Supplementary information

Characterization of two VAO-type flavoprotein oxidases from *Myceliophthora thermophile*

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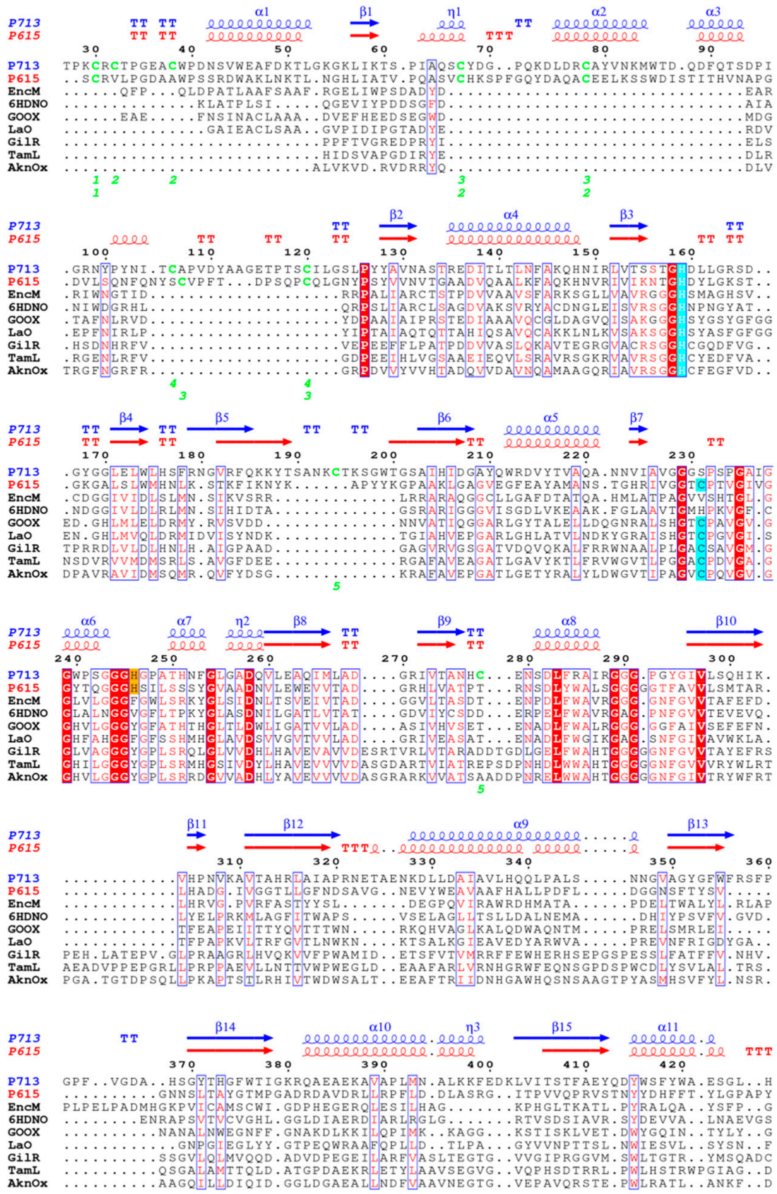
P713
P615
P713 TPKCRCTPGEACWPDNSVWEAFDKTLGKGKLIKTS.PIAQSCYDG..PQKDLDRCAYVNKMWTD.QDFQTSDPI
P615 ..SCRVLPGDAAWPSSRDWAKLNKTL.NGHLIATV.PQASVCHKSPFGQYDAQACEELKSSWDISTITHVNAPG
EncM ......QFP...QLDPATLAAFSAAF.RGELIWPSDADYD.............................EAR
6HDNO .............KLATPLSI.....QGEVIYPDDSGFD..............................AIA
GOOX ......EAE.....FNSINACLAAA.DVEFHEEDSEGWD..............................MDG
LaO ..............GAIEACLSAA.GVPIDIPGTADYE..............................RDV
GilR ..........................PPFTVGREDPRYI..............................ELS
TamL ..........................HIDSVAPGDIRYE..............................DLR
AknOx ..........................ALVKVD.RVDRRYQ..............................DLV
P713 .GRNYPYNI.TCAPVDYAAGETPTSCILGSLPYYAVNASTREDITLTLNFAKQHNIRLVTSSTGHDLLGRSD..
P615 .DVLSQNFQNYSCVPFT...DPSQPCQLGNYPSYVVNVTGAADVQAALKFAQKHNVRIVIKNTGHDYLGKST..
EncM .RIWNGTID...................RRPALIARCTSTPDVVAAVSFARKSGLLVAVRGGGHSMAGHSV..
6HDNO .NIWDGRHL...................QRPSLIARCLSAGDVAKSVRYACDNGLEISVRSGGHNPNGYAT..
GOOX .TAFNLRVD...................YDPAAIAIPRSTEDIAAAVQCGLDAGVQISAKGGGHSYGSYGFGG
LaO .EPFNIRLP...................YIPTAIAQTQTTAHIQSAVQCAKKLNLKVSAKSGGHSYASFGFGG
GilR .HSDNHRFV...................VEPEEFFLPATPDDVVASLQKAVTEGRGVACRSGGHCGQDFVG..
