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D3.2 Design and performance of integrate organic carbon recovery in whey management

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1. INTRODUCTION

1.1. Types of whey and their parameters

1.1.1. Types of whey

Whey is a by-product of the dairy industry from the production of cheese, curds and casein. For the purposes of this Deliverable, milk is defined as cow's milk, which is the world's dominant milk producer. According to recent research, milk contains about 10 000 different compounds. The basic components of whey, in simplified terms of dry matter 14% are lactose (5%) , fat (4%), protein (3.4%), minerals (0.9%), vitamins and other compounds such as hormones, peptides, urea. Proteins related to the formation of whey are divided into casein (2,8 %) and whey protein (0,6 %). Casein is the basis of cheese. Cheese used in statistics is divided into natural and processed. Natural cheese is hereinafter referred to as cheese. Processed cheese is not related to whey production. The casein for the production of most cheeses is precipitated from the milk using enzymes, known as rennet, and appropriate dairy micro-organisms are also added. The separation of the curds produces so-called sweet whey. Casein is also the basis of curds and fresh cheeses, where it is precipitated by increasing the acidity, lowering the pH below isoelectric point of pH = 4,5 by lactic acid, which is formed by the action of added lactic acid bacteria. Acid whey is formed after separation of the curds. Casein is also produced as a protein concentrate in two forms. Acid one, which is produced in a similar way to curds, except that it is then washed of all the other components of milk. Recently, however, it is produced on a large-scale by precipitation with the addition of inorganic acids, mainly hydrochloric acid, to form whey containing hydrochloric acid. Acid casein is mainly used for the production of sodium caseinate, as a protein concentrate to increase the protein content of foods and to improve their properties, especially emulsification and water binding.

The second type of casein is sweet one, which is produced by enzymatic precipitation, similar to cheese. It is washed of all the other components of milk to form sweet whey. Sweet casein, the production of which is marginal, is used mainly for the production of artificial horn – galalite. In the case of sweet whey, whey from the production of Dutch-type cheeses (e.g. eidam) can also be distinguished. In cheese making, at a certain stage in the processing of the casein-cheese curd, some of the whey (about 30 %) is removed and water is added, and after the final processing of the cheese curd, the remaining whey is separated and usually mixed with the other whey to produce whey with a lower dry matter content.

In the case of acid whey from curd production, it is possible to distinguish the whey from the production of 'thermo-curd'. Thermal curd is curd which contains a proportion of the nutritionally valuable whey protein, thereby increasing the yield of curd production. The whey protein in the production of conventional curd, when separated from the curd, leaves in the separated whey. As presented above, different types of cheese, curds and casein are produced, and moreover by different technologies. This produces different types of whey with different compositions. Depending on their composition, these types of whey are then suitable for further processing and use.

Both sweet and acid whey contain significant quantities of lactic acid bacteria. The whey contains the nutrients suitable for bacteria development and for the development of undesirable microorganisms, including pathogenic ones, with which it may have been contaminated during discharge from food production, storage and possible subsequent transport. Bacteria may then develop and further acidify the whey. It is necessary to take this into account when deciding whether appropriate sanitation and technological measures should be taken in connection with further processing.

Sweet whey, including whey from the production of Dutch-type cheeses, is largely further processed in the world mainly by drying, to produce protein concentrates, lactose and other acid whey components. These products are further used in the food, feed and pharmaceutical industries. Acid whey is poorly suited for drying due to its lactic acid content and its stickiness during drying (spray drying). However, in the world, when large volumes are processed, it is usually mixed with sweet whey, which predominates, and processed further as sweet whey.

1.1.2. Parameters of different types of whey

For some general overview 9 whey samples were obtained. The samples were 1 sweet whey, 1 sweet diluted whey (30 % water rinsing of cheese grains), 1 sweet concentrated whey by reverse osmosis, 1 dried sweet demineralized whey (90 % demineralization, electrodialysis), 2 acid whey, 2 acid whey from thermo-fermentation and 1 acid concentrated whey (reverse osmosis). After obtaining the sample, the cheese dust content was immediately determined as described. For further analysis, each sample was homogeneously separated and frozen at -18 °C.

Whey from any production contains so-called dust (fine particles of cheese, curd, casein). The amount of dust depends on the intensity of dust trapping in the cheese or curd production.

The reported dust figures also depend on the way in which the whey is sampled, e.g. being taken into storage containers, or being taken from storage containers where, for example, the dust is sedimented, or in the buoyancy.

Table 1: pH, density and dust values of whey samples

whey	Sample no.	pH	Density	Dust
			kg/m³	%
sweet	1	6,59	1,02	0,14
sweet diluted	2	6,56	1,02	0,02
sour	3	4,39	1,02	0,02
acid	4	4,45	1,02	0,55
acid thermocurds	5	4,23	1,02	0,01
acid thermocurds	6	4,47	1,02	0,01
demi sweet dry	7	-	-	-
acid concentrated	8	4,41	1,10	1,12
sweet concentrated	9	6,05	1,07	7,02

Table 2: Basic chemical composition of whey products

whey	Sample no.	Dry matter	ash	fat	protein	lactose
		%	%	%	%	%
sweet	1	6,84	0,50	0,06	0,62	5,21
sweet diluted	2	6,54	0,49	0,05	0,60	5,15
sour	3	6,24	0,60	0,00	0,40	4,23
acid	4	6,02	0,67	0,01	0,54	4,33
acid thermocurds	5	5,68	0,61	0,02	0,10	4,05
acid thermocurds	6	6,07	0,75	0,01	0,18	4,14
demi sweet dry	7	96,5	0,96	0,50	11,1	80,6
acid concentrated	8	16,3	2,32	0,36	0,40	11,9
sweet concentrated	9	16,5	1,97	0,14	2,42	11,0

Table 3: Nitrogen and whey protein content of whey samples

whey	Sample no.	total nitrogen (TN)	non-casein nitrogen (NCN)	Non-protein nitrogen (NPN)	whey protein (WP)[1]
		%	%	%	%
sweet	1	0,14	0,14	0,04	0,65
sweet diluted	2	0,13	0,13	0,04	0,59
sour	3	0,13	0,12	0,06	0,39
acid	4	0,13	0,13	0,05	0,51
acid thermocurds	5	0,09	0,09	0,07	0,11
acid thermocurds	6	0,09	0,09	0,06	0,19
demi sweet dry	7	2,28	2,07	0,54	9,76
acid concentrated	8	0,26	0,25	0,20	0,35
sweet concentrated	9	0,48	0,49	0,10	2,47

1 whey protein = (NCN - NPN) x 6,38

Table 4: Content of selected acids in whey samples

whey	**Sample no.**	**Formic acid**	**Citric acid**	**Phosphoric acid**	**Lactic acid**	**Succinic acid**	**Aspartic acid**	**Acetic acid**	**Glutamic acid**
		mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml
sweet	1	6	172	106	28	nd	9	nd	nd
sweet diluted	2	5	169	100	23	nd	nd	nd	nd
sour	3	12	15	190	812	18	20	75	19
acid	4	8	33	189	760	19	10	64	17
acid thermocurds	5	13	151	175	586	12	11	11	nd
acid thermocurds	6	9	11	191	656	12	13	83	25
demi sweet dry	7	9	315	58	40	nd	10	5	nd
acid concentrated	8	19	24	138	2247	31	30	170	46
sweet concentrated	9	4	517	315	104	13	14	21	24

Propionic or butyric acid is not listed in the table because it was not detected in any of the samples.
nd - not detected

1.1.3. Transport of whey

Whey of any kind is usually processed or disposed of elsewhere than where it originates. It is usually transported for further processing. However, the whey solids are low, its components are present in low concentrations, and water predominates (at least 94 %). Therefore, it is focused to reduce the water content and thus reduce transport costs. Transport is usually carried out by tanker trucks, usually stainless steel, with a minimum capacity of 5-10 000 Liters, but usually with a capacity of 20 000 Liters, with trailers up to 40 000 Liters. For short distances (tens of km, max. 80 km) whey can be transported in natural form. For longer distances, it is preferable to transport whey in concentrated form.

1.1.4. Thickening of whey

Before transport for further processing and before using reverse osmosis or evaporation, it is useful to de-dust the whey and skim off the fat as a valuable raw material that the dairy can use efficiently. At present, whey is most commonly concentrated by reverse osmosis to a dry matter content of up to about 18 %. This membrane process can not economically and technologically achieve higher dry matter contents. With such thickening, the development of microorganisms is already limited. The whey can also be thickened on multi-stage evaporators with reduced pressure.

Thickening, which does not require additional equipment and technology, is used up to about 30 % dry matter content. This is because lactose starts to crystallize at higher concentrations and at lower temperatures, especially in winter. The sedimenting crystals can then cause blockages in the tanker outlet pipes.

Where evaporators are available to allow higher concentration and rapid (expansion) cooling of the concentrated whey, the whey is concentrated to 45-65 % of dry matter. After expansion cooling, fine crystals of lactose are formed and further controlled crystallization results in further crystallization of fine crystals having a density close to or equal to that of the surrounding whey - mother liquor. This process ensures that the lactose crystals do not sediment significantly in the storage or transport containers. This allows the whey to be transported over long distances and in large volumes. Such high concentrations of dry matter or lactose also prevent the development of micro-organisms. In addition, the whey that has been evaporated has been pasteurized.

As regards the characteristics of acid whey in general, and the thickening process in particular, it is useful to note that acid whey is corrosive. The acid whey produced by lactic acid bacteria contains approximately 1 % lactic acid and a small amount of less than 0,1 % of other organic acids. Concentrated whey, however, contains 3 % lactic acid at 18 % of dry matter, concentrated to 30 % of dry matter and 5 % lactic acid, concentrated acid whey by evaporation 45 % of dry matter and 7 % lactic acid. The acid whey from casein production using inorganic hydrochloric acid contains approximately 1 % HCl. When

concentrated to 45 % dry matter or more in the evaporator, the whey is already very corrosive and the last stages of the evaporator must be made of molybdenum-alloyed stainless steel or so due to strong corrosivity.

1.2. Method of estimating whey production from cheese and curd production

Data on the production of whey and individual whey varieties in the world and in Europe and the Czech Republic are not systematically available. The International Dairy Federation (IDF) publishes only fragmentary data on the production of dried whey, i.e. sweet whey. IDF systematically compiles annual data from the major producing countries, mainly on the quantities of milk processed, selected dairy products and cheese. Cottage cheese is considered to be part of the cheese category [1,2]. The Czech-Moravian Dairy Association provides data for these statistics and also publishes data on the total quantity of milk purchased by dairies and on the total production of cheese and curds and other types of dairy products in dairies. No statistics were kept on whey production and processing and some data were only minimally and occasionally reported. [3] However, by choosing to simplify, it is possible to make some estimate of milk production from cheese and curd production and the resulting sweet and acid whey production. If data on the production of cheeses, or types of cheeses and curds, is available, the qualified estimate of sweet and acid whey production is easier and more accurate.

Milk requirement in kg = cheese production in kg + whey produced in kg

- 10 kg milk = 1 kg cheese + 9 kg sweet whey
- 10 kg milk + water = 1 kg cheese (Dutch type) + 12 kg sweet whey diluted
- 6 kg milk = 1kg cottage cheese, fresh cheese, cottage + 6 kg acid whey

Simplified:

- 1 kg of cheese is related to the production of 10 kg of sweet whey
- 1 kg of cottage cheese is related to the production of 6 kg of whey

For further illustration, graphs are presented showing what proportion of purchased milk is used for what type of dairy product in the world and in the Czech Republic. This can be used, using the above simplification, to make a rough estimate of whey production.

