

THESIS OF THE PHD DISSERTATION

**Processing whey and grape must by complex
membrane procedures**

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The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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1. Preliminaries and targets of the thesis

According to the material my examinations can be divided into two separate sections. The first one was the grape must. Due to the increasing energy prices and strict environmental criteria modern procedures become more and more significant in food industry. Membrane filtration is such an up-to-date technology that can be applied in dairy and soft drink industries as well. These membrane procedures have several advantages such as energy and raw material optimisation, improved product safety and the development of new healthier products.

Annual whey production is estimated to around 80 million tons, which contains 500.000 tons of protein with high biological value. In the past whey was considered to be a by-product or it was used for animal feeding. Since whey is rich in proteins, minerals and vitamins, recently more procedures have been developed to make it suitable for human consumption. Its high nutritional value has only been recognised in this century, however whey was commonly used in medication in the 18th and 19th centuries (HOFFMANN, 1961).

Membrane filtration of whey: advantages and applications

Profit increase: it can be achieved by using high value whey components in cheese production and other dairy processes.

Product safety and quality: Microfiltration as a substitution for pasteurisation for microbial safety. Setting permanent parameters of whey (defatting).

Product innovation: Improving product composition. Development of new, better products with special regard to process valuable whey compounds like lactose and whey protein besides keeping high quality standards.

My experiments were performed on whey that was by-product of mozzarella cheese coming from SAPORI D'ITALIA KFT'S Tök factory. By introducing membrane filtration the cheese production company could find a solution to utilise whey, which is available in high quantities.

Grape production, making must and wine is well-known in areas of appropriate climate. Both must and wine play an increasing role in our everyday life due to their nutritional properties. To make good wines grape and must quality is essential. Winemakers that recognised this fact focus on proper must treatment. Climate conditions have a huge impact on must and wine quality. In years with adverse weather musts are improved by adding sugar and treating colour deviations. In great vintages must has high sugar content, but in worse years it can be significantly lower. In latter case the increase of sugar content is needed.

However must is not only used in wine-making, the juice of grape is a popular soft drink and sweetener. Unfortunately it is impossible to store must for longer periods, because its fermentation begins within a few days or even hours depending on external factors. There are several procedures to preserve must, but most of them are based on chemical preservatives.

Today's consumer needs and the focus on healthy nutrition require delicious foodstuffs that are free from chemical additives and are rich in valuable compounds. This triggers the need for more gentle food industry procedures.

My must processing experiments have been carried out with musts of Kékfrankos and Furmint grape varieties. The aim of my thesis was to develop modern, complex membrane filtration procedures that can be applied for the gentle must processing and preservation. Furthermore this technology enables the production of valuable compounds from whey.

I set the following targets for my PhD thesis

- Examination of certain membrane parameters (trans-membrane pressure, recirculation volume-stream, temperature) during whey and grape must microfiltration.
- How efficient microbial stability can be attained by microfiltration of whey and grape must, which normally contain high number of microorganisms.
- Can microfiltration be used as a substitute for whey separation (defatting) and grape must clarification.

- Sensory comparison of untreated and microfiltered grape musts.
- Determination of the membrane's whey protein retaining properties during whey concentration by ultrafiltration.
- Impacts of the whey's fat content on ultrafiltration.
- Testing nanofiltration for the concentration of lactose in whey. Measuring the level of salts and minerals in the concentrate thus produced.
- Testing reverse osmosis for the high degree concentration of whey compounds.
- Enzymatic hydrolysis of lactose that come from model whey solutions.
- Testing to retain the membrane-reactor enzyme.
- Assembling a continuous-mode membrane reactor based on experimental results.
- Nanofiltration and reverse osmosis testing on grape must samples which are derived from pre-concentration with microfiltration.
- Concentration of samples with membrane distillation.
- Modelling nanofiltration and reverse osmosis according to the Rautenbach model
- Design of integrated small-scale equipment for grape must concentration
- Assembling complex procedures based on my experimental results and professional literature data.

2. Material and method

The raw materials used in my work were mozzarella whey from SAPORI D'ITALIA KFT and grape musts of Furmint and Kékfrankos grape varieties. In the membrane-bioreactor experiments I prepared solutions of different lactose content (7, 20%) from whey powder in order to model the original whey. For the enzymatic hydrolysis *Kluyveromyces Lactis* β -D-galactosidase enzyme was used.

