	R.GSTGEIGPAGPPGPPGLR.G + Hydroxylation (P) (P)
824.91	74	R.GSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
843.86	30	K.GEPGAPGENGTPGQTGAR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
851.37	74	K.GEPGAPGENGTPGQTGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
851.86	33	K.GEPGAPGENGTPGQTGAR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
900.40	35	R.GPNGDSGRPGEPGLMGPR.G + Deamidated (NQ); Oxidation (M); 2 Hydroxylation (P) (P)
904.90	54	R.GPPGNVGNPGVNGAPGEAGR.D + 3 Deamidated (NQ); 2 Hydroxylation (P) (P)
912.41	65	R.GPPGNVGNPGVNGAPGEAGR.D + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
912.90	53	R.GPPGNVGNPGVNGAPGEAGR.D + 3 Deamidated (NQ); 3 Hydroxylation (P) (P)
961.97	31	R.GERGPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
666.66	61	K.HGNRGEPGPAGAVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1004.47	51	K.GEPGAVGQPGPPGPSGEEGKR.G + 3 Hydroxylation (P) (P)
1026.49	57	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K)
1026.98	61	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K)
1034.48	49	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1034.98	58	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1066.06	109	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1068.02	53	K.GEPGVVGAPGTAGPSGPSGLPGER.G + 2 Hydroxylation (P) (P)
1076.02	39	K.GEPGVVGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1082.50	34	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1082.49	58	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1082.99	54	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 3 Deamidated (NQ); 2 Hydroxylation (P) (P)
1083.48	34	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 4 Deamidated (NQ); 2 Hydroxylation (P) (P)
1083.48	33	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 4 Deamidated (NQ); 2 Hydro (P) (P)

The highlighted peptides are unique peptides.

Table I-4 ***Continued.***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1131.06	68	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Hydroxylation (P) (P)
1131.56	48	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + Deamidated (NQ); 2 Hydro (P) (P)
1132.05	69	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Deamidated (NQ); 2 Hydro (P) (P)
798.72	35	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydro (P) (P)
1205.57	73	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1206.06	93	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + 2 Deamidated (NQ); Hydro (K) (K); Hydro (P) (P)
1208.12	37	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 3 Hydro (P) (P)
1208.60	63	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1213.04	61	K.GESGNKGEPGAVGQPGPPGPSGEEGK.R + Deamidated (NQ); 3 Hydro (P) (P)
1213.53	51	K.GESGNKGEPGAVGQPGPPGPSGEEGK.R + 2 Deamidated (NQ); 3 Hydro (P) (P)
1216.11	36	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydro (P) (P)
1216.60	51	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); 4 Hydro (P) (P)
1224.10	33	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1224.60	63	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
848.37	27	K.GQPGAPGVKGEPGAPGENGTPGQTGAR.G + 3 Deamidated (NQ); Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
1292.11	63	K.GENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydro (P) (P)
1295.14	40	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1303.13	79	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
693.09	27	K.GHNGLQGLPGLAGHHGDQGAPGAVGPAGPR.G + Deamidated (NQ); Hydro (P) (P)
924.12	26	K.GHNGLQGLPGLAGHHGDQGAPGAVGPAGPR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
929.12	33	K.GHNGLQGLPGLAGHHGDQGAPGAVGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1396.66	46	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1397.15	36	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1397.15	34	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + Deamidated (NQ); 3 Hydro (P) (P)
932.10	30	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1397.65	56	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
961.13	84	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1493.68	41	R.GPPGASGAPGPQGFQGPPGEPGEPGQTGPAGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1494.17	39	R.GPPGASGAPGPQGFQGPPGEPGEPGQTGPAGAR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
998.08	30	R.GPPGNVGNPGVNGAPGEAGRDGNPGNDGPPGR.D + 5 Deamidated (NQ); 5 Hydroxylation (P) (P)
1501.26	27	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 3 Hydroxylation (P) (P)
1502.17	32	R.GPPGASGAPGPQGFQGPPGEPGEPGQTGPAGAR.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
1509.25	41	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 4 Hydroxylation (P) (P)
1510.17	28	R.GPPGASGAPGPQGFQGPPGEPGEPGQTGPAGAR.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1517.25	57	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1517.74	56	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)

Table I-4 ***Continued***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1071.50	61	R.GPSGPPGPDGNKGEPGVVGAPGTAGPSGPSGLPGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
677.54	26	R.GLPGLKGHNGLQGLPGLAGHHGDQGAPGAVGPAGPR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1508.39	25	R.SGETGASGPPGFVGEKGPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 7 Hydroxylation (P) (P)
Collagen alpha-1(III) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
542.76	40	R.GPAGANGLPGEK.G + Deamidated (NQ); Hydroxylation (K) (K)
610.78	65	R.GQPGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); 2 Hydroxylation (P) (P)
637.29	61	R.GSPGGPGAAGFPGGR.G + 2 Hydroxylation (P) (P)
711.33	42	R.GPPGPPGTNGVPGQR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
718.84	55	R.GPPGPPGTNGVPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
719.33	67	R.GPPGPPGTNGVPGQR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
495.58	52	K.DGASGHPGPIGPPGPR.G + Hydroxylation (P) (P)
769.86	85	R.GETGPAGPSGAPGPAGSR.G + Hydroxylation (P) (P)
818.93	38	K.GEMGPAGIPGAPGLIGAR.G + Oxidation (M)
826.92	76	K.GEMGPAGIPGAPGLIGAR.G + Oxidation (M); Hydroxylation (P) (P)
828.38	29	K.GEPGSSGVDGAPGKDGPR.G + Hydroxylation (P) (P)
834.92	88	K.GEMGPAGIPGAPGLIGAR.G + Oxidation (M); 2 Hydroxylation (P) (P)
559.96	36	R.GVAGEPGRDGLPGGPGLR.G + Hydroxylation (P) (P)
840.40	73	R.GPAGANGLPGEKGPPGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
871.86	77	K.GENGVPGENGAPGPMGPR.G + 2 Deamidated (NQ); Oxidation (M); 2 Hydro (P) (P)
879.38	37	K.DGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
942.94	40	K.GEAGAPGIPGGKGDSGAPGER.G + 3 Hydroxylation (P) (P)
651.97	32	K.SGDRGETGPAGPSGAPGPAGSR.G + Hydroxylation (P) (P)
1002.95	38	R.GPPGPPGSNGNPGPPGSSGAPGK.D + Deamidated (NQ); 4 Hydroxylation (P) (P)
1011.44	55	R.GPPGPPGSNGNPGPPGSSGAPGK.D + 2 Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1041.56	65	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Hydroxylation (P) (P)
1042.05	98	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); Hydroxylation (P) (P)
700.04	64	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + 2 Hydroxylation (P) (P)
1050.05	75	R.GAPGPQGPPGAPGPLGIAGLTGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1093.97	68	R.GSDGQPGPPGPPGTAGFPGSPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
1098.50	62	K.GAAGPPGPPGSAGTPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
1098.99	94	K.GAAGPPGPPGSAGTPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1099.04	49	K.GEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Hydroxylation (P) (P)
1099.53	78	K.GEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Deamidated (NQ); Hydroxylation (P) (P)
1107.03	59	K.GEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Hydroxylation (K) (K); Hydro (P) (P)
1107.53	47	K.GEGGPPGAAGPAGGSGPAGPPGPQGVK.G + Deamidated (NQ); 2 Hydro (P) (P)
1112.49	27	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
1112.98	32	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 3 Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1114.48	79	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1115.03	57	K.GEGGPPGAAGPAGGSGPAGPPGPQGVK.G + 3 Hydroxylation (P) (P)
1120.49	31	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1120.98	61	R.GEQGPPGPAGFPGAPGQNGEPGAK.G + 3 Deamidated (NQ); 4 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-4 ***Continued***

Collagen alpha-1(III) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1122.48	74	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
748.66	54	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1122.48	73	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1200.03	45	R.GEPGPQGHAGAPGPPGPPGSNGSPGGK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1200.52	58	R.GEPGPQGHAGAPGPPGPPGSNGSPGGK.G + 2 Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1208.11	63	R.GPVGPSGPPGKDGASGHPGPIGPPGPR.G + Hydroxylation (P) (P)
1216.11	39	R.GPVGPSGPPGKDGASGHPGPIGPPGPR.G + 2 Hydroxylation (P) (P)
857.05	25	K.GETGAPGLKGENGVPGENGAPGPMGPR.G + 2 Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
858.07	41	K.GEGGPPGAAGPAGGSGPAGPPGPQGVKGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1293.07	40	K.GETGAPGLKGENGVPGENGAPGPMGPR.G + 2 Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1335.61	28	R.GFPGNPGAPGSPGPAGHQGAVGSPGPAGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1336.11	31	R.GFPGNPGAPGSPGPAGHQGAVGSPGPAGPR.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
1344.11	71	R.GFPGNPGAPGSPGPAGHQGAVGSPGPAGPR.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
961.11	29	R.GGPGGPGPQGPAGKNGETGPQGPPGPTGPSGDK.G + Deamidated (NQ); Hydroxylation (P) (P)
961.44	39	R.GGPGGPGPQGPAGKNGETGPQGPPGPTGPSGDK.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
984.48	29	R.GPTGPIGPPGPAGQPGDKGESGAPGVPGIAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
989.49	28	R.GPTGPIGPPGPAGQPGDKGESGAPGVPGIAGPR.G + 4 Hydroxylation (P) (P)
1484.23	64	R.GPTGPIGPPGPAGQPGDKGESGAPGVPGIAGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Strong glue (Pearls)

Table I-5 Protein identification of *strong glue (pearls)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α2(I)	*Bos taurus*	P02459	2555	51	44	9
Collagen α1(I)	*Bos taurus*	P02453	2507	44	41	1
Collagen α1(I)	Sus scrofa	A0A287A1S6	2367	42	16	5
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	2292	43	11	9

