

# 1 Camel and Bovine Chymosin: the Relationship Between their 2 Structures and Cheese Making Properties

## 3 Supplementary Material

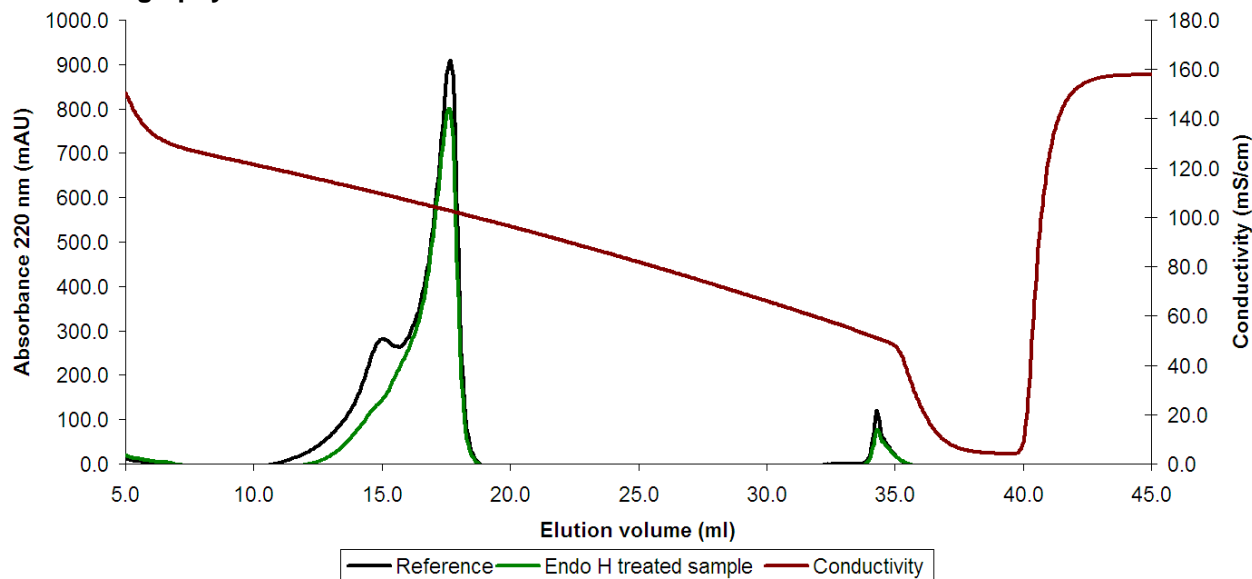
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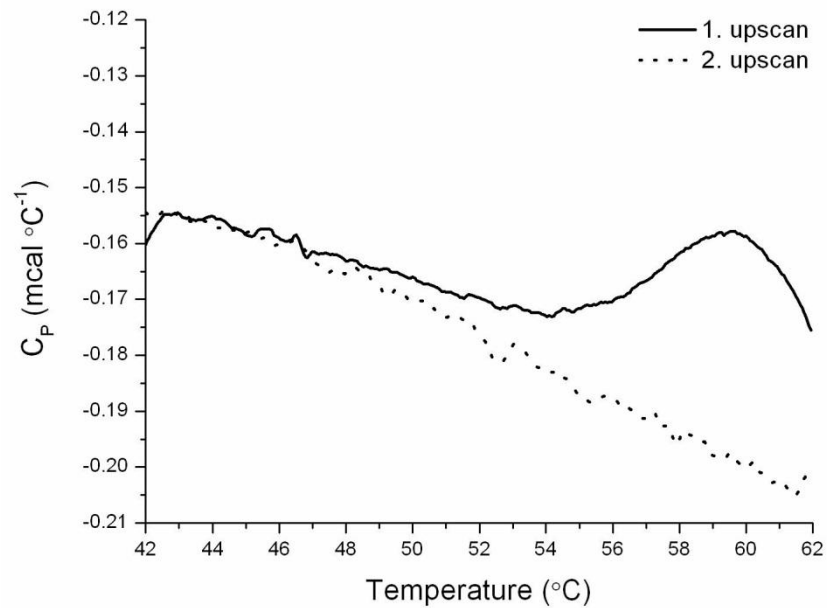
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### 13 Chromatography



