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Evaluating Protein Extraction Techniques from Lentils (*Lens Culinaris*): Comparing Ammonium Sulfate, Ultrasound-assisted and Conventional Extraction

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Introduction

Institute of Food Technology at University of Applied Sciences Weihenstephan-Triesdorf (HSWT) is the coordinator of ProxIMed, a PRIMA project with 17 partners from 10 countries. The project aims to explore and implement products with alternative proteins in Mediterranean region. The proteins are extracted from sustainable, plant-based sources and novel foods using innovative and environmentally friendly processing technologies. Selected proteins are further developed into customized products.
This study aims to compare and evaluate the efficiency of environmentally friendly protein extraction methods from lentils. The focus is on the applications of conventional extraction, ammonium sulfate precipitation, and ultrasound-assisted extraction. Effectiveness is assessed by protein yield and potential applications in the food industry.

Materials and Methods

Lens culinaris Medik.

Yellow and red ground lentilflour are treated with three different extraction methods:

Alkaline Solubilization/Isoelectric Precipitation (Al/IEP)

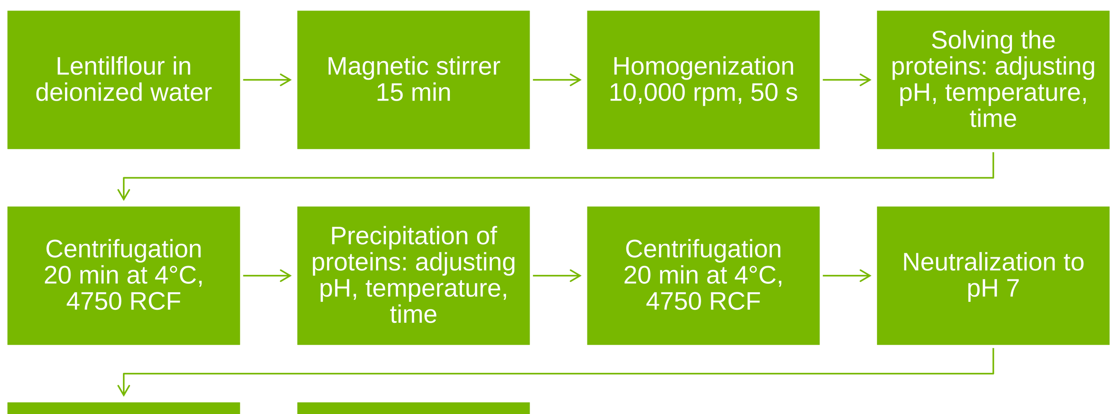


Fig. 1 Process flow of the conventional protein extraction (Al/IEP) from lentilflour

Ammonium Sulfate Precipitation (AS)

After alkaline solubilization, the proteins are precipitated using ammonium sulfate, with variations in salt concentration and extraction time. The salt is then removed by diafiltration (Vivaflow 50R, Sartorius, 100 MWCO HY), and the sample is subsequently freeze-dried.

Ultrasound-assisted Extraction (US)

The process flow is the same as for Al/IEP, but ultrasound (UP400St, Hielscher) is used instead of the homogenization step with Ultra Turrax.

Response Surface Methodology (RSM)

The response surface method is used to optimize the conventional extraction regarding pH, solvent-to-solid concentration (%w), extraction time, and temperature. The other methods are then adapted to the optimal parameters identified for conventional extraction.

Analysis

Protein content was measured using the Dumas method (Dumatherm, Gerhardt GmbH), and protein yield was calculated based on dry mass.

Results

Regression equation in uncoded units

Proteinyield = 66.29 + 4.34 pH + 0.373 Concentration + 0.078 Temperature - 0.1003 Time - 0.1958 pH*pH - 0.02265 Concentration*Concentration + 0.00333 Temperature*Temperature + 0.001117 Time*Time - 0.01457 Concentration*Temperature - 0.002158 Temperature*Time

Average for the equation across all blocks.

Eq. 1 Regression equation in uncided units for the results of alkaline solubilization

Factor	Solution	Adjusted values
pH	11	9
Concentration	0,5%	5%
Temperature	65°C	50°C
Time	30 min	30 min

Fig. 2 Solution of the target size optimization and adjusted values for alkaline solubilzation

