

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 µL of Nile Red (1 mg/mL) and stained for at least 1 h at room temperature. An aliquot of 5 µL stained milk was then mixed with 20 µL 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 nm 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

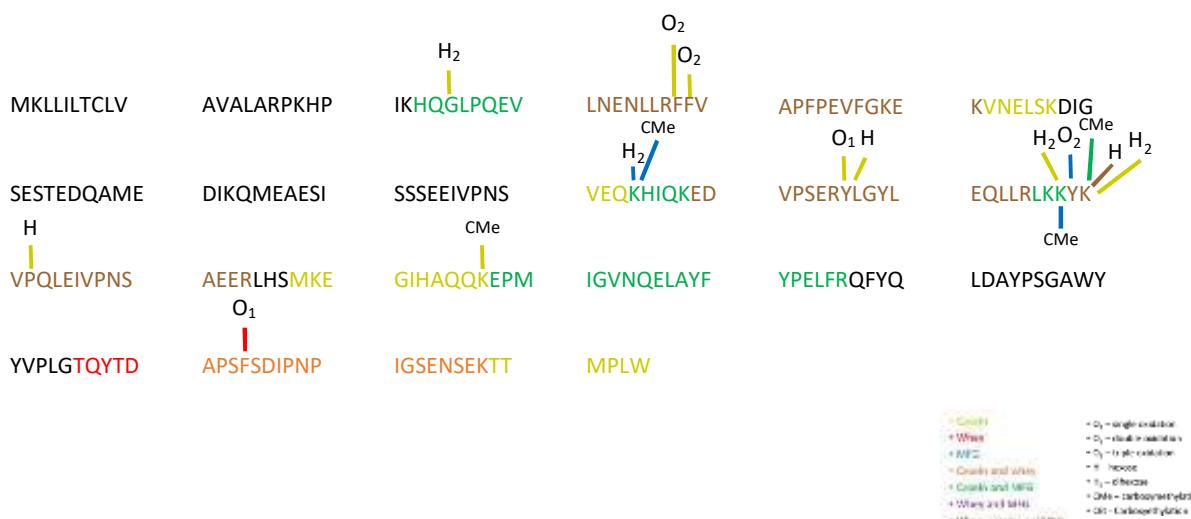
Modification scores, to determine the degree of protein modifications, were determined using an in-house software. Modifications were weighted according to number of modification changes. For example, each deoxidation was multiplied by 2 and trioxidation was multiplied by 3. These weighted scores were 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-Sidak 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.

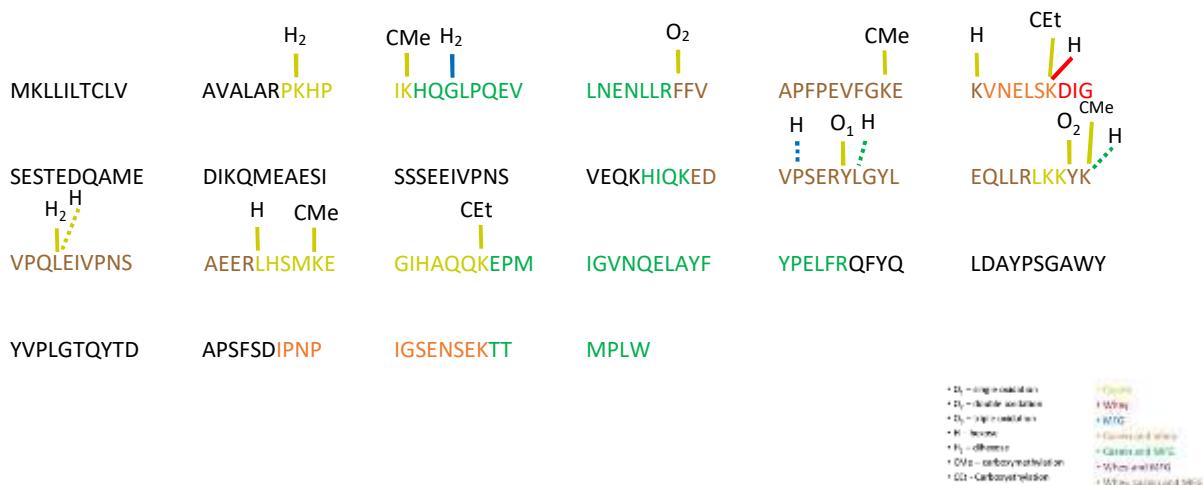
Key

- | | Observed in more than one sample | |
|------------------------|----------------------------------|-------------------------------------|
| • Casein | | • O ₁ – single oxidation |
| • Whey | | • O ₂ – double oxidation |
| • MFG | | • O ₃ – triple oxidation |
| • Casein and whey | | • H – hexose |
| • Casein and MFG | | • H ₂ – dihexose |
| • Whey and MFG | | • CMe – carboxymethylation |
| • Whey, casein and MFG | | • CEt - Carboxyethylation |