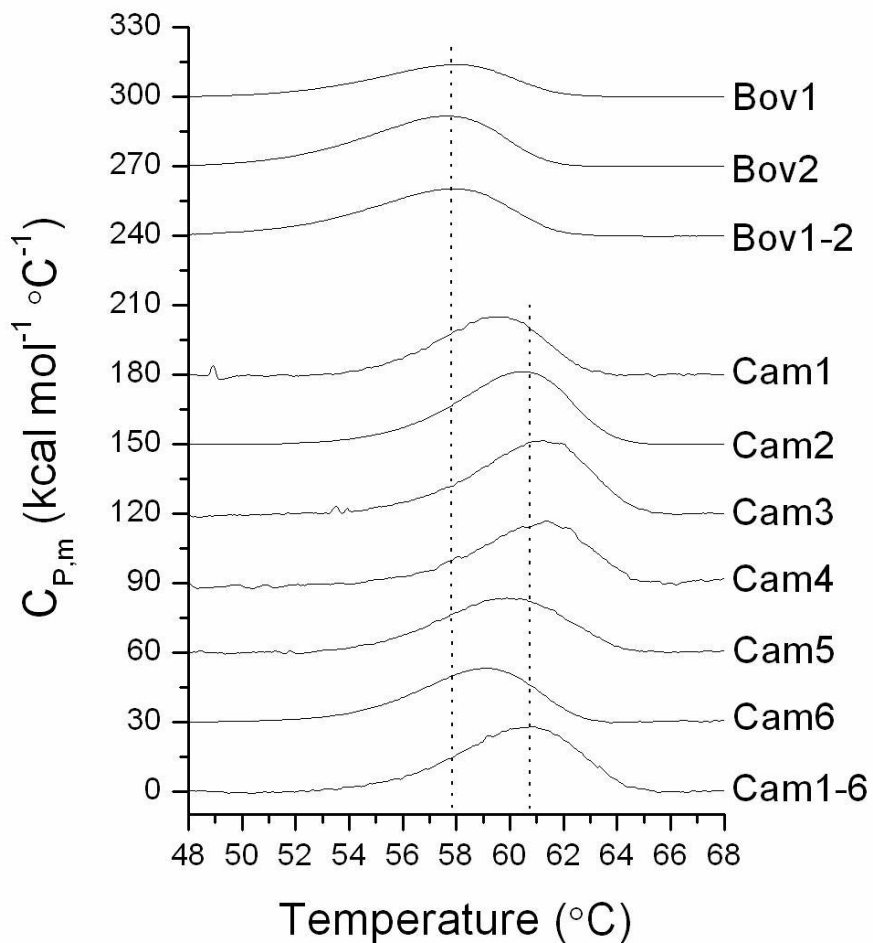
15 **Figure S1.** Representative chromatogram showing the FPLC separation of the commercial product of  
16 bovine chymosin against a salt gradient on a hydrophobic column. The black chromatogram shows the  
17 separated variants of the product. The green chromatogram shows the separation of the product pretreated  
18 with endoglycosidase H.

## 19 Differential Scanning Calorimetry



20

21 **Figure S2.** Raw DSC data for camel chymosin variant 5. The variants of bovine and camel chymosin  
22 denatured irreversibly upon heating. An endothermic contribution from denaturation can be seen in the  
23 first upscan after sample injection. This is absent in the second upscan.



