

## Abstract

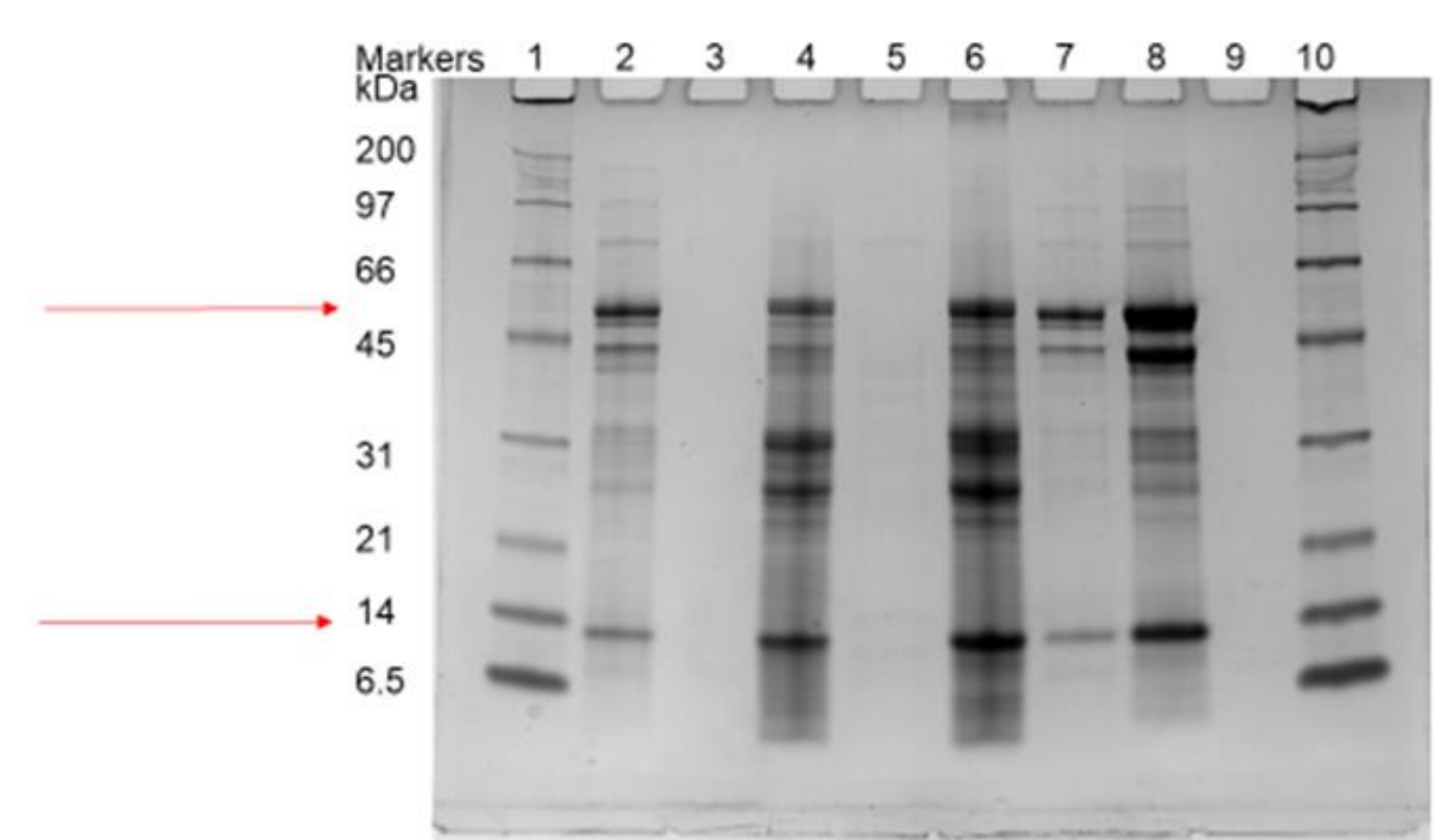
Processing of leafy side-streams has been researched for some time now, and feasibility was first illustrated for sugar beet leaves. This led to a patent which is licensed to Cosun.

We now demonstrated that our technologies on protein isolation can also be used on potentially toxic side-streams. This is now illustrated with tomato leaves. We aim expand this to potato leaves, and when possible cassava leaves.

## Background green leaves

Rubisco is the world's most ubiquitous protein, making it a worthwhile target in the protein transition. The total global potential for proteins from leaves is hard to estimate, as proteins from side streams and unused sources are not counted. We estimated the maximal potential for sugar beet based on the annual root production of around 75 t/ha (Factfish.com, 2014), resulting in nearly 29 t/ha of leaves available every year. This is equivalent to about 400-600 kg/ha of crude protein in the leaves, assuming an average of 22.8% crude protein on dry matter basis (Tamayo Tenorio, 2017). This value is fairly comparable to the protein production of soy (450-600 kg/ha) and cereals (570 kg/ha) (van Krimpen et al., 2013). However not all can be recovered as a Rubisco product.

