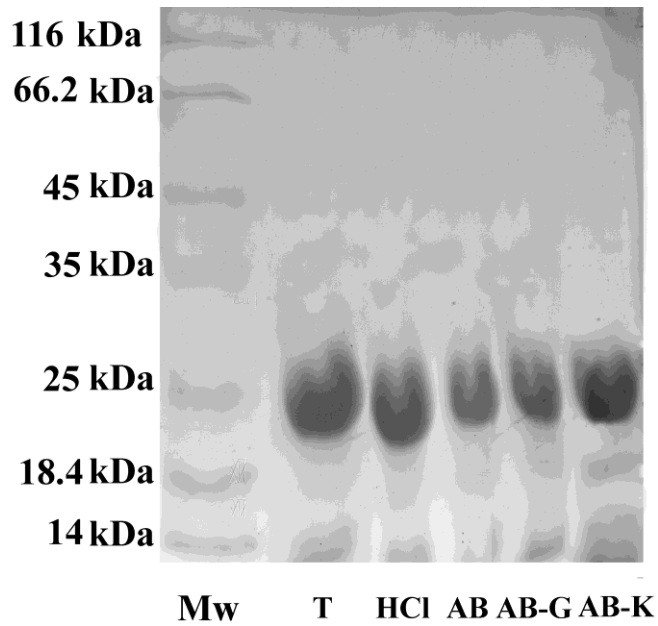


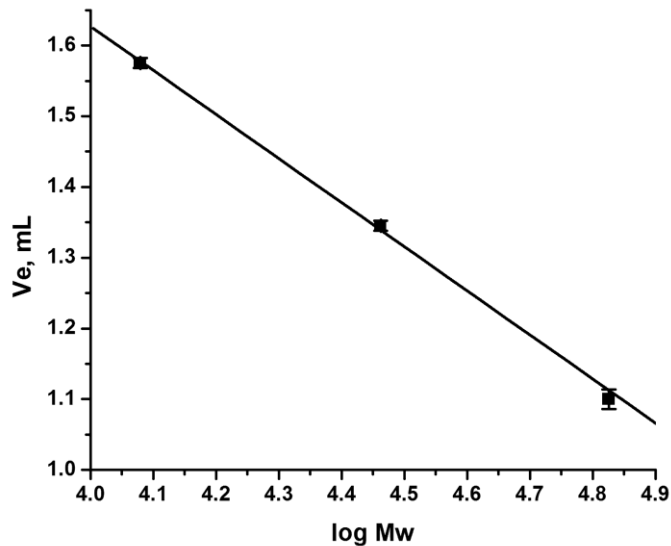
Supplementary material for the article:

Rašković, B.; Vatić, S.; Anđelković, B.; Blagojević, V.; Polović, N. Optimizing Storage Conditions to Prevent Cold Denaturation of Trypsin for Sequencing and to Prolong Its Shelf Life. *Biochemical Engineering Journal* **2016**, *105*, 168–176.

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Supplementary Figure S1. SDS PAGE analysis of the untreated trypsin (T) and trypsin samples after 7 freeze-thaw cycles. A) Trypsin dissolved in 1 mM HCl and B) Trypsin dissolved in 100 mM ammonium-bicarbonate (AB) with 0.3 M glycerol (AB-G) and 0.1 M lysine (AB-K).



Supplementary Figure S2. Calibration plot used to determine molecular weight of trypsin in gel filtration analysis. Bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome c (12.4 kDa) were used as molecular weight markers.

Table S1. BSA-specific peptides identified in the samples digested with differently treated trypsin (Untreated, HCl, AB-G and AB-K).

