

IMPACT OF THERMAL PROCESSING AND SIMULATED IN VITRO DIGESTION ON PHYTOCHEMICAL CONTENT, NUTRITIONAL PROFILE, AND BIOFUNCTIONAL PROPERTIES OF FENUGREEK SEEDS

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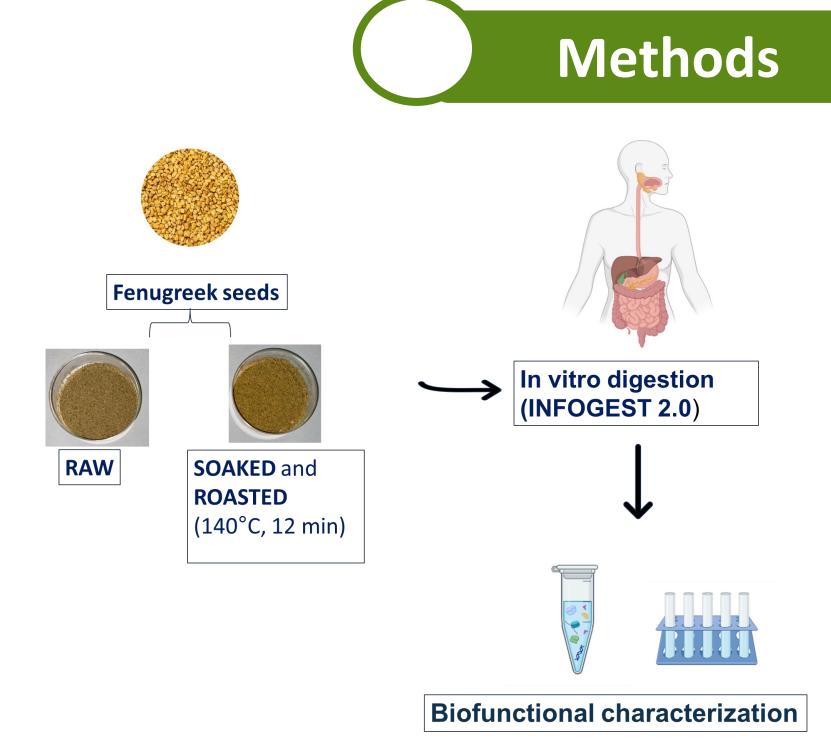
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Introduction

Fenugreek (*Trigonella foenum-graecum L.*) is a plant commonly used in traditional medicine and cooking for its health benefits. Its seeds contain natural compounds such as flavonoids and polyphenols, known for their antioxidant, anti-inflammatory, and antidiabetic effects.

Processing methods like soaking and roasting can enhance fenugreek's nutritional value but may also change its bioactive compounds. During digestion, these compounds can be further altered, influencing their stability and effectiveness.

In this study, we investigated how soaking, roasting, and simulated gastrointestinal digestion affect the antioxidant activity and phytochemical composition of fenugreek seeds. Molecular changes were also characterized using high-field Nuclear Magnetic Resonance (NMR) spectroscopy to better understand the behavior of key bioactive compounds.



- Phytochemical profile (polyphenols, flavonoids)
- Antioxidant activity (FRAP, ORAC)
- Enzyme inhibition (α-Amylase Lipase)
- NMR Spectroscopy

Results

Flavonoids exhibited remarkable stability
Processing lowers polyphenols, but intestinal digestion
greatly increases their bioaccessibility.

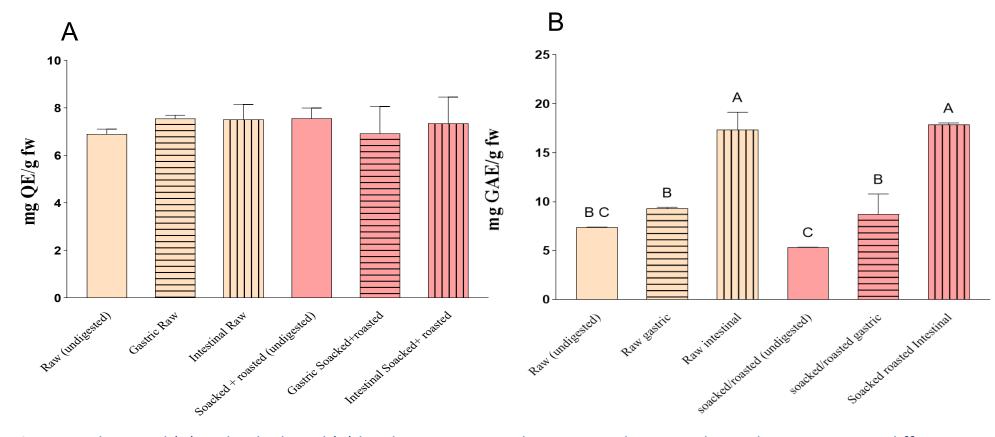


Figure 1. Flavonoid (A) and polyphenol (B) levels in raw, treated, gastric, and intestinal samples. Mean \pm SD; different letters indicate p < 0.05 (ANOVA + Tukey).

