Supplementary Material





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





Fig. S1C. High Calcium and phosphates, 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. S1. Mass spectra of proteolysed amelogenin by MMP-20. (A-E) Reflector mode, (F-J) Linear Mode.

Amelogenin (50.5 μ M) were incubated with 0.16 μ M of MMP-20 at 37 °C in a reaction buffer consists of 10 mM Tris buffer, 50 mM KCl, 1 μ M ZnCl₂, 60 μ M CaCl₂ at pH 7.4 with following composition of mineral ions– (A,F) No Calcium and Phosphates– no extra CaCl₂ or KH₂PO₄; (B, G) Low Calcium and Phosphates– 3.34 mM CaCl₂ and 2.29 mM KH₂PO₄; (C, H) High Calcium and Phosphates– 33.4 mM CaCl₂ and 22.9 mM KH₂PO₄; (D, I) High Calcium– 33.4 mM CaCl₂; (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 [Glu¹]-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 GIn->pyro-Glu@N-term	99
		SYGYEPMGGW	99
		VQPQPHQPMQPQPPVHP	99
		WYQSIRPPYP	99
		WYQSIRPPYPSYGYEPMGGW	99
		YEVLTPLK	99
		YQSIRPPYP	99
		YQSIRPPYPSYGYEPMGGW	99
		QQHPPTHTLQP	99
		PPMLPDLT	93.1

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHUQIIPVLSQQHPPTHTLQPHHLIPVVPAQQPVIPQQPMNPVPGQHSMTPI QHHQPNLPPPAQQPYQPQQPQQPQPPQPHQPNQPLPPQPPLPPMFPMQPLP PMLPDLTLEAMPSTDKTKREEVD

MGTWILFACLLGAAFAM**PLPPHPGHPGYINFSYE**VLTPLKWYQSIRPPYPSYGYEP MGGW**LHNQIIPYL**SQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMNPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP PMLP**DLTLEAWPSTDKTKREEVD**

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHUQIIEVLSQQHPPTHTLQPHHLPVVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPMLPPAQQPYQPQPQPQPQPQPQPQPQPQPQPVPPMQPLPPQPLPPMPPNQPLP PMLPDLTLEAWPSTDKTKKEEVD

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.	Sequence coverage
Low calcium	10	DLTLEAWPSTDKTKREEVD		99	
Low phosphate		LEAWPSTDKTKREEVD		99	MGTWILFACLLGAAFAMPLPPHPGHPGYINFS YEVLTPLKWYQSIRPPYPSYGYEF
1 hour		LHHQIIPVLS		99	
		PLPPHPGHPGY		99	PMLPDLTLEAWP STDKTKREEVD
		PLPPHPGHPGYINFS		99	
Low calcium	22.25	DLTLEAWPSTDKTKREEVD		99	
Low phosphate		LEAWPSTDKTKREEVD		99	
3 hour		LHHQIIPVLS		99	MGTWILFACLLGAAFAM PLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYP SYGYEP
		PLPPHPGHPGY		99	MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
		PLPPHPGHPGYIN		99	QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP DWLDDITIE8HDCTDUTUDEEUD
		PLPPHPGHPGYINF		99	PHLPDLILEAWPSIDKIKKEEVD
		PLPPHPGHPGYINFS		99	
		PLPPHPGHPGYINFSYE		99	
		WYQSIRPPYP		99	
		YEVLTPLK		99	
		YQSIRPPYP		99	
		LHHQIIP		67.2	
Low calcium	30.43	DLTLEAWPSTDKTKREEVD		99	MGTWILFACLLGAAFAM PLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP
Low phosphate		HHQPNLPPPAQQP		99	MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI
5 hour		INFSYEVLTPLK		99	QHRQPALFFFAQQF IQFQFYQFYQFYQFYYAFAQFLFFQFFLFFAFFAQFL PMI PDI TI FAWPSTDKTKRFFYD
		IQHHQPNLPPPAQQP		99	
		LEAWPSTDKTKREEVD		99	
		LHHQIIPVLS		99	
		LHHQIIPVLSQ		99	
		PLPPHPGHPGY		99	
		PLPPHPGHPGYIN		99	
		PLPPHPGHPGYINF		99	
		PLPPHPGHPGYINFS		99	
		PLPPHPGHPGYINFSYE		99	
		SYGYEPMGGW		99	
		WYQSIRPPYP		99	
		YQSIRPPYP		99	
		PPMLPDLT		82.1	

