Development of Hemp Protein Hydrolysates as a functional ingredient and its applications

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Introduction

- Hemp seed meal, a by-product of the hemp oil processing industry, is well known for its high protein contents and cost-effectiveness. However, compared to most plant proteins, hemp protein has relatively low solubility.
- To address the low solubility of hemp protein, this study used two common enzymes for hemp seed meal hydrolysis and then, explored the nutritional and functional properties.
- Additionally, the study investigated the effects of different hydrolysates concentrations when mixed with corn starch on antioxidant activity and starch digestibility.

Objectives

- Extract high-purity hemp protein isolate from hemp seed meal.
- Produce hemp protein hydrolysates (HPH) using bacterial and pepsin proteases.
- Characterize the nutritional and functional properties of the hydrolysates.
- Understand the interactions between Starch-HPH for the future development of healthier, more sustainable, and innovative foods

Methodology

Preparation of Protein Isolates:

- Defatting and isoelectric precipitation.

Enzymatic Hydrolysis of Hemp Protein Isolates:

- Bacterial protease: 55 ℃, pH 10.0.
- Pepsin protease: 37 ℃, pH 2.0.

Preparation of Starch-HPH complexes:

- HPH mixed with NCS (normal corn starch), WCS (waxy corn starch), and HACS (high amylose corn starch) at 0, 10, and 20% (w/w) ratios.

Characterizations:

- Characterization of intermolecular interactions and functional properties.
- Amino acid profiling, SDS-PAGE, Antioxidant activity, and starch digestibility.

Conclusion

- Hemp protein hydrolyzed with bacterial protease (HPHB) enhanced WHC and EC, while hemp protein hydrolyzed with pepsin (HPHP) improved OAC and FC.
- The total essential amino acids in HPH are higher than the recommended value by FAO/WHO (2013), which is 275 mg/g.
- The acidic and basic subunits of HPI were completely degraded by pepsin after 120 minutes and by bacterial protease after 60 minutes of hydrolysis.
- HPH at concentrations of 10% and 20% exhibits antioxidant properties when mixed with starch. Among these, 20%HPHP shown the highest antioxidant activity in starches with three different amylose content.
- The addition of 20%HPHP to HACS caused a significant 22% decrease in rapidly digestible starch. The highest increase in resistant starch values was about 10% with 10%HPHB in WCS.

Overall, bacterial protease is a better option for hydrolyzing HPI due to its shorter processing time, lower concentration requirements, and functional properties that are comparable to pepsin.

References

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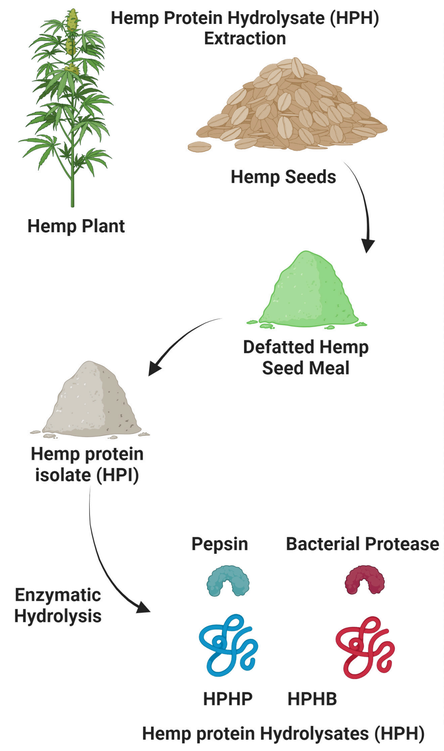

Figure 1. Experimental scheme of study.

Results

Table 1. Extraction Yield and Protein Content of HPI, HPHB, and HPHP, along with Intermolecular interactions and Functional Properties of HPHB and HPHP.

	Extraction yield (%)	Protein content (%)
HPI	31.40 ± 0.33	92.19 ± 0.44
HPHB	37.20 ± 0.80	91.95 ± 0.06
HPHP	63.73 ± 0.61	93.37 ± 0.03
Intermolecular interactions	**HPHB**	**HPHP**
Surface hydrophobicity (H_o)	1201.75 ± 42^{b}	3023.25 ± 43.5^{a}
Free SH (μmol/g)	3.74 ± 0.10^{b}	15.49 ± 0.00^{a}
Total SH (μmol/g)	20.62 ± 0.06^{b}	72.71 ± 1.03^{a}
SS bounds (μmol/g)	8.44 ± 0.08^{b}	28.61 ± 0.52^{a}
Functional Properties		
WHC (g/g)	1.12 ± 0.01^{a}	1.07 ± 0.02^{b}
OAC (g/g)	5.18 ± 0.03^{b}	5.46 ± 0.00^{a}
EC (mL/g)	47.83 ± 0.80^{a}	45.83 ± 0.52^{b}
FC (%)	28.00 ± 1.02^{b}	31.20 ± 0.75^{a}

Means in a column with different superscript letters are significantly different (p < 0.05). (WHC: Water holding capacity; OAC: Oil absorption capacity; EC: Emulsion capacity; FC: Foaming capacity).