Trypsin sample	Start-end	Peptide
HCl; AB-K	29-36	SEIAHRFK
AB-K	66-88	LVNELTEFAKTCVADESHAGCEK
Untreated; HCl	76-88	TCVADESHAGCEK
AB-K	89-100	SLHTLFGDELCK
Untreated; AB-G; AB-K	101–105	VASLR
Untreated; HCl; AB-K	106-117	ETYGDMADCCEK
Untreated	118-122	QEPER
Untreated; HCl; AB-G; AB-K	131–138	DDSPDLPK
Untreated	139–156	LKPDPNTLCDEFKADEKK
AB-G; AB-K	152-160	ADEKKFWGK
Untreated; AB-G; AB-K	157–160	FWGK
Untreated	161–167	YLYEIAR
Untreated; AB-G; AB-K	198–204	GACLLPK
AB-G	219-222	QRLR
Untreated; AB-G; AB-K	223–228	CASIQK
Untreated; AB-G; AB-K	229–232	FGER
Untreated; AB-G	236–241	AWSVAR
AB-G	242–245	LSQK
Untreated; AB-G; AB-K	246-248	FPK

AB-G; AB-K	246-266	FPKAEFVEVTKLVTDLTKVHK
Untreated; HCl; AB-G;	249-256	AEFVEVTK
AB-K		
Untreated; AB-G; AB-K	257-263	LVTDLTK
Untreated; AB-K	264-266	VHK
Untreated	264-280	VHKECCHGDLLECADDR
AB-G	281-285	ADLAK
AB-G	281-297	ADLAKYICDNQDTISSK
HCl; AB-K	286-297	YICDNQDTISSK
HCl; AB-G; AB-K	286-318	YICDNQDTISSKLECCDKPLLEKSHCIAEVEK
HCl	310-318	SHCIAEVEK
HCl; AB-G; AB-K	310-340	SHCIAEVEKDAIPENLPPLTADFAEDKDVCK
Untreated; AB-G; AB-K	341-346	NYQEAK
Untreated	361-374	HPEYAVSVLLRLAK
AB-G	372-386	LAKEYEATTLEECCA
HCl; AB-G	375-386	EYEATTLEECCA
Untreated; AB-K	413-436	QNCDQFEKLGEGYGFQNALIVRYTR
AB-G	434-436	YTR
Untreated; HCl; AB-G;	434-451	YTRKVPQVSTPTLVEVSR
AB-K		
Untreated; AB-G; AB-K	452-455	SLGK
Untreated; AB-G	456-459	VGTR
HCl; AB-G; AB-K	469-482	MPCTEDYLSLILNR

HCl	483-489	LCVLHEK
Untreated; AB-G; AB-K	490-495	TPVSEK
Untreated	496-498	VTK
Untreated; AB-G; AB-K	524-528	AFDEK
Untreated	524-547	AFDEKLFTFHADICTLPDTEKQIK
Untreated; HCl; AB-G; AB-K	529-548	LFTFHADICTLPDTEKQIKK
Untreated; AB-K	545-548	QIKK
Untreated	545-557	QIKKQTALVELLK
HCl	548-557	KQTALVELLK
AB-K	558-561	HKPK
Untreated; AB-G; AB-K	562-568	ATEEQLK
AB-G; AB-K	569-580	TVMENFVAFVDK
Untreated; AB-G; AB-K	581-597	CCAADDKEACFAVEGPK
Untreated; AB-G	588-597	EACFAVEGPK
Untreated; HCl; AB-G; AB-K	598-607	LVVSTQTAL

Table S2. Trypsin autolytic peptides found in all tested samples (Untreated, HCl, AB-G and AB-K). Peptides marked with asterisk were missing in the trypsin AB-K sample.

Start-end	Monoisotopic mass (m/z)	Peptide
113-114	260.197	LK
160-162	363.206	CLK
241-246*	633.320	QTIASN
67-72	659.383	SGIQVR
115-122	805.416	SAASLNSR
224-231*	906.504	NKPGVYTK
163-172	1020.503	APILSDSSCK
232-240	1111.560	VCNYVSWIK
149-159	1153.574	SSGTSYPDVLK
194-209	1495.615	DSCQGDSGGPVVCSGK
173-193	2193.994	SAYPGQITSNMFCAGYLEGGK
123-148*	2552.248	VASISLPTSCASAGTQCLISGWGNTK