



Antioxidant and anti-inflammatory potential of peptides derived from in vitro gastrointestinal digestion of germinated and heat-treated foxtail millet (*Setaria italica*) proteins

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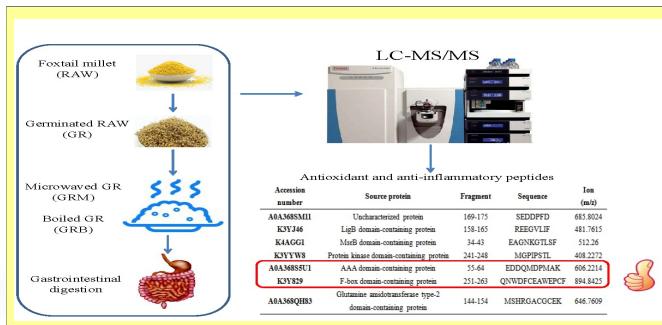
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Accepted article published in *J. Agric. Food Chem.* 2020, 68, 9415–9426.

Introduction

Germination is an economic and feasible approach to enhance the nutritional value of seeds. Gastrointestinal digestion is an inevitable process for food proteins in the human body. However, no study reported about the potential role of germinated and heated (boiling and microwave) foxtail millet protein as a source of dual antioxidant and anti-inflammatory peptides during its passage through the gastrointestinal tract. Here, seven novel peptides were identified using liquid chromatography with tandem mass spectrometry (LC-MS/MS) from the most potent F4 subfraction derived from boiled germinated millet, whereas two peptides (EDDQMDPMAK and QNWDFCEAWEPCF) were superior in inhibiting nitric oxide, tumor necrosis factor- α (reduced to 42.29 and 44.07%, respectively), and interleukin-6 (reduced to 56.59 and 43.45%, respectively) production in a RAW 264.7 cell model. This study is the first to report about the potential role of germinated and heated foxtail millet as a source of dual antioxidant and anti-inflammatory peptides.

1. Table of contents graphic.



4. Cell-based antioxidant activities of the synthesized peptides.

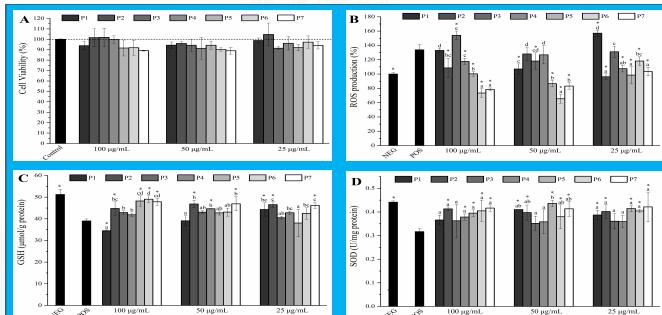


Fig.3. Cell-based antioxidant activities of the synthesized peptides at different concentrations. (A) Cell viability, (B) ROS production, (C) GSH content, and (D) SOD activity. P1–P7 represent the seven synthesized peptides.

5. Cell-based anti-inflammatory activities of the synthesized peptides.

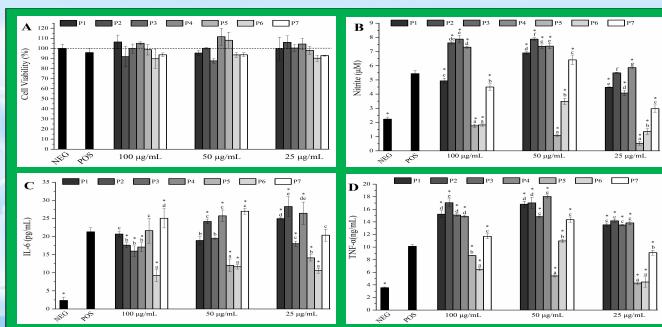


Fig.4. Cell-based anti-inflammatory activities of the synthesized peptides at different concentrations. (A) Cell viability, (B) NO production, (C) IL-6 secretion, and (D) TNF- α content. P1–P7 represent the seven synthesized peptides.

2. Purification of germinated foxtail millet peptides.

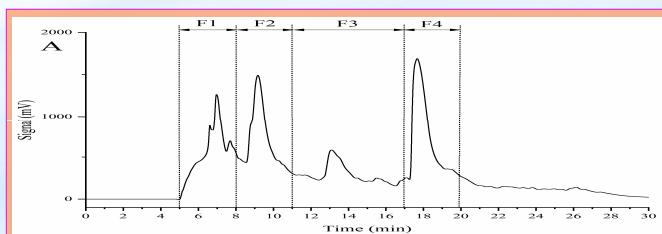


Fig.2. Representative chromatograms of the <3 kDa peptide fraction of RAW in vitro gastrointestinal digest obtained by semipreparative RP-HPLC, collected fractions were coded as F1–F4.

3. Identification of antioxidant and anti-inflammatory peptides.

Tab.1. Identification of peptides contained in F4 collected from the GRB.

Accession number	Source protein	Fragment	Sequence	Ion (m/z)
A0A368S111	Uncharacterized protein	169-175	SEDDPF	685.8024
K3YJ46	LigB domain-containing protein	158-165	REEGVLF	481.7615
K4AGG1	MsrB domain-containing protein	34-43	EAGNKGTLSF	512.26
K3YVW8	Protein kinase domain-containing protein	241-248	MGPPIPSTL	408.2272
A0A368S511	AAA domain-containing protein	55-64	EDDQMDPMAK	606.2214
K3V829	F-box domain-containing protein	251-263	QNWDFCEAWEPCF	894.8425
A0A368QH83	Glutamine amidotransferase type-2 domain-containing protein	144-154	MSHRGACGCEK	646.7609

Conclusion

This is the first study to report that bioactive peptides derived from the in vitro gastrointestinal digestion of germinated and heat-treated foxtail millet have the potential to modulate oxidative stress and inflammation. These findings will promote the consumption of germinated foxtail millet and provide the basis for the biological activity and cooking method of foxtail millet.