

Supplemental Material

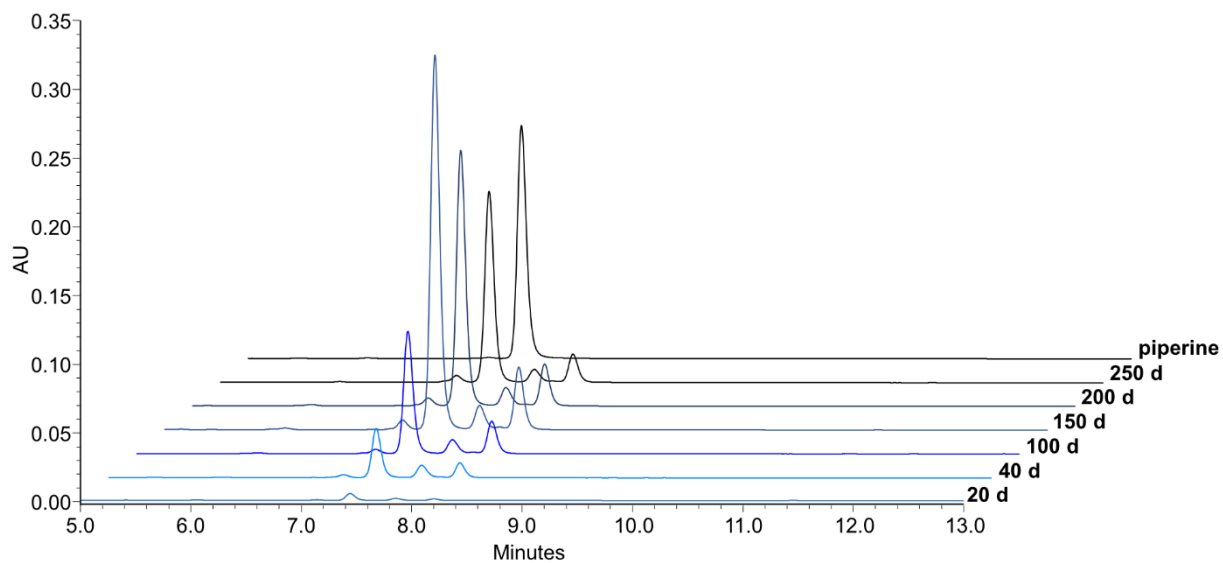


Figure S1 Accumulation of piperine during fruit development of black pepper. 5 μ l of a 10 mg/ml 90% MeOH extracted fresh fruits were analysed by LC-MS and product formation recorded at 340 nm. Days post anthesis are marked.

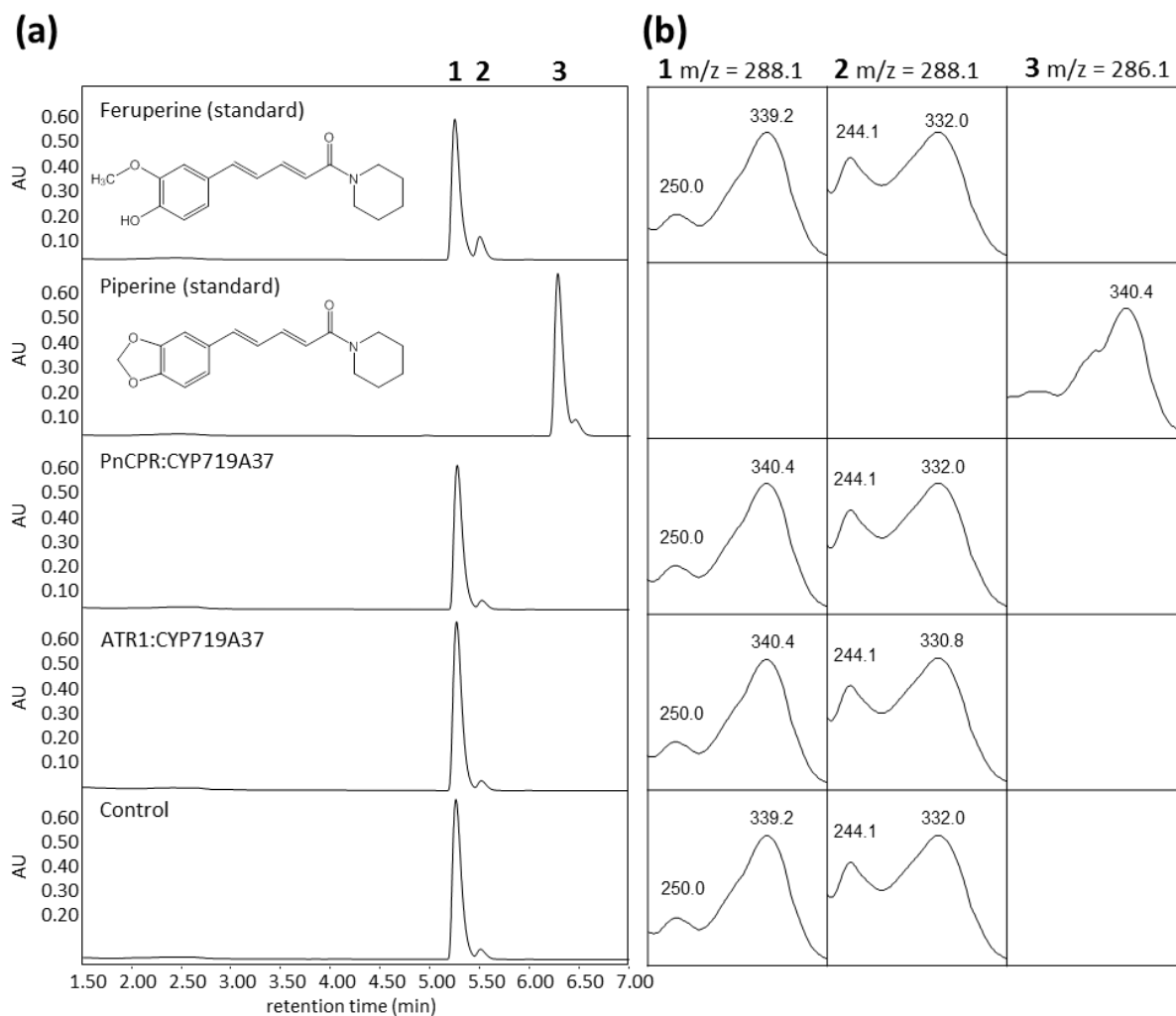


Figure S2 Microsomes from *CYP719A37*-transformed yeast were incubated