Microfiltration of whey and grape musts was performed on two pilot plant modules (one capillary and one ceramic) with 0,2 μm pore size. For whey experiments ultrafiltration was also applied on a 100 kDa pilot plant module. Nanofiltration experiments were done partly on a MILLIPORE YPROLAB 2 laboratory equipment. This latter device was used for concentration of the original whey and filtrates coming from whey micro- and ultrafiltration. Further nanofiltration and reverse osmosis experiments were performed on a DDS MINILAB 20 equipment with Trisep XN-45 nanofilter and DOW HR 30 reverse osmosis membrane. Following the microfiltration of grape must it was partly lead to a high pressure MFT Köln pilot plant reverse osmosis device to concentrate it. For membrane distillation measurements MD 020 CP 2N hydrophobic membrane was used from company Microdyn. The pore size of the membrane was 0,2 μm , meanwhile its active surface was 0,1 m^2 . Membrane bioreactor experiments were done with an Amicon (Millipore) laboratory stirring cell under 4 Bar pressure with nitrogen gas. To produce permeate I applied 10 kDa Pall and 30 kDa-os Nadir membranes. During the ultrafiltration (membrane bioreactor) after the enzymatic decomposition, a 5 kDa Nadir flat membrane was used.

Microbiology testing was performed using traditional surface spreading technique, meanwhile sensory analysis took place in an ISO certified laboratory. Further analytical tests were done on HPLC, refractometer and spectrophotometer. The determination of whey's fat and protein content standard procedures were applied.

3. The results

In my dissertation the membrane operations fit to process grape must and mozzarella whey were analysed, experimentally investigated, modelled and made designable. The procedures and devices were connected to have processes which result end-products while preserving the inner value of the elementary substances.

The essence of the new spare and environmental-friendly processes is the modern and exceedingly energy-saving membrane operations and devices:

- microfiltration, ultrafiltration, nanofiltration and reverse-osmosis
- and membrane distillation.

It was proved true in the course of microbiological experiments that microfiltration is fit to remove microorganisms in whey and grape must. In spite of the very large number of microbes in starting materials, microorganisms did not get over to the permeate. The most important aim of microfiltration adopted in my experiments was spare sterilisation and degreasing, and energy expenses saved during procedure are not to be neglected.

In ultrafiltration high protein concentration is to be achieved in case of cheese whey. The fat content of whey influences the successfulness of filtering, for this reason it is expedient to filter separated material in factories and with this fast blocking of membrane modules could be evaded. The retentate rich in proteins can be further concentrated by additional procedures (dia-filtration, evaporation, drying), and in this way whey protein powder of high value is to be obtained which can serve as elementary substance of medicines, food supplementary and various valuable, healthy food-products.

My experiments proved that concentrates with high protein and lactose contents is to be produced by nanofiltration of whey. Grape must could be concentrated successfully by nanofiltration with relatively high filtrate flux and end density the concentrate of which can be further thickened by membrane distillation into such state which end-product is to be kept without the customary preservation operations in a small place because of its high sugar content. Smaller proportion and slower pre-concentration could be attained by adopting

reverse osmosis but because the retention of it is higher than nanofiltration, end-product of better quality is to be obtained after membrane distillation concentrations.

Membrane reactor used after microfiltered whey is to be successfully adopted to make whey devoid of lactose. The reactor operates semi-continuously, so the cleaning and sterilisation procedures needed for standstills and enzyme loss can be avoided, and by this means time and money are saved.

Using the experimental results, the basis of such modern and complex membrane filtration procedures was worked out that is to be used to sparely process and preserve must, besides with this valuable end-products can be produced from whey as by-product.

3.1 New scientific results

1. Mozzarella whey and different grape musts were filtered with pilot plant ceramic and capillary micro-filtration equipment. The primary target was to separate the fat content of the whey and to remove the sediments of the grape musts (pre-treatment). I set the microbial sterilization of whey and improving sensory properties of grape must as secondary target. Based on experimental results I made the following statements:

- Micro-filtration with 0,2 µm pore-size filter retained 98,7% of fats and 100% of microorganisms from whey, thus this technique can successfully be applied for whey defatting and its microbial sterilization.
- According to sensory analysis results Microdyn 0,2 µm capillary module is recommended as first concentration step. External appearance of grape musts filtered with this device were scored to 73,6 points out of 100, meanwhile the untreated sample got 64 points. This verifies the efficiency of filtration. In terms of taste preferences filtrates of the capillary modules were ranked to first position with 75,75 points, in comparison with untreated samples there were minor differences in taste and smell intensity. Based on other tested parameters it can be stated that 0,2 µm pore size Microdyn ceramic module is not suitable for grape must pre-treatment.

2. In membrane distillation experiments an MD 020 CP 2N hydrophobic membrane with 0,2 μm pore size was used for concentrating model solutions (water, sugar-water solution) and different grape musts. Based on the experiments I stated that unlike nanofiltration and reverse osmosis with this technology the velocity of concentration is constant and is less dependant on the composition of the filtered solution. This is verified by the fact that flux was 2,4 $\text{kg}/(\text{m}^2\text{h})$ for water and 20 ref% sugar solution as well besides 30°C temperature difference. Grape musts of Kékfrankos and Furmint varieties were concentrated to 64 ref% besides identical experimental parameters (initial quantity and ref%, ΔT) during the same period of time.

