Extinct species identification from late Middle Pleistocene and earlier Upper Pleistocene bone fragments and tools not recognizable from their osteomorphological study by an enhanced proteomics protocol

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The formula used to calculate the frequency of modification

The frequency of modification is calculated according to the formula (1):

$$\text{Frequency of modification} = \frac{All\ Intensity\ of\ modified\ peptide}{All\ Intensity\ of\ modified\ peptide + intensity\ of\ control\ peptide} \times 100 \quad (1)$$

Table S4.1. Sequence of peptides used for calculation of deamidation and oxidation frequency. The table shows peptide sequence, PTMs, m/z, retention time and charges. The localization of modification is noted by bold and underline amino acids.

Protein	Peptide sequence	PTMs	*m/z*	Retention time (min)	Charge
COL1A1	GQAGV<u>M</u>GFPGPK	Oxidation (M)	581.2888	69.28	2
	G<u>Q</u>AGV<u>M</u>GFPGPK	Oxidation (M); Deamidated (Q)	581.7818	71.84	2
	GQAGV<u>M</u>GFPG<u>P</u>K	Oxidation (M); Oxidation (P)	589.2868	63.2	2
	G<u>Q</u>AGV<u>M</u>GFPG<u>P</u>K	Oxidation (M); Oxidation (P); Deamidated (Q)	589.7783	65.78	2
	GANGA<u>P</u>GIAGA<u>P</u>GF<u>P</u>GAR	3 Oxidation (M)	793.3881	74.9	2
	GA<u>N</u>GA<u>P</u>GIAGA<u>P</u>GF<u>P</u>GAR	3 Oxidation (M); Deamidated (N)	793.8801	75.6	2
COL1A2	GVVGPQGAR		420.7369	42.07	2
	GVVGP<u>Q</u>GAR	Deamidated (Q)	421.2286	45.76	2
	VGAPGPAGAR		426.7373	35	2
	VGAPG<u>P</u>AGAR	Oxidation (P)	434.7334	33.93	2
	GELGPVGNPGPAGPAGPR		800.4162	78	2
	GELGPVG<u>N</u>PGPAGPAGPR	Deamidated (N)	800.9085	82.06	2

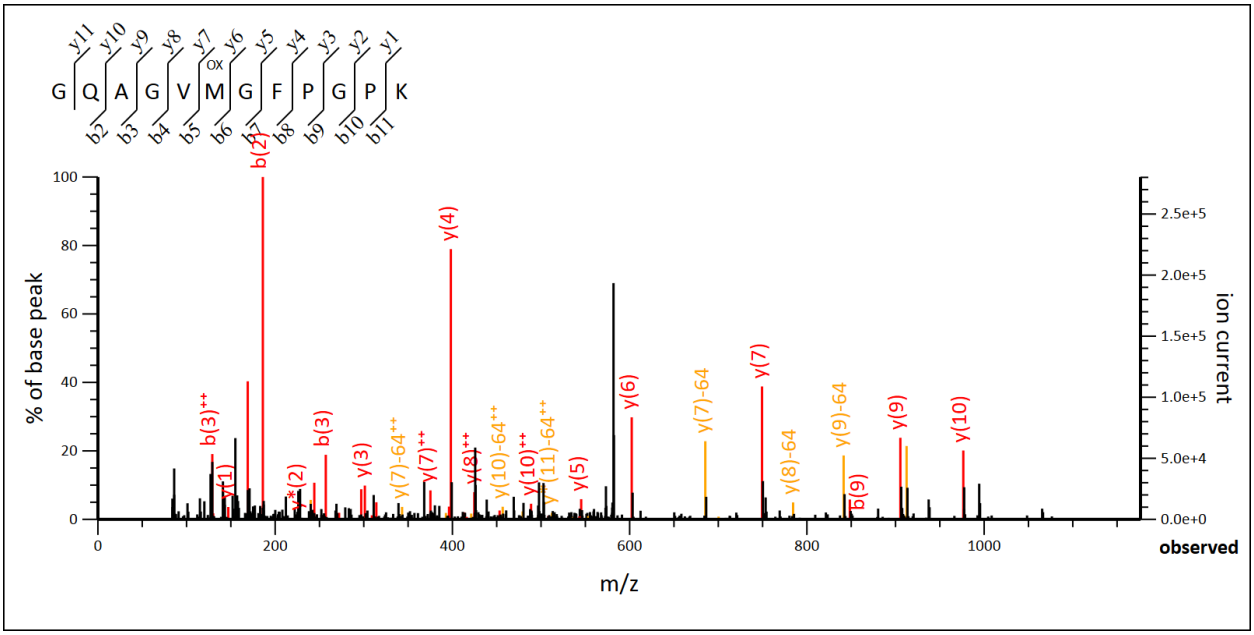


Figure S4.1. MS/MS Fragmentation of peptide GQAGVMGFPGPK.
Match to Query 3658: 1160.563088, from (581.288820, 2+), intensity (2194000.5000), rt in seconds (4049), scans (18446), index (10852)
Monoisotopic mass of neutral peptide Mr(calc): 1160.5649
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
M6 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
Ions Score: 51
Matches : 16/130 fragment ions using 30 most intense peaks

