Bioloģiski aktīvu peptīdu frakciju saturoši inovatīvi produkti no fermentētām siera sūkalām

Projekta līguma numurs: 1.1.1.3/1/24/A/169

Pārskats par periodu 01.03.2026 - 31.05.2026.

1. UZ PĀRSKATA PERIODU ATTIECINĀMĀS PROJEKTA AKTIVITĀTES LAIKA POSMĀ NO 01.03.2026. LĪDZ 31.05.2026.

1.1. turpināta SA1 izpilde (kultūru skrīnings),

1.1.1. no Latvijas Universitātes Mikroorganismu kultūru kolekcijas (LU MKK) uzglabātajiem 14 pienskābes baktēriju (LAB) un pieciem laktozi fermentējošajiem raugiem (LFY) atlasītas sešas bioloģiski aktīvos savienojumus (BAS) intensīvāk sintezējošās LAB kultūras, kuras vienlaikus uzkrāj peptīdu frakciju (PF) un viena perspektīvākā PF veidojoša LFY kultūra:

a) izstrādāts standartizēts protokols BAS un PF sintezējošo mikroorganismu kultivēšanai;
b) optimizēts protokols un iegūtu salīdzināmi dati atlasīto kultūru augšanu, antiolsidantu (AO) īpašības un BAS sintēzes aktivitātei;
c) raksturota sūkalu proteīna šķelšanas un PF uzkrāšanās dinamika 24 stundu ilgā fermentācijas periodā, izmantojot PAAG elektroforēzi;
d) veikto eksperimentu laikā raksturota LAB un LFY šūnu skaita dinamika.

1.1.2. turpināta datu uzkrāšana par a/s “Cesvaines piens” izmantoto siera ierauga kultūru ietekmi uz sūkalu bioķīmisko sastāvu

a) uzkrāta sistematizētu datu kopa par piena un siera sūkalu bioķīmisko sastāvu no 2025, gada jūlija līdz 2026. gada marta beigām;
b) aprobētas statistikas pieejas datu kopas analīzei;
c) sistematizēti dati par izmantoto rūpniecisko ieraugu īpašībām un sastāvu.

1.2. Turpināta SA2 izpilde (sūkalas saturošo barotņu un mikroorganismu augšanas apstākļu optimizācija)

1.2.1. pētītas iespējas ar vienkāršu barības vielu maisījumu un konkrētu savienojumu papildināšanu palīdzību stimulēt sūkalās pēc siera graudu atdalīšanas saglabājušos mikroorganismu aktivitāti

a) atlasīta sūkalu pēcfermentāciu potenciāli stimulējošo savienojumu kopa un izveidotas tos saturošo sūkalu barotņu formulācijas;
b) veikta eksperimentu sērija BAS un AO savienojumu sintēzes dinamikas noskaidrošanai papildinātās sūkalās pēcfermentācijas apstākļos, izmantojot ajtivizētas atlikušo industriālo oeraugu kultūras;
c) ar statistiskās analīzes metodēm noskaidroti būtiskākie pēcfermentācijas aktivitāti iespaidojošie faktori un izstrādāta pieeja tālākajai metodes optimizācijai.

1.3.Publicitātes pasākumi: ziņojumi starptautiskajās konferencēs

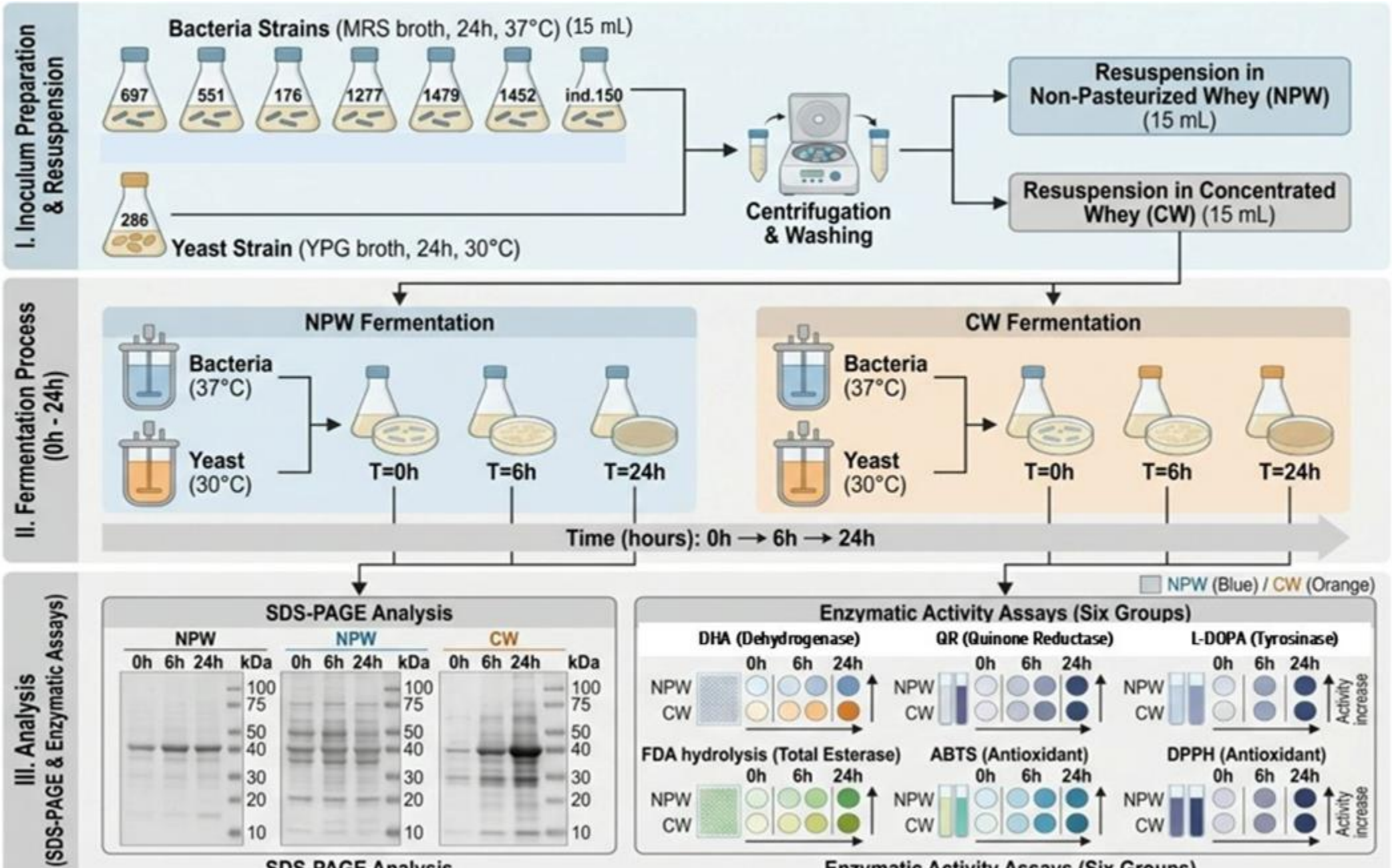
1.3.1. Dzīvības zinātņu konference "The COINS 2026“ Viļņas universitātē, Lietuvā;

1.3.2. konferencē *Biosystems Engineering* 2026 Tartu DzZU, Igaunijā.

2. TURPMĀKAJAI IZPĒTEI PERSPEKTĪVĀKO LAB un LFY KULTŪRU ATLASE

Projekta pirmā apakšaktivitāte paredz pakāpenisku izmantojamo mikroorganismu kultūra skaita samazināšanu, orientējoties uz lielāku potenciālu PF un BAS uzkrāšanā, izmantojot siera ražošanas procesā pēc pirmās koagulācijas fāzes veidoto (NPW), kā arī ar ultrafiltrāciju atdalot ūdeni iegūtas apm. pieckārtīgi koncentrēto sūkalu (CW) frakcijas. Sākotnējā fāzē tika pētītas 14 LAB un piecas LFY kultūras no LU MKK, salīdzinājumam izmantojot vienu industriālā ierauga kultūru. No tām turpmākajai analīzei atlasītas sešas BAS intensīvāk sitezējošas LAB un viena lielāku PF veidojoša LFY. Šajā posmā padziļināti raksturota atlasīto kultūru aktivitāte ar mērķi (1) turpināt perspektīvāko kultūru izvēli, samazinot izpētes objektu skaitu; (2) identificēt kandidātus kopkultūru veidošanai, lai optimizētu BAS un PF akumulāciju. Fermentācijā izmantotas pie 100^{o}C apstrādātās sūkalās, no kurām atdalīs potenciāli citu pārtikas produktu ražošanai izmantojama proteīna frakcija.

