Project Number	862834
Project acronym	AQUACOMBINE
Project title	Integrated on-farm Aquaponics systems for co-production of fish, halophyte vegetables, bioactive compounds, and bioenergy
Contract type	H2020-RUR-2019-1

Deliverable number	D5.9
Deliverable title	Report on the most optimal configuration for lipid and protein extraction with a proposed scale-up scheme.
Work package	WP5
Due date of deliverable	M36
Actual submission date	M36 – 04.10.2022
Start date of project	01.10.2019
Duration	48 months
Reviewer(s)	Mette Hedegaard Thomsen (AAU), Hinrich Uellendahl (FUAS)
Author/editor	Lukas Braem (UCL), Sylvia Fasse (BHV)
Contributing partners	UCL, BHV
Dissemination level	PU

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862834. Any results of this project reflect only this consortium's view and the European Commission is not responsible for any use that may be made of the information it contains.

Document history

Version no.	Date	Authors	Changed chapters
0.1	22/09/2022	Lukas Braem	First document version
0.2	27/09/2022	Lukas Braem	Second document version
0.3	27/09/2022	Sylvia Fasse	Third document version
0.4	29/09/2022	Mette Hedegaard Thomsen	Review of entire document
0.5	29/09/2022	Hinrich Uellendahl	Review of entire document
1.0	30/09/2022	Charlotte Holmene	Final document

Contributors

Part. No.	Part. short name	Name of the Contributor	E-mail
5	UCL	Lukas Braem	lukas.braem@uclouvain.be
5	UCL	Iwona Cybulska	Iwona.cybulska@uclouvain.be
4	BHV	Sylvia Fasse	sfasse@hs-bremerhaven.de
4	BHV	Axel Gottschalk	agottschalk@hs-bremerhaven.de

Table of Contents

List of Figures

List of Tables

1 Executive summary

This deliverable will report the computer aided process engineering tools used to support the extraction of protein from the green juice in solvent selection and solvent recovery. The progress of the work in the AQUACOMBINE project on the most optimal configuration for protein extraction with a proposed scale-up scheme in WP5 is on schedule. Simple centrifugation was found to deliver an increased protein fraction with reduced salt content, and it was chosen as the main extraction method. Freeze-dried protein enriched fractions were made using this extraction method and delivered to Riasearch for implementation in fish and shrimp feed in the feeding trials of WP7. The methodology and compositional data of the different input and output streams are currently drafted in a manuscript, which will be sent for publishing on schedule.

In collaboration with the BHV (Hochschule Bremerhaven) team, information on the lab-scale production of the protein enriched pellet was shared. A proposed upscaling using disc stack centrifuge will be further developed and simulations made towards the end of the project integrating results from all WPs.

2 Introduction

The rise in global population inevitably leads to an increased demand for proteins. With the high environmental cost of livestock more and more interest is being shown towards plant-based protein sources. Currently the main crops grown for protein extraction purpose include wheat, soybean, and corn but with the changing climate new protein sources are highly sought. In the AQUACOMBINE project we aim to establish *Salicornia* spp. as a novel source of plant-proteins with an aim to include protein-enriched extracts in the fish feed.

As part of the optimization of the combined aquaculture and halophyte farming process and the different processing streams of the AQUACOMBINE project. Each stream will be valorised for different goals. After harvesting of the fresh tips of *Salicornia* for human consumption there is a significant amount of fibrous residue left over which is not utilized. Here we juice that fraction and perform protein extraction on the recovered juice. In selecting a protein extraction method care was taken in the selection of solvents used in that method so that the end-fraction did not become unusable by chemical contamination.. We therefore chose to go with protein extraction based on a simple centrifugation step in order to make it easy to use and not requiring extensive technical skills or equipment. Indeed, for the solvent only water is used during the extraction process in the lab scale while it will be minimized or avoided in the upscaled proposition. The result is a protein-rich-fraction that contains polysaccharides and other molecules that might provide health benefits to the fish when used in their feed. Considering the design of a process flowsheet for protein extraction, a block scheme is given below (see Figure 1).