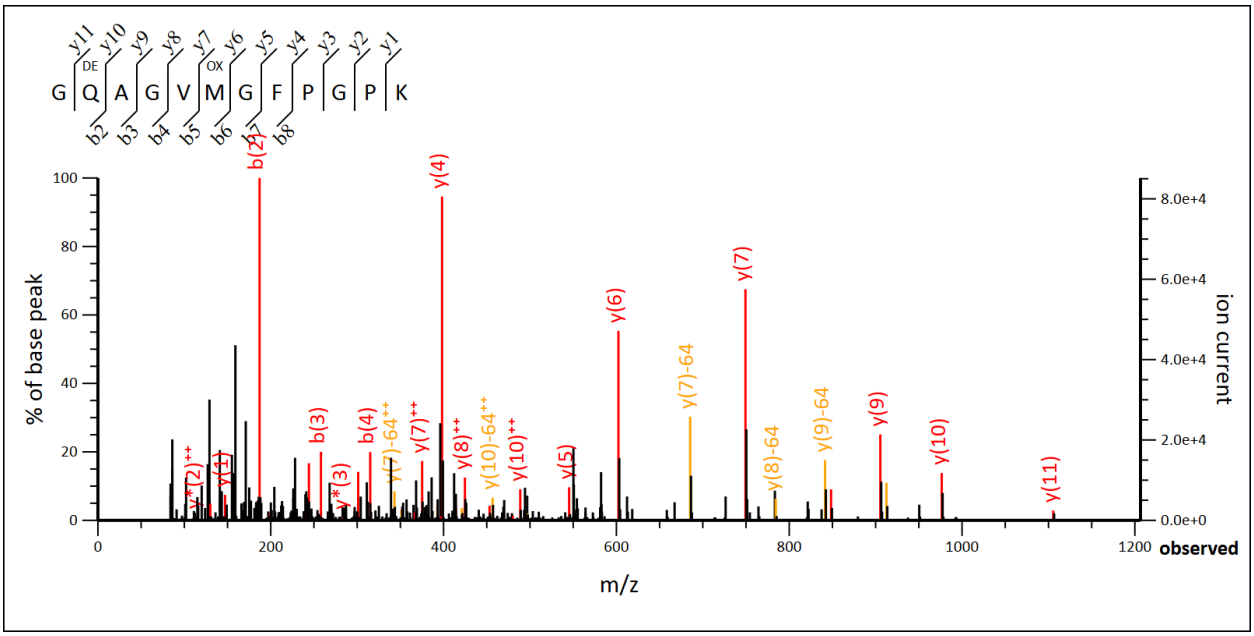


Figure S4.2. MS/MS Fragmentation of peptide GQAGVMGFPGPK.
Match to Query 3669: 1161.549048, from (581.781800, 2+), intensity (397039.8125), rt in seconds (4195), scans (19180), index (11525)
Monoisotopic mass of neutral peptide Mr(calc): 1161.5489
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
Q2 : Deamidated (NQ)
M6 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
Ions Score: 64
Matches : 18/130 fragment ions using 40 most intense peaks

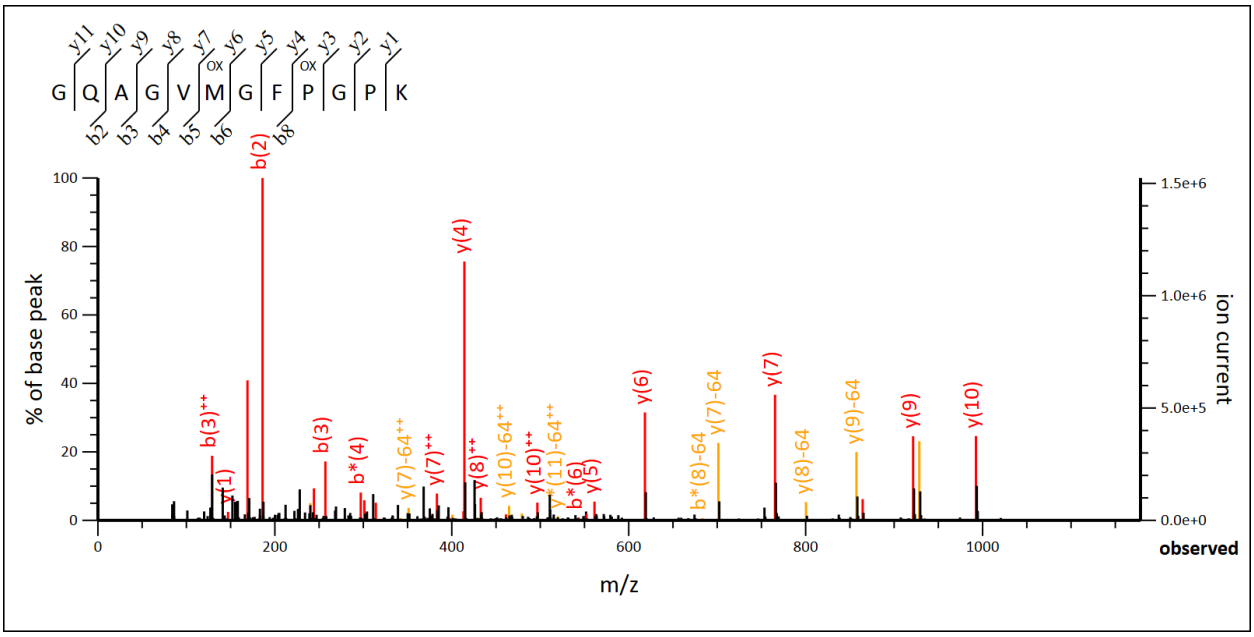


Figure S4.3. MS/MS Fragmentation of peptide GQAGVMGFPGPK.
Match to Query 3876: 1176.559048, from (589.286800, 2+), intensity (11115169.0000), rt in seconds (3718), scans (16772), index (9391)
onoisotopic mass of neutral peptide Mr(calc): 1176.5598
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
M6 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
P9 : Oxidation (P)
Ions Score: 58
Matches : 20/130 fragment ions using 40 most intense peaks