24

25 **Figure S3.** Melting curves of the variants of bovine (Bov1+2) and camel chymosin (Cam1+2+3+4+5+6),  
26 and the commercial products (Bov1-2+Cam1-6). For clarity, the curves are displaced in steps of 30,000  
27  $\text{kcal} / (\text{mol} \cdot ^\circ\text{C})$  along the vertical axis with respect to Cam1-6. The dashed lines indicate the melting  
28 point of the commercial products

29

30 Sequences

Pro-sequence

Bov AEITRIPLYK<sup>10p</sup>GSLSRKALK<sup>20p</sup>EHGLLED<sup>30p</sup>FLQ<sup>40p</sup>RQQYGISSKYSYSGF  
 Cam SGITRIPLHKGKTLRKALKERGLLED<sup>30p</sup>FLQRQQYAVSSKYSYSSL

Mature sequence

Bov	GEVASVPLTNYLD	SQYFGKIYLGTPPQE	FTVLFDTGSSD	FWVPSIYCKSNACKNHORFDP
Cam	GKVAREPLTSYLD	SQYFGKIYIGTPPQE	FTVVFDTGSSD	LWVPSIYCKSNVCKNHHRFDP
	* ** *	*	*	*

Bov	RKSSTFQNLGKPLS	IHYGTGSMQGI	GYDVTVTSNIVD	IQQTVGLSTQEPG	DVFTYA	EFD
Cam	RKSSTFRNLGKPLS	IHYGTGSM	EGFLGYDVTVTSNIVD	PNQTVGLSTEQPG	EVFTYSE	EFD
	*	** *	**	** *	*	*

Bov	GILGMAYPSLAS	EYSIPVFDNMMNRHLVAQD	LF SVYMDRNGQE	SMLTLGAI	DPSY	TGSL
Cam	GILGLAYPSLAS	EYSVPVFDNMMDRHLVA	RDLF SVYMDRNGQGSMLTLGAI	DPSY	TGSL	
	*	*	*	*		

Bov	HWVPVTVQQYWQFTVD	SVTISGVVVACE	GGCQAILD	TGTSKLVGPSSD	IILNIQQAIGATQ
Cam	HWVPVTLQQYWQFTVD	SVTINGVAVACVGGCQAILD	TGTSVLFVPSSD	IILKIQAIGATE	
	*	*	*	*	*

Bov	NQYGEFDIDCDNLSYMP	TVVFE	INGKMYPLTPSAYTSQ	DQGFCTSGFQSENHSQKWILGD
Cam	NRQYGEFDVNCGNLRSMP	TVVFE	INGRDYPLSPSAYTSKD	DQGFCTSGFQGDNNSLWILGD
	*	** *	**	*