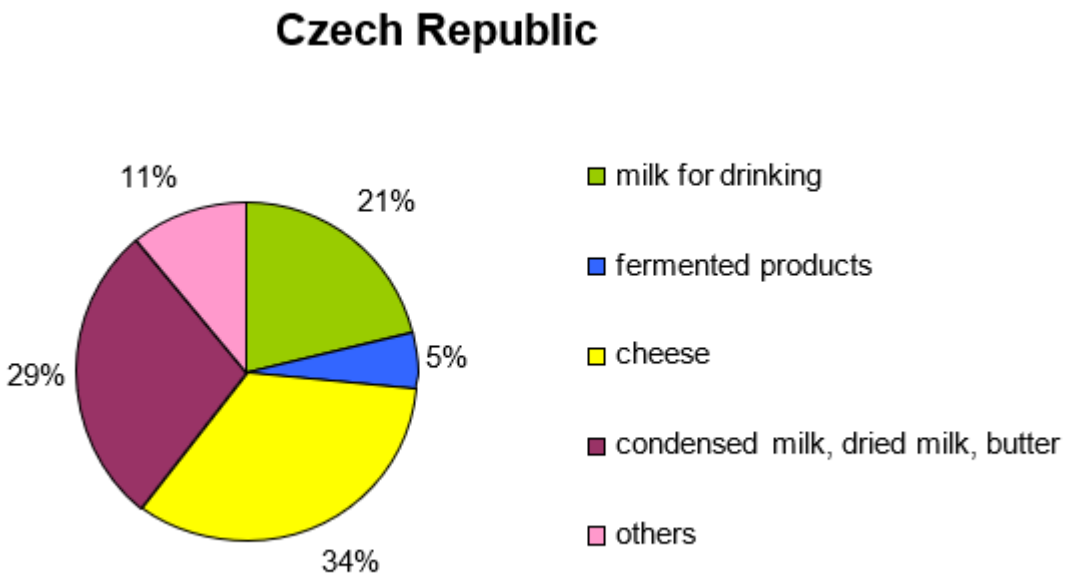


Figure 1: Milk distribution to products in the Czech Republic

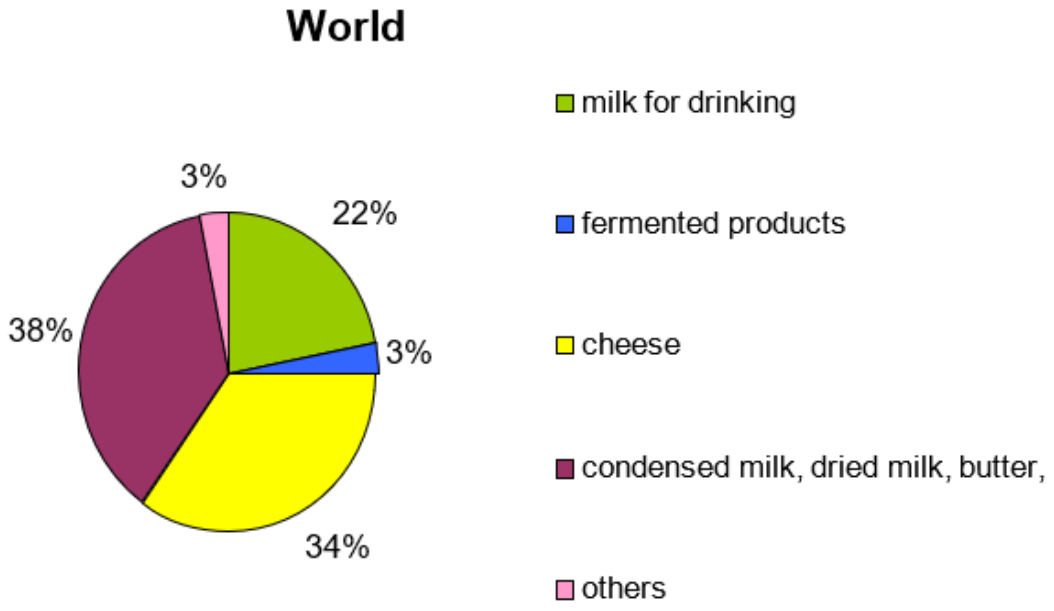


Figure 2: Milk distribution to products world-wide

1.3. Estimate of world whey production and in the European Union

World milk production or volumes of milk delivered to dairies in the world and in the EU28 [1,2] are shown in the following tables and figures. It is presented as an observed and statistically long-established, highly accurate figure. It can be used to check estimates of whey production and its trends.

Table 5: Cow's milk production worldwide [1] –

	2010	2015	2018	2019	2020	growth in 19-20	annual average growth in 10-20
	1000 t	1000 t	1000 t	1000 t	1000 t	%	%
World	599899	667273	701021	714781	735270	2,9	2,1
Asia	158093	190904	214435	225460	273824	5,5	4,2
EU28	149576	162958	166825	167801	170062	1,3	1,3
other European states	59059	57310	57793	57925	58716	1,4	0,1
North and Central America	112069	120662	126776	127148	129749	2	1,5
South America	60520	67066	65277	66120	68012	2,9	1,2
Africa	33933	36785	38680	39385	39787	1	1,6
Oceania	26648	31587	31234	30942	31120	0,6	1,6

Table 6: Cow's milk production worldwide [1] – annual average

	2010	2015	2018	2019	2020	growth in 10-20	annual average growth 10-20	ratio of world cheese production	acid whey production – estimate 2020
	1000 t	1000 t	1000 t	1000 t	1000 t	%	%	%	1000 t
World	17959	19946	21548	21908	22348	2	2,2	100	224200
Asia	697	897	1067	935	1138	22		5	11400
EU28	8368	8998	9497	9664	9852	1,9	1,6	44	99000
other European states	1290	1215	1156	1261	1308	3,7		6	13000
North and Central America	5395	6155	6820	6928	6998	1		31	70000
South America	1398	1564	1754	1745	1785	2,3		8	18000
Africa	294	387	506	552	559	1,3		3	5600
Oceania	607	897	751	736	720	-2,2		3	7200

We can make some simplified conclusions on whey production in 2020 from cheese production. It is produced worldwide 225 000 000 t of cheese form what EU28 produces 99 000 000 t which is 44% of world production. Trend in the last 5 years increasing with decreasing growth in the last two years.

The following tables shows a procedure for estimating how whey is used in the world and for what purpose. From the data on world and EU28 production of dried whey [4] it is possible to estimate what proportion of whey is processed by drying.

Table 7: Dried whey production in European Union

	dried whey dry matter content 95%					dried whey re-calculated for liquid whey dry matter content 6%				
	2016	2017	2018	2019	2020	2016	2017	2018	2019	2020
	1000 t	1000 t	1000 t	1000 t	1000 t	1000 t	1000 t	1000 t	1000 t	1000 t
EU 27				2114	2125				33824	34000
EU 28	2050	2100	2100	2180	2180	32800	33600	33600	34880	34880
Czech Republic	30	32	30	34	33	480	512	480	544	528
Denmark	29	37	39	66	81	464	592	624	1056	1296
Germany	360	345	316	310	312	5760	5520	5056	4960	4992
Spain		41	44	32	26		656	704	512	416
France	540	544	531	511	498	8640	8704	8496	8176	7968
Ireland	211	226	231	268	259	3376	3616	3696	4288	4144
Italy	194	210	223	251	240	3104	3360	3568	4016	3840
Lithuania	19	22	26	33	35	304	352	416	528	560
Netherlands	121	162	165	186	201	1936	2592	2640	2976	3216
Austria	37	35	42	42	25	592	560	672	672	400
Poland	166	164	285	288	309	2656	2624	4560	4608	4944
Portugal	18	19	13	21		288	304	208	336	0
Sweden	2	2	2	2	2	32	32	32	32	32
Great Britain	36	49	43	66	55	576	784	688	1056	880

Table 8: Dried whey production – main producers

	dried whey dry matter content 95%					dried whey re-calculated for liquid whey dry matter content 6%				
	2016	**2017**	**2018**	**2019**	**2020**	**2016**	**2017**	**2018**	**2019**	**2020**
	1000 t	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**
EU 27				2114	2125	0	0	0	33824	34000
EU 28	2050	2100	2100	2180	2180	32800	33600	33600	34880	34880
Russia	121	142	141	158	179	1936	2272	2256	2528	2864
Canada	34,3	37,6	37,8	35,7	34,7	548,8	601,6	604,8	571,2	555,2
USA	433	470	453	443	431	6928	7520	7248	7088	6896
Argentina	67	72	75	72	71	1072	1152	1200	1152	1136
Chile	25	26	27	27	32	400	416	432	432	512
Uruguay	16	19	16	15	16	256	304	256	240	256
New Zealand	17	15	16	13	15	272	240	256	208	240
Australia	50	50	48	52	49	800	800	768	832	784

Table 9 Ratio of whey used for drying in European Union

Year	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
	1000 t	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**	**1000 t**
dried whey calculated from cheese production (6%)	84000					90000			95000	97000	99000
dried whey re-calculated for normal liquid whey 6%							32800	33600	33600	34880	34880
whey ratio for drying %									35	36	35

there are no data available in empty cells

The above tables give a rough estimate of whey production, without distinguishing between species, for the world as a whole and the EU share.

Table 10: Summary of whey production in European Union

Production year 2020	**1000 t**	**Trend 2010 - 2020**
World milk	735270	growing at around 2% p.a. slowing down
EU28 milk	170062	growing at around 1% p.a.
EU28 cheese	9852	growing at around 1% p.a.
EU28 whey calculation	99000	resulting from cheese production
EU28 dried whey	2180	slightly increasing
EU28 liquid whey calc. from dried whey	34880	stagnating

Based on data on world and EU production of dried whey, it can be estimated that the share of whey processed by drying is 35%, a share that has been stable over the last 5 years. Another part of whey is processed into protein concentrates - whey and mixed one protein concentrates and for lactose production. It can be guessed that up to a further 1/3 of EU whey is processed into these products. This processing is carried out by large-scale production by specialist companies such as Borcullo - Holland, Lactoserum - France, DMV - Holland, MEGGLE - Germany, UK. In such plants, up to 10-20

000 t/day of whey is processed in one place and transported from distances of several hundred kilometers from all over Europe. It can therefore be guessed that the remaining 1/3 is used for feed or otherwise.

1.4. Estimate of total whey and acid whey production in the Czech Republic

The production of whey, especially acid whey, in the Czech Republic is not monitored and no systematic statistics are kept on it. A rough estimate can be derived from the production of cheese, individual types of cheese and curds [3]. The trend in production can also be traced from the production or purchase of raw milk for processing in dairies [3]. The processing of milk outside dairies and the production of cheese, so-called farm production, is up to 2% of total milk production in the Czech Republic, i.e. negligible.

Table 11: Cow's milk purchase into dairy-processing companies in the Czech Republic

Year	**2010**	**2015**	**2018**	**2019**	**2020**
	1000 t	**1000 t**	**1000 t**	**1000 t**	**1000 t**
Milk purchase	2683	3029	3078	3073	3183

Raw milk production between 2010 and 2020 increased by 18% of the 2010 value of 2 683 000 t to 3 181 000 t. The average annual growth was 1,7 % and the increase between 2019 and 2020 was 3,5 %.

Table 12: Cheese production in the Czech Republic in 1950 – 2020

Year	**Cheese**	**Curds**
	t	**t**
1950	13012	nd
1955	15281	nd
1960	23478	nd
1965	28219	nd
1970	39129	nd
1975	49792	nd
1980	58735	40430
1983	66510	40452
1985	70078	40480
1990	79632	45117
1995	52740	28837
2000	92406	28930
2004	94367	34368
2005	91720	29444
2010	81044	25550
2015	86142	34518
2017	94375	38923
2018	96870	38028
2019	96419	37893
2020	106369	41029

In the last 10 years, cheese production has increased by 31% from 81 044 t/year in 2010 to 106 369 t/year in 2020. The annual increase in cheese production in 2020 was 10%. Curd production increased by 60% over the same period from 25 550 t/year in 2010 to 41 029 t/year in 2020. The annual increase in curd production in 2020 was 8,5%.

1.4.1. Whey production in the Czech Republic

The very sparse published data on the production of individual types of cheese over the last 10 years shows the share of individual types of cheese in the total cheese production in the Czech Republic, as shown in the table below. The proportion of production of individual types of cheese has been stable over the long term, especially over the last ten years.

Table 13: Cheese production ratio in the Czech Republic

	semi hard	hard	extra hard	soft	fresh	total
%	60	8	7	17	8	100

Table 14: Whey production from cheese and curds

	semi hard	hard	extra hard	soft	fresh	total
kg/kg	12	10	10	9	8	6

Table 15: Production of cheese and curds in Czech Republic (2020)

2020	t
milk purchase	3183000
natural cheese production	106369
curds production	41029

Table 16: Sweet and acid whey production in Czech Republic (2020)

		semi hard	hard	extra hard	soft	fresh	curds
cheese curds	**t/year**	64000	8500	7500	18000	8500	41000
whey	**t/year**	768000	85000	75000	162000	68000	246000
Kind of whey		sweet diluted	sweet	sweet	sweet	acid	acid

We can make thus guess that the Czech Republic in 2020 will have produced approximately 1 200 000 t of sweet whey and approximately 300 000 t of acid whey. Over the last ten years, there is an increasing trend in the production of approximately 3% per year for sweet whey and 6% per year for acid whey. The future outlook, given the saturation of dairy product consumption, including cheese and curd, and the expected economic stagnation, a reduction in the growth rate of cheese and curd production, and possibly even stagnation, can be expected, i.e. a stagnation in the production of both sweet and acid whey. Their production ratio is expected to be maintained.

1.4.2. Estimations on the use of whey in the Czech Republic

In the last 10 years, very few and unsystematically published sources [3] indicate that 31 000 t/year of dried whey (95 % dry matter) and 36 000 t/year of concentrated whey (18 % dry matter - concentrated by reverse osmosis, or 30 % dry matter - concentrated by evaporation) were produced in the Czech Republic. The concentrated whey is transported abroad for further processing. For further calculations, it can be estimated that the average whey dry matter is 25 %. The quantity of concentrated whey transported can then be converted into natural whey with an original dry matter of 6 %. This is very likely to be cheese whey, i.e. sweet whey, but a mixture with sour whey cannot be ruled out.