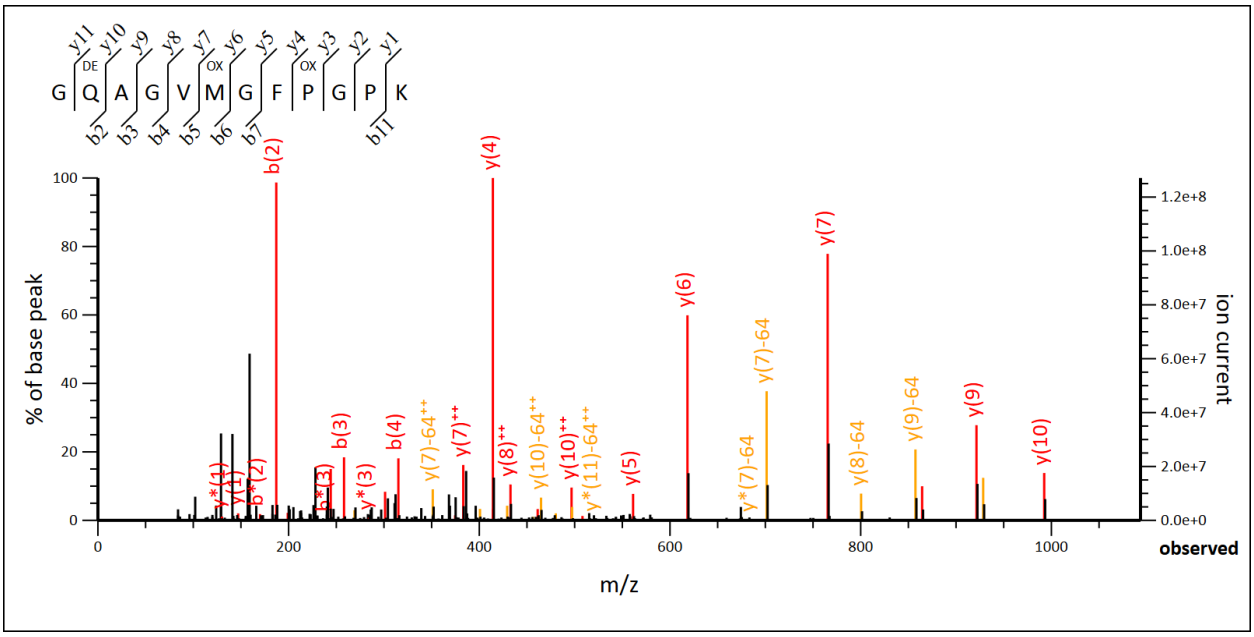


Figure S4.4. MS/MS Fragmentation of GQAGVMGFPGPK.
Match to Query 3898: 1177.542328, from (589.778440, 2+), intensity (1061965248.0000), rt in seconds (3842), scans (17401), index (9934)
Monoisotopic mass of neutral peptide Mr(calc): 1177.5438
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
Q2 : Deamidated (NQ)
M6 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
P9 : Oxidation (P)
Ions Score: 62
Matches : 23/130 fragment ions using 38 most intense peaks

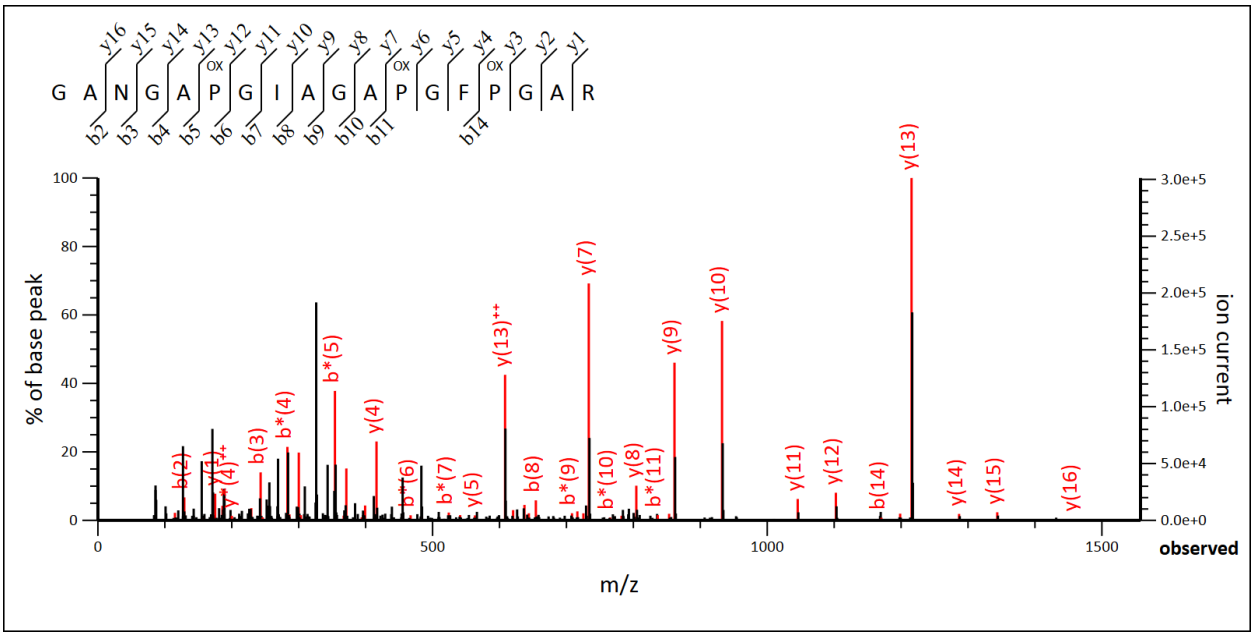


Figure S4.5. MS/MS Fragmentation of peptide GANGAPGIAGAPGFPGAR.
Match to Query 8761: 1584.765468, from (793.390010, 2+), intensity (2610150.7500), rt in seconds (4433), scans (20082), index (11948)
Monoisotopic mass of neutral peptide Mr(calc): 1584.7645
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
P6 : Oxidation (P)
P12 : Oxidation (P)
P15 : Oxidation (P)
Ions Score: 56
Matches : 34/132 fragment ions using 109 most intense peaks