Here, we show some results on tomato leaves (*Solanum lycopersicum*), the waste stream of tomato production. These are currently unutilized as a human food source. Tomato is part of the nightshade family and the leaves not only contain protein, but also toxins.

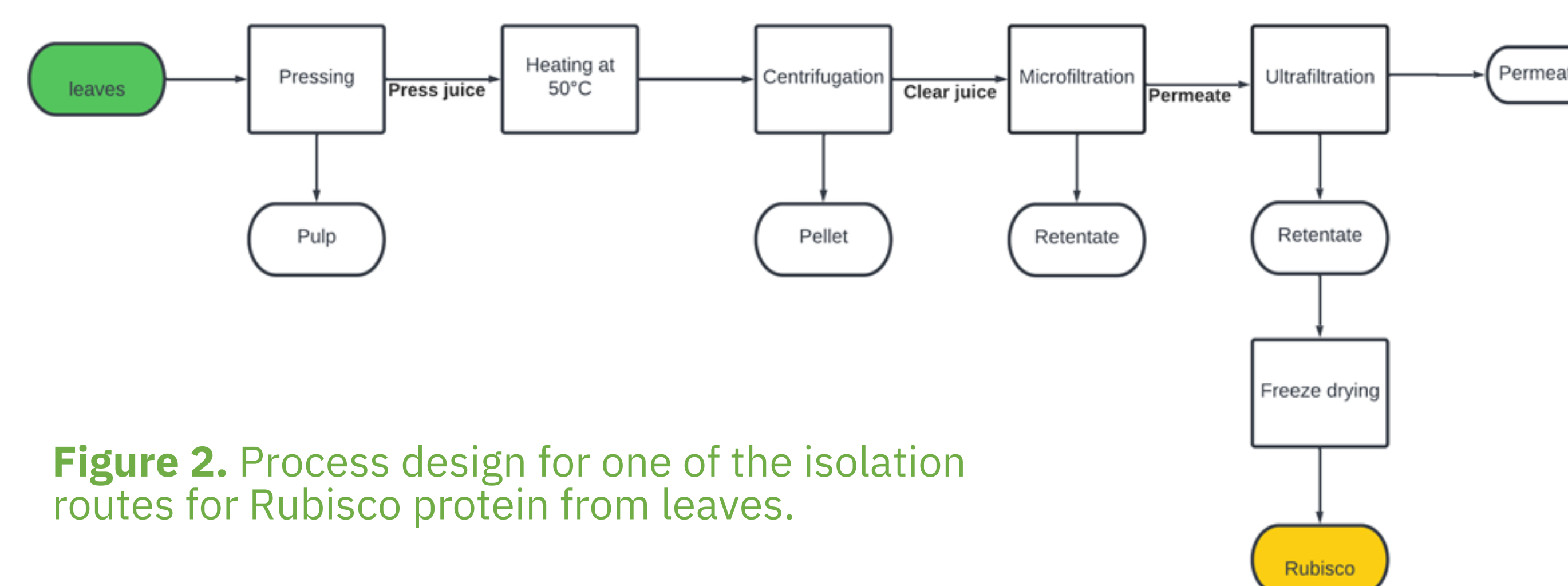


**Figure 1.** Liquid protein samples taken during processing. Rubisco is the main (soluble) protein in leaves and is shown on this SDS gel by protein bands at 50 kDa and 15 kDa, representing the large and small subunits of the enzyme.

## Methods

Protein isolation with simultaneous toxin removal was researched to obtain a safe protein isolate. Tomato plant leaf protein was isolated through three methods; by pH precipitation, ethanol precipitation, and by a combination of heat coagulation and filtration.

Protein yield and purity was determined and protein isolates were tested for the presence of tomatine and dehydrotomatine, two of the main toxins in tomato leaves.



