Protein modifications due to homogenisation and heat treatment of cow milk

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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 ≥ 98%, iodoacetamide, sucrose ≥ 99.5%, dithiothreitol, Fast green, Nile red and agarose were all obtained from Sigma-Aldrich (St Louis, MO, USA). Urea ≥ 99.5% and thiourea ≥ 99% from Acros Organics (China). Ammonium bicarbonate ≥ 99% from BDH Lab Supplies (Poole, England). Ammonium formate ≥ 99% from Fluka (India). Chloroform (≥ 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 µL of Fast Green FCF (1mg/mL) and 10 □L of Nile Red (1 mg/mL) and stained for at least 1 h at room temperature. An aliquot of 5□L stained milk was then mixed with 20 □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 ×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 µL of 50 mM ammonium bicarbonate using ultrasonication. For the whey, an aliquot of 100 µL 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 µL of methanol, 100 µL chloroform and 300 µL 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 µL 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 µL of 0.1 M ammonium bicarbonate. To reduce the proteins, 20 µL 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 µL 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 µg/µL) and 5 µg of trypsin (1 µg trypsin : 50 µg 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 µL 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 µL of 10 mM ammonium formate in 10% v/v acetonitrile and vortexed for one hour. The disks were then placed in 100 µL 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 (Phe or Tyr) and trioxidation of Phe; Search 3 included variable modifications of oxidation of His or Trp, dioxidation of Trp, trioxidation of Trp and tetraoxidation of Trp; search 4, oxidation of Cys or Met or Pro, deoxidation of Cys or Met and trioxidation of Cys; search 5, dehydration 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.

