Supplementary Material

Fig. S1A No Calcium and Phosphates (MS reflector mode)

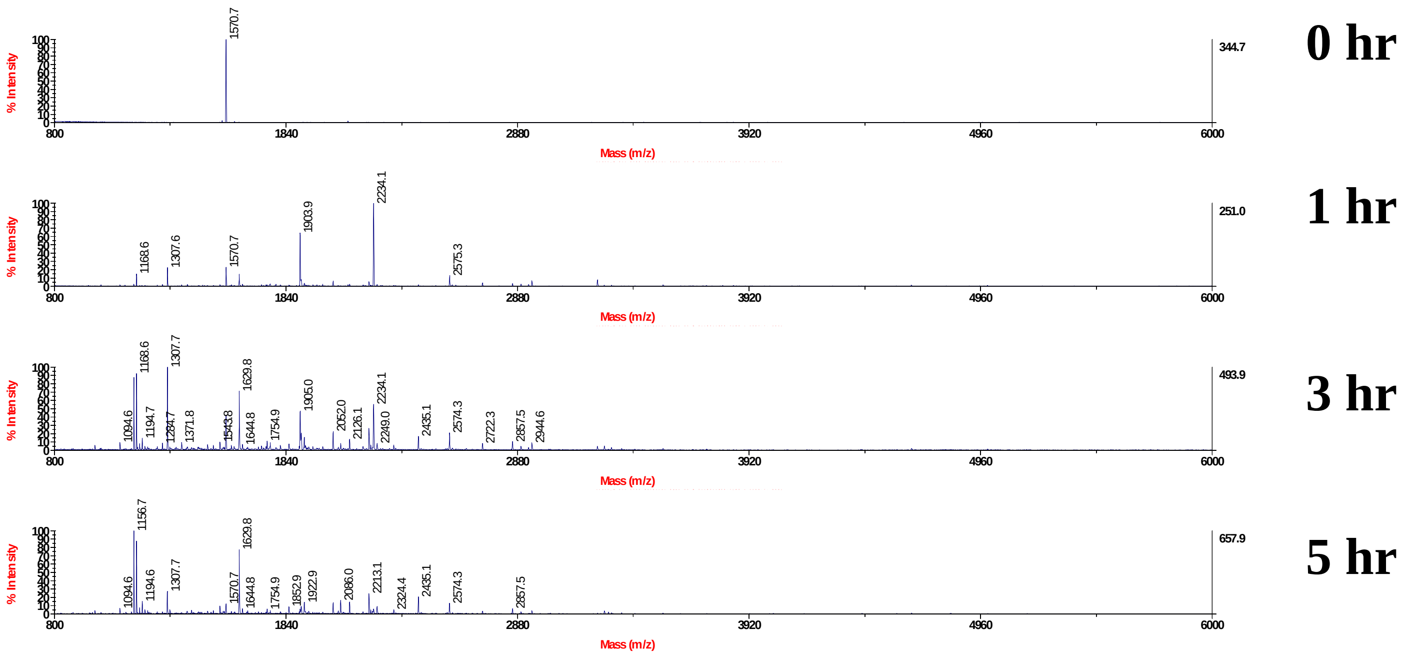


Fig. S1B. Low Calcium and phosphates PO4, (MS reflector mode)

