Uni ct AGRICOLTURA, ALIMENTAZIONE E AMBIENTE

GSICA SISS SISTAL SIMTREA Società Italiana di Microbiologia Agraria, Alimentare e Ambientale

28th Workshop on the Developments in the Italian Ph.D research on Food Science, Technology and Biotechnology

Catania, 18-20 September 2024

Exploring approaches to modify the techno-functionality of plant-based proteins

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INTRODUCTION

The increase in the global population and the growing awareness towards the sustainability impact of livestock farming have generated a large demand for alternative eco-friendly proteins that can meet the new nutritional needs. Plant-based proteins have emerged as promising candidates, and their use is already widespread. Utilization of plant proteins is not always straightforward; some strategies have been studied over time to improve their sensory, nutritional, and technological properties, including fermentation and high hydrostatic pressure (HPP) (Nasrabadi et al., 2021).

AIM: Investigate the techno-functional properties of selected plant-based proteins and test HPP and Fermentation as technologies for their modification.

MATERIALS AND METHODS

Pea protein isolate (77%) → 5%w/w dispersions ← **Milk whey protein concentrate (80%)**

↓

HPP

400 Mpa;10 min; *QFP* 35L-600, (*Avure* Technologies)

SSICA *Stazione Sperimentale per l'Industria delle Conserve Alimentari, Parma*

Pea protein isolate (77%) ↓ 10%w/w dispersion → + 2%w/w sucrose ← 20%w/w dispersion ↓ **Pumpkin seeds protein isolate (74%)**

↓

FERMENTATION

37°C; 18h; *Lactobacillus delbrueckii* subsp. *bulgaricus* (2214)

→ Freeze-drying ←

↓

TECHNO-FUNCTIONAL CHARACTERIZATION

WAC/OAC (g/g)

1g of powder + 10 mL of water/oil

↓

2 minutes of vortex agitation; centrifugation (3000 g x 20 min); pellet weighing.

$$WAC = \frac{water\ absorbed(g)}{dry\ sample\ (g)} ;\ OAC = \frac{oil\ absorbed(g)}{dry\ sample\ (g)}$$

Foaming Capacity (FC) and Stability (FS)

1% dispersion**

↓

Magnetic stirring*; High-speed homogenization (15000 rpm x 2 min) in graduated cylinder; Foam volume measured immediately and after 30 min.

$$FC = \left[\frac{(Vf - V0)}{V0}\right] \times 100\ ;\ FS = \left[\frac{(V30 - V0)}{(Vf - V0)}\right] \times 100$$

Emulsifying Capacity (EAI) and Stability (ESI)

15 mL of 1% dispersion + 5 mL sunflower oil **

↓

Magnetic stirring*; High-speed homogenization (12000 rpm x 2 min) in 50 mL centrifuge tubes. Absorbance measured at 500 nm.

$$EAI = \frac{(2 \times 2.303 \times A_0 \times DF)}{(C \times \varphi \times 10^4)}\ ;\ ESI\ (\%) = \left(\frac{A_t}{A_0}\right) \times 100$$

Solubility (%)

2% dispersion**

↓

Magnetic stirring*; centrifugation (10000g x 30 min); drying and weighing of the supernatant.

$$Solubility = \frac{Dry\ supernatant\ (g)}{Initial\ sample\ (g)} \times 100$$

*solubilization was conducted overnight with addition of sodium azide for HPP study, for 2h without antimicrobic for fermentation study.**For HPP study assessed at pH 7.0, for fermentation study, at pH 7.0 and 4.5.

