



## Ponovna uporaba odpadne kisle sirotke za ekstrakcijo bioaktivnih beljakovin z visoko dodano vrednostjo

### Reuse of waste acid whey for extraction of high added value bioactive proteins

Od stranskega produkta, ki se obravnava kot odpadke, do novih proizvodnih linij.  
From a by-product treated as waste to new product lines.



Upravičenec koordinator  
*Coordinating Beneficiary*  
ARHEL PROJEKTIRANJE  
IN INŽENIRING D.O.O.



Pridruženi upravičenec  
*Associated beneficiary*  
INŠTITUT ZA MLEKARSTVO IN PROBIOTIKE,  
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## Cilji projekta

Namen projekta je bil pokazati obetavne možnosti nadaljnje predelave kisle sirotke in njene ponovne uporabe, s čimer sirotko spremenimo iz potencialnega odpadka v nove produkte.

Cilj je bil dokazati, da lahko ostanek mlečnokislinske fermentacije mleka predstavlja priložnost za ustvarjanje novih prihodkov v mlekarnah, proizvajalcem mlečnih in sirotkinih sestavin ter obratom za biopredelavo.

Ključni poudarek projekta je bila optimizacija ekstrakcije laktoferina in prikaz različnih drugih možnih načinov kaskadne uporabe kisle sirotke kot živila ali vira hranil pred njeno končno predelavo v bioplinarni v bioplin.

Laktoferin je sirotkin protein s številnimi bioaktivnimi lastnostmi in visoko tržno vrednostjo.

## Project aims

The project aimed to show promising options for further acid whey processing and reuse by turning it from potential waste into new products.

The goal was to demonstrate that residue of lactic acid fermentation of the milk can represent an opportunity for new revenue generation in dairies, milk and whey ingredients producers, and bioprocessing plants.

The key project focus was the optimization of lactoferrin extraction and a demonstration of various other possible ways of cascade use of acid whey as a food or nutrient source before its final processing at the biogas plant into biogas.

Lactoferrin is a whey protein with many bioactive properties and high market value.



## Glavni rezultati

Vzpostavili smo nov demonstracijski sistem za dnevno predelavo 10.000 L sirotke. Delovno okolje v industrijskem merilu je omogočilo prvo obsežno in dolgoročno testiranje monolitnih kromatografskih kolon CIM® (Convective Interaction Media) za ekstrakcijo laktoferina. Izvedene aktivnosti so nam pomagale približati tehnologijo trgu.

Poleg selektivne izolacije več sirotkinih beljakovin smo dokazali možnost uporabe kisle sirotke kot gojišča za rast koristnih mlečnokislinskih bakterij in kvasovk ali kot surovine pri proizvodnji živil in kozmetike.

Vložili smo več patentnih prijav za pospešitev komercializacije.

## Main results

We established a new demonstration system for the daily processing of 10,000 L of whey. The industrial-scale environment enabled the first large-scale and long-term testing of CIM® (Convective Interaction Media) monolithic chromatographic columns for lactoferrin extraction. The activities helped bring the technology closer to the market.

In addition to the selective isolation of several whey proteins, we demonstrated the possibility of using acid whey as a culture medium for the growth of beneficial lactic acid bacteria and yeasts or as a raw material in the manufacture of food and cosmetics.

Several patent applications were filed to accelerate the commercialization.

Trajanje projekta / Project duration:

3. Julij 2017 – 31. Marec 2022 / 3rd July 2017 – 31st March 2022

Upravičeni stroški projekta / Eligible project costs: 4,439,001 €

Prispevek EU / EU contribution: 59,76 % končnih upravičenih stroškov projekta / of final project eligible costs



Evropska Komisija Finančni inštrument LIFE  
European Commission Financial Instrument LIFE