Collagen alpha-1(I) [*Bos taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
426.21	40	R.GFSGLDGAK.G
434.21	35	R.GFSGLDGAK.G + Hydroxylation (K) (K)
449.76	32	R.GVVGLPGQR.G + Hydroxylation (P) (P)
544.77	71	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
365.86	31	R.GRPGAPGPAGAR.G + 2 Hydroxylation (P) (P)
553.29	31	R.GVQGPPGPAGPR.G + Hydroxylation (P) (P)
553.78	46	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
581.29	37	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K)
581.29	74	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P)
589.28	63	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
589.28	66	R.GQAGVMGFPGPK.G + Oxidation (M); Hydroxylation (P) (P)
589.78	56	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (P) (P)
596.82	43	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
621.80	68	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.79	67	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
641.31	49	K.GEAGPSGPAGPTGAR.G
656.83	35	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.82	70	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
666.83	86	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
672.82	54	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
674.82	36	R.GPSGPQGPSGPPGPK.G + 2 Hydroxylation (P) (P)
718.34	64	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)
730.35	98	R.GSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)
780.91	45	R.GETGPAGPAGPIGPVGAR.G
793.88	60	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
803.91	43	R.GLTGPIGPPGPAGAPGDK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
539.26	32	R.GGPGSRGFPGADGVAGPK.G + 2 Hydroxylation (P) (P)
828.41	49	R.GFPGADGVAGPKGPAGER.G + Hydroxylation (P) (P)
845.89	43	K.DGEAGAQGPPGPAGPAGER.G
853.89	40	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
871.87	51	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P)
872.36	78	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)

Table I-5 ***Continued***

Collagen alpha-1(I) [*Bos taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
581.95	54	K.GARGSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)
872.85	63	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
880.36	85	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydro (P) (P)
906.94	30	R.VGPPGPSGNAGPPGPPGPAGK.E + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
605.29	38	R.VGPPGPSGNAGPPGPPGPAGK.E + Deamidated (NQ); 3 Hydroxylation (P) (P)
908.93	100	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
916.93	59	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); 2 Hydroxylation (P) (P)
988.50	60	K.SGDRGETGPAGPAGPIGPVGAR.G
1010.48	54	K.GEPGPTGIQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1037.00	89	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Hydroxylation (P) (P)
1045.00	89	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Hydroxylation (P) (P)
1053.00	90	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 3 Hydroxylation (P) (P)
1085.02	52	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
1093.03	65	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1099.98	76	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1100.48	94	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1107.98	58	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
1108.48	58	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1136.03	70	R.GFPGLPGPSGEPGKQGPSGASGER.G + 3 Hydroxylation (P) (P)
769.71	37	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Hydroxylation (K) (K); 2 Hydro (P) (P)
1154.56	35	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
772.68	80	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P)
773.01	26	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1162.06	57	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + 4 Hydroxylation (P) (P)
1236.11	27	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
829.74	32	R.GPPGSAGSPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydro (P) (P)
832.03	61	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydro(P) (P)
833.07	50	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
837.36	48	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
838.40	48	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1266.60	40	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 2 Hydroxylation (P) (P)
1267.10	36	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1274.60	52	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 3 Hydroxylation (P) (P)
1275.09	66	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1282.59	57	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
869.76	42	K.GDAGPPGPAGPAGPPGPIGNVGAPGPKGAR.G + 4 Hydroxylation (P) (P)
1345.13	76	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Hydroxylation (P) (P)
1352.12	36	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1353.13	93	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)

Table I-5 ***Continued***

Collagen alpha-1(I) [*Bos taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
907.08	33	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 5 Hydroxylation (P) (P)
946.47	41	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (P) (P)
1427.21	70	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
957.14	47	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1440.70	41	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 3 Hydroxylation (P) (P)
1056.49	32	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1061.82	42	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1062.15	30	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1067.15	34	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + 5 Hydroxylation (P) (P)
1067.49	26	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1075.80	51	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1076.13	45	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1081.46	42	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1134.19	27	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEGGPQGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
456.23	27	R.GHNGLDGLK.G + Deamidated (NQ)
542.78	41	R.GLVGEPGPAGSK.G + Hydroxylation (P) (P)
550.78	29	R.GLVGEPGPAGSK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
592.28	50	K.GGGPGPMGLMGPR.G
600.28	34	K.GGGPGPMGLMGPR.G + Hydroxylation (P) (P)
601.29	45	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
601.78	46	R.GEPGNIGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
601.79	29	R.GEPGNIGFPGPK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
604.84	60	R.IGQPGAVGPAGIR.G + Hydroxylation (P) (P)
605.33	53	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
608.28	27	K.GGGPGPMGLMGPR.G + Oxidation (M); Hydroxylation (P) (P)
611.81	51	R.GFPGTPGLPGFK.G + 3 Hydroxylation (P) (P)
611.80	33	R.GFPGTPGLPGFK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
619.80	47	R.GFPGTPGLPGFK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
631.31	47	R.GEAGPAGPAGPAGPR.G
634.34	104	R.GIPGPVGAAGATGAR.G + Hydroxylation (P) (P)
644.32	73	R.GFPGSPGNIGPAGK.E + 2 Hydroxylation (P) (P)
644.81	30	R.GFPGSPGNIGPAGK.E + Deamidated (NQ); 2 Hydroxylation (P) (P)
463.23	31	K.GETGLRGDIGSPGR.D + Hydroxylation (P) (P)
714.36	69	R.GIPGEFGLPGPAGAR.G + 2 Hydroxylation (P) (P)
722.36	30	R.GIPGEFGLPGPAGAR.G + 3 Hydroxylation (P) (P)
729.34	100	R.GDGGPPGATGFPGAAGR.T + Hydroxylation (P) (P)
737.33	91	R.GDGGPPGATGFPGAAGR.T + 2 Hydroxylation (P) (P)

Table I-5 ***Continued***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
746.85	49	R.SGETGASGPPGFVGEK.G + Hydroxylation (P) (P)
766.89	62	R.GEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
769.86	39	R.GAPGAIGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
781.91	75	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
790.88	73	R.GPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
800.42	36	K.GELGPVGNPGPAGPAGPR.G
808.41	75	K.GELGPVGNPGPAGPAGPR.G + Hydroxylation (P) (P)
824.91	63	R.GSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
851.36	82	K.GEPGAPGENGTPGQTGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
595.27	25	R.GPNGDSGRPGEPGLMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
602.31	26	K.RGSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
911.91	66	R.GPPGNVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
641.64	73	R.GERGPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
666.34	55	K.HGNRGEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
666.66	40	K.HGNRGEPGPAGAVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
504.00	29	K.HGNRGEPGPAGAVGPAGAVGPR.G + 2 Hydroxylation (P) (P)
1026.49	46	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K)
686.03	25	K.EGPVGLPGIDGRPGPIGPAGAR.G + Hydroxylation (P) (P)
1066.06	111	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1076.01	77	K.GEPGVVGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1082.50	38	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1131.06	85	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Hydroxylation (P) (P)
1131.55	38	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
798.71	28	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (P) (P)
1205.57	80	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1216.10	67	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydro (P) (P)
1224.10	82	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
847.72	54	K.GQPGAPGVKGEPGAPGENGTPGQTGAR.G + Deamidated (NQ); Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
1287.13	74	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1291.12	72	K.GENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + Hydroxylation (P) (P)
1292.10	68	K.GENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydro (P) (P)
1295.12	30	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + 3 Hydroxylation (P) (P)
1295.13	70	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
884.41	57	R.GSPGERGEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
889.75	41	R.GSPGERGEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
927.78	36	R.GAPGAIGAPGPAGANGDRGEAGPAGPAGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
955.79	46	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-5 ***Continued***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
960.80	31	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
961.12	50	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1501.18	27	R.GPPGASGAPGPQGFQGPPGEPGEPGQTGPAGAR.G + 4 Hydroxylation (P) (P)
1517.24	47	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1517.75	41	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1082.17	26	R.GPSGPPGPDGNKGEPGVVGAPGTAGPSGPSGLPGER.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1331.01	28	R.GEVGLPGLSGPVGPPGNPGANGLPGAKGAAGLPGVAGAPGLPGPR.G + Deamidated (NQ); 8 Hydroxylation (P) (P)
1497.73	36	R.SGETGASGPPGFVGEKGPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1503.06	38	R.SGETGASGPPGFVGEKGPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 6 Hydroxylation (P) (P)
1503.39	33	R.SGETGASGPPGFVGEKGPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + Deamidated (NQ); 6 Hydroxylation (P) (P)
Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
741.87	38	K.SAGISVPGPMGPSGPR.G + Oxidation (M)
773.90	55	R.GETGPAGPAGPVGPVGAR.G
585.60	28	R.EGAPGAEGSPGRDGAPGPK.G + 3 Hydroxylation (P) (P)
925.43	78	K.GEPGPTGVQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
981.49	39	K.SGDRGETGPAGPAGPVGPVGAR.G
1003.48	77	K.GEPGPTGVQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1060.99	56	K.GSPGADGPAGAPGTPGPQGIAGQR.G + 3 Hydroxylation (P) (P)
1086.02	45	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1143.52	26	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 3 Hydroxylation (P) (P)
1151.52	38	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 4 Hydroxylation (P) (P)
828.40	43	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
833.73	45	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
833.73	32	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1057.15	26	R.GSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGGK.G + 4 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
620.32	97	R.GIPGPAGAAGATGAR.G + Hydroxylation (P) (P)
637.31	76	R.GFPGSPGNVGPAGK.E + 2 Hydroxylation (P) (P)
677.34	61	K.GVGAGPGPMGLMGPR.G
727.37	80	R.GIPGEFGLPGPAGPR.G + 2 Hydroxylation (P) (P)
735.37	50	R.GIPGEFGLPGPAGPR.G + 3 Hydroxylation (P) (P)
739.84	32	R.TGETGASGPPGFAGEK.G + Hydroxylation (P) (P)
775.88	76	R.GPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
824.89	69	R.GPNGEVGSAGPPGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
890.41	82	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-5 ***Continued***

Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
929.47	40	R.IGPPGPSGISGPPGPPGPAGK.E + 4 Hydroxylation (P) (P)
946.96	88	R.GERGPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
1075.02	26	K.GEPGVLGAPGTAGPSGPSGLPGER.G + 2 Hydroxylation (P) (P)
1103.03	94	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 2 Hydroxylation (P) (P)
1111.03	43	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 3 Hydroxylation (P) (P)
1209.10	67	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1217.09	43	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1283.62	76	K.GENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (P) (P)
1284.11	60	K.GENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1075.85	49	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1076.18	31	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1493.05	28	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1503.71	31	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 7 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Strong glue (old manufacturing)

Table I-6 Protein identification of *strong glue (old manufacturing)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α2(I)	*Bos taurus*	P02459	2660	55	45	7
Collagen α1(I)	*Bos taurus*	P02453	932	45	42	_
Collagen α1(I)	*Sus scorfa*	A0A287A1S6	2250	42	7	4
Collagen α2(I)	*Sus scorfa*	A0A286ZWS8	2064	40	14	8
Collagen α1(I)	*Equus asinus*	B9VR88	2372	43	4	3
Collagen α2(I)	*Equus asinus*	B9VR89	2027	45	12	8

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
384.21	26	R.GAPGPAGPK.G + Hydroxylation (P) (P)
426.21	53	R.GFSGLDGAK.G
443.72	46	R.GSEGPQGVR.G
449.76	34	R.GVVGLPGQR.G + Hydroxylation (P) (P)
360.53	37	R.GRPGAPGPAGAR.G + Hydroxylation (P) (P)
544.77	60	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
365.86	36	R.GRPGAPGPAGAR.G + 2 Hydroxylation (P) (P)
553.78	58	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
581.29	56	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K)
581.78	44	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
589.28	58	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P); Oxidation (M)
589.28	56	R.GQAGVMGFPGPK.G + Hydroxylation (P) (P); Hydroxylation (K) (K)
589.78	68	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Oxidation (M)
589.78	43	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)
596.82	51	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
621.80	55	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.80	62	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
656.83	40	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.83	52	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
666.83	41	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
667.32	74	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)
672.82	50	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
718.34	70	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)
730.35	122	R.GSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)
780.91	87	R.GETGPAGPAGPIGPVGAR.G
788.91	46	R.GETGPAGPAGPIGPVGAR.G + Hydroxylation (P) (P)
793.88	80	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
795.91	56	R.GLTGPIGPPGPAGAPGDK.G + 2 Hydroxylation (P) (P)
803.90	57	R.GLTGPIGPPGPAGAPGDK.G + 2 Hydroxylation (P) (P); Hydroxylation (K) (K)
808.39	49	R.GGPGSRGFPGADGVAGPK.G + 2 Hydroxylation (P) (P)

Table I-6 ***Continued***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
828.40	35	K.GSPGEAGRPGEAGLPGAK.G + 3 Hydroxylation (P) (P)
845.89	58	K.DGEAGAQGPPGPAGPAGER.G
853.89	97	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
854.38	81	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)
871.87	26	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P)
872.36	80	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
872.36	75	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
872.86	43	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
872.86	67	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
880.36	79	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
880.85	64	K.GEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
908.93	45	R.GPPGPMGPPGLAGPPGESGR.E + Hydroxylation (P) (P); Oxidation (M)
908.93	74	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
916.93	89	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P); Oxidation (M)
924.93	62	R.GPPGPMGPPGLAGPPGESGR.E + 3 Hydroxylation (P) (P); Oxidation (M)
932.44	69	K.GEPGPTGIQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
932.93	68	K.GEPGPTGIQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
988.50	41	K.SGDRGETGPAGPAGPIGPVGAR.G
1002.49	48	K.GEPGPTGIQGPPGPAGEEGKR.G + Hydroxylation (P) (P)
1010.48	64	K.GEPGPTGIQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1010.98	56	K.GEPGPTGIQGPPGPAGEEGKR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
691.67	34	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Hydroxylation (P) (P)
1037.50	67	K.GAPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
1045.00	80	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 2 Hydroxylation (P) (P)
1053.00	80	K.GAPGADGPAGAPGTPGPQGIAGQR.G + 3 Hydroxylation (P) (P)
1067.49	32	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1067.98	36	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
717.66	25	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1085.03	30	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P); Hydro (K) (K)
1085.03	57	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
1093.03	52	R.GETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1099.98	92	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1100.48	74	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1100.97	36	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 4 Hydroxylation (P) (P)
1107.98	71	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P); Oxidation (M)
1108.47	44	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1108.47	45	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1116.53	39	R.GETGPAGRPGEVGPPGPPGPAGEK.G + 3 Hydroxylation (P) (P); Hydro (K) (K)

Table I-6 ***Continued***

Collagen alpha-1(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1128.53	40	R.GFPGLPGPSGEPGKQGPSGASGER.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1150.53	30	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1154.07	35	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + 3 Hydroxylation (P) (P)
1154.07	40	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + 2 Hydroxylation (P) (P); Hydro (K) (K)
1158.53	85	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P)
773.02	43	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 4 Hydro (P) (P)
1162.07	58	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + 4 Hydroxylation (P) (P)
1162.56	66	K.GDAGPPGPAGPAGPPGPIGNVGAPGPK.G + Deamidated (NQ); 4 Hydro (P) (P)
1166.52	63	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 5 Hydroxylation (P) (P)
1166.52	54	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + 4 Hydroxylation (P) (P); Hydro (K) (K)
1167.02	43	R.GEPGPPGPAGAAGPAGNPGADGQPGAK.G + Deamidated (NQ); 5 Hydro (P) (P)
832.03	41	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydro (P) (P)
1247.55	30	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydro (P) (P)
832.36	28	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 2 Deamidated (NQ); 3 Hydro (P) (P)
1249.11	72	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
837.36	28	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
837.36	29	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Hydroxylation (K) (K)
1257.11	80	K.GDRGETGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
842.69	26	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Oxidation (M)
1267.10	50	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1274.60	69	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 3 Hydroxylation (P) (P)
1275.09	62	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1283.10	59	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Hydroxylation (K) (K)
1337.14	29	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Hydroxylation (P) (P)
1345.13	80	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Hydroxylation (P) (P)
1345.63	59	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydro (P) (P)
1345.63	31	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydro (P) (P)
1352.62	29	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Hydroxylation (K) (K)
1353.12	93	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)
1353.62	87	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydrox (P) (P)
907.08	44	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 5 Hydroxylation (P) (P)
1361.13	72	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 4 Hydroxylation (P) (P)
920.43	27	K.QGPSGASGERGPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P); Oxidation (M)
1427.21	49	R.GLTGPIGPPGPAGAPGDKGEAGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
1432.70	44	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 2 Hydroxylation (P) (P)
960.79	26	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 3 Hydroxylation (P) (P)
1081.46	49	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)
1081.79	37	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 3 Deamidated (NQ); 5 Hydroxylation (P) (P); Oxidation (M)

Table I-6 ***Continued***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
393.20	27	R.GDQGPVGR.S
393.22	25	R.GATGPAGVR.G
405.22	35	K.GHAGLAGAR.G
542.78	48	R.GLVGEPGPAGSK.G + Hydroxylation (P) (P)
596.84	39	R.IGQPGAVGPAGIR.G
601.29	53	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
601.78	28	R.GEPGNIGFPGPK.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)
603.81	40	R.GFPGTPGLPGFK.G + 2 Hydroxylation (P) (P)
604.84	56	R.IGQPGAVGPAGIR.G + Hydroxylation (P) (P)
605.33	48	R.IGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
611.81	56	R.GFPGTPGLPGFK.G + 3 Hydroxylation (P) (P)
619.80	55	R.GFPGTPGLPGFK.G + 3 Hydroxylation (P) (P); Hydroxylation (K) (K)
631.3	63	R.GEAGPAGPAGPAGPR.G
634.34	107	R.GIPGPVGAAGATGAR.G + Hydroxylation (P) (P)
644.32	69	R.GFPGSPGNIGPAGK.E + 2 Hydroxylation (P) (P)
644.81	63	R.GFPGSPGNIGPAGK.E + Deamidated (NQ); 2 Hydroxylation (P) (P)
714.37	69	R.GIPGEFGLPGPAGAR.G + 2 Hydroxylation (P) (P)
722.36	57	R.GIPGEFGLPGPAGAR.G + 3 Hydroxylation (P) (P)
729.34	83	R.GDGGPPGATGFPGAAGR.T + Hydroxylation (P) (P)
737.34	89	R.GDGGPPGATGFPGAAGR.T + 2 Hydroxylation (P) (P)
746.85	58	R.SGETGASGPPGFVGEK.G + Hydroxylation (P) (P)
766.89	43	R.GEPGPAGAVGPAGAVGPR.G + Hydroxylation (P) (P)
769.86	64	R.GAPGAIGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
781.91	74	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
790.88	79	R.GPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
808.41	92	K.GELGPVGNPGPAGPAGPR.G + Hydroxylation (P) (P)
808.91	48	K.GELGPVGNPGPAGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
824.92	29	R.GSTGEIGPAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
851.37	86	K.GEPGAPGENGTPGQTGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
851.86	59	K.GEPGAPGENGTPGQTGAR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
584.29	26	R.GHNGLDGLKGQPGAPGVK.G + Deamidated (NQ); Hydroxylation (P) (P); 2 Hydroxylation (K) (K)
600.60	27	R.GPNGDSGRPGEPGLMGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P); Oxidation (M)
911.92	97	R.GPPGNVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
912.41	65	R.GPPGNVGNPGVNGAPGEAGR.D + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
926.42	37	K.GEPGAVGQPGPPGPSGEEGK.R + 3 Hydroxylation (P) (P)
961.97	86	R.GERGPPGESGAAGPTGPIGSR.G + Hydroxylation (P) (P)
666.67	34	K.HGNRGEPGPAGAVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1026.49	75	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (P) (P)
1026.99	62	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1028.55	40	K.EGPVGLPGIDGRPGPIGPAGAR.G + Hydroxylation (P) (P)
1034.49	48	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)

