

Supplementary Information for:

**Biological properties of almond proteins produced by aqueous and enzyme-assisted aqueous extraction processes from almond
cake**

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TABLE S1: Summary of resultant antioxidant peptides from the major almond protein subunits after subtilisin digestion *in silico* using BIOPEP database

Amandin subunit	ID	Sequence	Location	Name	Function	Activity	MI Mass	Chemical mass
Prunin-1	3317	HL	[81-82]	*	Antioxidative	Antioxidative	268.1420	268.3020
	7873	IY	[92-93]	peptide from soybean protein isolates: beta-conglycinin and glycinin	Antioxidative	Antioxidative	294.1470	294.3300
	8037	PWH	[429-431]	synthetic peptide	*	Antioxidative	438.1890	438.4720
	8224	VY	[439-440]	antioxidative peptide	Free radical scavenging	Antioxidative	280.1310	280.3030
Prunin-2	3317	HL	[72-73]	*	Antioxidative	Antioxidative	268.1420	268.3020
	7873	IY	[83-84]	peptide from soybean protein isolates: beta-conglycinin and glycinin	Antioxidative	Antioxidative	294.1470	294.3300
	8224	VY	[386-387]	antioxidative peptide	Free radical scavenging	Antioxidative	280.1310	280.3030

H – histidine; L – leucin; I – isoleucine; Y – tyrosine; P – proline; W – tryptophan; V – valine. Digestion simulation was conducted with subtilisin in the BIOPEP database ¹

TABLE S2: Overview of the presence of specific amino-acids suggested as important requirement for the α -glucosidase inhibition activity

Amandin subunit	Sequencie	R, L, S, T or Y at N-terminus	A or M at C-terminus	P close to C-terminus
Prunin-1	QQRRA	no match	A	no match
	RAL	R	no match	no match
	RNGIY	R	no match	no match
	RQL	R	no match	no match
Prunin-2	IPQNHA	no match	A	no match
	RADF	R	no match	no match
	RAL	R	no match	no match
	RIS	R	no match	no match
	RPS	R	no match	no match
	RQHIF	T	no match	no match
	TNTL	T	no match	no match
	TPHW	T	no match	no match
	NPS	no match	no match	P
	RPS	no match	no match	P

A – alanine; M – methionine; P – proline; R – arginine; K – lysine; S – serine; T – threonine; Y – tyrosine. The criteria used was suggested by Ibrahim *et al.*², that proposed the structural requirements for α -glucosidase inhibition as tri – to hexapeptides with serine S, T, Y, L or A as the ultimate N-terminal residue and P preferably at the penultimate C- terminal position while A or M at ultimate C-terminal position.

TABLE S3: Overview of the presence of specific amino-acids suggested as important requirement for the lipase inhibition activity

Amandin subunit	Sequence	Hydrophobicity (%)	Hydrophobic residues
Prunine-1	VAIPAG	83.33	A, P, G
	MAKAF	80.00	A, F
	AQAL	75.00	A, L
	RAL	66.67	A, L
	MANGL	60.00	M, A, G, L
	NAPQL	60.00	A, P, L
	GA	50.00	G, A
Prunine-2	L	100.00	L
	W	100.00	W
	AQAL	75.00	A, L
	ARL	66.67	A, L
	PAG	66.67	A, G
	RAL	66.67	A, L
	VNAPQL	66.67	A, P, L
	AG	50.00	A, G
	GA	50.00	A, G
	QNAF	50.00	A, F
	RADF	50.00	A, F

A – alanine; L – leucine; P – proline; F – phenylalanine; G – glycine; M – methionine; W – tryptophan. The criteria used was suggested by Ngoh et al. ³, that proposed the presence of hydrophobic residues as characteristic of a lipase inhibitor. To evaluate the hydrophobicity of the sequences, the platform: Peptide Hydrophobicity/Hydrophilicity Analysis Tool, was used.

Supplementary References:

1. Minkiewicz, P., Iwaniak, A. & Darewicz, M. BIOPEP-UWM Database of Bioactive Peptides: Current Opportunities. *Int. J. Mol. Sci.* **20**, 2–23 (2019).
2. Ibrahim, M. A., Bester, M. J., Neitz, A. W. H. & Gaspar, A. R. M. Structural properties of bioactive peptides with α -glucosidase inhibitory activity. *Chem Biol Drug Des* **91**, 370–379 (2018).
3. Ngoh, Y.-Y. & Gan, C.-Y. Enzyme-assisted extraction and identification of antioxidative and α -amylase inhibitory peptides from Pinto beans (*Phaseolus vulgaris* cv. Pinto). *Food Chem* **190**, 331–337 (2016).