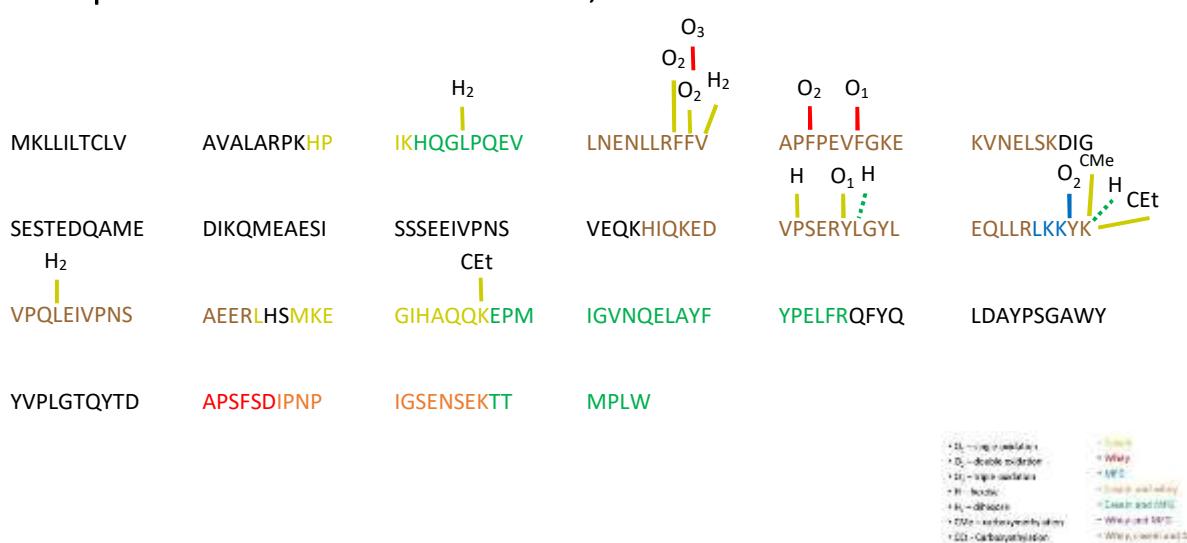
Alpha-S1-casein – Raw



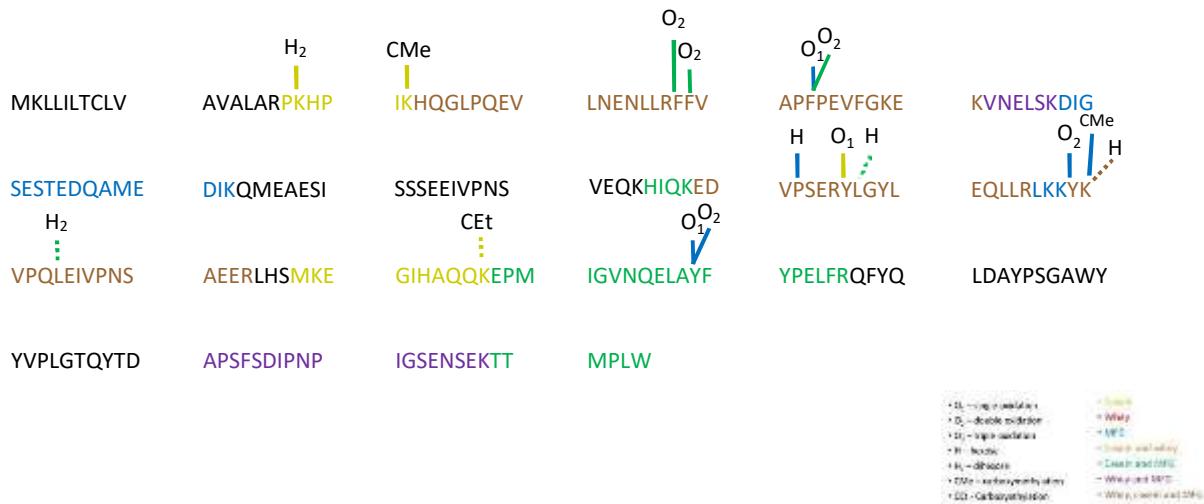
Alpha-S1-casein – Pasteurised



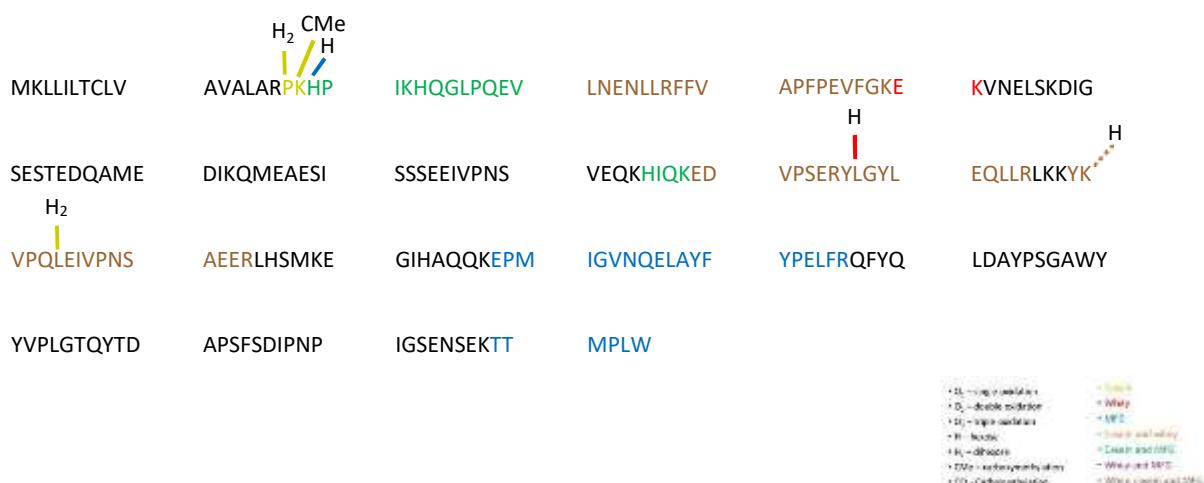
Alpha-S1-casein – 45°C, 0 bar



Alpha-S1-casein – 45°C, 350 bar



Alpha-S1-casein – 80°C, 0 bar



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

MKLLILTCLV	AVALARP KHP	IKHQGLPQE V H	LNE NLLRFF V	APFPEVFGKE	KVN ELSK DIG H2H2 H
SESTEDQAME	DIKQMEAESI	SSEEIVPNS	VEQ KHIQKED	VPSERYLGYL	EQ LLRLKKYK
VPQLEIVPNS	AEER LHS MKE	GIHAQQKEPM	IGVNQELAYF	YPELF FRQFYQ	LDA YPSGAWY
YVPLGTQYTD	APS FS DIPNP	IGSEN SEKTT	MPLW		

• S1 = single substitution
 • D2 = double substitution
 • D3 = triple substitution
 • H = Histidine
 • K = Arginine
 • Q = Asparagine
 • E = Glutamine
 • C = Cysteine
 • Y = Tyrosine
 • W = Tryptophan
 • F = Phenylalanine
 • I = Isoleucine
 • V = Valine
 • A = Alanine
 • M = Methionine
 • P = Proline
 • L = Leucine
 • D = Aspartic acid
 • N = Asparagine
 • G = Glycine
 • R = Arginine
 • S = Serine
 • T = Threonine
 • Cysteine and Methionine
 • Asparagine and Glutamine
 • Tyrosine and Phenylalanine
 • Histidine and Arginine
 • Alanine, Valine, Isoleucine, Leucine
 • Aspartic acid and Asparagine
 • Glutamic acid and Glutamine

