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Collagen as a source of bioactive peptides: A bioinformatics approach

Suleivys M. Nuñez [a], Fanny Guzmán [b], Pedro Valencia [c], Sergio Almonacid [c,d], Constanza Cárdenas [b,*]

[a] Programa de Doctorado en Biotecnología, Pontificia Universidad Católica de Valparaíso/Universidad Técnica Federico Santa María, Valparaiso, Chile
[b] Núcleo de Biotecnología Curauma (NBC), Pontificia Universidad Católica de Valparaíso, Valparaiso 2373223, Chile
[c] Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Valparaíso 2390123, Chile
[d] Centro Regional de Estudios en Alimentos Saludables (CREAS), Curauma, Valparaíso 2373223, Chile

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ABSTRACT

Background: Collagen is the most abundant protein in animals and can be obtained from residues of the food industry. Its hydrolysate has many desirable properties that make it suitable as an additive in foods and cosmetics, or as a component of scaffold materials to be used in biomedicine.
Results: We report here the characterization of type I collagen from five different sources, namely bovine, porcine, chicken, trout and salmon, as well as their hydrolysates by means of bioinformatics tools. As expected, the results showed that bovine and porcine collagen, as well as trout and salmon collagen, can be used interchangeably due to their high identity. This result is consistent with the evolution of proteins with highly identical sequences between related species. Also, 156 sequences were found as potential bioactive peptides, 126 from propeptide region and 30 from the central domain, according to the comparison with reported active sequences.
Conclusions: Collagen analysis from a bioinformatic approach allowed us to classify collagen from 5 different animal sources, to establish its interchangeability as potential additive in diverse fields and also to determine the content of bioactive peptides from its *in silico* hydrolysis.
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1. Introduction

Collagen is the most abundant and ubiquitous protein of animal origin, representing approximately 30% of the total. It is involved in the formation of fibrillar and microfibrillar networks of the extracellular matrix and basal membranes. Fibrillar proteins are the main protein components of bone, cartilage, tendon, skin and other forms of connective tissues [1,2].

Collagen is characterized by having a triple helix internal structure, which gives it mechanical strength and moisture retention properties. The main function of collagen is to maintain the structure of the animal tissues and to improve their strength and flexibility [3,4]. Twenty-eight different collagen types have been described, composed of 46 distinct polypeptide chains [5,6]. All of these collagens have a characteristic triple helix structure, but the length of the helix, and the size and nature of the non-helical portion varies from one type to another. Types I, II and III are the most abundant collagen types being responsible for tissue strength, elasticity and water retention, with type I being the most used [7,8,9]. Type I collagen is constituted mainly by two types of alpha chains (alpha 1 and alpha 2), although a third chain (alpha 3) has been reported in fish [10,11,12]. In procollagen each chain has an N-terminal and a C-terminal propeptide, which exhibit the greatest difference in sequence between species, and a central domain that forms the fibers in mature collagen. The central domain of collagen has a characteristic amino acid sequence, which contains repeats of Gly-Xaa-Yaa, where the X and Y are frequently Pro and 4-hydroxyproline (Hyp), respectively, and it is the domain that forms the highly stable triple helix structure [5,13].

Hydrolyzed collagen is composed of small peptides of low molecular weight (0.3–8 kDa) and can be produced from native collagen from bones, skin and connective tissue of animals (e.g., cattle, swine and fish). Collagen has an excellent biological compatibility, degradability and is weakly allergenic; due to its low molecular weight, hydrolyzed collagen is easily digested and absorbed in the human body [14].

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* Corresponding author.
E-mail address: constanza.cardenas@pucv.cl (C. Cárdenas).

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Collagen has a wide spectrum of applications; due to properties such as water holding capacity and antioxidant activity, it is used as additive in foods and nutricosmetics [3,4,15]. It has also the ability to form gels and antimicrobial activity with applications in biomaterials and wound healing [16,17,18]. Peptides obtained from hydrolyzed collagen are also reported, with a variety of activities, such as antioxidant anti-inflammatory [8,19,20], the ability to self-assemble and cell-penetrating activity and it is useful in the design of nanostructures and in drug delivery [21,22]. It has also been reported peptides with antifreezing and cryoprotecting activities are useful in the food industry and in biomedicine [23,24]. Another interesting application of antiarthritic peptides is their use in medicine and veterinary [25,26,27].

Using bioinformatics tools, we report here the characterization of collagen from five different animal sources, namely bovine, porcine, chicken, trout and salmon, in terms of its domains and physicochemical properties calculated *in silico* based on the reported sequences in Uniprot database.

Likewise, we did the *in silico* hydrolysis of the sequences using the enzyme subtilisin (Alcalase), characterizing these peptide sequences in terms of their properties and comparing them with peptide sequences that have been reported to have different biological activities.

As expected, the results showed that bovine and porcine collagen, as well as trout and salmon collagen, can be used interchangeably due to their high identity. This result is consistent with the evolution of proteins with highly identical sequences between related species. Additionally, in terms of potentially active peptides from subtilisin-hydrolyzed collagen, although there were differences in the cutting pattern, it was possible to obtain sequences clustered together with peptides of known activity, so that they could be potentially bioactive peptides.

