Protein modifications due to homogenisation and heat treatment of cow milk Jessica L Gathercole, Hanh TH Nguyen, Paul Harris, Mike Weeks and Mariza G Reis

SUPPLEMENTARY FILE

Supplementary methods and materials

Chemicals

Optima® LC-MS grade water, acetonitrile, methanol and formic acid were all obtained from Fisher Scientific (UK). Tris(2-carboxyethyl) phosphine \geq 98%, iodoacetamide, sucrose \geq 99.5%, dithiothreitol, Fast green, Nile red and agarose were all obtained from Sigma-Aldrich (St Louis, MO, USA). Urea \geq 99.5% and thiourea \geq 99% from Acros Organics (China). Ammonium bicarbonate \geq 99% from BDH Lab Supplies (Poole, England). Ammonium formate \geq 99% from Fluka (India). Chloroform (\geq 99.1%) from VWR (Paris, France). Trypsin was obtained from Promega (Madison, WI, USA) and Empore C18 discs were from Supelco (Bellefonte, PA, USA).

Microstructural analysis

The microstructural analysis of milk samples was carried out using an inverted confocal laser scanning microscope (CLSM) (Fluoview FV10i, Olympus, Auckland, New Zealand). Milk sample (1 mL) was mixed with 10 μ L of Fast Green FCF (1mg/mL) and 10 \Box L of Nile Red (1 mg/mL) and stained for at least 1 h at room temperature. An aliquot of 5 \Box L stained milk was then mixed with 20 \Box L of a low melting point agarose solution before deposition onto a cavity microscope slide and covered with a

0.17 mm thick coverslip (ProSciTech, QLD, Australia). The $\times 60$ water-immersion objective and numerical aperture of 1.0 were used. The excitation/emission wavelengths were set at 480 nm/ 500-530 mm and 635 nm/660–710nm for Nile Red and Fast Green FCF, respectively. At least six images were taken for each milk sample and the typical images are presented in the results section.

Sample preparation for proteomics

Cream was separated from the homogenised milk using a sugar gradient according to a method

based on Lee *et al.* (Lee & Sherbon, 2002). Briefly, 20 mL of milk was placed under 30 mL of 50 g/L sucrose using a glass pipette. The samples were centrifuged at 14 500 g at 4 °C for 15 min (Kubota 7000 centrifuge). Immediately after removal of the tubes, the cream was collected and dried on a Whatman no 1 filter paper at room temp. The skimmed milk was separated into casein and whey using ultracentrifugation as published previously (Gathercole et al., 2017) by centrifuging at 100 000 g for 1 h to limit the changes that occur due to acid precipitation. During acidification, the acid reduces the amount of calcium bonding on κ -casein which leads to changes in the casein micelle structure (Li & Zhao, 2019).

To denature the proteins, a small amount of cream and casein (separately) was dissolved in 100 μ L of 50 mM ammonium bicarbonate using ultrasonication. For the whey, an aliquot of 100 μ L of each whey sample was taken. Equivalent amounts of solute to produce a 7 M urea, 2 M thiourea and 50 mM dithiothreitol solution was added to each sample. They were then shaken overnight at 25 °C on a temperature-controlled thermomixer (Thermoshaker, Acon Scientific) at 600 rpm. To isolate the proteins, methanol-chloroform extraction was done according to the method by Wessel and Flügge (Wessel & Flügge, 1984). Briefly, 400 μ L of methanol, 100 μ L chloroform and 300 μ L water were added to each sample vortexing briefly after each addition. The mixture was centrifuged for 1 min at 13 000 *g* and the top aqueous layer was removed. An additional 400 μ L of methanol was added and after mixing, centrifuged for 2 min at the same speed. After removal of the organic layer the precipitated proteins were left to air dry.

The protein precipitate was dissolved in 60 μ L of 0.1 M ammonium bicarbonate. To reduce the proteins, 20 μ L 100 mM tris(2-carboxyethyl)phosphine was added and the samples were incubated for 45 min at 56 °C on a thermomixer. The proteins were then alkylated by the addition of 20 μ L of 150 mM iodoacetamide in 50 mM ammonium bicarbonate and incubated in the dark at room temperature for 30 minutes on the thermomixer. Trypsin (Promega) was dissolved in Promega trypsin buffer (to a concentration of 1 μ g/ μ L) and 5 μ g of trypsin (1 μ g trypsin : 50 μ g of protein)