with 100 μ M feruperine (1) and its isomer side product (2) for up to 24 hours (shown). No product formation (piperine, 3) was observed, neither with co-transformed reductases, ATR1 nor with PnCPR1. Corresponding UV-spectra are shown on the right. Piperine and empty vector controls are also shown.

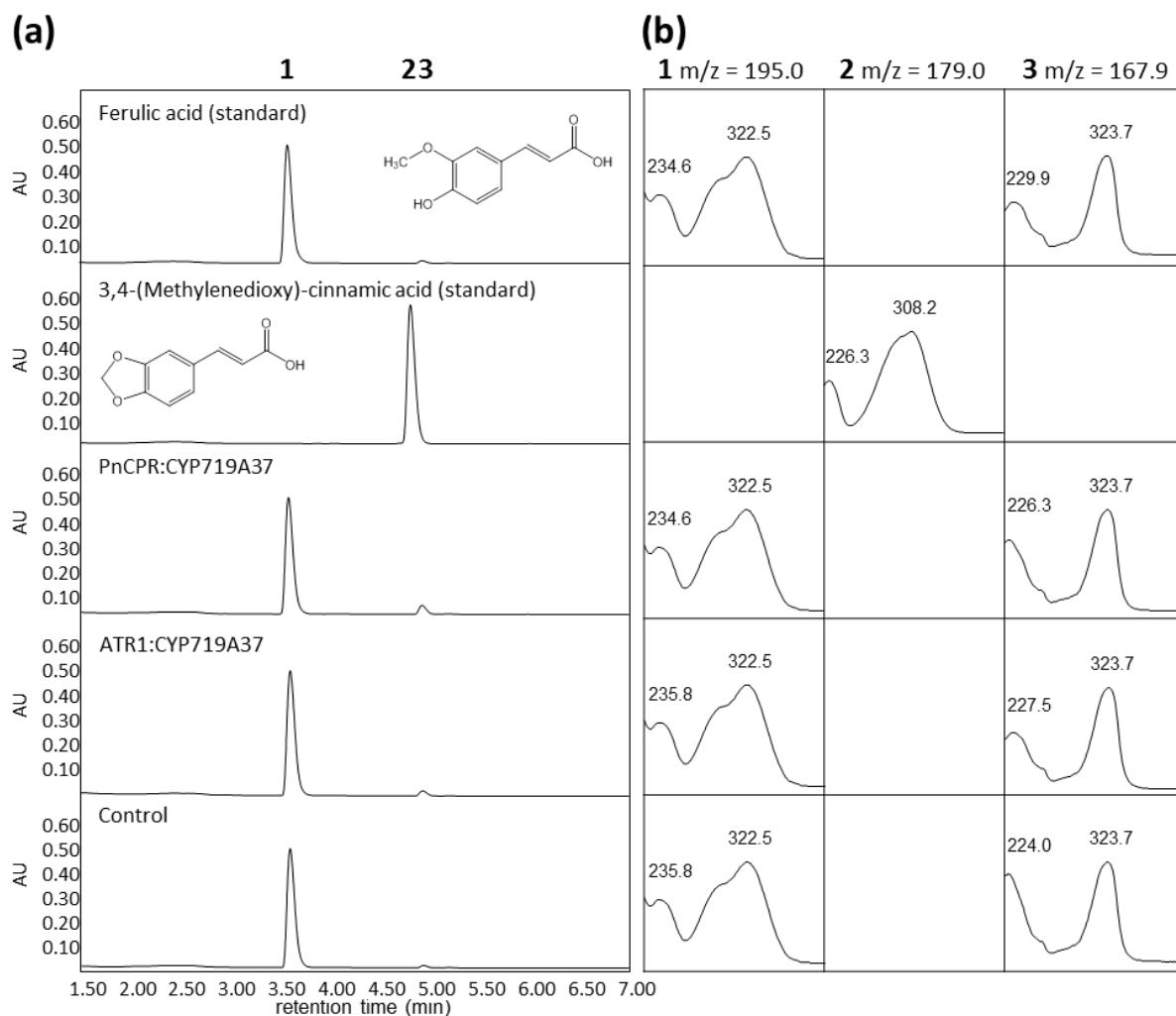


Figure S3 Microsomes from CYP719A37-transformed yeast were incubated with 100 μ M ferulic acid (1) for up to 24 hours (shown). No product formation (3,4-methylenedioxy cinnamic acid, 2) was observed, neither with co-transformed reductases, ATR1 nor with PnCPR1. Corresponding UV-spectra are shown on the right. The minor compound (3) is also detected in the standard and is not identical to the expected product. Ferulic acid and empty vector controls are also shown.