Regression equation in uncoded units

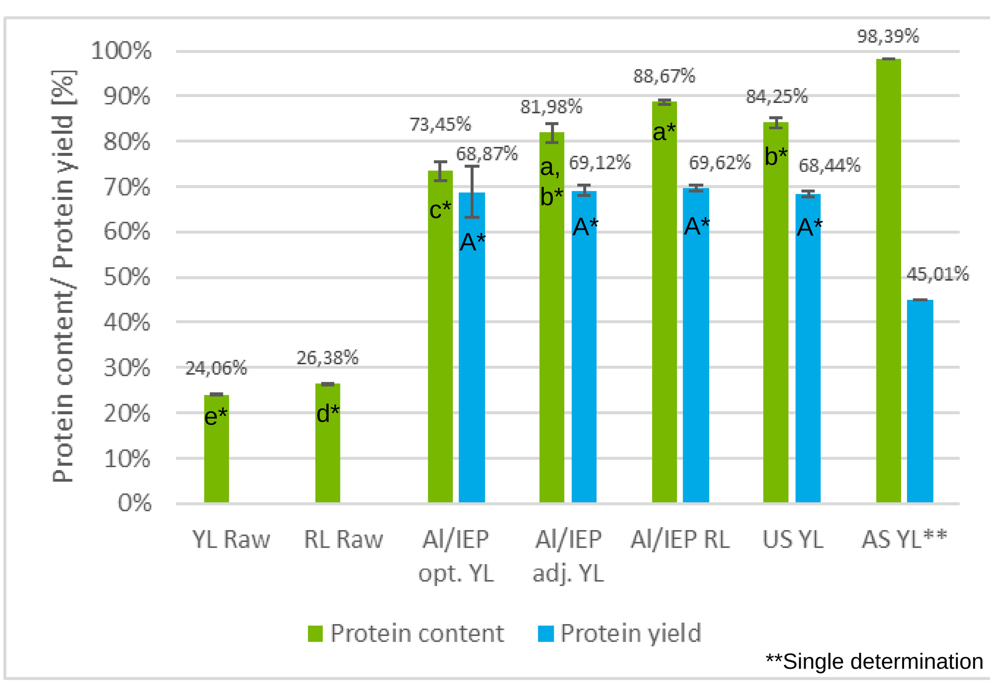
Proteinyield = 359.6 - 134.4 pH + 0.452 Temperature - 0.0733 Time + 15.57 pH*pH + 0.00301 Temperature*Temperature - 0.1438 pH*Temperature

Average for the equation across all blocks.

Eq. 2 Regression equation in uncided units for the results of isoelectric precipitation

Factor	Solution	Adjusted values
pH	5	5
Temperature	4°C	RT
Time	30 min	30 min

Fig. 3 Solution of the target size optimization and adjusted values for isoelectric precipitation



*samples with different lowercase or uppercase letter have a significant difference according to Tukey`s Test α=0,05

Fig. 4 Comparison of protein content and yield across test series

Alkaline solubilization and **isoelectric precipitation** were each examined separately, with alkaline solubilization optimized first.
For the optimization of **alkaline solubilization**, a Central Composite Design was applied with the following parameter ranges: pH 7-11, concentration (w/w) 5-20%, temperature 20-50°C, and time 60-120 minutes. This yielded an R^2 of 85.05%.
The optimization of **isoelectric precipitation** was conducted using the Box-Behnken design to focus on the set minimum and maximum values, avoiding overfitting. Parameters included pH 4-5, temperature 4-50°C, and time 30-90 minutes, achieving an R^2 of 82.42%.
The **regression equations in uncoded units** are shown in Eq. 1 and 2, highlighting the significant parameters, quadratic effects, and interactions.
Fig. 2 and 3 present the **target size optimization** solutions for each method. Parameter adjustments were guided by practical considerations and insights from the literature [1].

For **ammonium sulfate precipitation** preliminary trials were conducted, testing a salt concentration of 80%.

For the optimization of **ultrasound-assisted extraction**, a Box-Behnken design was applied. The parameter ranges were pH 7-10, temperature 20-60°C, amplitude 20-100%, and energy input 1200-2500 Ws/g dry matter. Currently, the best results were achieved with a pH of 10, temperature of 40°C, amplitude of 60, and an energy input of 2500 Ws/g dry matter.

This study compares the potential of US and AS extraction to Al/IEP in terms of protein content and yield.
The results showed no significant differences in protein yield across methods. The highest protein yield of 69.62% was achieved using Al/IEP on red lentil flour (Al/IEP RL). Regarding protein content, Al/IEP RL also achieved the highest level at 88.67%, with no significant difference between red and yellow lentils for this method. This suggests that red lentil flour results can likely be replicated with yellow lentil flour under similar conditions.
No significant difference was found between **US extraction** and Al/IEP adj. YL, indicating that ultrasound does not provide a substantial efficiency advantage over the conventional method.
Initial tests of **AS precipitation** at 80% concentration resulted in a high protein content of 98.39% but a lower yield of 45.01%, suggesting limited efficiency of this method. In contrast, Alsohaimy et al. (2007) reported a protein yield of 93% using a similar approach [2]. These findings suggest further research is required to fully evaluate the effectiveness of AS precipitation.

Conclusion

With **conventional extraction**, a protein content of 88.67% was achieved for red lentils. **Ammonium sulfate precipitation** yielded an even higher protein content of 98.39%, though it resulted in a lower protein yield compared to other methods. In the food industry, efficiency often prioritizes methods that provide greater product yield in a single process. Despite its advantages – such as high solubility, protein structure stabilization, purity, and low cost – ammonium sulfate precipitation requires time-intensive desalting steps, like ultrafiltration or dialysis [3, 4]. Therefore, further research is necessary to assess the method's practical viability.
Ultrasound-assisted extraction, on the other hand, showed no significant improvement in protein content or yield compared to conventional extraction. This suggests that cell disruption is sufficiently achieved without ultrasound.
Overall, lentil proteins demonstrate strong potential for use in milk alternatives and other high-quality foods due to their valuable sensory and technofunctional properties [5]. To better evaluate the applicability of each method in the food industry, a detailed analysis of the technofunctional properties resulting from each extraction technique is recommended.

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