

## **SUPPLEMENTARY MATERIAL**

### **New pipeline for analysing fruit proteolytic products used as digestive health nutraceuticals**

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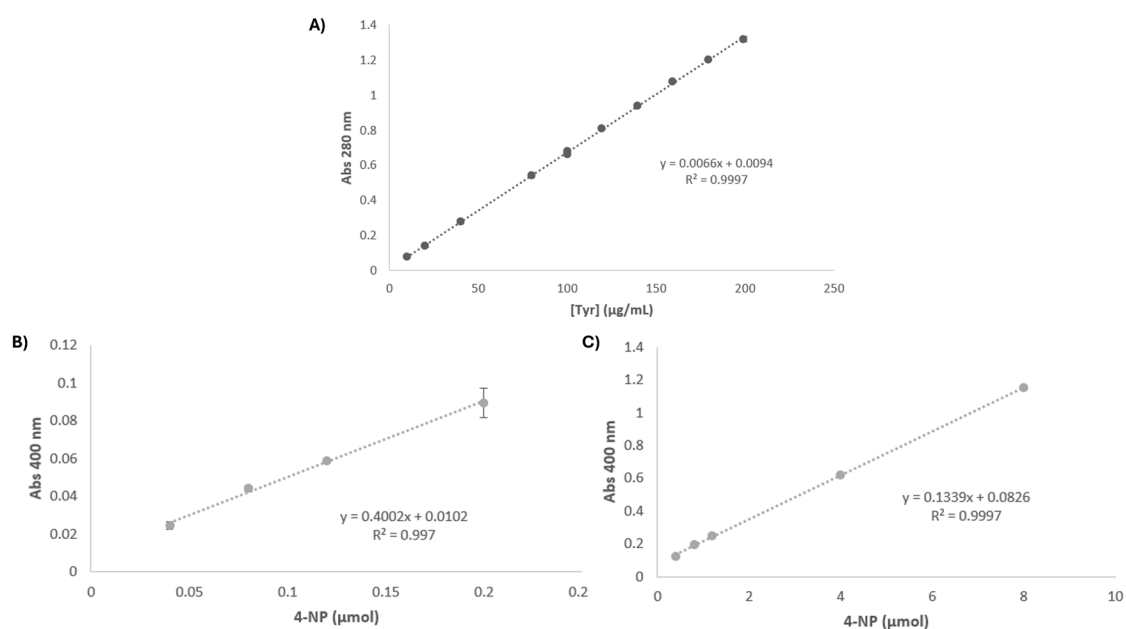
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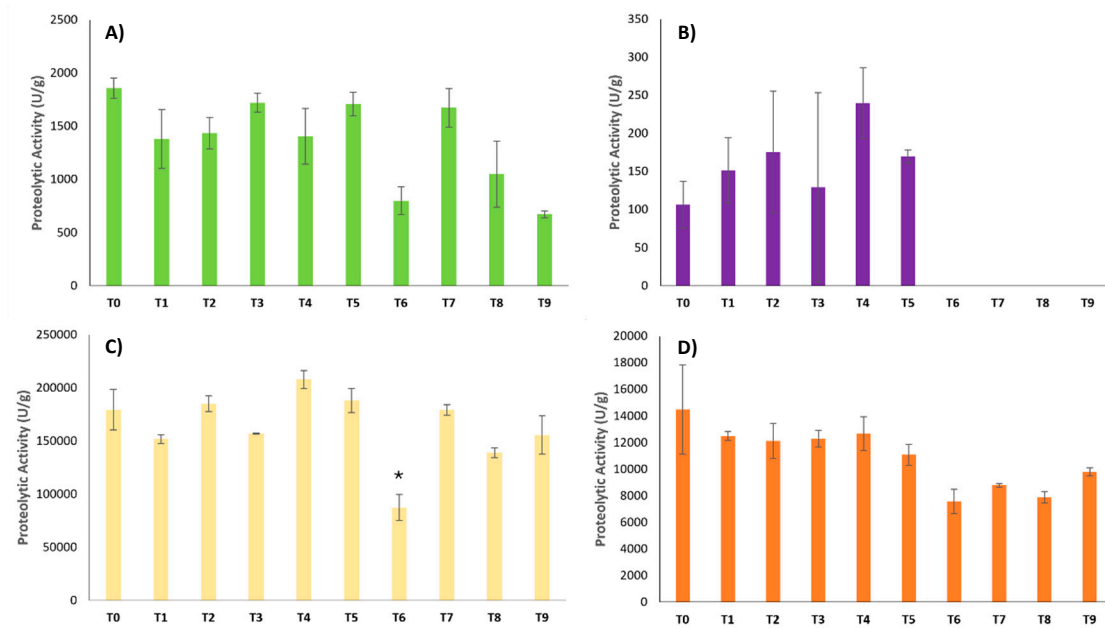
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**Figure S1.** Calibration curves to quantify proteolytic activity. A) Tyrosine (Tyr) calibration curve to calculate Casein Digestion Units (CDU). B) 4-nitrophenol calibration curve (4-NP) to enzymatic units' calculation from 0,04 - 0,3  $\mu\text{mol}$  4-NP and C) for 0,4 - 8  $\mu\text{mol}$  4-NP.