LIFE16 ENV/SI/000335 LIFE for Acid Whey

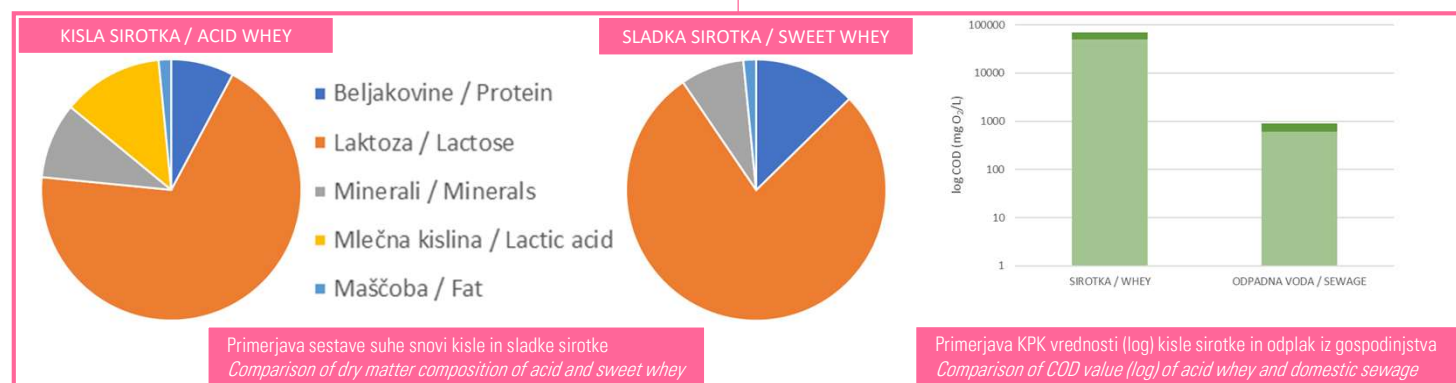


## Ozadje projekta in obstoječe stanje

Stranski proizvod mlečne industrije, kislá sirotka (KS), je ostanek proizvodnje mlečnih izdelkov, kot so sveži sir, skuta in grški jogurt. Kislá sirotka se po kemijski sestavi razlikuje od sladke sirotke - SS (ostanek proizvodnje sira) in ima nižji pH, vsebuje manjšo količino beljakovin in laktoze ter večjo količino kalcija, fosforja in mlečne kisline. Vse to otežuje nadaljnjo predelavo (različne tehnike ločevanja, sušenje) ali krmljenje živali. Kislá sirotka zato pogosto predstavlja okoljski problem ali strošek za mlekarne pri zagotavljanju njene obdelave. Poleg tega sirotka predstavlja 90 % mleka, ki se uporablja pri proizvodnji sirov s KPK (Kemijska Potreba po Kisiku) 50.000 – 70.000 mg O<sub>2</sub>/L, kar je približno 80-krat več kot običajne gospodinske odplake (600 - 900 mg O<sub>2</sub>/L).

## Project background and State of the Art

The by-product of the dairy industry, acid whey (AW), is a residual of the production of dairy products like fresh curd cheese, cottage cheese, quark, and Greek yoghurt. From the chemical composition point of view, acid whey differs from sweet whey - SW (residual after the cheese production) and has a lower pH, contains a lower quantity of proteins and lactose, and a higher quantity of calcium, phosphorous and lactic acid. All this makes it more difficult for further processing (different separation techniques, drying) or animal feeding. Acid whey, therefore, often represents an environmental problem or a cost for dairies in ensuring its treatment. Furthermore, the whey represents 90% of the milk used in the production of cheeses with a COD (Chemical Oxygen Demand) of 50,000 – 70,000 mg O<sub>2</sub>/L, which is about 80 times higher than usual domestic sewage (600 – 900 mg O<sub>2</sub>/L).



Mleko vsebuje veliko vrst beljakovin in peptidov, ki so pomembni za zdravje ljudi. Medtem ko se kazeinski delež mlečnih beljakovin ohrani v siru, sirotka zadrži do 20 % preostalih beljakovin. Poleg tega so sirotkine beljakovine bogate z aminokislinami, kot so levcin, valin, izolevcin in cistein, ki so bistvenega pomena za pravilno presnovo, živčni sistem in homeostazo. Glavne beljakovine sirotke so  $\beta$ -laktoglobulin, glikomakropeptid,  $\alpha$ -laktalbumin, imunoglobulini, goveji serumski albumin, laktoferin, laktoperoksidaza in peptoni (vodotopni polipeptidi in aminokislinae). Laktoferin je pritegnil veliko pozornosti zaradi vsestranskih, blagodejnih učinkov na zdravje.



Milk contains many types of proteins and peptides that are of interest to human health. While the casein portion of milk proteins is retained in cheese, the whey keeps up to 20% of the remaining proteins. In addition, whey proteins are rich in amino acids such as leucine, valine, isoleucine, and cysteine, which are essential for proper metabolism, the nervous system, and homeostasis. The main whey proteins are  $\beta$ -lactoglobulin, glycomacropeptide,  $\alpha$ -lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase, and peptones (water-soluble polypeptides and amino acids). Lactoferrin attracted much attention due to its versatile, beneficial health effects.



Različne kromatografske elucije s posameznimi proteini. / Various chromatographic elutions containing specific proteins

Čeprav je na trgu več tehnik ločevanja beljakovin, kot sta membranska filtracija in delčna ionsko izmenjevalna kromatografija, imajo določene omejitve. V primeru, ko je na primer njihova procesna zmogljivost visoka (membranski procesi), to običajno rezultira v nizki čistosti produkta, medtem ko visoka učinkovitost čiščenja (selektivnost) običajno zahteva dolge procesne čase (ionski izmenjevalci).

While there are several protein separation techniques on the market, such as membrane separations or particulate ion-exchange chromatography, they have certain limitations. For example, if their processing capacity is high (membrane processes), this usually results in low purification efficiency, while high purification efficiency usually demands long processing times (particulate ion exchangers).

Monolitne kromatografske kolone CIM® (Convective Interaction Media) predstavljajo najsodobnejšo tehnologijo za čiščenje in separacijo velikih biomolekul. V projektu smo tehnologijo prvič uporabili za izolacijo posameznih beljakovin iz stranskih produktov živilskopredelovalne industrije.



CIM® (Convective Interaction Media) monolithic chromatographic columns present the State of the Art technology for purifying large biomolecules. In the project, the technology was used for the first time to isolate individual proteins from the by-products of the food processing industry.