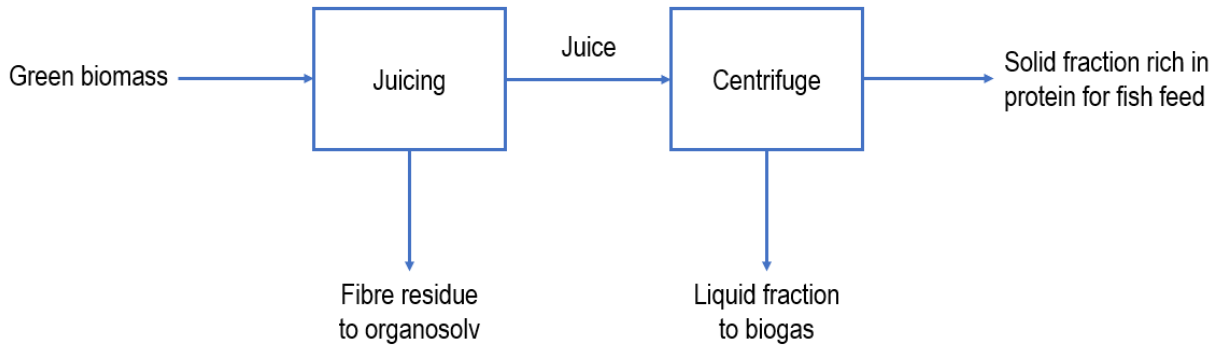

Figure 1. Process flowsheet scheme considered for the scale-up of the protein recovery

3 Methodology

3.1 Material

The material used in this study was fresh *Salicornia ramosissima* plants harvested by Riasearch (Portugal) in June 2021, shipped fresh in cooled boxes to the UCL lab. Harvested biomass was mostly the succulent green leafy part of the plant, with smaller branches included. Big woody stems were not used in the extraction procedure as the lab-scale juicer would not be able to process them. A JAZZUNO screw-type juicer was used to separate the juice and the fibre (Figure 1). The nitrogen content of the untreated juice, measured by Kjeldahl and the combustion method, was 1.8% of the dry weight which converts into 11.25% dry weight for protein content using the Jones-factor of 6.25 (Jones, 1941).

3.2 Centrifugation method

The centrifugation method relies on the difference in density of the suspended solids in the juice of *Salicornia*. Proteins precipitate out of an aqueous phase when their interaction with this phase is reduced. In the case of *Salicornia* the high internal salt concentration can cause a reduction in the amount of available water to keep proteins in solution. This causes these proteins to precipitate out of the solution when centrifugation is applied in a process called salting out. Up to 500 ml of juice was placed into a 750mL Thermo Scientific Polypropylene bottle then they were centrifuged at 3000 RPM for 25 min followed by decanting of the supernatant and a resuspension of the pellet with 50 ml of water. This redissolved pellet was then freeze dried and analysed for its composition.

3.3 Analysis

Establishing a protein measurement methodology that is based on total nitrogen (N) analysis was a key objective of the project. The Kjeldahl method for nitrogen content measurement was chosen. In short, the Kjeldahl protocol starts by breaking down the bonds between the amino acids in the proteins by boiling the sample at 430º in sulphuric acid in the presence of a catalyser tablet (K2SO4 salts + Se) (Figure 1).

The nitrogen atoms found in the amino acid backbone are oxidized and trapped in the form of ammonium sulphate. The sample is then introduced into the distillation unit where sodium hydroxide is added which leads to the formation of ammonia. Using steam, the ammonia is triggered to escape the solution in gas form (Figure 2). This gas is condensed and trapped in a boric acid solution which is subsequently titrated with a strong acid (HCl) in order to determine the original amount of nitrogen molecules in the sample. From this estimated amount of nitrogen, the protein content is then calculated by multiplying it with a Jones factor of 6.25 (Jones, et al 1941).