2. Materials and methods

2.1. Sequences used for analysis

The study involved sequences at three different levels that will be analyzed in detail in the following list:

1. Sequences of collagen type I from five different sources were obtained from UniProt database for alpha 1 and alpha 2 chains (Table 1). Annotations defining domains and other features are available only for some of the sequences but we used them transversally according to sequence similarity and based on multiple alignment made with the Geneious 9.1.8 package (http://www.geneious.com) (Supplementary Figures S1 and S2). In order to make the comparison of the complete sequences of the five sources, in this work only alpha 1 and alpha 2 chains were included, since the sequence for alpha 3 chain of salmon and trout are not found in databases. There is only one fragment for trout, reported by Saito et al.[12]. NCBI accession BAA33381).
2. Sequences of peptides resulting from subtilisin (EC 3.4.21.62) (Alcalase) hydrolysis of the collagen sequences. Subtilisin is an alkaline protease being reported to have affinity for diverse amino acids (Phe, Tyr, Trp, Leu, Ala, Glu, Met, Ser and Lys, [28,29]). Hydrolyses were simulated through BIOPEP server [30] for alpha 1 and alpha 2 collagen chains. The specificity of the enzyme defined in BIOPEP has the amino acids Tyr, Phe, Leu, Trp and Ser in their Cterminus and Val in its Nterminus as cutting sequence (http://www.uwm.edu.pl/biochemia/index.php/en/biopep). For the analysis, peptides were classified for each sequence and domain. Redundant or shorter sequences of three residues were eliminated (Table S1).
3. Sequences of peptides from different sources with reported activities (summarized in Table 2).

2.2. Calculation of descriptors and physicochemical properties

Protparam tool through Expasy web server [31] was used to calculate amino acid contents. The analyses were made through Rstudio (Rstudio team 2019) using specific R functions or packages (R CoreTeam 2019), as described below:

Physicochemical properties and indices were calculated with the Peptides package [32]. The definitions of descriptors calculated are presented in Table S2.

The variables were scaled through `scale` function in R, and subsequently, to orthogonalize the space and reduce the number of variables, a principal component analysis (PCA) was made using the function `prcomp` in R, with the scaled data. The number of principal components (PC) was selected according to their eigenvalue and the variance explained.

2.3. Clustering analysis

To perform the clustering analysis the new dataset obtained by PCA was used; in the case of collagen domains the data were clustered directly using the package pheatmap

[33] in Rstudio, with the default parameters of the program, that is a hierarchical clustering with Euclidean distance and complete-linkage method [34].

Clustering analysis of the bioactive peptides in conjunction with collagen-hydrolyzed peptides was made by kmeans analysis, with

Table 1
Summary of collagen sequences used with accession number, length, and defined domains limits, SP: signal peptide; Nterm: N-terminal peptide; Colldom: Collagen or central domain and Cterm: C-terminal peptide. The last column shows the number of peptides obtained for each sequence for *in silico* hydrolysis with subtilisin.

Source	UniProt accession number	Length	Domains				# Peptides by subtilisin hydrolysis
	Alpha 1 chain		SP	Nterm	Colldom	Cterm	
Bovine	P02453	1463	1–22	23–161	162–1217	1218–1463	175
Porcine	A0A287A1S6	1466	1–22	23–164	165–1620	1221–1466	184
Chicken	P02457	1453	1–22	23–151	152–1207	1208–1453	167
Trout	Q910C0	1449	1–22	23–146	147–1203	1204–1449	176
Salmon	A0A1S3R8F9	1449	1–22	23–161	162–1217	1218–1463	169
	Alpha 2 chain		SP	Nterm	Colldom	Cterm	
Bovine	P02465	1364	1–22	23–79	80–1117	1118–1364	185
Porcine	A0A1S7J1Y9	1366	1–20	21–79	80–1119	1120–1366	185
Chicken	P02467	1363	1–22	23–77	78–1118	1119–1363	176
Trout	O93484	1356	1–22	23–72	73–1113	1114–1356	179
Salmon	A0A1S3Q205	1356	1–24	25–72	73–1113	1114–1356	173

Table 2
Summary of the peptides with reported activities used in the clustering analysis to compare with collagen peptides.

Activity	# Peptides	Source
Antifreezing peptides	55	[41,42,43]
Antioxidant peptides	180	[24,44,45,46,47]
Cell penetrant peptides	1180	CPP2 Database
Gel peptides	32	[48,49,50,51,52,53]
Drug Delivery peptides	1487	SATP Database
Antimicrobial peptides	653	APD Database
Antimicrobial peptides	3980	DRAMP Database

tidyverse and factoextra packages [35,36] determining initially the optimal number of clusters by gap_stat method, and the dendrogram was constructed with pheatmap [33].

3. Results and discussion

3.1. Collagen domain analysis

Collagen from the five sources and their basic information are presented in Table 1.

The analysis was carried out on the complete collagen sequences, and not only on the central domain, which is the one found in mature collagen coming from food byproducts, taking advantage of the fact that we carried out an in silico analysis. Additionally, the subsequent analysis will allow us to determine sequences with potential bioactivity that come from the propeptide domains that do not have an amino acid composition bias.