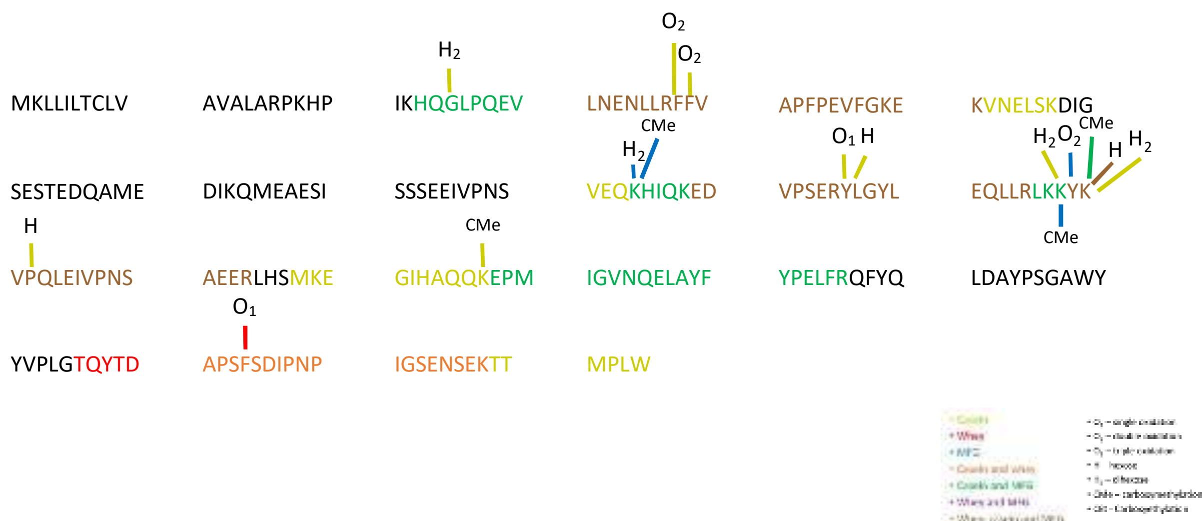
Key

Observed in more than one sample

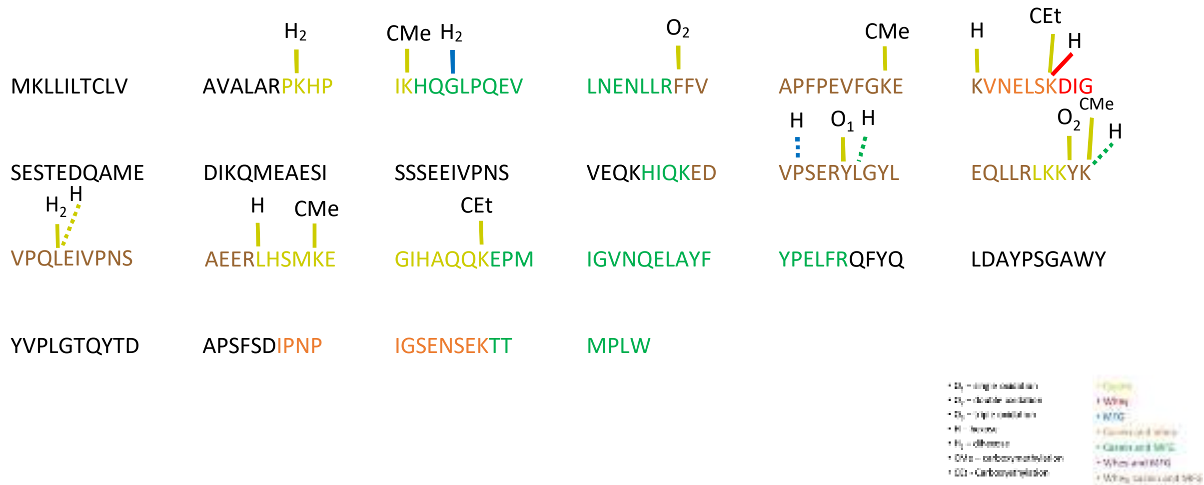
- Casein
- Whey
- MFG
- Casein and whey
- Casein and MFG
- Whey and MFG
- Whey, casein and MFG

- O_1 – single oxidation
- O_2 – double oxidation
- O_3 – triple oxidation
- H – hexose
- H_2 – dihexose
- CMe – carboxymethylation
- CEt - Carboxyethylation