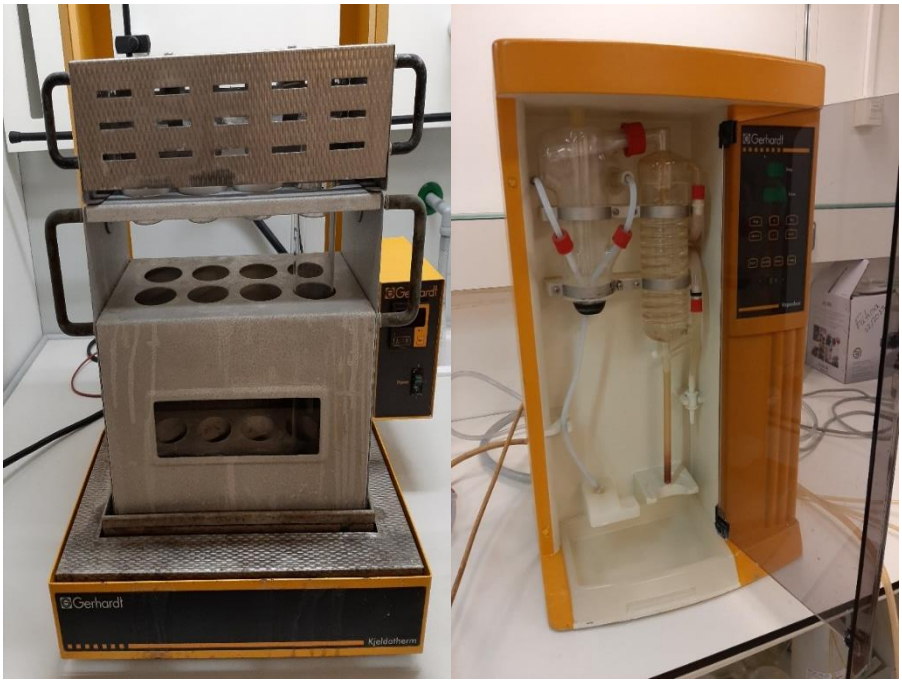

Figure 2: Gerhardt Block Digestion and distillation system

3.4 Calculations

The total solids content in the sample is calculated using the following formula:

$$\%TS = \frac{Wb-Wa}{Ws} \times 100$$

With:
%TS: Total solids weight fraction (%)
W_a: Weight of the crucible (g)
W_b: Weight of the crucible and the sample after drying at 105°C for 12 h (g)
W_s: Weight of the wet sample (g)

The Salicornia dry weight is calculated using the average total solids content as determined by the dry weight analysis.

$$Salicornia\_DW = \frac{\boldsymbol{Weights}salicornia\ x\ \%TS}{100}$$

With:
Salicornia DW: Salicornia dry weight (g)
$Weight_{Salicornia}$: Weight of air-dry Salicornia sample introduced in the extraction (g)
TS: Total solid

The nitrogen concentration in the sample is calculated with the following formula:

$$Nitrogen\ content\ (\frac{gN}{g_{Salicornia\_DM}}) = \frac{(V1 - V0) \times N \times 14.007}{m} x \frac{1}{1000} \times (\frac{L}{mL}) =)$$

With:
V_1: Volume of HCl added during the titration of the sample (mL)

V_0: Volume of HCl added during the titration of the blank (mL)

N: Molality of the HCl solution (mol/L)

14.007: Atomic mass of nitrogen (g/mol)

m: Mass of the sample (g)

The protein content is then calculated using a Jones' Factor of 6.25 (average for plants) (Mariotti *et al.*, 2008).

$$Proteins\ (\frac{gprotein}{g_{Salicornia\_DM}}) = Nitrogen\ (\frac{gN}{g_{Salicornia\_DM}}) \times 6.25(\frac{gprotein}{gN})$$

3.5 Computer Aided Process Engineering (CAPE)

To support the scale-up of the recovery of protein from the green juice towards future industrial scale, computer aided process engineering (CAPE) tools are used.

The tools in use within the AQUACOMBINE project are commercial software like the AspenOne Engineering [ASP22] including several tools for process flowsheet simulation (beside others), the environmental life cycle impact assessment software UMBERTO [UMB22] linked to the ECOINVENT [ECO22] database, the Cost Engineering Suite [COS22] capable to estimate CAPEX, OPEX, NPV, RoI etc, as well as Microsoft Excel [EXC22].

Excel acts as platform that integrates the other software tools via interfaces for automatic data transfer.

At this point in time, the modelling and simulation is ongoing to find the optimal configuration and operating conditions of the integrated whole biorefinery, including the protein recovery. Final results will be reported towards the end of the project.

4 Results and Discussion

4.1 Juicing step

Biomass was introduced into the juicer, whose parameters are summarized in Table 1 and the resulting fibrous and liquid fraction were further analysed (Figure 3).

Table 1. Lab scale juicer Parameters overview

INSTRUMENT:	LAB-SCALE JUICER
LENGTH	26 cm
WIDTH	17,5 cm
HEIGHT	21,5 cm
ENERGY CONSUMPTION	200 W
OVERHEATING PAUZE	Every 30 min (for 5 min)
PROCESSING CAPACITY	10 kg biomass per hour

Figure 3: Fibre fraction (Left) and Juice fraction (Right) after biomass juicing

After processing with a lab-scale screw-type juicer the fibre and juice fraction composition were examined. Table 2 shows the juicing results starting from 1 kg of fresh *Salicornia* biomass. The juice wet weight is more than double that of the wet fibres, with a juice wet weight of 512.259 ± 33.6g while the fibre wet weight was 237.4 ± 11.8g. However, after drying it was observed that the differences in weight between both fractions was reduced. With 40.5 ± 1.1g observed for the fibre dry weight and 48.3 ± g for the juice dry weight. Based on these results the juice density was calculated as 0.0943 g/ml as the juice volume was 500.42 ml.