## Postopek obdelave kisle sirotke

Prvotni načrt projekta je predvideval izolacijo treh beljakovin kisle sirotke (laktoperferin, laktoperoksidaza in Ig-G) ter transport preostanka sirotke v bioplinarno za proizvodnjo bioplina. Pilotni testi nadaljnjih biotehnoloških pretvorb deproteinizirane kisle sirotke pa so pokazali odlične rezultate. Zato smo nadgradili kaskadno pot predelave sirotke demonstracijskega sistema.

Po odstranitvi laktoferina iz kisle sirotke smo prikazali več zaporednih korakov izrabe sirotke pred oddajo ostankov v anaerobno predelavo v bioplin. Prikazali smo nadaljnjo separacijo preostalih beljakovin sirotke (laktoperoksidaza,  $\beta$ -laktoglobulin,  $\alpha$ -laktalbumin, Ig-G, osteopontin) in kalcijevega laktata ter uporabo preostale sirotke za gojenje različnih mlečnokislinskih bakterij in kvasovk. Iz pridobljenih izolatov smo ustvarili prehranska dopolnila, kozmetične izdelke in izdelek uporaben v predelavi organskih odpadkov.

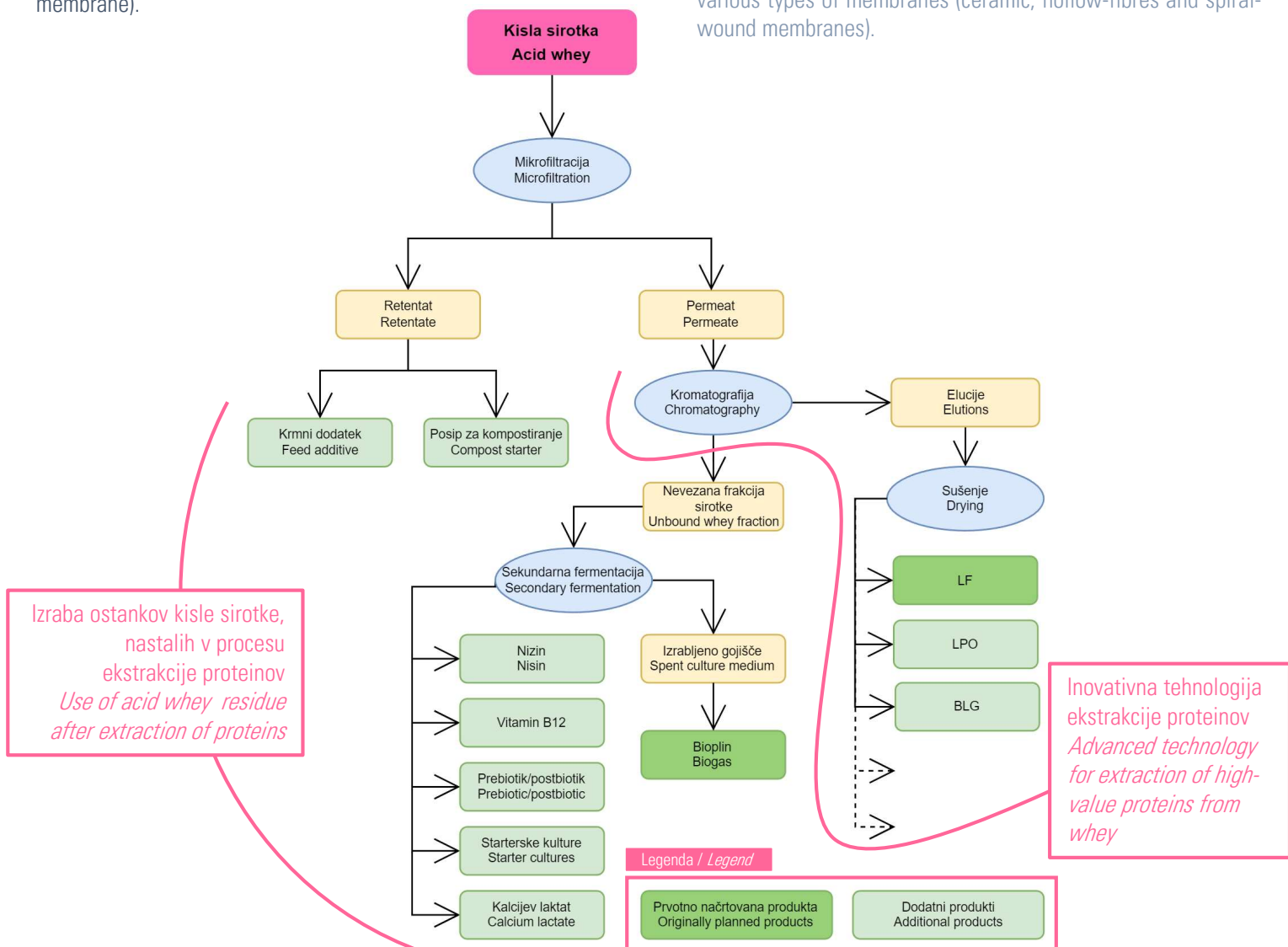
Poleg celotne linije za predelavo laktoferina je bila tako dodana še različna oprema za polni izkoristek sirotkega potenciala. Demonstracijskemu obratu smo dodali procesne enote pripravljalnih in zaključnih postopkov obdelave produktov, kot je preprosta bioreaktorska posoda, centrifuga, industrijski zamrzovalni sušilnik in filtrirni sistemi za različne vrste membran (keramične, votla vlakna in spiralno navite membrane).