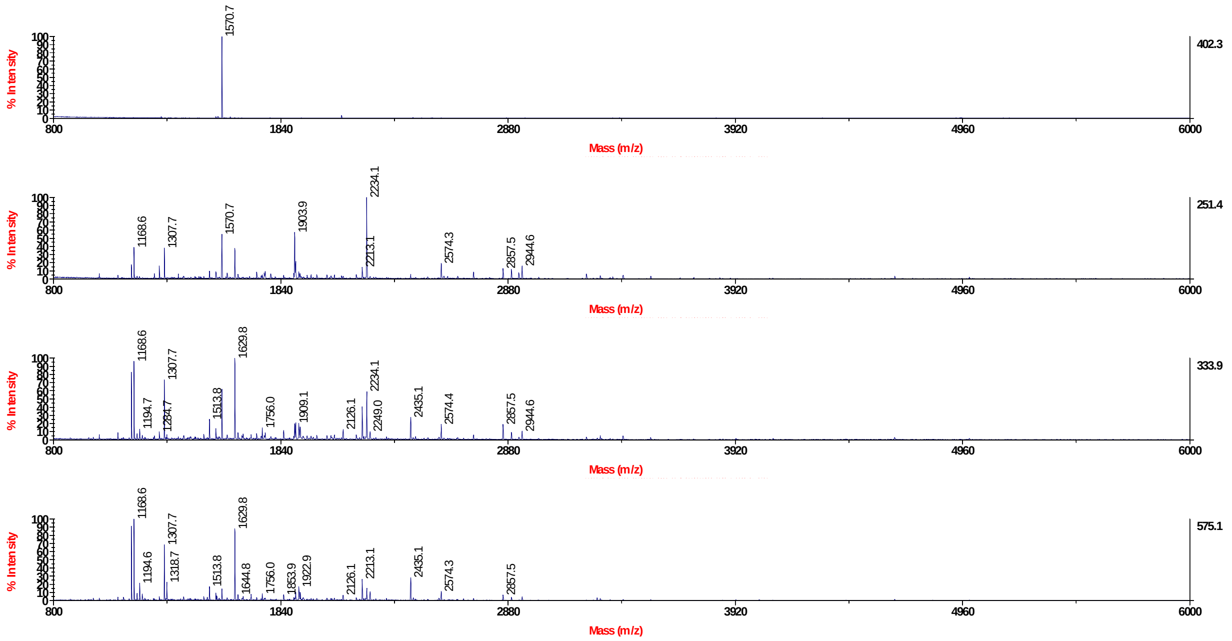


Fig. S1C. High Calcium and phosphates, MS reflector mode

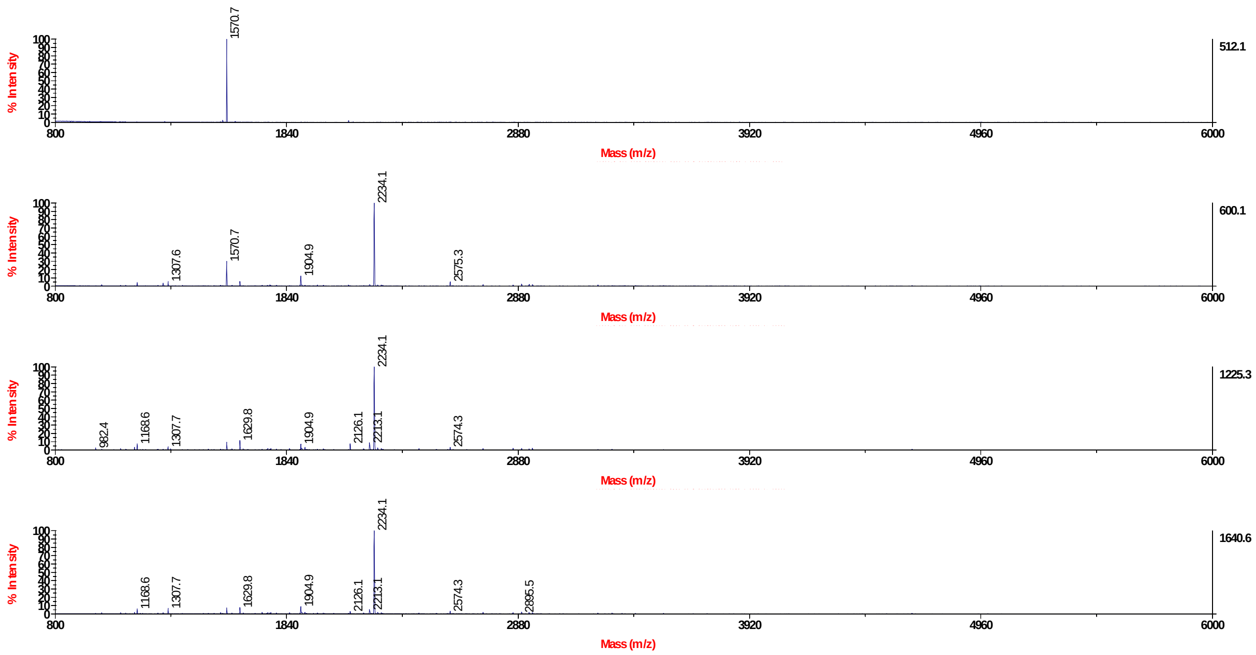


