Biocracking of sugar beet leaves targeting on Rubisco

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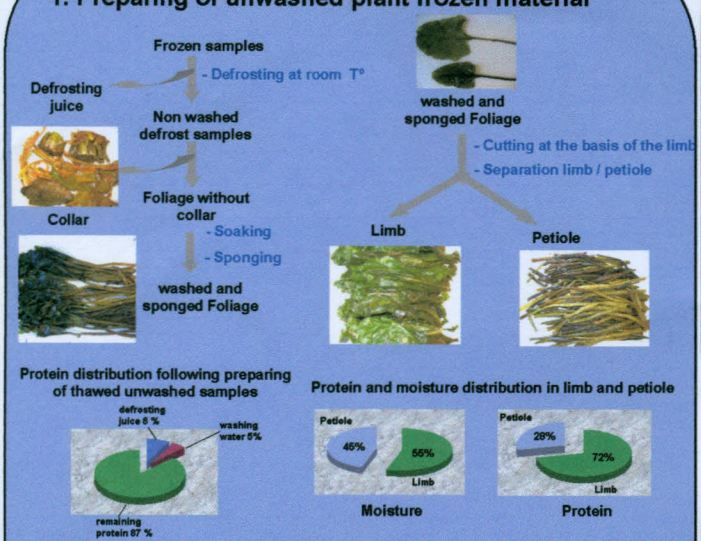
INTRODUCTION

Leaf protein (LP) is potentially the most abundant of all the protein sources. Protein can be extracted satisfactory from many, but not all, species of leaf. Sugar beet (*Beta vulgaris*) leaves which are largely wasted, are one of the two most abundant by-products in the temperate zone. They represent potential source of protein isolates. About 45 T leaves are produced per ha. That represents 40 to 45 % of the biomass of that culture. There are many processes for leaves bio-cracking. Nature and properties of LP isolates depend on protocol of their preparation. We will focus on those with respect to soft conditions, in accordance with the environment and human consumption. For that purpose it will be avoided, as much as possible, any aggressive chemical and use rather mechanical, temperature and pH effect to fractionate sugar beet leaves. It is believed that rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39) could be the main product when "cracking" leaves into proteins, fibber, vitamins, pigments, and other products. Rubisco from different sources presents exceptional emulsifying and whipping properties interesting for agro-food and cosmetic industries. This compound has a molecular weight close to 550 kDa and a general structure L8S8: eight large sub-units (L) with molecular weights around 55 kDa and eight small sub-units (S) with molecular weights close to 12,5 kDa.

Our goal is to throw the basis of sugar beet Rubisco valorisation following its extraction by solubilization. Optimisation of this valorisation need to master extraction and purification process. It needs, as well, the characterization and optimisation of functional properties of extracted proteins, and the determination of the main production-transformation process parameters. In addition, it is necessary to have a thorough knowledge of extracted co-products. This poster deals with the establishment of sugar beet top cracking process targeting on proteins, mainly rubisco. HPLC and SDS-PAGE analyses of extracted proteins were performed. Amino acid composition of the "white protein" is compared with that of "green" and unfractionated protein.

MAIN LEAVES BIO-CRACKING STEPS

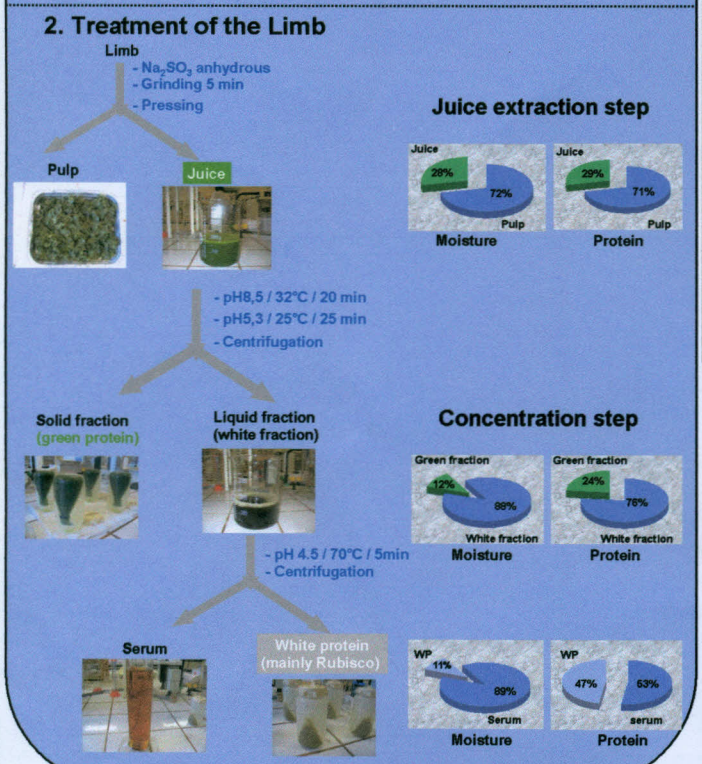
1. Preparing of unwashed plant frozen material