Table 2. Fibre and Juice parameters after processing of 1 kg of fresh Salicornia with a screw-type juicer.

PARAMETERS:	WET WEIGHT	DRY WEIGHT	DENSITY
FIBER	237.4 ± 11.8g	40.5 ± 1.1g	/
JUICE	512.259 ± 33.6g	48.3 ± 3.5g	1.024 g/ml

4.2 Centrifugation

In order to keep the processing simple and therefore widely applicable, the juice was subsequently centrifuged in order to concentrate the proteins within the pellet. No solvents were added to the juice as protein precipitation was considered sufficient with the native juice conditions. Due to the halophytic nature of *Salicornia ramosissima* the plant contains very high levels of salt which can be found within the plant juice. Most of this salt remains in the supernatant (70% of DW) while the pellet contains only 30% of DW in salt. Centrifugation therefore serves a double function in concentrating the nutritionally positive proteins within the pellet while keeping some of the abundant salt out.

Centrifugation was carried out in a Heraeus Megafuge 40 from Thermo Scientific, see material and methods while the equipment details are listed below in Table 3.

Table 3. Centrifuge parameters overview

Instrument	Thermo Scientific Heraeus Megafuge 40
Dimensions (H x W x D)	360 x 550 x 670 mm
Weight	86 kg
Power Usage	1700 W
Centrifuge Tube	Thermo Scientific Polypropylene 750 ml bottle
Tube Holder	TX-750 Round Buckets

Pellets were then transferred to bottles suitable for freeze-drying. Due to limitations of the centrifugation setup the pellets had to be resuspended in 50 ml of ultrapure water per bottle to facilitate the transfer. Some properties of the supernatant and pellet, after centrifugation of 0,5L of juice, are listed in Table 4 These are the result from three repeats of extraction. Protein recovery is in % DW of total protein content in fresh *Salicornia* plants.

Table 4. Supernatant and pellet parameters after processing of 500 ml of Salicornia ramossisima juice.

PARAMETERS:	WET WEIGHT	DRY WEIGHT	DENSITY	PROTEIN CONTENT	PROTEIN RECOVERY
SUPERNATANT	472.87 ± 1.63g	5.79 ± 0.45g	0.012 g/ml	9.324 % DW	31.85%
PELLET	27.13 g ± 1.63g	4.05 ± 0.43g	0.149 g/ml	16.738 % DW	39.67%

Comparing to different crops in literature shows that our protein recovery lies higher than carrot (20.9%) and sugarbeet (14.6%), less then mangold (53.1%) and lucerne (52.1%), and somewhat close to the one observed for beetroot (35.5%) and cabbage (37.4%) (Nynäs, et al 2021). In comparison to grasses such as ryegrass and red clover with recovery values of respectively 38% and 32%, our recovery of 39.67% is very much comparable (Rasmus, et al 2019).