Bov	VFIREYYSVFD	RANNLVGLAKAI
Cam	VFIREYYSVFD	RANNRVGLAKAI
		*

X acidic	N glycosylated
X basic	* Difference

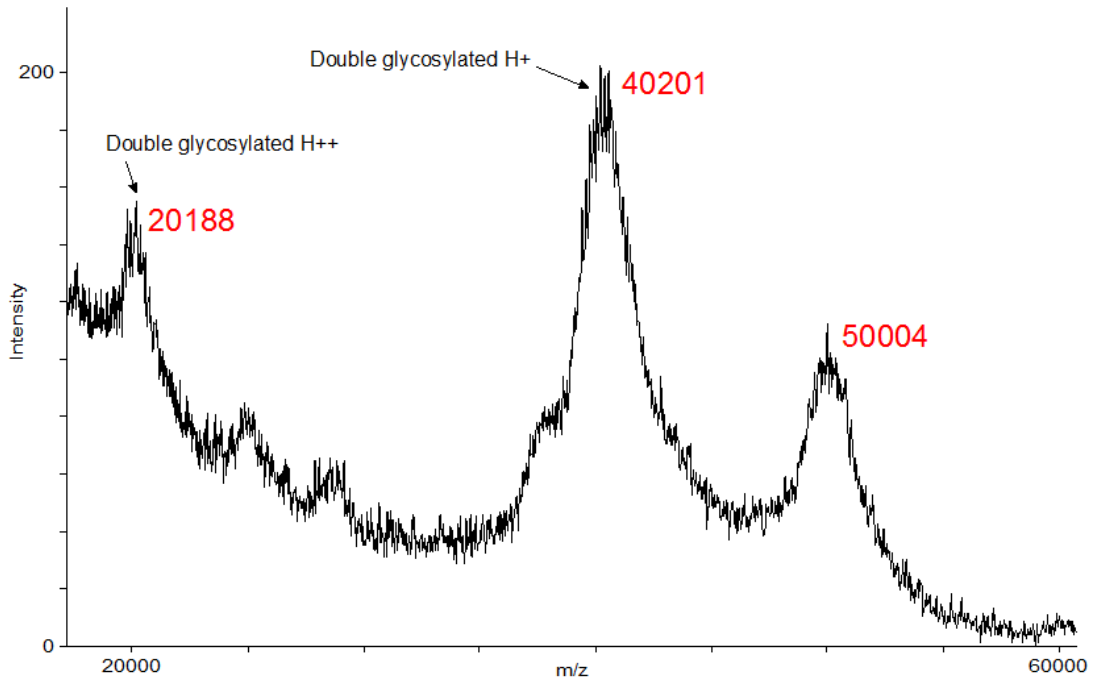
Bovine κ-casein

Cas	QEQNQE	QPIRCEKDERFFS	DKIAKYIPIQYVLSR	YPSYGLNYYQQK	PPVALINNQFLPYPY	
Cas	YAKPAAVR	SPAQILQWQVLSNTVPAK	SQCQAQPTTMA	RHPHLSFMAIPPKKNQDKTE	IP	
Cas	TINTIASG	EPTSTPTT	EAVESTVATLED	SP	VI	SPPEINTVQVTSTAV

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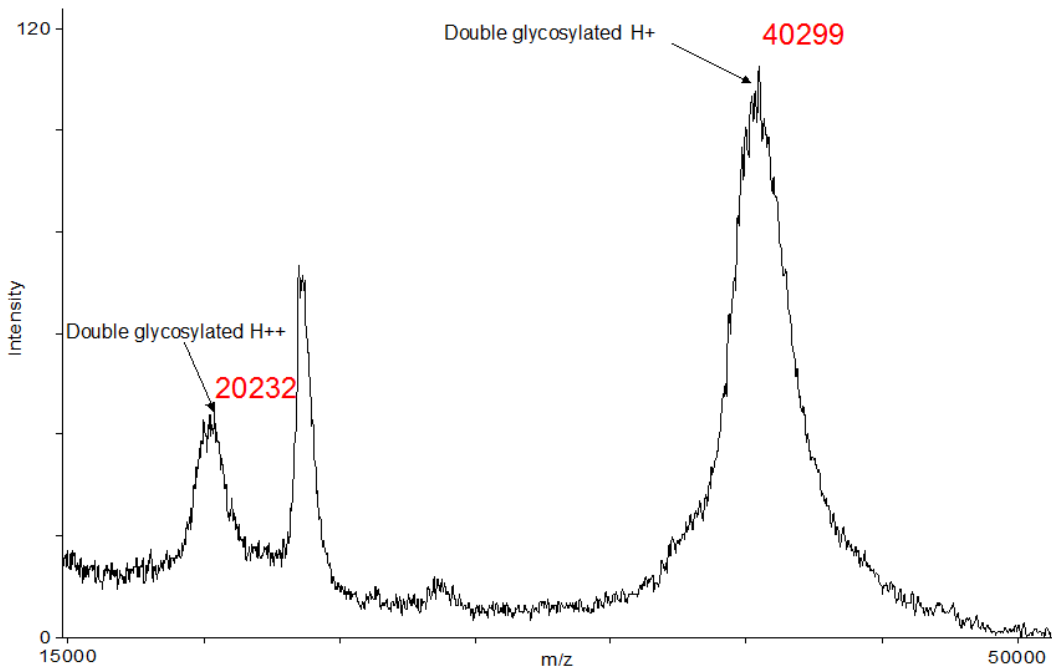
32 **Figure S4.** (top) Alignment of the sequences of the prosegment and mature protein of bovine and camel  
 33 chymosin based on Uniprot entries P00794 and Q9GK11, respectively. (bottom) The sequence of bovine  
 34 κ-casein based on Uniprot entry P02668. The *Phe105-Met106* bond cleaved by chymosin is marked with  
 35 arrowheads.