## Acid whey treatment process

The original project plan envisaged the isolation of three whey proteins from acid whey (lactoferrin, lactoperoxidase, and Ig-G) and the transportation of this whey to a biogas plant for the production of biogas. However, pilot tests on further biotechnological conversions of deproteinized acid whey have shown excellent results. That's why we upgraded the whey processing cascade of the demonstration system.

After the removal of lactoferrin from acid whey, we demonstrated several sequential steps of whey utilization before taking the residues to anaerobic processing to biogas. We demonstrated the further separation of the remaining whey proteins (lactoperoxidase,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, Ig-G, osteopontin) and calcium lactate and the use of the remaining whey for the cultivation of various lactic acid bacteria and yeasts. From the obtained isolates, we created nutritional supplements, cosmetic products and products for use in organic waste processing.

In addition to the entire lactoferrin processing line, various other equipment was set up to achieve full use of whey. Upstream and downstream bioprocessing units were added to the demonstration plant, such as a simple bioreactor vessel, centrifuge, industrial freeze-dryer, and filtration systems for various types of membranes (ceramic, hollow-fibres and spiral-wound membranes).



Shema predelave kisle sirotke v uporabne produkte. Predstavljeni so prvotno načrtovani produkti v sklopu projekta in naknadno predstavljeni produkti.  
*Scheme of processing acid whey into useful products. The originally planned products within the project and additionally presented products are included.*

## Optimizacija celotnega postopka izolacije laktoferina je bila pomemben cilj projektnih aktivnosti

Optimizacijo smo izvajali v vseh korakih procesa:

- Opredelitev metode ekstrakcije laktoferina in laktoperoksidaze (patentna prijava EP18204674).
- Optimizacija korakov priprave sirotke za povečanje kapacitete kromatografske kolone za vezavo laktoferina in njene zaščite za ohranjanje dolgoročnega delovanja (patentna prijava P-202100196).
- Optimizacija uporabe pufernih raztopin z vidika optimalne izrabe surovin in preprečevanja nastajanja odpadkov (Patentna prijava P-202100230).
- Optimizacija in standardizacija zgoščevanja elucije beljakovin in razsoljevanja za doseganje zelo čistih izolatov.
- Optimizacija postopkov sušenja beljakovinskih izolatov za doseganje zahtevanih granulacij, zmanjšanje izgub in stroškov sušenja.
- Optimizacija spremljajoče logistike in postopkov, kot so čiščenje, spremljanje procesov in nadzorna analitika.
- Povečanje kapacitete procesa z nadgradnjo sistema iz osemilitrske na štiridesilitrsko monolitno kromatografsko kolono.

## Optimization of the entire lactoferrin isolation process was an important goal of the project activities

The optimization was carried out in all steps of the process:

- Definition of lactoferrin and lactoperoxidase extraction method (Patent application EP18204674).
- Optimisation of the whey pre-filtration steps to increase the chromatographic column capacity for lactoferrin binding and its protection to maintain long-term operation (Patent application P-202100196).
- Optimisation of the buffer solutions application from the point of view of optimal use of raw materials and prevention of waste generation (Patent application P-202100230).
- Optimisation and standardization of the protein elution concentration and desalination to achieve highly pure isolates.
- Optimisation of protein isolates drying processes to achieve required granulations, and reduce powder losses and drying costs.
- Optimisation of the accompanying logistics and procedures such as cleaning, process monitoring, and control analytics.
- Increasing the capacity of the process by upgrading the system from an eight-liter to a forty-liter monolithic chromatographic column.

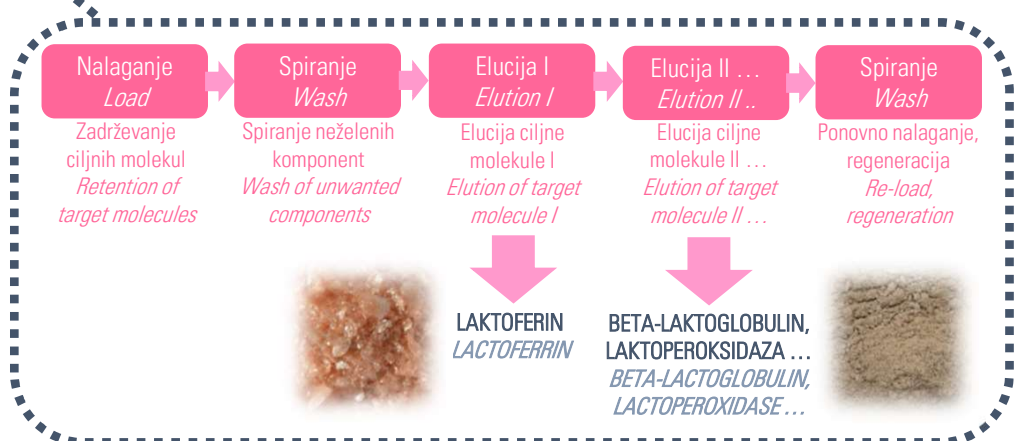


### SEKVENČNA SELEKTIVNA EKSTRAKCIJA PROTEINOV

Uporaba monolitnih kromatografskih kolon. Zadrževanje in sproščanje proteinov s kontroliranjem slanosti in pH mobilne faze - pufernih raztopin.

