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# Interactions between proteins, phytic acid and calcium in Fava Bean concentrate

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## INTRODUCTION

Alternative legume proteins such as fava bean become of a huge interest for their economic and ecologic advantages as well as for their health benefits. They are rich in protein and a good source of essential nutrients and minerals. However, these advantages can be partially impaired due to their high phytic acid (PA) content. This natural polyphosphate is a major antinutrient in plant-based foods, as it can bind minerals (particularly calcium) and proteins, thereby reducing protein digestibility and subsequent mineral bioavailability. Complexes formed are insoluble and unavailable for absorption, thus lowering the nutritional value of these pulses. Molecular interactions are influenced by the environmental medium including pH, ionic strength and molar concentrations modulating the stability of these complexes during protein extraction. Protein/phytic acid/calcium complexes stability is of high relevance for food processing affecting not only structure but also functional and nutritional properties of proteins in legume-based foods. In this context, the present study investigates possible associations between fava bean proteins (FP), phytate (PA), and calcium (Ca) involved in concentrate ingredient.

## I - INGREDIENT

Table 1: Comparison of protein, phytic acid and calcium contents of fava bean concentrate powder.

	Concentrate
Processing method	Dry processing (air classification)
Protein (g/100 g)	51.7 ± 1.8
Phytic acid (PA) (g/100 g)	3.2 ± 0.7
Total calcium (mg/100 g)	127.1