		membrane-anchored region															
CYP719A37	(Pni)	-----M	EQAQWVDPAL	FSAFVSIIEF	FLGMFLGRIS	LGVGKGAAPR	SPSSTEWFDG										
CYP719A26	(Pme)	-----M	EQAQWVDPAL	LPAFVGIIEF	FLGMFFGRSS	LGAGKGAAPR	STSSTEWFDG										
CYP719A1	(Cja)	-----MEMN	PLLVCATVAI	VFATTTIIRI	LF-----	-----SSS	SLPQMKWESG										
CYP719A5	(Eca)	-----M	EESLWVVTAT	VVVVFAIAKL	LK-----	-----KSS	SISTMEWPKG										
pCYP77A2	(Atr)	-----	-MALLYLFSI	LSALLFCYLF	LS-----	-----SR	KTKALKLFPG										
CYP77A4	(Ath)		MFPLISFSPT	SLDFTFFAII	ISGFVFIIR	WN-----	-----SN	SKKRLNLEPG									
											: * *						
CYP719A37	(Pni)		PPKLPIIGNL	HQLNKGGLV	HHKLAKLAQS	YDRAMTIWVG	SWGPMIVVSD	ADLAWEVLVT									
CYP719A26	(Pme)		PPKLPIIGNL	HQLNKGGLV	HHNLAKLAQS	YDRAMTIWVG	SWGPMIVVSD	ADLAWEVLVT									
CYP719A1	(Cja)		PRKLPIIGNL	HQLG--DDVL	HVALAKLAKV	HGSVMTIWIW	SWRPVIVISD	IEKAWEVLVN									
CYP719A5	(Eca)		PKKLPIIGNL	HQLG--GEAF	HVVLANLAKI	HGTVMTIWIW	AWRPVIVISD	IDKAWEVLVN									
pCYP77A2	(Atr)		PKGWPLVGNL	LQIARSGKPF	VFIVNELSQK	YGPIFTLKMG	AR-TLIVISS	AELAHEALIQ									
CYP77A4	(Ath)		PPGWVFNGL	QQFARSGKPF	FEYAEDLTKT	YGPIFTLRMG	TR-TMIILSD	ATLVHEALIQ									
			*	:::***	*	:	..	.	*	:	..	::*:*	:	..	::*:*	.	::*:*
CYP719A37	(Pni)		KSPDFAGRVL	SKLS-HLFNA	DYNTVVAYDA	GPQWQSLRRG	LQHGPLGPAH	VSAQARFHEE									
CYP719A26	(Pme)		KSPDFAGRVL	SKLS-HLFNA	NYNTVVAYDA	GPQWQSLRRG	LQHGPLGPAH	VSAQARFHEE									
CYP719A1	(Cja)		KSADYGARM	PEIT-KIASA	SWHTISTSDA	GSFQNVVRKG	LQSGAMGPLN	VAAQNYQYQR									
CYP719A5	(Eca)		KSSDYAGRDF	PEIT-KIISA	NWKNISCSDS	GPFWQNLKRG	LQSGALAPLN	VISQYQLQER									
pCYP77A2	(Atr)		KGQTFANRPA	ETPTRAIFSS	HKKTVNSAEY	GPLWRSLLRN	MVQGMILTPAK	LRAFRPIRRK									
CYP77A4	(Ath)		RGALFASRPA	ENPRTTIFSC	NKFTVNAAKY	GPVWRSLLRN	MVQNMLSSTR	LKEFGKLRQS									
			..	:: *	:	..	.	*	::*:*	:	..	::*:*	:	..	::*:*		
CYP719A37	(Pni)		DMKLLVSDMM	RAAKKGGNG	VVEPLAYVRR	ATIRFLSRLC	FGEAFNDEAF	VEGMDEAVEE									
CYP719A26	(Pme)		DMKLLVSDMM	RAAQKGGNG	VVEPLAYVRR	ATIRFLSRLC	FGEAFNDEAF	VEGMDEAVEE									
CYP719A1	(Cja)		DMKRLIKAMS	DEAAN--NNG	IVKPLDHKK	NTVRLTLRLI	FGQAFDDNKF	IESMHYIED									
CYP719A5	(Eca)		DMKNLITSMQ	EKASK--NNG	ILKPLDYLKE	ETIRLLSRLI	FGQSFNDENF	VKGVHLALDD									
pCYP77A2	(Atr)		ALDKLVERLA	AESKS---G	LVRVLSNCRF	TVFRILLCMC	FGVEMDENMI	EKVDVSLKVV									