Lactadherin (MFGM_Bovine) - Raw

MPCPRLLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	HLGFMGLQR W	APELARLHQT O1	GIVNAWTSGN	YDKNPWIQVN O1	LMRKMWVTGV
VTQGASRAGS	AEYL KTFKV A	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVR LVPI	ICHR GCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYAR LD O1 O3	NQ GKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGV TW	TEY KDPGASE	SKIFPGNMDN	NSHKK NIFET	PFQAR FVRIQ	PVAWHNRITL
RVELLGC					

• S1 = single substitution
 • D2 = double substitution
 • D3 = triple substitution
 • H = Histidine
 • K = Arginine
 • Q = Asparagine
 • E = Glutamine
 • C = Cysteine
 • Y = Tyrosine
 • W = Tryptophan
 • F = Phenylalanine
 • I = Isoleucine
 • V = Valine
 • A = Alanine
 • M = Methionine
 • P = Proline
 • L = Leucine
 • D = Aspartic acid
 • N = Asparagine
 • G = Glycine
 • R = Arginine
 • S = Serine
 • T = Threonine
 • Cysteine and Methionine
 • Asparagine and Glutamine
 • Tyrosine and Phenylalanine
 • Histidine and Arginine
 • Alanine, Valine, Isoleucine, Leucine
 • Aspartic acid and Asparagine
 • Glutamic acid and Glutamine

Lactadherin (MFGM_Bovine) - Past

• O₁ = single substitution
 • O₂ = double substitution
 • O₃ = triple substitution
 • H = hydroxyl
 • K = carbonyl
 • D/G = carboxymethyl residue
 • C/D = carbosypholide
 • E = ester
 • W = Wile
 • M/T = M/T
 • C = C-terminal amide
 • L = Lysine and W/G
 • M/H = Methionine and M/T
 • M/H = Methionine and W/G

MPCPRLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI O ₃
ADSQISASSM	HLGFMGLQRW O ₁ O ₁	APELARLHQ O ₃	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	AEYLKTFKVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELN GC O ₄	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
RVELLGC					

Lactadherin (MFGM_Bovine) -45°C, 0 bar

• O₁ = single substitution
 • O₂ = double substitution
 • O₃ = triple substitution
 • H = hydroxyl
 • K = carbonyl
 • D/G = carboxymethyl residue
 • C/D = carbosypholide
 • E = ester
 • W = Wile
 • M/T = M/T
 • C = C-terminal amide
 • L = Lysine and W/G
 • M/H = Methionine and M/T
 • M/H = Methionine and W/G

MPCPRLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	HLGFMGLQRW	APELARLHQ	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	AEYLKTFKVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL O ₁ O ₂
ETQYVRLVPI	ICHRGCTLRF	ELLGCELN GC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
RVELLGC					

Lactadherin (MFGM_Bovine) - 45°C, 350 bar

• D₁ = single residue
 • D₂ = double residue
 • D₃ = triple residue
 • H = Histidine
 • K = Glutamine
 • D/G = Aspartic acid/Asparagine
 • E/R = Glutamic acid/Glutamine
 • C/S/T = Cysteine/Cysteine
 • W/F = Tryptophan/Phenylalanine
 • M/V/I = Methionine/Isoleucine/Valine
 • Y/L = Tyrosine/Leucine
 • P = Proline
 • A = Alanine
 • G = Glycine
 • N = Asparagine
 • Q = Glutamine
 • H = Histidine
 • K = Glutamine
 • D/G = Aspartic acid/Asparagine
 • E/R = Glutamic acid/Glutamine
 • C/S/T = Cysteine/Cysteine
 • W/F = Tryptophan/Phenylalanine
 • M/V/I = Methionine/Isoleucine/Valine
 • Y/L = Tyrosine/Leucine
 • P = Proline
 • A = Alanine
 • G = Glycine

MPCPRLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	HLGFMGLQRW	APELARLHQQT	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

• D₁ = single residue
 • D₂ = double residue
 • D₃ = triple residue
 • H = Histidine
 • K = Glutamine
 • D/G = Aspartic acid/Asparagine
 • E/R = Glutamic acid/Glutamine
 • C/S/T = Cysteine/Cysteine
 • W/F = Tryptophan/Phenylalanine
 • M/V/I = Methionine/Isoleucine/Valine
 • Y/L = Tyrosine/Leucine
 • P = Proline
 • A = Alanine
 • G = Glycine
 • N = Asparagine
 • Q = Glutamine
 • H = Histidine
 • K = Glutamine
 • D/G = Aspartic acid/Asparagine
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 • W/F = Tryptophan/Phenylalanine
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 • A = Alanine
 • G = Glycine

MPCPRLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	HLGFMGLQRW	APELARLHQQT	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, 350 bar

• D₁ - single methylation
 • D₂ - double methylation
 • D₃ - triple methylation
 • H - hydroxyl
 • K₁ - dibasic
 • D₁₂ - methoxy methylation
 • CMe - carbamoylation

• Lysine
 • Tyrosine
 • MTC
 • Gluconidic acid
 • Galactosidic acid
 • Glucosidic acid
 • Methyl glucosidic acid
 • Methyl galactosidic acid

MPCPRLAAL	FCSSGLFAAS	GDFCDSSLCL	HGGTCLLNED	RTPPFYCLCP	EGFTGLLCNE
TEHGPCFPNP	CHNDAECQVT	DDSHRGDVFI	QYICKCPLGY	VGIHCETTCT	SPLGMQTGAI
ADSQISASSM	H ₁ GF ₂ M ₁ GLQRW	APELARLHQ ₂ T	GIVNAWTSGN	YDKNPWIQVN	LMRKMWVTGV
VTQGASRAGS	A ₁ EYLKTF ₂ KVA	YSTDGRQFQF	IQVAGRSGDK	IFIGNVNNSG	LKINLFDTPL
ETQYVRLVPI	ICHRGCTLRF	ELLGCELNGC	TEPLGLKDNT	IPNKQITASS	YYKTWGLSAF
SWFPYYARLD	NQGKFNAWTA	QTNSASEWLQ	IDLGSQKRVT	GIITQGARDF	GHIQYVAAYR
VAYGDDGVTW	TEYKDPGASE	SKIFPGNMDN	NSHKKNIFET	PFQARFVRIQ	PVAWHNRITL
RVELLG					