36 **Mass spectra**



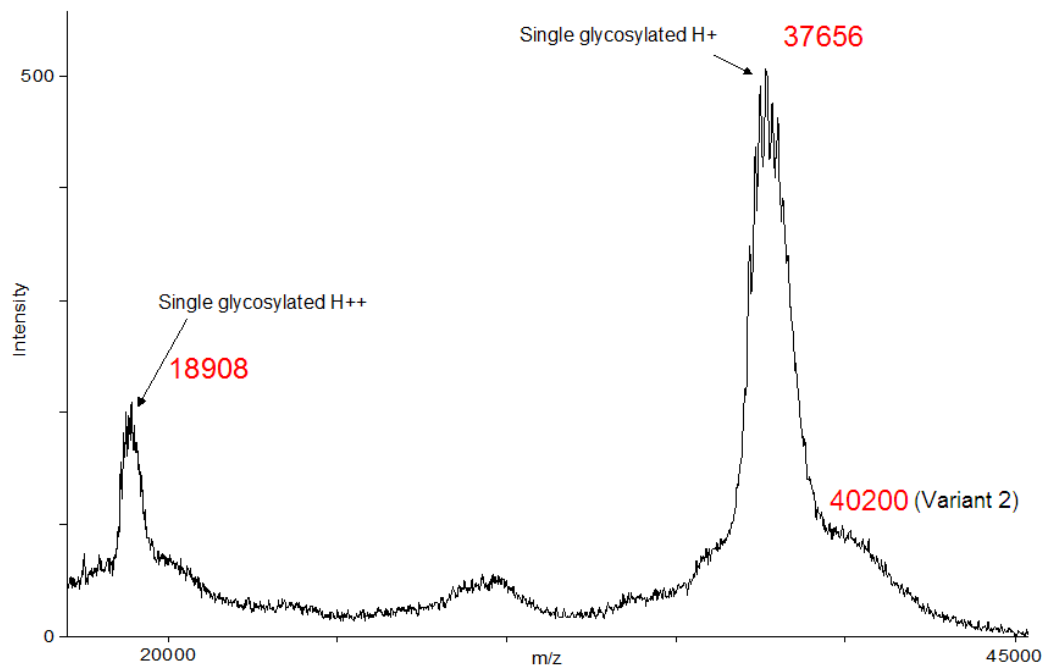
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38 **Figure S5.** Mass spectra of camel chymosin variant 1.