was added to each sample which were incubated overnight at 37°C with shaking. The digests were dried in a centrifugal concentrator and resuspended in 100 μ L of 10 mM ammonium formate, pH 10. To clean the sample, three 2 mm x 2 mm disks of Empore C18 material was used for each sample. The disks were conditioned for 1 min each with acetonitrile followed by methanol and then water. Three disks were then placed directly into each sample and incubated to bind the peptides to Empore disks for 2.5 hrs at room temperature with vortexing. Prior to eluting the Empore disks were rinsed in 0.1% formic acid. The peptides were eluted in two fractions. Initially the disks were placed in 100 μ L of 10 mM ammonium formate in 10% v/v acetonitrile and vortexed for one hour. The disks were then placed in 100 μ L of 10 mM ammonium formate in 50% v/v acetonitrile for one hour. The disks were discarded, and each eluent was dried using a centrifugal concentrator and stored at -20 °C until LC-MS/MS analysis.

Protein and peptide identification

LC-MS/MS files were converted into Mascot generic format (mgf) and imported into ProteinScape (Version 4.0.3 315, Bruker Daltonics). The mgf of both the Empore fractions were combined into one file prior to protein database searches. Spectra was compared against the SwissProt *Bos Taurus* database using Mascot and ProteinExtractor and six different sets of modifications. For all searches, semitrypsin was selected as the enzyme allowing for up to 2 missed cleavages. The peptide tolerance was 0.1 Da and the MS/MS tolerance was 0.6 Da was used. All searches contained fixed carbamidomethyl of Cys and variable deamidation (Asn or Gln) and phosphorylation off Ser or Thr. In addition to these modifications, search 1 included variable modification of hexose and dihexose on the N-terminus or Lys, carboxymethyl of Lys, and carboxyethyl of Lys; search 2, variable modifications for oxidation (Phe or Tyr), dioxidation of Phe; Search 3 included variable modifications of oxidation of His or Trp, dioxidation of Cys or Met and trioxidation of Cys; search 4, oxidation of Ser and Thr, and didehydro of Ser or Thr or Tyr; and search 6, cysteine to dehydroalanine, pyroglutamate

from Gln or Asn and amino loss from N-terminus of Cys. The searches were then compiled into one file and peptide lists were exported into Microsoft Excel for further analysis. Proteins observed only in the homogenised milk cream fraction were analysed with Panther (Mi, Muruganujan, Ebert, Huang, & Thomas, 2018; Thomas et al., 2006) to determine molecular and biological functions.

Protein modification analysis

Modification scores, to determine the degree of protein modifications, were determined using an in- house software. Modifications were weighted according to number of modification changes. For example, each deoxidation was multiplied by 2 and trioxidation was multiplied by 3. These weighted scores were used to calculate the modification scores by obtaining the ratio of number of modifications observed and the number of times the amino acid was observed, as reported previously (Dyer et al., 2010; Gathercole et al., 2017; Lassé et al., 2015). Modifications scores were calculated for each type and group of modifications (e.g. carboxymethylation, oxidation of cysteine and total oxidation) as well as a total modification score. The average of the three replicates was used in further analysis. The Sparkline function in Microsoft Excel was used to screen, for modifications which differed between milk treatments. The most abundant types of modifications were investigated further to determine if the modification site or area of the protein was consistent. One-way ANOVA was done on total cysteine oxidation, total proline oxidation and total oxidation for all three fractions. If the modification score means for treatments were significantly different according to ANOVA, pairwise comparisons were run using the Holm-Skdak method (SigmaPlot version 13.0, Dundas Software ltd, Germany) to determine significant differences between treatments.

Supplementary Figure S1. Comparison of α -S1-casein, lactadherin, β - lactoglobulin and xanthine dehydrogenase/oxidase protein modification locations according to milk fraction and processing.

Key

- Casein
- Whey
- MFG
- Casein and whey
- Casein and MFG
- Whey and MFG
- Whey, casein and MFG

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Observed in more than one sample

- O₁ single oxidation
- O₂ double oxidation
- O₃ triple oxidation
- H hexose
- H₂ dihexose
- CMe carboxymethylation

When and MHG

• CEt - Carboxyethylation

Alpha-S1-casein – Raw



Alpha-S1-casein – Pasteurised



Alpha-S1-casein – 45°C, 0 bar

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SESTEDQAME H ₂	DIKQMEAESI	SSSEEIVPNS CEt	VEQKHIQKED	VPSERYLGYL	EQLLRLKKYK
VPQLEIVPNS	AEERLHSMKE	GIHAQQKEPM	IGVNQELAYF	YPELFRQFYQ	LDAYPSGAWY
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Alpha-S1-casein – 45°C, 350 bar