B-Lactoglobulin - Raw

• D₁ - single methylation
 • D₂ - double methylation
 • D₃ - triple methylation
 • H - hydroxyl
 • K₁ - dibasic
 • D₁₂ - methoxy methylation
 • CMe - carbamoylation

• Lysine
 • Tyrosine
 • MTC
 • Gluconidic acid
 • Galactosidic acid
 • Glucosidic acid
 • Methyl glucosidic acid
 • Methyl galactosidic acid

MKCLLLALAL	TCGAQALIVT	QTMKGLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLRVYVE	ELKPTPEGDL	EILLQKWENG
H ECAQKKIIAE	C _{Et} C _{Et} ^{H₂} KTKIPAVFKI	CMe DALNENKVLV	LDTDYKKYLL
FCMENSAEPE	QLACQCLVR	TPEVDDEALE	KFDKALKALP
MHIRLSFNPT	QLEEQCHI		

B-Lactoglobulin - Past

MKCLLLALAL	TCGAQALIVT	QTMKGLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLRVYVE	ELKPTPEGDL	EILLQKWENG
ECAQKKIIAE	KTKIPAVFKI	DALNENKVLV	LDTDYKKYLL
FCMENSEAEP O ₁ MHIRLSFNPT	QLACQCLVR H O ₂	TPEVDDEALE	KFDKALKALP

- D₁ = single methylation
- D₂ = double methylation
- D₃ = triple methylation
- H = hydroxyl
- K₁ = dibasic
- D₁D₂ = methoxy methylation
- CMe = carbamoylation
- Lysine
- Alanine
- Methionine
- Arginine
- Tyrosine
- Glutamine and Asparagine
- Aspartic acid and Glutamic acid
- Methionine and MTO
- Methionine and MTO

B-Lactoglobulin - 45°C, 0 bar

MKCLLLALAL	TCGAQALIVT	QTMKGLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLRVYVE	ELKPTPEGDL	EILLQKWENG
ECAQKKIIAE	KTKIPAVFKI	DALNENKVLV	LDTDYKKYLL
FCMENSEAEP O ₂ MHIRLSFNPT	QLACQCLVR H O ₃	TPEVDDEALE	KFDKALKALP

- D₁ = single methylation
- D₂ = double methylation
- D₃ = triple methylation
- H = hydroxyl
- K₁ = dibasic
- D₁D₂ = methoxy methylation
- CMe = carbamoylation
- Lysine
- Alanine
- Methionine
- Arginine
- Tyrosine
- Glutamine and Asparagine
- Aspartic acid and Glutamic acid
- Methionine and MTO
- Methionine and MTO

B-Lactoglobulin - 45°C, 350 bar

MKCLLLALAL	TCGAQALIVT	QTM K GLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLR VYVE	ELKPTPEGDL	EILLQ KWENG
ECAQKKII AE	KTKIPAVFKI	DALNENK VLV	LDTDYKKYLL
FCMENSAEPE	QSLACQCLV R	TPEVDDALE	KFDK ALKALP
O_2		CMe	CEt
MHIRLSFNPT	QLEEQCHI		

- L - single methionine
- D₂ - double methionine
- D₁ - triple methionine
- H - histidine
- K₂ - dilysine
- D₂₁ - methionine residue
- CMe - carbamoylmethionine
- CEt - carbamoylalanine
- Wres -
- MTC -
- ExoH -
- Wres -
- MTC -
- ExoH and Wres -
- Lysine and Wres -
- Methionine and MTC -
- Methionine and Wres -

B-Lactoglobulin - 80°C, 0 bar

MKCLLLALAL	TCGAQALIVT	QTM K GLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLR VYVE	ELKPTPEGDL	EILLQ KWENG
ECAQ K KIIAE	KTKIPAVFKI	DALNENK VLV	LDTDYKKYLL
FCMENSAEPE	QSLACQCLVR	TPEVDDALE	KFDK ALKALP
H ₂	O_2O_1		
MHIRLSFNPT	QLEEQCHI		

- L - single methionine
- D₂ - double methionine
- D₁ - triple methionine
- H - histidine
- K₂ - dilysine
- D₂₁ - methionine residue
- CMe - carbamoylmethionine
- CEt - carbamoylalanine
- Wres -
- MTC -
- ExoH -
- Wres -
- MTC -
- ExoH and Wres -
- Lysine and Wres -
- Methionine and MTC -
- Methionine and Wres -

B-Lactoglobulin - 80°C, 350 bar

• D₁ = single methionine
 • D₂ = double methionine
 • D₃ = triple methionine
 • P = Proline
 • H₁ = histidine
 • D₂H = histidine methionine
 • C_{Et} = carbamoyl ethyl side chain
 • CMe = carbamoyl methyl side chain
 • D₁W = tryptophan
 • W = Tryptophan
 • MTC = methionine residue
 • Easylab and WIC = Methionine residue
 • MHC and MTC = Methionine residue and WIC

MKCLLLALAL	TCGAQALIVT	QTM K GLDIQK	VAGTWYSLAM
AASDISLLDA	QSAPLR V YVE	ELKPTPEGDL	EILLQKWENG
ECAQ K KIIAE	^{CEt} KTKIPAVFKI	^{CMe}	DALNENKVLV
FCMENSAEPE	QSLACQCLVR	TPEVDDALE	LDTDYKKYLL
^{O₂} MHIRLSFNPT	QLEEQCHI		^{CMe} KFD K ALK ALP

AA 1-360

Xanthine dehydrogenase/oxidase – 1-Raw

• D₁ = single methionine
 • D₂ = double methionine
 • D₃ = triple methionine
 • P = Proline
 • H₁ = histidine
 • D₂H = histidine methionine
 • C_{Et} = carbamoyl ethyl side chain
 • CMe = carbamoyl methyl side chain
 • D₁W = tryptophan
 • W = Tryptophan
 • MTC = methionine residue
 • Easylab and WIC = Methionine residue
 • MHC and MTC = Methionine residue and WIC