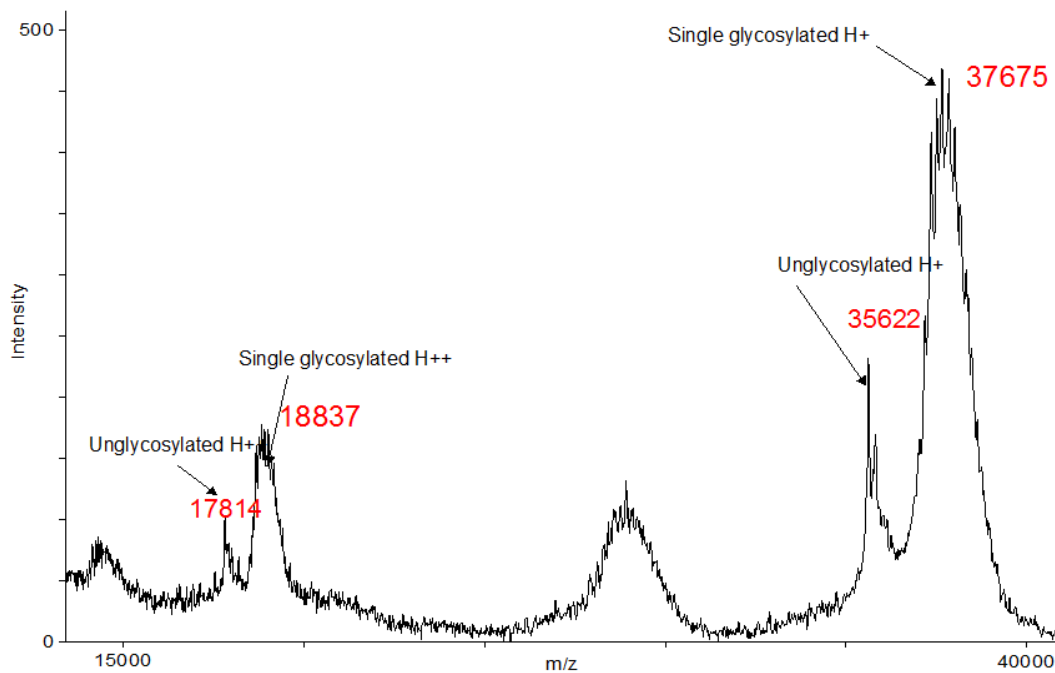


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40 **Figure S6.** Mass spectra of camel chymosin variant 2.

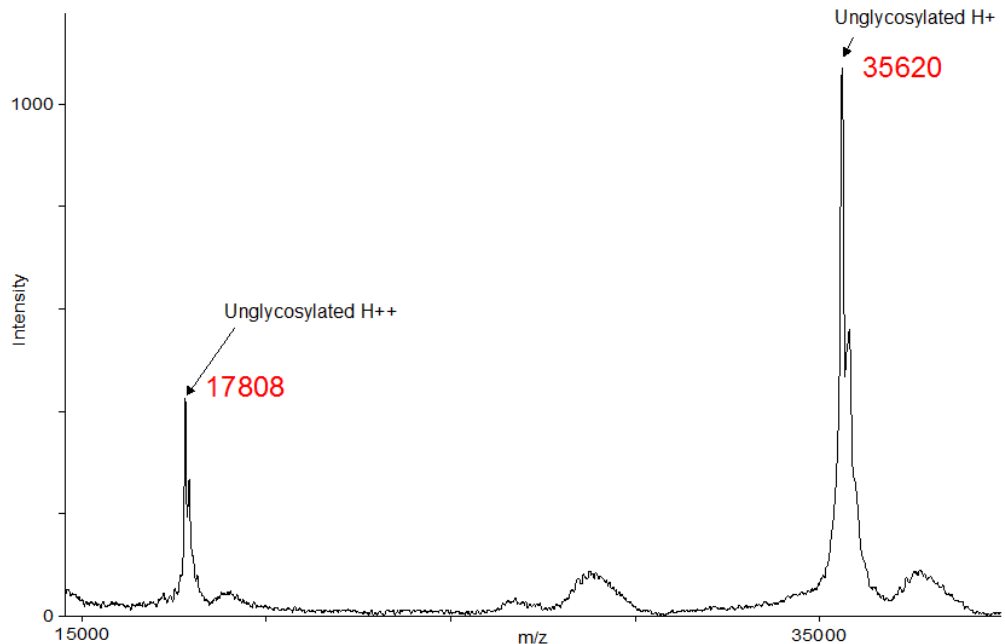


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42 **Figure S7.** Mass spectra of camel chymosin variant 3.