Multiple alignment between the collagen sequences showed a high percentage of identity, with >76% for the alpha 1 chains and >68% for the alpha 2 chains with chicken collagen being the most different one (Figures S1, S2 and Table 3).

A close view of the domains showed that N-terminal peptides and central or collagen domain have the lowest values of identity among species (Figure S3).

An amino acid composition analysis showed a bias for both chains, with a high content of glycine and proline, a characteristic of collagen, corresponding mainly to the central domain (Figures S4 and S5).

Additionally, a matrix was constructed with the sequences corresponding to the domains of alpha 1 and alpha 2 chains of the five species chosen, and 80 descriptors calculated with Peptides package in Rstudio, having a matrix of 40 rows × 80 columns. PCA reduced the number of variables to ten PCs according to their variance (90%) and these new sets were used to perform the clustering analysis (Fig. 1). As can be seen, the groups are formed for the domains for both chains alpha 1 and alpha 2, with the exception of the N terminal peptide that forms two separate clusters, indicating that this domain is the most different in the two chains and in the five species; also, the central domains for both alpha and alpha 2 chains are in the same cluster interspersed, confirming their high similarity that was expected.

Table 3
Identity percentage between alpha 1 chains (upright matrix) and alpha 2 chains (down left matrix) for the compared collagens.

	Bovine	Porcine	Chicken	Trout	Salmon
Bovine		97.14	89.78	76.37	76.78
Porcine	95.39		90.01	76.76	77.23
Chicken	83.58	83.07		78.78	79.26
Trout	68.49	68.42	68,84		98.14
Salmon	68.93	68.79	68,84	96.31	

3.2. Subtilisin hydrolysis and clustering analysis

Subtilisin (Alcalase) is a protease frequently used for collagen, which, being a fibrillar protein and due to its wide compositional bias, is not easy to hydrolyze. Subtilisin is a low cost, microbial, commercial protease widely used due to its broad specificity and the high degree of hydrolysis that can be achieved in a relatively short time under moderate conditions. Subtilisin from different sources has been used in numerous studies about collagen hydrolysis, obtaining peptides with antifreeze, antioxidant and antihypertensive activities, among others [3,18,37,38,39]. In this report, alpha 1 and alpha 2 chains of the five sequences (Table 1) were used to generate peptides by subtilisin hydrolysis on BIOPEP server [30]. Despite the high similarity between the sequences, there are only few peptides shared between the five types of collagen. From the 1178 peptides obtained from both chains (Table 1), 480 are unique.

To determine the potential bioactivity of collagen peptides, 831 peptides from hydrolyzed collagen and 7567 bioactive peptides characterized with 80 descriptors were used to construct the matrix for the analysis (8546 × 80). PCA reduced the variables to nine PCAs with >90% of the total variance explained.

Due to the high number of cases, a kmeans cluster was made to establish a relationship between collagen-hydrolyzed peptides, and bioactive peptides. The optimal number of clusters according gap_stat method was 14 (Figure S6). The dendrogram constructed in pheatmap for these 14 clusters is shown in Fig. 2, and the analysis of cluster members is summarized in Fig. 3.

First of all, clusters formed mainly by collagen peptides (4 and 10), were excluded. An analysis of the dendrogram showed associations between clusters, having four groups. The first one was formed by clusters 8, 2 and 12 with a high number of CPP and DD peptides, with 14 peptides from alpha 1 chain and 24 from alpha 2 chain, and then associated with these activities. The second group was formed by clusters 3, 11 and 13 having a high number of antimicrobial peptides (APD and DRAMP) and AFPs, with 57 from alpha 1 chain and 48 from alpha 2 chain. A third group was formed by clusters 1, 6 and 9 having also a high number of antimicrobial peptides, these two groups without having a clear association with a specific activity, containing 38 sequences form alpha 1 chain and 31 from alpha 2 chain. A fourth group was formed by clusters 7 and 14 with a high number of antioxidant peptides with 17 from alpha 1 chain and 16 from alpha 2 chain. Excluding the sequences of two residues, because they are very frequent and not very specific, the potential bioactive peptides from hydrolyzed collagen are 101 from alpha 1 chain and 97 from alpha 2 chain.

It is worth noting that in the DRAMP and APD databases there are many cases in which a sequence has several activities simultaneously, so there is no sequence specificity, this can cause a bias in the analysis with the presence of peptides from these databases in virtually all clusters.

Table 4 summarizes the collagen peptides from the central domain belonging to the four groups mentioned above, and Table S3 the peptides from the propeptide domains.