MTADELVFFV	NGKKVVEKNA	DPETTLLAYL	RRKLGLRGTK	LGCGE ^G CGA	CTVMLSKYDR
LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSHG	SQCGFCTPGI
VMSMYTLLRN	QPEPTVEEIE	DAFQGNLCRC	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC
MNQKKDHTVT	LSPSLFNPEE	FMP ^L DPTQEP	IFPP ^E ELLRLK	DVPPKQLRFE	GER V TWIQAS
TLKELLDLK	QHPEAKLVVG	NTEIGIEMKF	KNQLFPMIIC	PAWIPELN ^A	EHGPEGISFG
AACALSSVEK	TLLEAVAKLP	TQKTEVFR G V	LEQLRW ^F FAGK	QVK S VASLG ^G	NIITASPISD

AA 361-720

Xanthine dehydrogenase/oxidase -2- Raw

+ D ₁ = single residue	- Exons
+ D ₂ = double residue	+ Wt/Sp
+ D ₃ = triple residue	+ MTC
+ H = Histidine	= Exons and splice
+ K = Glutamine	+ Exons and Wt/Sp
+ D ₄ = tetraresidue residue	+ MTC and MTC
+ GLC = Carboxyterminus	+ Wt/Sp, Exons and Wt/Sp

LNPVF <small>M</small> ASGT	KLTIVSRGTR	RTVPM <small>D</small> H <small>T</small> FF	PSYRK <small>T</small> LLGP	EEILLSIEIP	YSREDEFFSA
FKQASR <small>R</small> REDD	IAKVTGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSKFWNE
KLLQDV <small>C</small> AGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCGKLDP
TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDIPR <small>Y</small> E
NELFLRLVTS	TRAHAKIKSI	DVSEAQK <small>V</small> PG	FVCFLSADDI	PGSNETGLFN	DETVFAKDTV
TCVGHIIGAV	VADTPEHAER	AAHVVK <small>V</small> TYE	DLPAIITIED	AIKNNSFYGS	ELK <small>I</small> EKGDLK

AA 721-1080

Xanthine dehydrogenase/oxidase – 3-Raw

+ D ₁ = single residue	- Exons
+ D ₂ = double residue	+ Wt/Sp
+ D ₃ = triple residue	+ MTC
+ H = Histidine	= Exons and splice
+ K = Glutamine	+ Exons and Wt/Sp
+ D ₄ = tetraresidue residue	+ MTC and MTC
+ GLC = Carboxyterminus	+ Wt/Sp, Exons and Wt/Sp

KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNAME	KTQSFVAKML
GVPVNRLILVR	VKRMGGGF <small>G</small> GG	KETRSTLVSV	AVALAAYKTG	HPVRCMLDRN	EDMLITGGRH
PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLHSIMER	ALFHMDNCYK	IPNIRGTGRL
CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQRLE
GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL
IHVYTDSLVL	VSHGGTEMGQ	GLHTK <small>M</small> VQVA	SKALKIPIISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1320

Xanthine dehydrogenase/oxidase – 4-Raw

• D₁ = single residue
• D₂ = double residue
• D₃ = triple residue
• H = Histidine
• K = Lysine
• D₄ = Aspartic acid
• E₁ = Glutamic acid
• C = Cysteine
• W = Tryptophan
• MTC = Methionine residue
• E = Tyrosine residue
• F = Phenylalanine
• G = Glycine
• A = Alanine and Valine
• S = Serine and Threonine
• P = Proline
• I = Isoleucine and Leucine
• R = Arginine
• Y = Tyrosine and Phenylalanine
• D = Asparagine and Aspartate
• N = Glutamine and Glutamate

VSTD IY GQAV	YEACQT IL KR	LEPFKKKNPD	GSWEDWVMAA	YQDR V SLTT	GFYRTPNLGY
SFETNSGN AF	HYFTYGVACS	EVEIDCLTG D	HKNLRTDIVM	DVGSSLNP AI	DIGQVEGAFV
QGLGLFTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRD CP NK	KAIYASK AVG
EPPLFLGASV	FFAIKDAIR A	ARAQHTNNNT	KELFRLDSPA	TPEKIRNA CV	DKFTTLCVTG
APGNCKPWSL	RV				

AA 1-360

Xanthine dehydrogenase/oxidase – 1-Past

• D₁ = single residue
• D₂ = double residue
• D₃ = triple residue
• H = Histidine
• K = Lysine
• D₄ = Aspartic acid
• E₁ = Glutamic acid
• C = Cysteine
• W = Tryptophan
• MTC = Methionine residue
• E = Tyrosine residue
• F = Phenylalanine
• G = Glycine
• A = Alanine and Valine
• S = Serine and Threonine
• P = Proline
• I = Isoleucine and Leucine
• R = Arginine
• Y = Tyrosine and Phenylalanine
• D = Asparagine and Aspartate
• N = Glutamine and Glutamate

M TADELVFFV	NG KKVV EKNA	DPET TLLAYL	RRKL GLRGTK	LGC GEGGCCA	CTVMLS KYDR
LQDKIIHFSA	NACLAPICTL	HHVA VTT VEG	IGSTKTRLHP	VQERIAKSHG	SQCGFCTPGI
VMSMYTLLRN N	QPEPTV EEIE	DAFQGN LCR C	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC O₂
MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPP ELLRLK	DVPPKQLRFE	GER VTWIQAS
TLKELLDLKA	QHPEAK LVVG	NTEIGIEM KF	KNQLFP MIIC	PAWIPELN AV	EHGPEGISFG
AACALSSVEK	TLLEAVAKLP	TQKTEVFR GV	LEQLRW FAGK	QVKSVASLGG	NIITASPISD

AA 361-720

Xanthine dehydrogenase/oxidase -2- Past

+ D ₁ = single residue	- Exons
+ D ₂ = double residue	+ Wtseq
+ D ₃ = triple residue	+ MTC
+ H = Histidine	+ Exons and introns
+ K = Glutamine	+ Exons and Wtseq
+ D ₄ = methionine residue	+ Methionine MTC
+ C ₁ = Carboxyterminus	+ Methionine, carboxy and Wtseq