Fig. S1D High Calcium , MS reflector mode

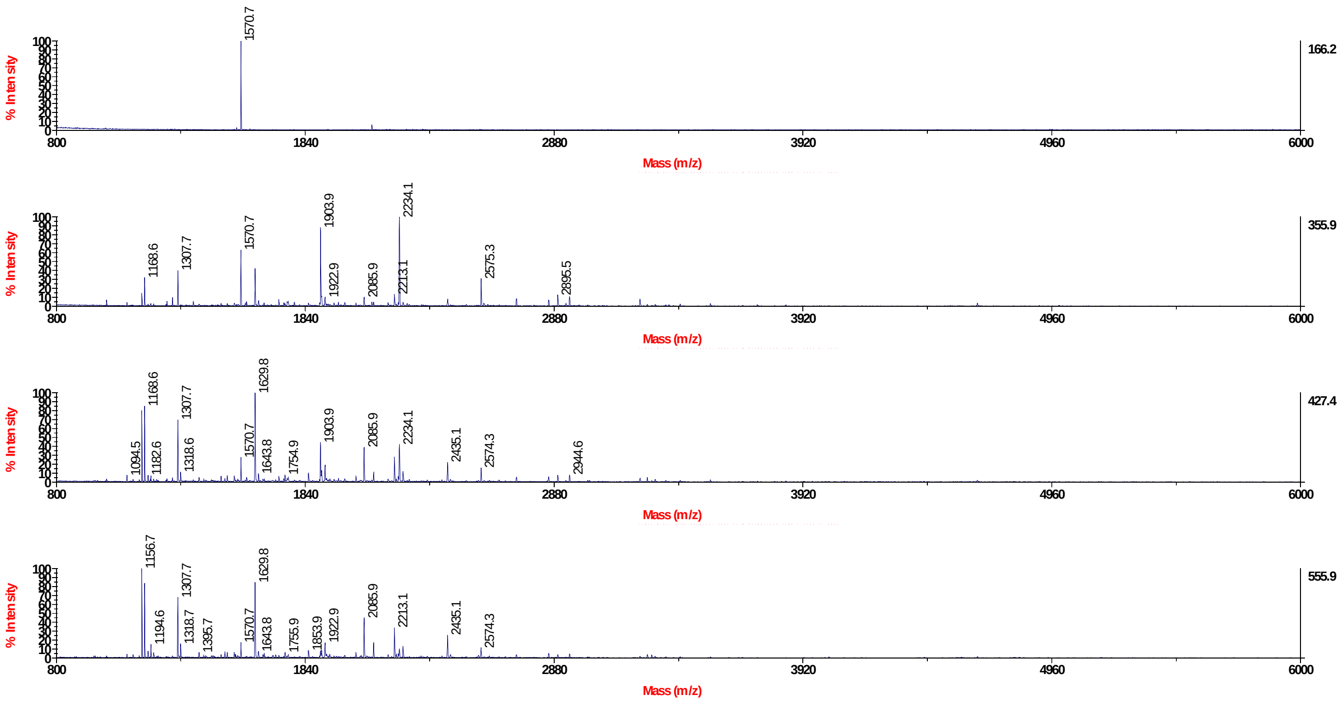


Fig. S1E. High phosphates, MS reflector mode

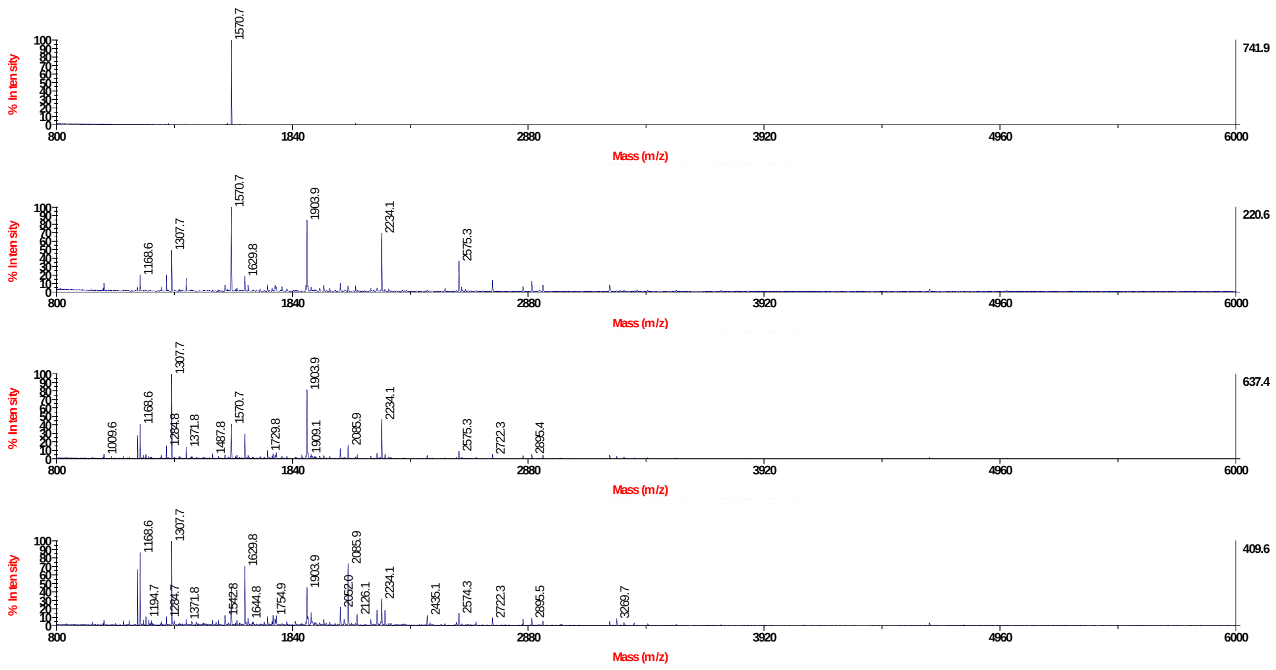