EKSPERIMENTA SHĒMA LAB UN LFY ĪPAŠĪBU RAKSTUROŠANAI



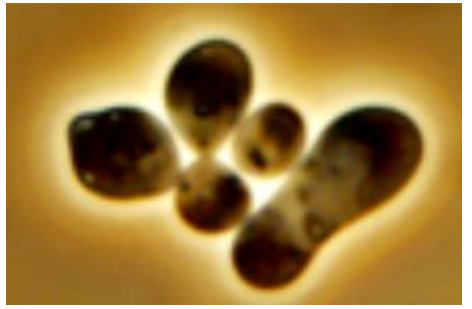
SĀKOTNĒJĀ SKRĪNINGĀ IEKĻAUTIE LAB CELMI

	Collection No.	Species	Full name	Description/origin
1	LMKK 697	*Lactiplantibacillus plantarum*	Lactiplantibacillus (Lactobacillus) plantarum DSM 20205, ATCC 8014	Food testing, quality control, organic acid production. DSM < ATCC < E. McCoy, 17-5 (Lactobacillus arabinosus).
2	LMKK 549	*Lactobacillus delbrueckii*	Lactobacillus delbrueckii	LU MBI
3	LMKK 551	*Lactiplantibacillus plantarum*	Lactiplantibacillus plantarum (Lactobacillus plantarum)	LU MBI Rīga, skābbarība
4	LMKK 547	*Lacticaseibacillus paracasei*	Lacticaseibacillus paracasei subsp. paracasei (Lactobacillus casei subsp. alactosus) VKPM B-4079	Rīga, raudzēta cukurbiešu sula
5	LMKK 176	*Lactococcus lactis subsp. lactis*	Lactococcus lactis subsp. lactis (Microbacterium lacticum 392) 153	Rīga, 1961., epifīts, lucernas virszemes daļas no Pierīgas
6	LMKK 1156	*Lacticaseibacillus rhamnosus*	Lacticaseibacillus (Lactobacillus) rhamnosus (Crystal)	Rīga, 2011., Padēļu ezera sapropelis
7	LMKK 1454	*Lacticaseibacillus rhamnosus*	Lacticaseibacillus (Lactobacillus) rhamnosus (Crystal)	Rīga, 2014., bišu zarnu trakts
8	LMKK 1458	*Lactobacillus delbrueckii*	Lactobacillus delbrueckii	Rīga, 2015.
9	LMKK 1277	*Lactobacillus johnsonii*	Lactobacillus johnsonii (Crystal)	Rīga, 2012., skābbarība
10	LMKK 799	*Lactobacillus helveticus*	Lactobacillus helveticus DSM 20075, ATCC 15009	DSMZ, Emmental (Swiss) cheese, Serological group A. DSM < ATCC < P.A. Hansen, Lh12 < S. Orla-Jensen, 12 (Thermobacterium helveticum). Tipa kultūra
11	LMKK 657	*Lactobacillus acidophilus*	Lactobacillus acidophilus (Crystal)(Lactococcus cremoris (L. lactis subsp. cremoris)) (Crystal)	Rīga, 2002, piemasījums pie maizes rauga Saccharomyces minor MSCL 575
12	LMKK 798	*Lactococcus lactis subsp. lactis*	Lactococcus lactis subsp. lactis	Rīga, 2008.
13	LMKK 1479	*Limosilactobacillus fermentum*	Limosilactobacillus (Lactobacillus) fermentum (API)	Rīga, 2015., biogāzes reaktors
14	LMKK 1452	*Lacticaseibacillus paracasei*	Lacticaseibacillus (Lactobacillus) paracasei (Crystal)	Rīga, 2014., bišu zarnu trakts
15	Cesvaine	FC150	Firmas Hansen izmantotie industriālie ieraugi; *lactococcus lactis* subtipu maisījums	
16	Cesvaine	FC170		

SĀKOTNĒJĀ SKRĪNINGĀ IEKĻAUTIE LFY CELMI

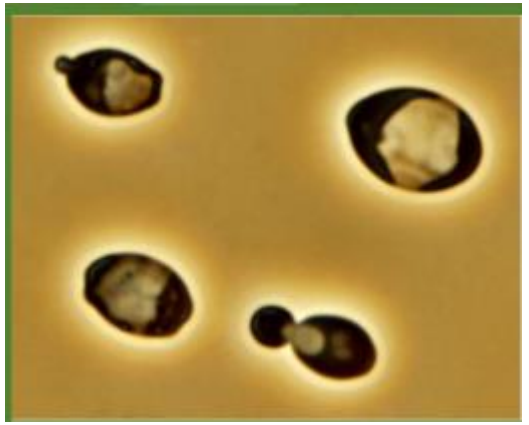
Kluyveromyces marxianus 79
Kluyveromyces marxianus 282
Kluyveromyces marxianus 285
Kluyveromyces marxianus 286
Kluyveromyces marxianus 831

Y1

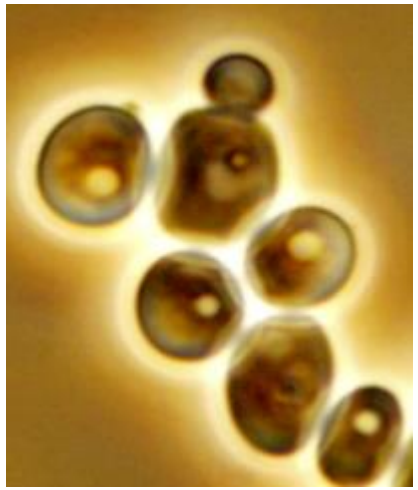


Y2

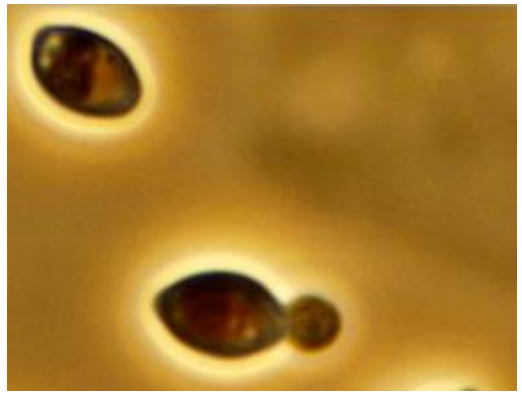
Y3

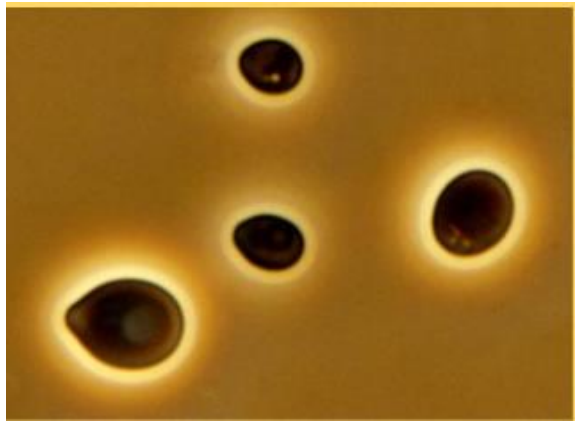


Y5



Y4





TURPMĀKAJIEM PĒTĪJUMIEM ATLASĪTIE MIKROORGANISMU CELMI
to īpašības un izvēles kritēriji

Strain no.	Strain ID	Species	Type	Main Tricine-SDS-PAGE observation	Proteolytic activity / peptide formation	Conclusion
1	697	*Lactiplantibacillus plantarum*	Bacteria	Main whey protein bands remained mostly unchanged from 0 to 24 h in both whey types. No strong new low-molecular-weight bands.	Low	Limited visible whey protein hydrolysis during 24 h fermentation.
2	551	*Lactiplantibacillus plantarum*	Bacteria	Similar band pattern at 0, 6, and 24 h. Major bands remained stable.	Low	Weak proteolytic effect; little evidence of protein degradation.
3	176	*Lactococcus lactis subsp. lactis*	Bacteria	Major bands remained visible. Minor changes may be present, but not strong.	Low to moderate	Some possible proteolysis, but not clearly strong by Tricine-SDS-PAGE.
4	1277	*Lactobacillus johnsonii*	Bacteria	More visible low-molecular-weight bands/smearing around ~15–30 kDa, especially in highlighted areas.	Moderate to high	One of the better bacterial strains for peptide formation/protein hydrolysis.
5	1479	*Limosilactobacillus fermentum*	Bacteria	Clearer low-molecular-weight region/smearing, especially in concentrated whey.	Moderate	Shows evidence of peptide formation; promising strain for whey protein hydrolysis.
6	1452	*Lacticaseibacillus paracasei*	Bacteria	Band profile mostly stable over fermentation time. No obvious disappearance of main whey bands.	Low	Limited detectable proteolytic activity under tested conditions.
7	Industrial 150	*Industrial bacterial strain*	Bacteria	Main bands remained stable across 0, 6, and 24 h. Changes were not very clear.	Low	Weak visible proteolysis; not a strong peptide producer in this experiment.
8	286	*Kluyveromyces marxianus*	Yeast	Broad low-molecular-weight bands/smears around ~15–35 kDa, especially visible in highlighted region.	High / most visible	Yeast showed the strongest apparent low-molecular-weight peptide/protein pattern; most promising, but bands may also include yeast biomass proteins.