Table I-6 ***Continued***

Collagen alpha-2(I) [*Bos Taurus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1034.98	66	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + 2 Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)
1066.06	98	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1074.06	45	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 4 Hydroxylation (P) (P)
1076.02	63	K.GEPGVVGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1082.00	40	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1082.50	52	R.GAPGPDGNNGAQGPPGLQGVQGGK.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
1131.06	73	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + 2 Hydroxylation (P) (P)
1131.56	30	R.GYPGNAGPVGAAGAPGPQGPVGPVGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
1205.57	64	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)
804.05	36	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)
808.70	31	K.GESGNKGEPGAVGQPGPPGPSGEEGK.R + 2 Hydroxylation (P) (P); Hydroxylation (K) (K)
809.03	38	K.GESGNKGEPGAVGQPGPPGPSGEEGK.R + Deamidated (NQ); 3 Hydroxylation (P) (P)
1224.11	71	R.GEVGLPGLSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Hydroxylation (K) (K)
848.05	28	K.GQPGAPGVKGEPGAPGENGTPGQTGAR.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P); Hydroxylation (K) (K)
1287.13	65	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + Hydroxylation (P) (P); Hydroxylation (K) (K)
1291.62	66	K.GENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (P) (P)
1292.11	72	K.GENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1295.13	75	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + 2 Hydroxylation (P) (P); Hydroxylation (K) (K)
1303.13	31	R.GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK.G + 3 Hydroxylation (P) (P); Hydroxylation (K) (K)
1391.16	28	R.GAPGAIGAPGPAGANGDRGEAGPAGPAGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
697.09	32	K.GHNGLQGLPGLAGHHGDQGAPGAVGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1396.66	42	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + 3 Hydroxylation (P) (P)
1397.16	45	K.GEQGPAGPPGFQGLPGPAGTAGEAGKPGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
961.13	53	K.GPKGENGPVGPTGPVGAAGPSGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (P) (P); Hydroxylation (K) (K)
997.10	26	R.GPPGNVGNPGVNGAPGEAGRDGNPGNDGPPGR.D + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1509.25	76	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 4 Hydroxylation (P) (P)
1517.25	56	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1525.24	27	K.GPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 6 Hydroxylation (P) (P)
1071.18	39	R.GPSGPPGPDGNKGEPGVVGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1503.06	28	R.SGETGASGPPGFVGEKGPSGEPGTAGPPGTPGPQGLLGAPGFLGLPGSR.G + 6 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-6 ***Continued***

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
925.43	26	K.GEPGPTGVQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
654.66	48	K.SGDRGETGPAGPAGPVGPVGAR.G
1003.48	65	K.GEPGPTGVQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1086.02	76	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1143.52	26	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 3 Hydroxylation (P) (P)
1151.52	49	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 4 Hydroxylation (P) (P)
1152.02	42	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
833.73	37	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
620.32	89	R.GIPGPAGAAGATGAR.G + Hydroxylation (P) (P)
637.31	75	R.GFPGSPGNVGPAGK.E + 2 Hydroxylation (P) (P)
727.37	73	R.GIPGEFGLPGPAGPR.G + 2 Hydroxylation (P) (P)
735.37	36	R.GIPGEFGLPGPAGPR.G + 3 Hydroxylation (P) (P)
739.84	30	R.TGETGASGPPGFAGEK.G + Hydroxylation (P) (P)
775.88	72	R.GPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
890.41	94	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
602.30	28	K.RGPNGEVGSAGPPGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
904.41	48	K.GEPGAAGPQGPPGPSGEEGK.R + 2 Hydroxylation (P) (P)
946.96	82	R.GERGPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
1073.99	35	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1074.48	56	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + 2 Deamidated (NQ); 2 Hydro (P) (P)
1103.04	50	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 2 Hydroxylation (P) (P)
1111.03	30	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 3 Hydroxylation (P) (P)
1217.10	48	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydroxylation (P) (P); Hydroxylation (K) (K)
1360.62	27	R.GYPGNPGPAGAAGAPGPQGAVGPAGKHGNR.G + 3 Deamidated (NQ); 4 Hydroxylation (P) (P); Hydroxylation (K) (K)
1498.38	32	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 6 Hydroxylation (P) (P)
Collagen alpha-1(I) [*Equus asinus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
765.90	56	R.GEAGPAGPAGPIGPVGAR.G
884.95	44	R.GPPGPVGPPGLAGPPGESGR.E + Hydroxylation (P) (P)
892.95	56	R.GPPGPVGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
900.95	41	R.GPPGPVGPPGLAGPPGESGR.E + 3 Hydroxylation (P) (P)
1094.51	26	R.GETGPAGRPGEAGPPGPPGPAGEK.G + 3 Hydroxylation (P) (P)
824.41	26	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-6 ***Continued***

Collagen alpha-2(I) [*Equus asinus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
724.83	63	R.AGETGASGPPGFAGEK.G + Hydroxylation (P) (P)
794.93	32	R.GEPGPVGSVGPVGAVGPR.G
802.92	43	R.GEPGPVGSVGPVGAVGPR.G + Hydroxylation (P) (P)
836.86	51	K.GEPGAPGENGTPGQAGAR.G + 2 Deamidated (NQ); 3 Hydroxylation (P) (P)
868.91	44	R.GPPGAVGAPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
902.44	43	R.TGPPGPSGISGPPGPPGAAGK.E + 2 Hydroxylation (P) (P); Hydroxylation (K) (K)
602.96	30	K.RGPNGEPGSTGPAGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1073.07	79	R.GLPGVAGSLGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1150.57	65	R.GYPGNAGPVGAVGAPGPHGPVGPTGK.H + 2 Hydroxylation (P) (P)
1151.06	52	R.GYPGNAGPVGAVGAPGPHGPVGPTGK.H + Deamidated (NQ); 2 Hydroxylation (P) (P)
1210.11	57	R.GEVGLPGLSGPVGPPGNPGANGLTGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1218.11	56	R.GEVGLPGLSGPVGPPGNPGANGLTGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Hydroxylation (K) (K)
1371.15	37	R.GAPGAVGAPGPAGANGDRGEAGAAGPAGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Equus asinus*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1500.26	40	K.GPSGEPGTAGPPGTPGPQGLLGAPGILGLPGSR.G + 5 Hydroxylation (P) (P)
1477.06	26	R.AGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGLLGAPGILGLPGSR.G + 6 Hydroxylation (P) (P)
1482.72	36	R.AGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGLLGAPGILGLPGSR.G + Deamidated (NQ); 7 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Fish glue (flakes)

Table I-7 Protein identification of *fish glue (flakes)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein Name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)	*Sus scrofa*	A0A287A1S6	3107	54	52	8
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	3026	55	47	19
Collagen α1(III)	*Sus scrofa*	F1RYI8	1345	33	29	15

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
426.21	44	R.GFSGLDGAK.G
434.21	30	R.GFSGLDGAK.G + Hydroxylation (K) (K)
441.76	26	R.GVVGLPGQR.G
449.76	32	R.GVVGLPGQR.G + Hydroxylation (P) (P)
542.75	43	R.EGAPGAEGSPGR.D
544.77	71	R.GFPGADGVAGPK.G + Hydroxylation (P) (P)
545.29	52	R.GVQGPPGPAGPR.G
553.29	36	R.GVQGPPGPAGPR.G + Hydroxylation (P) (P)
553.78	39	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
374.53	40	R.GRPGPPGPAGAR.G + 2 Hydroxylation (P) (P)
581.28	56	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K)
581.78	44	R.GQAGVMGFPGPK.G + Deamidated (NQ); Hydroxylation (K) (K)
588.82	50	R.GVPGPPGAVGPAGK.D + Hydroxylation (P) (P)
589.28	63	R.GQAGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
589.28	69	R.GQAGVMGFPGPK.G + Oxidation (M); Hydroxylation (P) (P)
589.78	56	R.GQAGVMGFPGPK.G + Deamidated (NQ); Oxidation (M); Hydroxylation (P) (P)
596.82	66	R.GVPGPPGAVGPAGK.D + 2 Hydroxylation (P) (P)
597.28	29	R.GQAGVMGFPGPK.G + Oxidation (M); Hydroxylation (K) (K); Hydroxylation (P) (P)
621.80	67	K.GLTGSPGSPGPDGK.T + Hydroxylation (P) (P)
629.79	70	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
648.83	44	R.GFPGLPGPSGEPGK.Q
656.31	59	K.GETGPSGPAGPTGAR.G
656.83	51	R.GFPGLPGPSGEPGK.Q + Hydroxylation (P) (P)
664.82	63	R.GFPGLPGPSGEPGK.Q + 2 Hydroxylation (P) (P)
664.83	41	R.GFPGLPGPSGEPGK.Q + Hydroxylation (K) (K); Hydroxylation (P) (P)
666.83	51	R.GPSGPQGPSGPPGPK.G + Hydroxylation (K) (K)
666.83	63	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
667.32	44	R.GPSGPQGPSGPPGPK.G + Deamidated (NQ); Hydroxylation (P) (P)
672.82	49	R.GFPGLPGPSGEPGK.Q + 3 Hydroxylation (P) (P)
674.83	48	R.GPSGPQGPSGPPGPK.G + 2 Hydroxylation (P) (P)
718.34	71	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)
722.35	79	R.GSAGPPGATGFPGAAGR.V + Hydroxylation (P) (P)
730.34	112	R.GSAGPPGATGFPGAAGR.V + 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued***