Fig. S1F No Calcium and Phosphates MS linear mode

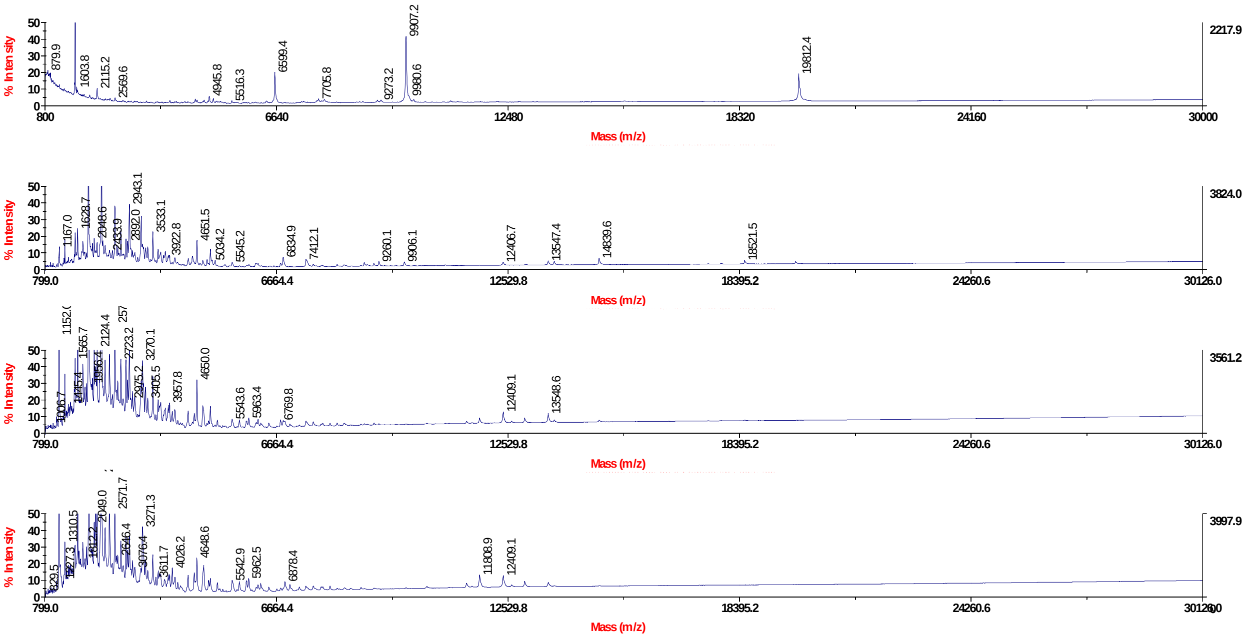
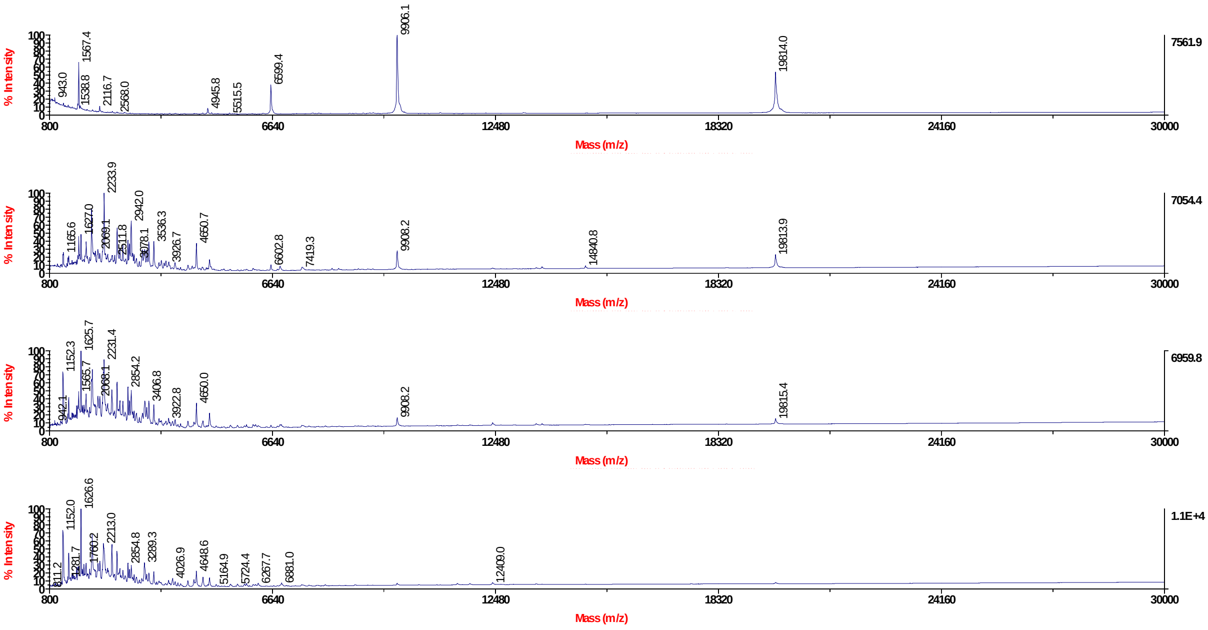


