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Enzymatically modified, pea protein-starch Maillard induced conjugates: A novel functionally improved food ingredient

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INTRODUCTION

Plant proteins are gaining increased attention as important functional ingredients to enhance the organoleptic properties (taste, texture, aroma, mouthfeel) of processed foods (1). However, the major drawback of plant proteins are their inferior functionality and digestibility owing to harsh commercial extraction conditions. Enzymatic modification of the proteins is an alternative "clean" approach to enhance their techno-functionality in a controlled approach, but their use is limited owing to loss in protein structures beyond certain degrees of hydrolysis (DH). Previous research has shown some improvement in legume protein functional properties at moderate hydrolysis (2,3,4). This study aims to further improve the protein functional properties by conjugating hydrolyzed proteins with the available starch fraction in pea protein-enriched flour making the process highly economically feasible and sustainable.

HYPOTHESIS AND OBJECTIVES

Hypothesis:

- Conjugation of hydrolyzed proteins with hydrophilic starch/dextrins will lead to an optimum hydrophobic-hydrophilic balance and increase their amphiphilicity leading to enhanced functional properties
- High DH coupled with starch conjugation will improve the techno-functionality of proteins

Objectives:

- ✓ Hydrolysis of the protein and starch fractions followed by heat-induced Maillard conjugation in a single reaction vessel ("One-pot") to obtain a variety of conjugates
- ✓ Comparative analysis of surface (Zeta potential) and functional properties (foaming, emulsification, water and oil holding properties) at pH 4, 7 and 10 of the raw samples, controls, and hydrolyzed conjugates.

MATERIALS AND METHODS

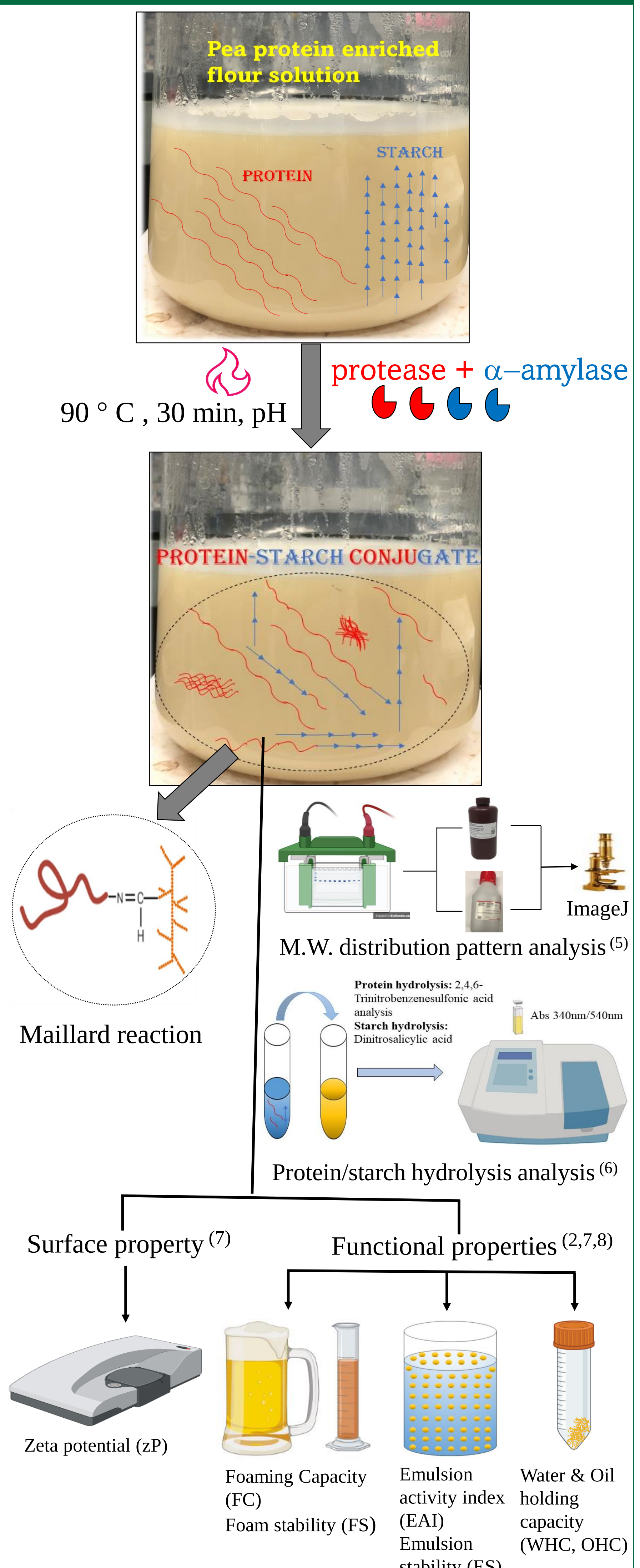


Figure 1. Schematic outline of "One-pot" conjugation

Statistical analysis

- Data → Mean ± Standard Deviation of triplicates
- One-way ANOVA + Tukey Post hoc test
- Different letters indicate a significant difference ($p < 0.05$)