It must be noted that in the sequence analysis post-translational modifications, such as the formation of hydroxyproline, a very important amino acid in collagen, cannot be taken into account. In this case there are two major biases: on the one hand the effect of this amino acid specifically on the activity of the

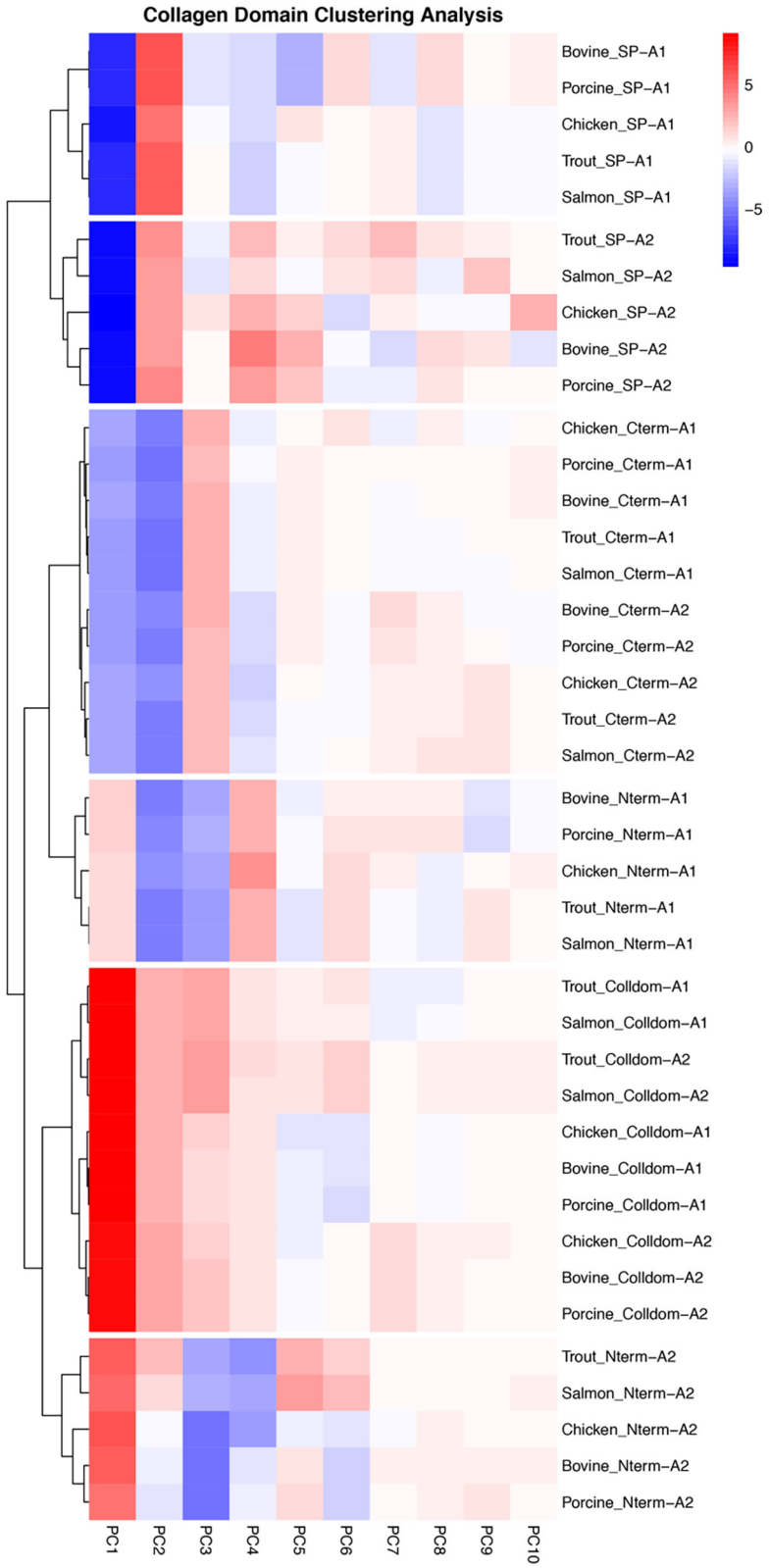


Fig. 1. Clustering analysis for collagen domains with ten principal components obtained from 80 initial variables (supplemental Table S2). Analysis was made with pheatmap package as implemented in Rstudio.

sequences is not taken into account, and on the other hand the potentially active sequences found, may have a different behavior if the amino acid considered is hydroxyproline.

Many peptides derived from hydrolyzed collagen have been reported, with biomedical applications as can be seen in the reviews of Liu et al. [13], Gomez-Guillen et al. [18] and Felician

