ROLE OF *AMARANTHUS CAUDATUS* (KIWICHA) PROTEIN AS SOURCE OF MULTIFUNCTIONAL PEPTIDES

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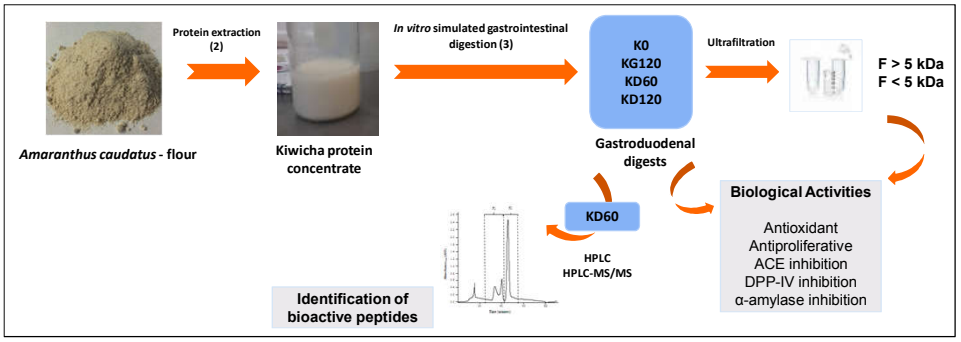
INTRODUCTION

- The multifactorial origin of many chronic diseases provides a new framework for the development of multifunctional foods.
- Bioactive peptides have become very popular because of their demonstrated multiple activities (1).
- No data on the potential role of *Amaranthus caudatus* (kiwicha) proteins as source of bioactive peptides have been reported.

OBJECTIVE

To evaluate the effect of gastrointestinal digestion *in vitro* simulating physiological conditions on the release of multifunctional peptides from the protein of kiwicha (*Amaranthus caudatus*)

MATERIALS AND METHODS



RESULTS

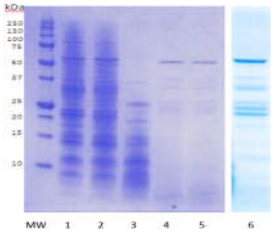


Figure 1. Characterization of the kiwicha protein digests obtained after an *in vitro* simulated gastrointestinal digestion by SDS-PAGE under reducing conditions. MW: molecular weight marker; Lane 1: kiwicha protein concentrate (KPC), Lane 2: KPC digest at time 0 (K0), Lane 3: KPC gastric digest at 120 min (KG120), Lane 4: KPC gastroduodenal digest at 60 min (KD60), Lane 5: KPC gastroduodenal digest at 120 min (KD120), Lane 6: Digestion blank with digestive enzymes.

Kiwicha proteins are completely degraded during the intestinal phase

Table 1. Angiotensin converting enzyme inhibitory and antioxidant activity, colon cancer cells viability, dipeptidyl peptidase IV and α-amylase inhibitory activities of gastric and gastroduodenal digests of kiwicha protein concentrate and their fractions > and < 5 kDa

Digest	Fraction	Angiotensin converting enzyme inhibition (IC_{50}, μg protein/mL)	Antioxidant activity (ORAC, μmol Trolox equivalents/mg protein)	DPP-IV inhibition (IC_{50} – mg protein/mL)	α-amylase inhibition (IC_{50} – mg protein/mL)	Caco-2 cell viability inhibition (IC_{50} – mg protein/mL)	
						Blank	Sample
KG120	Whole digest	39.00 ± 2.99^{a}	1.51 ± 0.01^{b}	0.66 ± 0.01^{d}	n.d.$^{+}$	n.d.$^{+}$	0.72 ± 0.01^{e}
KD60	Whole digest	81.00 ± 10.53^{b}	4.28 ± 0.11^{e}	0.32 ± 0.01^{b}	2.73 ± 0.02^{d}	1.24 ± 0.04^{g}	0.08 ± 0.002^{a}
	F > 5 kDa	133.74 ± 2.55^{c}	0.99 ± 0.03^{a}	0.46 ± 0.01^{c}	n.d.*	0.46 ± 0.01^{d}	0.33 ± 0.03^{c}
	F < 5 kDa	75.61 ± 5.77^{b}	3.02 ± 0.13^{d}	0.45 ± 0.03^{c}	1.81 ± 0.05^{b}	0.80 ± 0.01^{f}	n.d.*
KD120	Whole digest	88.01 ± 13.96^{b}	3.03 ± 0.06^{d}	0.28 ± 0.01^{ab}	2.45 ± 0.01^{c}	1.24 ± 0.04^{g}	0.14 ± 0.01^{ab}
	F > 5 kDa	181.85 ± 6.12^{d}	0.98 ± 0.08^{a}	0.68 ± 0.07^{d}	n.d.*	0.46 ± 0.01^{d}	0.19 ± 0.01^{b}
	F < 5 kDa	83.90 ± 6.81^{b}	2.27 ± 0.12^{c}	0.19 ± 0.01^{a}	0.84 ± 0.03^{a}	0.80 ± 0.01^{f}	1.76 ± 0.11^{h}

$^{a-g}$: Different lowercase letters in each biological activity indicate significant differences among samples (p<0.05, Duncan test)
n.d.$^{+}$: No inhibitory effect observed at the highest concentration used (4 mg/mL)
n.d.*: No inhibitory effect observed at the highest concentration used (2.5 mg/mL)

-The resulting peptides after gastric digestion show potent ACE inhibition, while duodenal digestion improves ORAC, DPP-IV, α-amylase and Caco-2 cell viability inhibitory activities.