3. My membrane reactor experiments verified that 5kDa Nadir membrane retains 100% of β -galactosidase enzymes (in spite of 5 and 10 kDa Pall membranes that do not). As enzyme activity cannot be measured in the filtrate of membrane reactors I ran HPLC tests to justify that in membrane bioreactor whey samples with 7% dry matter, 50 g/L enzyme and 20% dry matter, 120 g/L enzyme can successfully be hydrolysed besides 100% retention of β -galactosidase enzyme.

4. I made a complex technology for processing whey as a dairy by-product (Figure 1). The procedure, which is a combination of membrane techniques, is based on the following results. As a first step I sterilized the whey and separated 98,7% of fats on a pilot plant capillary equipment with 0,2 μm pore size. This operation combines two traditional technology steps. In the membrane bioreactor lactose was removed from whey which can be then lead into three parallel lines where ultrafiltration, nanofiltration and reverse osmosis are applied to produce the desired finished products:

- With the ultra- and nanofiltration of micro-filtered filtrate we get two finished products. The 100 kDa ultrafiltration membrane retained 75% of proteins, which can be explained with whey proteins of smaller molecule size. Nanofiltration of UF permeate results in high lactose concentrate. These mixtures can be powdered to obtain valuable raw materials for pharmaceutical and nutritional applications.
- During the nanofiltration of micro-filtered whey on a 400 Da R75A Millipore membrane I achieved 92% lactose retention besides 4 times concentration ratio. I

increased the lactose content of whey up to 21%. Whey proteins and lactose can be concentrated with nanofiltration. The concentrates thus obtained can be further used as raw material for ice-cream production.

- In reverse osmosis experiments I measured the following flux increase during concentration compared to the initial whey: defatted filtrate 16%; defatted and protein free filtrate 57%; permeate of nanofiltration 84%. Thus I verified that during reverse osmosis fat content plays a smaller role in filtrate flux decrease than protein and lactose content do. Fat globules with micrometer size have less impact on osmotic properties. Further experiments justified that reverse osmosis is suitable for the concentration of valuable whey components, since the decrease of conductivity of RO permeate of MF filtrate was 93% even in case of double concentration. This complex membrane filtration can be ideal for small plants, because the concentrate can be lead to cheese production to increase the yield of cheese.

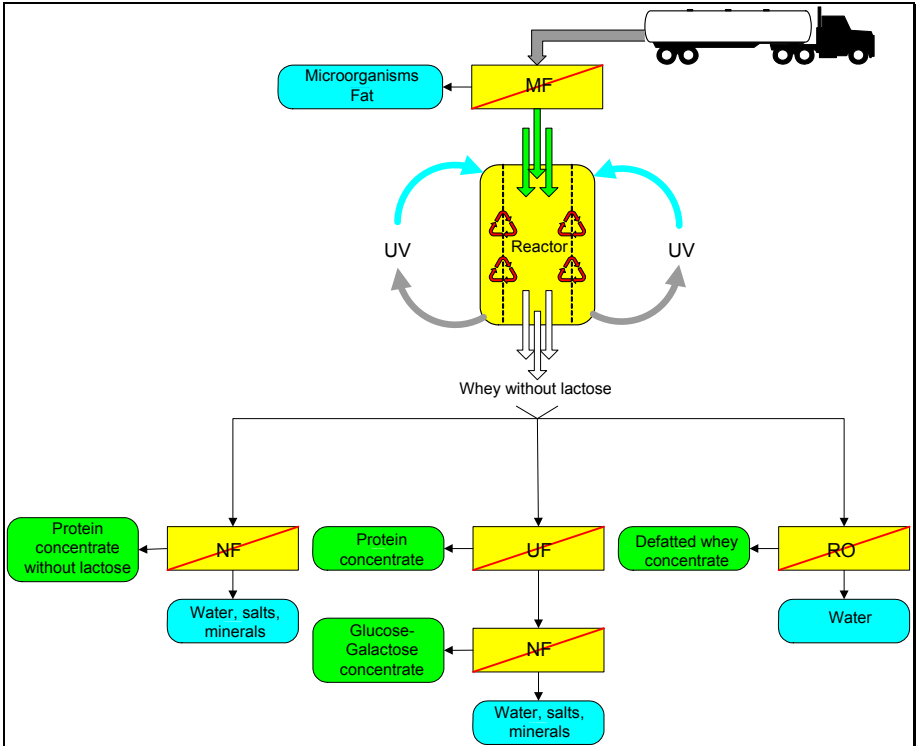


Figure 1. New complex membrane separation procedures for whey utilisation

5. I established a complex procedure for the concentration of grape musts (Figure 2). The technique, which consists of gradual concentration steps, is based on the following results. As a first step I sterilised and removed the clarified the grape must of a worse vintage on a capillary micro-filter with 0,2 µm pore size. Then the filtrate was concentrated on a pilot plant MFT-Köln CD-1 reverse osmosis equipment up to 22,5 ref%. The initial material was then concentrated to 64 ref% with membrane distillation using 0,2 µm pore size hydrophobic membrane. This end-product is suitable for winery and soft drink technologies or even for direct consumption.