FERMENTATION

Pea proteins

Treatment	pH	Solubility (%)	EAI (m^2/g)	ESI (%)	FC (%)	FS (%)	WAC(g/g)	OAC(g/g)
Control	4.5	22,1±0,6 bc	12,4±1,0 a	52,3±1,7 a	64,0±6,9 a	14,8±4,6 b	2,3±0,09 a	1,43±0,04 a
Control	7.0	26,8±1,7 a	13,6±1,5 a	86,3±16,5 a	60,0±6,9 a	44,7±4,7 a		
Fermented	4.5	19,9±1,4 c	6,7±1,7 b	59,7±30,7 a	30,7±2,3 b	0,0±0,0 c	2,40±0,00 a	1,34±0,02 b
Fermented	7.0	25,8±1,9 ab	14,3±1,4 a	78,6±9,5 a	41,3±2,3 b	51,5±2,6 a		
Variable					**Significance**			
treatment		0,099	0,015	0,989	0,000	0,087	0,118	0,025
pH		0,000	0,001	0,035	0,296	0,000	/	/
treatment * pH		0,492	0,004	0,491	0,039	0,001	/	/

Pumpkin seeds proteins

Treatment	pH	Solubility (%)	EAI (m^2/g)	ESI (%)	FC (%)	FS (%)	WAC(g/g)	OAC(g/g)
Control	4.5	20,7±0,9 bc	3,9±0,5 c	143,3±53,8 a	0,0±0,0 b	0,0±0,0	1,74±0,04 a	2,15±0,04 a
Control	7.0	27,9±2,7 a	15,7±0,1 a	23,5±1,2 b	0,0±0, b	0,0±0,0		
Fermented	4.5	19,2±1,5bc	5,1±0,7 c	83,7±3,4 ab	0,0±0,0 b	0,0±0,0	1,75±0,03 a	2,02±,11 a
Fermented	7.0	28,1±1,1 a	6,9±0,6 b	82,9±11,6 ab	13,3±2,3 a	0,0±0,0		
Variable					**Significance**			
Treatment		0,523	0,000	0,992	0,000	/	0,773	0,131
pH		0,000	0,000	0,012	0,000	/	/	/
Treatment * pH		0,401	0,000	0,013	0,000	/	/	/

Effect of fermentation:

- No effect on solubility and WAC
- Positive effect on foaming capacity for pumpkin proteins; negative effect for pea proteins
- Negative effect on OAC and emulsifying capacity
  →Hydrolysis, and changes in secondary and tertiary structures (Emkani et al.,2022)

HPP

Pea and milk whey proteins

Protein	Treatment	Solubility (%)	EAI (m^2/g)	ESI (%)	FC (%)	FS (%)	WAC(g/g)	OAC(g/g)
Whey	CTR	98,7±1,9 b	15,9±4,0 a	63,8±31,3 a	104,8±16,4 a	41,1±5,36 a	nd	1,59±0,12 a
	HPP	100,0±0,0 a	19,8±1,4 a	88,3±8,0 a	103,3±14,0 a	41,2±6,83 a	nd	2,55±0,29 a
Pea	CTR	26,6±1,8 c	9,4±2,0 b	55,1±6,4 a	60,8±18,3 b	57,2±19,76 a	4,00±0,47 a	1,72±1,25 a
	HPP	25,7±0,4 c	15,4±0,2 a	72,3±30,5 a	60,0±8,7 b	66,4±8,45 a	4,67±0,71 a	2,72±0,16 a
Variable					**Significance**			
Treatment		0,825	0,002	0,126	0,891	0,491	0,245	0,030
Protein		<0,001	0,001	0,348	0,000	0,008	nd	0,704
Treatment*protein		0,302	0,431	0,779	0,968	0,500	nd	0,954

- HPP showed effect only on EAI, ESI and OAC with slight increasing of their values → unfolding of proteins, and exposure of polar and non-polar residues (Wang et al., 2022).

CONCLUSIONS

- HPP showed slight effect on some of the techno-functional properties (EAI and OAC);
- LAB fermentation induced changes in some techno-functional properties (e.g., foaming and emulsifying capacity and stability), depending on the specific sample;
- Specific analysis are needed, to better understand the degree and type of proteins modification (e.g., proteolysis, digestibility) and address a more targeted approach.

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IPSUS *The project is part of IPSUS project "Climate-smart food innovation using plant and seaweed proteins from upcycled sources" (ERA-NETs SUSFOOD2 and FOSC Joint Call).*

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