### SEQUENCED SELECTIVE PROTEIN EXTRACTION

Application of monolithic chromatographic columns. Proteins are captured or released by controlling salinity and pH of the mobile phase – buffer solutions.

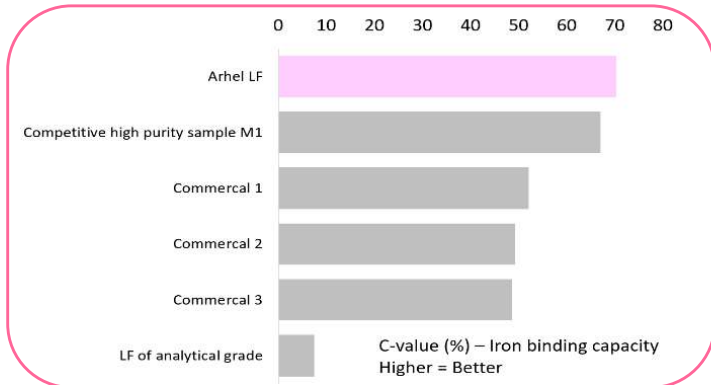


**Najsodobnejša tehnologija frakcioniranja proteinov - CIM® (Convective Interaction Media) monolitne kromatografske kolone**, za katero je značilna visoka zmogljivost obdelave in visoka ločljivost, ki omogoča obdelavo velike količine sirotke v kratkih kontaktnih časih s kromatografskim nosilcem in ustvarja izjemno visoko čistost in aktivnost izoliranih beljakovin.

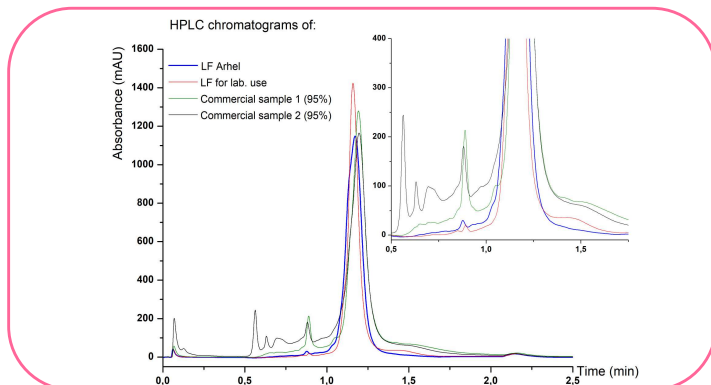
**State-of-the-art protein fractionation technology - CIM® (Convective Interaction Media) monolithic chromatographic columns**, characterised by high-performance capacity for processing a large quantity of whey in short contact times and high resolution, which ensures exceptionally high purity and activity of isolates.

## Visoka kakovost laktoferina

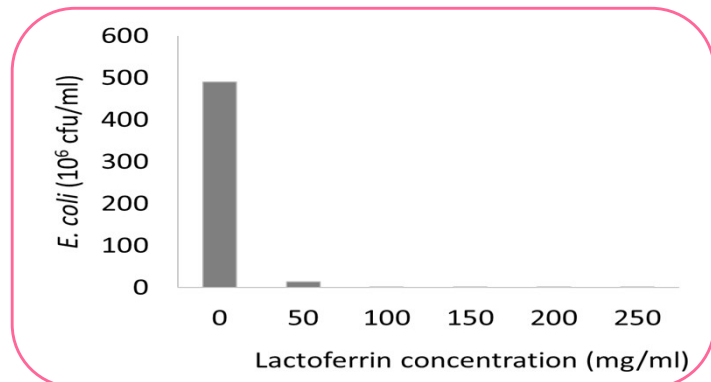
- Več kot 98 % čisti izvlečki!
- Visoko optimizirana metoda za ekstrakcijo laktoferina z največjo ohranjeno sposobnostjo vezave železa (C vrednost 68 – 76 %)!
- Ohranjena proteinska struktura s specifičnimi biološkimi funkcionalnostmi!
- Visoka topnost beljakovine!



Visoka ohranjena sposobnost laktoferina za vezavo železa.  
Antimikrobno in antioksidativno delovanje.  
*High preserved capacity of lactoferrin for iron binding.  
Antimicrobial and antioxidant activity.*



Visoka čistost, ohranjena struktura.  
Več kot 95% frakcija laktoferina.  
*High purity, preserved structure.  
More than 95% lactoferrin fraction.*



Protimikrobno delovanje proti patogenim bakterijam.  
Zaviranje rasti *E. coli* O157 H7 med 6-urno inkubacijo pri 37°C.  
*Antimicrobial action against pathogenic bacteria.  
Growth inhibition of *E. coli* O157 H7 during 6-hour incubation at 37°C.*

## High quality of lactoferrin

- More than 98% pure extracts!
- Highly optimized method for lactoferrin extraction with the highest retained iron-binding capacity (C value 68 – 76%)!
- Retained protein structure with specific biological functionalities!
- High protein solubility!