## II - METHODS

### Solubility in aqueous solutions as function of pH

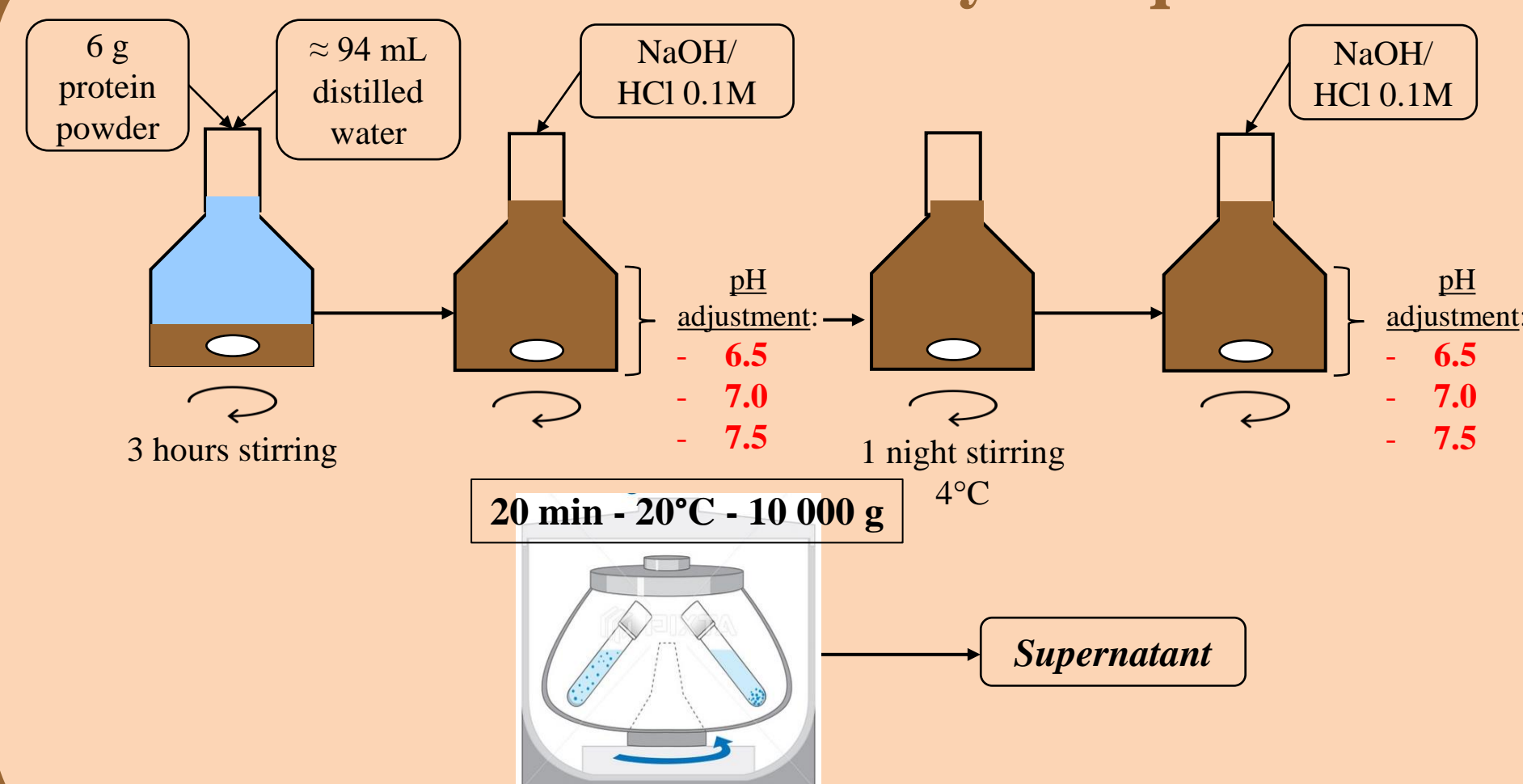


Figure 1: Protocol for concentrate ingredient solubilization in distilled water.

### Analysis:

- Nitrogen assay by Kjeldahl method: **protein concentrations**. N = 5.5 [1]
- Total phosphorus, free phosphorus and phytic acid** contents determination by Megazyme Phytic Acid kit (K-PHYT).
- Calcium concentration** by Flame Atomic Absorption Spectrometry.

## III - RESULTS

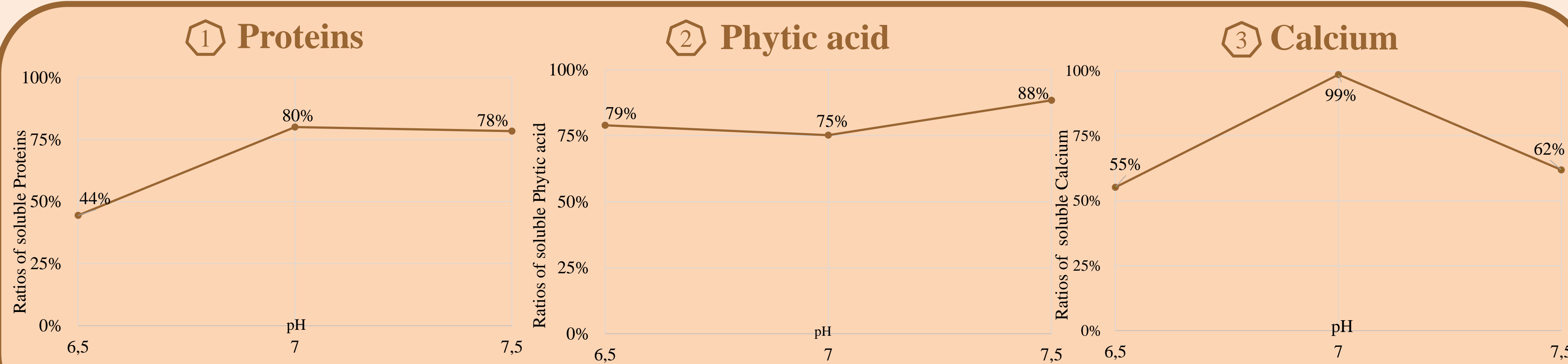
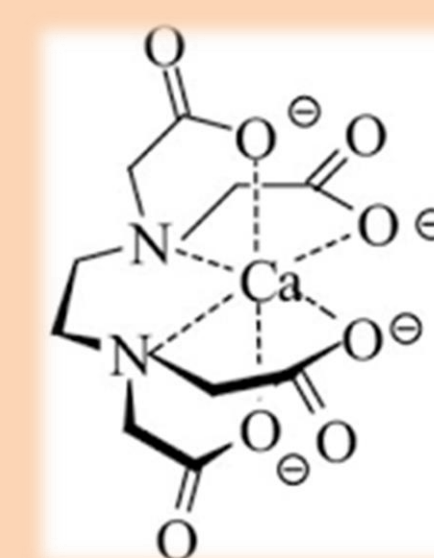


Figure 2: Comparison of percentages of soluble protein (1), phytic acid (2) and total calcium (3) in fava bean concentrate supernatants as function of pH.