Approximately 500 000 t/year of liquid sweet whey is processed into dried whey. 150 000 t/year of sweet and acid whey is processed into concentrated whey for foreign processing. It can be estimated that this is 70 000 kg of acid whey and 80 000 kg of sweet whey.Of the total estimated quantity of sweet whey produced of 1 200 000 t/year, approximately 500 000 t of sweet whey/year are dried and 80 000 t sweet whey/year are concentrated and transported for further processing. Of the total estimated quantity of acid whey of 300 000 t acid whey/year, approximately 70 000 t acid whey/year are concentrated

and transported for further processing. It can therefore be estimated that 600 000 t/year of sweet whey and 230 000 t/year of acid whey remain to be processed in the Czech Republic. These quantities are most likely to be used for feeding purposes.

1.5. Brief description of whey and acid whey use

Whey is processed into many products using sub-processes as shown in the following diagram [5,6]

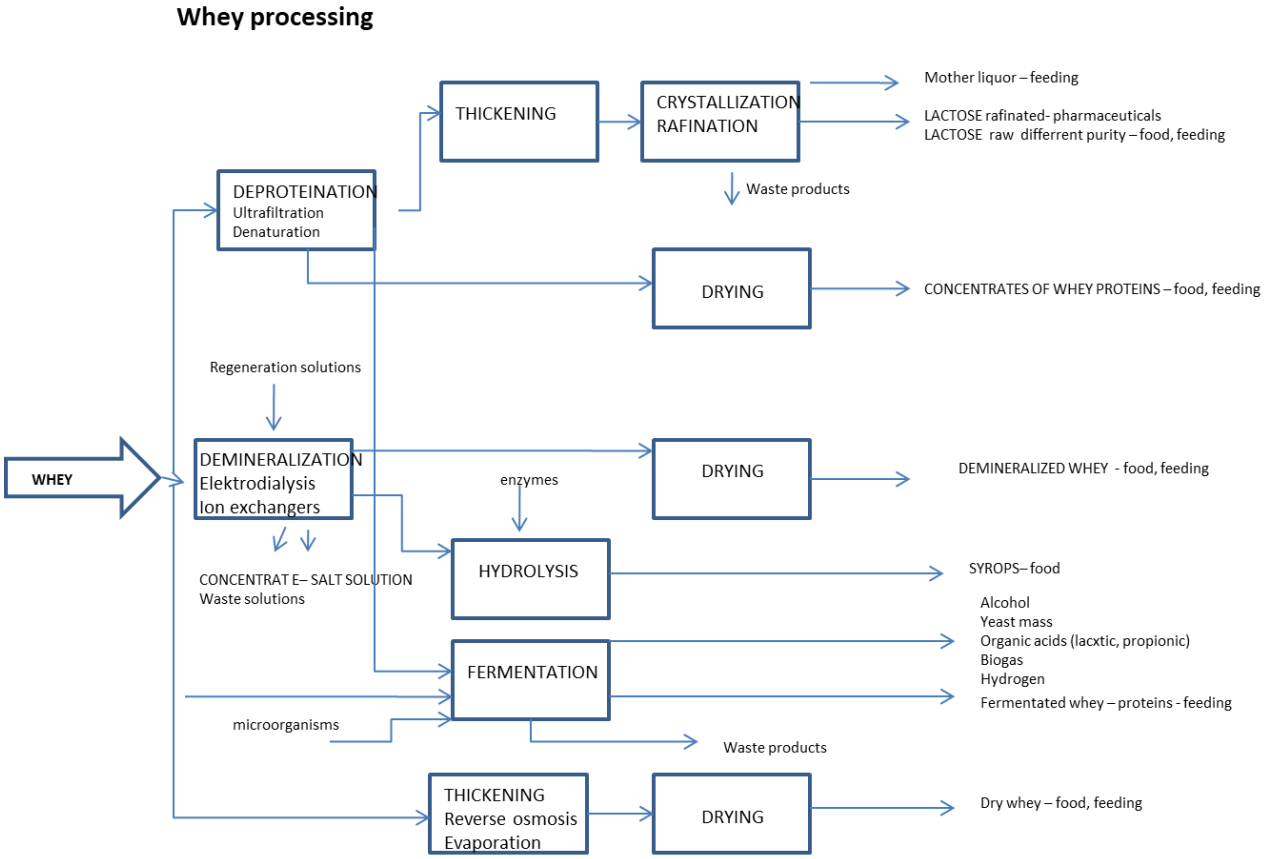


Figure 3: Main whey processing

1.5.1. Examples of manufacturing processes for selected most common processing options

- ***Transportation of non-thickened whey for feed purposes - feed drink.*** Usually does not include heat treatment, usually does not include cheese dust capture. Includes storage in the dairy, includes storage at the user's premises. Suitable for fattening pigs. Less suitable for feeding cattle - inoculation diet required.
- ***Whey thickening*** (sweet, acid) - using reverse osmosis, thickening to 18 % dry matter for transport for further processing.
  using evaporation under reduced pressure - tube evaporators - thickening to 30 % dry matter.
  Typically includes post-production storage, fat separation by centrifugation, dust separation, thickening, storage, transport and storage at the user's site.
- ***Drying of whey*** (especially sweet whey, practically not acid whey, neutralization would be necessary). Concentration (natural whey or transported pre-thickened whey, possible mixing to 45 - 60 % by evaporation and spray drying – dry matter content up to 95 %.
  Usually includes post-production storage, fat separation by centrifugation, dust separation, thickening, drying, packaging, storage, transport and storage at the user's premises.
  Non-caking dried whey with controlled crystallized lactose can be produced.
  It is possible to produce dried whey demineralized by electrodialysis, or by ion exchangers; producing by-products such as concentrate from electrodialysis or regeneration wastewater from ion exchangers.

1.5.2. Separation of suitable substances for food-processing and pharmacy industry

- ***Lactose*** - usually from sweet whey. In acid whey, the lactose content is reduced by the action of microorganisms. Lactose can also be produced from a suitable mixture of sweet and acid whey – processes: pre-treatment by bleaching, thermal denaturation, ultrafiltration.
  Lactose production (different types, alpha, beta, different purities) includes high whey concentration by evaporation, separation and purification crystallization procedures, drying, treatment, packaging. By-products are mother liquors and other waste substances.
- ***Sweet syrups*** based on the enzymatic breakdown of lactose into simple sugars. It is used in the beverage industry. The production of sweetening syrups involves skimming of whey usually by ultrafiltration, demineralization by e.g. electrodialysis and subsequent enzymatic hydrolysis, hickening or drying.
- ***Caseinomacropeptide*** – food-processing industry – emulsification, growth promotion of probatics.
- ***Ethanol*** - production includes whey separation, fermentation by lactose utilizing yeasts, separation of yeast mass as a by-product, distillation, rectification.
- ***Silage microbial cultures*** - production consists mainly of cementation with selected microbial cultures.
- ***Feed biomass*** - production involves fermentation by suitable microorganisms (yeasts), usually thickening and drying.
- ***Lactic acid*** - production involves fermentation by microorganisms that produce lactic acid. Further separation and purification of lactic acid by crystallization of lactic acid, better and more efficiently by esterification, hydrolysis, distillation. The production, by fermentation of lactose in whey, competes significantly with the fermentation of hydrolyzed corn grain starch.
- ***Organic acids*** (e.g. propionic acid, or ferments containing propionic acid, which has antimicrobial, antifungal effects)
- ***Xanthan*** - production consists of fermentation of whey by suitable microorganisms to produce the polysaccharide xanthan - its presence significantly increases viscosity - lubricant, drilling rigs, toothpaste.
- ***Methane*** - production consists of fermentation by methanogenic microorganisms, gas purification.
- ***Hydrogen*** - production consists of fermentation of whey by sui table microorganisms (e.g. *Clostridia* strains), gas purification.
- ***Hydrogel, fertilizer*** - whey is a water substitute and mineral supplier.

The above whey processing options are selected examples. When choosing to apply these and any others, economic effects play a major role. These must include the cost of processing or disposing of all by-products and the cost of minimizing the environmental impact.

2. ACID WHEY MANAGEMENT IN THE PROJECT

2.1. Identified technological challenges

It was identified several technological challenges throughout organic carbon recovery. At first, we decided to find some wastes with rich organic carbon content in food processing industry. During thorough literature research we identified huge potentials in dairy industry despite of the fact that beverage industry showed also several promising options. Acid whey management seems to be promising business in organic carbon recovery business as it was depicted in Chapter 1. Acid whey is recently considered as a waste and this paradigm could be changed in our Deliverable. We identified several main challenges throughout acid whey business-line that were investigated in our project:

a. application of electrospun nanofibrous membranes (ENM) as a pre-treatment before nanofiltration unit (replacement of centrifuge) – removal of fats and casein
b. thickening of acid whey by nanofiltration in pilot-scale unit
c. acid whey thickening by ENM
d. application of acid whey to field to supply organic carbon into soil
e. extraction of valuable compounds from acid whey

We decided to verify all hypotheses throughout lab-scale into pilot-scale and recommendation to full-scale.

2.2. Description of technological background

We were utilizing workshop and laboratory of ASIO TECH to complete all laboratory and pilot-scale units and its control commissioning and performed there most of the technological analyses unless it was analyzed somewhere else since very specific parameters has been identified. First acid whey analyses have been analyzed by project partners IRTA and IASP, however price and conditions of transport were too expensive and thus we decided to use local laboratories. Design, installation and completion of all testing units have been performed using ISO procedures of ASIO TECH.

2.3. Lab-scale tests

Lab-scale tests served to analyze several kinds of acid whey to understand the product that will be used for all tests throughout project. Acid whey has pH around 4.6 and it is quite unstable in the room temperature. Whey decay starts after several hours and thus whey has to be cooled for 4 °C or conservated for further management. We contracted two acid whey producers for its supply for our purposes and started to cooperate mainly with MADETA Jindřichův Hradec since they have very good acid whey management, proper for our project. We performed thorough analysis of acid whey from both producers with cooperation of IRTA, IASP and local laboratories.

Table 17: Analysis of whey from Dairy plant A

Parameter/Whey	Sweet whey	Acid whey	Permeate	Thickened whey
TS (%)	7,13	6,15	0,11	18,83
VSS (%)	6,21	5,48	0,09	17,27
TP (µg/g)	183	300	0	426
N-NH_4 (mg/L)	153	231	4	406
N-NO_2 (mg/L)	nd	nd	nd	nd
N-NO_3 (mg/L)	59	60	4	90
K (mg/L)	1258	1400	224	3397
Proteins (%)	1,02	1,02	0,05	2,82
TN (mg/L)	1603	1597	81	4423
TON (mg/L)	1450	1366	77	4017
TOC (mg O_2/L)	220172	15580	259	45400
Fats (%)	0,03	0,01	0,01	0,20

Table 18: Analysis of acid whey from dairy plant MADETA, grabbed sample

Parameter	Unit	Value
TSS	%	18
TC	%	7,42
TN	%	0,23
TH	%	1,23
TS	%	0,02
TP	g/kg	1,95
K	g/kg	1,71
N-NH_4	g/kg	0,24
N-NO_2	mg/kg	< 10
N-NO_3	mg/kg	< 10
pH	.	4,24

We used acid whey for MADETA for our lab-scale and pilot-scale experiments. Acid whey was cooled for its transport and left to heat to the room temperature for lab-scale and pilot-scale experiments for its better permeability through membranes. Our agreement with MADETA enables us to perform testing at their facility and thus we do not need to care

about transport and logistics problems. We performed several tests there and several tests were performed at our pilot-plant workshop in Brno. Acid whey was then transported via IBC containers of 1 m^3 and sold for contracted price to us.

As we can see from the results of acid whey analyses then it is very rich not only on organic carbon content, but also for nutrient content (N, P and K) and thus it can have very good fertilizing potential. Several full-scale tests have been performed to demonstrate fertilizing potential of acid whey – see Chapter 3. Tests to verify proper membrane pore size for hypotheses defined in Chapter 2.1 were carried out using the lab-scale unit. The lab-scale unit serves to define proper material, pore size and working conditions for the pilot-scale. We operate with samples of membranes of several squared centimeters and thus many materials and pore sizes can be tested to choose proper membranes.

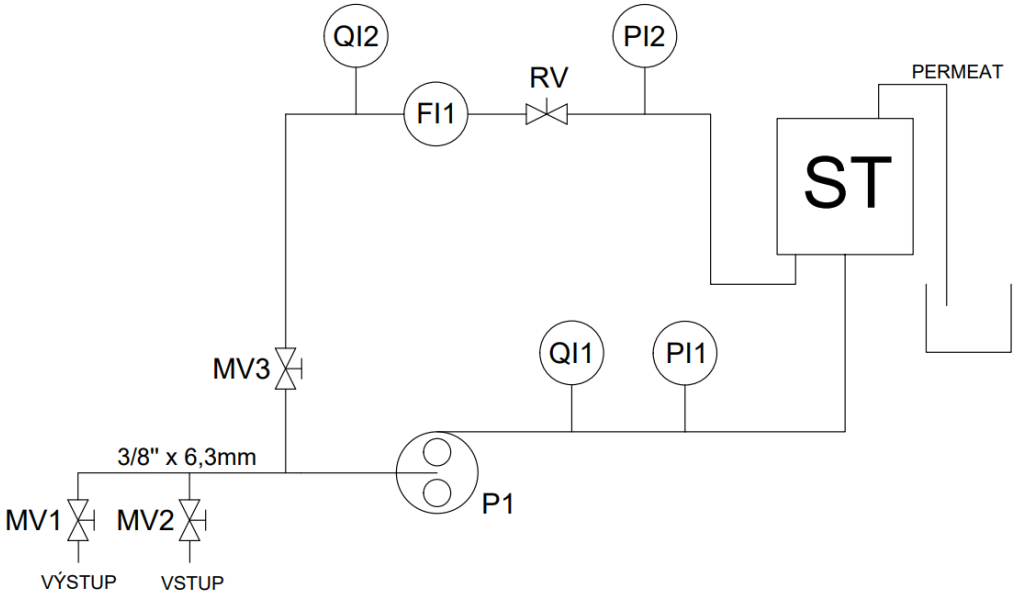


Figure 4: Scheme of lab-scale testing unit



Figure 5: Pictures of lab-scale unit

The main task was to compare a content of casein, fat and other components in raw whey from the milk production of the dairy plant MADETA after centrifugation separation process. Second task included to determine whey composition after the filtration process by two types of nanofibrous membranes to choose the most suitable nanofibrous membrane.