LNPVFMASGT	KLTIVSRGTR	RTVPMMDHTFF	PSYRK TLLGP	EEILLSIEIP	YSREDEFFSA
FK QASRREDD	IAKVTCGM RV	LF QPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSK FWNE
K LLQDVCAGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCGKLDP
TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDIPR YE
N EFLRLVTS	TRAHAKIKSI	D VSEA QK VPG	FVCFLSADDI	PGSNETGLFN	DETVFAKDTV
TCVGHIIGAV	VADTPEHAER	AAHVVK VTYE	D LPAIITIED	AIKNNSFYGS	EL KIEKGDLK

AA 721-1080

Xanthine dehydrogenase/oxidase – 3-Past

+ D ₁ = single residue	- Exons
+ D ₂ = double residue	+ Wtseq
+ D ₃ = triple residue	+ MTC
+ H = Histidine	+ Exons and introns
+ K = Glutamine	+ Exons and Wtseq
+ D ₄ = methionine residue	+ Methionine MTC
+ C ₁ = Carboxyterminus	+ Methionine, carboxy and Wtseq

KGFSEADNVV	SGELYIGGQD	HFILETHCTI	AIPKGEEGEM	ELFVSTQNAME	KTQSFVAKML
GVPVNRLILVR	VKRMGGGF _{GG}	KETR STLVSV	AVALAAY KTG	HPVRCMLDRN	EDMLITGGRH
PFLARYKVGF	M K TGTIVALE	V DHYSNAGNS	RDLHSIMER	ALFHMDNCYK	IPNIRGTGRL
CKTNLSSNTA	F RGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQR LE
GFSVPRCWDE	ECLKSSQYYAR	KSEVDKFNKE	NCWKKR GLCI	IPTKFGISFT	VPFLNQAGAL
IHVYTDSLVL	VSHGGTEMGQ	GLHTK MVQVA	SKALKIPIISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1320

Xanthine dehydrogenase/oxidase – 4-Past

- D₁ = single residue
- D₂ = double residue
- D₃ = triple residue
- H = Histidine
- K = Lysine
- C = Cysteine
- D/G = Aspartic acid/Asparagine
- E/R = Glutamic acid/Glutamine
- W/F = Tryptophan/Phenylalanine
- M/P = Methionine/Proline
- Y/V = Tyrosine/Valine

VSTDIFYGQAV	YEACQTILKR	LEPFKKKNPD	GSWEDWVMAA	YQDR V SLTT	G FYRTPNLGY
SFETNSGNAF	HYFTYGVACS	EVEIDCLTGD	HKNLRTDIVM	DVGSSLNPAl	DIGQVEGAFV
QGLGLFTLEE	LHYSPEGSLH	TRGPSTYK I P	A FGSIPTEFR	VSLLRDCPNK	KAIYASK AVG
EPPLFLGASV	FFAIKDAIRA	ARAQHTNNNT	KELFRLDSPA	TPE K RNACV	DK F TTLCVTG
APGNCKPWSL	R V				

AA 1-360

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

- D₁ = single residue
- D₂ = double residue
- D₃ = triple residue
- H = Histidine
- K = Lysine
- C = Cysteine
- D/G = Aspartic acid/Asparagine
- E/R = Glutamic acid/Glutamine
- W/F = Tryptophan/Phenylalanine
- M/P = Methionine/Proline
- Y/V = Tyrosine/Valine

MTADELVFFV	N GKKVVEKNA	DPETTLLAYL	RRKLGRLGTK	LGC G EGCGA	CTVMLSKYDR
LQDKIIHFSA	NACLAPICTL	HHVAVTTVEG	IGSTKTRLHP	VQERIAKSHG	SQCGFCTPGI
VMSMYTLLRN	QPEPTVEEIE	DAF Q GNLRC	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC O ₂
MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRFE	GER V TWIQAS
TLKELLDLK	QHPEAK L VVG	NTEIGIEMKF	KNQLFPMIIC O ₂	PAWIPELN	EHGPEGISFG
AACALSSVEK	TLLEAVAKLP	TQKTEVFR G V	LEQLRW F AGK	QVKSVASLGG	NIITASPISD

AA 361-720

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

• D₁ = single residue
• D₂ = double residue
• D₃ = triple residue
• P₁ = Proline
• H₂ = Histidine
• D₇₂ = Aspartic acid residue
• G₁₂ = Glutamic acid residue

• E₁ = Glutamate
• W₁ = Tryptophan
• M₁ = Methionine
• C₁ = Cysteine and cysteine residue
• L₁ = Leucine and Valine
• V₁ = Alanine and Isoleucine
• A₁ = Alanine and Valine
• F₁ = Phenylalanine and Tyrosine
• Y₁ = Tyrosine and Phenylalanine
• S₁ = Serine and Threonine
• T₁ = Threonine and Serine
• D₁₂ = Asparagine and Aspartate
• G₇₂ = Glutamine and Glutamate

LNPVF <small>M</small> ASGT	KLTIVSRGTR	RTVPM <small>D</small> H <small>T</small> FF	PSYRK <small>T</small> LLGP	EEILLSIEIP	YSREDEFFSA
FKQASR <small>R</small> REDD	IAKVTGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSKFWNE
KLLQDVCAGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCGKLDP
TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDIPR <small>Y</small> E
NELFLRLVTS	TRAHAKIKSI	DVSEAQK <small>V</small> PG	FVCFLSADDI	PGSNETGLFN	DETVFAKDTV
TCVGHIIGAV	VADTPEHAER	AAHVVK <small>V</small> TYE	DLPAIITIED	AIKNNSFYGS	ELK <small>I</small> EKGDLK

AA 721-1080

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

• D₁ = single residue
• D₂ = double residue
• D₃ = triple residue
• P₁ = Proline
• H₂ = Histidine
• D₇₂ = Aspartic acid residue
• G₁₂ = Glutamic acid residue

• E₁ = Glutamate
• W₁ = Tryptophan
• M₁ = Methionine
• C₁ = Cysteine and cysteine residue
• L₁ = Leucine and Valine
• V₁ = Alanine and Isoleucine
• A₁ = Alanine and Valine
• F₁ = Phenylalanine and Tyrosine
• Y₁ = Tyrosine and Phenylalanine
• S₁ = Serine and Threonine
• T₁ = Threonine and Serine
• D₁₂ = Asparagine and Aspartate
• G₇₂ = Glutamine and Glutamate