### Techniques to investigate complexes

**Calcium chelation** by **EDTA** addition



**Protein removal (> 5 kDa)** by **Ultrafiltration**



Ultrafiltration processing

Millipore Kwick Lab Cassette Pellicon® 2 Mini Ultrafiltration Biomax® Module filtration area 0.1 m<sup>2</sup>, NMWCO 5 kDa - P ≈ 2-2.5 bar

## IV - HYPOTHESIS

### Influence of EDTA on concentrates

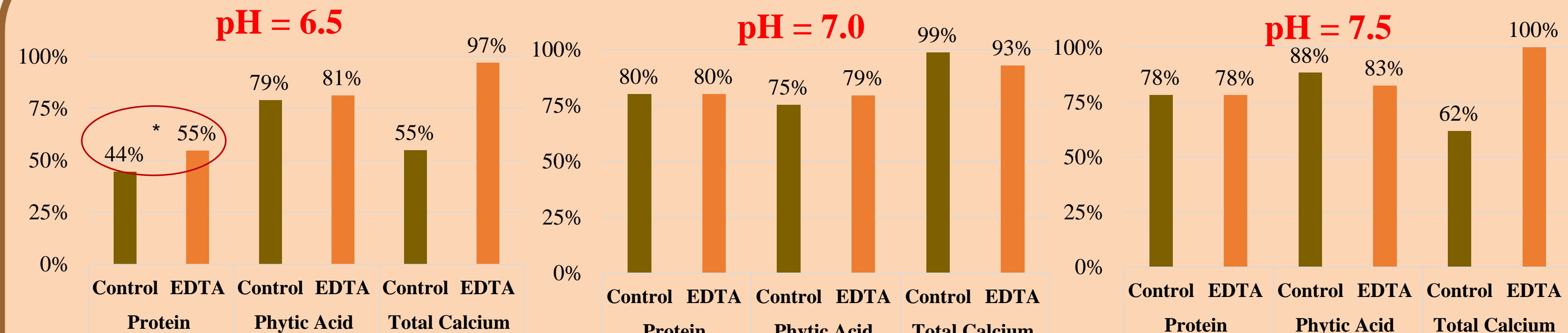


Figure 3: Comparison of ratios of soluble protein, phytic acid and total calcium on fava bean concentrate supernatants with or without EDTA addition in function of pH.