Table 19: Whey parameters before and after filtration and after centrifugation (3 replications, standard deviation)

	Raw acid whey before centrifuge	Acid whey after centrifuge	Acid whey after membrane (SPURTEX MFRF)	Acid whey after membrane (SPURTEX MF)
pH	4.4 ± 0.1	4.3 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Conductivity, mS.cm^{-1}	7.0 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.4 ± 0.1
Dry matter, %	6.5 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.2 ± 0.1
Total protein, g/kg	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
N, g/kg	0.2 ± 0.1	0.08 ± 0.01	0.09 ± 0.01	0.15 ± 0.01
SS, mg/l	780 ± 20	130 ± 20	440 ± 20	600 ± 20
Fats, mg/l	1,0 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1

The first membrane (Spurtex MFRF) with a pore size 250 nm showed better results than the Spurtex MF membrane. We obtained two solutions – permeate (filtrate) which can be used for the NF process and retentate (concentrate), where concentrations of main parameters were higher than in permeate and raw whey and comparable with concentrations in whey after centrifuge. This proves that nanofibrous microfiltration membranes can also be used for whey thickening.

During testing on lab-scale unit we verified three main hypotheses defined in Chapter 2.1:

1. application of ENM as a pre-treatment before nanofiltration unit (replacement of centrifuge) – removal of fats and casein
2. thickening of acid whey by nanofiltration in pilot-scale unit
3. acid whey thickening by ENM

Fat and casein are necessary to be removed from solution because of the following nanofiltration process because the membrane material is sensitive to those components at such high concentrations.

General comparison of a centrifuge process and nanofibrous filtration in the light of first hypothesis shows that nanofibrous filtration is more effective than centrifugation as supported also by nutrient analyses. Centrifuge showed lower concentration of sludge than nanofiltration from pre-treated whey.

Table 20: Comparison table for the removal of fat and casein and the nutrient content (3 replications, standard deviation)

Removal of fats and casein		
Compound	**Pre-treated whey centrifuge**	**ENM Permeate**
Fats [mg/L]	8 ± 3	2.1 ± 0.8
Non-dissolved matters [mg/L]	500 ± 50	210 ± 20
General dry matter [%]	6.0 ± 0.6	5.6 ± 0.6
Organic dry matter [%]*	87 ± 9	87 ± 9
Inorganic dry matter, [%]*	13 ± 1	13 ± 1

* - from general dry matter

Nutrients		
Nutrient	Pre-treated whey centrifuge	ENM Permeate
P [mg/kg]	720 ± 70	710 ± 60
K [mg/kg]	1740 ± 160	1700 ± 150
C [%]	2.34 ± 0.06	2.32 ± 0.05
N-NH_4 [mg/kg]	121 ± 6	120 ± 8
N-org. [mg/kg]	660 ± 40	590 ± 35

We can thus conclude that nanofibrous membranes are mainly appropriate for fat removal and partially casein removal too. The second hypothesis verified that ENM membranes could be used for thickening. The comparison of the quality parameters of the input whey with the filtrated and the concentrated whey have been done based on the balance

equations. Fats and non-dissolved matter showed promising thickening results whilst other parameters did not show satisfactory results.

Table 21: Thickening of fats and casein by means of ENV SPURTEX MFRF membrane (3 replications, standard deviation)

Compound	Feed	Retentate	Concentration, %
Fats [mg/L]	24 ± 10	35 ± 14	**45**
Non- dissolved matters [mg/L]	380 ± 40	485 ± 50	**27**
General dry matter [%]	5.5 ± 0.5	5,7 ± 0,6	**4**
Organic dry matter [%]*	87 ± 9	87 ± 9	**0**
Inorganic dry matter, [%]*	13 ± 1	13 ± 1	**0**

* - from general dry matter

Thickening of dry matter showed 12% dry matter thickening by nanofibrous membrane, compared to 14% by centrifugation and 16% by nanofiltration unit at MADETA Jindřichův Hradec. Although results with nanofibrous membrane were worse it gives some chance to achieve better results by test replications and optimizations in the future.

Nanofiltration process is widely used for acid whey thickening. According to the 3rd hypothesis it can be presumed that a dry matter concentration of 18-24% can be achieved within certain range of operating conditions. We achieved similar results in the comparison with MADETA industrial nanofiltration unit as it can be seen in Table 22. This result was achieved by centrifuge pre-treated whey as feed for pilot-plant unit.

Table 22: Thickening of acid whey by nanofiltration after centrifuge (3 replications, standard deviation)

Concentration of dry matter, non-dissolved matters			
Compound	**Feed (liqduid from centrifuge)**	**Lab-scale concentrate**	**Madeta concentrate**
Non- dissolved matters [mg/L]	500 ± 50	1170 ± 117	570 ± 60
General dry matter [%]	6.0 ± 0.6	14 ± 2	16 ± 2
Organic dry matter [%]*	87 ± 9	90 ± 9	92 ± 10
Inorganic dry matter [%]*	13 ± 1	10 ± 1	8 ± 1

* - from general dry matter

Concentration of nutrients			
Nutrient	**Feed (liquid from centrifuge)**	**Lab-scale concentrate**	**Madeta concentrate**
P [mg/kg]	720 ± 70	1650 ± 150	1530 ± 140
K [mg/kg]	1740 ± 160	1880 ± 170	1800 ± 160
C [%]	2.34 ± 0.06	5.69 ± 0.09	6.33 ± 0.06
$N\text{-}NH_4$ [mg/kg]	121 ± 6	105 ± 6	107 ± 6
N-org. [mg/kg]	660 ± 40	1650 ± 100	1645 ± 115

Once we used a filtrate from nanofibrous module as a feed whey, the efficiency was a bit lower (Table 23.)

Table 23: Thickening of acid whey by nanofiltration after ENM module (3 replications, standard deviation)

Concentration of dry matter, non-dissolved matters			
Compound	**Feed (Permeate from ENM)**	**Lab-scale concentrate**	**Madeta concentrate**
Non- dissolved matters [mg/L]	210 ± 20	340 ± 34	570 ± 60
General dry matter [%]	5.6 ± 0.6	12 ± 2	16 ± 2
Organic dry matter [%]*	87 ± 9	91 ± 10	92 ± 10

Concentration of nutrients			
Nutrient	**Feed (Permeate from ENM)**	**Lab-scale concentrate**	**Madeta concentrate**
P [mg/kg]	710 ± 60	1250 ± 110	1530 ± 140
K [mg/kg]	1700 ± 150	1520 ± 140	1800 ± 160
C [%]	2.32 ± 0.05	4.76 ± 0.09	6.33 ± 0.06

Inorganic dry matter [%]*	13 ± 1	9 ± 1	8 ± 1

* - from general dry matter

$N\text{-}NH_4$ [mg/kg]	120 ± 8	76 ± 4	107 ± 6
N-org. [mg/kg]	590 ± 35	1330 ± 80	1645 ± 115

Conductivity was comparable in every solution but the highest one was observed in case of concentrate when was used a centrifuge pre-treated whey as feed whey. Chloride content was quite lower in filtrates than in concentrates. The highest density was in the case of concentrate from MADETA and nanofiltration.

2.4. Main conclusions and recommendations to pilot scale

In general, the test results showed success, however we expected a higher concentration of dry matter through the nanofiltration process and we can conclude that all hypotheses can be tested in full-scale about possible scale-up into full operation scale. Tests for determination of the most appropriate conditions for acid whey thickening under various conditions on our own pilot scale will be described in Chapter 3.

3. PILOT SCALE EXPERIMENTS

3.1. Hypothesis and test design of the acid whey management in the pilot-plants

We identified several main challenges throughout acid whey business-line that were investigated in our project:

a. application of electrospun nanofibrous membranes (ENM) as a pre-treatment before nanofiltration unit (replacement of centrifuge) – removal of fats and casein
b. thickening of acid whey by nanofiltration in pilot-scale unit
c. acid whey thickening by ENM
d. application of acid whey to field to supply organic carbon into soil
e. extraction of valuable compounds from acid whey

3.2. Microfiltration of thickened acid whey

Pre-treatment serves to remove fats and remaining casein from acid whey. We expected that our electrospun nanofibrous membranes (ENM) could work more efficiently than centrifuge. The pilot plant with performance of ca. 1 m3/h was constructed. We conducted commissioning that included pre-treatment optimization, process parameters optimization and material development. All key performance indicators showed good match in commissioning phase. We operated the pilot-plant for one week at full-scale application to compare results from pilot-plant and real plant, including pre-treatment (centrifugation x electrospun nanofibrous membranes - ENM). We tested several nanofibrous membranes:SPURTEX MFRF with average pore size 250 nm was chosen as the best fitted membrane. This membrane is made from polyurethane and has a supporting layer from polyester non-woven textile. We conducted two comparative runs with the same acid whey for a week period with real full-scale centrifuge and with our submerged module of 6 m2. The centrifuge worked with an average flow Q = 9 m3/h of acid whey and produced ca. 50 L/h of excessive sludge.

Table 24: Comparison of fat removal by centrifugation and ENM (3 replications, standard deviation)

		Feed acid whey	Pretreated whey after centrifugation	Permeate after ENM membrane
Fats	**mg/L**	0.17 ± 0.02	0.12 ± 0.01	0.10 ± 0.01
TSS	**%**	6.2 ± 0.6	4.7 ± 0.5	6.4 ± 0.6

We can observe that permeate after electrospun nanofibrous membranes can achieve slightly better performance, but no significant. Further analyses of VSS, dry residue, pH, conductivity, TC, TN, TP, TK, $N\text{-}NH_4$, TON were performed to verify results and confirm mass balances.

We made a thorough economic analysis of our processes and compared it to existing full-scale plant. Our pre-treatment (ENM) showed comparable treatment results as centrifuge in the fat removal parameter, but much better turbidity results than centrate from centrifuge. Turbidity was analysed and it showed that mainly Ca. Mg and phosphates are main compounds responsible for that. Economic analyses magnified to 100 m³ acid whey operation demonstrated that centrifuge has 25 EUR cheaper operational costs than ENM. If we summarize all pros and cons then we can state that

ENM is promising competitive technology to centrifuge serving for acid whey stream pre-treatment before nanofiltration thickening.

We installed electrometers, chemical and demi-water consumption for comparative calculations at a full-scale unit and a pilot-scale nanofiltration unit to compare operational costs. Pilot-scale operational unit showed 200 EUR cheaper operational costs than full-scale unit for treatment of 100 m^3 of product.

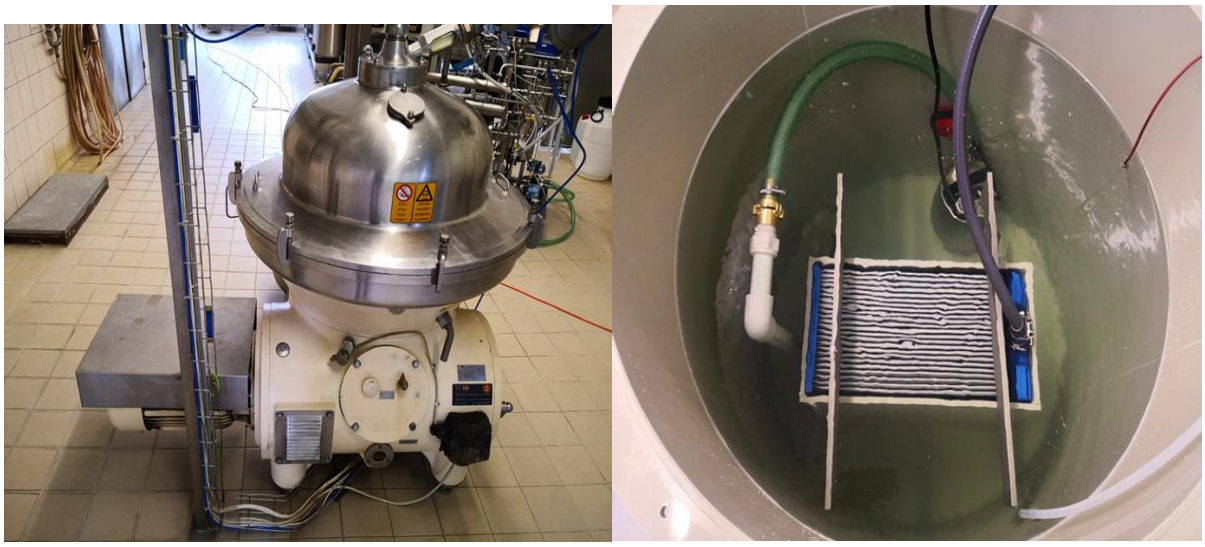


Figure 6: Pre-treatment technologies, centrifuge (left), ENM unit (right)

As it was already discussed in previous objective ENM can serve for effective fat removal from acid whey. It demonstrated slightly better fat removal (10-20%) than centrifuge and achieved better turbidity results than centrate. We made thorough analyses of both supernatants. Ca, Mg salts and phosphates were identified as main compounds responsible for this turbidity. ENM membrane had ca. 40-50% better removal of above-mentioned compounds and thus no turbidity was observed.