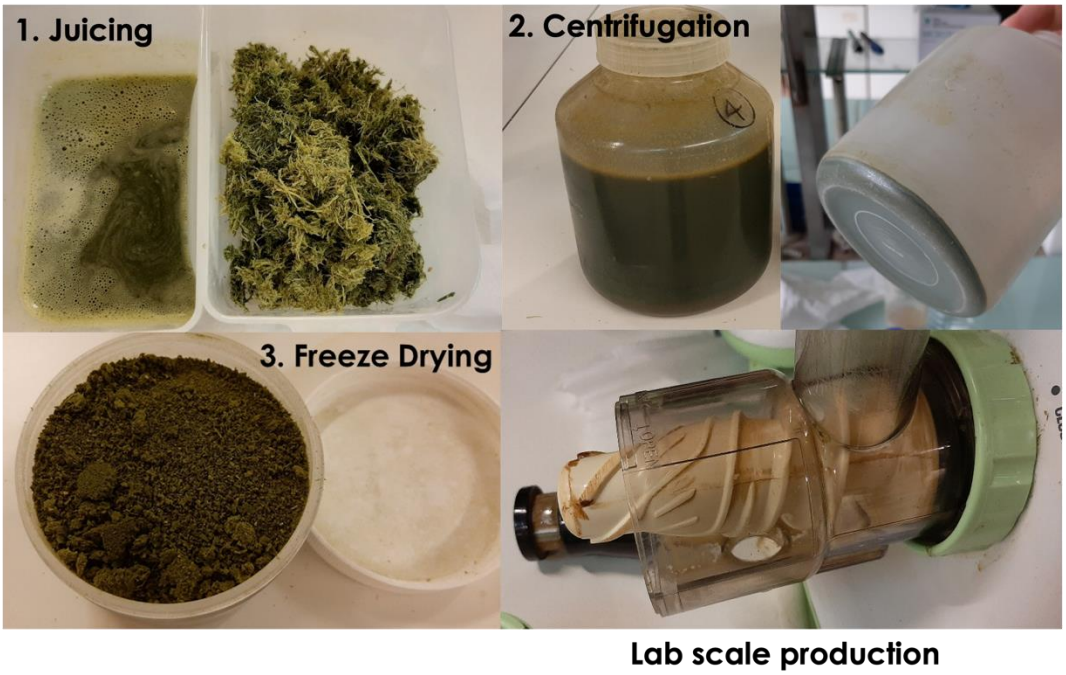

Figure 4: Overview of centrifugation method for lab-scale protein enrichment

4.3 Simulation and proposed scale-up scheme

As the general feasibility has been proven and optimized in lab-scale experiments, the next step is the scale-up to industrial scale. Regarding scale-up, equipment available at technical scale is considered only, in order to guarantee the technical feasibility. Modelling and simulation enable to calculate mass- and energy balances that are required for the conceptual sizing of equipment as well as the conceptual process design resulting in process flowsheet schemes. To conclude the optimal configuration, the enrichment via centrifugation has to be investigated to find the optimal operating conditions in the scope of the whole integrated AQUACOMBINE biorefinery. This includes e.g. operating conditions (temperature and pressure levels) and protein yields, their environmental impact (e.g. toxicity, health impact, greenhouse gas emissions), and the economic performance (e.g. CAPEX, OPEX).

In the laboratory scale, the first step is the juicing of the green biomass. For the scale-up it is aimed to implement a juicer, wherein higher flow rates of green biomass can be processed, depending on the production capacity of the biorefinery. Recovery rates in the juice and fibre fractions are taken as reference points for the larger scale. After juicing, the fibre residues are collected and send to other processes, e.g. organosolv.

From the benchtop centrifuge used in the laboratory operated in batch mode, the scale-up will be performed considering continuous operation at future industrial scale. This will be a disc stack centrifuge, which is capable of handling a wide range solid ratio. The scale-up factors from laboratory centrifuges to disc stack centrifuges are well described in literature and will ensure that the technical feasibility is given in the industrial scale [AMB59].

5 Conclusions and Outlook

Our goal in this work was to develop an efficient and cost-effective method for protein extraction from the juice of fresh Salicornia that can be scaled up. The centrifugation method was chosen for its simplicity and ease in use. It reduces the salt level in the protein-enriched fraction and is a scalable method. For the scale-up a disc stack centrifuge will be used, capable of handling a wide range solid ratio. Simulations will be carried out using computer aided process engineering tools, including commercial tools like AspenOne Engineering Suite, UMBERTO (LCA), ECOINVENT (LCA) and the Cost Engineering Suite as well as MS-Excel.

6 Bibliography

[AMB59] C. M. Ambler, “The theory of scaling up laboratory data for the sedimentation type centrifuge,” *Biotechnol. Bioeng.*, vol. 1, no. 2, pp. 185–205, 1959, doi: 10.1002/jbmte.390010206.

[ASP22] AspenONE Engineering (28.09.2022), see: www.aspentech.com/en/products/full-product-listing

[COS22] Cost Engineering Suite (28.09.2022), source: www.supren.eu

[ECO22] ecoinvent (28.09.2022), see: www.ifu.com/de/umberto/ecoinvent-datenbank/

[EXC22] Microsoft Excel (28.09.2022), see: www.microsoft.com/de-de/microsoft-365/excel

[UMB22] Umberto (28.09.2022), see: www.ifu.com/de/umberto/oekobilanz-software/

[Jones, et al 1941] Jones, D. B. (1941). Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. US Department of Agriculture - circ. 183. Washington, DC.

[Nynäs, et al 2021] Nynäs, Anna-Lovisa & Newson, William & Johansson, Eva. (2021). Protein Fractionation of Green Leaves as an Underutilized Food Source—Protein Yield and the Effect of Process Parameters. Foods. 10. 2533. 10.3390/foods10112533.

[Rasmus, et al 2019] Cour, Rasmus & Schjoerring, Jan & Jørgensen, Henning. (2019). Enhancing Protein Recovery in Green Biorefineries by Lignosulfonate-Assisted Precipitation. Frontiers in Sustainable Food Systems. 3. 10.3389/fsufs.2019.00112.