KGFSEADNVV	SGELYIGGQD	HFILETHCTI	AIPKGEEGEM	ELVSTQNAME	KTQSFVAKML
GVPVNRLILVR	VKRMGGGF <small>G</small> GG	KETRSTLVSV	AVALAAYKTG	HPVRCMILDRN	EDMLITGGRH
PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLHSIMER	ALFHMDNCYK	IPNIRGTGRL
CKTNLSSNTA	FRGFGGPOAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQRLE
GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL
IHVYTDSLVL	VSHGGTEMGQ	GLHTK <small>M</small> VQVA	SKALKIPISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1320

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

- D_1 - single substituent
- D_2 - double substituent
- D_3 - triple substituent
- E_1 - ketone
- E_2 - aldehyde
- EWG - electron-withdrawing group
- LBG - leaving group

VSTDIYQQAV	YEACQTLKRV	LEPFKKKKNPD	GSWEDWVMAA	YQDRVSLSTT	GFYRTPNLGY
SFETNSGNAF	HYFTYGVACS	EVEIDCLTGD	HKNLRTDIVM	DVGSSLNPAT	DIGQVEGAFV
QGLGLFTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRDCPNK	KAIYASKAVG
EPPLFLGASV	FFAIKDARA	ARAQHTNNNT	KELFRLDSPA	TPEKIRNACV	DKFTTLCVTG
APGNCKPWSL	RV				

AA 1-360

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

- Si = single covalent
- Di = double covalent
- Tri = triple covalent
- R = Resonance
- H₂ = diatomic
- 2H₂ = tetraatomic molecule
- CH₃ = carbon atom bonded to three hydrogen atoms

MTADELVFFV	NGKKVVEKNA	DPETTLAYL	RRKLGLRGTK	LGCGEGGCGA	CTVMLSKYDR
O ₂ LQDKIIHFS	NACLAPICTL	HHAVTTVEG	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	NIITASPID

AA 361-720

Xanthine dehydrogenase/oxidase -2- 45°C, 350 bar

• D₁ = single substitution
• D₂ = double substitution
• D₃ = triple substitution
• H = Histidine
• R = Arginine
• C = Cysteine
• G = Glycine
• D = Aspartic acid
• E = Glutamic acid
• W = Tryptophan
• M = Methionine
• Y = Tyrosine
• F = Phenylalanine
• I = Isoleucine and Valine
• L = Leucine and Alanine
• V = Alanine and Valine
• P = Proline
• S = Serine
• T = Threonine
• N = Asparagine
• Q = Glutamine
• K = Lysine
• D₄ = carbonyl substitution
• D₅ = carbonyl reduction

LNPVFMASGT	KLTIVSRGTR	RTVPMMDHTFF	PSYRKTLGP	EEILLSIEIP	YSREDEFFSA
FKQASRREDD	IAKVTGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSKFWNE
KLLQDVCAGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCGKLDP
TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDIPRYE
NELFLRLVTS	TRAHAKIKSI	DVSEAQKVPG	FVCFLSADDI	PGSNETGLFN	DETVFAKDTV
TCVGHIIGAV	VADTPEHAER	AAHVVVKVTYE	DLPAIITIED	AIKNNSFYGS	ELKIEKGDLK

AA 721-1080

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

• D₁ = single substitution
• D₂ = double substitution
• D₃ = triple substitution
• H = Histidine
• R = Arginine
• C = Cysteine
• G = Glycine
• D = Aspartic acid
• E = Glutamic acid
• W = Tryptophan
• M = Methionine
• Y = Tyrosine
• F = Phenylalanine
• I = Isoleucine and Valine
• L = Leucine and Alanine
• V = Alanine and Valine
• P = Proline
• S = Serine
• T = Threonine
• N = Asparagine
• Q = Glutamine
• K = Lysine
• D₄ = carbonyl substitution
• D₅ = carbonyl reduction

KGFSEADNVV	SGELYIGGQD	HFILETHCTI	AIPKGEEGEM	ELFVSTQNAME	KTQSFVAKML
GVPVNRLILVR	VKRMGGGF GG	KETRSTLVSV	AVALAAYKTG	HPVRCMLDRN	EDMLITGGRH
PFLARYKVGF	MKTGTIVALE	VDHYSNAGNS	RDLHSIMER	ALFHMDNCYK	IPNIRGTGRL
CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQRLE
GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL
IHVYTDSVL	VSHGGTEMGQ	GLHTKMQVVA	SKALKIPISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1320

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

Legend:
• D₁ = single methionine
• D₂ = double methionine
• D₃ = triple methionine
• H = Histidine
• I = Isoleucine
• M = Methionine
• V = Valine and Isoleucine
• W = Tryptophan and Tyrosine
• Y = Tyrosine and Methionine
• C = Cysteine and Methionine
• E = Glutamate and Aspartate
• F = Phenylalanine
• G = Glycine
• K = Lysine and Arginine
• P = Alanine and Serine
• R = Arginine
• S = Alanine and Threonine
• T = Threonine and Serine
• U = Cysteine and Methionine
• X = Methionine and Tyrosine

VSTD I YGQAV	YEACQT I LKR	LEPFKKKNPD	GSWEDWVMAA	YQDR V SLSTT	GFYRTPNLGY
SFETNSGN A F	HYFTYGVACS	EVEIDCLTG D	HKNLRTDIVM	DVGSSLNP A I	DIGQVEGAFV
QGLGLFTLEE	LHYSPEGSLH	TRGPSTYKIP	AFGSIPTEFR	VSLLRD C PNK	KAIYASK A VG
EPPLFLGASV	FFAI K DAIRA	ARAQHTNNNT	KELFRLDSPA	TPEKIRNA C V	DKFTTLCVTG
APGNCKPWSL	RV				

AA 1-360

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

Legend:
• D₁ = single methionine
• D₂ = double methionine
• D₃ = triple methionine
• H = Histidine
• I = Isoleucine
• M = Methionine
• V = Valine and Isoleucine
• W = Tryptophan and Tyrosine
• Y = Tyrosine and Methionine
• C = Cysteine and Methionine
• E = Glutamate and Aspartate
• F = Phenylalanine
• G = Glycine
• K = Lysine and Arginine
• P = Alanine and Serine
• R = Arginine
• S = Alanine and Threonine
• T = Threonine and Serine
• U = Cysteine and Methionine
• X = Methionine and Tyrosine