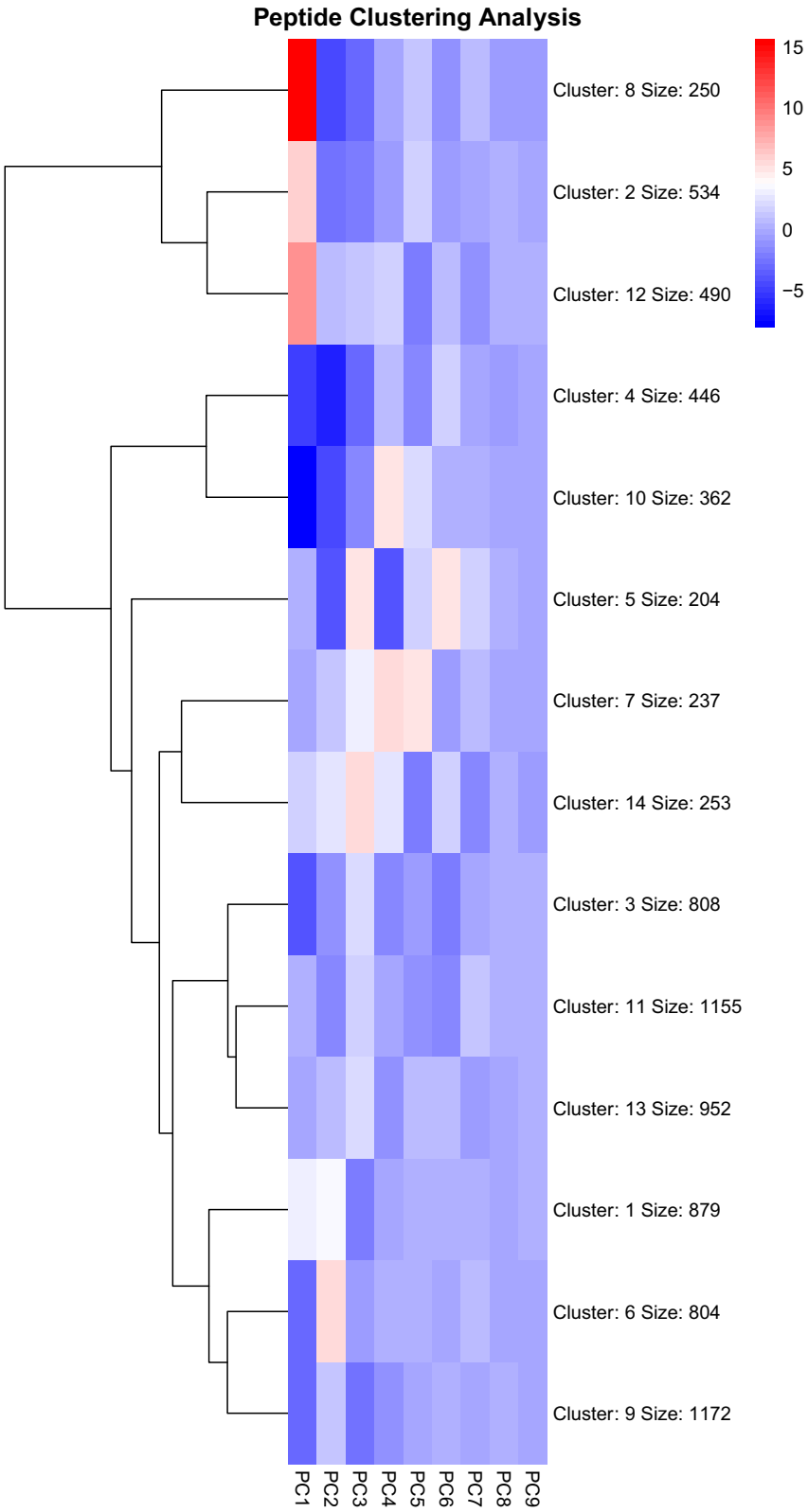
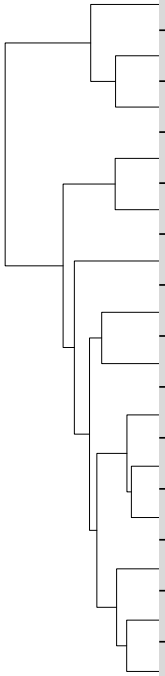


Fig. 2. Kmeans clustering for the 8546 peptides with the first nine principal components. Analysis was made with pheatmap in Rstudio.



Cluster	AFP	Antiox	CPP	Gel	DD	APD	DRAMP	Col_A1	Col_A2	Size
8			158		81		8	2	1	250
2		1	245	1	174	8	76	9	20	534
12		4	198		132	2	148	3	3	490
4	2	11	20	2	26	14	48	145	178	446
10		10		1	8	1	8	148	186	362
5		30	17	4	19	7	59	42	26	204
7		28	40		86	12	56	9	6	237
14		26	36	6	133	3	31	8	10	253
3	14	9	27		142	95	465	33	23	808
11	1	6	40		178	106	813	7	4	1155
13	18	24	70	7	175	114	506	17	21	952
1		7	181	4	136	50	494	2	5	879
6	4	17	94		125	85	443	23	13	804
9	16	16	54	7	72	156	825	13	13	1172
Total	**55**	**180**	**1180**	**32**	**1487**	**653**	**3980**	**461**	**509**	**8546**

Fig. 3. Summary of the clusters obtained by Kmeans analysis as presented in Fig. 2, for the 8546 peptides with nine PCAs. Each column is associated with a set of peptides with a particular reported activity, AFP: antifreezing peptides, Antiox: antioxidant peptides, CPP: cell penetrating peptides, Gel: gel forming peptides, DD: drug delivery peptides from SATPDB database, APD: antimicrobial peptides from APD database only, DRAMP: antimicrobial peptides from DRAMP database, including some peptides shared with APD database, Col_A1 and Col_A2 correspond to the hydrolyzed peptides from alpha 1 and alpha 2 collagen chains, respectively.

Table 4
Peptides from hydrolyzed collagen associated with the selected clusters. Peptides are labeled according to the chains alpha 1:A1 or alpha 2:A2, and the first letter from the collagen source: bovine (B), porcine (P), chicken (C), trout and salmon (TS).