Fig. S1G. Low Calcium and phosphates PO4, MS linear mode



* Note that y-axis in Fig. S1F is shown up to 50% (not 100%), which gives better view of the low abundance peaks.

Fig. S1H. High Calcium and phosphates,

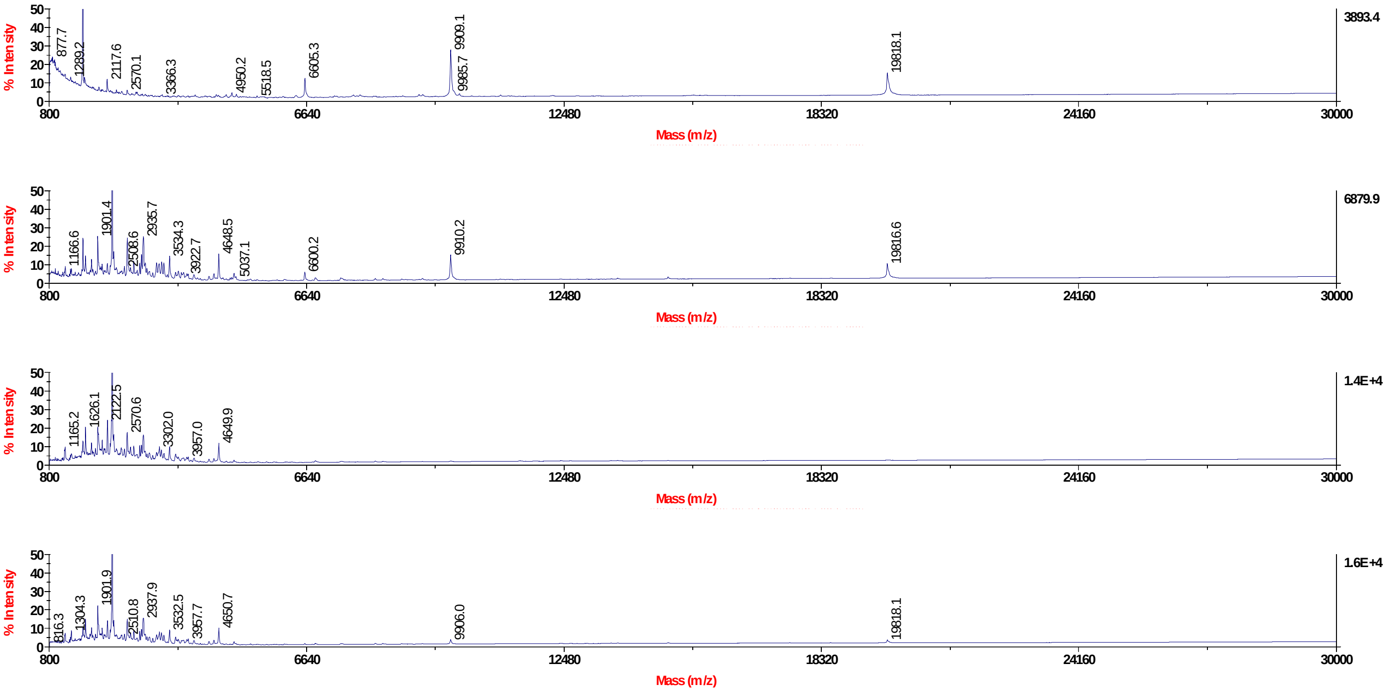


Fig. S1I High Calcium , MS linear mode

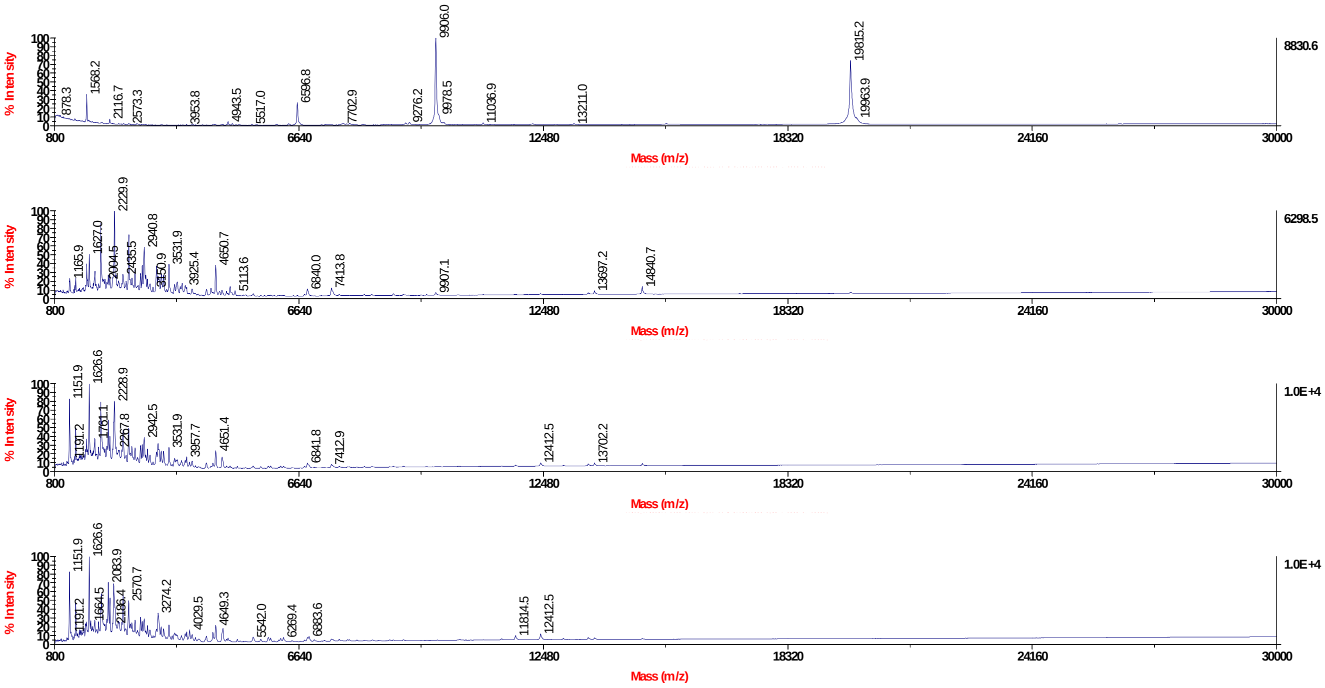


Fig. S1J. High phosphates, MS linear mode

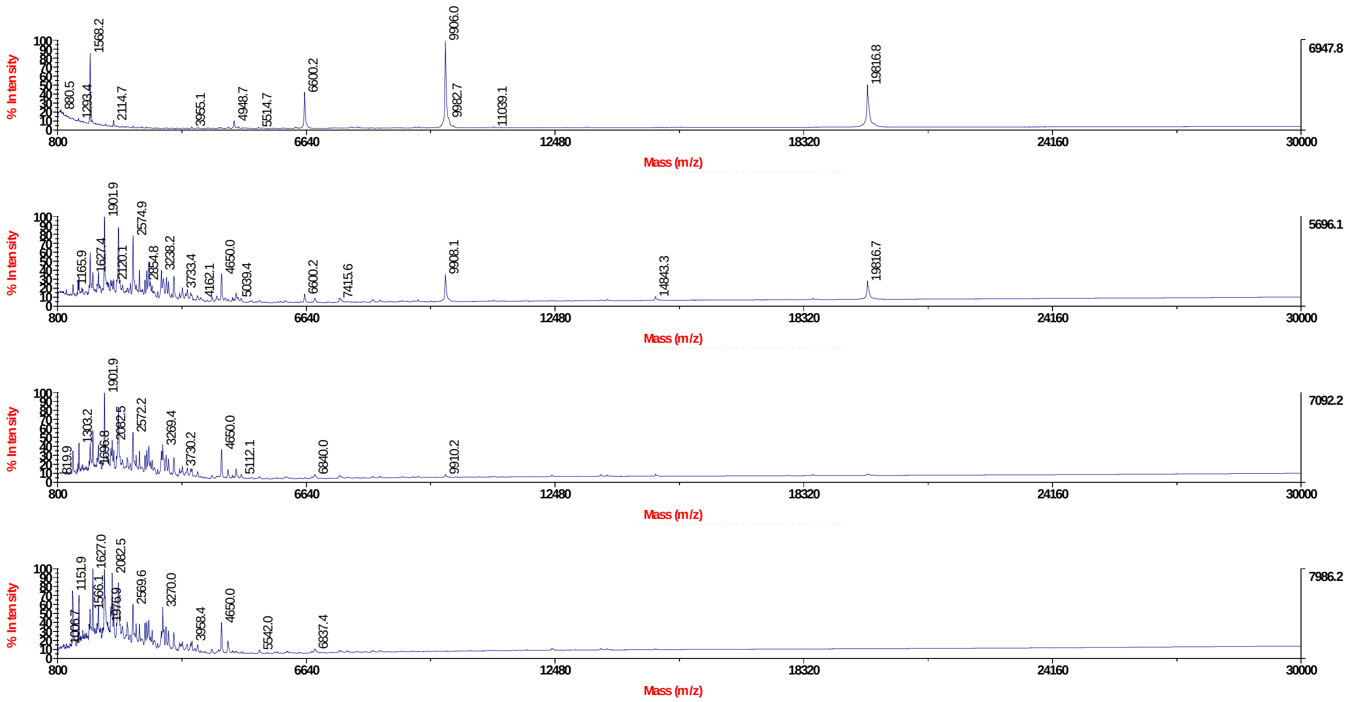
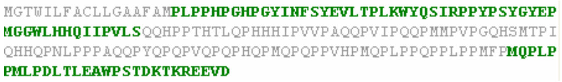