TURPMĀKAJIEM PĒTĪJUMIEM ATLASĪTIO MIKROORGANISMU CELMU IZCELSME

#	MSC #	Nomenclature name, identification	Source	Place, time and source of distribution	Notes
1	697	*Lactiplantibacillus (Lactobacillus) plantarum* DSM 20205, ATCC 8014	DSMZ	N/A	Food testing, quality control, organic acid production. DSM < ATCC < E. McCoy, 17-5 *(Lactobacillus arabinosus).*
2	551	*Lactiplantibacillus plantarum (Lactobacillus plantarum)*	LU MBI	Riga, silage	Can be used for silage fermentation
3	176	*Lactococcus lactis subsp. lactis* (Crystal) *(Microbacterium lacticum* 392) 153, 392	LU MBI	Aboveground parts of Lucerne, 1961, from Pieriga.	Stimulates plant growth and development, increases sugar beet yield. Synthesizes group B vitamins and heteroauxin.
4	1277	*Lactobacillus johnsonii* (Crystal)	LMKK	Riga, silage, 2012	
5	1479	*Limosilactobacillus (Lactobacillus)* fermentum (API)	LMKK	Riga, 2015, biogas reactor	
6	1452	Lacticaseibacillus (Lactobacillus) paracasei (Crystal)	LU BF	Riga, 2014, bee intestinal tract	
8	286	Kluyveromyces marxianus 1215	LU MBI	N/A	For the determination of nicotinamide (vitamin PP)

3. BAS UN AO SINTĒZE ATLASĪTAJĀS MIKROORGANISMU KULTŪRĀS

Šajā pētījumu posmā pēc iepriekš attēlotās eksperimentu shēmas tika pārbaudītas skrīniingā atlasīto 6 LAB un vienas LFY kultūru BAS un AO veidojošā aktivitāte sūkalu preparātos, kuru raksturoja pēc mikroorganismu enzīmu vai enzīmu kompleksu spējas mijiedarbībā ar krāsu veidojošiem substrātiem mainīt reakcijas maisījuma absorbcijas intensitāti pie noteikta gaismas viļņa garuma.

QR – hinona reduktāze. Raksturo antioksidanta īpašības.

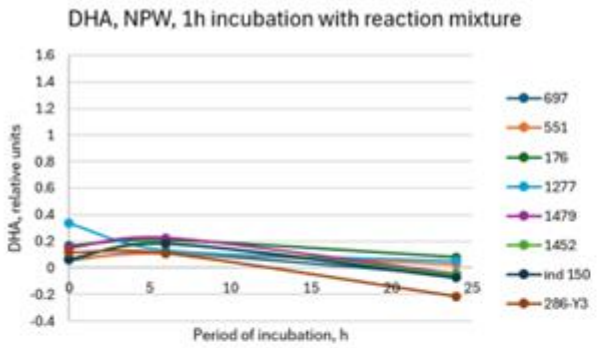
DHA – dehidrogenāze. Raksturo kopējo enzimātisko aktivitāti.

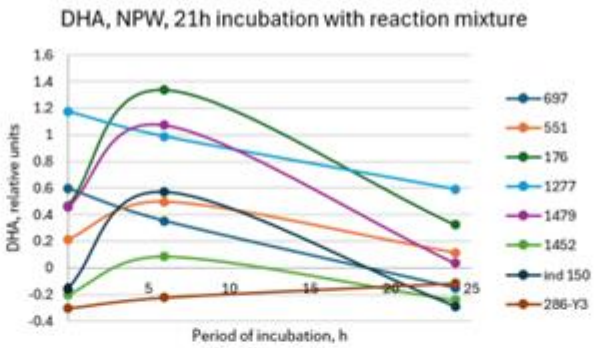
L-DOPA – levo-dopamīna sintēze. BAS sintēze.

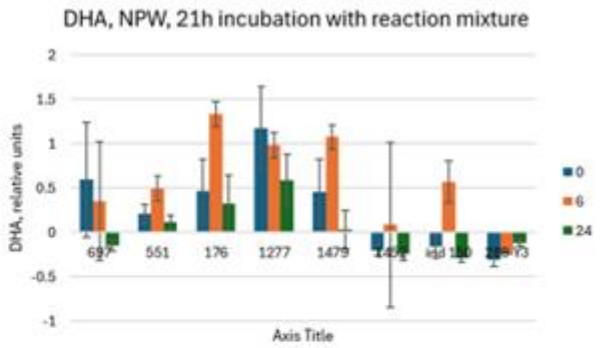
FDA – fuorescīna diacetāta hidrolīze. Augšanas aktivitātes vispārējs raksturojums.

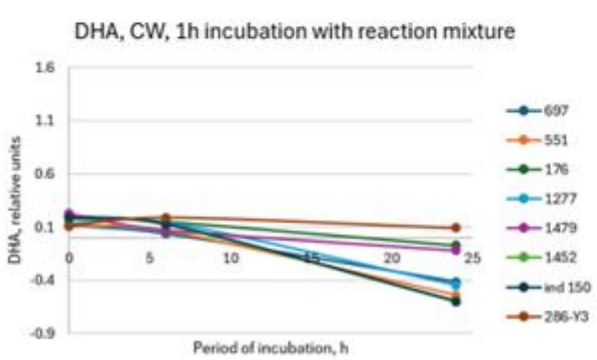
DPPH un ABTS atkrāsošanas inhibīcija. Aktīvā skābekļa radikālu (ROS) saistīšana, AO aktivitāte.

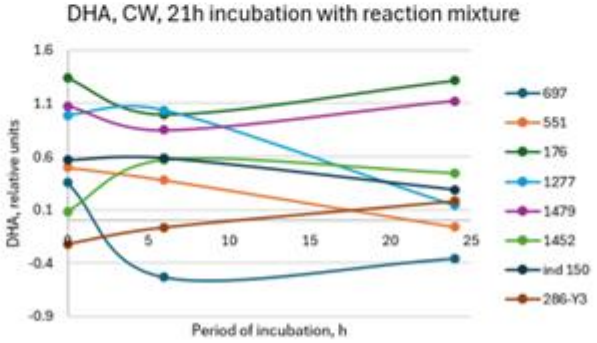
DHA, DEHIDROGENĀŽU, AKTIVITĀTES DINAMIKA