TamL .RGENLRFV...................GDPEEIHLVGSAAEIEQVLSRAVRSGKRVAVRSGGHCYEDFVA..
AknOx TRGFNGRFR...................GRPDVVYVVHTADQVVDAVNQAMAAGQRIAVRSGGHCFEGFVD..
P713 .GYGGLELWLHSFRNGVRFQKKYTSANKCTKSGWTGSAIHIDGAYQWRDVYTVAQA.NNVIAVGGGSPSPGAIG
P615 .GKGALSLWMHNLK.STKFIKNYK.......APYYKGPAAKLGAGVEGFEAYAMANS.TGHRIVGGTCPTVGIVG
EncM .CDGGIVIDLSLMN.SIKVSRR.............LRRARAQGGCLLGAFDTATQA.HMLATPAGVVSHTGL.G
6HDNO .NDGGIVLDLRLMN.SIHIDTA.............GSRARIGGGVISGDLVKEAAK.FGLAAVTGMHPKVGF.C
GOOX ED.GHLMLELDRMY.RVSVDD.............NNVATIQGGARLGYTALELLDQGNRALSHGTCPAVGV.G
LaO EN.GHLMVQLDRMIDVISYNDK.............TGIAHVEPGARLGHLATVLNDKYGRAISHGTCPGVGI.S
GilR TPRRDLVLDLHNLH.AIGPAAD.............GAGVRVGSGATVDQVQKALFRRWNAALPLGACSAVGM.G
TamL NSDVRVVMDMSRLS.AVGFDEE.............RGAFAVEAGATLGAVYKTLFRVWGVTLPGGACPDVGA.G
AknOx DPAVRAVIDMSQMR.QVFYDSG.............KRAFAVEPGATLGETYRALYLDWGVTIPAGVCPQVGV.G
P713 GWPSGGHGPATHNFGLGADQVLEAQIMLAD....GRIVTANHC...ENSDLFRAIRGGG.PGYGIVLSQHIK.
P615 GYTQGGGHSILSSSYGVAADNVLEWEVVTAD....GRHLVATPT...RNSDLYWALSGGGGGTFAVVLSMTAR.
EncM GLVLGGGFGWLSRKYGLSIDNLTSVEIVTAD....GGVLTASDT...ENPDLFWAVRGGG.GNFGVVTAFEFD.
6HDNO GLALNGGVGFLTPKYGLASDNILGATLVTAT....GDVIYCSDD...ERPELFWAVRGAG.PNFGVVTEVEVQ.
GOOX GHVLGGGYGFATHTHGLTLDWLIGATVVLAD....ASIVHVSET...ENADLFWALRGGG.GGFAIVSEFEFN.
LaO GHFAHGGFGFSSHMHGLAVDSVVGVTVVLAD....GRIVEASAT...ENADLFWGIKGAG.SNFGIVAVWKLA.
GilR GLVAGGGYGPLSRQLGLVVDHLHAVEVAVVDESRTVRLVTARADDTGDLGELFWAHTGGGGGNFGVVTAYEFRS
TamL GHILGGGYGPLSRMHGSIVDYLHAVEVVVVDASGDARTVIATREPSDPNHDLWWAHTGGGGGNFGVVVRYWLRT