Fig. S1. Mass spectra of proteolysed amelogenin by MMP-20. (A-E) Reflector mode, (F-J) Linear Mode.

Amelogenin (50.5 µM) were incubated with 0.16 µM of MMP-20 at 37 °C in a reaction buffer consists of 10 mM Tris buffer, 50 mM KCl, 1 µM $ZnCl_2$, 60 µM $CaCl_2$ at pH 7.4 with following composition of mineral ions− (A,F) No Calcium and Phosphates− no extra $CaCl_2$ or KH_2PO_4; (B, G) Low Calcium and Phosphates− 3.34 mM $CaCl_2$ and 2.29 mM KH_2PO_4; (C, H) High Calcium and Phosphates− 33.4 mM $CaCl_2$ and 22.9 mM KH_2PO_4; (D, I) High Calcium− 33.4 mM $CaCl_2$; (E) High Phosphates− 22.9 mM KH2PO4. Each Figure has four panels which stand for amelogenin digests collected at 0, 1, 3, 5 h. The peak at m/z 1570.7 Da in all spectra corresponds to [Glu^1]-fibronopeptide B (m/z = 1570.68 Da), which was spiked in MALDI matrix for internal mass calibration.

Conditions	Protein score	Amelogenin peptides identified	Modifications	Conf.
No calcium	24	DLTLEAWPSTDKTKREEVD		99
No phosphate		LEAWPSTDKTKREEVD		99
1 hour		LHHQIIPVLS		99
		LTLEAWPSTDKTKREEVD		99
		MLPDLTLEAWPSTDKTKREEVD		99
		MQPLPPMLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		WYQSIRPPYPSYGYEPMGGW		99
No calcium	14	DLTLEAWPSTDKTKREEVD		99
No phosphate		LEAWPSTDKTKREEVD		99
3 hour		LHHQIIPVLS		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
No calcium	52.42	DLTLEAWPSTDKTKREEVD		99
No phosphate		HHQPNLPPPAQ		99
5 hour		HHQPNLPPPAQQP		99
		HHQPNLPPPAQQPYQPQP		99
		INFSYEVLTPLK		99
		IQHHQPNLPPPAQQP		99
		IQHHQPNLPPPAQQPYQPQP		99
		LEAWPSTDKTKREEVD	Oxidation(W)@4	99
		LHHQIIPVLS		99
		LHHQIIPVLSQ		99
		MPLPPHPGHPGY		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QSIRPPYPSYGYEPMGGW	Gln->pyro-Glu@N-term	99
		SYGYEPMGGW		99
		VQPQPHQPMQPQPPVHP		99
		WYQSIRPPYP		99
		WYQSIRPPYPSYGYEPMGGW		99
		YEVLTPLK		99
		YQSIRPPYP		99
		YQSIRPPYPSYGYEPMGGW		99
		QQHPPTHTLQP		99
		PPMLPDLT		93.1

Sequence coverage



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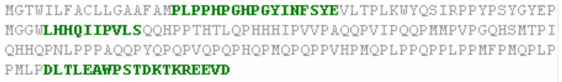
PMLPDLTLEAWPSTDKTKREEVD



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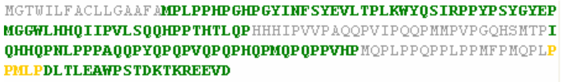
MGGW**LHHQIIPVLS**QQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP
PMLP**DLTLEAWPSTDKTKREEVD**



MGTWILFACLLGAAFA**MPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP**
MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTP**I**
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPL**P**
PMLPDLTLEAWPSTDKTKREEVD

Table S1. Amelogenin peptides identified from “No Calcium and No Phosphate” samples. All peptides identified with Conf<99 have been manually verified by inspecting their MS/MS spectra. In sequence coverage pictures, the sequences in green are associated with high confident (Conf.=99) peptides identified, while those in yellow or red are associated with low confident peptides identified (not shown in table).

Conditions	Protein score	Amelogenin peptides identified	Modifications	Conf.
Low calcium	10	DLTLEAWPSTDKTKREEVD		99
Low phosphate		LEAWPSTDKTKREEVD		99
1 hour		LHHQIIPVLS		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYINFS		99
Low calcium	22.25	DLTLEAWPSTDKTKREEVD		99
Low phosphate		LEAWPSTDKTKREEVD		99
3 hour		LHHQIIPVLS		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		WYQSIRPPYP		99
		YEVLTPLK		99
		YQSIRPPYP		99
		LHHQIIP		67.2
Low calcium	30.43	DLTLEAWPSTDKTKREEVD		99
Low phosphate		HHQPNLPPPAQQP		99
5 hour		INFSYEVLTPLK		99
		IQHHQPNLPPPAQQP		99
		LEAWPSTDKTKREEVD		99
		LHHQIIPVLS		99
		LHHQIIPVLSQ		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
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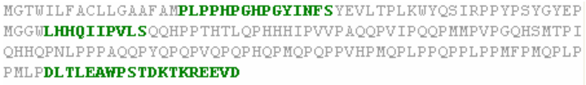
		YQSIRPPYP		99
		PPMLPDLT		82.1

