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Study of microbial fermentations of the seed of Mediterranean carob (*Ceratonia siliqua*, L.)

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INTRODUCTION & AIM

Plant-based foods are essential to the human diet and offer solutions to health, environmental, and economic challenges. Plant proteins, especially when fermented, become more nutritionally complete. Controlled microbial fermentation enhances food quality and safety, suppresses anti-nutritional factors like phytic acid, and enables the development of innovative, functional food products. The objective of this study was to investigate the microbial fermentation of carob seeds (*Ceratonia siliqua* L.), a xyrophytic plant widely distributed across Mediterranean countries, and their potential applications in the food industry.

METHOD

The carob seeds were primarily selected and dried and then fermented in lab-scale solid-state type fermentation by lactic acid bacteria (LAB), *Saccharomyces cerevisiae* (yeast) and *Aspergillus oryzae* (fungus) for four days. The fermented carob seeds were then analyzed for the following: a) in-vitro digestibility was tested by applying appropriate proteolytic enzymes and measuring pH decrease, b) concentration of phytic acid was calculated through a biochemical assay that included measurements of free and total phosphorus and c) protein electrophoresis was applied to analyze the degree of fragmentation of the fermented proteins from carob seeds.

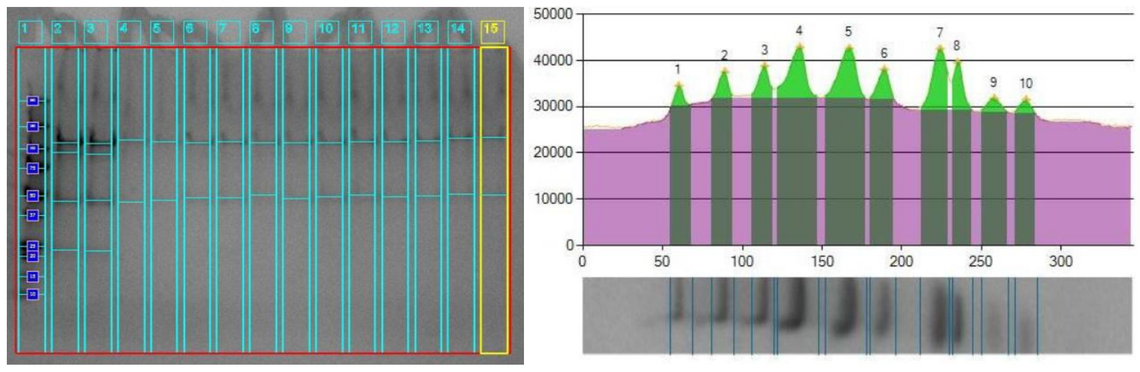


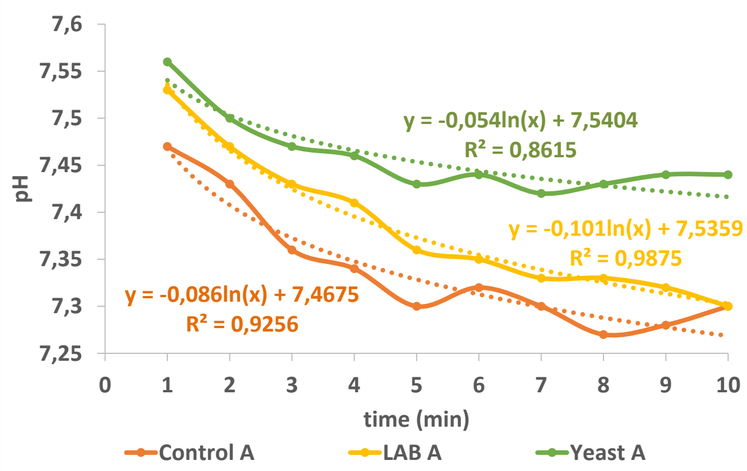
Figure 1: Gel analysis with the MiniBIS Pro (DNS-Bio-Imaging Systems) and the software GelCapture MiniBIS 1.0.0.1 for identification of the MW of protein bands.

RESULTS & DISCUSSION

The results have shown a mild proteolytic activity exerted by the microbial fermentation of the carob seeds which was evident in the in-vitro digestibility study and the decrease of the concentration of phytic assay. However, protein electrophoretic patterns have not revealed new protein zones as evidence of protein hydrolysis due to seed fermentation.

pH (0h)			
t (min)	Control A	LAB A	Yeast A
0	7,96	7,97	7,95
1	7,47	7,53	7,56
2	7,43	7,47	7,5
3	7,36	7,43	7,47
4	7,34	7,41	7,46
5	7,3	7,36	7,43
6	7,32	7,35	7,44
7	7,30	7,33	7,42
8	7,27	7,33	7,43
9	7,28	7,32	7,44
10	7,30	7,30	7,44

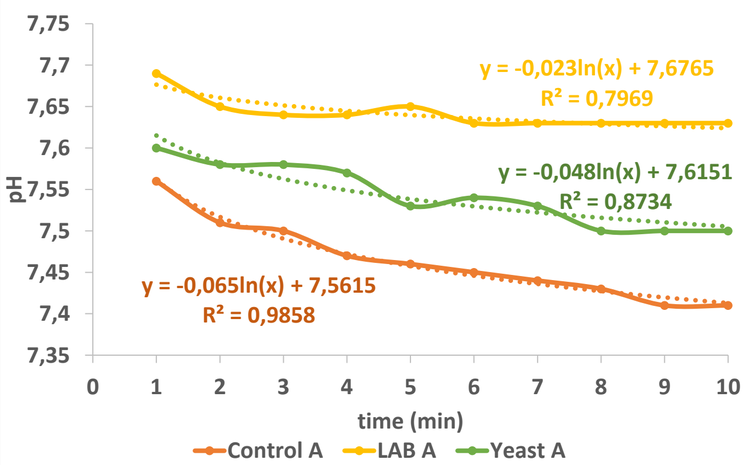
Table 1: pH results of Carob seeds samples (t_{0h}).