Protein distribution following preparing of thawed unwashed samples

Protein and moisture distribution in limb and petiole

2. Treatment of the Limb



STATEMENT OF THE EXTRACTION PROCESS

Statement of juice extraction from 100 kg frozen sugar beet leaves

Plant material preparation

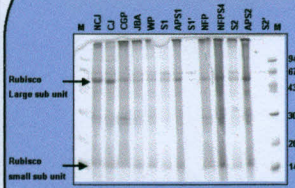
Products	Weight (kg)	H_2O content (%)	Dry material (DM) (kg)	Sum of DM per step (kg)	Yield of dry material (%)	% of eq* protein/DM	Weight of eq protein (g)	Sum of eq protein per step (g)	Yield of eq protein (%)
Frozen foliar sample	100	-	-	-	-	-	-	-	-
Defrosting juice	0,3** 23	92,9 +-4.10⁻²	1,6 +-8.10⁻²	-	19	6,9	110 +-8	-	8
Collar	12,1	-	-	-	-	-	-	-	-
Washing water	146,1	99,7 +-1.10⁻²	0,5 +-3.10⁻²	-	6	17,7	73 +-8	-	5
Petiole	29,7	87,2 +-0,3	3,8 +-0,1	8,4	45	9,7	370 +-41	1330	28
Limb	29,7	84,6 +-0,3	4,6 +-0,1		55	20,9	960 +-2		72

* Eq protein = equivalent protein; ** 0,3 = weight of soil

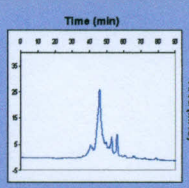
Statement of the treatment of grinded limb

Products	Weight (kg)	H_2O content (%)	Dry material (kg)	Sum of DM per step(kg)	Yield of DM (%)	% of eq protein/DM	Weight of eq protein (g)	Sum of eq protein per step (g)	Yield of eq protein (%)
Limb	27,9	84,6 +-0,3	4,3 +-0,1	4,3	1	20,9	900	900	1
Pulp	12,3	76,9 +-1,6	2,8 +-0,1	3,9	65 91	21,9	620 +-24	870	69 96
Juice	13,7	91,9 +-3.10⁻²	1,1 +-3.10⁻²		26	22,7	250 +-9		27

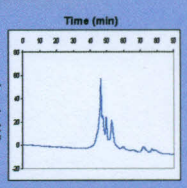
HPLC AND SDS PAGE ANALYSES



SDS-PAGE (12,5%) analysis of extracted proteins from sugar beet leaves

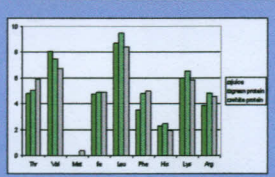


Gel permeation HPLC chromatogram of Sigma standard Rubico



Gel permeation HPLC chromatogram of sugar beet leaves white protein

AMINO ACIDS COMPOSITION OF EXTRACTED PROTEINS



- Presence of all essential amino acids (AA) in the white protein.
- Leu, Val, Lys are the most representative essential AA in all cases.
- Thr, Ile Phe, Arg present intermediate proportions.
- His and especially Met are the less representative essential AA.

CONCLUSION AND OUTLOOK

- Determination of suitable washing conditions to limit material lost
- Establishment of suitable harvesting and storage conditions of the leaves
- Determination of optimal grinding conditions
- Determination of optimal pressing process
- Determination of a reproducible process of leaves juice extraction and concentration of its compounds
- Determination of a protocol for Rubisco analysis by SDS-PAGE and HPLC
- White protein (Rubisco) presents a nutritive value as it contains all essential amino acids
- Green protein may be good as supplement for animal feeding, especially in cereal diets in which Lysine is nutritionally limiting
- Coming exceptional techno-functional analysis of the extracts would asses their potential use in higher added value non food domain

REFERENCES

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