Sequence coverage



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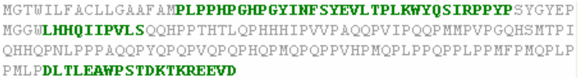
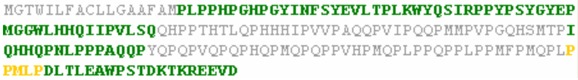
MGGW**LHHQIIPVLS**QQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP
PMLP**DLTLEAWPSTDKTKREEVD**



MGTWILFACLLGAAFAM**PLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYP**SYGYEP
MGGW**LHHQIIPVLS**QQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP
PMLP**DLTLEAWPSTDKTKREEVD**



MGTWILFACLLGAAFAM**PLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP**
MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTP**I**
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPL**P**
PMLPDLTLEAWPSTDKTKREEVD

Table S2. Amelogenin peptides identified from “Low Calcium and Low Phosphate” samples. All peptides identified with Conf.<99 have been manually verified by inspecting their MS/MS spectra. In sequence coverage pictures, the sequences in green are associated with high confident (Conf.=99) peptides identified, while those in yellow or red are associated with low confident peptides identified (not shown in table).

Conditions	Protein score	Amelogenin peptides identified	Modifications	Conf.
High calcium	34	DLTLEAWPSTDKTKREEVD		99
1 hour		LEAWPSTDKTKREEVD		99
		LHHQIIPVLS		99
		LTLEAWPSTDKTKREEVD		99
		MLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QSIRPPYPSYGYEPMGGW	Gln->pyro-Glu@N-term	99
		TLEAWPSTDKTKREEVD		99
		WYQSIRPPYP		99
		WYQSIRPPYPSYGYEPMGGW		99
		YEVLTPLK		99
High calcium	99.97	AWPSTDKTKREEVD		99
3 hour		DLTLEAWPSTDKTKREEVD	Oxidation(W)@7	99
		FSYEVLTPLK		99
		HHQPNLPPPAQQP		99
		HHQPNLPPPAQQPYQPQP		99
		HSMTPIQHHQPNLPPPAQQP		99
		HSMTPIQHHQPNLPPPAQQPYQPQP		99
		INFSYEVLTPLK		99
		IQHHQPNLPPPAQ		99
		IQHHQPNLPPPAQQP		99
		IQHHQPNLPPPAQQPYQPQP		99
		LEAWPSTDKTKREEVD	Oxidation(W)@4	99
		LHHQIIPVLS		99
		LHHQIIPVLSQ		99
		LHHQIIPVLSQQHPPTHT		99
		LHHQIIPVLSQQHPPTHTLQP		99
		LHHQIIPVLSQQHPPTHTLQPHH		99
		LKWYQSIRPPYP		99
		LTLEAWPSTDKTKREEVD		99
		MLPDLTLEAWPSTDKTKREEVD		99
		MMPVPGQHSMTPIQ		99
		MPLPPHPGHPGY		99
		MPLPPHPGHPGYINFS		99
		MQPLPPMLPDLT		99
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		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		PPHPGHPGY		99
		QQHPPTHTLQPHHHIPVVPAQQPVIPQQP		99
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		TLEAWPSTDKTKREEVD		99
		VLTPLKWY		99
		VQPQPHQPMQPQPPVHP		99
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		YEVLTPLK		99
		YEVLTPLKWY		99
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		YQSIRPPYPSYGYEPMGGW		99
		PPMLPDLT		96.5
		YQSIRPPYP		96.2
		YEVLTPLKW		95.9
		IRPPYPSYGYEPMGGW		93.6
High calcium	56.11	DLTLEAWPSTDKTKREEVD		99
5 hour		HHQPNLPPPAQQP		99
		HHQPNLPPPAQQPYQPQP		99
		INFSYEVLTPLK		99
		IQHHQPNLPPPAQQP		99
		IQHHQPNLPPPAQQPYQPQP		99
		LEAWPSTDKTKREEVD	Oxidation(W)@4	99
		LHHQIIPVLS		99
		LHHQIIPVLSQ		99
		LHHQIIPVLSQQHPPTHT		99
		LKWYQSIRPPYP		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
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		WYQSIRPPYP		99
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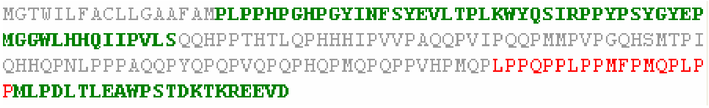
Sequence coverage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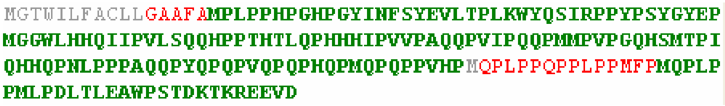
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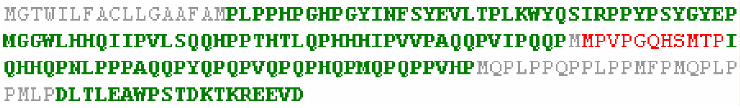
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PMLPDLTLEAWPSTDKTKREEVD

Table S3. Amelogenin peptides identified from “High Calcium” samples. All peptides identified with Conf.<99 have been manually verified by inspecting their MS/MS spectra. In sequence coverage pictures, the sequences in green are associated with high confident (Conf.=99) peptides identified, while those in yellow or red are associated with low confident peptides identified (not shown in table).