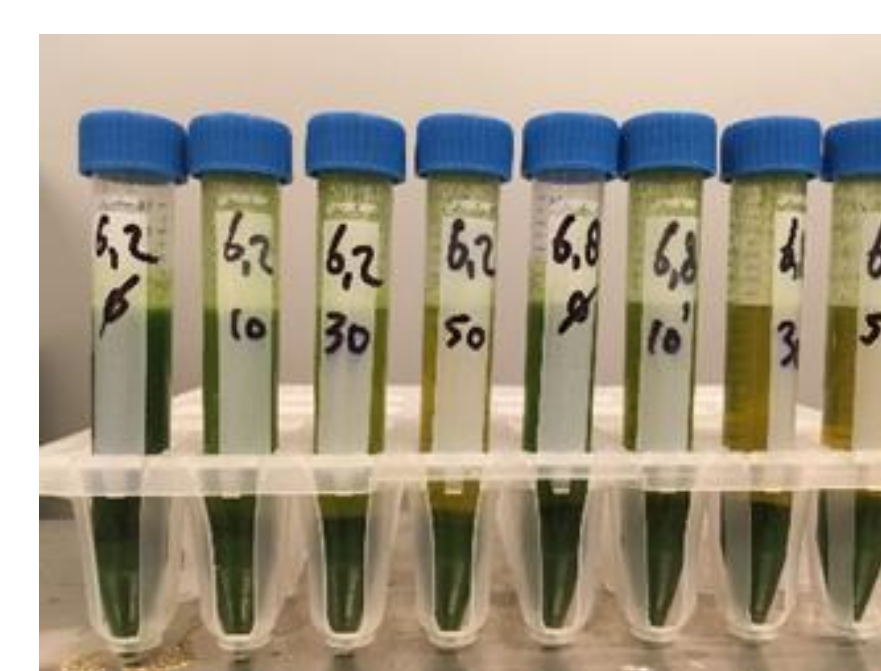
**Figure 2.** Process design for one of the isolation routes for Rubisco protein from leaves.



**Figure 3.** Tomato growth in green houses



**Figure 4.** Pressing tomato leaves



**Figure 5.** Precipitation of green material from the press juice

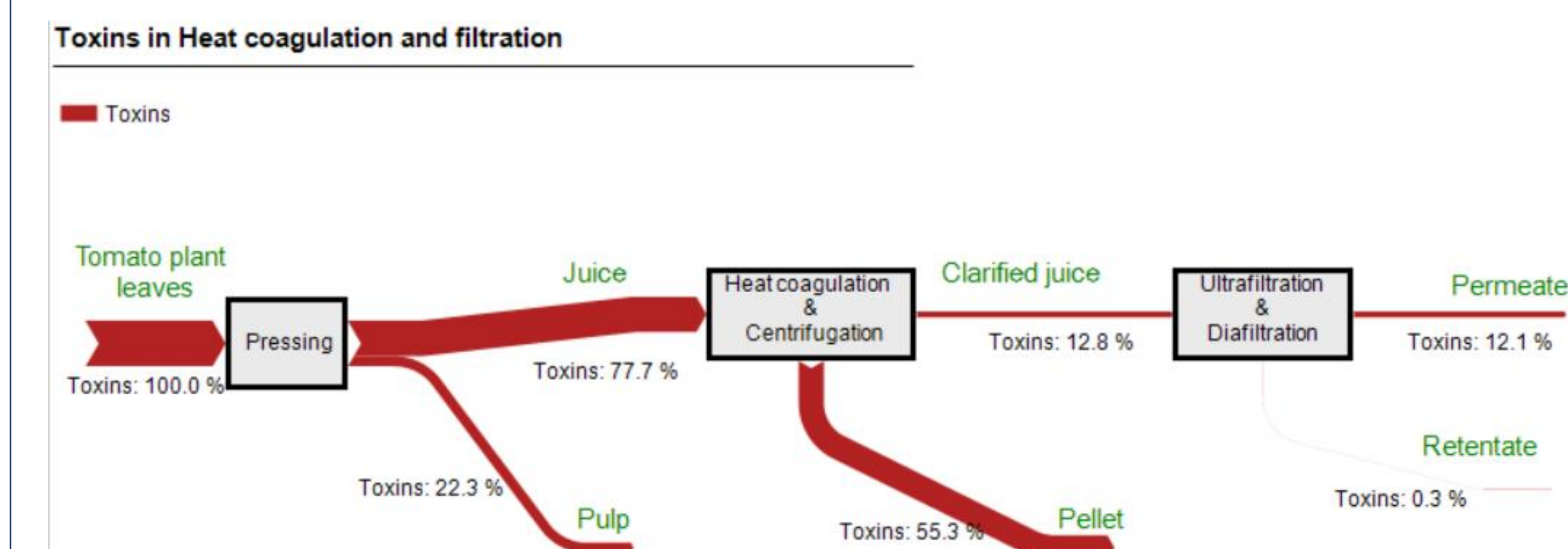
## Highlights

Up to 10% of protein was extracted from tomato leaves, being mainly Rubisco protein.

Both filtration and ethanol precipitation removed over 99% of (hydro)tomatine.

Isoelectric precipitation removed the least amount of toxins (97%).

Filtration and isoelectric precipitation gave highest protein purity.



**Figure 6.** Toxin distribution in selected tomato plant protein isolation process

## Publication results

Bruins ME, Speranza P & Sato ACK (2023) Green Leaves. Book chapter in: Our Future proteins.

Free download at

<https://vuuniversitypress.com/product/ourfutureproteins/?lang=en>

Liese HW, Valkenburg TTA, America AHP, Scholten E & Bruins ME (2023) Toxin removal during protein extraction from tomato leaves, Innovative Food Science & Emerging Technologies, 88: 103454

<https://doi.org/10.1016/j.ifset.2023.103454>.