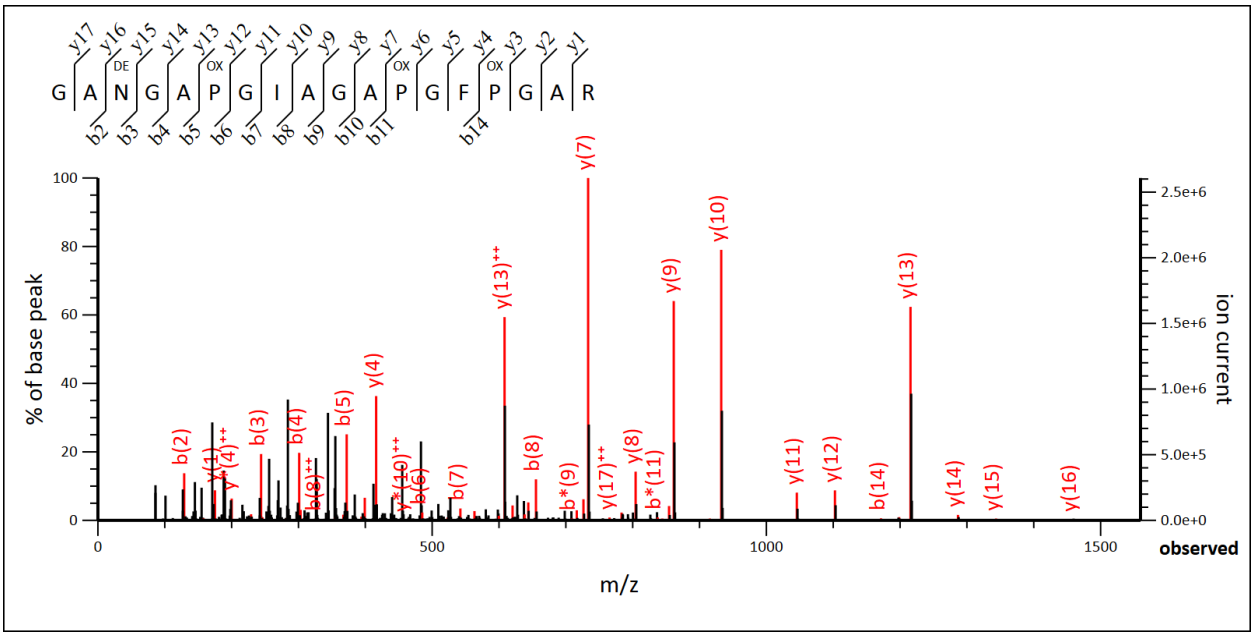


Figure S4.6. MS/MS Fragmentation of peptide GANGAPGIAGAPGFPGAR.
Match to Query 8787: 1585.746188, from (793.880370, 2+), intensity (28993934.0000), rt in seconds (4847), scans (22142), index (13839)
Monoisotopic mass of neutral peptide Mr(calc): 1585.7485
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
N3 : Deamidated (NQ)
P6 : Oxidation (P)
P12 : Oxidation (P)
P15 : Oxidation (P)
Ions Score: 64
Matches : 31/132 fragment ions using 91 most intense peaks

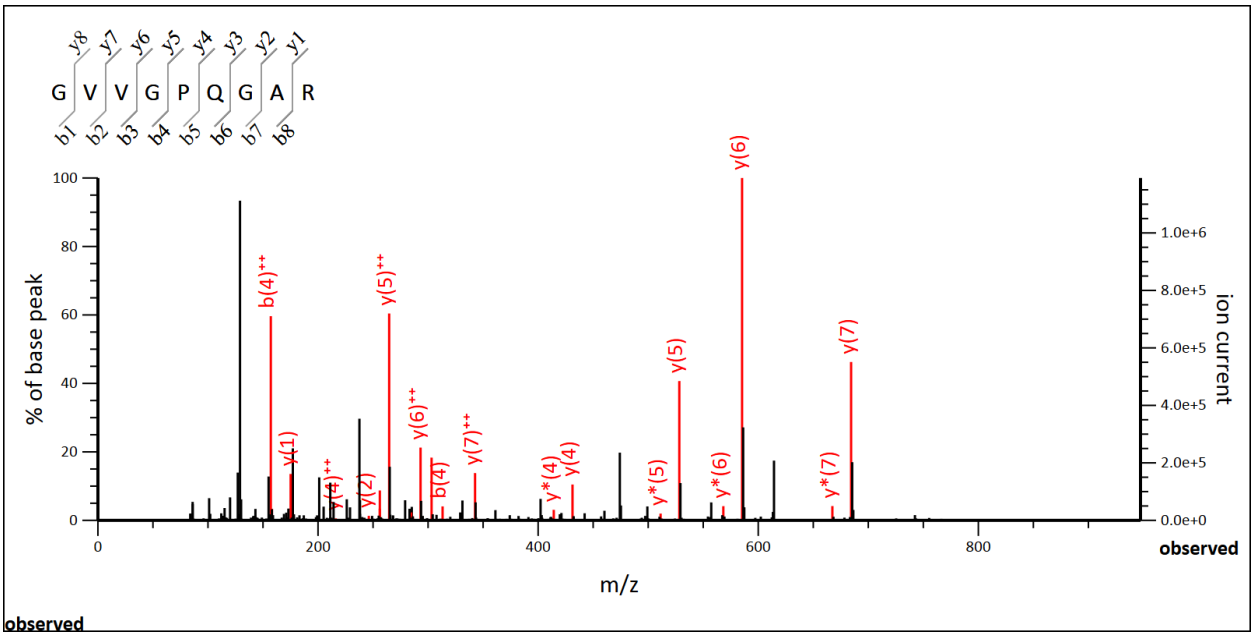


Figure S4.7. MS/MS Fragmentation of peptide GVVGPQGAR.
Match to Query 611: 839.460488, from (420.737520, 2+), intensity (6347722.0000), rt in seconds (2506), scans (10638), index (4130)
Monoisotopic mass of neutral peptide Mr(calc): 839.4614
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 33
Matches : 9/54 fragment ions using 14 most intense peaks