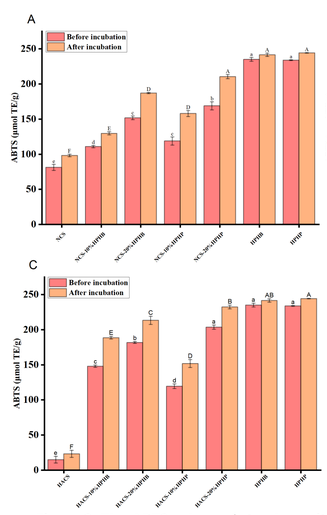

Figure 3. The ABTS values of starches with different amylose content and Starch-HPH complexes. (A) normal corn starch, (B) waxy corn starch, (C) high amylose corn starch. Bars with different letters have mean values that are significantly different (p<0.05).

Table 2. Amino acid compositions, the nutritional profile of HPI, HPHB and HPHP.

Amino acids (mg/g)	Aspartic acid (Asp)	Glutamic acid (Glu)	Serine (Ser)	Histidine (His)	Glycine (Gly)	Threonine (Thr)	Arginine (Arg)	Alanine (Ala)	Tyrosine (Tyr)	Valine (Val)	Methionine (Met)	Phenylalanine (Phe)	Isoleucine (Ile)	Lysine (Lys)	Leucine (Leu)	Proline (Pro)	EAA	NEEA	Total AA
HPI	46.80 ± 1.87^{a}	129.59 ± 2.77^{b}	35.39 ± 1.90^{a}	21.18 ± 1.13^{a}	14.62 ± 0.53^{a}	22.95 ± 1.00^{b}	87.69 ± 3.78^{c}	28.82 ± 1.27^{c}	24.70 ± 1.39^{a}	33.17 ± 4.39^{a}	16.37 ± 5.06^{a}	33.29 ± 1.38^{a}	27.15 ± 1.30^{a}	30.08 ± 1.19^{a}	42.78 ± 1.56^{a}	30.35 ± 5.62^{a}	314.67 ± 19.94^{b}	310.28 ± 15.01^{b}	624.95 ± 34.83^{b}
HPHB	52.83 ± 0.32^{a}	154.81 ± 5.74^{a}	40.08 ± 0.67^{a}	15.17 ± 1.08^{c}	14.99 ± 0.15^{a}	22.91 ± 0.64^{b}	99.97 ± 0.52^{b}	33.00 ± 0.92^{b}	24.56 ± 0.12^{a}	33.18 ± 0.59^{a}	19.20 ± 2.00^{a}	32.90 ± 1.61^{a}	29.54 ± 0.55^{a}	29.04 ± 8.49^{a}	45.49 ± 1.04^{a}	23.67 ± 3.07^{a}	327.39 ± 6.92^{a}	343.94 ± 6.22^{a}	671.32 ± 9.66^{ab}
HPHP	52.31 ± 3.73^{a}	140.30 ± 10.88^{ab}	39.40 ± 3.51^{a}	17.86 ± 0.88^{b}	15.74 ± 1.41^{a}	25.65 ± 1.10^{a}	100.14 ± 8.51^{a}	34.24 ± 2.96^{a}	28.23 ± 2.21^{a}	34.48 ± 4.65^{a}	18.51 ± 2.81^{a}	33.96 ± 2.52^{a}	30.72 ± 2.40^{a}	28.23 ± 8.15^{a}	46.40 ± 3.99^{a}	31.30 ± 4.52^{a}	335.95 ± 30.14^{a}	341.53 ± 20.93^{a}	677.48 ± 51.06^{a}
FAO/WHO (2013) requirement for adult				16		25			41	40	23		30	48	61		275		

Different letters in the same column denote statistically significant differences (p < 0.05). EAA – Essential amino acids; NEAA – Non-essential amino acids; AA – Amino acids.