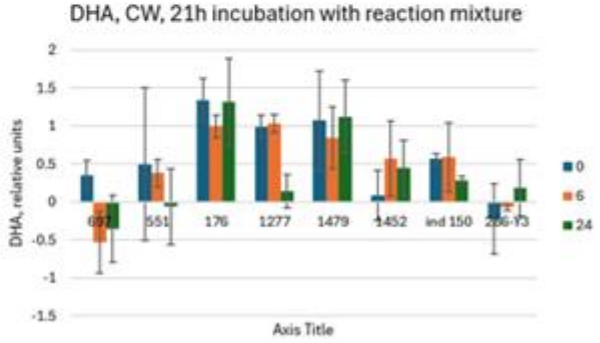






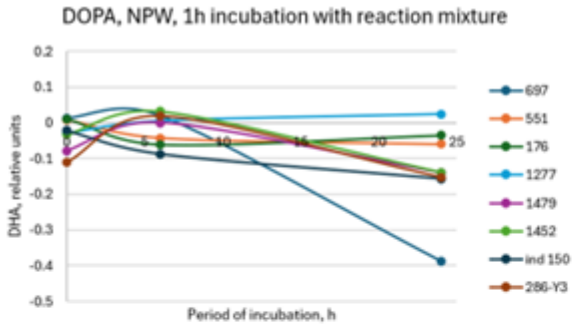


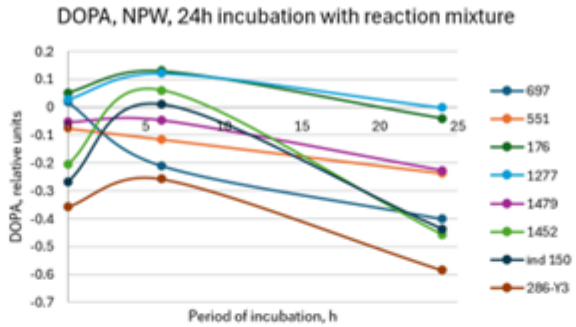


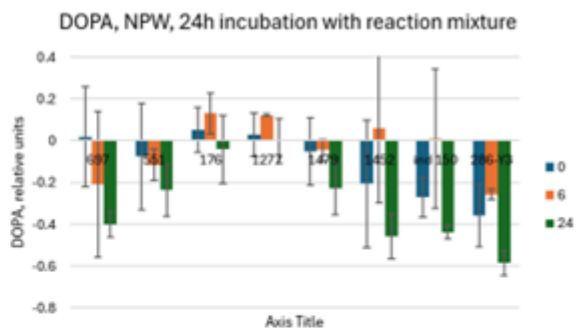


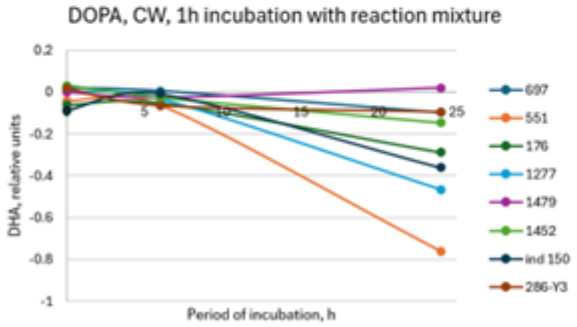
- The DHA assay demonstrated clear differences among the tested isolates.
- On NPW little activity was observed during the initial incubation period, as most values remained close to zero. However, after longer incubation, several isolates exhibited a marked increase in activity.
- LAB **176**, **1277**, and **1479** showed the strongest responses, suggesting a greater general metabolic activity over time.
- CW produced more variable results. While some isolates maintained positive activity, others showed reductions or even negative values after prolonged incubation.
- Overall, the DHA results indicate that activity is strongly influenced by both incubation time and growth conditions, with isolates **176**, **1277**, and **1479** displaying the most pronounced responses.

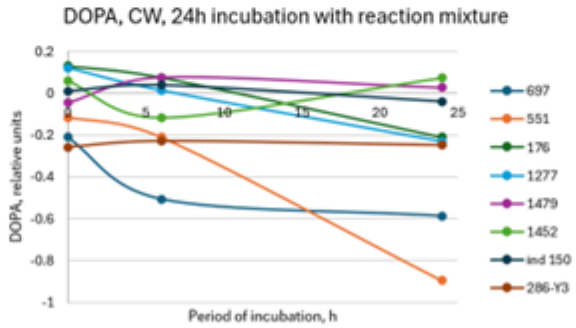
L-DOPA, LEVODOPAMĪNA SINTĒZES AKTIVITĀTES DINAMIKAL-

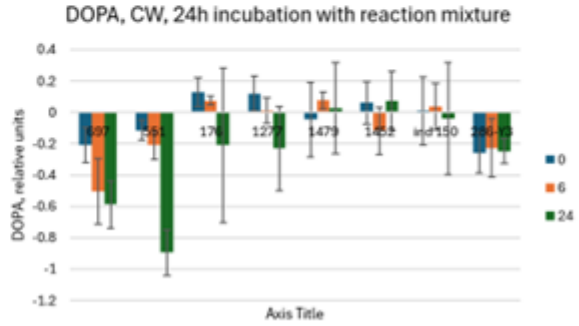






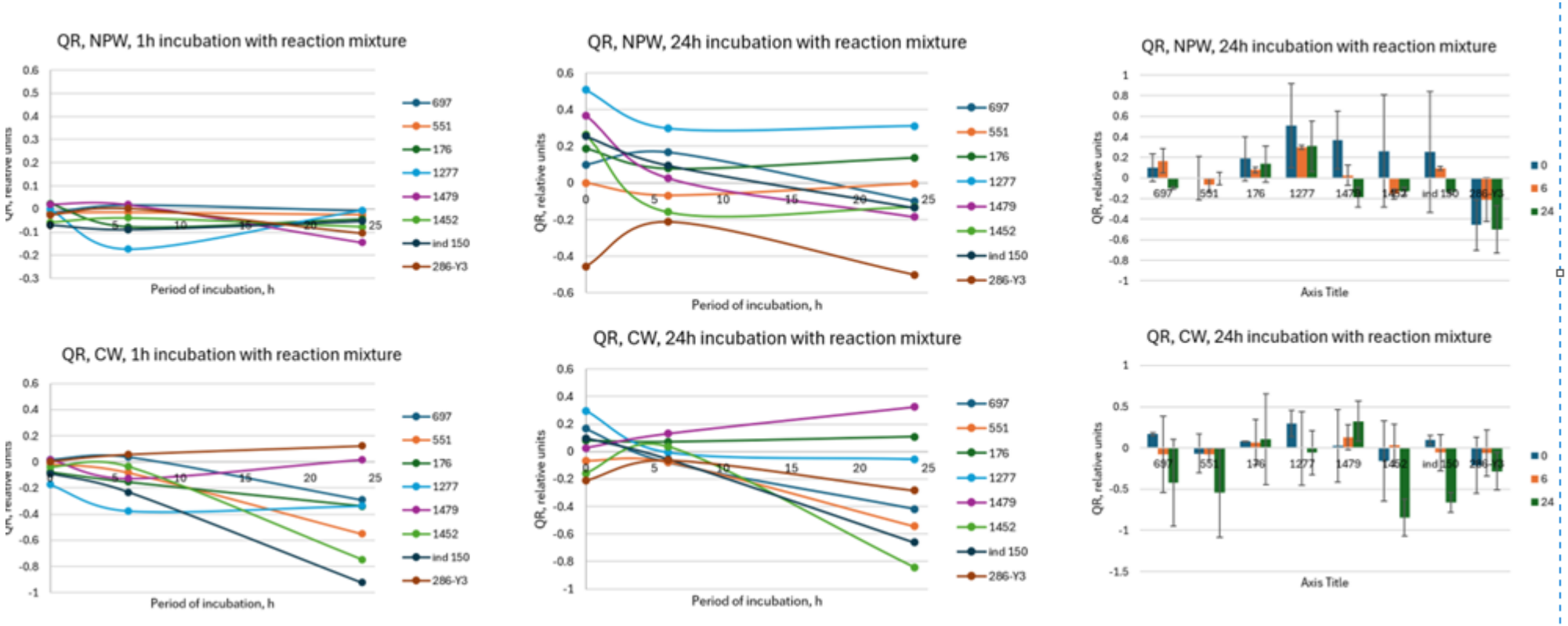






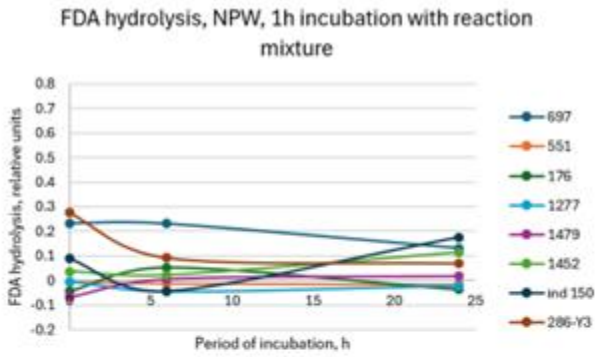
- The DOPA assay revealed a generally decreasing trend over time.
- In NPW most isolates showed negligible activity during extended incubation.
- The effect was even more pronounced in CW, where several isolates, particularly **551** and **ind150**, exhibited substantial reductions in activity.
- **DOPA assay shows depletion of the measured compound during incubation**.