AknOx GHVLGGGYGPLSRRDGVVADHLYAVEVVVVDASGRARKVVATSAADDPNRELWWAHTGGGGGNFGIVTRYWFRT
P713 ...........VHPNVKAVTAHRLAIAPRNETAENKDLLDAIAVLHQQLPALS.....NNGVAGYGFWFRSFP
P615 ...........LHADG.IVGGTLLGFNDSAVG..NEVYWEAVAAFHALLPDFL.....DGGNSFTYSV.....
EncM ...........LHRVG.PVRFASTYYSL.......DEGPQVIRAWRDHMATA......PDELTWALYL.RLAP
6HDNO ...........LYELPRKMLAGFITWAPS.....VSELAGLLTSLLDALNEMA.....DHIYPSVFV.GVD.
GOOX ...........TFEAPEIITTYQVTTTWN.....RKQHVAGLKALQDWAQNTM.....PRELSMRLEI.....
LaO ...........TFPAPKVLTRFGVTLNWKN.....KTSALKGIEAVEDYARWVA.....PREVNFRIGDYGA..
GilR PEH.LATEPV.GLPRAAGRLHVQKVVFPWAMID..ETSFVTVMRRFFEWHERHSEPGSPESSLFATFFV.NHV.
TamL AEADVPPEPGRLLPRPPAEVLLNTTVWPWEGLD..EAAFARLVRNHGRWFEQNSGPDSPWCDLYSVLAL.TRS.
AknOx PGA.TGTDPSQLLPKAPTSTLRHIVTWDWSALT..EEAFTRIIDNHGAWHQSNSAAGTPYASMHSVFYL.NSR.
P713 GPF..VGDA..HSGYTHGFWTIGKRQAEAEKAVAPLMN.ALKKFEDKLVITSTFAEYQDYWSFYWA.ESGL..H
P615 ..........GNNSLTAYGTMPGADRDAVDRLLRPFLD.DLASRG..ITPVVQPRVSTNYYDHFFT.YLGPAPY
EncM PLPELPADMHGKPVICAMSCWI.GDPHEGERQLESILHAG.......KPHGLTKATL.PYRALQA..YSFP..G
6HDNO ........ENRAPSVTVCVGHL.GGLDIAERDIARLRGLG.......RTVSDSIAVR.SYDEVVA..LNAEVGS
GOOX ..........NANALNWEGNFF.GNAKDLKKILQPIMK..KAGG...KSTISKLVET.DWYGQIN..TYLY..G
LaO ............GNPGIEGLYY.GTPEQWRAAFQPLLD..TLPA...GYVVNPTTSL.NWIESVL..SYSN..F
GilR ..........SSGVLQLMVQQD.ADVDPEGEILARFVASLTEGTG..VVGIPRGGVM.SWLTGTR..YMSQADC
TamL ..........QSGALAMTTQLD.ATGPDAEKRLETYLAAVSEGVG..VQPHSDTRRL.PWLHSTRWPGIAG..D
AknOx ..........AAGQILLDIQID.GGLDGAEALLNDFVAAVNEGTG..VEPAVQRSTE.PWLRATL..ANKF..D