**Figure S2.** Stability test in long-term conditions (25 °C, 60 % relative humidity) using Z-L-Lys-ONp hydrochloride assay during 6 months for K2 (A), F2 (B), P4 (C), and PAP2 (D). T0, start. T1, 1 week. T2, 2 weeks. T3, 3 weeks. T4, 1 month. T5, 1.5 months. T6, 2 months. T7, 2.5 months. T8, 3 months. T9, 6 months. \*  $p < 0.001$ , significant difference with respect to T0. Horizontal bar to compare bars at both ends.



**Figure S3.** Comparison of A) *Ficus carica* ficin amino acid sequences and B) identity percentages.

Ficin_2a	MASLTSLSSLLLLLVLVAAADGSSFEESNPIRLVSDGLRDFHSQLLQVVGNTRHLSF	60
Ficin_2b	MASLTSLSSLL-LLVLVAAADGSSFEESNPIRLVSDGLRDFHSQLLQVVGNTRHLSF	59
Ficin_2c	MASLTSLSSLL-LLVLVAAADGSSFEENPIRLVSDGLRDFHSQLLQVVGNTRHLSF	59
Ficin_isoform_D	-----	0
Ficin_3	-MDLTVFTILT---IVVCLALS---YP---P-----LTSSRTDVEVREMF	36
Ficin_1c	-MALTTFVTILT---IVLCLALS---YP---P-----LTSSRTDVEVREMF	36
Ficin_1b	-MALTTFVTILT---IVLCLALS---YP---P-----LTSSRTDAEVREMF	36
Ficin_1a	-MALTTFVTILT---IVLCLALS---YP---P-----LTSSRTDAEVREMF	36
Ficin_2a	TRFASKYGKRYETEEIEIKLRFEIFSENKIIKSSNR-KGLPYTLAVNQFADWTWEEFKQH	119
Ficin_2b	TRFASKYGKRYETEEIEIKLRFEIFSENKIIKSSNR-KGLPYTLAVNQFADWTWEEFKQH	118
Ficin_2c	TRFASKYGKRYETEEIEIKLRFEIFSENKIIKSSNR-KGLPYTLAVNQFADWTWEEFKQH	118
Ficin_isoform_D	-----	0
Ficin_3	VKWMATHGRAYNSLEEERREFEIKDNLRFVDEHNAIQNRTYKVGMMNFSMDTDEEYRRD	96
Ficin_1c	VNMWATHGRAYNSLGEERREFEIKDNLRFVDEHNAIQNRTYKVGMMNFMADMTNEEYRRK	96
Ficin_1b	VNMWATHGRAYNSLGEERREFEIKDNLRFVDEHNAIQNRTYKVGMMNTFADMTNEEYRRK	96
Ficin_1a	VNMWATHGRAYNSLGEERREFEIKDNLRFVDEHNAIQNRTYKVGMMNTFADMTNEEYRRK	96
Ficin_2a	KLGAQNCSATN-----KGSHKLTDDVLPKTKDWRETGIVSPVKNGQSGCSCWTFSTTGA	174
Ficin_2b	KLGAQNCSATN-----KGSHKLTDDVLPKTKDWRETGIVSPVKNGQSGCSCWTFSTTGA	173
Ficin_2c	KLGAQNCSATN-----KGSHKLTDDVLPKTKDWRETGIVSPVKNGQSGCSCWTFSTTGA	173
Ficin_isoform_D	-----LPETVDWRSKGAVDPIRDQKGCSCWVFSVTVV	33
Ficin_3	MLGTRSDPEL-KTKVDSSRYAPHAASLPETVDWRSKGAVNPIRNGKCGSCWTFSTVAV	156
Ficin_1c	MLGARVDPPELINTKVASSRYAPHAASLPETVDWIRIQGAVNPIRNGRCGSCWAFSVVAV	155
Ficin_1b	MLGARVDPPELIKTKVASSRYAPHAASLPETVDWIRIQGAVNPIRNGRCGSCWAFSVVAV	156
Ficin_1a	MLGARVDPPELIKTKVASSRYAPHAASLPETVDWIRIQGAVNPIRNGRCGSCWAFSVVAV	156
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Ficin_2a	LEAAYKQAFGKDISLSEQLVDCAGAFNNFGCNGGLPSQAFEYIKYNGGLETEEAYPYTG	234
Ficin_2b	LEAAYKQAFGKDISLSEQLVDCAGAFNNFGCNGGLPSQAFEYIKYNGGLETEEAYPYTG	233
Ficin_2c	LEAAYKQAFGKDISLSEQLVDCAGAFNNFGCNGGLPSQAFEYIKYNGGLETEEAYPYTG	233
Ficin_isoform_D	VEGIVKIVTDLPSSLSEQLLDCAPSYKDLGCKGGWMDKAYDYIINKRGITSESQNPYTA	93
Ficin_3	VEGITKIVTNKLPSSLSEQLLDCAPSYQNLGCKGGWNNRAYDYIINKRGITSQSNPYTA	215