- High MW peptides present marked α-amylase and Caco-2 cell inhibitory activity while the low MW ones stand out for antioxidant, ACE and DPP-IV inhibitory activities

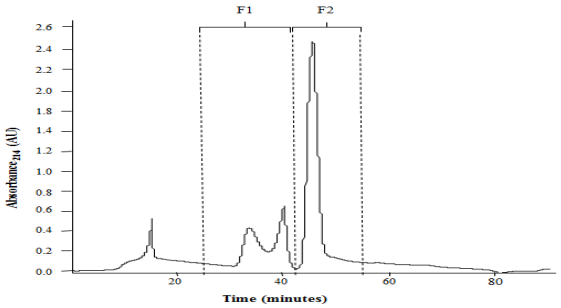


Figure 2. Fractionation by semi-preparative RP-HPLC of the gastroduodenal digest at 60 min (KD60). Collected fractions are termed with F followed by a number.

Table 2. Antioxidant activity and angiotensin converting enzyme, dipeptidyl peptidase IV, α-amylase and colon cancer Caco-2 cells viability inhibitory activities of fractions collected by RP-HPLC from gastroduodenal digests of kiwicha protein concentrate obtained after incubation with pepsin for 120 min and pancreatin for 60 min (KD60)

Fraction	Angiotensin converting enzyme inhibition (IC_{50}, μg protein/mL)	Antioxidant activity (ORAC, μmol Trolox equivalents/mg protein)	DPP-IV inhibition (IC_{50}, mg protein/mL)	α-amylase inhibition (IC_{50}, mg protein/mL)	Caco-2 cell viability inhibition (IC_{50}, mg protein/mL)
F-1	359.47 ± 0.52^{b}	1.56 ± 0.13^{a}	0.38 ± 0.04^{b}	0.42 ± 0.03^{a}	1.17 ± 0.14^{a}
F-2	81.47 ± 7.61^{a}	4.47 ± 0.39^{b}	0.18 ± 0.01^{a}	1.17 ± 0.05^{b}	0.87 ± 0.08^{a}

$^{a-b}$: Different lowercase letters in each column indicate significant differences among samples (p<0.05, Duncan test)

Table 3. Peptides identified by HPLC-MS/MS in the fractions F-1 and F-2 collected from kiwicha protein concentrate digested with pepsin for 120 min and pancreatin for 60 min (KD60)

Fraction	Observed mass	Calculated mass	Sequence	Fragment	Source protein	NCBI Accession number
F-1	669.30	669.26	YESGSQ	f(130-135)	11S Globulin	CAA57633.1
	505.17	505.16	GGEDE	f(139-143)	11S Globulin	CAA57633.1
	615.31	615.29	NRPET	f(479-483)	11S Globulin	CAA57633.1
	614.39	614.34	QQQLV	f(254-258)	Acetylate synthase	AAK50821.1
	517.29	517.22	ACDIP	f(606-610)	Acetylate synthase	AAK50821.1
F-2	807.40	807. 45	FLISCLL	f(22-28)	11S Globulin	CAA57633.1
	630.25	630.32	TALEPT	f(56-61)	11S Globulin	CAA57633.1
	776.52	776.45	HVIKPPS	f(288-294)	11S Globulin	CAA57633.1
	924.49	924.40	SVFDEELS	f(402-409)	11S Globulin	CAA57633.1
	945.33	945.36	ASANEPDEN	f(81-89)	Albumin 1	1JLY_B
	677.26	677.26	VEEGNM	f(103-108)	Albumin 1	1JLY_B
	748.40	748.40	DFIILE	f(56-61)	Polyamine oxidase	AAM43922.1
	630.24	630.32	EVEAAI	f(133-138)	Polyamine oxidase	AAM43922.1

13 potentially multifunctional peptide sequences have been identified in the kiwicha digests

CONCLUSIONS

- **The role of kiwicha proteins as source of multifunctional peptides released under *in vitro* conditions simulating gastrointestinal digestion is confirmed.**
- **These proteins might be used as ingredients for functional foods to prevent and/or manage chronic diseases related to oxidative stress, hypertension and/or diabetes.**

REFERENCES

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