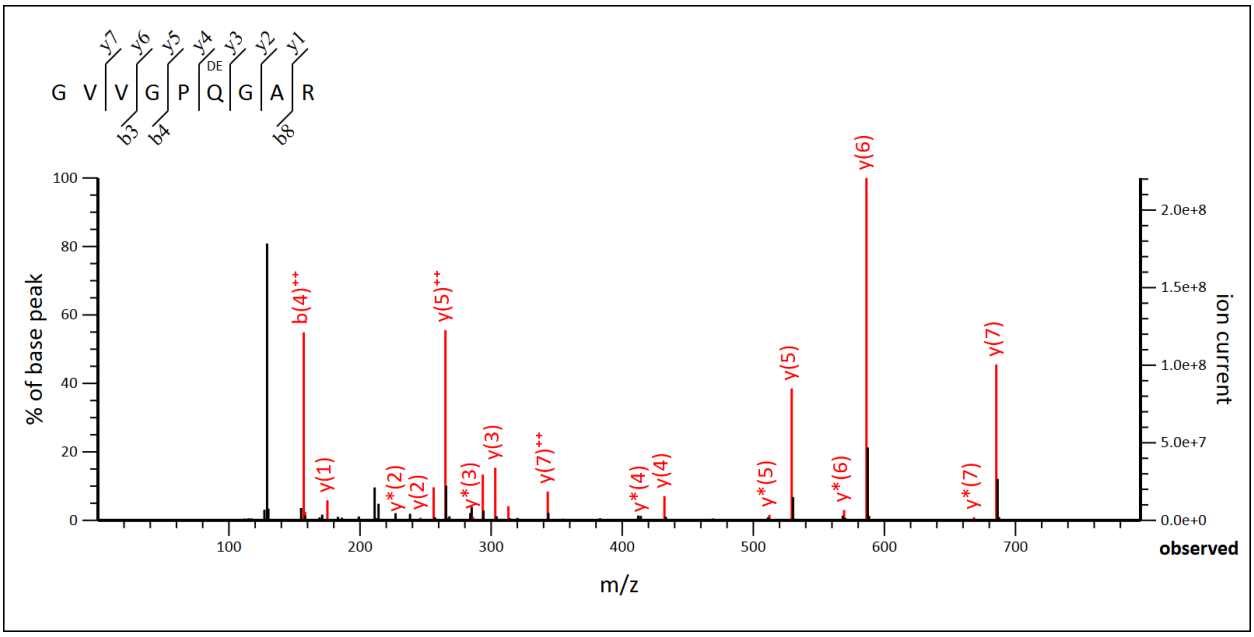


Figure S4.8. MS/MS Fragmentation of peptide GVVGPQGAR.
Match to Query 617: 840.441508, from (421.228030, 2+), intensity (944276864.0000), rt in seconds (2753), scans (11892), index (5150)
Monoisotopic mass of neutral peptide Mr(calc): 840.4454
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
Q6 : Deamidated (NQ)
Ions Score: 47
Matches : 12/54 fragment ions using 18 most intense peaks

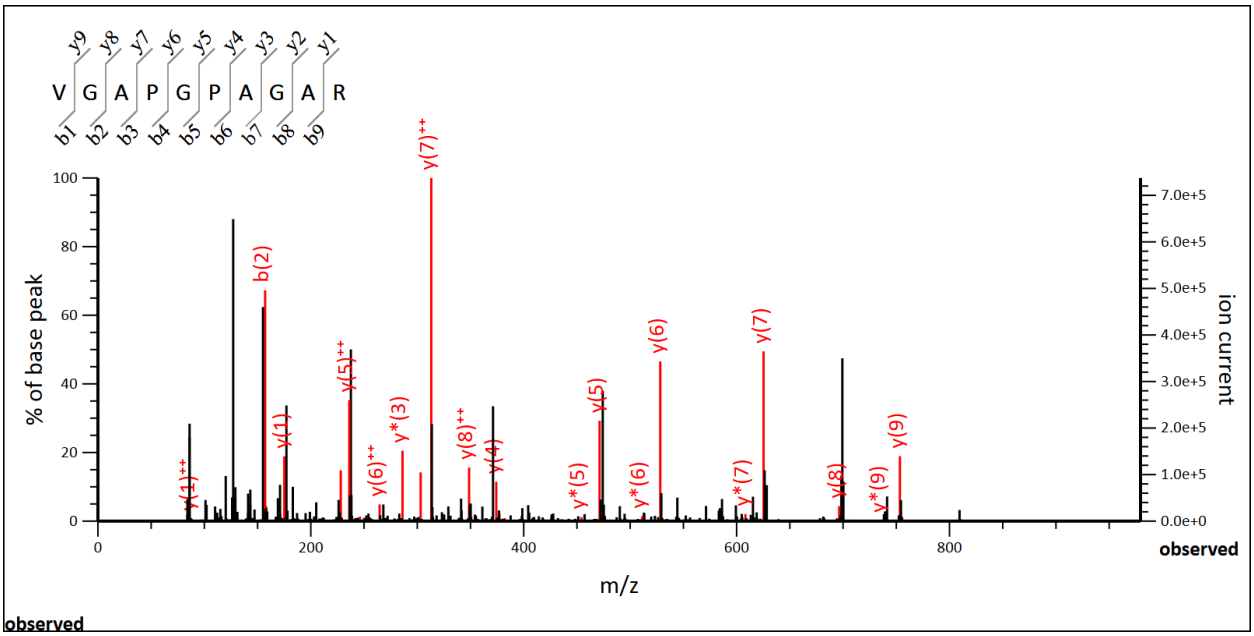


Figure S4.9. MS/MS Fragmentation of peptide VGAPGPAGAR.
Match to Query 609: 851.460788, from (426.737670, 2+), intensity (3472103.2500), rt in seconds (2618), scans (11240), index (4563)
Monoisotopic mass of neutral peptide Mr(calc): 851.4613
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 32
Matches : 14/54 fragment ions using 52 most intense peaks