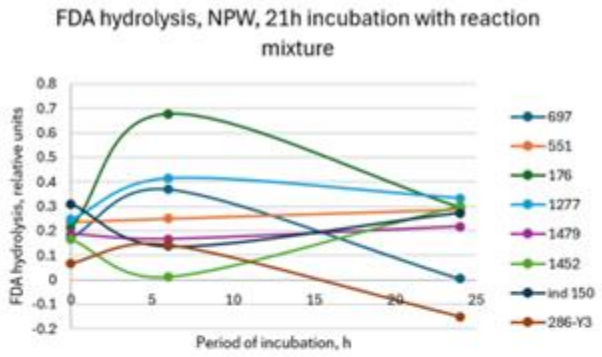
QR, HINONA REDUKTĀZES AKTIVITĀTES DINAMIKAL-

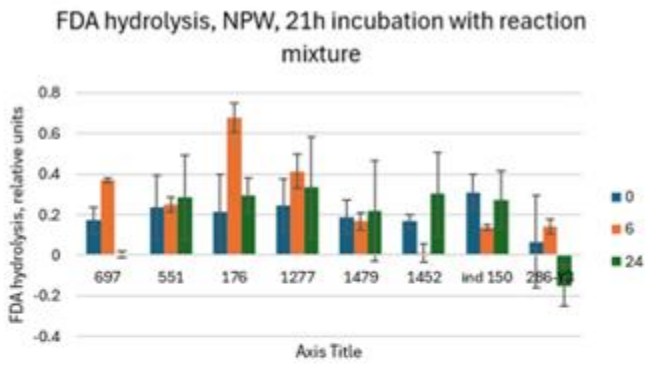


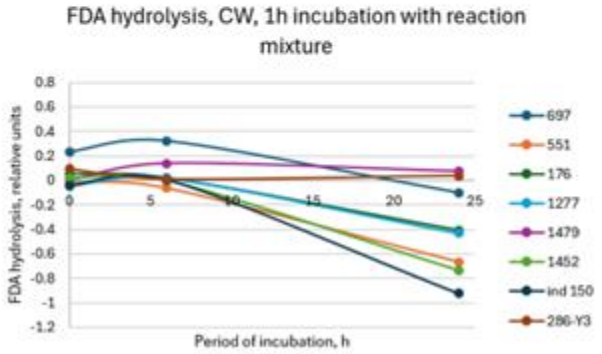
- QR assay highlighted significant differences among the isolates.
- In NPW, most isolates showed minimal activity during the early incubation with gradual increse after extended incubation,
- Strain **1277** displayed most positive response.
- In CW QR variability increased considerably. Strain **1479** exhibited one of the strongest positive results, but strains **1452 and ind150** showed the biggest decrease.

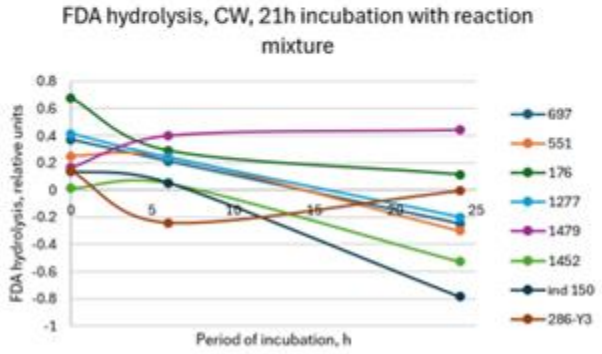
FDA, FUORESCĪNA DIACETĀTA HIDROLĪZES AKTIVITĀTES DINAMIKA

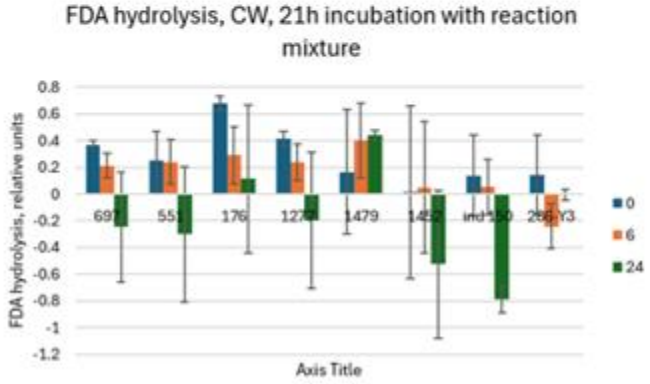






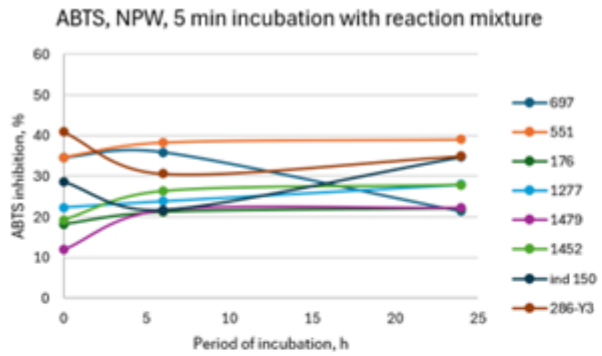


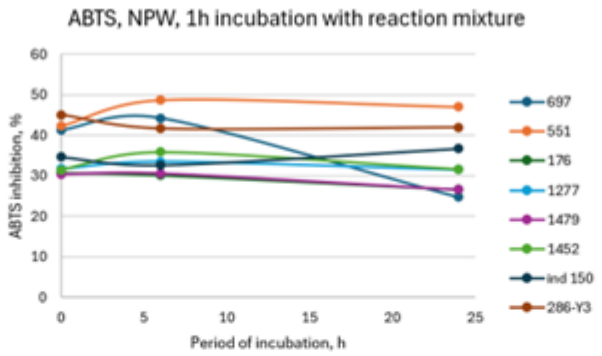


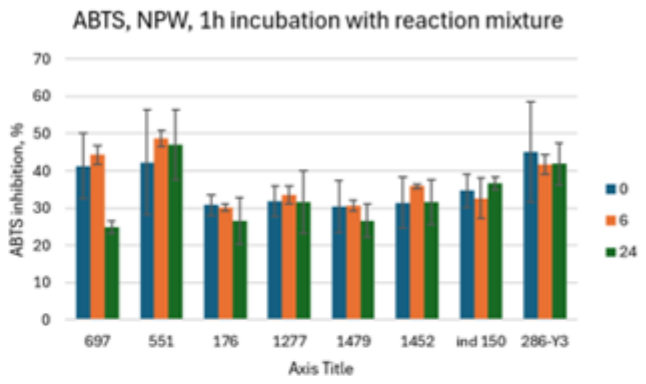


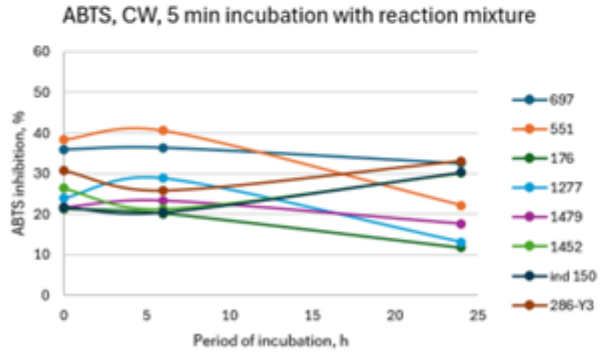
- FDA hydrolysis showed a moderate increase in activity in NPW .
- Among all tested strains, **MSC 176 exhibited the highest values,** suggesting strong metabolic activity.
- CW samples were variable; several strains experienced decline during extended incubation. Ind 150 showed the most pronounced decrease, but 1479 showed a significant increase, followed by stagnation
- CW might impose stress-reducing metabolic performance for some strains.