Alpha 1			**Alpha 2**		
Label	**Sequence**	**Cluster**	**Label**	**Sequence**	**Cluster**
			A2_BPC9	RGERGL	2
			A2_B26	KGIRGH	2
			A2_P31	KGIR	2
Label	**Sequence**	**Cluster**	**Label**	**Sequence**	**Cluster**
A1_BP30	PQPPQEKAHDGGRY	13	A2_BP16	MGPRGF	11
A1_BP44	VPGDL	3	A2_C41	KAADF	13
A1_TS13	DMGF	11	A2_C38	HNGL	13
A1_TS39	IAQPAQEKAPDPF	13			
A1_TS43	IEIRAEGNS	13			
A1_B37	TGIS	3			
A1_C43	PAGQ	11			
A1_C65	PRGDKGETGEQGDRGMKGHRGF	13			
A1_S13	PGADGAAGPKGGPGERGGAG	13			
Label	**Sequence**	**Cluster**	**Label**	**Sequence**	**Cluster**
A1_BPCTS26	VMGF	6	A2_TS64	QGAI	9
A1_BP47	VRGL	9	A2_TS83	VGEPGRL	9
A1_TS5	AGQL	9	A2_P61	VGPAGIR	9
A1_TS81	VAGAS	9			
A1_P2	AGIS	9			
A1_C7	ARGL	9			
A1_C58	PGPPGPAGKQGS	6			
A1_C69	QGPPGPPGAPGEQGPS	9			
A1_C74	TGPIGPPGPAGAPGDKGEAGPPGPAG	9			
A1_C84	VPGNAGAPG	9			
Label	**Sequence**	**Cluster**	**Label**	**Sequence**	**Cluster**
A1_C62	PPGAPGPQGF	7	A2_TS54	PGHL	7

et al. [3], as well as additives in the food industry such as those reported by Wang and Damodaran [38,40].

4. Conclusions

Using bioinformatics tools we classified collagen from different animal sources based on their physicochemical properties and other features obtained through the Expasy web server, and with the use of the Peptide package in Rstudio.

The space of bioactive peptides is highly diverse, and it is not possible so far to establish a clear association between the properties of a peptide and its specific activity. However, the use of descriptors covering a wide variety of properties, to characterize each sequence and the orthogonalization of the space by means of principal component analysis, permitted to establish a connection between collagen peptides and peptides reported to have particular activities. By means of clustering analysis of peptides with more than three residues, we found 24 peptides from collagen that may have cell penetrating activity: 3 from the central domain and 21 from the propeptides, 85 peptides that may have a potential antioxidant activity: 12 from central domain and 73 from propeptides, and two groups with 38 and 9 peptides that may have antimicrobial activity: 15 from central domain and 32 from propeptide. This set of peptides could be tested for the predicted activities, keeping in mind that antioxidant, gel forming or antimicrobial activities are quite useful for additives in the food industry.

Collagen is an ingredient widely used in the food, cosmetic industries, and as scaffold in tissue engineering, and sometimes it is not easy to obtain it from a single source. According to our results, collagen from bovine and porcine are highly similar, and could be used interchangeably or even as a mix, as well as trout and salmon, meanwhile chicken collagen is closer to bovine and porcine collagen being the most different among the species considered.

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Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejbt.2020.09.009.