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
733.87	49	K.SAGISVPGPMGPSGPR.G
741.87	26	K.SAGISVPGPMGPSGPR.G + Oxidation (M)
757.90	40	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ)
765.90	42	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
773.89	47	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
773.90	75	R.GETGPAGPAGPVGPVGAR.G
781.40	36	K.DGLNGLPGPIGPPGPR.G + 3 Hydroxylation (P) (P)
781.89	57	K.DGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
785.88	30	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
793.88	64	K.GANGAPGIAGAPGFPGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
539.93	43	R.GFSGLDGAKGDAGPAGPK.G + Hydroxylation (K) (K)
828.40	52	K.GSPGEAGRPGEAGLPGAK.G + 3 Hydroxylation (P) (P)
828.41	30	R.GFPGADGVAGPKGPAGER.G + Hydroxylation (P) (P)
845.89	107	K.DGEAGAQGPPGPAGPAGER.G
853.88	100	K.DGEAGAQGPPGPAGPAGER.G + Hydroxylation (P) (P)
574.94	25	R.EGAPGAEGSPGRDGAPGPK.G + Hydroxylation (P) (P)
580.27	27	R.EGAPGAEGSPGRDGAPGPK.G + 2 Hydroxylation (P) (P)
871.87	82	K.GEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P)
872.36	53	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 2 Hydro (P) (P)
872.36	70	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
585.60	38	R.EGAPGAEGSPGRDGAPGPK.G + 3 Hydroxylation (P) (P)
879.87	70	K.GEPGSPGENGAPGQMGPR.G + Oxidation (M); 3 Hydroxylation (P) (P)
880.36	85	K.GEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydro (P) (P)
900.93	93	R.GPPGPMGPPGLAGPPGESGR.E + Hydroxylation (P) (P)
604.96	30	R.VGPPGPSGNAGPPGPPGPAGK.E + 3 Hydroxylation (P) (P)
908.93	88	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); Hydroxylation (P) (P)
908.93	88	R.GPPGPMGPPGLAGPPGESGR.E + 2 Hydroxylation (P) (P)
916.93	96	R.GPPGPMGPPGLAGPPGESGR.E + Oxidation (M); 2 Hydroxylation (P) (P)
924.93	27	R.GPPGPMGPPPGLAGPPGESGR.E + Oxidation (M); 3 Hydroxylation (P) (P)
925.43	92	K.GEPGPTGVQGPPGPAGEEGK.R + 2 Hydroxylation (P) (P)
925.92	47	K.GEPGPTGVQGPPGPAGEEGK.R + Deamidated (NQ); 2 Hydroxylation (P) (P)
981.49	54	K.SGDRGETGPAGPAGPVGPVGAR.G
1003.48	59	K.GEPGPTGVQGPPGPAGEEGKR.G + 2 Hydroxylation (P) (P)
1037.01	86	K.GSPGADGPAGAPGTPGPQGIAGQR.G
1045.49	61	K.GSPGADGPAGAPGTPGPQGIAGQR.G + Deamidated (NQ); Hydroxylation (P) (P)
1051.49	55	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Hydroxylation (P) (P)
1053.00	93	K.GSPGADGPAGAPGTPGPQGIAGQR.G + 2 Hydroxylation (P) (P)
1054.03	37	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S
1059.49	42	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 2 Hydroxylation (P) (P)
1062.02	44	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
711.99	44	R.GEPGPPGPAGFAGPPGADGQPGAK.G + Hydroxylation (K) (K); 2 Hydro (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued***

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1067.49	45	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1075.49	45	R.GEPGPPGPAGFAGPPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1078.02	50	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
1086.02	44	R.GESGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
1091.98	49	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 3 Hydroxylation (P) (P)
730.01	39	R.GETGPAGRPGEAGPPGPPGPAGEK.G + Hydroxylation (K) (K); 2 Hydro (P) (P)
1094.51	41	R.GETGPAGRPGEAGPPGPPGPAGEK.G + 3 Hydroxylation (P) (P)
1099.98	87	K.GDAGAPGAPGSQGAPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1099.99	42	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 3 Hydroxylation (P) (P)
1100.48	69	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1107.98	80	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
1108.48	46	K.GDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
757.35	62	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 2 Hydroxylation (P) (P)
757.68	26	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + Deamidated (NQ); 2 Hydrox (P) (P)
1139.57	88	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K)
1143.52	43	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 3 Hydroxylation (P) (P)
762.68	38	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + Hydroxylation (K) (K); 2 Hydro(P) (P)
1144.01	31	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1147.57	80	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); Hydro (P) (P)
768.01	55	R.GEPGPPGPAGAAGPAGNPGADGQPGGK.G + 4 Hydroxylation (P) (P)
770.71	84	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 3 Hydroxylation (P) (P)
770.71	32	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); 2 Hydro (P) (P)
1155.57	75	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 3 Hydroxylation (P) (P)
1155.57	57	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + Hydroxylation (K) (K); 2 Hydrox (P) (P)
1163.56	58	K.GDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 4 Hydroxylation (P) (P)
797.75	62	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ)
803.08	45	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
807.04	26	R.GPSGPQGPSGPPGPKGNSGEPGAPGSK.G + Hydroxylation (K) (K); 3 Hydro (P) (P)
808.41	28	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 2 Hydro (P) (P)
812.40	54	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S
1220.11	47	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 3 Hydro (P) (P)
817.73	38	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (P) (P)
1228.12	41	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 4 Hydro (P) (P)
823.07	29	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + Hydroxylation (K) (K); Hydroxylation (P) (P)
1234.09	71	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 2 Hydroxylation (P) (P)
1236.11	61	R.GPPGSAGAPGKDGLNGLPGPIGPPGPR.G + Deamidated (NQ); 5 Hydro (P) (P)
826.70	30	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); Hydroxylation (P) (P)
828.40	48	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 3 Hydroxylation (P) (P)
831.70	44	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + 3 Hydroxylation (P) (P)
832.03	27	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued***

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
832.03	50	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 2 Hydroxylation (P) (P)
833.73	53	K.GDRGESGPAGPPGAPGAPGAPGPVGPAGK.S + 4 Hydroxylation (P) (P)
837.36	49	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 3 Hydroxylation (P) (P)
837.36	57	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1258.61	53	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (P) (P)
842.36	32	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Oxidation (M); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
842.69	30	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); Oxidation (M); 4 Hydroxylation (P) (P)
1266.60	63	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
1266.61	85	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 2 Hydroxylation (P) (P)
1267.10	67	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1274.60	62	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + 3 Hydroxylation (P) (P)
1275.09	57	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1282.59	73	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
865.43	27	K.GDAGPPGPAGPTGPPGPIGSVGAPGPKGAR.G + Hydro (K) (K); 2 Hydro(P) (P)
1329.13	91	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G
1337.13	102	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Hydroxylation (P) (P)
1344.13	36	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1345.13	95	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 2 Hydroxylation (P) (P)
1352.12	31	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
901.75	26	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 4 Hydroxylation (P) (P)
1353.13	91	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + 3 Hydroxylation (P) (P)
1353.63	87	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydro (P) (P)
907.08	27	R.GAPGDRGEPGPPGPAGFAGPPGADGQPGAK.G + 5 Hydroxylation (P) (P)
923.77	28	K.QGPSGPSGERGPPGPMGPPGLAGPPGESGR.E + Oxidation (M); Hydro (P) (P)
955.46	36	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 2 Hydroxylation (P) (P)
956.47	40	R.GLTGPIGPPGPAGAPGDKGETGPSGPAGPTGAR.G + Hydroxylation (P) (P)
960.79	31	R.GVPGPPGAVGPAGKDGEAGAQGPPGPAGPAGER.G + 3 Hydroxylation (P) (P)
1442.21	82	R.GLTGPIGPPGPAGAPGDKGETGPSGPAGPTGAR.G + 2 Hydroxylation (P) (P)
1031.50	40	K.GEPGDAGAKGDAGPPGPAGPTGPPGPIGSVGAPGPK.G + 2 Hydroxylation (K) (K); Hydroxylation (P) (P)
1075.80	63	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); 5 Hydroxylation (P) (P)
1076.14	47	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); 5 Hydroxylation (P) (P)
1080.81	61	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Oxidation (M); 5 Hydroxylation (P) (P)
1081.14	48	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1081.46	37	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + 2 Deamidated (NQ); Oxidation (M); 5 Hydroxylation (P) (P)
1086.47	34	R.GANGAPGNDGAKGDAGAPGAPGSQGAPGLQGMPGER.G + Deamidated (NQ); Oxidation (M); Hydroxylation (K) (K); 5 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued.***