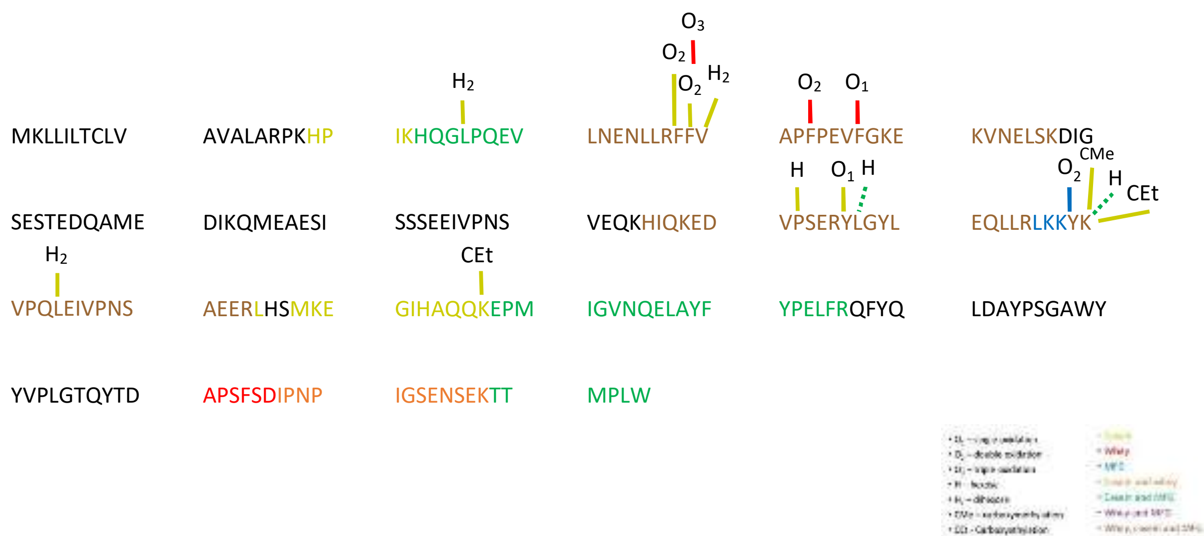
Alpha-S1-casein – Raw



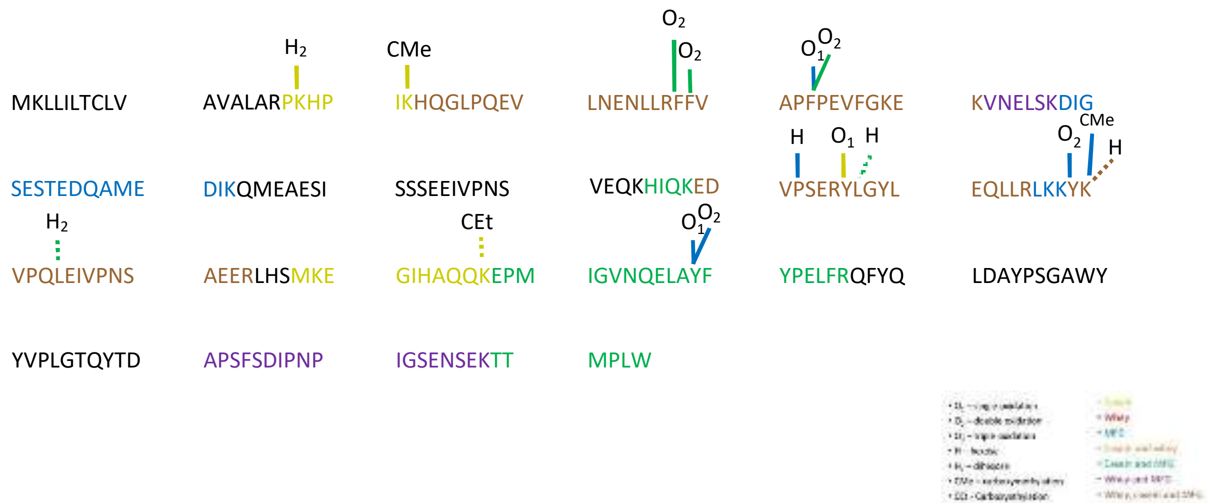
Alpha-S1-casein – Pasteurised



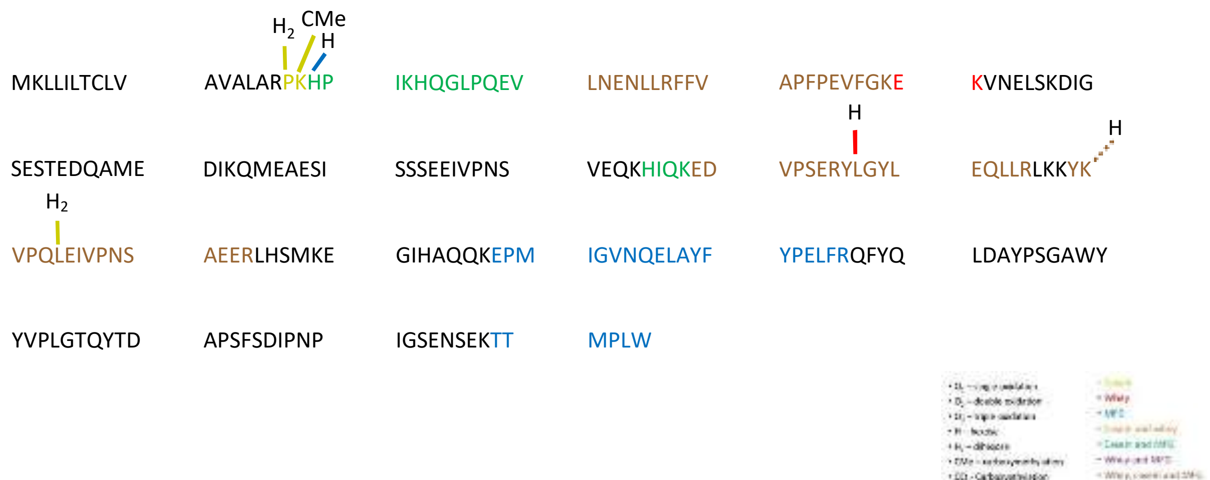
Alpha-S1-casein – 45°C, 0 bar



Alpha-S1-casein – 45°C, 350 bar



Alpha-S1-casein – 80°C, 0 bar



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

MKLLILTCLV	AVALARPKHP	IKHQGLPQEV	LNENLLRFFV	APFPEVFGKE	KVNELSKDIG
SESTEDQAME	DIKQMEAESI	SSSEEIVPNS	VEQKHIQKED	VPSERYLGYL	EQLLRLKKYK
VPQLEIVPNS	AEERLHSMKE	GIHAQQKEPM	IGVNQELAYF	YPELFRQFYQ	LDAYPSGAWY
YVPLGTQYTD	APSFSDIPNP	IGSENSEKTT	MPLW		

H

H_2 H_2 H H

Lactadherin (MFGM_Bovine) - Raw

MPCPRLLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	HLGFMGLQRW	APELARLHQT	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	AEYLKTFKVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAARY
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
RVELLGC					