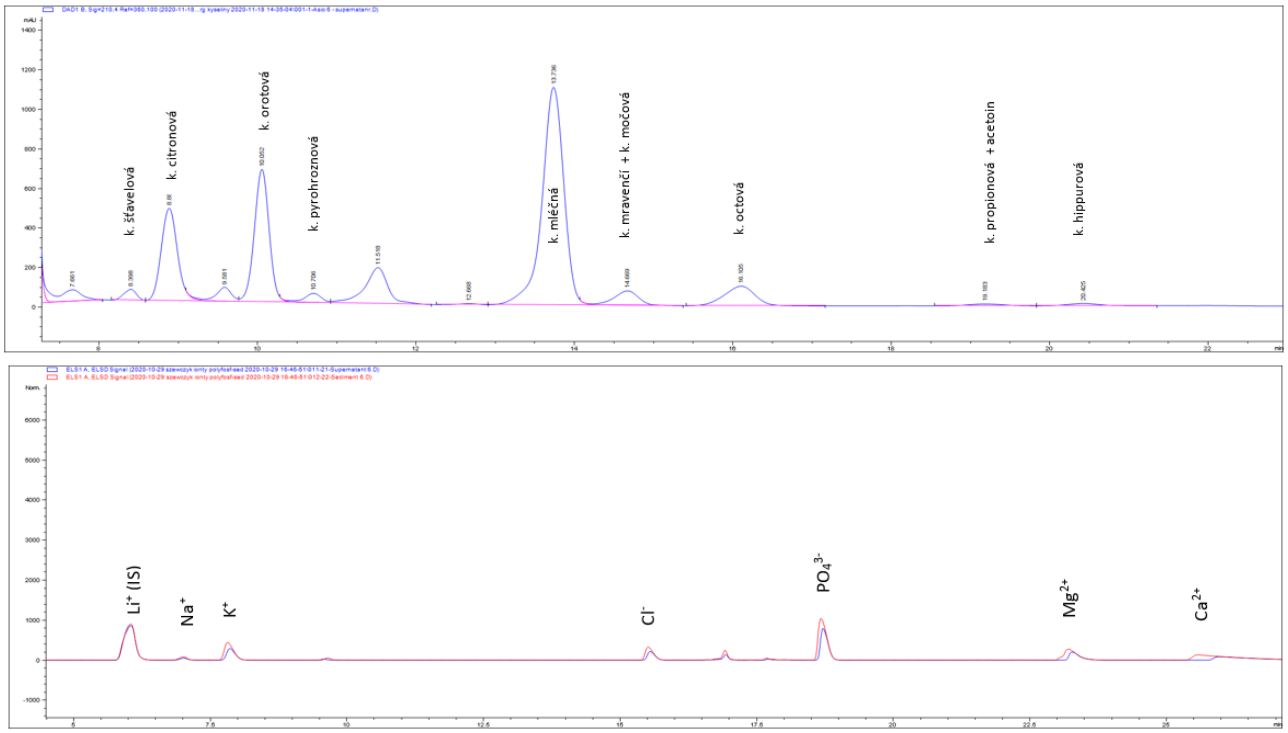


Figure 7: Chomatograms from analyses of supernatants

Our tests thus led to the following conclusions:

- Electrospun nanofibrous membrane can replace centrifugation as acid whey pre-treatment before acid whey thickening by nanofiltration (fat removal efficiency)
- It was demonstrated effective acid whey thickening up to 18% TSS by our nanofiltration pilot-plant (as it is recently usual at the full-scale plants)
- Variable conditions (different pressure and temperature) of acid whey thickening at our pilot-plant were carried out to indicate proper range for full-scale operation

Furthermore, detailed analysis of streams at full-scale plant have been conducted.

Table 25: Material balance at full-scale nanofiltration plant for acid whey thickening

	TN	TK	TP	$N\text{-}NH_4$	TSS	pH
	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	-
Inert from centrifuge	658	1726	704	115,6	6,41	4,08
Sludge from centrifuge	690	857	401	91,8	3,4	4,20
Centrate from centrifuge = inlet to nanofiltration	893	1611	694	74,8	6,09	4,10
Thickened acid whey	2225	1634	1613	119,0	17,69	4,06
Permeate from nanofiltration	204	1595	300	153	0,94	3,94
Permeate from reverse osmosis	16	90	<5	<10	0,21	4,45
Concentrate from reverse osmosis	721	5057	1054	486	3,81	

3.3. Thickening of acid whey by electrospune nanofibrous membranes

This hypothesis expected to use pre-treated acid whey as source for different types of electrospun nanofibrous membranes to verify whether we can achieve some level of acid whey thickening transferable into full-scale. Several ENM membranes have been tested by different material and porosity. Final results proving that our hypothesis was wrong were carried out with ENMs SPURTEX RFMF 250 indicating that average pore size is about 250 nm. We tried to test ENMs in the pore size range from 150 to 400 nm. 150 nm is the technical limit of ENMs production and membranes with lower price is impossible to produce.

Acid whey was circulated in 1 m^3 storage tank until transmembrane pressure reached 0.4 bar and a minimum level of concentrate in the storage tank was achieved.



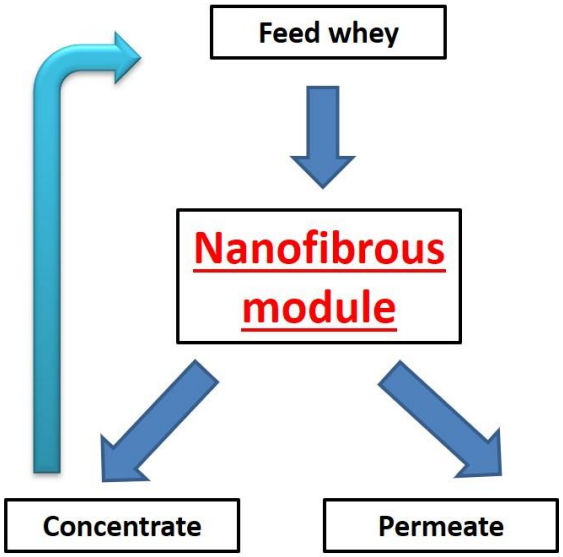


Figure 8: Scheme of pilot-plant test for acid whey thickening via ENMs

Table 26: Comparison of thickening via ENMs and at full-scale nanofiltration unit (3 replications, standard deviation)

Compound	Unit	Retentate from ENM	Retentate from full-scale unit

Non-dissolved matters	**mg/L**	530 ± 50	1100 ± 111
General dry matter	**%**	6.0 ± 0.6	16.5 ± 1.5
Organic dry matter	**% of DM**	87 ± 8	91 ± 10
Inorganic dry matter	**% of DM**	12 ± 1	9 ± 1

Comparison of our results with the data obtained from the full-scale unit revealed a difference in the dry matter content of more than two times, which indicates that ENM is ineffective for thickening whey. We have to admit that our hypothesis was wrong and any ENMs membranes are possible to use for acid whey thickening.

3.4. Thickening of acid whey by nanofiltration

Although most of the companies do not thicken acid whey from 6 to 18%, it can have some business potential to thicken whey in future. Except of environmental issues (gasoline consumption, CO_2 emissions, etc.) there can be observed an economical issue since three times more concentrated acid whey means three times lower costs from logistics point of view. Furthermore, permeate from nanofiltration can be treated via reverse osmosis and treated water can be reused and save costs at the acid whey processing plant. Our business exploration showed that there are several companies that already thicken acid whey, but the number of full-scale replications is very promising.

We built-up a pilot-plant nanofiltration unit (performance up to 3 m^3/h) that can use pre-treated acid whey and thicken it up to TSS = 18%. The pilot unit is suitable for semi-operational tests of the membrane process - nanofiltration. The unit is used to test water softening, partial demineralization and for food purposes (e.g. to concentrate milk whey) and can operate in manual or automatic mode. The NF unit consists of one NF membrane 3838 installed in a stainless-steel housing, a safety candle filter, flow, conductivity and pressure measurement. The unit operates in cross-flow mode. The original solution is split under pressure into a more concentrated flow (retentate or concentrate) and a purified flow-permeate. To ensure the required pressure and flow of the solution in the system, two pumps are used. The possibility of remote access to the unit via a router is made. All materials in contact with the medium are suitable for drinking water and food production. The pilot-plant unit was commissioned with tap water and then tested with pre-treated acid whey at different pressure (10 to 18 bars) and temperature (10 – 25 °C). The full-scale comparison against a real unit was processed for period of one week.

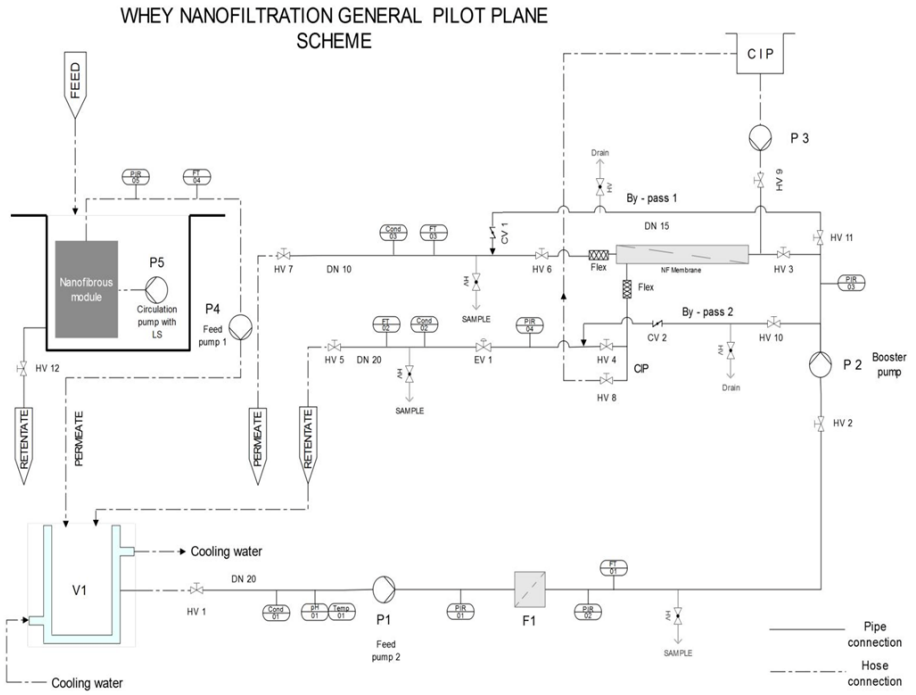


Figure 9: Scheme of nanofiltration pilot-plant

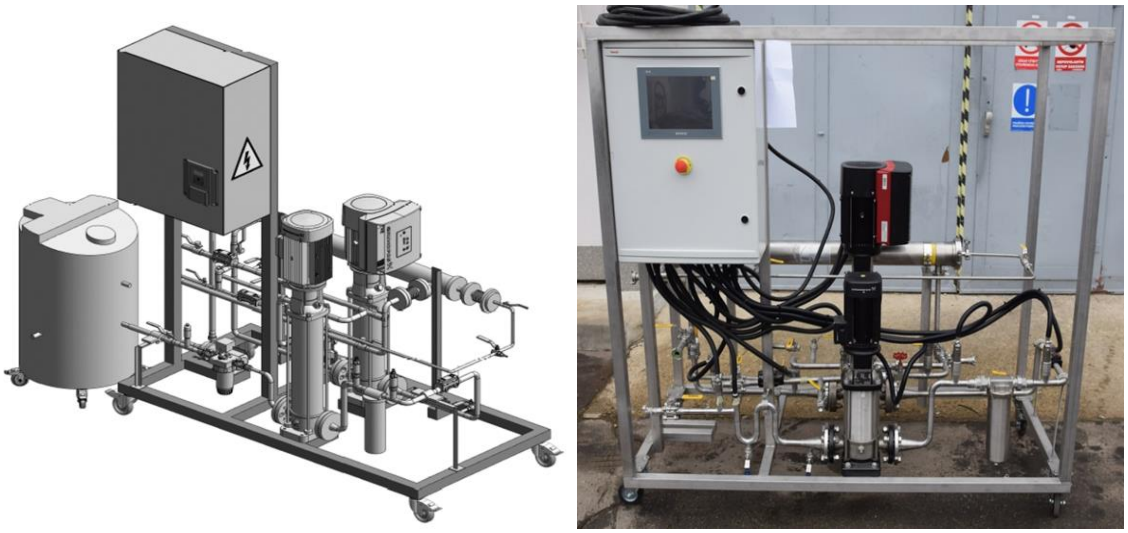


Figure 10: Pictures of nanofiltration pilot-plant

Table 27: Thickening of acid whey by nanofiltration at pilot-scale (first column with ENM pre-treatment, second column with centrifuge pre-treatment) (3 replications, standard deviation)

Compound	Unit	Concentrate from lab scale NF	Concentrate from pilot scale NF	Concentrate from full-scale NF
Non-dissolved matters	**mg/L**	800 ± 80	950 ± 95	1100 ± 111
General dry matter	**%**	17.5 ± 1.8	15.8 ± 1.5	16.5 ± 1.5
Organic dry matter	**% of DM**	92 ± 10	91 ± 10	91 ± 10
Inorganic dry matter	**% of DM**	8 ± 1	9 ± 1	9 ± 1

The difference in dry matter content was less than 15%, which indicates the effectiveness of the pilot nanofiltration unit for thickening of acid whey. Further analyses of pH, conductivity, TC, TN, TP, TK, N-NH_4, TON were performed to verify results and confirm mass balances.