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1124.54	26	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGAR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1129.87	53	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGAR.G + 4 Hydro(P) (P)
1130.19	61	R.GNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGAR.G + Deamidated (NQ); 4 Hydroxylation (P) (P)
1222.58	31	R.TGDAGPVGPPGPPGPPGPPGPPSGGFDFSFLPQPPQEK.A + 2 Hydroxylation (P) (P)
1227.92	34	R.TGDAGPVGPPGPPGPPGPPGPPSGGFDFSFLPQPPQEK.A + 3 Hydroxylation (P) (P)
1371.97	28	R.GEQGPAGSPGFQGLPGPAGPPGEAGKPGEQGVPGDLGAPGPSGAR.G + Deamidated (NQ); 6 Hydroxylation (P) (P)
1061.26	29	R.GETGPAGRPGEAGPPGPPGPAGEKGSPGADGPAGAPGTPGPQGIAGQR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
435.23	37	R.GPSGPQGIR.G + Deamidated (NQ)
456.23	34	R.GHNGLDGLK.G + Deamidated (NQ)
542.78	65	R.GLVGEPGPAGSK.G + Hydroxylation (P) (P)
590.82	29	R.TGQPGAVGPAGIR.G
591.31	47	R.TGQPGAVGPAGIR.G + Deamidated (NQ)
598.82	37	R.TGQPGAVGPAGIR.G + Hydroxylation (P) (P)
599.31	39	R.TGQPGAVGPAGIR.G + Deamidated (NQ); Hydroxylation (P) (P)
601.29	64	R.GEPGNIGFPGPK.G + 2 Hydroxylation (P) (P)
603.81	34	R.GFPGTPGLPGFK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
611.81	35	R.GFPGTPGLPGFK.G + 3 Hydroxylation (P) (P)
620.32	100	R.GIPGPAGAAGATGAR.G + Hydroxylation (P) (P)
631.32	57	R.GEAGPAGPAGPAGPR.G
637.31	74	R.GFPGSPGNVGPAGK.E + 2 Hydroxylation (P) (P)
677.34	62	K.GVGAGPGPMGLMGPR.G
693.33	53	K.GVGAGPGPMGLMGPR.G + Oxidation (M); Hydroxylation (P) (P)
727.37	81	R.GIPGEFGLPGPAGPR.G + 2 Hydroxylation (P) (P)
729.34	115	R.GDGGPPGATGFPGAAGR.I + Hydroxylation (P) (P)
735.37	53	R.GIPGEFGLPGPAGPR.G + 3 Hydroxylation (P) (P)
737.34	75	R.GDGGPPGATGFPGAAGR.I + 2 Hydroxylation (P) (P)
739.84	44	R.TGETGASGPPGFAGEK.G + Hydroxylation (P) (P)
762.36	58	R.GAPGAVGAPGPAGANGDR.G + 2 Hydroxylation (P) (P)
762.85	40	R.GAPGAVGAPGPAGANGDR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
774.89	93	R.GEPGPAGSVGPAGAVGPR.G + Hydroxylation (P) (P)
775.88	83	R.GPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
781.91	67	K.GAAGLPGVAGAPGLPGPR.G + 3 Hydroxylation (P) (P)
783.87	89	R.GPPGESGAAGPAGPIGSR.G + 2 Hydroxylation (P) (P)
808.41	62	K.GELGPVGNPGPAGPAGPR.G + Hydroxylation (P) (P)
824.40	69	R.GPNGEVGSAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
824.90	85	R.GPNGEVGSAGPPGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued*****.**

Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
873.93	91	R.GPPGAVGNPGVNGAPGEAGR.D + Hydroxylation (P) (P)
874.42	60	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); Hydroxylation (P) (P)
881.92	111	R.GPPGAVGNPGVNGAPGEAGR.D + 2 Hydroxylation (P) (P)
882.42	82	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); 2 Hydroxylation (P) (P)
889.92	89	R.GPPGAVGNPGVNGAPGEAGR.D + 3 Hydroxylation (P) (P)
890.41	78	R.GPPGAVGNPGVNGAPGEAGR.D + Deamidated (NQ); 3 Hydroxylation (P) (P)
902.46	65	K.RGPNGEVGSAGPPGPPGLR.G + 2 Hydroxylation (P) (P)
902.95	60	K.RGPNGEVGSAGPPGPPGLR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
904.41	42	K.GEPGAAGPQGPPGPSGEEGK.R + 2 Hydroxylation (P) (P)
614.65	40	R.IGPPGPSGISGPPGPPGPAGK.E + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
921.47	49	R.IGPPGPSGISGPPGPPGPAGK.E + 3 Hydroxylation (P) (P)
619.98	46	R.IGPPGPSGISGPPGPPGPAGK.E + 4 Hydroxylation (P) (P)
929.47	27	R.IGPPGPSGISGPPGPPGPAGK.E + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
946.96	68	R.GERGPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
636.97	26	R.GERGPPGESGAAGPAGPIGSR.G + 2 Hydroxylation (P) (P)
671.67	77	K.HGNRGEPGPAGSVGPAGAVGPR.G + Hydroxylation (P) (P)
671.99	43	K.HGNRGEPGPAGSVGPAGAVGPR.G + Deamidated (NQ); Hydroxylation (P) (P)
1014.53	28	K.EGPAGLPGIDGRPGPIGPAGAR.G + Hydroxylation (P) (P)
1022.53	31	K.EGPAGLPGIDGRPGPIGPAGAR.G + 2 Hydroxylation (P) (P)
1025.99	47	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Hydroxylation (K) (K)
1026.49	83	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K)
1034.49	79	R.GEVGPAGPNGFAGPAGAAGQPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1058.06	99	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 2 Hydroxylation (P) (P)
710.99	30	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); Hydroxylation (P) (P)
710.99	30	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); Hydroxylation (P) (P)
1066.06	108	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
1073.49	45	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + 2 Hydroxylation (P) (P)
1073.99	66	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
716.66	28	R.GAPGPDGNNGAQGPPGPQGVQGGK.G + 2 Deamidated (NQ); 2 Hydro (P) (P)
1075.03	83	K.GEPGVLGAPGTAGPSGPSGLPGER.G + 2 Hydroxylation (P) (P)
1083.02	95	K.GEPGVLGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1095.04	90	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + Hydroxylation (P) (P)
1103.03	70	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 2 Hydroxylation (P) (P)
1103.53	33	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + Deamidated (NQ); 2 Hydro (P) (P)
1111.03	45	R.GYPGNPGPAGAAGAPGPQGAVGPAGK.H + 3 Hydroxylation (P) (P)
788.69	29	K.GESGNKGEPGAAGPQGPPGPSGEEGK.R + Hydroxylation (P) (P)
794.02	39	K.GESGNKGEPGAAGPQGPPGPSGEEGK.R + 2 Hydroxylation (P) (P)
798.39	40	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Hydroxylation (P) (P)
1197.57	126	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydro (K) (K)
1201.10	49	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 3 Hydro (P) (P)
1205.07	102	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Hydroxylation (K) (K); Hydro (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued*****.**

Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1205.57	82	R.GEVGPAGPNGFAGPAGAAGQPGAKGER.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1208.61	53	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + 4 Hydroxylation (P) (P)
1209.09	54	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1209.10	53	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); 4 Hydro (P) (P)
1216.60	50	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1217.09	68	R.GEVGLPGVSGPVGPPGNPGANGLPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1283.12	107	K.GENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Hydroxylation (P) (P)
1283.61	79	K.GENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Deamidated (NQ); Hydro (P) (P)
1284.11	77	K.GENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
1383.66	26	R.GAPGAVGAPGPAGANGDRGEAGPAGPAGPAGPR.G + 2 Hydroxylation (P) (P)
923.11	47	R.GAPGAVGAPGPAGANGDRGEAGPAGPAGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
703.59	27	K.GHNGLQGLPGLAGHHGDQGAPGPVGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
703.59	26	K.GHNGLQGLPGLAGHHGDQGAPGPVGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
1410.67	60	K.GEQGPAGPPGFQGLPGPAGTAGEVGKPGER.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
955.14	58	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
955.47	76	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
955.47	47	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
1433.19	43	K.GPKGENGPVGPTGPVGAAGPAGPNGPPGPAGSR.G + 2 Deamidated (NQ); Hydroxylation (K) (K); Hydroxylation (P) (P)
968.10	31	R.GPPGAVGNPGVNGAPGEAGRDGNPGSDGPPGR.D + Deamidated (NQ); 4 Hydroxylation (P) (P)
973.10	28	R.GPPGAVGNPGVNGAPGEAGRDGNPGSDGPPGR.D + 5 Hydroxylation (P) (P)
973.43	26	R.GPPGAVGNPGVNGAPGEAGRDGNPGSDGPPGR.D + Deamidated (NQ); 5 Hydroxylation (P) (P)
1486.69	29	R.GPPGAVGAPGPQGFQGPAGEPGEPGQTGPAGAR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1494.19	32	R.GPPGAVGAPGPQGFQGPAGEPGEPGQTGPAGAR.G + 4 Hydroxylation (P) (P)
1509.25	67	K.GPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 4 Hydroxylation (P) (P)
1517.25	53	K.GPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1613.28	70	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + 3 Hydroxylation (P) (P)
1621.26	39	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + 4 Hydroxylation (P) (P)
1086.51	29	R.GPSGPPGPDGNKGEPGVLGAPGTAGPSGPSGLPGER.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
848.68	26	R.GLPGLKGHNGLQGLPGLAGHHGDQGAPGPVGPAGPR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1217.19	26	R.GSQGSQGPAGPPGPPGPPGPPGPSGGGYDFGYEGDFYR.A + 2 Hydro(P) (P)
1222.53	39	R.GSQGSQGPAGPPGPPGPPGPPGPSGGGYDFGYEGDFYR.A + 3 Hydro(P) (P)
1227.86	55	R.GSQGSQGPAGPPGPPGPPGPPGPSGGGYDFGYEGDFYR.A + 4 Hydro (P) (P)
1321.00	38	R.GEVGLPGVSGPVGPPGNPGANGLPGAKGAAGLPGVAGAPGLPGPR.G + Deamidated (NQ); Hydroxylation (K) (K); 6 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued*****.**

Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
1326.34	35	R.GEVGLPGVSGPVGPPGNPGANGLPGAKGAAGLPGVAGAPGLPGPR.G + Deamidated (NQ); 8 Hydroxylation (P) (P)
1487.72	36	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 4 Hydroxylation (P) (P)
1493.06	26	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 5 Hydroxylation (P) (P)
1498.38	32	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + 6 Hydroxylation (P) (P)
1498.38	37	R.TGETGASGPPGFAGEKGPSGEPGTAGPPGTPGPQGILGAPGFLGLPGSR.G + Hydroxylation (K) (K); 5 Hydroxylation (P) (P)
Collagen alpha-1(III) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
521.76	32	K.GEAGAPGIPGGK.G + 2 Hydroxylation (P) (P)
549.77	48	R.GEAGSPGIPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
556.77	32	R.GLAGPPGMPGAR.G + 2 Hydroxylation (P) (P)
564.77	40	R.GLAGPPGMPGAR.G + Oxidation (M); 2 Hydroxylation (P) (P)
602.29	49	R.GQPGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
610.29	49	R.GQPGVMGFPGPK.G + Oxidation (M); 2 Hydroxylation (P) (P)
637.30	69	R.GSPGGPGAAGFPGGR.G + 2 Hydroxylation (P) (P)
645.29	64	R.GSPGGPGAAGFPGGR.G + 3 Hydroxylation (P) (P)
704.82	44	R.GPPGPPGTNGAPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
480.90	46	R.GERGEAGSPGIPGPK.G + 2 Hydroxylation (P) (P)
495.57	38	K.DGASGHPGPIGPPGPR.G + Hydroxylation (P) (P)
496.23	34	K.GENGKPGPSGLNGER.G + 2 Deamidated (NQ); Hydroxylation (P) (P)
500.91	42	K.DGASGHPGPIGPPGPR.G + 2 Hydroxylation (P) (P)
761.86	53	R.GETGPAGPAGAPGPAGSR.G + Hydroxylation (P) (P)
813.37	57	K.GEVGPAGSPGPSGSPGQR.G + 2 Hydroxylation (P) (P)
556.60	27	R.GAPGANGLPGEKGPAGER.G + Deamidated (NQ); 2 Hydroxylation (P) (P)
835.90	34	K.GEMGPAGIPGAPGLMGAR.G + 2 Hydroxylation (P) (P)
560.62	39	R.GVAGEPGRDGVPGGPGLR.G + 2 Hydroxylation (P) (P)
565.95	65	R.GVAGEPGRDGVPGGPGLR.G + 3 Hydroxylation (P) (P)
878.38	31	K.GENGLPGENGAPGPMGPR.G + Deamidated (NQ); Oxidation (M); 2 Hydroxylation (P) (P)
878.89	47	K.DGSPGEPGANGLPGAAGER.G + 3 Hydroxylation (P) (P)
902.45	52	R.GPPGPQGLPGLAGAAGEPGR.D + 3 Hydroxylation (P) (P)
636.30	27	K.GDSGAPGERGPPGAVGPSGPR.G + 2 Hydroxylation (P) (P)
961.94	37	K.DGPPGPPGSSGAPGSPGVSGPK.G + 4 Hydroxylation (P) (P)
655.64	28	K.NGDRGETGPAGPAGAPGPAGSR.G + Hydroxylation (P) (P)
1037.52	98	K.GSPGPQGPPGAPGPGGISGITGAR.G + 2 Hydroxylation (P) (P)
1045.52	48	K.GSPGPQGPPGAPGPGGISGITGAR.G + 3 Hydroxylation (P) (P)
1085.48	74	R.GSDGQPGPPGPPGTAGFPGSPGAK.G + 5 Hydroxylation (P) (P)
1095.51	53	K.GPAGPPGPPGAAGTPGLQGMPGER.G + 4 Hydroxylation (P) (P)
1103.51	64	K.GPAGPPGPPGAAGTPGLQGMPGER.G + Oxidation (M); 4 Hydroxylation (P) (P)
748.33	31	K.GEDGKDGSPGEPGANGLPGAAGER.G + 3 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Table I-7 ***Continued***.

Collagen alpha-1(III) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
748.66	40	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
748.66	39	K.GEDGKDGSPGEPGANGLPGAAGER.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
749.67	33	R.GEHGPPGPAGFPGAPGQNGEPGAK.G + 4 Hydroxylation (P) (P)
750.00	49	R.GEHGPPGPAGFPGAPGQNGEPGAK.G + Deamidated (NQ); Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
1184.57	43	K.GEGGPPGIAGQPGGTGPPGPPGPQGVK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1192.57	54	K.GEGGPPGIAGQPGGTGPPGPPGPQGVK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
975.14	27	R.GAPGEKGEGGPPGIAGQPGGTGPPGPPGPQGVK.G + 4 Hydroxylation (P) (P)
980.47	31	R.GAPGEKGEGGPPGIAGQPGGTGPPGPPGPQGVK.G + 2 Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
994.16	43	R.GPTGPIGPPGPAGQPGDKGESGAPGLPGIAGPR.G + 4 Hydroxylation (P) (P)
994.16	34	R.GPTGPIGPPGPAGQPGDKGESGAPGLPGIAGPR.G + 4 Hydroxylation (P) (P)
999.49	34	R.GPTGPIGPPGPAGQPGDKGESGAPGLPGIAGPR.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
1056.49	58	R.GGAGPPGPEGGKGPAGPPGPPGAAGTPGLQGMPGER.G + 5 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Sturgeon fish glue (pearls)

Table I-8 Protein identification of sturgeon *fish glue (pearls)* MS/MS spectra by Mascot MS/MS Ion search using COLLE dataset, considering deamidation on Gln and Asn, oxidation on Met, pyro-Glu formation at Gln at the N-terminus of Identified Peptides as variable modifications.

Protein name	Taxonomy	Protein identifier	Protein Score	Sequence coverage (%)	No. of Peptides	No. of Unique Peptides
Collagen α1(I)/ Partial	*Scyliorhinus canicula*	D0PQF7	586	13	11	8
Collagen α1(I)	*Sus scrofa*	A0A287A1S6	425	11	6	3
Collagen α2(I)	*Sus scrofa*	A0A286ZWS8	205	8	5	4

Collagen alpha-1(I) [*Scyliorhinus canicula*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
447.72	48	R.GYNGLDGAK.G
448.21	52	R.GYNGLDGAK.G + Deamidated (NQ)
542.75	68	R.EGAPGAEGAPGR.D + Hydroxylation (P) (P)
550.75	38	R.EGAPGAEGAPGR.D + 2 Hydroxylation (P) (P)
579.81	50	R.GFPGSEGLIGPK.G
587.81	37	R.GFPGSEGLIGPK.G + Hydroxylation (K) (K)
594.29	45	R.GQPGVMGFPGPK.G + Hydroxylation (K) (K)
602.29	38	R.GQPGVMGFPGPK.G + Hydroxylation (K) (K); Hydroxylation (P) (P)
602.81	36	R.GFPGTPGLPGIK.G + Hydroxylation (K) (K); 3 Hydroxylation (P) (P)
610.29	52	R.GQPGVMGFPGPK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
721.84	49	R.GEPGAQGLPGVSGER.G + 2 Hydroxylation (P) (P)
561.27	28	K.GATGEPGRPGEAGLPGAR.G + 2 Hydroxylation (P) (P)
849.41	31	K.GATGEPGRPGEAGLPGAR.G + 3 Hydroxylation (P) (P)
943.95	30	R.GQPGVMGFPGPKGAAGEPGK.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1033.48	40	R.GESGSPGPAGFAGPPGADGQPGAK.G
1041.47	66	R.GESGSPGPAGFAGPPGADGQPGAK.G + Hydroxylation (P) (P)
1049.47	76	R.GESGSPGPAGFAGPPGADGQPGAK.G + 2 Hydroxylation (P) (P)
1057.46	64	R.GESGSPGPAGFAGPPGADGQPGAK.G + 3 Hydroxylation (P) (P)
1206.09	55	R.GPPGPSGSPGKDGASGLPGPIGPPGPR.G + Hydroxylation (K) (K); 2 Hydroxylation (P) (P)
1214.09	33	R.GPPGPSGSPGKDGASGLPGPIGPPGPR.G + 4 Hydroxylation (P) (P)
1222.09	45	R.GPPGPSGSPGKDGASGLPGPIGPPGPR.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)
883.74	33	R.GAPGERGESGSPGPAGFAGPPGADGQPGAK.G + Hydroxylation (P) (P)
899.74	33	R.GAPGERGESGSPGPAGFAGPPGADGQPGAK.G + 4 Hydroxylation (P) (P)
905.07	34	R.GAPGERGESGSPGPAGFAGPPGADGQPGAK.G + Hydroxylation (K) (K); 4 Hydroxylation (P) (P)

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
629.79	69	K.GLTGSPGSPGPDGK.T + 2 Hydroxylation (P) (P)
666.82	51	R.GPSGPQGPSGPPGPK.G + Hydroxylation (P) (P)
718.34	45	R.GEPGPAGLPGPPGER.G + 3 Hydroxylation (P) (P)
845.89	68	K.DGEAGAQGPPGPAGPAGER.G
654.66	31	K.SGDRGETGPAGPAGPVGPVGAR.G

The highlighted peptides are unique peptides.

Table I-8 ***Continued***.