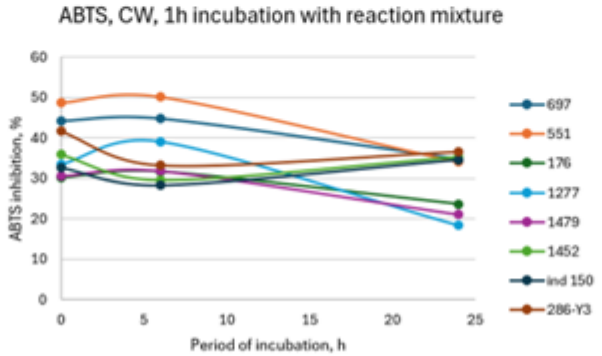
ABTS, 2,2'-AZINOBIS-3-ETILBENZILTIAZOLĪNSULFOSKĀBES KRĀSAS MAIŅAS TESTS

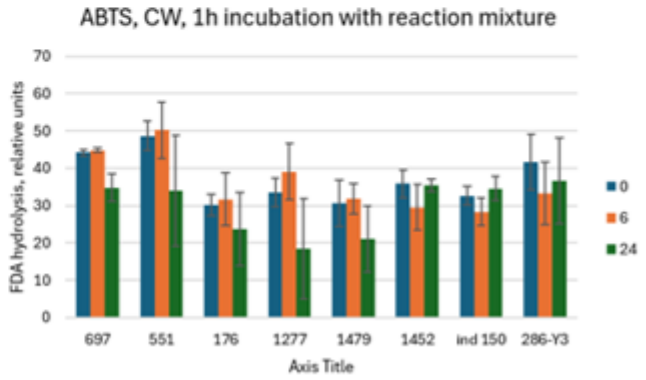






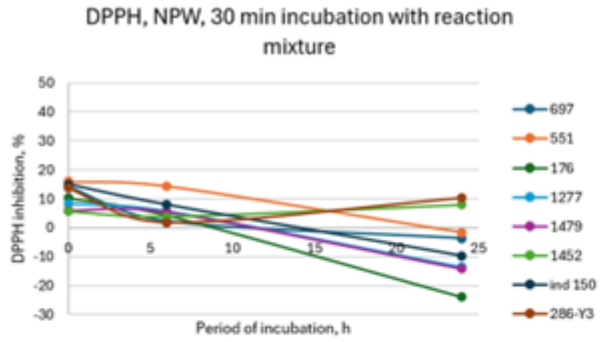


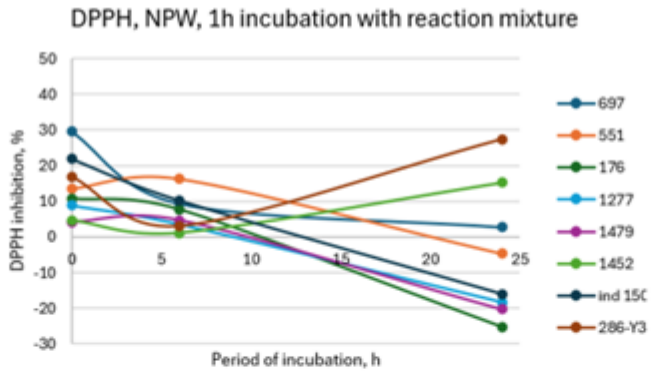


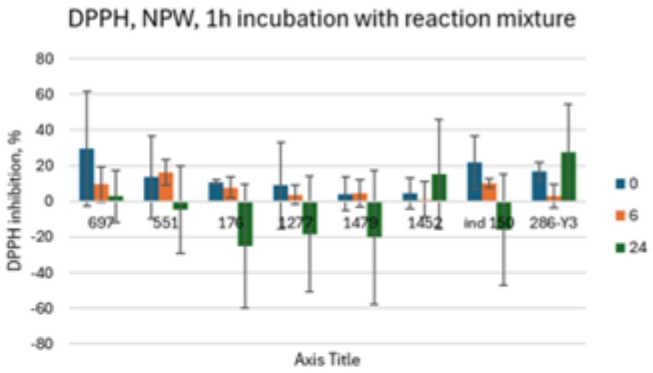


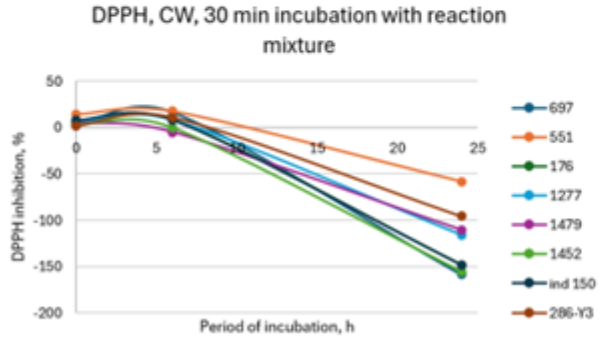
- Demonstrated a relatively stable antioxidant activity across all isolates and incubation periods. **Changes over time were generally small.**
- Most strains maintained positive values both in NPW and CW. The strongest activities were observed in strains 551, 286 (Yeast), **and 697**, while 1479 and 1277 generally showed lower values.

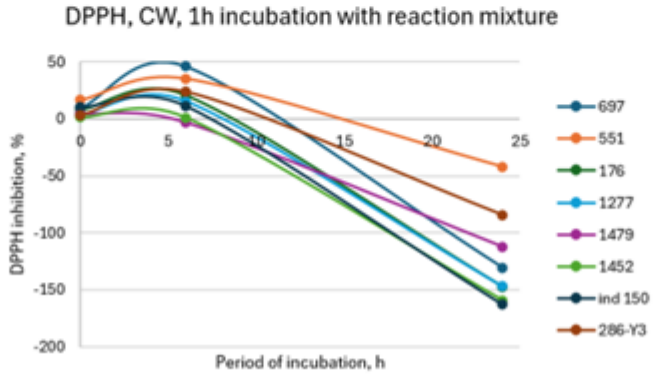
DPPH, 2,2-DIFENIL-6-PIKROHIDRAZĪNA KRĀSAS MAIŅAS TESTS

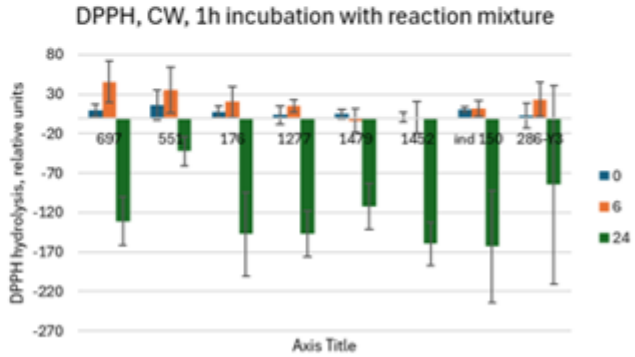












- In NPW, many isolates initially displayed positive antioxidant activity, which declined substantially with time.
- In CW, the declines were even greater, with several isolates exhibiting strongly negative values at the final point.
- Strains **ind150** and **1452** showed particularly severe reduction in AO activity during incubation.
- DPPH and ABTS tests should only be done by initial reading at point «0» and then after an hour.

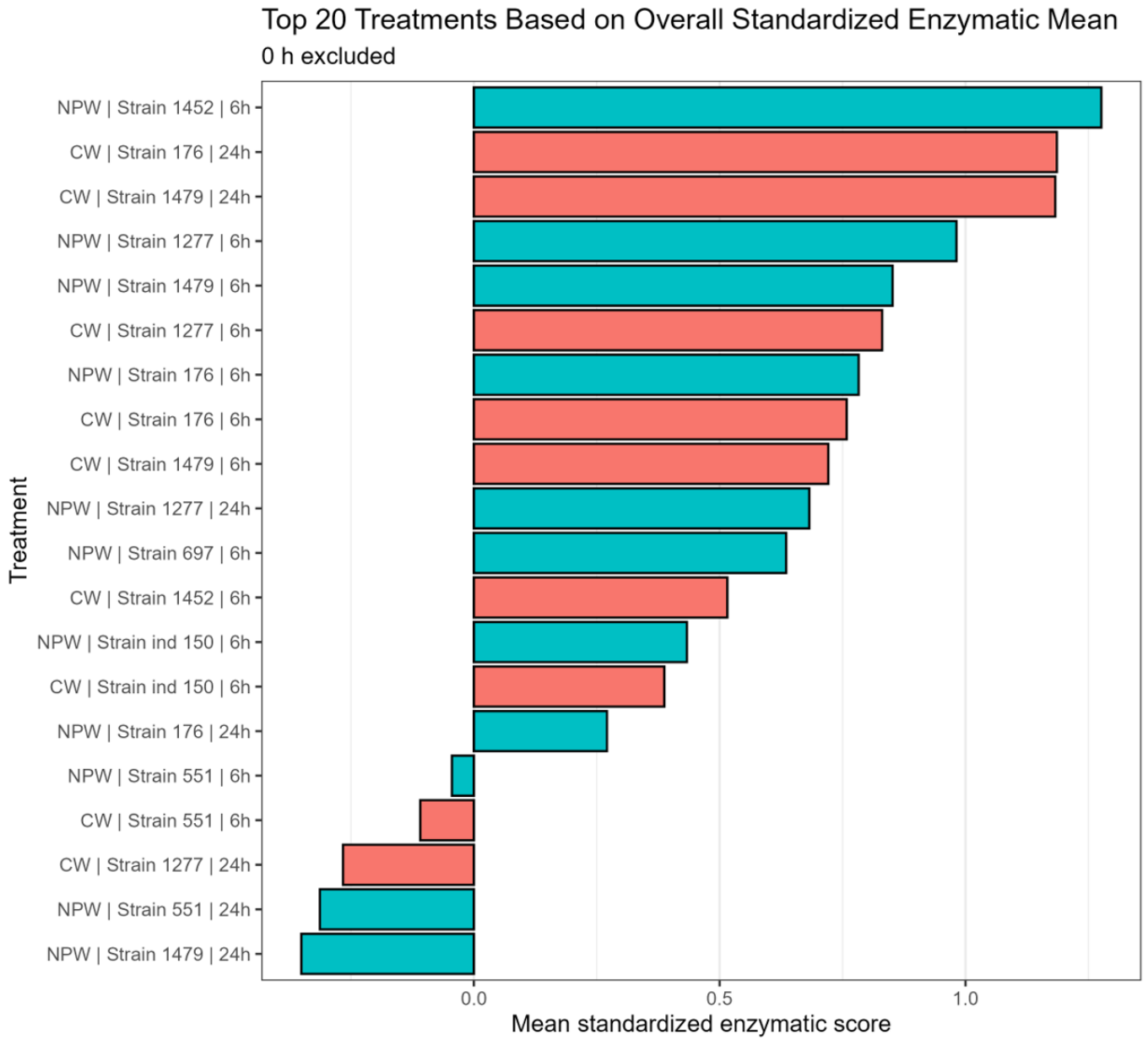
ENZĪMU AKTIVITĀTES DINAMIKAS MĒRĪJUMU KOPSAVILKUMS FERMENTĒTOS SŪKALU PREPARĀTOS, IZMANTOJOT ATLASITOS MIKROORGANISMU CELMUS