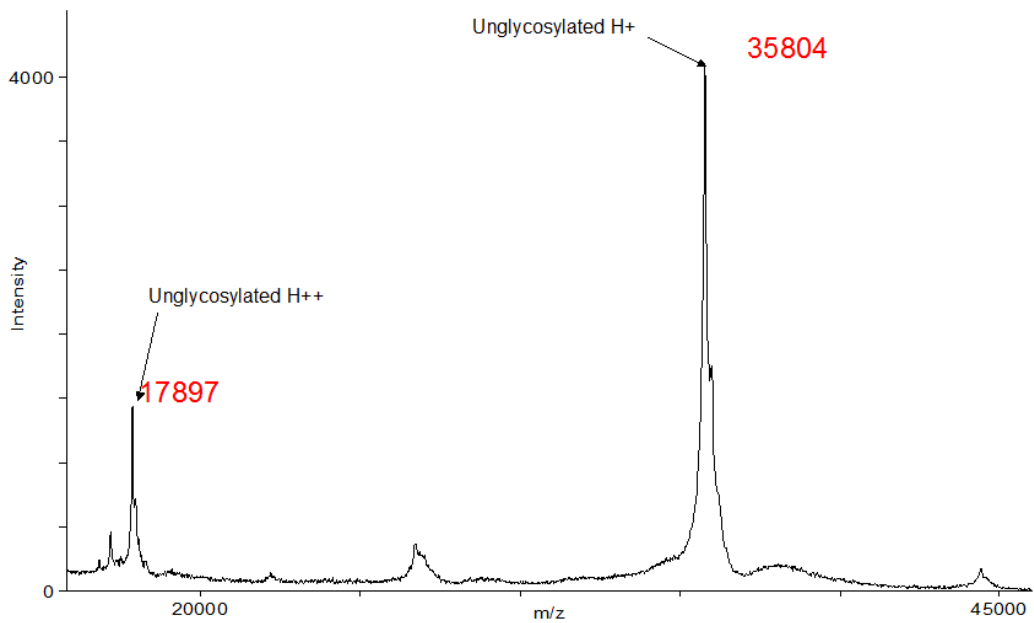
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44 **Figure S8.** Mass spectra of camel chymosin variant 4.



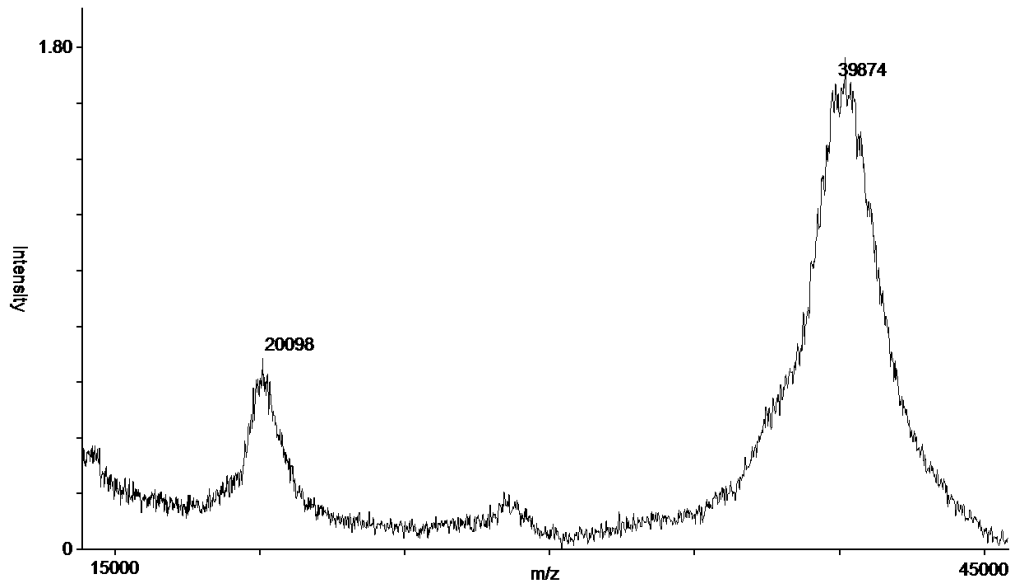
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46 **Figure S9.** Mass spectra of camel chymosin variant 5.



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48 **Figure S10.** Mass spectra of camel chymosin variant 6.



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50 **Figure S11.** Mass spectra of crystals of camel chymosin variant 2.