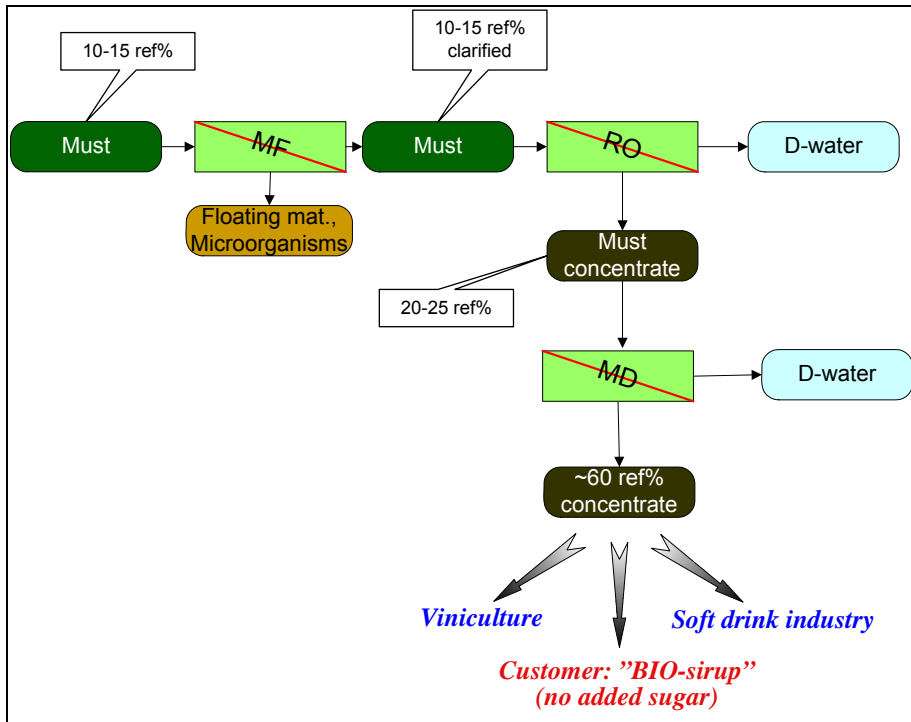


Figure 2. New complex membrane separation techniques for grape must concentration

4. Conclusions and recommendations

4.1. Conclusions

During my research projects I worked out a complex procedure for whey that comes from cheese production: processing and eliminating lactose.

- Micro-filter with 0,2 μm pore size is suitable for the efficient separation of fats found in whey, furthermore it can be used for whey sterilisation.
- Membranes of 100 kDa can retain 75% of whey proteins. Higher retention can be achieved with the application of membranes of lower cut off value.
- Nanofiltration retained 91% of whey even in case of four times concentration, consequently to retain lactose from whey 400 Da or equivalent membranes seem to be ideal.
- The Retention of β -galactosidase enzyme does not only depend on cut off value, but on the type of membrane too. With good membrane selection 100% of β -galactosidase enzyme can be retained, thus the membrane bioreactor can be operated profitably.

During my research projects I worked out a complex procedure for the concentration of grape must.

- Microfilter with 0,2 μm pore size is suitable for sterilization and clarification of grape must.
- I managed to obtain concentrate above 20 ref% with reverse osmosis, which can be used for improving grape musts of low sugar content (poor vintages). Such a concentrate belongs to the category of grape musts that are suitable raw materials for high quality wines.
- Grape musts can be further concentrated with membrane distillation above 60 ref%. The velocity of concentration is determined by the temperature difference

between the two sides of the membrane. The process can be optimised by choosing the right temperature.

4.2. Recommendations

- For the complex processing of whey with membrane techniques further pilot plant and industrial experiments and relevant calculations are needed to optimise and coordinate different steps in the technology.
- Elimination of lactose from whey with membrane bioreactor is just the result of a laboratory experiment that shows the success of membrane techniques. Further experiments are needed to implement the technique in industry scale.
- For the complex processing of grape must with membrane techniques further pilot plant and industrial experiments and relevant calculations are needed to optimise and coordinate different steps in the technology.

5. List of publications related to the dissertation

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