3.5. Valorization of acid whey (lactoferrin extraction)

Lactoferrin is part of acid whey. It is a multifunctional protein with a molecular mass of about 80 kDa and one of the components of the immune system of the body that is widely represented in various secretory fluids, such as milk, saliva, tears. Apart from its main biological function, namely binding and transport of iron ions, lactoferrin also has antibacterial, antiviral, antiparasitic, catalytic, anti-cancer, and anti-allergic functions and properties.

We used membrane technologies for lactoferrin pre-treatment and thickening in the solution to produce a purified product applicable in the pharmaceutical and food-processing industries. Membrane technologies – microfiltration, ultrafiltration and reverse osmosis have been used in a technological train. The technological process is under optimization, but first results show very promising outputs – lactoferrin concentration up to 6% with high purity of the product.

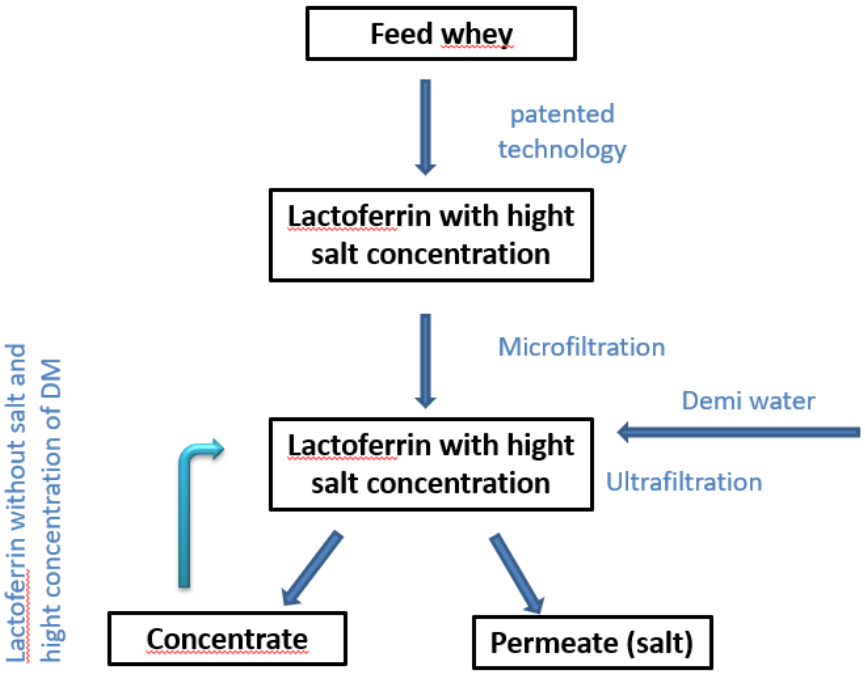


Figure 11: Technological scheme of lactoferrin extraction

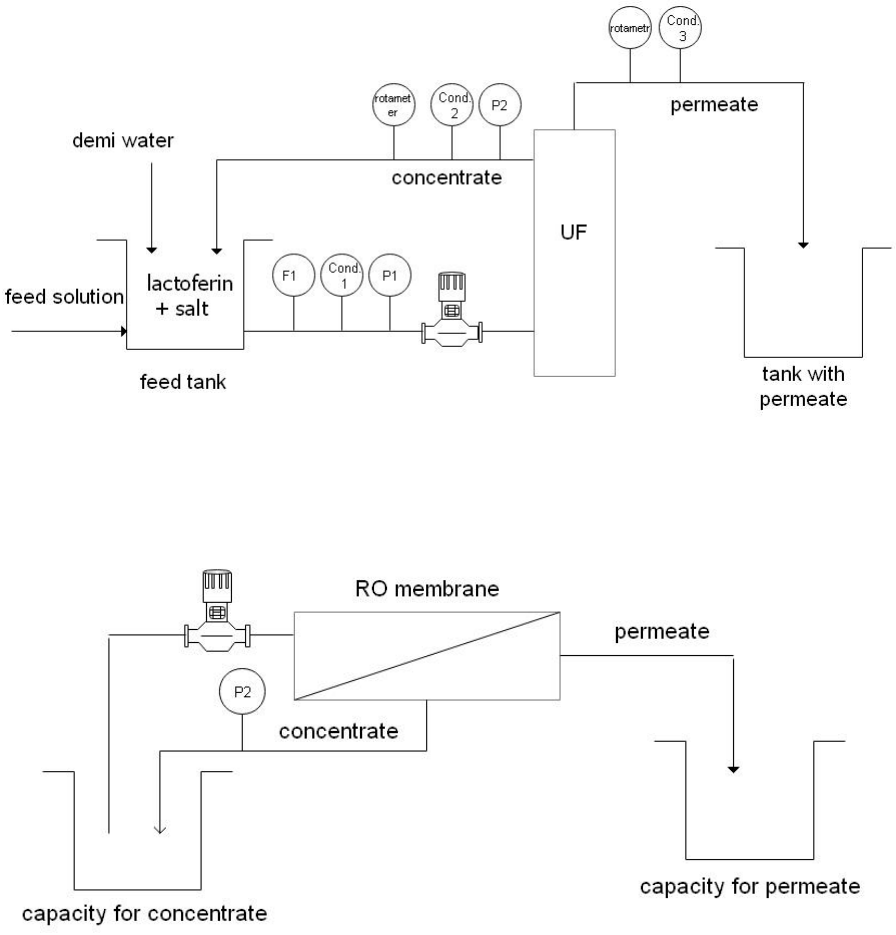


Figure 12: Technological schemes of technical solution



Figure 13: Pilot-plant for lactoferrine concentration

3.6. Valorization of acid whey as animal fodder

We expected to investigate acid whey utilization for animal fodder application throughout the Circular Agronomics Project. We visited one company in the Czech Republic to discuss possible investigations for extension of acid whey supply to this kind of industry. Several meetings with this company led to the conclusion that it is well-established process for tens of years, esp. in Belgium and also Czech Republic, where is expected that ca. 1/3 of acid whey is used as animal fodder. The whole process of animal feeding by acid whey is certified for feeding of pigs and thoroughly monitored by State Veterinary Administration that regularly checks hygiene, transport, etc. Pigs are fed by a feed mixture mixed with acid whey. Acid whey addition improves increment of pigs during fattening compared to other fodders. Acid whey is enhanced by addition of acids (lactic acid, phosphoric acid, acetic acid, formic acid, etc.) to improve the digestion of pigs and also to conserve the acid whey. Conservated acid whey can last more than three days without any quality worsening. Usual dosage of thickened acid whey (18% TSS) is ca. 2 L/day/pig. Acid whey is used as animal fodder from 25 to 120 kg of weight of pigs (ca. 90 days). The business model is different in every country – in some countries acid whey is sold as product whereas in other countries acid whey is considered as waste and companies treating it are paid to hand over acid whey. We have not found any possibility of useful research helpful for Circular Agronomics Project except of this rough market search.

3.7. Valorization of acid whey as soil conditioner

A case study at South Moravia was focused on application of acid whey for maize production at last two seasons. Several hypotheses have been verified:

- Acid whey effect on lettuce production
- Maximum possible dose of acid whey for plants

- Acid whey effect on maize production
- Ion exchange resin application for carbon limitation

Flower-pot tests with lettuce (*Lactuca sativa*) were performed during 2021 with 6 kg of soil per every flower-pot and one lettuce plant per every flower-pot. Thickened acid whey (TSS=18%) has been added into ten flower-pots up to 3 g N/pot with step 300 mg N/flower-pot at the start of the test once lettuce was ensconced. Flower-pots were protected against beetles and insects by construction from non-woven textile (Figure 14) and plants have been regularly watered if no rain occurred. Lettuce was harvested after ten weeks and weighted. We could observe linear dependency on the biomass growth with addition of acid whey as can be seen at Figure 15.



Figure 14: Tests for acid whey effect on lettuce production

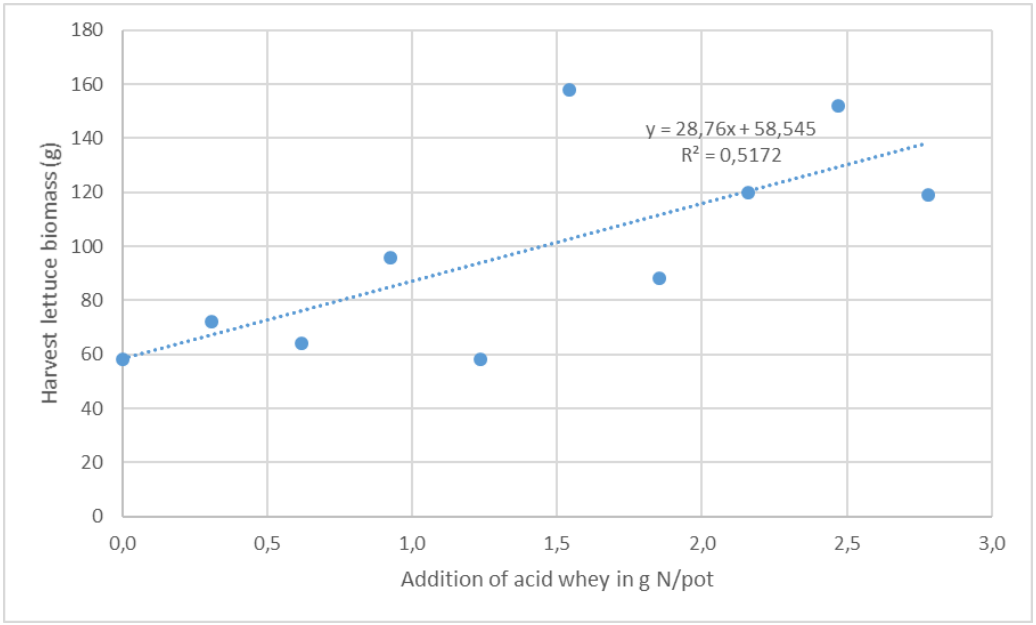


Figure 15: Dependency of acid whey addition to lettuce biomass growth

Maximum possible dose of acid whey for lettuce (*Lactuca sativa*) has been tested in 2022 for possible replication to the field. We used the same condition as in 2021, only dosage of thickened acid whey (TSS=18%) has been increased up to 6 g N/pot (step of acid whey addition was 500 mg N/flower-pot). Lettuce was harvested after ten weeks and weighted (Figure 17).



Figure 16: Tests for maximum possible dose of acid whey for plants

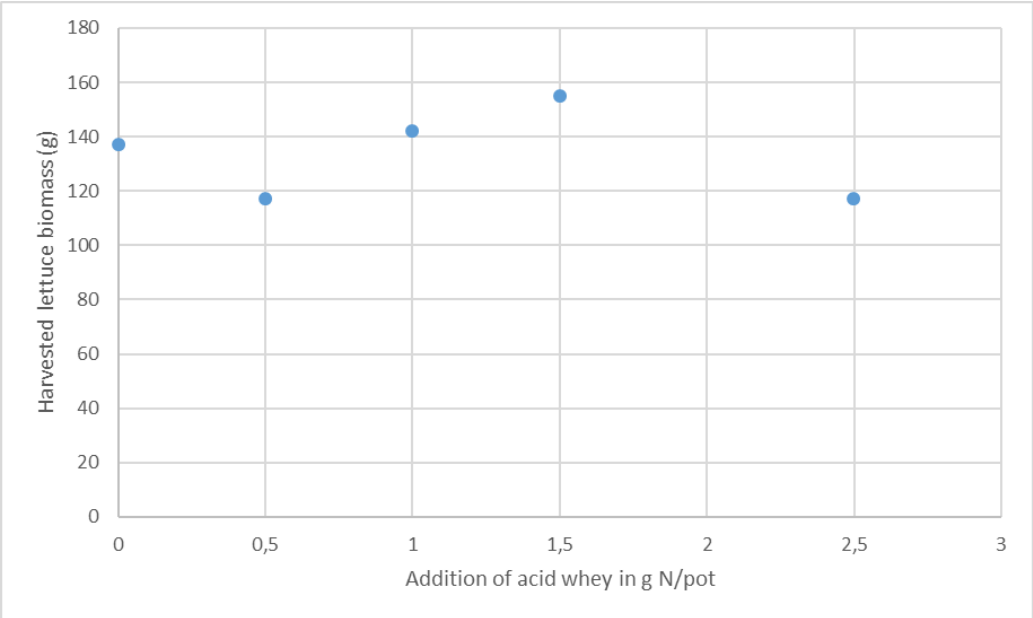


Figure 17: Determination of maximum admissible addition of acid whey addition per lettuce plant

We performed tests about the maximum admissible addition of acid whey also in period 2021 with cabbage and lettuce. Those tests aimed to find approximate levels of whey addition. Results shown that it can be ranged somewhere between 2.5 – 3 g N/flowerpot. Results from 2022 showed that first estimation were very close since all lettuce with higher

concentration than 2.5 g N/flowerpot was dead. Similar results can be seen also at Fig. 15 where all lettuce survived except of lettuce with acid whey addition 3 g N/flowerpot.