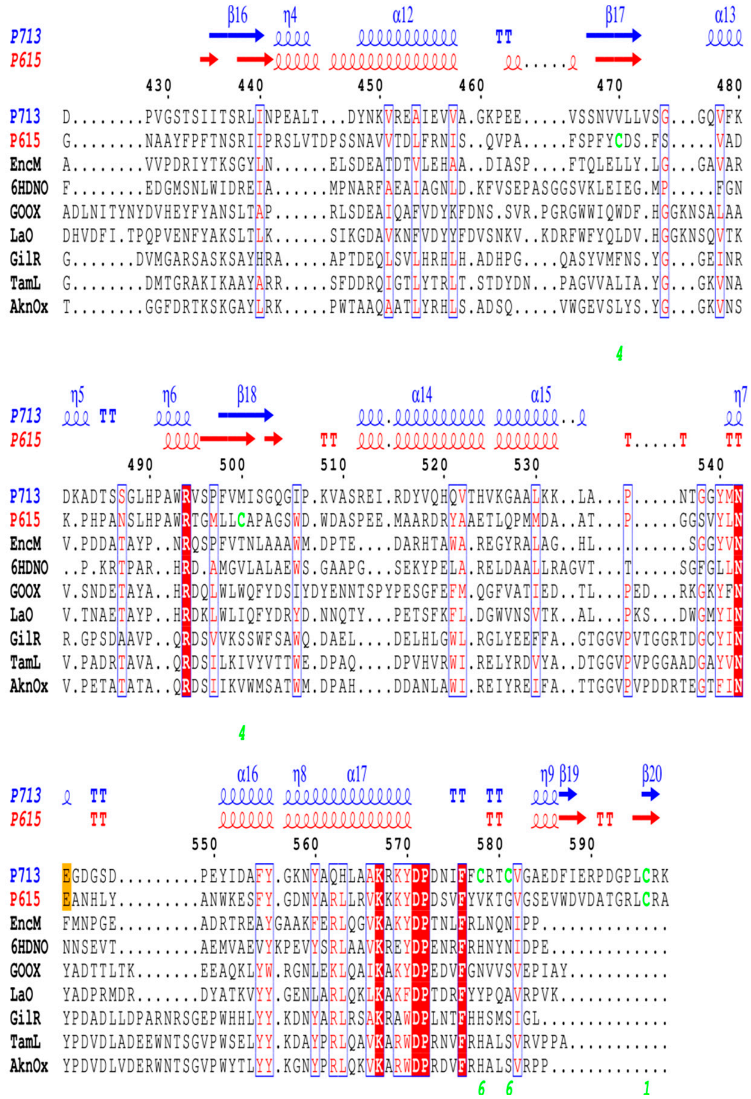

Figure S1: Multiple sequence alignment. Structure–based alignment of MtVAO713 and MtVAO615, EncM (PDB code 3w8w) [22], 6-hydroxy-D-nicotine oxidase (6HDNO, PDB code 2bvf) [23], glucooligosaccharide oxidase (GOOX, PDB code 1ZR6) [4], lactose oxidase (LaO, PDB code 3rj8), oxidoreductase GilR (PDB code 3POP) [25], S. sp. 307-9 tirandamycin oxidase (TamL, PDB code 2Y08) [24] and aclacinomycin oxidoreductase (AckOx, PDB code 2IPI) [8]. The structural alignment was made with Promals3D [38]. The indicated secondary structure elements are those obtained from the crystal structure of MtVAO713 and MtVAO615. Residues involved in FAD binding have a cyan background color, identical residues have a red background color and similar residues have a red color. Residues with orange background are likely to be involved in catalysis. Disulfide linkages are indicated in green italics below the sequences. The figure was created with ESPript [39].

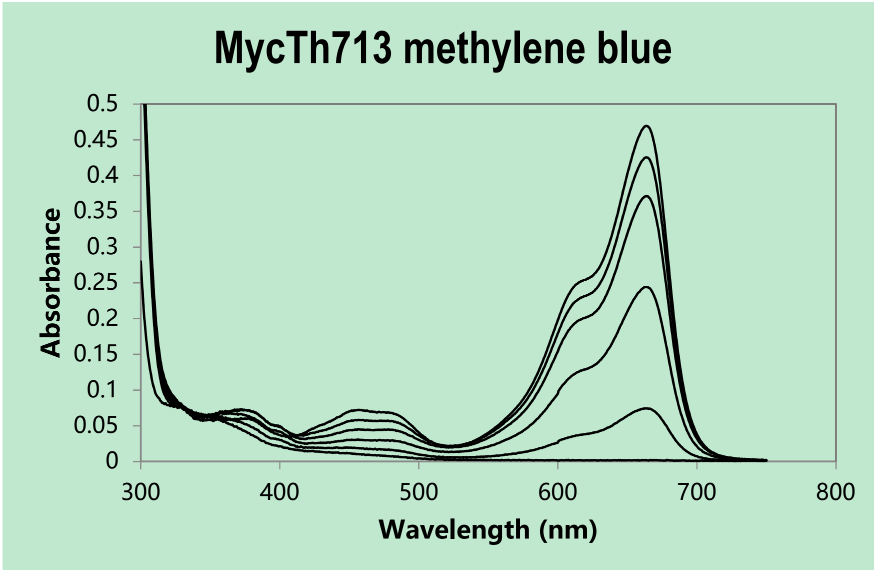

Fig S2: Spectrum of 5 µM MtVAO713 in the redox potential determination experiment with xanthine oxidase. The collected spectra are shown between time point 0 and time point at 34 minutes.