O_1 O_1 O_1 O_3 O_2

Lactadherin (MFGM_Bovine) - Past

MPCPRLLAAL FCSSGLFAAS GDFCDSSLCL HGGTCLLNED RTPPFYCLCP EGFTGLLCNE

TEHGPCFPNP CHNDAECQVT DDSHRGDVFI QYICKCPLGY VGIHCETTCT SPLGMQTGAI

O_3

ADSQISASSM HLGFMGLQRW APELARLHQT GIVNAWTSGN YDKNPWIQVN LMRKMWVTGV

O_1 O_1 O_3

VTQGASRAGS AEYLKTFKVA YSTDGRQFQF IQVAGRSGDK IFIGNVNNSG LKINLFDTPL

ETQYVRLVPI ICHRGCTLRF ELLGCELNGC TEPLGLKDNT IPNKQITASS YYKTWGLSAF

O_4

SWFPYYARLD NQGKFNAWTA QTNSASEWLQ IDLGSQKRVT GIITQGARDF GHIQYVAAYR

VAYGDDGVTW TEYKDPGASE SKIFPGNMDN NSHKKNIFET PFQARFVRIQ PVAWHNRITL

RVELLGC

Lactadherin (MFGM_Bovine) -45°C, 0 bar

MPCPRLLAAL FCSSGLFAAS GDFCDSSLCL HGGTCLLNED RTPPFYCLCP EGFTGLLCNE

TEHGPCFPNP CHNDAECQVT DDSHRGDVFI QYICKCPLGY VGIHCETTCT SPLGMQTGAI

ADSQISASSM HLGFMGLQRW APELARLHQT GIVNAWTSGN YDKNPWIQVN LMRKMWVTGV

VTQGASRAGS AEYLKTFKVA YSTDGRQFQF IQVAGRSGDK IFIGNVNNSG LKINLFDTPL

O_1O_2

ETQYVRLVPI ICHRGCTLRF ELLGCELNGC TEPLGLKDNT IPNKQITASS YYKTWGLSAF

SWFPYYARLD NQGKFNAWTA QTNSASEWLQ IDLGSQKRVT GIITQGARDF GHIQYVAAYR

VAYGDDGVTW TEYKDPGASE SKIFPGNMDN NSHKKNIFET PFQARFVRIQ PVAWHNRITL

RVELLGC

Lactadherin (MFGM_Bovine) - 45°C, 350 bar

MPCPRLLAAL FCSSGLFAAS GDFCDSSLCL HGGTCLLNED RTPPFYCLCP EGFTGLLCNE

TEHGPCFPNP CHNDAECQVT DDSHRGDVFI QYICKCPLGY VGIHCETTCT SPLGMQTGAI

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

RVELLGC

Lactadherin (MFGM_Bovine) - 80°C, 0 bar

MPCPRLLAAL FCSSGLFAAS GDFCDSSLCL HGGTCLLNED RTPPFYCLCP EGFTGLLCNE

TEHGPCFPNP CHNDAECQVT DDSHRGDVFI QYICKCPLGY VGIHCETTCT SPLGMQTGAI

ADSQISASSM HLGFMGLQRW APELARLHQT GIVNAWTSGN YDKNPWIQVN LMRKMWVTGV

VTQGASRAGS AEYLKTFKVA O_1 YSTDGRQFQF IQVAGRSGDK IFIGNVNNSG LKINLFDTPL

ETQYVRLVPI ICHRGCTLRF ELLGCELNGC TEPLGLKDNT IPNKQITASS YYKTWGLSAF

SWFPYYARLD NQGKFNAWTA QTNSASEWLQ IDLGSQKRVT GIITQGARDF GHIQYVAAYR

VAYGDDGVTW TEYKDPGASE SKIFPGNMDN NSHKKNIFET PFQARFVRIQ PVAWHNRITL

RVELLGC

Lactadherin (MFGM_Bovine) - 80°C, 350 bar