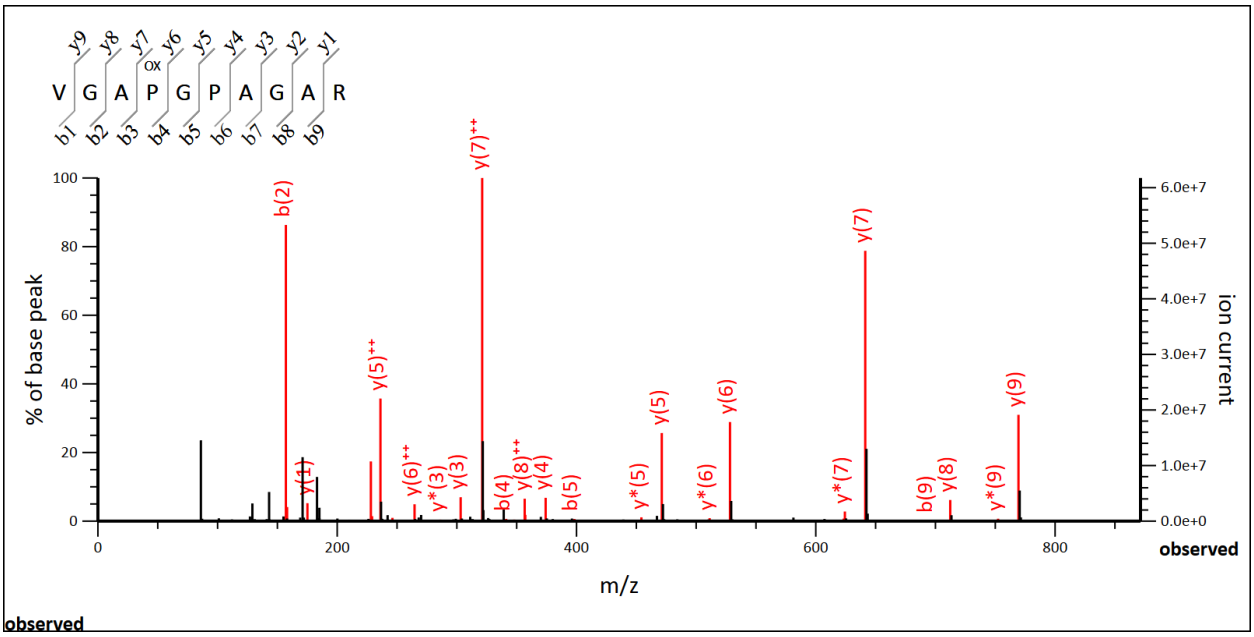


Figure S4.10. MS/MS Fragmentation of peptide VGAPGPAGAR.
Match to Query 805: 867.454448, from (434.734500, 2+), intensity (381743040.0000), rt in seconds (2051), scans (8323), index (2194)
Monoisotopic mass of neutral peptide Mr(calc): 867.4563
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
P4 : Oxidation (P)
Ions Score: 54
Matches : 25/54 fragment ions using 60 most intense peaks

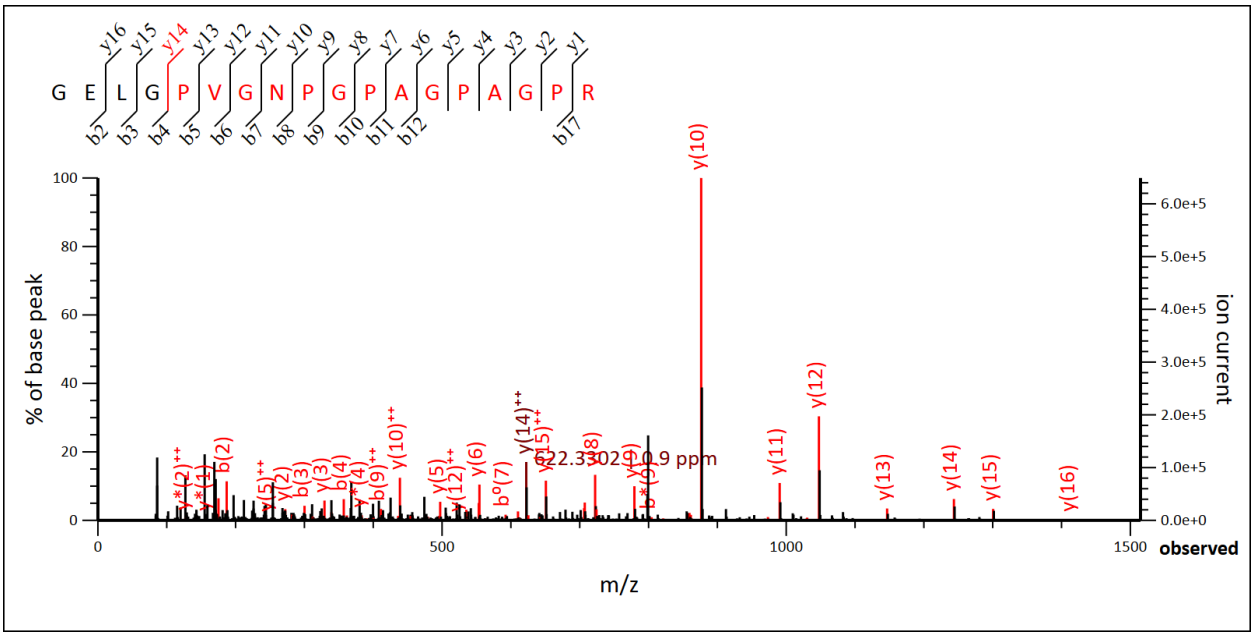


Figure S4.11. MS/MS Fragmentation of peptide GELGPVGNPGPAGPAGPR.
Match to Query 8447: 1598.816868, from (800.415710, 2+), intensity (1912708.8750), rt in seconds (4728), scans (21954), index (13746)
Monoisotopic mass of neutral peptide Mr(calc): 1598.8165
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 62
Matches : 11/156 fragment ions using 14 most intense peaks