### OPTIMIZIRAN TEHNOLOŠKI POSTOPEK OMOGOČA ODLIČNO KAKOVOST LAKTOFERINA (LF)

*THE OPTIMIZED TECHNOLOGICAL PROCESS GIVES  
EXCELLENT LACTOFERRIN (LF) QUALITY*

Preizkušeni različni načini  
sušenja elucije z LF  
*Different ways of drying the  
elution with LF were tested*



Elucija z LF  
*Elution with LF*

→ Sušenje z razprševanjem  
*Spray drying*



Sušenje / *Drying*



Posušen produkt / *Dried product*

→ Sušenje z zamrzovanjem (Liofilizacija)  
*Freeze drying (Lyophilization)*



Sušenje / *Drying*



Posušen produkt / *Dried product*

→ Sušenje v vrtničnoslojni komori  
*Drying in a fluid-bed coater*



Sušenje / *Drying*



Posušen produkt / *Dried product*

## Demonstracija izrabe ostanka kisle sirotke po ekstrakciji proteinov

### IZKORIŠČANJE UPORABNIH SNOVI V OSTANKU KISLE SIROTKE PO MIKROFILTRACIJI

V projektu smo demonstrirali dve nadaljnji uporabi frakcije sirotke, ki je preostala po pripravi kisle sirotke na kromatografski postopek. Poleg drugih snovi je frakcija vsebovala veliko proteinov in primarno mlekarstvo kulturo.

Predstavili smo možnost izdelave beljakovinsko bogatega praškastega produkta uporabnega kot krmni dodatek. Pri izdelavi smo opredelili optimalne postopke inaktivacije mikroorganizmov in sušenja.

Potencial primarne mlečnokislinske flore preostanka sirotke smo izkoristili za razvoj inovativnega postopek priprave laktofermentacijskega posipa (LAKTOPRO) za pospeševanje in usmerjanje procesa kompostiranja (patentna prijava P-202100089).



1 Sušenje inokuliranega substrata / *Drying of the inoculated substrate*  
2 Zapakiran posip / *Packaged starter*  
3 Predstavitve produkta na promocijskem dogodku / *Presentation of the product at a promotional event*

### BIOTEHNOLOŠKA PRETVORBA OSTANKOV KISLE SIROTKE S SEKUNDARNO FERMENTACIJO

Po ekstrakciji izbranih proteinov ostane sirotka z manjšo vsebnostjo proteinov in pretežno nespremenjenimi drugimi lastnostmi. Vsebuje laktozo in ostale hranilne snovi, ki so uporabne za gojenje mlečnokislinskih bakterij in kefirnih zrn.

Optimizirali smo gojenje kefirnih zrn na laboratorijskem nivoju in ga uspešno prenesli na pilotni nivo. Po inaktivaciji in liofilizaciji kefirnih zrn in gojišča smo pripravili stabilen praškast produkt, uporaben kot prehransko dopolnilo s prebiotičnimi in postbiotičnimi lastnostmi (patentna prijava P-202200052).

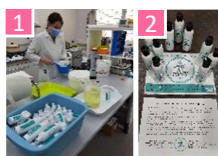


1 Inkubacija kefirnih zrn a) v laboratoriju in b) na pilotnem nivoju / *Incubation of kefir grains a) in the laboratory and b) on pilot scale*  
2 Odcejanje kefirnih zrn a) v laboratoriju in b) na pilotnem nivoju / *Draining of kefir grains a) in the laboratory and b) on pilot scale*

V izrabljenem gojišču smo uspešno oborili in posušili kalcijev laktat, ki je uporaben v prehranski in farmacevtski industriji.

### OSTALE MOŽNOSTI IZRABE SIROTKE

Kislo sirotko ali ostanke procesa ekstrakcije proteinov smo uporabili kot kakovostno sestavino šampona.



1 Priprava šampona / *Shampoo preparation*  
2 Predstavitve izdelka na konferenci / *Presentation of the product at the conference*

### ANAEROBNA DIGESTIJA SIROTKE

Ostanki procesa ekstrakcije proteinov in sekundarne fermentacije imajo še vedno visok energijski potencial. V ko-digestiji sirotke z blatom čistilne naprave se je v poskusih bistveno povečal izplen metana.

## Demonstration of the utilization of acid whey residue after protein extraction

### UTILISATION OF USEFUL SUBSTANCES IN THE RESIDUE OF ACID WHEY AFTER MICROFILTRATION

In the project, we demonstrated two further uses of the whey fraction remaining after the acid whey preparation for the chromatographic process. In addition to other substances, the fraction contained a lot of protein and a primary dairy culture.

We presented the possibility of producing a protein-rich powder product useful as a feed additive. During production, we defined optimal procedures for inactivating microorganisms and drying.

We used the potential of the primary lactic acid flora of the residual whey to develop an innovative process for the preparation of lactofermentation starter (LAKTOPRO) to accelerate and direct the composting process (patent application P-202100089).

### BIOTECHNOLOGICAL CONVERSION OF ACID WHEY RESIDUES BY SECONDARY FERMENTATION

After extracting the selected proteins, whey remains with lower protein content and mostly unchanged other properties. It contains lactose and other nutrients for growing lactic acid bacteria and kefir grains.