### Ultrafiltration influence on concentrates

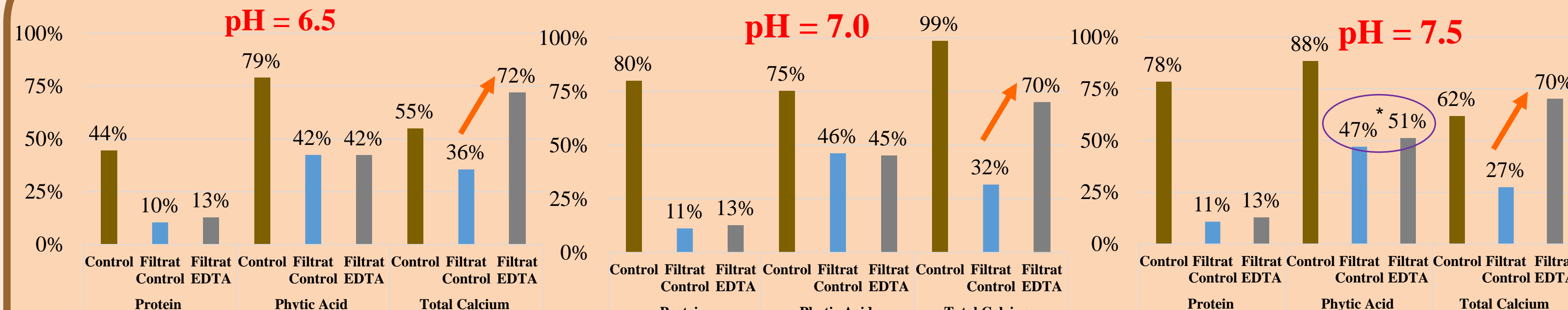
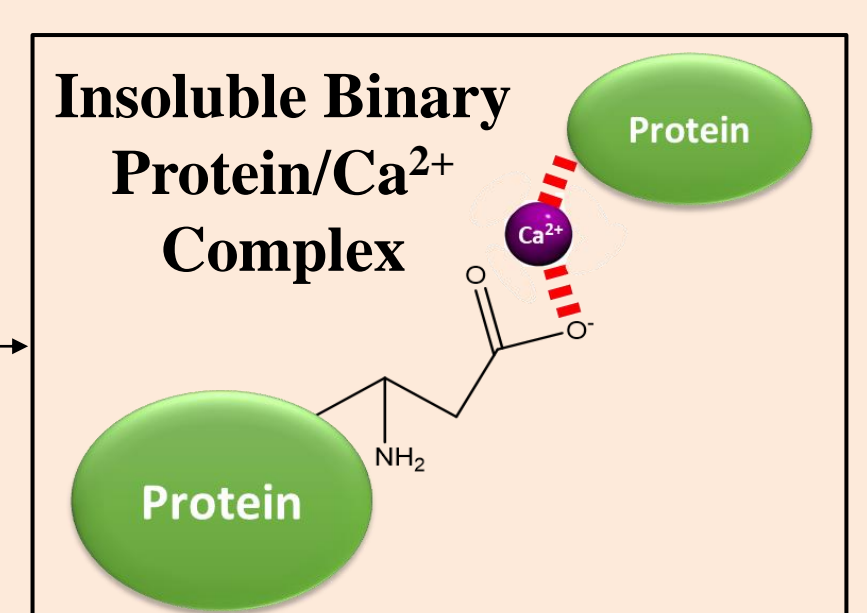


Figure 4: Comparison of ratios of soluble protein, phytic acid and total calcium on fava bean concentrate supernatants control vs filtrates with or without EDTA addition in function of pH.

### pH 6.5:

- Protein EDTA ratio ≥ 25%.**
- Insoluble Binary Proteins/Ca<sup>2+</sup> complexes.**
- pH > pI (≈ 4-5): proteins negatively charged.



### pH 7.5:

- ≈ 40% soluble PA/Protein/(Ca<sup>2+</sup>) complexes.
- PA EDTA Filtrate ratio ≥ 8.5%.**
- Small % of soluble Ternary complexes.**
- ≈ 90% of soluble Binary PA/Protein complexes.