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	Protoin	Amelogenin pentidos identificat	Andifications	Conf
High colours	Protein score	Amerogenin peptides identified N	NOUITICATIONS	cont.
nign calcium	54			99
11001		IHHOIPVIS		99
		LTLEAWPSTDKTKREEVD		99
	1	MLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QSIRPPYPSYGYEPMGGW G	GIn->pyro-Glu@N-term	99
		TLEAWPSTDKTKREEVD		99
		WYQSIRPPYP		99
		VEVITELK		99
High calcium	99.97			99
3 hour	55.57	DITIFAWPSTDKTKRFFVD	xidation(W)@7	99
Siloui		FSYEVLTPLK	xidation(W/e/	99
		HHQPNLPPPAQQP		99
		HHQPNLPPPAQQPYQPQP		99
		HSMTPIQHHQPNLPPPAQQP		99
		HSMTPIQHHQPNLPPPAQQPYQPQP		99
		INFSYEVLTPLK		99
		IQHHQPNLPPPAQ		99
		IQHHQPNLPPPAQQP		99
	-	IQHHQPNLPPPAQQPYQPQP		99
	1	LEAWPSTDKTKREEVD C	Dxidation(W)@4	99
	-	LHHQIIPVLS		99
				99
				99
	-			99
				99
				99
		MIPDITIFAWPSTDKTKRFFVD		99
		MMPVPGQHSMTPIQ		99
		MPLPPHPGHPGY		99
		MPLPPHPGHPGYINFS		99
		MQPLPPMLPDLT		99
		MQPLPPMLPDLTLEAWPSTDKTKREEVD		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTP		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		PPHPGHPGY		99
		QQHPPTHTLQPHHHIPVVPAQQPVIPQQP		99
		QSIRPPIPSIGIEPINGGW		99
				99
				99
				99
				00
		WYOSIRPPYP		99
		WYQSIRPPYPSYG		99
		WYQSIRPPYPSYGYEPMGGW		99
		YEVLTPLK		99
		YEVLTPLKWY		99
		YQPQPVQPQPHQPMQPQPPVHP		99
		YQSIRPPYPSYGYEPMGGW		99
		PPMLPDLT		96.5
		YQSIRPPYP		96.2
		YEVLTPLKW		95.9
		IRPPYPSYGYEPMGGW		93.6
High calcium	56.11	DLILEAWPSTDKTKREEVD		99
5 nour	-	HHQPNLPPPAQQP		99
	_	HHQPNLPPPAQQPYQPQP		99
				99
	-			99
			Dividation(W)@4	90
			xidation(w)@4	99
		I HHOUPVISO		99
		LHHOIIPVLSOOHPPTHT		99
		LKWYQSIRPPYP		99
		PLPPHPGHPGY		99
		PLPPHPGHPGYIN		99
		PLPPHPGHPGYINF		99
		PLPPHPGHPGYINFS		99
		PLPPHPGHPGYINFSYE		99
		PLPPHPGHPGYINFSYEVLTPLK		99
		QQHPPTHTLQP		99
		QQHPPTHTLQPHH		99
		QQHPPTHTLQPHHHIPVVPAQQPVIPQQP		99
		QSIRPPYPSYGYEPMGGW		99
		SYGYEPMGGW		99
		VQPQPHQPMQPQPPVHP		99
		WYQSIRPPYP		99
		YEVLTPLK		99
		YEVLTPLKWY		99
		YQSIRPPYPSYGYEPMGGW		99
	1	YQSIRPPYP		960

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP FMLPDLTLEAWPSTDKTKREEVD

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPMFPMQPLP 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).

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP PMLPDLTLEAWPSTDKTKREEVD

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 GIn->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
		PLPPHPGHPGYINFSYEVLTPLK	99
		QSIRPPYPSYGYEPMGGW	99
		VLTPLKWY	99
		WYQSIRPPYP	99
		YEVLTPLKWY	99
		YQSIRPPYP	99
		YQSIRPPYPSYGYEPMGGW	99
		YEVLTPLK	99
		QSIRPPYP	93

MGTWILFACLLGAAFAM**PLPPHPGHPGYINFS**YEVLTPLKWYQSIRPPYPSYGYEP MGGW**LHHQIIPVLS**QQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI 2HHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP P**MLPDLTLEAWPSTDKTKREEVD**

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPVQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFFMQPLP PMLPDLTLEAWPSTDKTKREEVD

MGTWILFACLLGAAFAM**PLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP** MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP PMLPDLTLEAWPSTDKTKREEVD

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
		FAWPSTDKTKRFFVD		99
		INFSYEVLTPLK		99
		LHHOIIPVLS		99
		LKWYOSIRPPYP		99
				99
				99
		PIPPHPGHPG		99
				99
				99
				99
				99
				00
				99
				99
		QSIRPPTPSTGTEPIVIGGVV		99
				99
				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

MGTWILFACLLGAAFAM**PLPPHPGHPGYINFS**YEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP PMLPDLTLEAWPSTDKTKREEVD

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPI QHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLP PMLPDLTLEAWPSTDKTKREEVD

MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYEVLTPLKWYQSIRPPYPSYGYEP MGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMPVPGQH3MTPI QHHQPNLPPPAQQPYQPQPVQPQPQPQPQPQPPQPPHPMQPLPPQPPLPPMFFMQPLP 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).