Collagen alpha-1(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
832.03	28	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
837.36	25	K.GDAGPAGPKGEPGSPGENGAPGQMGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P); Oxidation (M)
Collagen alpha-2(I) [*Sus scrofa*]		
M (Obs.)	**Ion Score**	**Identified Peptide**
727.37	34	R.GIPGEFGLPGPAGPR.G + 2 Hydroxylation (P) (P)
631.64	29	R.GERGPPGESGAAGPAGPIGSR.G + Hydroxylation (P) (P)
671.67	33	K.HGNRGEPGPAGSVGPAGAVGPR.G + Hydroxylation (P) (P)
1066.06	82	R.GLPGVAGSVGEPGPLGIAGPPGAR.G + 3 Hydroxylation (P) (P)
923.11	26	R.GAPGAVGAPGPAGANGDRGEAGPAGPAGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)

The highlighted peptides are unique peptides.

Appendix II

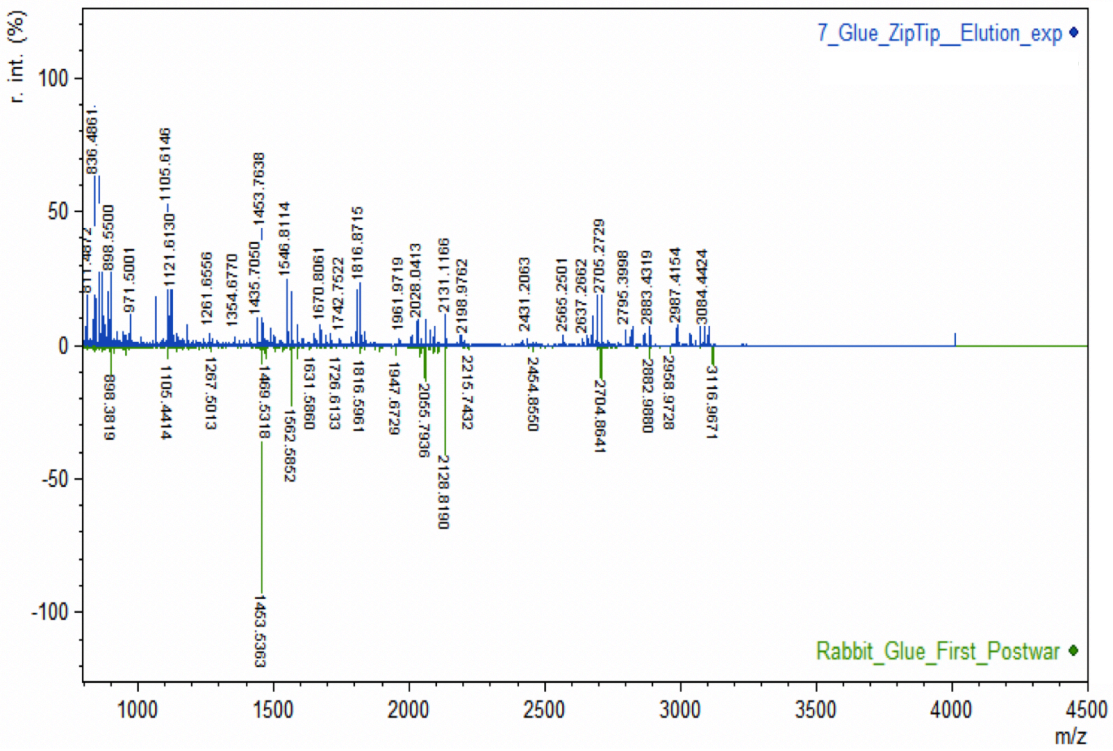


Figure II-1 Mass spectra of rabbit glue (7) after ZipTip versus mass spectra of rabbit glue (of first postwar) obtained by MALDI-TOF

Table II-1 Matched masses of rabbit glue (7)-ZipTip with rabbit glue (of first post war) 'reference' sample and identified protein and peptides according to MS/MS results and Mascot tool

Rabbit Glue (7)-ZipTip vs Rabbit Glue (of first post war)				
***m/z* MALDI**	**M (exp.) Orbit Trap**	**Protein**	**U**	**Peptide**
836.33	836.43	CO1A2	U	R.GLPGIKGHNGLQGLPGLAGQHGDQGAPGAVGPAGPR.G + 2 Deamidated (NQ); 2 Hydroxylation (P) (P)
898.38	898.48	CO1A1	_	R.GVVGLPGQR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1105.44	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]

[1]this peptide is common/shared between several species, namely *Oryctolagus cuniculus, Equus asinus, Capra hircus, Bos taurus* and *Sus scrofa*.

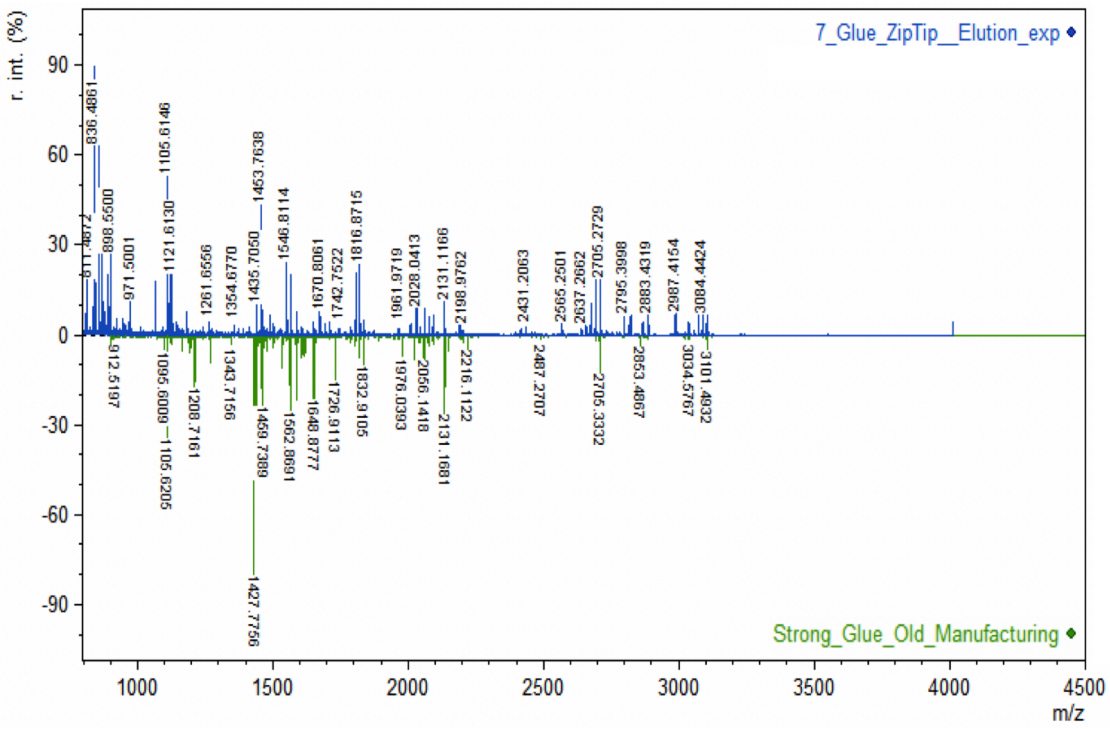


Figure II-2 Mass spectra of rabbit glue (7) after ZipTip versus mass spectra of strong glue (old manufacturing) obtained by MALDI-TOF

Table II-2 Matched masses of rabbit glue (7)-ZipTip with strong glue (old manufacturing) 'reference' sample and identified protein and peptides according to MS/MS results and Mascot tool

Rabbit- Glue (7)-ZipTip vs Strong Glue (old manufacturing)				
***m/z* MALDI**	**M (exp) Orbit Trap**	**Protein**	**U**	**Peptide**
1104.60	1104.57	CO1A1	_	R.GVQGPPGPAGPR.G + Hydroxylation (P) (P)
1105.62	1105.55	CO1A1	_	R.GVQGPPGPAGPR.G + Deamidated (NQ); Hydroxylation (P) (P)[1]
1239.60	1239.66	CO1A1		R.GVVGLPGQRGER.G + Hydroxylation (P) (P)[2]
1435.73	1435.67	CO3A1- *Bos taurus*	U	R.GPPGPPGTNGVPGQR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)
1500.78	1500.69	CO1A1- *Oryctolagus cuniculus*	U	R.GSPGPPGATGFPGAAGR.V + 3 Hydroxylation (P) (P)
1655.73	1655.79	CO1A1- Ctenopharyngodon idella	U	K.GSPGTPGIAGAPGFPGPR.G + 4 Hydroxylation (P) (P)
1706.70	1706.75	CO1A1	_	K.DGEAGAQGPPGPAGPAGER.G + Deamidated (NQ); Hydroxylation (P) (P)[3]
2199.03	2199.06	CO1A1	_	R.GETGPAGRPGEVGPPGPPGPAGEK.G + 2 Hydroxylation (P) (P)[2]
2689.34	2689.24	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 2 Hydroxylation (P) (P)[2]
2705.13	2705.23	CO1A1	_	R.GFSGLQGPPGPPGSPGEQGPSGASGPAGPR.G + Deamidated (NQ); 3 Hydroxylation (P) (P)[3]
3100.49	3100.38	CO1A1		R.GLPGPPGAPGPQGFQGPPGEPGEPGASGPMGPR.G + Deamidated (NQ); 6 Hydroxylation (P) (P)

[1]this peptide is common/shared between several species, namely *Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus, Oryctolagus cuniculus.* [2]within the group of (*Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected in all but protein Collagen α1(I) of *Capra hircus*. [3]within the group of (*Bos taurus, Sus scrofa, Capra hircus*, *Ovis aries*, *Equus asinus* and *Oryctolagus cuniculus*) this peptide has been detected in all but protein Collagen α1(I) of *Ovis aries*. [4]this peptide is common/shared between several species, namely *Ctenopharyngodon Idella*, *Danio rerio* and *Carassius auratus*.

5 Bibliography

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