Conditions	Protein score	Amelogenin peptides identified	Modifications	Conf.
High phosphate	16	DLTLEAWPSTDKTKREEVD		99
1 hour		LEAWPSTDKTKREEVD		99
		LHHQIIPVLS		99
		LTLEAWPSTDKTKREEVD		99
		MLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
High phosphate	42.18	DLTLEAWPSTDKTKREEVD		99
3 hour		INFSYEVLTPLK		99
		LEAWPSTDKTKREEVD		99
		LHHQIIPVLS		99
		LTLEAWPSTDKTKREEVD		99
		MQPLPPMLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QSIRPPYPSYGYEPMGGW	Gln->pyro-Glu@N-term	99
		SYGYEPMGGW		99
		TLEAWPSTDKTKREEVD		99
		WYQSIRPPYP		99
		YEVLTPLKWY		99
		YQSIRPPYP		99
		YEVLTPLK		99
		QSIRPPYP		83.5
High phosphate	34.32	DLTLEAWPSTDKTKREEVD		99
5 hour		INFSYEVLTPLK		99
		LEAWPSTDKTKREEVD		99
		LHHQIIPVLS		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
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		QSIRPPYPSYGYEPMGGW		99
		VLTPLKWY		99
		WYQSIRPPYP		99
		YEVLTPLKWY		99
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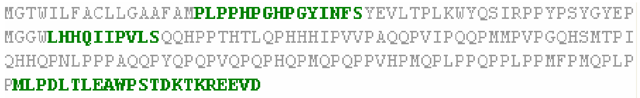
		QSIRPPYP		93

Sequence coverage



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MGGW**LHHQIIPVLS**QQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP
P**MLPDLTLEAWPSTDKTKREEVD**

MGTWILFACLLGAAFAM**PLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP**
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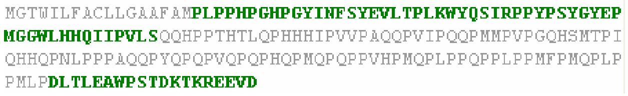

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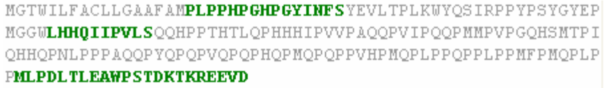
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP
PMLP**DLTLEAWPSTDKTKREEVD**

Table S4. Amelogenin peptides identified from "High Phosphate" samples. All peptides identified with Conf.<99 have been manually verified by inspecting their MS/MS spectra. In sequence coverage pictures, the sequences in green are associated with high confident (Conf.=99) peptides identified, while those in yellow or red are associated with low confident peptides identified (not shown in table).

Conditions	Protein score	Amelogenin peptides identified	Modifications	Conf.
Hig calcium	16	DLTLEAWPSTDKTKREEVD		99
High phosphate		LEAWPSTDKTKREEVD		99
1 hour		LHHQIIPVLS		99
		LTLEAWPSTDKTKREEVD		99
		MLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
Hig calcium	56	AWPSTDKTKREEVD		99
High phosphate		DLTLEAWPSTDKTKREEV		99
3 hour		DLTLEAWPSTDKTKREEVD	Oxidation(W)@7	99
		EAWPSTDKTKREEVD		99
		INFSYEVLTPLK		99
		LHHQIIPVLS		99
		LKWYQSIRPPYP		99
		LTLEAWPSTDKTKREEVD		99
		MQPLPPMLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPG		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QSIRPPYPSYGYEPMGGW		99
		SYGYEPMGGW		99
		TLEAWPSTDKTKREEVD		99
		WPSTDKTKREEVD		99
		WYQSIRPPYP		99
		WYQSIRPPYPSYGYEPMGGW		99
		YEVLTPLK		99
		YQSIRPPYP		99
		YQSIRPPYPSYGYEPMGGW		99
Hig calcium	55.28	AWPSTDKTKREEVD		99
High phosphate		DLTLEAWPSTDKTKREEVD	Oxidation(W)@7	99
5 hour		EAWPSTDKTKREEVD		99
		INFSYEVLTPLK		99
		LEAWPSTDKTKREEVD	Oxidation(W)@4	99
		LHHQIIPVLS		99
		LHHQIIPVLSQ		99
		LKWYQSIRPPYP		99
		LTLEAWPSTDKTKREEVD		99
		MLPDLTLEAWPSTDKTKREEVD		99
		MQPLPPMLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPG		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QQHPPTHTLQPHHHIPVVPAQQPVIPQQP		99
		SYGYEPMGGW		99
		TLEAWPSTDKTKREEVD		99
		VQPQPHQPMQPQPPVHP		99
		WYQSIRPPYP		99
		WYQSIRPPYPSYGYEPMGGW		99
		YEVLTPLK		99
		YQSIRPPYPSYGYEPMGGW		99
		YQSIRPPYP		93.1

Sequence coverage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MGGW**LHHQIIPVLS**QQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
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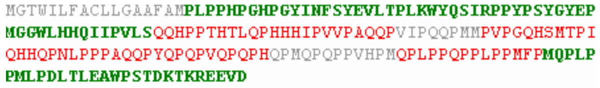
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MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFP**MQPLP**
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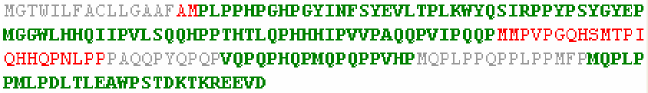
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QHHQPNLPPPAQQPYQPQP**VQPQPHQPMQPQPPVHP**MQPLPPQPPLPPMFP**MQPLP**
PMLPDLTLEAWPSTDKTKREEVD

Table S5. Amelogenin peptides identified from “High Calcium and High Phosphate” samples. All peptides identified with Conf.<99 have been manually verified by inspecting their MS/MS spectra. In sequence coverage pictures, the sequences in green are associated with high confident (Conf.=99) peptides identified, while those in yellow or red are associated with low confident peptides identified (not shown in table).