Acid whey effect on maize production has been tested in two years period. We used 20 testing plots of area 10 x 3 m in 2021 in four replications. Dosage of thickened acid whey (TSS = 18%) was as follows (0 kg N/ha, 30 kg N/ha, 70 kg N/ha, 110 kg N/ha, 150 kg N/ha, respectively). Field was prepared by local farmer and maize seeds (Type DEKALB: 31377) were manually seeded in four rows per one testing plot with distance 75 centimeters per row and 16 cm of distance in row between two seeds. Plants were treated by herbicide (Laudis) with dosage 2.25 L/ha. Acid whey was added manually (Day 46) by watering pots. Maize was harvested (Day 178) by combine harvester. Maize yield was averaged per every dose as depicted at Figure 18.

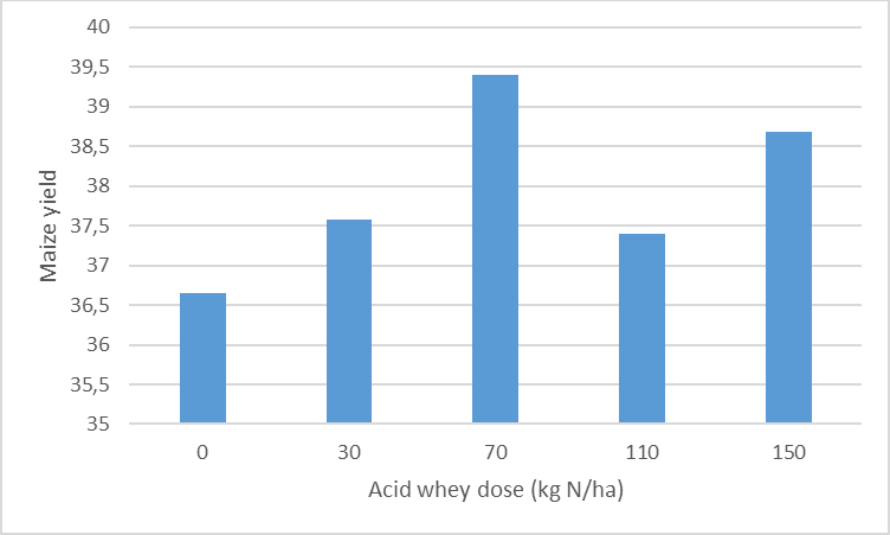


Figure 18: Maize yield dependency on the different acid whey dose – 2021

Acid whey addition effect has been studied also in 2022. We used 24 testing plots of area 10 x 3 m in 2022 in eight replications. Dosage of thickened acid whey (TSS = 18%) was as follows (0 kg N/ha, 80 kg N/ha, 160 kg N/ha, respectively). Field was prepared by local farmer and maize seeds (Type DEKALB: 31377) were seeded by combine seeder in four rows per one testing plot with distance 75 centimeters per row and 16 cm of distance in row between two seeds. Plants were treated by herbicide (Laudis) with dosage 2.25 L/ha. Acid whey was added manually (Day 52) by watering pots. We added tap water to all testing pots to keep balance of liquid added per plot for ion exchange resin tests. Maize was harvested (Day 148) by combine harvester. Maize yield was averaged per every dose as depicted at Figure 19.

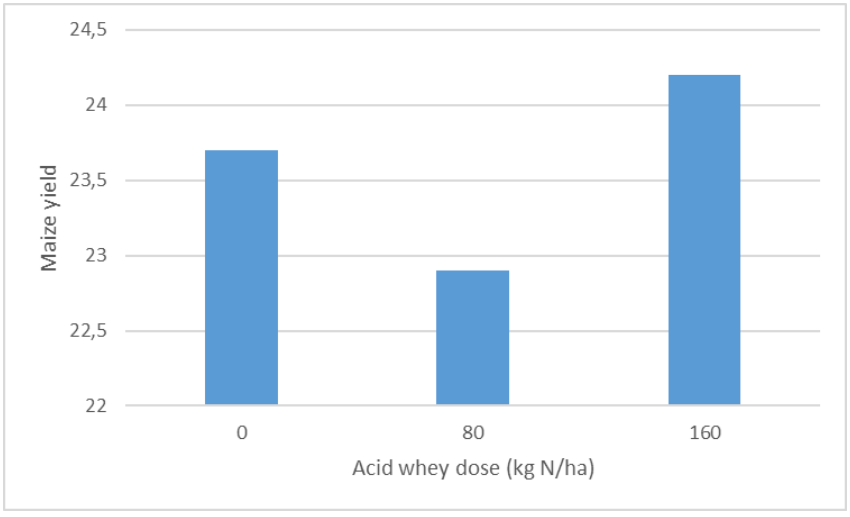


Figure 19: Maize yield dependency on the different acid whey dose – 2022

Based on current knowledge of soil nitrogen transformations and soil stoichiometry [7] it can be assumed that the application of organic and inorganic fertilizers with elevated nitrogen content to soil may create an environment where carbon is the limiting nutrient. In such an environment, microbial nitrogen use efficiency will be reduced and microbial decomposers will begin to release increasing amounts of ammoniacal nitrogen [8]. Released mineral nitrogen implies reduced input use efficiency and can pose a potential problem for soil quality [9, 10]. And water quality [11]. Therefore, one of the bases for assessing the sustainability of agricultural systems and farming techniques should be data describing the conversion, transformation and distribution of nitrogen in the soil [12]. The method of Binkley & Matson [13] modified in Novosádová *et al.* [14] will be used to determine mineral nitrogen leaching into the subsoil. The mineral nitrogen will be captured from the eluate using ion exchange grains (IER) placed in special discs made of PVC ring. This PVC ring is sealed on both sides with UHELON 130T mesh.

a. For the capture of $N\text{-}NO_3$, Purolite A-520E
b. for the capture of $N\text{-}NH_4$, Purolite C100E.

The mineral nitrogen thus captured will be determined according to the method of Peoples [15] on a Behr S3 Stream Distillation Unit. The amount of mineral nitrogen captured will be related to the soil area or per unit weight of the ion exchange unit [16]. The IER discs has been applied to the experimental plots in the soil at a depth of 25 cm on Day 35 in a total of 24 units (4 replications, 6 variants). Discs has been removed from the soil and subjected to laboratory evaluation prior to harvest (Day 146). We analyzed obtained data with software Statistica 14. Analysis of variance (ANOVA) was done with subsequent post-hoc LSD test ($p < 0,05$).

No significant differences were observed. Therefore, results of our method did not refute null hypothesis. Whey application did not significantly influence the amount of nitrogen leaking to a subsoil within any treatment.

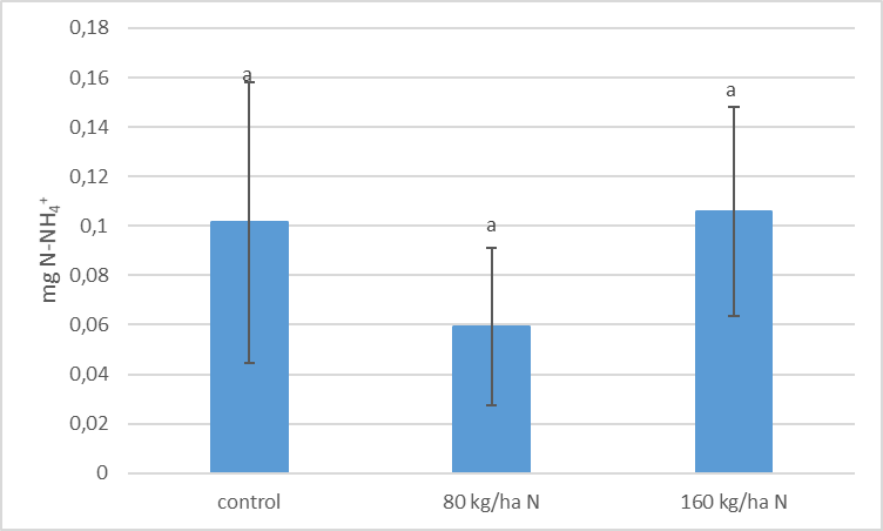


Figure 20: N-NH4+ leaked from topsoil

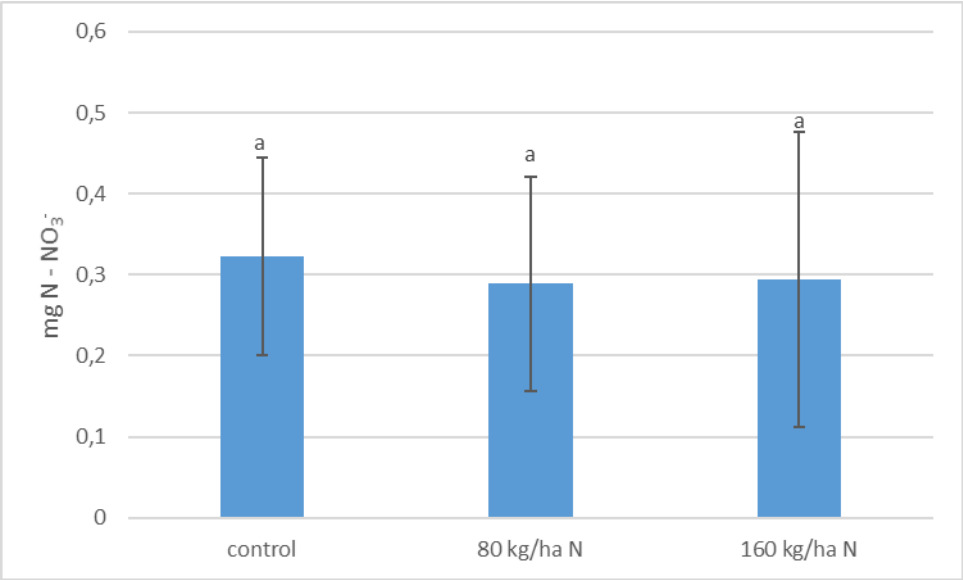


Figure 21: N-NO3- leaked from topsoil

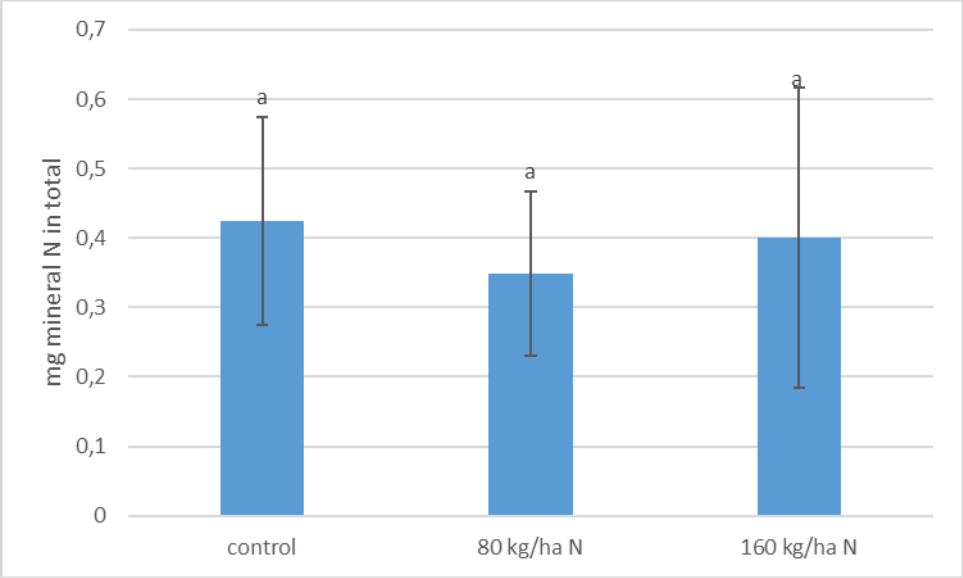


Figure 22: Mineral nitrogen leaked from a topsoil

Table 28: Comparison between nitrogen leakage from topsoil

Treatment	N-NH_4 (mg)	N-NO_3 (mg)	N mineral (mg)
Control	0.101 ± 0.057	0.323 ± 0.121	0.424 ± 0.150
80 kg/ha N	0.059 ± 0.032	0.289 ± 0.132	0.348 ± 0.119
160 kg/ha N	0.106 ± 0.042	0.294 ± 0.183	0.400 ± 0.216

Acidic whey has been in many studies highlighted to be a good alternative for conventional fertilizers [17, 18]. Proper application of this soil amendment can lead to increases in microbial biomass and microbial activity [19]. In one pot experiment has whey application increased soil carbon content and microbial biomass compared to all other treatments [18]. Based on published papers about soil stoichiometry [7] we can conclude that with increased carbon sequestration there is most likely an increase in a soil nitrogen content to occur. IT can be hypothesized that some nitrogen applied on the experimental site in form of acidic whey could be mineralized and subsequently assimilated by soil microorganisms. Such assimilated nitrogen does not represent an environmental risk.