Lipase inhibition drops sharply after intestinal digestion. α -Amylase inhibition appears only after intestinal digestion, favoring raw seeds.

Table 1. Lipase and α -amylase inhibition (800 μ g/mL) by raw and treated samples under digestion (mean \pm SD).

Sample treatment	Digestive Condition	Pancreatic Lipase Inhibition (%)	α-Amylase Inhibition (%)
Raw	Undigested	$82.64 \pm 12{,}28$	
Soaked and Roasted	J	$7.98 \pm 2{,}76$	
Raw	Gastric Digestion	$40.14 \pm 5{,}84$	
Soaked and Roasted	<u> </u>	$51.01 \pm 1,90$	
Raw	Intestinal		73.17 ± 9.66
Soaked and Roasted	Digestion		25.05 ± 1.73

NMR analysis showed that soaking and roasting had minimal effect. intestinal digestion produced new small metabolites, amino acids, sugars, and partially hydrolyzed lipids.

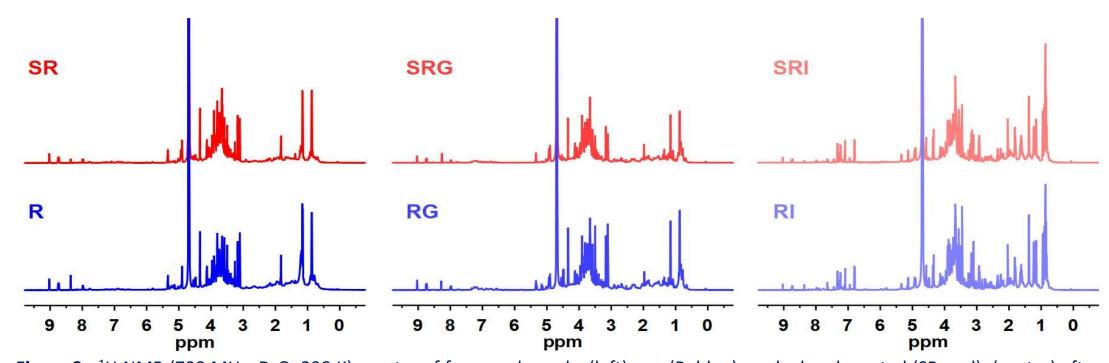


Figure 2. ¹H NMR (700 MHz, D₂O, 298 K) spectra of fenugreek seeds: (left) raw (R, blue), soaked and roasted (SR, red), (centre) after simulated gastric digestion (RG, blue, SRG, red), and (right) after simulated intestinal digestion (RI, blue, SRI, red).

Intestinal digestion significantly boosts the antioxidant activity of fenugreek seeds, while soaking and roasting have minimal effect.

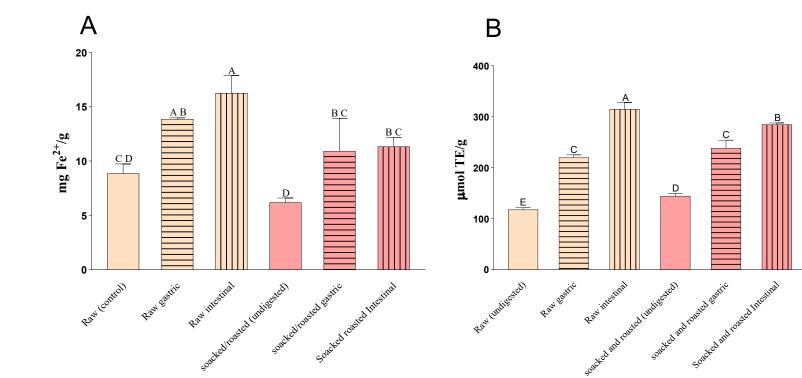


Figure 3. Antioxidant activity of fenugreek seeds (FRAP and ORAC) under different treatments (mean \pm SD; p < 0.05).

Conclusion

- Fenugreek's activity depends on processing and digestion.
- Flavonoids remain stable, while polyphenols peak intestinally.
- Digestion releases sugars, amino acids, and fatty acids.
- Lipase inhibition is lost; α -amylase inhibition emerges intestinally, strongest in raw extracts.
- Intestinal digestion drives bioactivity.

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