M T ADELVFFV	NGKKVV E KNA	DPET T LLAYL	RRKLGLRGTK	LGC G EGGCGA	CTVMLSKYDR
LQDKIIHFSA	NACLAPICTL	HHVA V TTVEG	IGSTKTRLHP	VQERIAKSH G	SQCGFCTPGI
VMSMYTLLRN	QPEPTVEEIE	DAFQ G NLCR C	TGYRPILQGF	RTFAKNGGCC	GGNGNNPNCC 
MNQKKDHTVT	LSPSLFNPEE	FMPLDPTQEP	IFPPELLRLK	DVPPKQLRFE	GER V TWIQAS 
TLKELL D LKA	QHPEAK L VVG	NTEIGIEMKF	KNQLFP M IIC	PAWIPELN A V	EHGPEGISFG
AACALSSVEK	TLLEAVAKLP	TQKTEVFR G V	LEQLRW F AGK	QVK S VASLG G	NIITASPISD

AA 361-720

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

+ D ₁ = single residue	- D ₁ = double residue
+ D ₂ = triple residue	- D ₂ = double residue
+ P ₁ = Proline	- P ₁ = Proline
+ H ₁ = Histidine	- H ₁ = Histidine
+ D ₃ = methionine thioether	- D ₃ = methionine thioether
+ C ₁ = carbamoyl residue	- C ₁ = carbamoyl residue

LNPVF <small>M</small> ASGT	KLTIVSRGTR	RTVPM <small>D</small> H <small>T</small> FF	PSYRK <small>T</small> LLGP	EEILLSIEIP	YSREDEFFSA
FKQASR <small>R</small> REDD	IAKVTGMRV	LFQPGSMQVK	ELALCYGGMA	DRTISALKTT	QKQLSKFWNE
KLLQDV <small>C</small> AGL	AEELSLSPDA	PGGMIEFRRT	LTLSFFFKFY	LTVLKKLGKD	SKDKCGKLDP
TYTSATLLFQ	KDPPANIQLF	QEVPNGQSKE	DTVGRPLPHL	AAAMQASGEA	VYCDDIPR <small>Y</small> E
NELFLRLVTS	TRAHAKIKSI	DVSEAQK <small>V</small> PG	FVCFLSADDI	PGSNETGLFN	DETVFAKDTV
TCVGHIIGAV	VADTPEHAER	AAHVVK <small>V</small> TYE	DLPAIITIED	AIKNNSFYGS	ELK <small>I</small> EKGDLK

AA 721-1080

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

+ D ₁ = single residue	- D ₁ = double residue
+ D ₂ = triple residue	- D ₂ = double residue
+ P ₁ = Proline	- P ₁ = Proline
+ H ₁ = Histidine	- H ₁ = Histidine
+ D ₃ = methionine thioether	- D ₃ = methionine thioether
+ C ₁ = carbamoyl residue	- C ₁ = carbamoyl residue

KGFSEADNVV	SGELYIGGQD	HFYLETHCTI	AIPKGEEGEM	ELFVSTQNAME	KTQSFVAKML
GVPVN <small>R</small> ILVR	VKRMGGGF <small>G</small>	KETRSTLVSV	AVALAAYKTG	HPVRCMLDRN	EDMLITGGRH
PFLARYKVGF	MKTG <small>T</small> IVALE	VDHYSNAGNS	RDLHSIMER	ALFHMDNCYK	IPNIRGTGRL
CKTNLSSNTA	FRGFGGPQAL	FIAENWMSEV	AVTCGLPAEE	VRWKNMYKEG	DLTHFNQRLE
GFSVPRCWDE	CLKSSQYYAR	KSEVDKFNKE	NCWKKRGLCI	IPTKFGISFT	VPFLNQAGAL
IHVYTDSLVL	VSHGGTEMGQ	GLHTK <small>M</small> VQVA	SKALKIPIISK	IYISETSTNT	VPNSSPTAAS

AA 1081-1332

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

+ D ₁ – single residue	- E ₁ –
+ D ₂ – double residue	- E ₂ –
+ D ₃ – triple residue	- E ₃ –
+ P ₁ – Proline	- F ₁ –
+ H ₁ – Histidine	- G ₁ –
+ D ₄ – Aspartic acid residue	- L ₁ – Leucine and Isoleucine
+ S ₁ – Cysteine/thiol	- M ₁ – Methionine and Tryptophan
+ C ₁ – Cysteine/cysteine	- W ₁ –
+ A ₁ – Alanine	- K ₁ –
+ T ₁ – Threonine	- D ₁ –
+ V ₁ – Valine	- E ₂ –
+ N ₁ – Asparagine	- F ₂ –
+ Y ₁ – Tyrosine	- G ₂ –
+ D ₅ – Aspartic acid residue	- L ₂ – Leucine and Isoleucine
+ H ₂ – Histidine	- M ₂ – Methionine and Tryptophan
+ D ₆ – Aspartic acid residue	- W ₂ –
+ S ₂ – Cysteine/cysteine	- K ₂ –
+ C ₂ – Cysteine/cysteine	- D ₃ –
+ A ₂ – Alanine	- E ₃ –
+ T ₂ – Threonine	- F ₃ –
+ V ₂ – Valine	- G ₃ –
+ N ₂ – Asparagine	- L ₃ – Leucine and Isoleucine
+ Y ₂ – Tyrosine	- M ₃ – Methionine and Tryptophan

VSTD IYGQAV	YEAC QTILKR	LEPF KKKNPD	GSWEDWVMAA	YQDR VSLSTT	GFYRTPNLGY
SFETNSGN AF	HYFT YGVACS	EVEIDCL TG D	HKNLRTD IVM	DVGSSLNP AI	DIGQVEGA FV
QGLGLFT LEE	LHYSPEG SLH	TRGPSTY KIP	AFGSIPTE FR	VSLLRD CPNK	KAIYASK AVG
EPPLFLGAS V	FFAI KDAIRA	ARAQHTNN NNT	KELFRLD SPA	TPEKIRNA CV	DK FTTLCVTG
APGNCKPWS L	RV				