References

[1] Hashim P, Mohd Ridzwan MS, Bakar J, et al. Collagen in food and beverage industries. Int Food Res J 2015;22:1–8.
[2] Roy BC, Omana DA, Betti M, et al. Extraction and characterization of gelatin from Bovine Lung. Food Sci Technol Res 2017;23:255–66. https://doi.org/10.3136/fstr.23.255.
[3] Felician FF, Xia C, Qi W, et al. Collagen from marine biological sources and medical applications. Chem Biodivers 2018;15:e1700557. https://doi.org/10.1002/cbdv.201700557. PMid:29521032.
[4] León-López A, Morales-Peñaloza A, Martínez-Juárez VM, et al. Hydrolyzed collagen—sources and applications. Molecules 2019;24:4031. https://doi.org/10.3390/molecules24224031. PMid:31703345.
[5] Ricard-Blum S. The collagen family. Cold Spring Harb Perspect Biol 2011;3. https://doi.org/10.1101/cshperspect.a004978. PMid:21421911.
[6] Shoulders MD, Raines RT. Collagen structure and stability. Annu Rev Biochem 2009;78:929–58. https://doi.org/10.1146/annurev.biochem.77.032207.120833. PMid:19344236.
[7] Silvipriya K, Kumar K, Bhat A, et al. Collagen: animal sources and biomedical application. J Appl Pharm Sci 2015:123–7. https://doi.org/10.7324/JAPS.2015.50322.
[8] Hong H, Fan H, Chalamaiah M, et al. Preparation of low-molecular-weight, collagen hydrolysates (peptides): current progress, challenges, and future perspectives. Food Chem 2019;301:125222. https://doi.org/10.1016/j.foodchem.2019.125222. PMid:31382108.
[9] Sorushanova A, Delgado LM, Wu Z, et al. The collagen suprafamily: from biosynthesis to advanced biomaterial development. Adv Mater 2019;31:1801651. https://doi.org/10.1002/adma.201801651. PMid:30126066.
[10] Gistelinck C, Gioia R, Gagliardi A, et al. Zebrafish collagen type I: molecular and biochemical characterization of the major structural protein in bone and skin. Sci Rep 2016;6:21540. https://doi.org/10.1038/srep21540. PMid:26876635.
[11] Muralidharan N, Jeya Shakila R, Sukumar D, et al. Skin, bone and muscle collagen extraction from the trash fish, leather jacket (Odonus niger) and their characterization. J Food Sci Technol 2013;50:1106–13. https://doi.org/10.1007/s13197-011-0440-y. PMid:24426022.
[12] Saito M, Takenouchi Y, Kunisaki N, et al. Complete primary structure of rainbow trout type I collagen consisting of α1(I)α2(I)α3(I) heterotrimers. Eur J Biochem 2001;268:2817–27. https://doi.org/10.1046/j.1432-1327.2001.02160.x. PMid:11358497.
[13] Liu D, Nikoo M, Boran G, et al. Collagen and gelatin. Annu Rev Food Sci Technol 2015;6:527–57. https://doi.org/10.1146/annurev-food-031414-111800. PMid:25884286.
[14] Sibilla S, Godfrey M, Brewer S, et al. An overview of the beneficial effects of hydrolysed collagen as a nutraceutical on skin properties: scientific background and clinical studies. Open Nutraceuticals J 2015;8:29–42. https://doi.org/10.2174/1876396001508010029.
[15] Avila Rodríguez MI, Rodríguez Barroso LG, Sánchez ML. Collagen: a review on its sources and potential cosmetic applications. J Cosmet Dermatol 2018;17:20–6. https://doi.org/10.1111/jocd.12450. PMid: 29144022.
[16] Li L, Yu F, Zheng L, et al. Natural hydrogels for cartilage regeneration: modification, preparation and application. J Orthop Transl 2019;17:26–41. https://doi.org/10.1016/j.jot.2018.09.003. PMid:31194006.
[17] Razavi M, Qiao Y, Thakor AS. Three-dimensional cryogels for biomedical applications. J Biomed Mater Res Part A 2019;107:2736–55. https://doi.org/10.1002/jbm.a.36777. PMid:31408265.
[18] Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP. Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocoll 2011;25:1813–27. https://doi.org/10.1016/j.foodhyd.2011.02.007.
[19] Wang J, Luo D, Liang M, Zhang T, Yin X, Zhang Y, et al. Spectrum-effect relationships between high-performance liquid chromatography (HPLC) fingerprints and the antioxidant and anti-inflammatory activities of collagen peptides. Molecules 2018;23:3257. https://doi.org/10.3390/molecules23123257. PMid:30544714.
[20] Tkaczewska J, Bukowski M, Mak P. Identification of antioxidant peptides in enzymatic hydrolysates of carp (Cyprinus Carpio) skin gelatin. Molecules 2018;24:97. https://doi.org/10.3390/molecules24010097. PMid:30597854.
[21] Yamazaki CM, Nakase I, Endo H, et al. Collagen-like cell-penetrating peptides. Angew Chem Int Ed 2013;52:5497–500. https://doi.org/10.1002/anie.201301266. PMid:23592529.
[22] Masuda R, Yamamoto K, Koide T. Cellular uptake of IgG using collagen-like cell-penetrating peptides. Biol Pharm Bull 2016;39:130–4. https://doi.org/10.1248/bpb.b15-00548. PMid:26725435.
[23] Du L, Betti M. Chicken collagen hydrolysate cryoprotection of natural actomyosin: mechanism studies during freeze-thaw cycles and simulated digestion. Food Chem 2016;211:791–802. https://doi.org/10.1016/j.foodchem.2016.05.092. PMid:27283698.
[24] Wu R, Wu C, Liu D, et al. Antioxidant and anti-freezing peptides from salmon collagen hydrolysate prepared by bacterial extracellular protease. Food Chem 2018;248:346–52. https://doi.org/10.1016/j.foodchem.2017.12.035. PMid:29329864.
[25] König D, Oesser S, Scharla S, et al. Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women—a randomized controlled study. Nutrients 2018;10:97. https://doi.org/10.3390/nu10010097. PMid:29337906.
[26] Dressler P, Gehring D, Zdzieblik D, et al. Improvement of functional ankle properties following supplementation with specific collagen peptides in athletes with chronic ankle instability. J Sports Sci Med 2018;17:298–304. https://doi.org/10.1016/j.jbmt.2018.09.037. PMid:29769831.
[27] Dobenecker B, Reese S, Jahn W, et al. Specific bioactive collagen peptides (PETAGILE ®) as supplement for horses with osteoarthritis: A two-centred study. J Anim Physiol Anim Nutr (Berl) 2018;102:16–23. https://doi.org/10.1111/jpn.12863. PMid:29623685.
[28] Ellaiah P, Srinivasulu B, Adinarayana K. A review on microbial alkaline proteases. J Sci Ind Res 2002;61:690–704.
[29] Valencia P, Pinto M, Almonacid S. Identification of the key mechanisms involved in the hydrolysis of fish protein by Alcalase. Process Biochem 2014;49:258–64. https://doi.org/10.1016/j.procbio.2013.11.012.
[30] Minkiewicz, Iwaniak, Darewicz. BIOPEP-UWM database of bioactive peptides: current opportunities. Int J Mol Sci 2019;20:5978. https://doi.org/10.3390/ijms20235978. PMid:31783634.