We optimized the cultivation of kefir grains at the laboratory level and successfully transferred them to the pilot level. After inactivation and lyophilization of kefir grains and culture medium, we prepared a stable powder product, used as a food supplement with prebiotic and postbiotic properties (patent application P-202200052).

Calcium lactate, useful in the food and pharmaceutical industry, was successfully precipitated and dried out of the spent culture medium.

### OTHER POSSIBILITIES OF USING WHEY

Acid whey or residues from the protein extraction process was used as a quality shampoo ingredient.

### ANAEROBIC DIGESTION OF WHEY

Residues from the protein extraction and secondary fermentation process still have a high energy potential. In the co-digestion of whey with the sewage treatment plant sludge, methane production significantly increased in the experiments.

## Vzpostavitev proizvodnih prostorov

### Demonstracijski obrat

V Komendi v Sloveniji je bil zgrajen nov demonstracijski obrat s trenutno dnevno zmogljivostjo predelave 10.000 L sirotke. Demonstracijski proizvodni obrat ima vso spremljajočo opremo za ekstrakcijo laktoferina (mikrofiltracija, kromatografija s puferskim sistemom, ultrafiltracija, sušenje) in različno drugo opremo, ki omogoča popoln izkoristek sirotke. Nadaljnja predelava sirotke je možna z opremo, kot je bioreaktorska posoda, centrifuga, industrijski liofilizator in filtracijski sistemi za različne membrane (keramične, votle-vlaknaste in spiralno navite membrane).

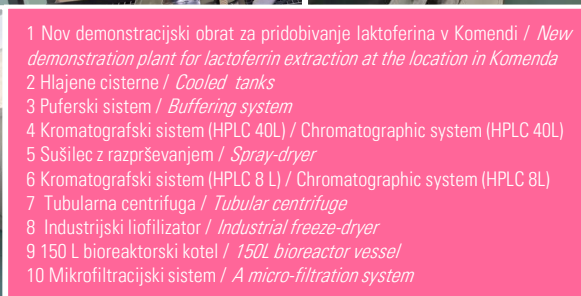
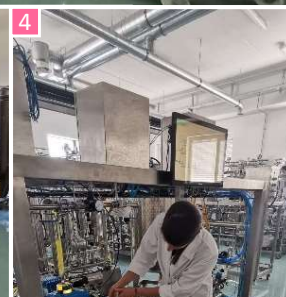
Poleg začetne proizvodnje bo demonstracijski sistem podpiral tudi razvoj novih preparativnih postopkov za izolacijo molekul.

## Establishment of production premises

### Demonstration plant

In Komenda, Slovenia, a new demonstration plant was constructed with a current daily processing capacity of 10,000L of whey. The demonstration production plant has all accompanying equipment for the extraction of lactoferrin (microfiltration, chromatography with buffer system, ultrafiltration, drying) and various other equipment which enable full utilization of whey. Further whey processing is possible with equipment such as bioreactor vessel, centrifuge, industrial freeze-dryer, and filtration systems for various membranes (ceramic, hollow-fibres and spiral-wound membranes).

In addition to initial production, the demonstration system will also support the development of new preparative procedures for isolating biomolecules.



- 1 Nov demonstracijski obrat za pridobivanje laktoferina v Komendi / *New demonstration plant for lactoferrin extraction at the location in Komenda*
- 2 Hlajene cisterne / *Cooled tanks*
- 3 Puferjski sistem / *Buffering system*
- 4 Kromatografski sistem (HPLC 40L) / *Chromatographic system (HPLC 40L)*
- 5 Sušilec z razprševanjem / *Spray-dryer*
- 6 Kromatografski sistem (HPLC 8 L) / *Chromatographic system (HPLC 8L)*
- 7 Tubularna centrifuga / *Tubular centrifuge*
- 8 Industrijski liofilizator / *Industrial freeze-dryer*
- 9 150 L bioreaktorski kotel / *150L bioreactor vessel*
- 10 Mikrofiltracijski sistem / *A micro-filtration system*



## Razvojni laboratorij

Vzpostavljen je bil razvojni laboratorij, kjer smo izvajali optimizacije postopka gojenja kefirnih zrn, zgoščevanje raztopin na rotavaporju, izdelavo šampona z dodatkom sirotke ter sušenje z razprševanjem in liofilizacijo različnih surovin.

## Development Laboratory

A development laboratory was established, where we optimised the process of growing kefir grains, the concentration of solutions on a rotavapor, production of shampoo with the addition of whey, and spray drying and lyophilisation of various raw materials.



- 1 Delo v razvojnem laboratoriju / *Work in the developmental laboratory*
- 2 Liofilizacija in sušenje z razprševanjem / *Freeze drying and spray drying*
- 3 Gojenje kefirnih zrn / *Kefir grains cultivation*
- 4 Zgoščevanje vzorca z rotavaporjem / *Concentration of a sample with rotavapor*
- 5 Priprava šampona s sirotko / *Preparation of shampoo with whey*
- 6 Priprava prehranskega dopnila iz kefirnih zrn / *Preparation of food additive from kefir grains*

## Kontrolni laboratorij

Vzpostavljen je bil kontrolni laboratorij, kjer smo izvajali nadzorno analitiko posameznih korakov obravnave sirotke na demonstracijskem sistemu.