Ficin_1c	VESITKIVTDELPSLSEQLVDCAKSARDLGRGGWMTKAYDYIINKGGITSQSNPYTA	216
Ficin_1b	VEGISKIVTDELPSLSEQLVDCATSYKNLGCSCGGWMTKAYDYIINKGGITSQSNPYTA	216
Ficin_1a	VEGITKIVTDELPSLSEQLVDCATSYKNLGCSCGGWMTKAYDYIINKGGITSQSNPYTA	216
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Ficin_2a	KNGLCKFSAENAVQVVKSV-NITLGADELKHAVGIVRPVSVAFQ-VVRGFHFYKDGVF	292
Ficin_2b	KNGLCKFSAENAVQVVKSV-NITLGADELKHAVGIVRPVSVAFQ-VVRGFHFYKDGVF	291
Ficin_2c	KNGLCKFSAENAVQVVKSV-NITLGADELKHAVGIVRPVSVAFQ-VVRGFHFYKDGVF	291
Ficin_isoform_D	RKGAQCKRKAQEVATISSYEDVPADNEALKKAVA-NQPVSVDAIDASSAAVKQYSGVF	152
Ficin_3	RKGQCNKSAQSVATIDRYENVRNNENALKKAVA-NQPVSVDAIDASSAAVKQYSGVF	274
Ficin_1c	RKGCNVTKASQSVATIDRYESVPRNNENALKKAVA-NQPVSVTIEAGGRAFLQYKSGVF	275
Ficin_1b	RKGCNCKDLASQIVATIDSSEYHVRNNENALKKAVA-NQPVSVTIEAGGRAFLQYKSGVF	275
Ficin_1a	RKGCNCKDLASQIVATIDSSEYHVRNNENALKKAVA-NQPVSVTIEAGGRAFLQYKSGVF	275
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Ficin_2a	TSDTGCGSTSQDVNHAVLAVGYGVENGVPYWLKINSWGADWIDGYFMELG----KNMCG	348
Ficin_2b	TSDTGCGSTSQDVNHAVLAVGYGVENGVPYWLKINSWGADWIDGYFMELG----KNMCG	347
Ficin_2c	TSDTGCGSTSQDVNHAVLAVGYGVENGVPYWLKINSWGADWIDGYFMELG----KNMCG	347
Ficin_isoform_D	VG----SCGVANDHAVVVVGYGTESGVNVLVRNSWGTNNGEYGMKLERDTAHPAGCKG	208
Ficin_3	TG----SCGTGLDHAVVVVGYGTQNGVDYWLVRNSWGTNNGEYGMKLRQNVAPAGCKG	330
Ficin_1c	TG----PCGTGLDHAVVAIGYGSSENDVDYWLVRNSWG--NWGERGYIKIQRNVAPAGCKG	330
Ficin_1b	TG----SCGTGLDHAVVAIGYGSSENDVDYWLVRNSWGTNNGEYGMKLERDTAHPAGCKG	331
Ficin_1a	TG----SCGTGLDHAVVAIGYGSSENDVDYWLVRNSWGTNNGEYGMKLRQNVAPAGCKG	331
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Ficin_2a	VATCASYPIV-----	358
Ficin_2b	VATCASYPIV-----	357
Ficin_2c	VATCASYPIV-----	357
Ficin_isoform_D	IAMESTYPVKAC-----	220
Ficin_3	IAMQATYPVKKGKTSNKPWAYEVDAAEMVVA	362
Ficin_1c	IAMHSTYPVKK--TSTKPYWAYEVDAAEMVALA	360
Ficin_1b	IAMQSTYPVKK--TSTKPYWAYEVDAAEMVAVA	361
Ficin_1a	IAMQSTYPVKK--TSTKPYWAYEVDAAEMVAVA	361
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B)

	Ficin_2a	Ficin_2b	Ficin_2c	Ficin_isoform_D	Ficin_3	Ficin_1c	Ficin_1b	Ficin_1a
Ficin_2a	100.00%	99.72%	99.16%	42.45%	38.11%	37.80%	37.99%	38.30%
Ficin_2b	99.72%	100.00%	99.44%	42.92%	38.53%	38.23%	38.41%	38.72%
Ficin_2c	99.16%	99.44%	100.00%	42.92%	38.53%	38.23%	38.41%	38.72%
Ficin_isoform_D	42.45%	42.92%	42.92%	100.00%	77.73%	70.18%	71.23%	71.23%
Ficin_3	38.11%	38.53%	38.53%	77.73%	100.00%	84.12%	84.72%	84.72%
Ficin_1c	37.80%	38.23%	38.23%	70.18%	84.12%	100.00%	92.78%	93.89%
Ficin_1b	37.99%	38.41%	38.41%	71.23%	84.72%	92.78%	100.00%	98.34%
Ficin_1a	38.30%	38.72%	38.72%	71.23%	84.72%	93.89%	98.34%	100.00%