CYP77A4	(Ath)		AMDKLIERTK	SEARDN--DG	LIWVLKNARF	AAFCILLEMC	FGIEMDEETI	EKMDEILKTV									
			..	::*:	:	..	::*:	*	::*:	*	::*:	::*:	:	..	::*:		
CYP719A37	(Pni)		TIRATG----	HARILDAFYF	TRHLPRIIRS	FMNTVAAKKK	IESLVRPLLS	----RPAPPG									
CYP719A26	(Pme)		TIGATG----	HARILDAFYF	TRHLPRIIRS	FIDTVNAKKK	IESLVRPLLS	----RPAPPG									
CYP719A1	(Cja)		IIRISG----	YARLAEAFFY	AKYLPSHKKA	EREAFVVKCR	VEELVRPLLS	----SKPPTN									
CYP719A5	(Eca)		LVRISG----	YASLADAFKF	CENLPSHKKS	IREVHEVNER	VVNLVKPYLV	----KNPPTN									
pCYP77A2	(Atr)		LITTLEPRIDD	FLPILQAFV	KKVAHAKHVR	KEQLETLPLL	IRSRKKTIQN	----AMSAPF									
CYP77A4	(Ath)		LMTVDPRIDD	YLPILAPFFS	KERKRALEVR	REQVDYVVG	IERRRAIQN	PGSDKTASSF									
			:	..	:	..	:	..	:	..	:	..	:	..	:		
CYP719A37	(Pni)		SYLHFLSLTD	AP-----E	SMIIFRIFEV	YLGVDSTAS	TTTWALAFLV	SNQQAQEKLH									
CYP719A26	(Pme)		SYLHFLSLTD	AP-----E	NMIIFRIFEV	YLGVDSTAS	TTTWALAFLV	SNQQAQEKLH									
CYP719A1	(Cja)		SYLYFLLSQN	FE-----E	EVIIFCIFEI	YLGVDSTSS	TTTWALAYLI	REQGAQEKLY									
CYP719A5	(Eca)		TYLYFLLSQK	FS-----D	EVIISAVLEV	YLGVDSTAS	TAVWALTFLV	REPRVQEKLY									
pCYP77A2	(Atr)		AYIDSLFDLD	MG--QGPPE	DMLVTLCAEF	LNCGTDATAT	AVEWAMAHLV	ADSEIQTRVR									
CYP77A4	(Ath)		SYLDTLFDLK	IEGRKTPPSN	EELVTLCESE	LNCGTDATGT	AIEWGIAQLI	ANPEIQSRLY									
			::*	* ..	:	..	*	::*:*	:	..	::*:*	:	..	::*:*	:	..	
CYP719A37	(Pni)		NELAQYCASQ	NNQNIKAEDV	GKLSYLLGVV	KETMRMKPIA	PLAVPHKTLK	ETMLDGKRVV									
CYP719A26	(Pme)		NELAQYCASQ	NNQIIKADDV	GKLSYLLGVV	KETMRMKPIA	PLAVPHKTLK	ETMLDGKRVV									
CYP719A1	(Cja)		QDIRMTLGDV	D--LVKIEDV	NKLYLQGVV	KETMRMKPIA	PLAIPHKTAK	ETTLMTGKVA									
CYP719A5	(Eca)		KEIIDLTGGE	R--SVKVEDV	SKLEYLQAVM	KETMRMKPIA	PMAIPHKTSR	DTSLMGKVVN									
pCYP77A2	(Atr)		AEIDR-VSPD	H--RVNDEDV	EKMEYLQAFV	KETLRKHPEPT	YFLLTHAVTE	KSLGGYDIP									
CYP77A4	(Ath)		DEIKSTVGDD	R--RVDEKDV	DKMVFLOAFV	KELLRKHPEPT	YFSLTHAVME	TTTLGAYDIP									
			::	.	:	..**	*	::*:*	*	::*:*	*	::*:*	:	..*	.	::*:*	
CYP719A37	(Pni)		AGTTVVVNLV	AVHYNPKLWP	EPEQFRPERF	VIGASGGNGG	GSSEYMLQSY	LPFGGMRAC									
CYP719A26	(Pme)		AGTTVVVNLV	AVHYNPKLWP	EPEQFRPERF	VVGASGGNGG	GSSEYMLQSY	LPFGGMRSC									
CYP719A1	(Cja)		KGTRIMVNLV	ALHNNQNIWP	DPYKFMPERF	LEG---ETGT	AYNKAMEQSF	LPFSAGMRIC									
CYP719A5	(Eca)		KGTSIMVNLV	AIHNNPKVFP	EPYKFIPERF	LQQG--ESKY	GDIKEMEQLS	LPFSAGMRIC									