RESULTS

Table 1: DH, Zeta Potential (mV) analysis of raw, control and enzymatically hydrolyzed conjugates at pH 4, 7, and 10; ImageJ densitometry analysis of Coomassie Blue (CB) and Fuchsin sulfite (Fuchsin) stained conjugates.

Sample	Name	Hydrolysis (%)		Zeta potential (mV)			% Intensity	
		DH	DE	pH 4	pH 7	pH 10	Fuchsin	CB
Raw sample	PPEF	-	-	8.19 ± 0.4^{ab}	-24.2 ± 0.5^{bc}	-27.3 ± 1.1^{f}	3.5	4.1
Trypsin control	Tryp0	-	-	8.3 ± 0.2^{b}	-28.6 ± 0.4^{ab}	-35.2 ± 2.4^{abcde}	4.9	6.8
Trypsin conjugates (Tryp conjugates)	Tryp2.5	2.5 ± 0.3^{a}	-	3.4 ± 0.2^{ab}	-28.2 ± 0.5^{ab}	-35.9 ± 1.2^{abcd}	5.0	5.5
	Tryp9.9	9.9 ± 2.3^{abcd}	-	2.7 ± 0.3^{ab}	-28.4 ± 0.5^{ab}	-36.6 ± 0.5^{abc}	5.1	5.2
	Tryp10.8	10.8 ± 0.8^{abcd}	-	2.4 ± 0.3^{ab}	-27.5 ± 0.3^{abc}	-34.9 ± 0.4^{abcde}	5.1	5.4
	Tryp17.7A	17.7 ± 1.25^{cde}	>1	-1.3 ± 1.5^{ab}	-20.9 ± 0.2^{c}	-29.8 ± 0.9^{def}	5.0	5.2
	Tryp19.4A	19.4 ± 4.7^{ce}	>1	0.9 ± 1.0^{ab}	-20.6 ± 0.8^{c}	-29.3 ± 0^{def}	5.0	4.4
	Tryp21.7A	21.7 ± 4.2^{de}	>1	1.8 ± 0.2^{ab}	-21.1 ± 0.1^{c}	-29.4 ± 0.8^{def}	5.1	4.4
	Tryp24A	24.1 ± 0.9^{e}	>1	0.2 ± 1.1^{ab}	-20.7 ± 0.1^{c}	-28.7 ± 0.7^{ef}	5.1	5.3
	Tryp25.9	25.9 ± 2.9^{e}	-	0.8 ± 0.7^{ab}	-29.6 ± 0.5^{ab}	33.2 ± 0.1^{abcdef}	5.1	5.1
Papain control	Pap0	-	-	8.4 ± 0.2^{b}	-28.5 ± 1.4^{ab}	-30.6 ± 1.2^{cdf}	5.1	5.5
Papain conjugates (Pap conjugates)	Pap3.3	3.3 ± 0.7^{ab}	-	-0.7 ± 0.4^{ab}	-30.4 ± 0.4^{ab}	-37.5 ± 0.3^{ab}	5.1	5.3
	Pap5.2	5.2 ± 1.0^{ab}	-	-1.2 ± 0.1^{ab}	-30.5 ± 1.3^{ab}	-34.7 ± 0.6^{abcde}	5.1	4.3
	Pap8.1	8.1 ± 0.9^{abc}	-	-1.3 ± 0.0^{ab}	-31.1 ± 0^{ab}	-34.8 ± 1.0^{abcde}	5.1	4.5
	Pap8.3A	8.3 ± 3.6^{abc}	>1	-0.7 ± 0.0^{ab}	-31.5 ± 1.4^{a}	-34.4 ± 0.4^{a}	5.1	4.1
	Pap9.1	9.1 ± 0.5^{abc}	-	-0.6 ± 0.0^{ab}	-31.9 ± 0.2^{a}	-33.1 ± 0.5^{abcde}	5.1	5.0
	Pap9.8A	9.8 ± 0.25^{abcd}	>1	-1.9 ± 0.4^{ab}	-33 ± 0.4^{a}	39.1 ± 1.5^{abcdef}	5.2	5.1
	Pap10.2	10.2 ± 0.3^{abcd}	-	-2.3 ± 1.9^{ab}	-32.2 ± 0.1^{a}	-38.9 ± 0.4^{a}	5.2	5.1
	Pap14.8A	14.8 ± 1.9^{bcde}	>1	-0.4 ± 0.4^{ab}	26.2 ± 5.0^{abc}	-31.3 ± 4.0^{bcdef}	5.0	4.8
	Pap18.7A	18.7 ± 1.0^{cde}	>1	-3.2 ± 1.0^{a}	-21.2 ± 0.7^{c}	-29.2 ± 0.1^{ef}	5.0	5.0