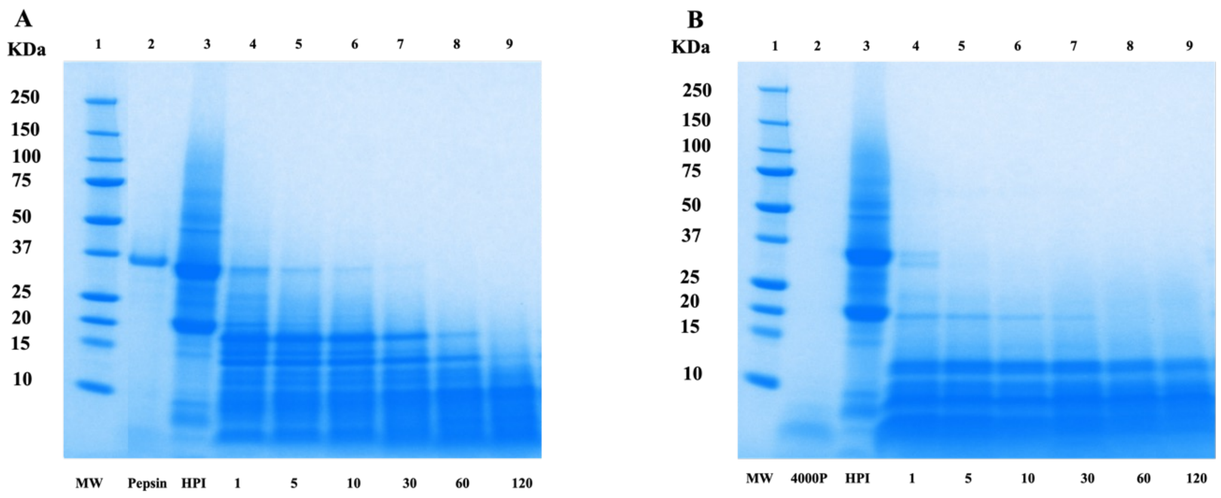

Figure 2. SDS-PAGE profiles of HPI, protease, and its hydrolysates. (A) Lane 1: protein molecular weight marker; Lane 2: pepsin; Lane 3: hemp protein isolate; Lane 4–9: HPI treated by 2% pepsin for 1, 5, 10, 30, 60, and 120 min, respectively. (B) Lane 1: protein molecular weight marker; Lane 2: bacterial protease; Lane 3: hemp protein isolate; Lane 4–9: HPI treated by 0.5% bacterial protease for 1, 5, 10, 30, 60, and 120 min, respectively.

Table 3. Starch digestibility of starches with different amylose content and Starch-HPH complexes.

	NCS			WCS			HACS		
	RDS (g/100g)	SDS (g/100g)	RS (g/100g)	RDS (g/100g)	SDS (g/100g)	RS (g/100g)	RDS (g/100g)	SDS (g/100g)	RS (g/100g)
Control	85.96 ± 0.22^{a}	12.59 ± 1.43^{a}	32.68 ± 1.03^{b}	89.89 ± 0.19^{a}	13.53 ± 2.76^{a}	29.70 ± 1.03^{ab}	59.82 ± 0.49^{a}	14.38 ± 1.11^{b}	57.63 ± 1.03^{b}
10%HPHB	84.30 ± 0.96^{b}	9.39 ± 0.58^{a}	30.89 ± 1.03^{c}	85.96 ± 0.56^{b}	11.12 ± 2.06^{ab}	32.68 ± 1.03^{a}	57.06 ± 1.61^{b}	12.24 ± 0.30^{b}	53.47 ± 1.78^{cd}
20%HPHB	78.47 ± 0.19^{c}	3.10 ± 0.86^{b}	26.73 ± 0.00^{d}	77.32 ± 0.54^{d}	6.61 ± 1.00^{b}	29.70 ± 1.03^{ab}	51.15 ± 0.51^{c}	12.19 ± 0.45^{b}	50.50 ± 1.03^{d}
10%HPHP	82.91 ± 0.54^{b}	9.58 ± 2.87^{a}	35.65 ± 0.00^{a}	79.04 ± 0.07^{c}	14.10 ± 1.46^{a}	32.08 ± 1.78^{a}	48.59 ± 1.01^{d}	17.56 ± 1.42^{a}	61.79 ± 1.10^{a}
20%HPHP	75.44 ± 0.44^{d}	10.826 ± 0.75^{a}	32.08 ± 0.00^{bc}	73.00 ± 0.30^{e}	9.86 ± 2.76^{ab}	28.52 ± 0.00^{b}	46.48 ± 0.08^{d}	14.80 ± 1.55^{ab}	55.25 ± 1.78^{bc}

RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. Bars with different letters have mean values that are significantly different (p<0.05).