Alpha-S1-casein – 80°C, 0 bar



Alpha-S1-casein – 80°C, 350 bar – *no* cream



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VTQGASRAGS	AEYLKTFKVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
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ADSQISASSM	HLGFMGLQRW	APELARLHQT	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	AEYLKTFKVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
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ADSQISASSM	HLGFMGLQRW	APELARLHQT	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	AEYLKTFKVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
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VTQGASRAGS		YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL

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SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
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B-Lactoglobulin - Raw

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TLKELLDLKA	QHPEAKLVVG	NTEIGIEMKF	KNQLFPMIIC	PAWIPELNAV	EHGPEGISFG
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KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNAM	KTQSF\	/AKML
GVPVNRILVR	VKRMGGGFGG	KETRSTLVSV	AVALAAYKTG	HPVRCMLDRN	EDMLI	ſGGRH
PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLSHSIMER	ALFHMDNCYK	IPNIRG	TGRL
CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	i DLTHFI	NQRLE
GFSVPRCWDE	ECLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNG	QAGAL
IHVYTDGSVL	VSHGGTEMGQ	GLHTKMVQVA	SKALKIPISK	IYISETSTNT	VPNSSI	PTAAS

AA 10	AA 1081-1320									
	Xanthine dehydrogenase/oxidase – 4-Past						+ MPC + Excellent along + Easeward WC + Wile, and WC + Wile, state and WC			
	VSTDIYGQAV	YEACQTILKR	LEPFKKKNPD	GSWEDWVMAA	YQDRVSLST	T GFYRTF	PNLGY			
	SFETNSGNAF	HYFTYGVACS	EVEIDCLTGD	HKNLRTDIVM	DVGSSLNPA	I DIGQVI	EGAFV			
	QGLGLFTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRDCPN	K KAIYAS	KAVG			
	EPPLFLGASV	FFAIKDAIRA	ARAQHTNNNT	KELFRLDSPA	TPEKIRNAC	/ DKFTTL	.CVTG			
	APGNCKPWSL	RV								

AA 1	-360					• D conjo contratori • D deut in coldurica	- Excess + Wikey
Xan	ithine deh	ydrogenas	se/oxidase	e−1-45°C,	0 bar	 10, - Orgi- real-Main 11, - Neconi 14, - Ofoccosi 150 c - renticip voltasfedori 10, - Oarkospettylation 	+ Mrg - Excellented alony + Eacety and WC + White and MPG + When, stores and MCs
	MTADELVFFV	NGKKVVEKNA	DPETTLLAYL	RRKLGLRGTK	LGCGEGGCG	GA CTVM	LSKYDR
	LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSH	g sqcgf	CTPGI
	VMSMYTLLRN	QPEPTVEEIE	DAFQGNLCRC	TGYRPILQGF	RTFAKNGGC	C GGNG	NNPNCC O2
	MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRF	e gervt	I WIQAS
	TLKELLDLKA	QHPEAKLVVG	NTEIGIEMKF	KNQLFPMIIC O2	PAWIPELNA	V EHGPE	GISFG
	AACALSSVEK	TLLEAVAKLP	TQKTEVFRGV	LEQLRWFAGK	QVKSVASLG	G NIITAS	PISD

AA 3	61-720				;	0. – ungle unit-stori D. – deutels sektories	- Cases
Xar	nthine deh	9), - taple califitati H - Necce H - Obscase N - Obscase 201 - Calificasen y Eralificas 201 - Calificasen y Erali	+ MPC + Excellent along + Excellent WC + When and MPC + When, second MPC + When, second and MPC				
	LNPVFMASGT	KLTIVSRGTR	RTVPMDHTFF	PSYRKTLLGP	EEILLSIEIP	YSREDE	FFSA
	FKQASRREDD	IAKVTCGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLS	KFWNE
	KLLQDVCAGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKC	GKLDP
	TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGE	A VYCDD	IPRYE
	NELFLRLVTS	TRAHAKIK <mark>SI</mark>	DVSEAQKVPG	FVCFLSADDI	PGSNETGLFN	DETVF	AKDTV