Increase

Increase

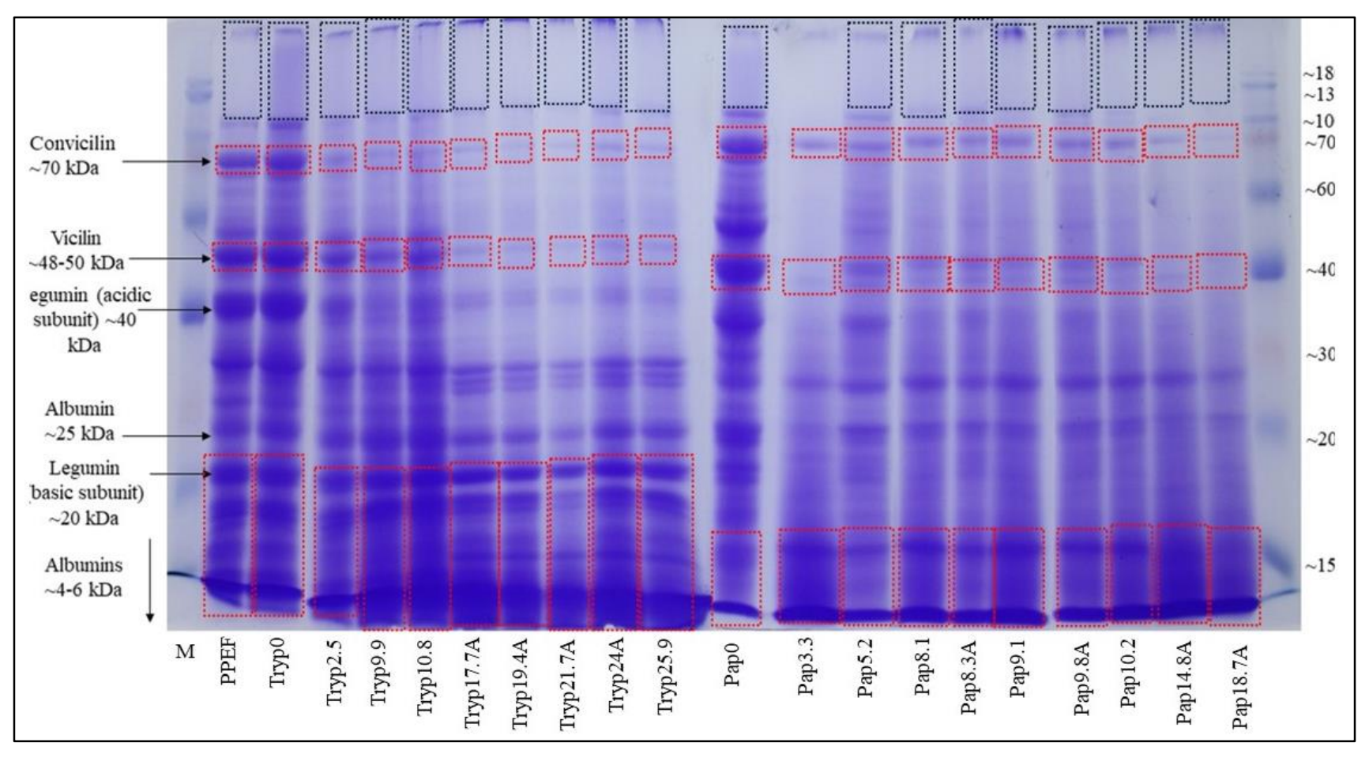


Figure 2. Coomassie Blue stain of PPEF, control and hydrolyzed conjugates

Surface charge (conjugates) > Surface charge (PPEF) at pH 7, pH 10
Intensity of **conjugates' smear** (Fuchsin + Coomassie Blue) > **Intensity** of **PPEF smear**
Disappearance of **high MW protein bands** + **appearance** of **low MW bands** with **increasing DH** → hydrolysis