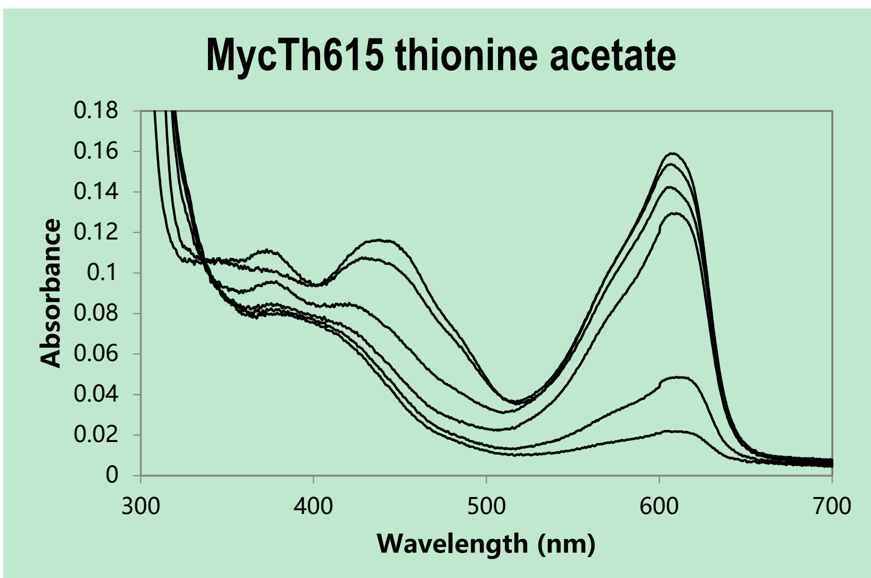

Fig S3: Spectrum of 10 µM MtVAO615 in the redox potential determination experiment with xanthine oxidase. The collected spectra are shown between time point 0 and time point at 88 minutes.

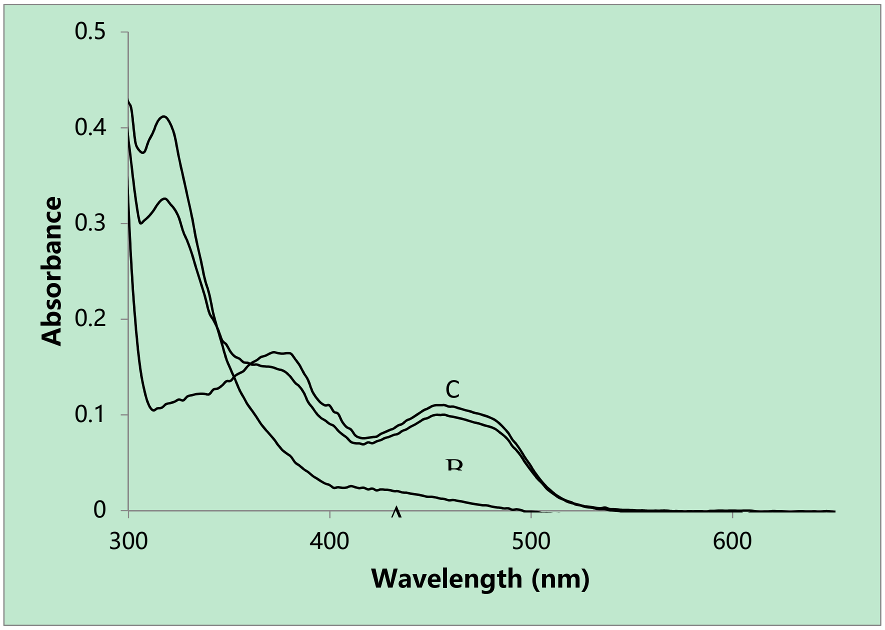

Fig S4: Deconvoluted absorption spectra of reduced (A), intermediate (B) and oxidized (C) MtVAO713 during reoxidation by molecular oxygen as measured by double-mixing stopped-flow spectrophotometry, after a delay time of 1.0 s.