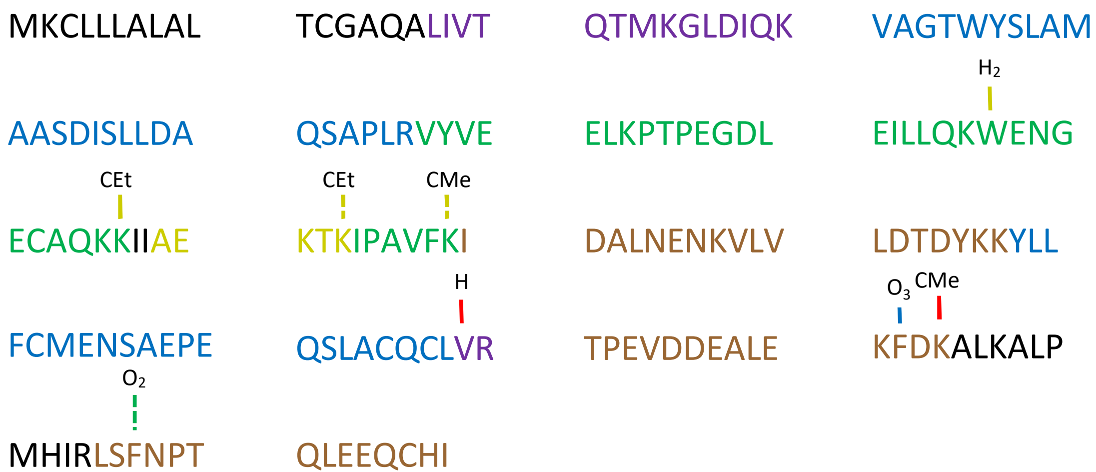
MPCPRLLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	HLGFMGLQRW	APELARLHQT	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	AEYLKTFKVA (O_1, O_2)	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
RVELLGC					

B-Lactoglobulin - Raw

MKCLLLALAL	TCGAQALIVT	QTMKGLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLRVYVE	ELKPTPEGDL	EILLQKWENG
ECAQKKIIAE (H)	KTKIPAVFKI (CEt, CEt, H_2, CMe)	DALNENKVLV (CMe)	LDTDYKKYLL
FCMENSAEPE	QSLACQCLVR	TPEVDDEALE	KFDKALKALP (CMe, CMe)
MHIRLSFNPT (O_3)	QLEEQCHI		

B-Lactoglobulin - Past

B-Lactoglobulin - 45°C, 0 bar



B-Lactoglobulin - 45°C, 350 bar

MKCLLLALAL TCGAQALIVT QTMKGLDIQK VAGTWYSLAM

AASDISLLDA QSAPLRVYVE ELKPTPEGDL EILLQKWENG

CEt CEt CMe

ECAQKKIIAE KTKIPAVFKI DALNENKVLV LDTDYKKYLL

H CMe CEt

FCMENSAEPE QSLACQCLVR TPEVDDEALE KFDKALKALP

O_2

MHIRLSFNPT QLEEQCHI

B-Lactoglobulin - 80°C, 0 bar

MKCLLLALAL TCGAQALIVT QTMKGLDIQK VAGTWYSLAM

AASDISLLDA QSAPLRVYVE ELKPTPEGDL EILLQKWENG

ECAQKKIIAE KTKIPAVFKI DALNENKVLV LDTDYKKYLL

FCMENSAEPE QSLACQCLVR TPEVDDEALE KFDKALKALP

H_2 O_2 O_1

MHIRLSFNPT QLEEQCHI

B-Lactoglobulin - 80°C, 350 bar

MKCLLLALAL	TCGAQALIVT	QTMKGLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLRVYVE	ELKPTPEGDL	EILLQKWENG
ECAQKKIIAE	KTKIPAVFKI (CEt, CMe)	DALNENKVLV	LDTDYKKYLL
FCMENSAEPE	QSLACQCLVR	TPEVDDEALE	KFDKALKALP (CMe)
MHIRLSFNPT (O_2)	QLEEQCHI		

AA 1-360

Xanthine dehydrogenase/oxidase – 1-Raw

MTADELVFFV	NGKKVVEKNA	DPETTLLAYL	RRKLGLRGTK	LGCGEGGCGA	CTVMLSKYDR
LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSHG	SQCGFCTPGI
VMSMYTLLRN	QPEPTVEEIE	DAFQGNLCRC	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC
MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRFE	GERVTWIQAS (O_2)
TLKELLDLKA	QHPEAKLVVG	NTEIGIEMKF	KNQLFPMIIC	PAWIPELNAV	EHGPEGISFG
AACALSSVEK	TLLEAVAKLP	TQKTEVFRGV	LEQLRWFAGK	QVKSVASLGG	NIITASPISD