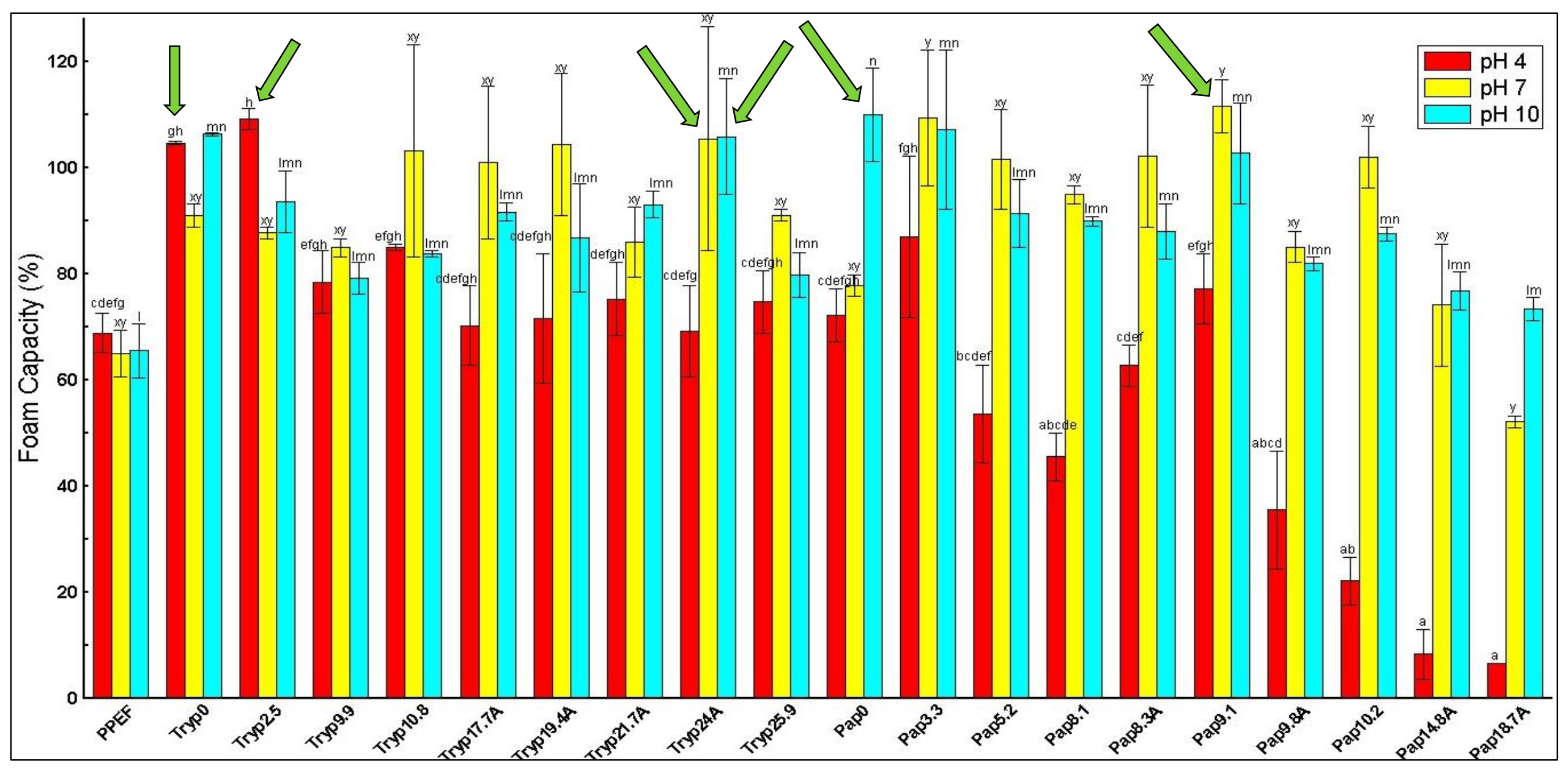