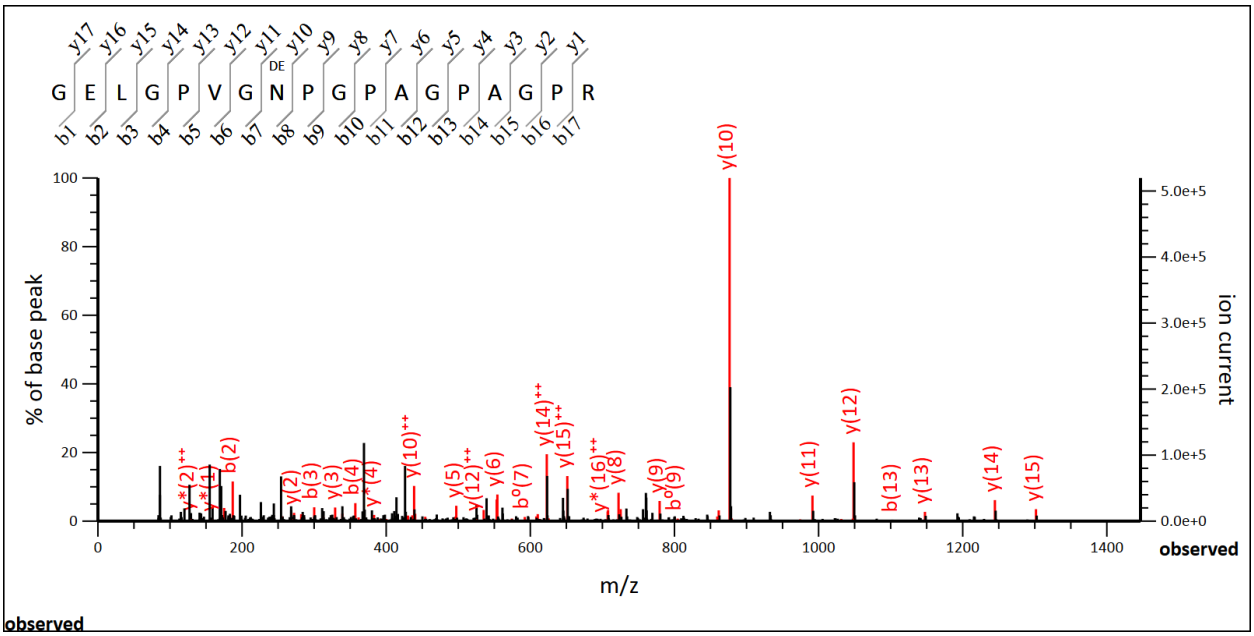


Figure S4.12. MS/MS Fragmentation of peptide GELGPVGNPGPAGPAGPR.
Match to Query 9268: 1599.802228, from (800.908390, 2+), intensity (1478661.2500), rt in seconds (4874), scans (22591), index (14582)
Monoisotopic mass of neutral peptide Mr(calc): 1599.8005
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
N8 : Deamidated (NQ)
Ions Score: 43
Matches : 8/156 fragment ions using 13 most intense peaks

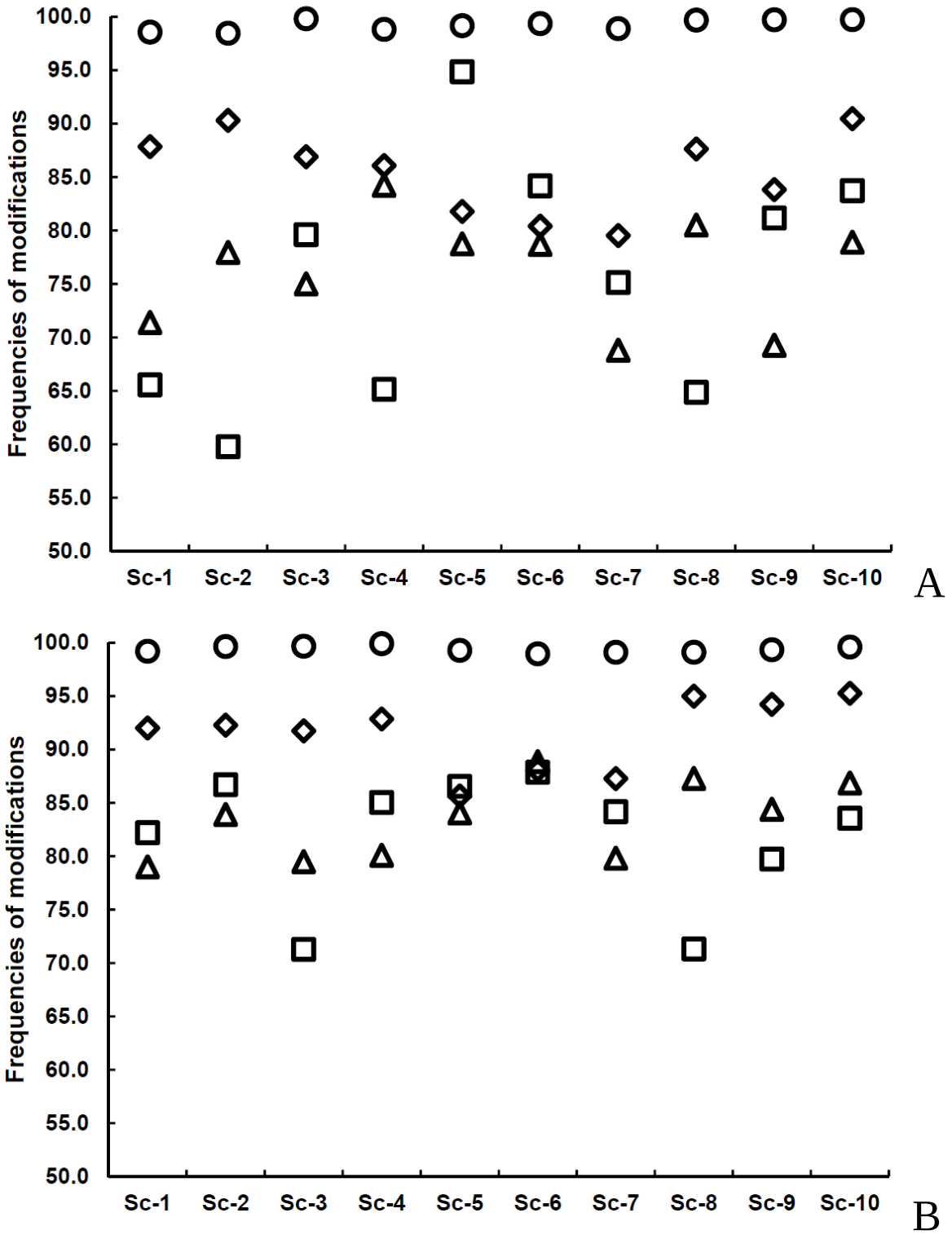
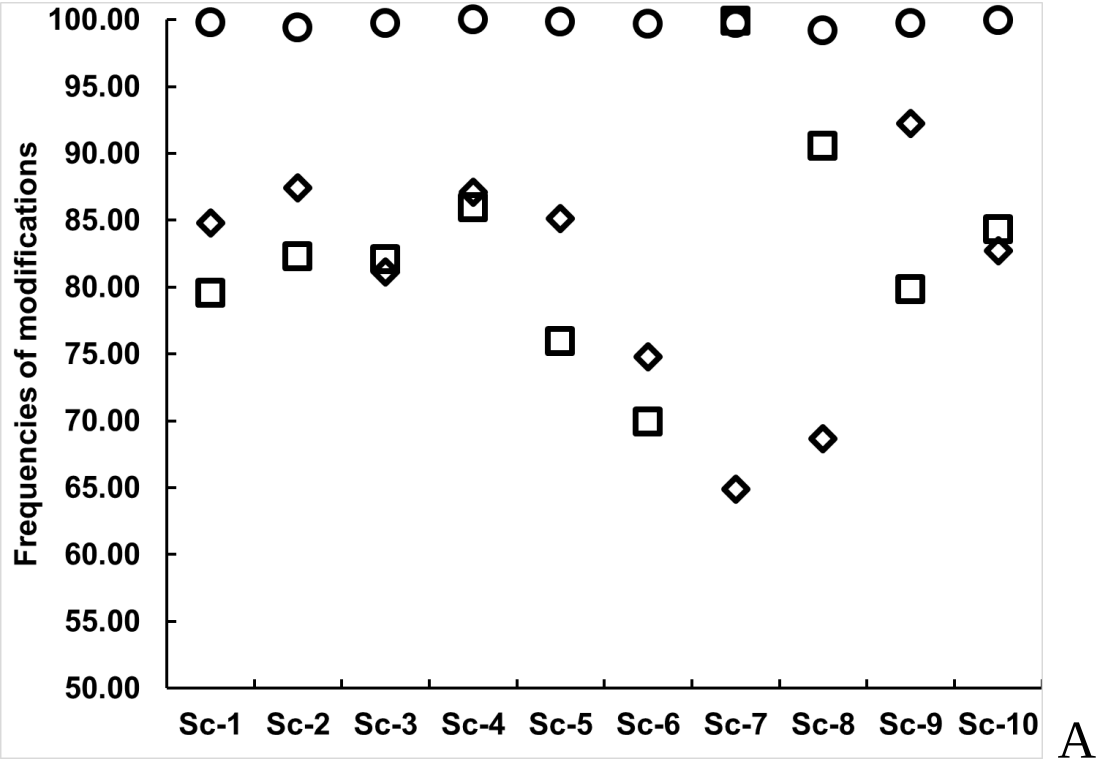