Graph 1: Alteration of pH of Carob seeds samples during fermentation (t_{0h}).

pH (72h)			
t (min)	Control A	LAB A	Yeast A
0	7,96	7,96	8,01
1	7,56	7,69	7,6
2	7,51	7,65	7,58
3	7,5	7,64	7,58
4	7,47	7,64	7,57
5	7,46	7,65	7,53
6	7,45	7,63	7,54
7	7,44	7,63	7,53
8	7,43	7,63	7,50
9	7,41	7,63	7,50
10	7,41	7,63	7,50

Table 2: pH results of Carob seeds samples (t_{72h}).



Graph 2: Alteration of pH of Carob seeds samples during fermentation (t_{72h}).

Samples		A (655 nm)	ΔAphosphorus	c (Phosphorus)	c (Phytic Acid)
Carob seed control	(FP)	0,096	0,466	0,255	0,9047
	(TP)	0,562			
Oat flour control	(FP)	0,113	0,581	0,318	1,1280
	(TP)	0,694			
Carob seed + dH_2O	(FP)	0,120	0,148	0,081	0,2873
	(TP)	0,268			
Carob seed + LAB	(FP)	0,322	0,077	0,042	0,1495
	(TP)	0,399			
Carob seed + Yeast	(FP)	0,484	0,175	0,096	0,3398
	(TP)	0,659			
Carob seed + Fungus	(FP)	0,076	0,197	0,108	0,3825
	(TP)	0,273			

Table 3: Results of the absorption and concentrations of phosphorus and phytic acid in carob seed flour samples.

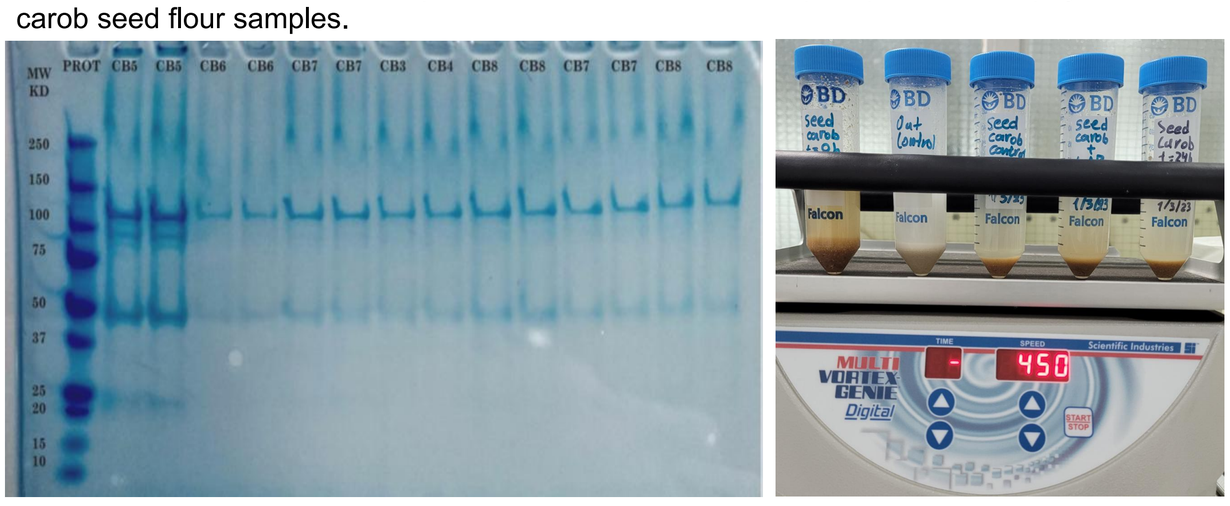


Image 1: Protein bands of fermented carob seeds in SDS-PAGE electrophoresis gel.
Column 1: PROT: reference proteins with standard molecular weights (MW in KD)
Columns 2-3: CB5: carob seeds milled (untreated sample)
Columns 4-5: CB6: carob seeds milled and hydrated for 3 days without microorganisms
Columns 6-7: CB7: carob seeds milled and fermented with LAB for 3 days
Column 8: CB3: carob seeds milled and fermented with LAB for 3 days
Column 9: CB4: carob seeds milled and fermented with yeasts for 3 days
Columns 10-11: CB8: carob seeds milled and fermented with yeasts for 3 days

Number of Zone	Carob seeds control (MW kDa)	Carob seed + dH_2O (MW kDa)	Carob seeds + LAB (MW kDa)	Carob seeds + Yeast (MW kDa)
1	110.84	110.84	110.84	110.84
2	94.58			
3	45.95	46.29	44.87	45.09
4	24.09			

Table 4: Molecular weights of proteins detected in SDS-PAGE electrophoresis.

CONCLUSION

The observed protein fragmentation cannot be attributed exclusively to microbial fermentation, and further investigation is needed to differentiate the effects of microbial activity from those caused by endogenous seed enzymes or hydration-induced modifications. Further research is needed to establish the optimum conditions for solid-state microbial fermentations of carob seeds to obtain a more digestible source of protein.

ACKNOWLEDGEMENTS

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