## Control laboratory

A control laboratory was established, where we carried out a control analysis of the individual steps of whey treatment on the demonstration system.



- 1 Kontrolni laboratorij / *Control laboratory*
- 2 Centrifugiranje vzorcev / *Centrifugation of samples*
- 3 Izvajanje mikrobioloških analiz s števcem kolonij / *Microbiological analyses with colony counter*
- 4 Analize vzorcev na PATfix HPLC sistemu / *Analysis of samples on the analytical PATfix HPLC system*
- 5 Laminarij za izvajanje mikrobioloških analiz / *Laminar Flow Cabinet for carrying out microbiological analyses*

## Koristi in potencial uporabe inovativne tehnologije ekstrakcije proteinov

### ODLIČEN TEHNOLOŠKI PROCES

- Visoki izkoristki ekstrakcije (>99 %) z najvišjo čistostjo beljakovin in ohranjeno aktivnostjo → Zadovoljevanje zahtevnih potreb strank
- Prilagodljivost tehnologije za ekstrakcijo več frakcij sirotkinih beljakovin glede na poslovni fokus in tržne priložnosti
- Hiter in stroškovno učinkovit postopek
- Nadaljnje možnosti predelave sirotke (npr. proizvodnja koncentrata sirotkinih beljakovin ali uporaba kot bioprocenjski medij) zaradi nespremenjenih lastnosti sirotke po ločitvi posamezne beljakovine!

### POMEN SIROTKINIH BELJAKOVIN NARAŠČA

- Sirotkine beljakovine imajo pomembne prehranske in funkcionalne lastnosti s pozitivnimi učinki na zdravje!
- Trg sestavin živalskih beljakovin je rastoči trg!
- Cene sirotkinih beljakovin rastejo z višjo stopnjo čistosti!

### TEHNOLOGIJA EKSTRAKCIJE BELJAKOVIN OMOGOČA ODLIČNE, ZAŽELENE KARAKTERISTIKE BELJAKOVIN

- Več kot 98% čisti izvlečki!
- Visoko optimizirana metoda za ekstrakcijo laktoferina z najvišjo kapaciteto zadrževanja železa!
- Ohranjena proteinska struktura s specifičnimi biološkimi funkcionalnostmi!
- Visoka topnost beljakovin!

### POSLOVNE PRILOŽNOSTI ZA MLEKARNE IN PREDELOVALCE

- Odprava sirotke kot odpada proizvodnje!
- Optimizacija vrednosti sirotke kot surovine!
- Racionalna uporaba odvečnega mleka (pred UTH pasterizacijo)!
- Dostop do lastno proizvedenih sirotkinih beljakovin!
- Zagotavljanje novih poti za proizvodnjo novih izdelkov!

## Tržno usmerjeni rezultati projekta

- Opredeljena visoka proizvodna zmogljivost za ekstrakcijo LF.
- Optimizirana uporaba pufernih raztopin pri proizvodnji LF – manjši vpliv na okolje.
- Definirana kaskadna ekstrakcija več beljakovin sirotke in ponovna uporaba deproteinizirane sirotke pred anaerobno pretvorbo v bioplin.
- Tehnologija pripravljena za vstop na trg.
- Vzpostavljen demonstracijski objekt blizu ciljev projekta (10 m<sup>3</sup> sirotke/dan).
- Opredeljene obetavne možnosti za nadaljnjo predelavo kisle sirotke in ponovno uporabo – razviti različni novi izdelki.

## Benefits and applicability potential of the innovative protein extraction technology

### EXCELLENT TECHNOLOGY PROCESS

- High extraction yields (>99%) with the highest protein purity and retained activity → Meeting demanding customers' needs
- The flexibility of the technology to extract several whey protein fractions depending on the business focus and market opportunities
- High-speed and cost-effective process
- Further whey processing options (e.g. production of WPC or use as a bioprocessing media) due to unchanged whey characteristics after separation of individual protein!

### THE IMPORTANCE OF WHEY PROTEINS IS GROWING

- Whey proteins have important nutritional and functional properties with positive health impacts!
- The market of animal protein ingredients is a growing market!
- Whey protein prices increase with higher levels of purity!

### THE PROTEIN EXTRACTION TECHNOLOGY ENABLES EXCELLENT, DESIRABLE PROTEIN CHARACTERISTICS

- More than 98% pure extracts!
- Highly optimised method for lactoferrin extraction with the highest retained iron binding capacity!
- Retained protein structure with specific biological functionalities!
- High protein solubility!

### BUSINESS OPPORTUNITIES FOR DAIRIES AND PROCESSORS

- Elimination of whey as waste production flow!
- Optimisation of the value of whey as raw material!
- Rational use of excess milk (before UTH pasteurization)!
- Access to own produced whey proteins!
- Provision of novel routes to produce new products!

## Market-oriented results of the project

- Defined high production capacity for LF extraction.
- Optimised buffer solutions use in LF production – decreased environmental impact.
- Defined cascade extraction of several whey proteins and deproteinized whey reuse before anaerobic conversion to biogas.
- Technology prepared for market entry.
- Established process facility close to project goals (10 m<sup>3</sup> whey/day).
- Defined promising options for further acid whey processing and reuse – various new products developed.