TCVGHIIGAV VADTPEHAER AAHVVKVTYE DLPAIITIED AIKNNSFYGS ELKIEKGDLK

AA 72	1-1080					• D singler	unit di mi	- Causara
Xanthine dehydrogenase/oxidase – 3-45°C, 0						 10, - trajer 11, - trajer 11, - texes 14, - decas 25, - decas 25, - Cartos 	nalistan ministratista ministratista	+ MPC - Excellenced alloyy - Easiery and WPC + White and MPC + White, successed 2005
	KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNA	М	KTQSFV	AKML
	GVPVNRILVR	VKRMGGGFGG	KETRSTLVSV	AVALAAYKTG	HPVRCMLD	RN	EDMLIT	GGRH
	PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLSHSIMER	ALFHMDNC	ΥK	IPNIRGT	GRL
	CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYK	ŒG	DLTHFN	QRLE
	GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT		VPFLNQ	AGAL
	IHVYTDGSVL	VSHGGTEMGQ	GLHTKMVQVA	SKALKIPISK	IYISETSTNT		VPNSSP ⁻	TAAS

AA 1081-1320	A 1081-1320							
Xanthine dehydrogenase/oxidase – 4-45°C, 0 bar							 Constraint alway Constraint with alway Constraint WC When and MPU When, constraint bits 	
VSTDIY	GQAV	YEACQTILKR	LEPFKKKNPD	GSWEDWVMAA	YQDRVSLSTT	GFYRTP	NLGY	
SFETNS	GNAF	HYFTYGVACS	EVEIDCLTGD	HKNLRTDIVM	DVGSSLNPA	DIGQVE	GAFV	
QGLGL	FTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRDCPNK	KAIYASK	AVG	
EPPLFL	GASV	FFAIKDAIRA	ARAQHTNNNT	KELFRLDSPA	TPEKIRNACV	DKFTTL	CVTG	
APGNO	KPWSL	RV						

AA 1-360				• 51, - 0 • 0, - 0	ngto contrations cuto existentica	- Laurenter		
Xanthine dehydrogenase/oxidase – 1-45°C, 350 bar								
MTADELVFFV O ₂	NGKKVVEKNA	DPETTLLAYL	RRKLGLRGTK	LGCGEGGCGA	CTVML	SKYDR		
LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSHG	SQCGF	CTPGI		
VMSMYTLLRN	QPEPTVEEIE	DAFQGNLCRC	TGYRPILQGF	RTFAKNGGCC	GGNGN	INPNCC		
MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRFE	GERVT	WIQAS		

LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSHG	SQCGFCTPGI
VMSMYTLLRN	QPEPTVEEIE	DAFQGNLCRC	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC
MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRFE	GERVTWIQAS
TLKELLDLKA	QHPEAKLVVG	NTEIGIEMKF	KNQLFPMIIC	PAWIPELNAV	EHGPEGISFG
AACALSSVEK	TLLEAVAKLP	TQKTEVFRGV	LEQLRWFAGK	QVKSVASLGG	NIITASPISD

AA 3	61-720				• 0, va	fronteston + Wiley		
Xanthine dehydrogenase/oxidase -2- 45°C, 350 bar								
	LNPVFMASGT	KLTIVSRGTR	RTVPMDHTFF	PSYRKTLLGP	EEILLSIEIP	YSREDEFFSA		
	FKQASRREDD	IAKVTCGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSKFWNE		
	KLLQDVCAGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCGKLDP		
	TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDIPRYE		
	NELFLRLVTS	TRAHAKIKSI	DVSEAQKVPG	FVCFLSADDI	PGSNETGLFN	DETVFAKDTV		
	TCVGHIIGAV	VADTPEHAER	AAHVVKVTYE	DLPAIITIED	AIKNNSFYGS	ELKIEKGDLK		

AA 721-1080				• 11	regio contratore: Institución	- Caurtan * Milana
Xanthine del	nydrogena	ase/oxidas	se – 3-45°C	, 350 bar	ngi e nadridnam noom Rocane mark en verhele doe Gark asett ykirke	+ MPC + Construct + Calebra + White PT + White PT
KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNAM	KTQSF\	/AKM

AA 721-1080

de a	+ William
	+ 84710
	+ Except and alloy
	+ Ealety and W/C
Interior	+ White and MPUL
lation	+ Wites, cause and \$800

KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNAM	KTQSFVAKML	
GVPVNRILVR	VKRMGGGFGG	KETRSTLVSV	AVALAAYKTG	HPVRCMLDRN	EDMLITGGRH	
PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLSHSIMER	ALFHMDNCYK	IPNIRGTGRL	
CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQRLE	
GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL	
IHVYTDGSVL	VSHGGTEMGQ	GLHTKMVQVA	SKALKIPISK	IYISETSTNT	VPNSSPTAAS	

AA 1081-1320								
Xar	nthine deł	, 350 bar	realities - EUTC Constraint of alway ale - Constraint of alway ale - Constraint WIC Kommittelector - White and MTC appropriates - White, constraint (MC)					
	VSTDIYGQAV	YEACQTILKR	LEPFKKKNPD	GSWEDWVMAA	YQDRVSLSTT	GFYRTPNLGY		
	SFETNSGNAF	HYFTYGVACS	EVEIDCLTGD	HKNLRTDIVM	DVGSSLNPAI	DIGQVEGAFV		
	QGLGLFTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRDCPNK	KAIYASKAVG		
	EPPLFLGASV	FFAIKDAIRA	ARAQHTNNNT	KELFRLDSPA	TPEKIRNACV	DKFTTLCVTG		
	APGNCKPWSL	RV						

AA 1-360				- 11, 1 D	
Xanthine d	- Tigle real Value + MTS Notox + Descentional delay - Obscise - Descentional WFC Promotion workelicities + Whole and MTS Promotion workelicities + Whole and MTS - Carlossengalistics + Whole and MTS				
MTADELVF	FV NGKKVVEKNA	DPETTLLAYL	RRKLGLRGTK	LGCGEGGCGA	CTVMLSKYDR
LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSHG	SQCGFCTPGI
VMSMYTLL	RN QPEPTVEEIE	DAFQGNLCRC	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC O2
MNQKKDH	TVT LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRFE	GERVTWIQAS
TLKELLDLKA	QHPEAKLVVG	NTEIGIEMKF	KNQLFPMIIC	PAWIPELNAV	EHGPEGISFG
AACALSSVE	K TLLEAVAKLP	TQKTEVFRGV	LEQLRWFAGK	QVKSVASLGG	NIITASPISD

AA 3	61-720				• 11,	gir unitation autos colducion	- Lauren * Wilson
Xar	nthine deh	lydrogena	se/oxidase	e -2- 80°C,	0 bar	ale constant cons locates tectores, eschelistor articasen ylation	+ MPC + Excess of alway + Eastward WC + White and MPC + When, screen and MPC
	LNPVFMASGT	KLTIVSRGTR	RTVPMDHTFF	PSYRKTLLGP	EEILLSIEIP	YSREDE	FFSA
	FKQASRREDD	IAKVTCGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSK	FWNE
	KLLQDVCAGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCG	GKLDP
	TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDI	PRYE
	NELFLRLVTS	TRAHAKIKSI	DVSEAQKVPG	FVCFLSADDI	PGSNETGLFN	DETVFA	KDTV
	TCVGHIIGAV	VADTPEHAER	AAHVVKVTYE	DLPAIITIED	AIKNNSFYGS	ELKIEKO	GDLK

AA	721-1080				• 11, - 004 • 10, - dev	de unit-den - Engen de la soldariza - Millan
Ха	nthine deł	nydrogena	se/oxidase	e – 3-80°C,	0 bar	in national and an interference of the second and t
	KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNAM	KTQSFVAKML
	GVPVNRILVR	VKRMGGGFGG	KETRSTLVSV	AVALAAYKTG	HPVRCMLDRN	EDMLITGGRH
	PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLSHSIMER	ALFHMDNCYK	IPNIRGTGRL
	CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQRLE
	GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL
	IHVYTDGSVL	VSHGGTEMGQ	GLHTKMVQVA	SKALKIPISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1332		11 sangle contrations 13 daubits coldenias	- Laure • When			
Xanthine de	Kanthine dehydrogenase/oxidase – 4-80°C, 0 bar					
VSTDIYGQAV	YEACQTILKR	LEPFKKKNPD	GSWEDWVMAA	YQDRVSLSTT	GFYRTPI	NLGY
SFETNSGNAF	HYFTYGVACS	EVEIDCLTGD	HKNLRTDIVM	DVGSSLNPAI	DIGQVE	GAFV
QGLGLFTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRDCPNK	KAIYASK	AVG
EPPLFLGASV	FFAIKDAIRA	ARAQHTNNNT	KELFRLDSPA	TPEKIRNACV	DKFTTLC	CVTG
APGNCKPWSL	RV					