6h						
NPW	30min	5min	21h	21h	21h	21h
	DPPH	ABTS	DHA	DOPA	FDA	QR
697	2.20493	44.2621	0.35267	-0.20933	0.37	0.16633
551	14.3969	48.7038	0.49567	-0.11533	0.24933	-0.06833
176	4.28016	30.1786	1.33567	0.13033	0.677	0.07867
1277	5.57717	33.4828	0.98833	0.12133	0.41367	0.29667
1479	5.31777	30.612	1.074	-0.046	0.16633	0.02633
1452	3.76135	35.8662	0.085	0.06033	0.01267	-0.15767
ind 150	7.9118	32.6162	0.56767	0.00967	0.137	0.09567
286-Y3	1.68612	41.6621	-0.22433	-0.25733	0.14233	-0.21133

CW						
	DPPH	ABTS	DHA	DOPA	FDA	QR
697	16.2127	44.8579	-0.53133	-0.506	0.21167	-0.07967
551	17.7691	50.1663	0.378	-0.21067	0.241	-0.07833
176	8.94942	31.6953	0.993	0.075	0.28933	0.069
1277	8.69001	39.1162	1.03233	0.01333	0.23967	-0.006
1479	-5.05837	31.8578	0.848	0.07567	0.401	0.12933
1452	-0.1297	29.5286	0.56867	-0.11733	0.05067	0.035
ind 150	8.17121	28.2828	0.586	0.038	0.05233	-0.05733
286-Y3	10.7652	33.2662	-0.06767	-0.22633	-0.24133	-0.06333

- When all assays are considered together, **Strain 176, 1277, and 1479** emerge as the strongest performers.
- **Strain 176** consistently demonstrated high metabolic and enzymatic activity, particularly in the DHA and FDA hydrolysis assays.
- **Strain 1277** showed strong responses in both the DHA and QR assays, while **strain 1479** exhibited substantial QR activity and generally positive performance across several measurements.
- In contrast, isolate **ind150** was among the least stable performers. It frequently displayed declining activity, particularly in the FDA hydrolysis and DPPH assays, suggesting greater sensitivity to incubation conditions.
- **Isolate 286-Y3** showed intermediate performance, with results varying depending on the assay used.
- Comparing the two experimental conditions, **NPW** generally supported more favorable biological activity, whereas **CW** often resulted in greater variability and more pronounced declines during prolonged incubation. **This suggests that NPW may provide a more suitable environment for maintaining metabolic and enzymatic activity in the tested isolates.**

ENZĪMU AKTIVITĀTES DINAMIKAS MĒRĪJUMU KOPSAVILKUMS FERMENTĒTOS SŪKALU PREPARĀTOS, IZMANTOJOT ATLASITOS MIKROORGANISMU CELMUS



The highest overall standardized enzymatic activity was shown for strain 1452 (*Lacticaseibacillus (Lactobacillus) paracasei*) after 6h fermentation in NPW, followed by strains 176 (*Lactococcus lactis subsp. lactis)* and 1479 (*Limosilactobacillus (Lactobacillus) fermentum)* fermented in CW for 24h.

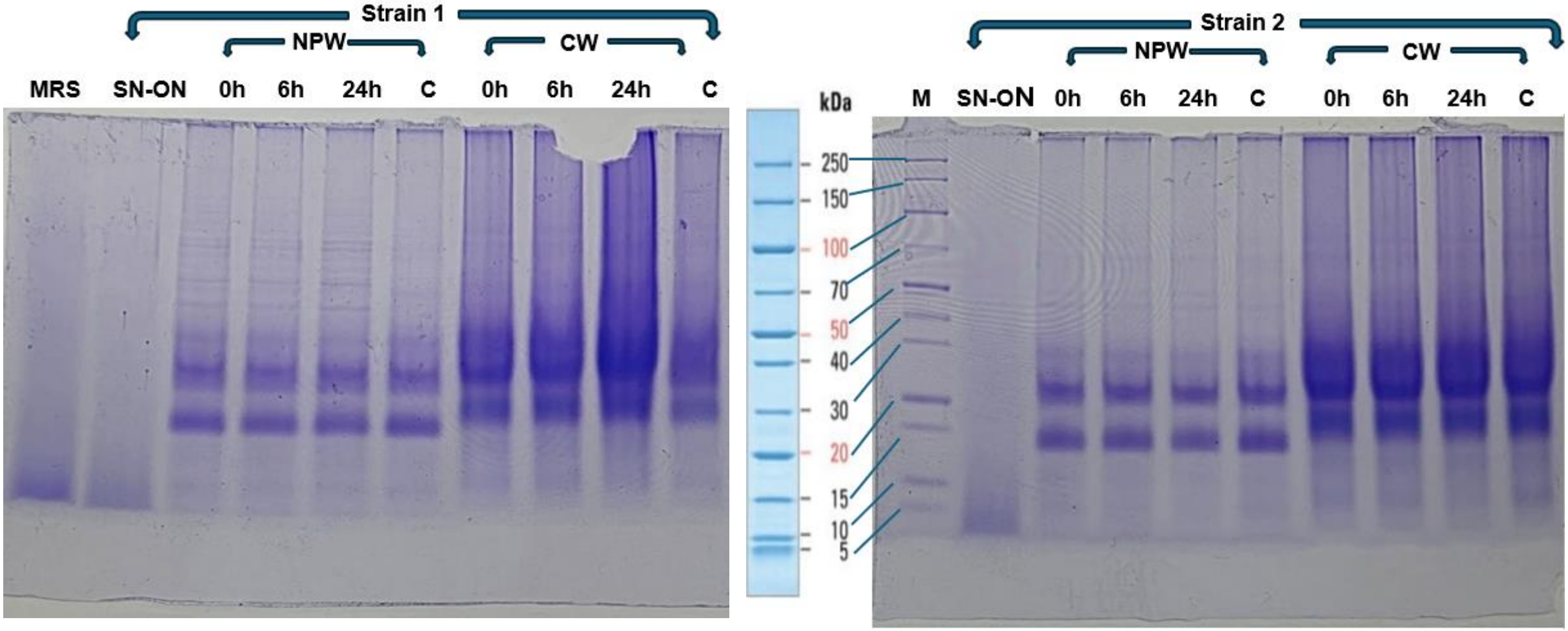
697 - *Lactiplantibacillus (Lactobacillus) plantarum DSM 20205, ATCC 8014*
551 - *Lactiplantibacillus plantarum (Lactobacillus plantarum)*
176 - *Lactococcus lactis subsp. lactis*
1277 - *Lactobacillus johnsonii*
1479 - *Limosilactobacillus (Lactobacillus) fermentum*
1452 - *Lacticaseibacillus (Lactobacillus) paracasei*
Ind 150 – Industrial bacteria strain
286-Y3 - *Kluyveromyces marxianus* 1215

4. PROTEĪNA UN PEPTĪDU FRAKCIJAS AR ATLASĪTAJĀM LAB UN LFY KULTŪRĀM FERMENTĒTAO SŪKALU PREPARĀTOS

10% poliakrilamīda denaturējošā gēla (dPAAG) elektroforēzes rezultāti Na-dodecilsulfāta (SDS) klātbūtnē Tric;ina buferī liecina, ka sūkalu fermentācija ar atlasītajiām mikroorganismu kultūrām radīja izmaiņas sūkalu proteīna sadalījumā, kuras galvenokārt bija redzamas kā zemas molekulmasas peptīdu zonas pveidošanās un vairāku lielākas molekulmasas proteīnu zonu koncentrācijas samazināšanās vai izzušana. Lielākā daļa LAB celmu 24 stundu laikā galvenos sūkalu proteīnus pilnībā nesašķēla. Starp testētajām kultūrām lielāko proteolītisko vai peptīdu producēšanas aktivitāti uzrādīja *Lactobacillus johnsonii* MCS 1277, *Limosilactobacillus fermentum* MCS 1479 un it īpaši raugs *Kluyveromyces marxianus* MCS 286.