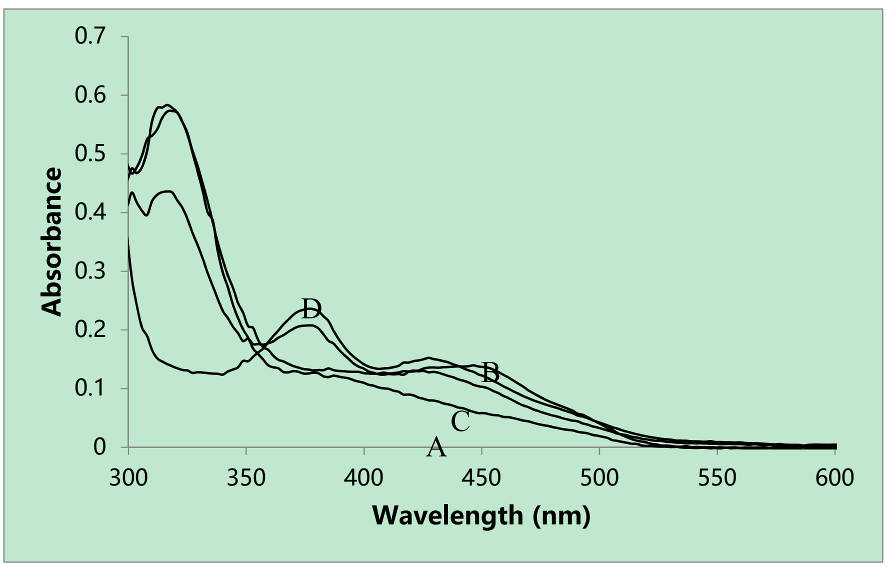

Fig S5: Deconvoluted absorption spectra of reduced (A), intermediate 1 (B), intermediate 2 (C) and oxidized (D) MtVAO615 during reoxidation by molecular oxygen as measured by double-mixing stopped-flow spectrophotometry, after a delay time of 1.0 s.

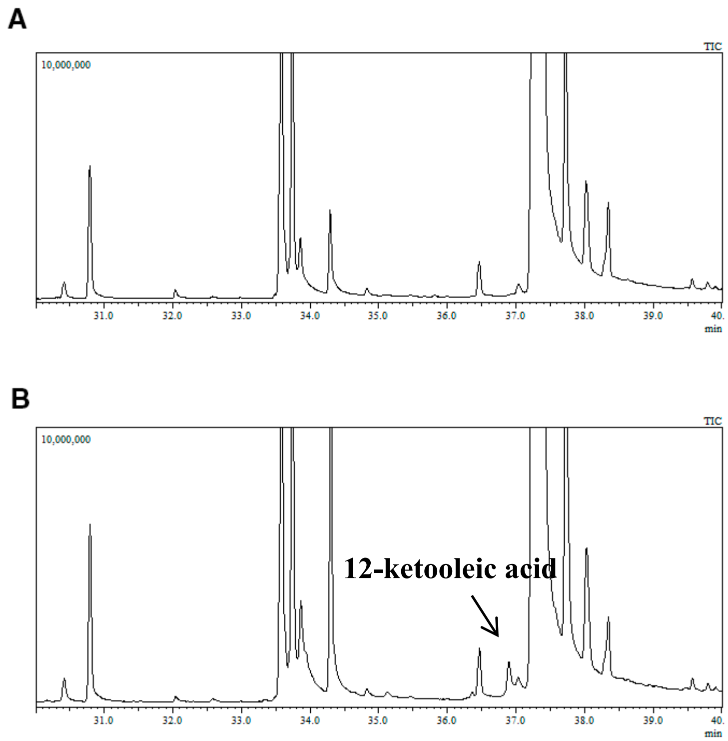

Fig S6: GC spectra of ricinoleic acid conversion by MtVAO713. A) Negative control without MtVAO713. B) Conversion with MtVAO713 present.

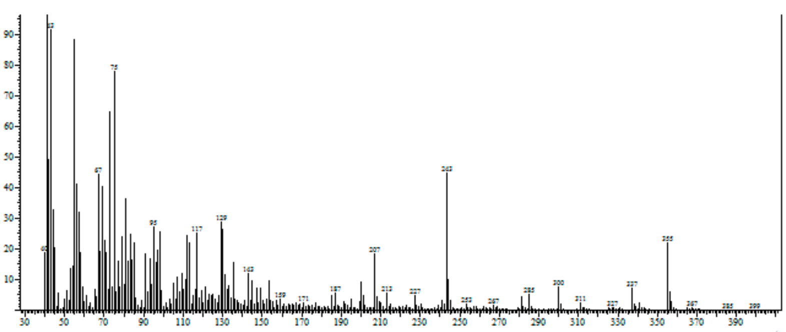

Figure S7: MS spectrum of the selected peak corresponding to 12-ketooleic acid.