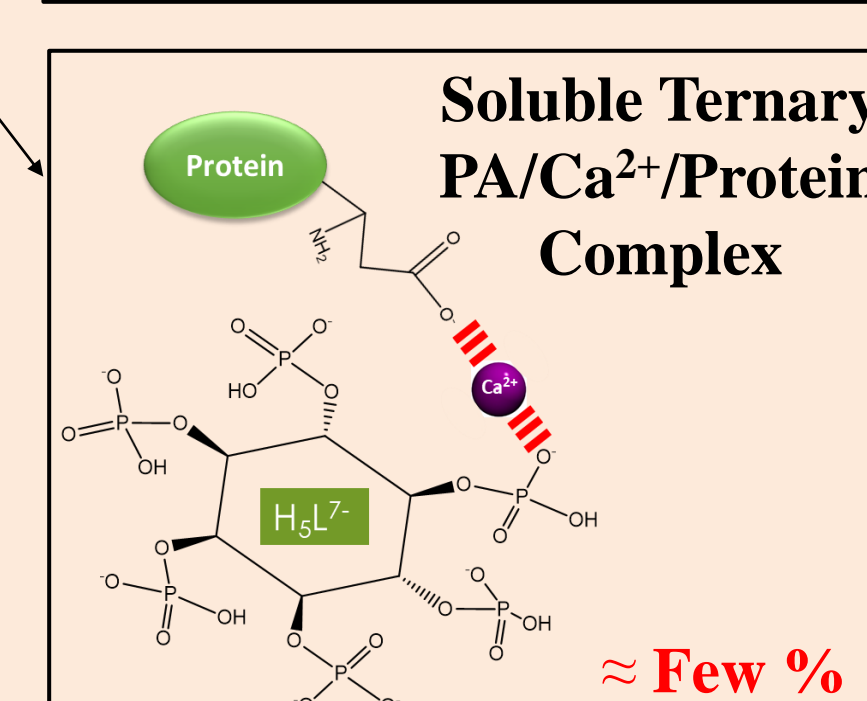
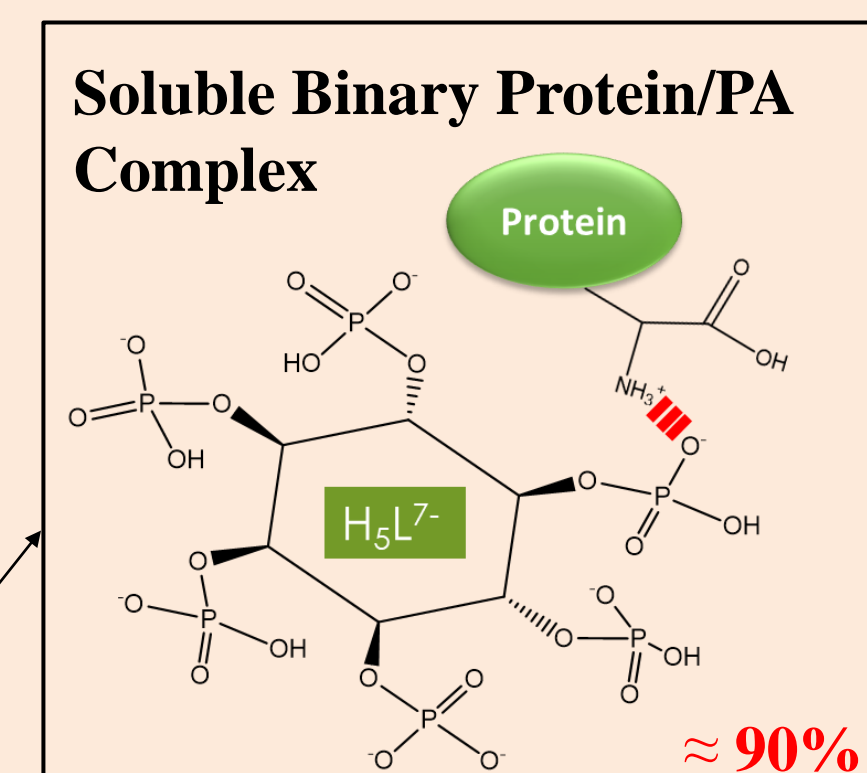


Figure 5: Proposed mechanisms of interactions between fava bean proteins, phytic acid and calcium depending on pH conditions.

## CONCLUSION

This study investigates possible mechanisms of interaction between phytic acid (PA), calcium (Ca) and fava bean proteins from concentrate (FPC). For the range of pH considered (6.5 to 7.5), results show the increase of the amount of soluble protein with pH raising. In addition, insoluble binary FPC/Ca complexes have been considered at pH 6.5 with no variation of phytic acid contents. The ultrafiltration process on concentrate highlights that around 40% of FPC/PA/(Ca) complexes are soluble. Even more, at pH 7.5, it should be considered a small percentage of ternary soluble complexes. Further work is needed to clarify these phenomena depending on ingredient process history. The role of protein fractions in such interaction represents an area of interest.

## References

- [1] Sosulski, F. W., & Holt, N. W. (1980). Amino acid composition and nitrogen-to-protein factors for grain legumes. *Canadian Journal of Plant Science*, 60(4), 1327-1331.