Figure S4.13. Deamidation and oxidation frequencies for peptides form COL1A1 for bone powder fraction (A) and the demineralization residual fraction (B). Circles: oxidation on proline (GQAGVMGFPGPK); squares: glutamine deamidation (GQAGVMGFPGPK); triangle glutamine deamidation (GQAGVMGFPGPK); diamonds asparagine deamidation (GANGAPGIAGAPGFPGAR).



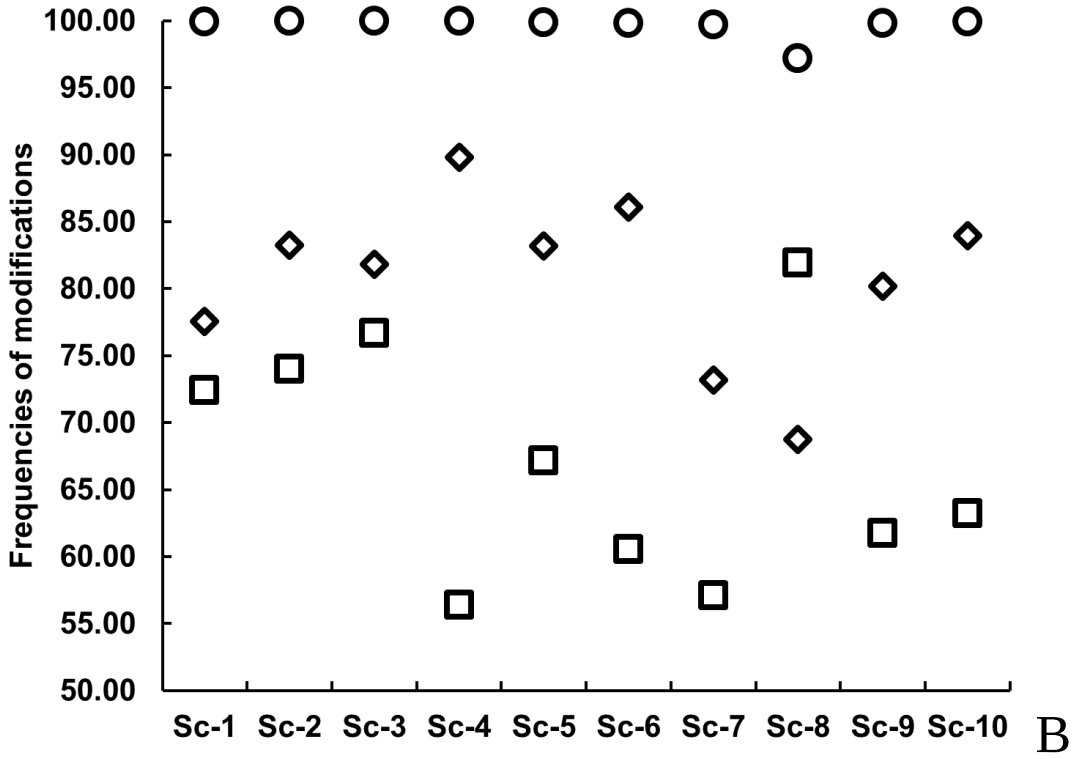


Figure S4.14. Deamidation and oxidation frequencies for peptides form COL1A2 for bone powder fraction (A) and the demineralization residual fraction (B). Circles: oxidation on proline (VGAPGPAGAR); squares: glutamine deamidation (GVVGPQGAR); diamonds asparagine deamidation (GELGPVGNPGPAGPAGPR).