# Valorization of collagen and keratin by-products from leather industry to increase the quality of production from orchard

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The increasingly intensive promotion of organic agriculture keeps up-to-date research for the recovery of proteins (primarily nitrogen carriers) from various secondary sources and their validation for use in plant culture (Zhao, 2022).

The natural leather processing industry, through the specificity of the processes, is an important source of protein by-products, collagen and keratin, which can be exploited for agricultural use (Stefan, 2021), due to the high nitrogen content and the wide spectrum of amino acids with potential of protection against abiotic stress and stimulation of plant growth (Abdelkader, 2023).

Many research studies are still in progress, both for improving the processes of obtaining hydrolysates of collagen and keratin (Szopa, 2023; Mengistu, 2023), and those related to the use of these hydrolysates to increase the yields of agricultural crops (Ambrosini, 2021; Chen, 2022). The extraction of collagen as gelatin is also currently being studied (Ramli, 2023).

In the present research gelatin, collagen hydrolysates and keratin hydrolysate were extracted for protein combinations dedicated to association with plant extracts with fungicidal effects, in order to develop and validate a multifunctional bio-pesticide for orchard protection and to increase production.

## Materials and methods

Bovine leather and sheep wool by-products have been used for collagen and keratin extracts as gelatin and collagen and keratin hydrolysates.

Gelatin and collagen hydrolysates from residual untanned leather by-products were prepared by thermal and enzymatic hydrolysis. Also, collagen hydrolysate was prepared by alkaline and enzymatic hydrolysis of residual bovine-tanned leather. Keratin hydrolysate was prepared by alkaline and enzymatic hydrolysis from degreased residual sheep wool.

The protein extracts were analyzed physico-chemically to evaluate the most significant characteristics, for applications in the agricultural field: total nitrogen and amino nitrogen content, molecular weight, gelatin texture, surface tension and contact angles, particle size distribution in protein hydrolysates, amino acid content.

The combined protein extracts, as part of a biopesticide prototype, in two experimental variants were tested in the orchard on fruit of the cherry species, the Skeena variety, compared to an untreated control and a variant with standard treatment.

#### **Results and discussions**

The average molecular mass of gelatin was determined by SDS Page electrophoresis with processing of the migration gel on the viewing camera. Figure 1 shows the migration gel, with the gelatin sample on line 12, the buffer on line 14 and the marker on line 15, detected automatically, as well as the details recorded by the viewing camera for the gelatin sample on line 12.



Figure 1. SDS Page electrophoresis of gelatin: (a) the gel after decolorization, (b) the molecular mass profile of the peptides, recorded by the viewing camera

After processing the data recorded by the viewing camera, an average molecular weight of gelatin of 127 kDa was obtained, in accordance with the amino nitrogen content (< 0.2% of total nitrogen).

The amino acid profile and content in protein extracts determined by HPLC is shown in the Figure 2. A very wide profile of amino acids, including essential ones, capable of penetrating cell membranes is observed.



Figure 2. Amino acid profile of protein extracts: (a) gelatin extracted from untanned leather, (c) collagen hydrolysate extracted from tanned leather, (c) collagen hydrolysate extracted from

untanned leather, (d) keratin hydrolysate

In figure 3, the fruit production of the cherry species, the Skeena variety, is presented for the tested treatment variants: 1 - the untreated control, 2 - treatment with the biopesticide prototype containing a protein combination consisting of gelatin and collagen hydrolysate extracted from untanned leather, 3 - treatment with the biopesticide prototype containing a protein combination consisting of gelatin extracted from untanned leather, collagen hydrolysate extracted from tanned leather and keratin hydrolysate, 4 - treatment with a standard product (Serenade® ASO).



Figure 3. The influence of treatments with biopesticide variants containing protein combinations,

on fruit production

The treatments containing protein combinations was shown to increase fruit production compared to both the standard treatment and the untreated control.  $\vec{x}$ 

### Conclusions

The composites based on protein hydrolysates and gelatins have amino acids and polypeptides suitable for the immediate and delayed release of organic nitrogen necessary for plant stimulation and nutrition.

The use of collagen and keratin from the by-products of the leather industry for agricultural applications is a viable alternative to synthetic products.

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