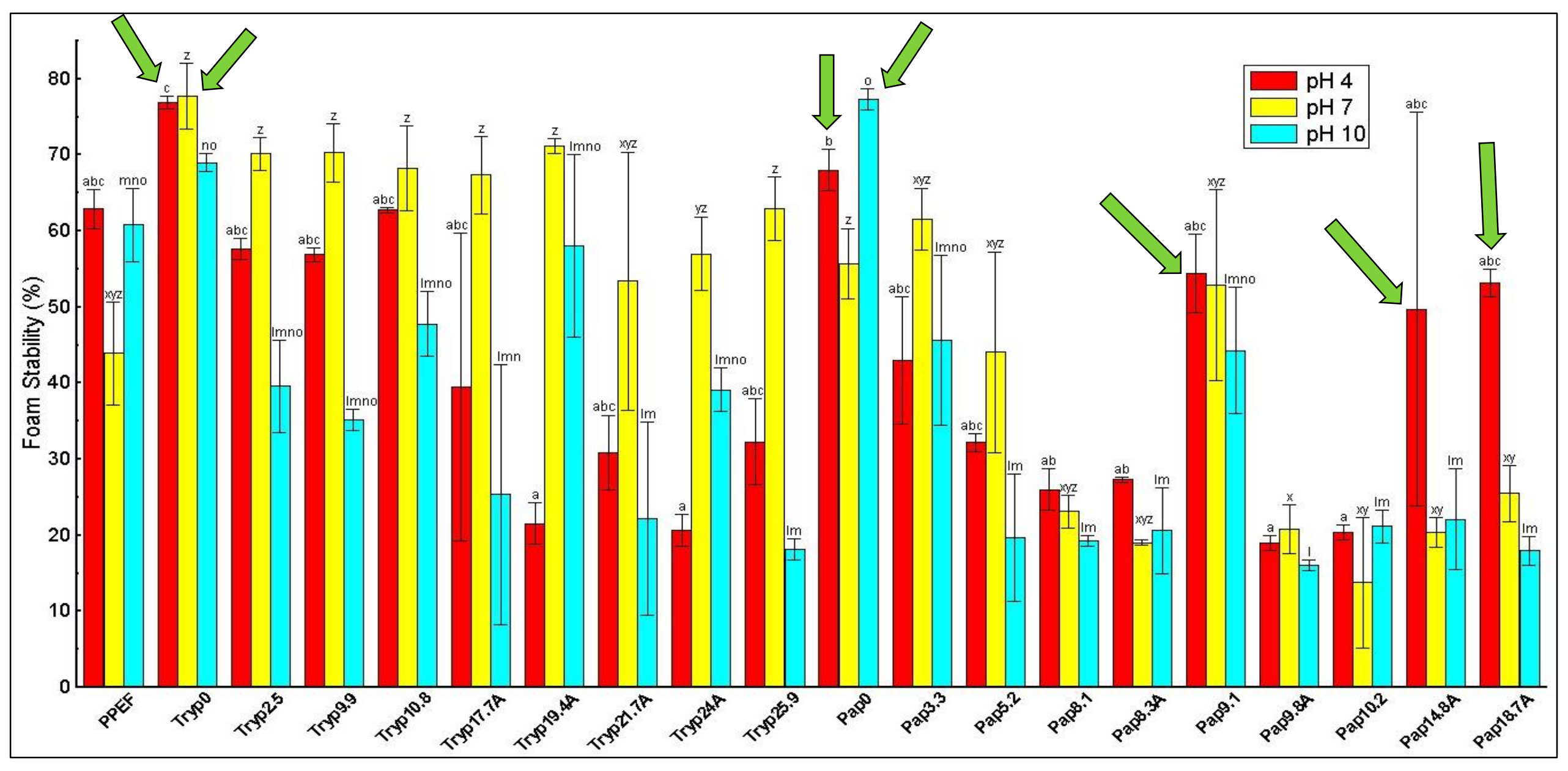