pCYP77A2	(Atr)		EGVNVDFYTP	SISDDPKLWG	DPERFRPERF	VDKMDGDDDA	DLTGKVGKVM	MPFGVGRRIC									
CYP77A4	(Ath)		AGVNVVEYLP	GISEDPRINW	NPKKFDPRDF	MLGKE---DA	DITGISGVKM	IPFGVGRRIC									
			*	..	:	..**	*	::*:*	*	::*:*	*	::*:*	:	..*	.	::*:*	
CYP719A37	(Pni)		AGMEVGKLVQ	AMVVANLVMS	FKWLPEAEGK	MPDLAEDMTF	VLMKKPLAA	KIVPRA--									
CYP719A26	(Pme)		AGMEVGKLVQ	AMVVANLVMA	FKWLPEEEGK	MPDLAEDMTF	VLMKKPLAA	KIVPRA--									
CYP719A1	(Cja)		AGMDLGLKQF	AFALANLVNA	FKWSCVEEGK	LPDMGELSFS	VLLMKTPLAA	RIAGRNV--									
CYP719A5	(Eca)		AGMELGLKQY	GFSLASLVEA	FKWTCVADGK	LPDLSEDHCF	ILLMKNPLEA	RITPRTQL									
pCYP77A2	(Atr)		PGLGLGMLHI	SIMVATMVQQ	FEWLPNPVAV	RVIDMSEKFEF	TVLMKTPLLA	IAQPRKK--									
CYP77A4	(Ath)		PGLAMATIHV	HLMLARMVQE	FEWCAHPGGS	EIDFAGKLEF	TVVMKNPLRA	MVKPRI--									
			::*	..	:	..**	*	::*:*	*	::*:*	*	::*:*	:	..*	.	::*:*	
aromatic region																	
CYP719A37	(Pni)		AGTTVVVNLV	AVHYNPKLWP	EPEQFRPERF	VIGASGGNGG	GSSEYMLQSY	LPFGGMRAC									
CYP719A26	(Pme)		AGTTVVVNLV	AVHYNPKLWP	EPEQFRPERF	VVGASGGNGG	GSSEYMLQSY	LPFGGMRSC									
CYP719A1	(Cja)		KGTRIMVNLV	ALHNNQNIWP	DPYKFMPERF	LEG---ETGT	AYNKAMEQSF	LPFSAGMRIC									
CYP719A5	(Eca)		KGTSIMVNLV	AIHNNPKVFP	EPYKFIPERF	LQQG--ESKY	GDIKEMEQLS	LPFSAGMRIC									
pCYP77A2	(Atr)		EGVNVDFYTP	SISDDPKLWG	DPERFRPERF	VDKMDGDDDA	DLTGKVGKVM	MPFGVGRRIC									
CYP77A4	(Ath)		AGVNVVEYLP	GISEDPRINW	NPKKFDPRDF	MLGKE---DA	DITGISGVKM	IPFGVGRRIC									
			*	..	:	..**	*	::*:*	*	::*:*	*	::*:*	:	..*	.	::*:*	
binding domain																	
CYP719A37	(Pni)		AGMEVGKLVQ	AMVVANLVMS	FKWLPEAEGK	MPDLAEDMTF	VLMKKPLAA	KIVPRA--									
CYP719A26	(Pme)		AGMEVGKLVQ	AMVVANLVMA	FKWLPEEEGK	MPDLAEDMTF	VLMKKPLAA	KIVPRA--									
CYP719A1	(Cja)		AGMDLGLKQF	AFALANLVNA	FKWSCVEEGK	LPDMGELSFS	VLLMKTPLAA	RIAGRNV--									
CYP719A5	(Eca)		AGMELGLKQY	GFSLASLVEA	FKWTCVADGK	LPDLSEDHCF	ILLMKNPLEA	RITPRTQL									
pCYP77A2	(Atr)		PGLGLGMLHI	SIMVATMVQQ	FEWLPNPVAV	RVIDMSEKFEF	TVLMKTPLLA	IAQPRKK--									
CYP77A4	(Ath)		PGLAMATIHV	HLMLARMVQE	FEWCAHPGGS	EIDFAGKLEF	TVVMKNPLRA	MVKPRI--									
			::*	..	:	..**	*	::*:*	*	::*:*	*	::*:*	:	..*	.	::*:*	

Figure S4 Amino acid sequence of CYP719A37 (MT643912). Characteristic features and relevant amino acids are shown.