[31] Gasteiger E. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucl Acids Res 2003;31:3784–8. https://doi.org/10.1093/nar/gkg563. PMid:12824418.
[32] Osorio D, Rondon-Villareal P, Torres R. Peptides: a package for data mining of antimicrobial peptides. R J 2015;7:4–14. https://doi.org/10.32614/RJ-2015-001.
[33] Kolde R. pheatmap:Pretty Heatmaps 2019. R package version 1.0.12. https://CRAN.R-project.org/package=pheatmap.
[34] Maechler M, Rousseeuw P, Struyf A, et al. cluster: Cluster Analysis Basics and Extensions. 2019. R package version 2.1.0. https://svn.r-project.org/R-packages/trunk/cluster.
[35] Wickham H, Averick M, Bryan J, et al. Welcome to the Tidyverse. J Open Source Softw 2019;4:1686. https://doi.org/10.21105/joss.01686.
[36] Kassambara A, Mundt F. factoextra: Extract and Visualize the Results of Multivariate Data Analyses 2019. R package version 1.0.6. https://CRAN.R-project.org/package=factoextra.
[37] Damodaran S. Inhibition of ice crystal growth in ice cream mix by gelatin hydrolysate. J Agric Food Chem 2007;55:10918–23. https://doi.org/10.1021/jf0724670. PMid: 18044830.
[38] Wang S, Damodaran S. Ice-structuring peptides derived from bovine collagen. J Agric Food Chem 2009;57:5501–9. https://doi.org/10.1021/jf900524v. PMid:19480387.
[39] Aguilar-Toalá JE, Hernández-Mendoza A, González-Córdova AF, et al. Potential role of natural bioactive peptides for development of cosmeceutical skin products. Peptides 2019;122:170170. https://doi.org/10.1016/j.peptides.2019.170170. PMid: 31574281.
[40] Damodaran S, Wang S. Ice crystal growth inhibition by peptides from fish gelatin hydrolysate. Food Hydrocoll 2017;70:46–56. https://doi.org/10.1016/j.foodhyd.2017.03.029.
[41] Bang J, Lee J, Murugan R, et al. Antifreeze peptides and glycopeptides, and their derivatives: potential uses in biotechnology. Mar Drugs 2013;11:2013–41. https://doi.org/10.3390/md11062013. PMid: 23752356.
[42] Kim H, Lee J, Hur Y, et al. Marine antifreeze proteins: structure, function, and application to cryopreservation as a potential cryoprotectant. Mar Drugs 2017;15:27. https://doi.org/10.3390/md15020027. PMid: 28134801.
[43] Surís-Valls R, Voets IK. Peptidic antifreeze materials: prospects and challenges. Int J Mol Sci 2019;20:5149. https://doi.org/10.3390/ijms20205149. PMid: 31627404.
[44] Samaranayaka AGP, Li-Chan ECY. Food-derived peptidic antioxidants: a review of their production, assessment, and potential applications. J Funct Foods 2011;3:229–54. https://doi.org/10.1016/j.jff.2011.05.006.
[45] Intiquilla A, Jiménez-Aliaga K, Guzmán F, et al. Novel antioxidant peptides obtained by alcalase hydrolysis of *Erythrina edulis* (pajuro) protein. J Sci Food Agric 2019;99:2420–7. https://doi.org/10.1002/jsfa.9449. PMid: 30362128.
[46] Chen N, Chen J, Yao B, et al. QSAR study on antioxidant tripeptides and the antioxidant activity of the designed tripeptides in free radical systems. Molecules 2018;23:1407. https://doi.org/10.3390/molecules23061407. PMid: 29890782.
[47] Yang XR, Qiu YT, Zhao YQ, et al. Purification and characterization of antioxidant peptides derived from protein hydrolysate of the marine bivalve mollusk *Tergillarca granosa*. Mar Drugs 2019;17:1–16. https://doi.org/10.3390/md17050251. PMid: 31035632.
[48] Echalier C, Jebors S, Laconde G, et al. Sol–gel synthesis of collagen-inspired peptide hydrogel. Mater Today 2017;20:59–66. https://doi.org/10.1016/j.mattod.2017.02.001.
[49] Worthington P, Pochan DJ, Langhans SA. Peptide hydrogels – versatile matrices for 3D cell culture in cancer medicine. Front. Oncol. 2015;5. https://doi.org/10.3389/fonc.2015.00092. PMid: 25941663.
[50] Koutsopoulos S. Self-assembling peptide nanofiber hydrogels in tissue engineering and regenerative medicine: progress, design guidelines, and applications. J Biomed Mater Res Part A 2016;104:1002–16. https://doi.org/10.1002/jbm.a.35638. PMid: 26707893.
[51] Jonker AM, Löwik DWPM, van Hest JCM. Peptide- and protein-based hydrogels. Chem Mater 2012;24:759–73. https://doi.org/10.1021/cm202640w.
[52] Yan C, Pochan DJ. Rheological properties of peptide-based hydrogels for biomedical and other applications. Chem Soc Rev 2010;39:3528. https://doi.org/10.1039/b919449p. PMid: 20422104.
[53] Boden N, Aggeli A, Buckland T. Beta sheet forming peptides and gels made thereof. Patent 0132974 A1, 2002.