Figure 3. Foam Capacity (%) of PPEF, control and hydrolyzed conjugates at pH 4, 7 and 10.

- **High FC at pH 4: Tryp0** (104.7% ± 0.3%), **Tryp2.5** (109.2% ± 2.0%)
- **Highest FC: Pap9.1** (pH 7: 111.7% ± 5.0%)

Figure 4. Foam Stability (%) of PPEF, control and hydrolyzed conjugates at pH 4, 7 and 10.

- **Highest FS: Tryp0** (pH 4: 76.9% ± 0.9%, pH 7: 77.8% ± 4.4%) **Pap0** (pH 10: 77.3% ± 1.4%)
- **Pap conjugates:** FS (pH 7, 10) > FS (pH 4)



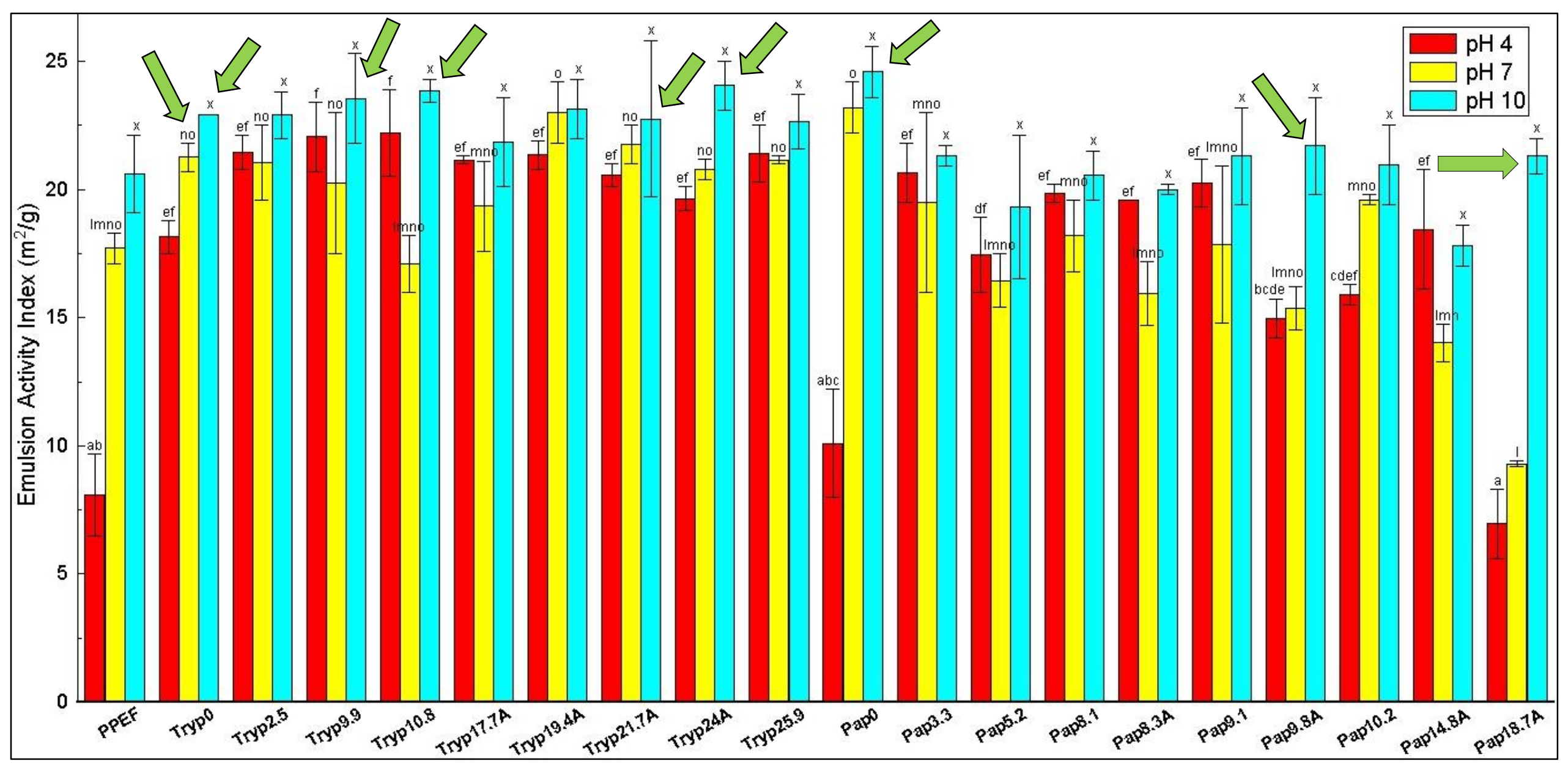
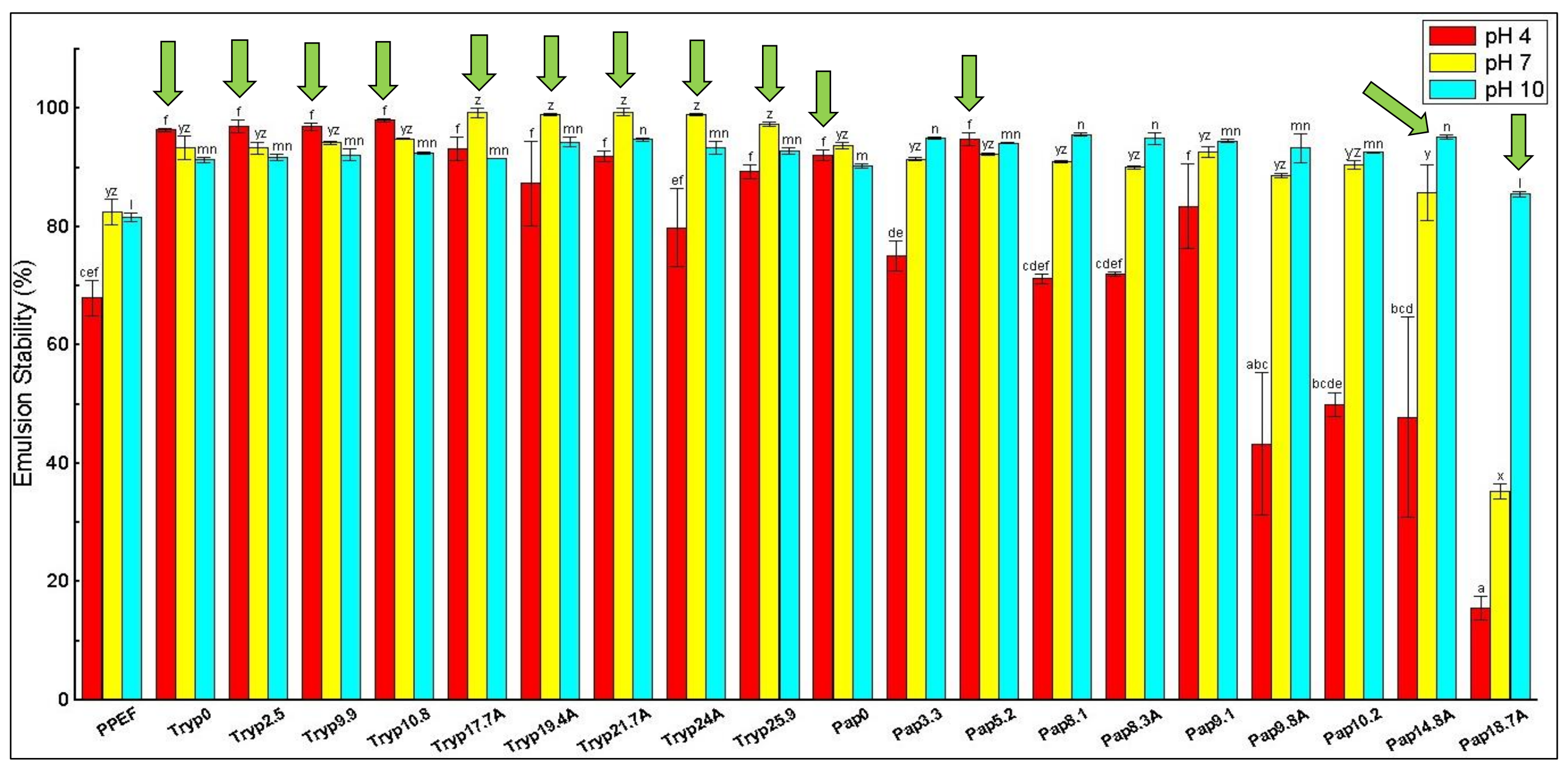