In this field trial with acidic whey applications, we did not find any significant difference in mineral nitrogen leaking through a soil profile compared to the control. However, it is important to state that capacity of cultivated soil to bind mineral nitrogen is very dependent on local conditions.

3.8. Recommendations to full-scale

Our tests with ENM led to conclusion that it can be applicable at full-scale instead of centrifuge to remove casein, fats and turbidity form acid whey sample. Pilot-scale results showed promising results supported by economic analysis in the Chapter 4. However, it will need long-term testing to evaluate correctly life-time of membrane and mechanism of membrane clogging and fouling. One-week test cannot serve as valid supplement of full-scale application. ENM are not proper for acid whey thickening, mainly due to pore size.

Thickening of acid whey by nanofiltration is possible and it looks like the best business-line based on our project results. Most of the dairy plants have no acid whey thickening from 6% TSS to 18% TSS and thus pays undue costs for waste management with acid whey. Thickening of acid whey would decrease logistic costs and offer valuable product with further possible valorization scenarios.

Three conceptual studies of acid whey valorization have been tested. Animal fodder application is widely used throughout whole world and thus we did not reinvent wheel there. Lactoferrin business line is based on several technological steps to receive pure product for pharmacy. Several years of optimization is necessary until this process is completely finished. Acid whey application as soil conditioner is widely used in the Eastern Europe and offers interesting alternative of soil enhancement by organic carbon and nutrients. We demonstrated direct impact on plant growth with maximum admissible dosage determination. Acid whey implementation has however several limitations – it can be used only seasonally and due to higher content of dissolved salts in the medium cannot be applicable every year.

4. FINAL DESIGN OF EXPERIMENTAL UNITS

4.1. Comparison with real full-scale plant (one-week simultaneous operation)

We performed one-week comparative study of full-scale application and our pilot-plant applications in MADETA Jindřichův Hradec. Detailed data about technological parameters and efficiencies can be found in Chapter 3 and 4.2.

4.2. Conceptual study

The evaluated site is MADETA Jindřichův Hradec – Czech biggest milk-processing industry company located at South Bohemia. The whole plant consists from several parts that process milk. We evaluated the part of the plant that is connected with drying processes and we balanced the acid whey management there. The schematic process flow of the acid whey management is depicted in Figure 23.

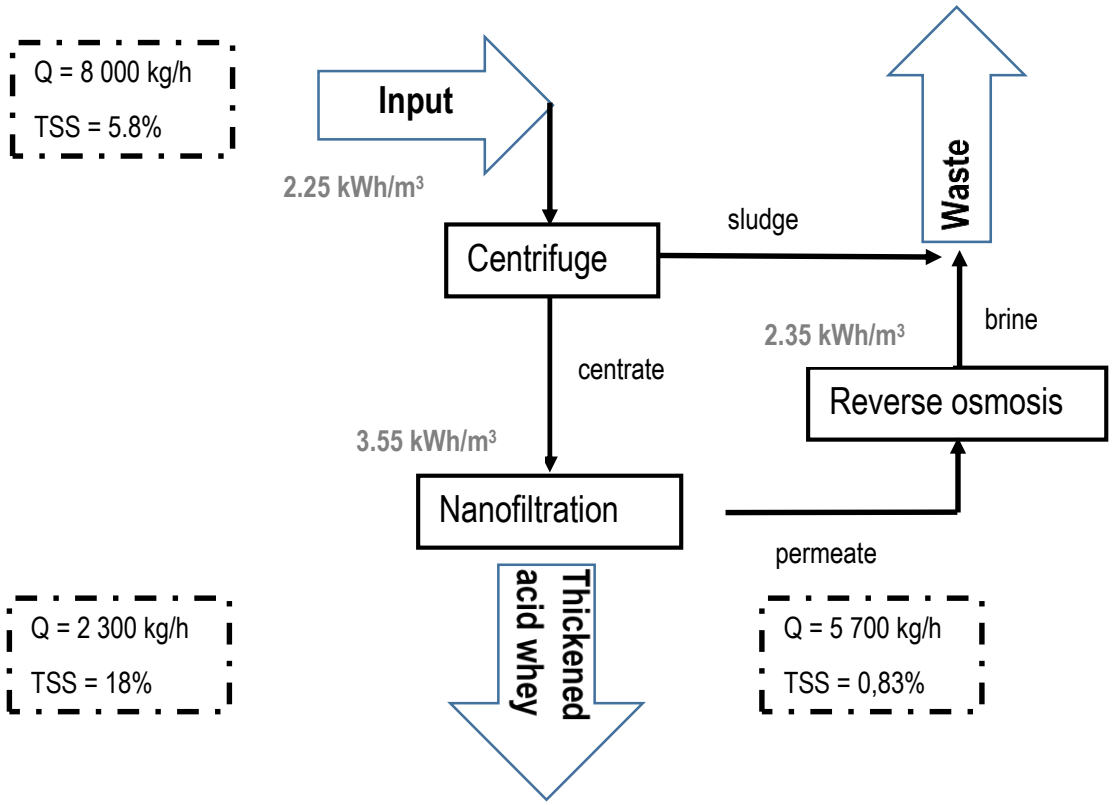


Figure 23: Site overview with relevant parameters

Fresh acid whey of pH = 4.4 – 4.6 is processed through pre-treatment to remove remaining proteins and other sediments to decrease the sediment volume under 0.02% via centrifugation. Another target of the pre-treatment is the removal of fats below 0.06% to pervent membrane fouling. Acid whey is pasteurized for 15 s at 52-54 °C to kill all bacteria present in the acid whey (eventually H_2O_2 (20 ppm) or NaHS (50 ppm) is added). Acid whey is cooled in the storage tank to 27 – 30°C before it is pumped into the nanofiltration unit. Average capacity of the nanofiltration unit is 8 000 kg/h. TSS concentration of acid whey is around 5.8%. Nanofiltration produces ca. 5700 kg/h of permeate (TSS = 0.83 %) and 2300 kg/h of retentate (TSS = 18%).

Thickened acid whey (TSS = 18%) is further processed outside of the company. It serves for pig feedstock nearby or it is sold and transported abroad. The permeate is pumped into the reverse osmosis unit. The reverse osmosis unit produces water for membrane CIP and retentate is mixed with excessive sludge from the centrifuge and discharged as waste.

Technical parameters for all hyptheses have been discussed in Chapter 3 thoroughly. Thus we focus here only for cost aspects for possible scale-up.

Cost estimations include either direct data measured at full-scale plant (8000 kg/h of acid whey) or data measured at our pilot-plants with electrospun nanofibrous membranes or at our nanofiltration pilot-plant (up to 3 m^3/h). The text contains detailed explanation how costs were estimated. Since price of the energy is being rapidly increased then we will not use EUR unit for calculations, but only kWh or volume units (L, kg, m^3) will be used.

<u>Comparative study of pre-treatment: Electrospun nanofibrous membranes vs. centrifuge</u>

We compared the treatment of 100 m^3 raw acid whey treated by centrifugation and via ENM pilot-plant. Centrifugation is well-known robust and reliable technology in food processing industry with fully automatic systems and automatic rinse of water. Its disadvantage is the higher energy consumption. Our microfiltration ENM pilot-plant unit is automated as well and is low in energy consumption. However, it is necessary to conduct CIP (cleaning-in-place) frequently. Both units were equipped by electrometer for period of one week to compare electricity consumption and by flowmeter to perform mass balance analyses.

Centrifuge worked with an average electricity consumption of 2.25 kWh/m^3 and average water consumption for rinsing = 55 L/m^3. The ENM unit worked with an average electricity consumption of 0.6 kWh/m^3 and average water consumption for rinsing = 140 L/m^3. Furthermore, the ENM pilot-plant unit needed chemicals for cleaning – 26 g of citric acid/m^3 and 52 g/m^3 of alkaline product.

It is very difficult to estimate investment costs recently since prices of whatever technological unit are valid for few weeks only and drastically increased. We can roughly guess that the complete supply of a centrifuge with fully automatic operation and rinsing, control panel, piping and other basic accessories for a capacity of 10 m^3/h could cost around 300 000 EUR. A fully equipped and automated unit of electrospun nanofibrous membranes for the same volume (10 m^3/h) of acid whey could cost around 250 000 EUR. All prices depend on the level of automation, control and installed instrumentation. Those rough guesses were estimated for basic automation, control and instrumentation.

Application of nanofibrous membranes for acid whey thickening

Long-term tests proved that electrospun nanofibrous membranes cannot be used in any modification for effective acid whey thickening. This technology does not have the desired efficiency for practical application and thus we do not recommend to scale-up this technology into full-scale. Analysis of CAPEX and OPEX is thus irrelevant.

Application of commercial nanofiltration membranes for acid whey thickening to TSS=18%

We compared treatment of 100 m^3 pre-treated acid whey treated by the commercial nanofiltration unit and via our nanofiltration pilot-plant. Nanofiltration is the key technology in food processing industry for acid whey thickening. The full-scale unit is fully-automated including CIP. It has the benefit that it can be used for permeate from reverse osmosis for cleaning to save the costs. Our pilot-plant unit was operated in semi-automated operation including also CIP. The pilot-plant unit had very basic instrumentation only. Both units were equipped by electrometer for the period of one week to compare electricity consumption and by flowmeter to perform mass balance analyses.

Full-scale unit worked with average inlet 9100 L/h, producing 6500 L/h of permeate (TSS = 0.94%) and 2600 L/h of concentrate (TSS = 17.7%). Average electricity consumption was 3.55 kWh/m^3 and water consumption 50 L/m^3. Consumption of chemicals include neutral enzyme-based product 160 g/m^3, 300 g/m^3 of alkaline product 1, 160 g/m^3 of alkaline product 2 and 150 g/m^3 of acidic product. Reverse osmosis (for mass balance calculations) with average inlet 6500 L/h produced 3600 L/h of permeate (TSS = 0.21%) and 2900 L/h of brine (TSS = 3.81%) with addition of citric acid and potassium hydroxide in the cleaning protocol. Average energy consumption was 2.35 kWh/m^3. The investigated week did not include application of CEB (chemically enhanced backwash) that is carried out several times per year too. Our pilot-plant unit had an average energy consumption 0.49 kWh/m^3 and a water consumption of 300 L/m^3. The design of the test included the same recovery and chemical consumption for both technologies. However, we have to admit that it is incorrect to make some conclusions based on the comparison of both units from energy consumption point of view. The full-scale unit needs to have a fully-automated system with analyses of all risks and many necessary accessories to enable trouble-free process whereas our pilot-plant unit included only very basic instrumentation necessary for technology verification.

The estimation of investment costs for ca. 10 000 L/h unit equipped by full automation, control and necessary instrumentation with reverse osmosis unit and reverse osmosis permeate reuse could be in the range of 4 000 000 EUR for both full-scale applications. Again, the market is very unstable and this rough guess can be changed in several weeks significantly.

4.3. Replication potential throughout Czech Republic and EU

The deliverable opens several novel or innovative business lines that can be used by industry partners. The overall worldwide production of whey is estimated as 225 000 000 t in 2020 (99 000 000 t in EU) and thus we can observe a huge business potential there to recent valorisation scenarios. We focused on the market in the Czech Republic and could research that there are 18 huge companies producing acid whey that is only rarely thickened to 18% and thus we can find interesting business there. Valorisation scenarios with thickened acid whey show also better economics for whatever further use. There is huge replication potential and possibly replicated also to other European Union countries.

We found interesting data about field application of acid whey. It can open novel business for organic carbon recovery and soil improvement. The limitating factor will be the distance between the dairy company and field since logistic costs seem to be main decisive factor. It can create locally viable seasonal cooperation's between farmers and acid whey producers, but it is limited to soil conditioner needs during the year.

Other valorisation scenarios proved already working business-lines (pig fattening) or necessary long time till trouble-free full-scale operations (lactoferrin extraction and separation).

5. CONCLUSIONS

Our tests with ENM led to the conclusion that it can be applicable at full-scale instead of centrifuge to remove casein, fats and turbidity form acid whey samples. Pilot-scale results showed promising results supported by economic analysis in the Chapter 4. However, it will need long-term testing to evaluate correctly life-time of membrane and mechanism of membrane clogging and fouling. One-week test cannot serve as valid supplement of full-scale application.

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