AA 361-720

Xanthine dehydrogenase/oxidase -2- Raw

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

Xanthine dehydrogenase/oxidase – 3-Raw

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

Xanthine dehydrogenase/oxidase – 4-Raw

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

Xanthine dehydrogenase/oxidase – 1-Past

MTADELVFFV NGKKVVEKNA DPETTLLAYL RRKLGLRGTK LGCGEGGCGA CTVMLSKYDR

LQDKIIHFSA NACLAPICTL HHVAVTTVEG IGSTKTRLHP VQERIAKSHG SQCGFCTPGI

VMSMYTLLRN QPEPTVEEIE DAFQGNLCRC TGYRPILQGF RTFAKNGGCC GGNGNNPNCC

O_2

MNQKKDHTVT LSPSLFNPEE FMPLDPTQEP IFPPELLRLK DVPPKQLRFE GERVTWIQAS

TLKELLDLKA QHPEAKLVVG NTEIGIEMKF KNQLFPMIIC PAWIPELNAV EHGPEGISFG

AACALSSVEK TLLEAVAKLP TQKTEVFRGV LEQLRWFAGK QVKSVASLGG NIITASPISD

AA 361-720

Xanthine dehydrogenase/oxidase -2- Past

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

Xanthine dehydrogenase/oxidase – 3-Past

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	ECLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL
IHVYTDGSVL	VSHGGTEMGQ	GLHTKMVQVA	SKALKIPISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1320

Xanthine dehydrogenase/oxidase – 4-Past

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

Xanthine dehydrogenase/oxidase – 1-45°C, 0 bar

MTADELVFFV NGKKVVEKNA DPETTLLAYL RRKLGLRGTK LGCGEGGCGA CTVMLSKYDR

LQDKIIHFSA NACLAPICTL HHVAVTTVEG IGSTKTRLHP VQERIAKSHG SQCGFCTPGI

VMSMYTLLRN QPEPTVEEIE DAFQGNLCRC TGYRPILQGF RTFAKNGGCC GGNGNNPNCC

MNQKKDHTVT LSPSLFNPEE FMPLDPTQEP IFPPELLRLK DVPPKQLRFE GERVTWIQAS (O_2)

TLKELLDLKA QHPEAKLVVG NTEIGIEMKF KNQLFPMIIC PAWIPELNAV EHGPEGISFG

AACALSSVEK TLLEAVAKLP TQKTEVFRGV LEQLRWFAGK (O_2) QVKSVASLGG NIITASPISD

AA 361-720

Xanthine dehydrogenase/oxidase -2- 45°C, 0 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

Xanthine dehydrogenase/oxidase – 3-45°C, 0 bar

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

Xanthine dehydrogenase/oxidase – 4-45°C, 0 bar

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

Xanthine dehydrogenase/oxidase – 1-45°C, 350 bar

MTADELVFFV NGKKVVEKNA DPETTLLAYL RRKLGLRGTK LGCGEGGCGA CTVMLSKYDR

O_2

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 361-720

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

Xanthine dehydrogenase/oxidase – 3-45°C, 350 bar

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

Xanthine dehydrogenase/oxidase – 4-45°C, 350 bar

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

Xanthine dehydrogenase/oxidase – 1-80°C, 0 bar

MTADELVFFV NGKKVVEKNA DPETTLLAYL RRKLGLRGTK LGCGEGGCGA CTVMLSKYDR

LQDKIIHFSA NACLAPICTL HHVAVTTVEG IGSTKTRLHP VQERIAKSHG SQCGFCTPGI

VMSMYTLLRN QPEPTVEEIE DAFQGNLCRC TGYRPILQGF RTFAKNGGCC GGNGNNPNCC

O_2

MNQKKDHTVT LSPSLFNPEE FMPLDPTQEP IFPPELLRLK DVPPKQLRFE GERVTWIQAS

TLKELLDLKA QHPEAKLVVG NTEIGIEMKF KNQLFPMIIC PAWIPELNAV EHGPEGISFG

AACALSSVEK TLLEAVAKLP TQKTEVFRGV LEQLRWFAGK QVKSVASLGG NIITASPISD

AA 361-720

Xanthine dehydrogenase/oxidase -2- 80°C, 0 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

Xanthine dehydrogenase/oxidase – 3-80°C, 0 bar

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

Xanthine dehydrogenase/oxidase – 4-80°C, 0 bar

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