Figure 5. Emulsion Activity Index (m^2/g) of PPEF, control, and hydrolyzed conjugates at pH 4, 7, and 10.

- **Highest EAI of conjugates (pH 10):** 17.8% ± 0.8% - 24.6% ± 1.0%
- **EAI (pH 7, 10):** Comparable

Figure 6. Emulsion Stability (%) of PPEF, control, and hydrolyzed conjugates at pH 4, 7, and 10.

- **High ES (pH 4):** Tryp (68% ± 3.0% - 98% ± 0.3 %) Pap conjugates (83.5% ± 7.2% - 94.8% ± 1.1%)
- **ES (pH 7, 10**): Comparable ($p<0.05$)
- **Highest ES (pH 7): Tryp21.7A** (99.4% ± 0.7%), **Tryp24A** (99.0% ± 0.3%)



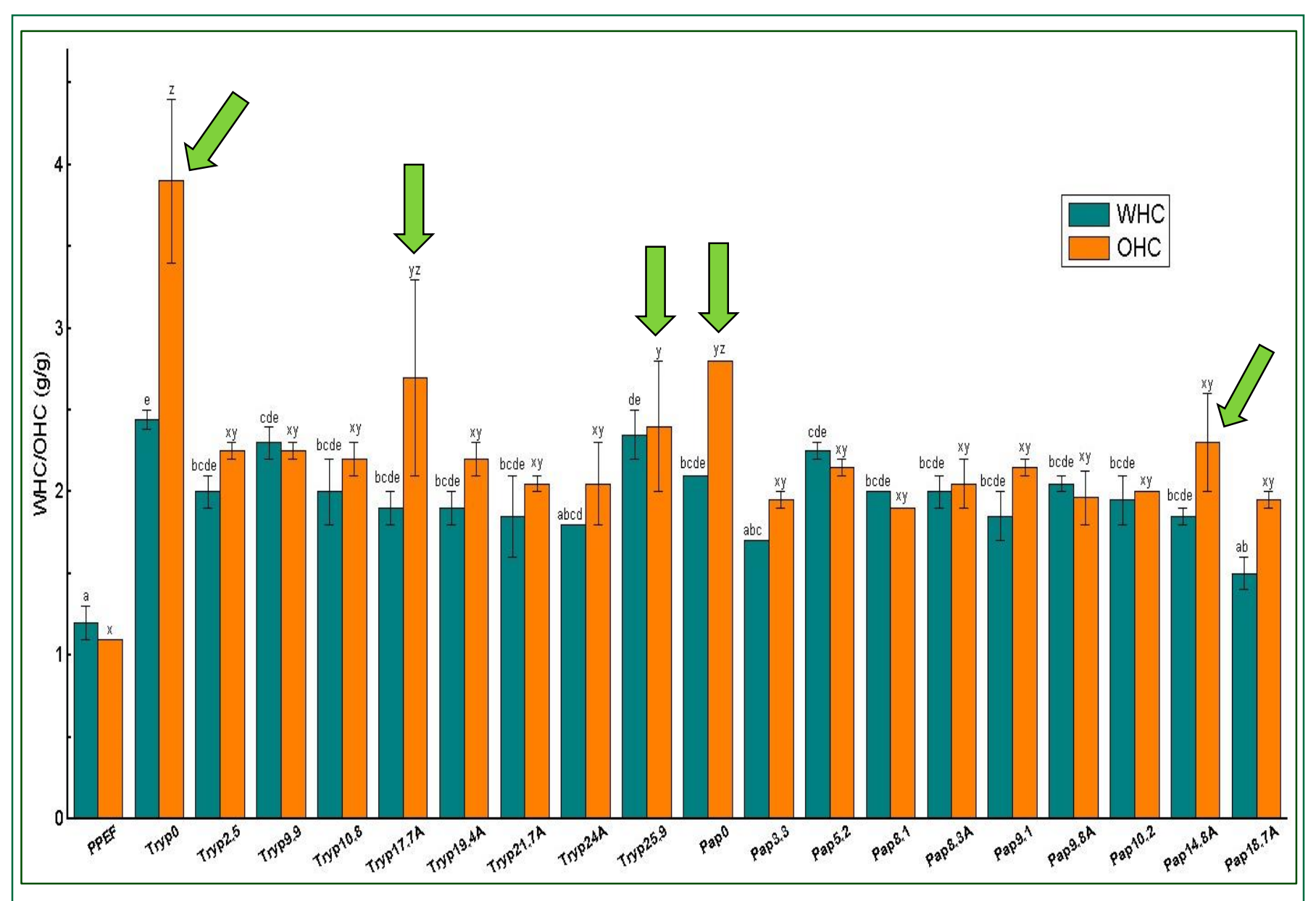


Figure 7: Water and Oil holding capacities of PPEF, control and hydrolyzed conjugates.

- **WHC + OHC of conjugates →** Significantly higher ($p<0.05$) than PPEF
- **Highest OHC + WHC:** Tryp0 (OHC: 3.9 ± 0.5 g/g; WHC: 2.4 ± 0.1 g/g)

CONCLUSIONS

- Foam Capacity (FC) of both Tryp and Pap conjugates at pH 7 and pH 10 were greater than PPEF
- Tryp conjugates showed high FC at pH 4 and FS at pH 4, pH 7 and pH 10 as compared to the Pap conjugates
- FC of both Tryp and Pap conjugates were comparable at pH 7 and pH 10.
- EAI of both Tryp and Pap conjugates were greater than PPEF at pH 4
- EAI of Tryp conjugates (pH 7, pH 10) was greater than EAI of Pap conjugates (pH 7, pH 10)
- ES of Tryp conjugates (pH 4, pH 7 and pH 10) and Pap conjugates (pH 7, pH 10) were significantly higher than PPEF
- ES of Tryp conjugates (pH 4 and pH 7) was greater than ES of Pap conjugates at pH 4 and pH 7)
- High foaming and emulsification at pH 4 indicate potential use of the conjugates as functional ingredients in the beverage industry especially in fruit juice, coffee, or other drinks having a low pH range.
- High WHC and OHC of the conjugates show probable use in the alternate protein industry as meat extenders/burger patties
- Most of the conjugates with high degrees of protein hydrolysis showed increased functionality, and the majority of the conjugates in general reported high techno-functional properties thus validating our hypothesis

FUTURE STUDY

In our future research studies, we aim to investigate the following:

- Effects of increased degrees of starch hydrolysis on the functional properties of conjugates using a pea starch-rich fraction
- Scaling up the process on an industrial scale for the formulation of food prototypes and comparison with available products in the market

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