Salīdzinot ar NPW ,CW fermentācijā veidojās intensīvākas, bet daļēji pārklājošās proteīnu zonas, kas apgrūtina rezultātu interpretāciju. Kopumā dPAAG gēli liecina, ka vairākas, bet ne visas atlasītās kultūras spēj veidot peptīdu frakcijas sūkalu fermentācijas laikā.

SŪKALU PROTEĪNS PĒC FERMENTĀCIJAS. AR CBB-R250 KRĀSOTS 10% dPAAG-TRICĪNA GĒLS



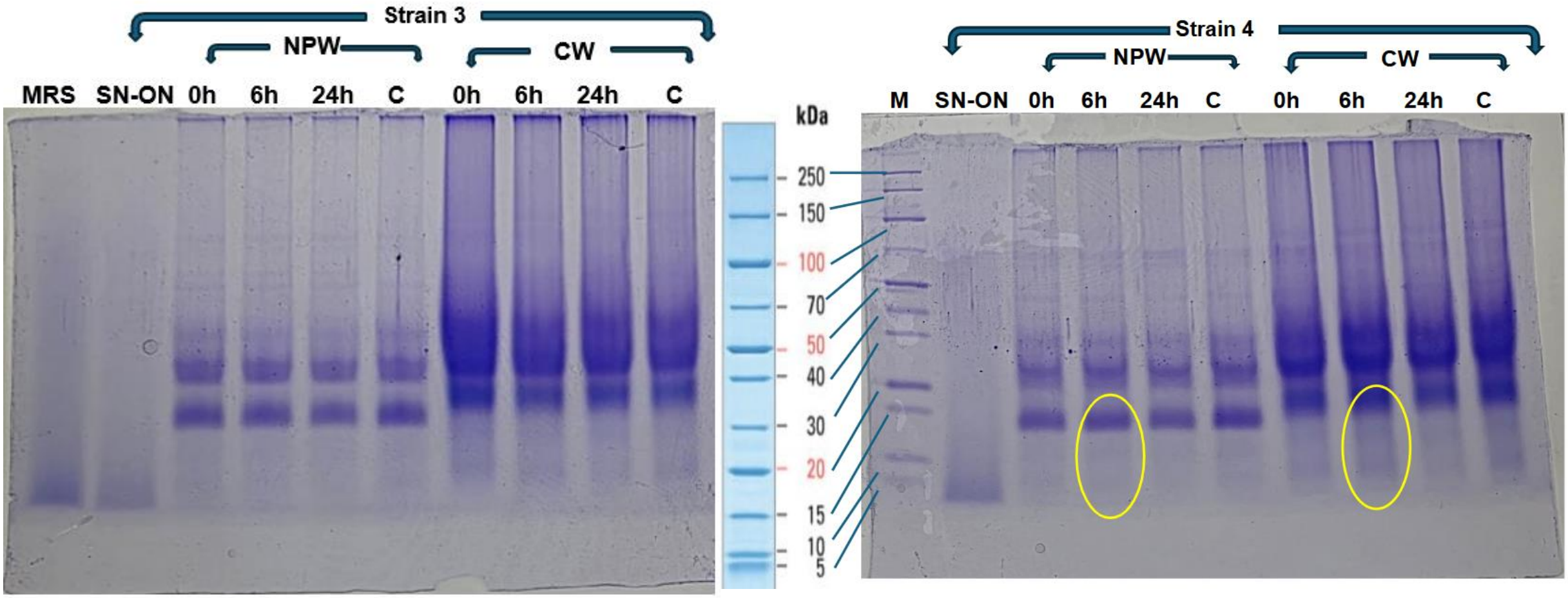
M = Marker NPW = Non-pasteurized autoclaved whey CW = Conc autoclaved whey SN-ON= Supernatant of overnight culture

C = uninoculated whey 0, 6, 24 hours = incubation period MRS= MRS media uninoculated

Strain 1 = 697 strain *Lactiplantibacillus plantarum*

Strain 2 = 551 strain *Lactiplantibacillus plantarum*

SŪKALU PROTEĪNS PĒC FERMENTĀCIJAS. AR CBB-R250 KRĀSOTS 10% dPAAG-TRICĪNA GĒLS



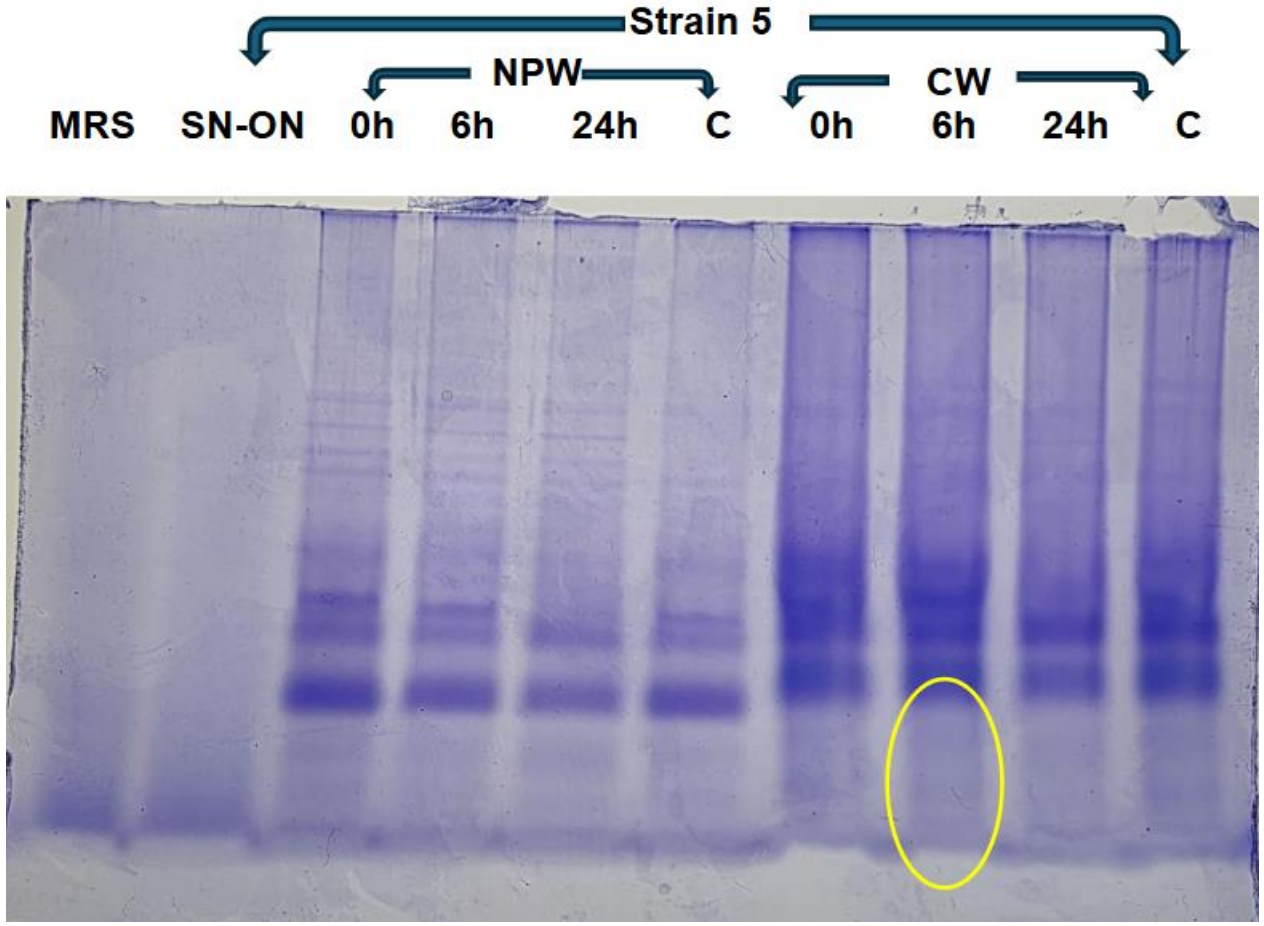
M = Marker NPW = Non-pasteurized autoclaved whey CW = Conc autoclaved whey SN-ON= Supernatant of overnight culture

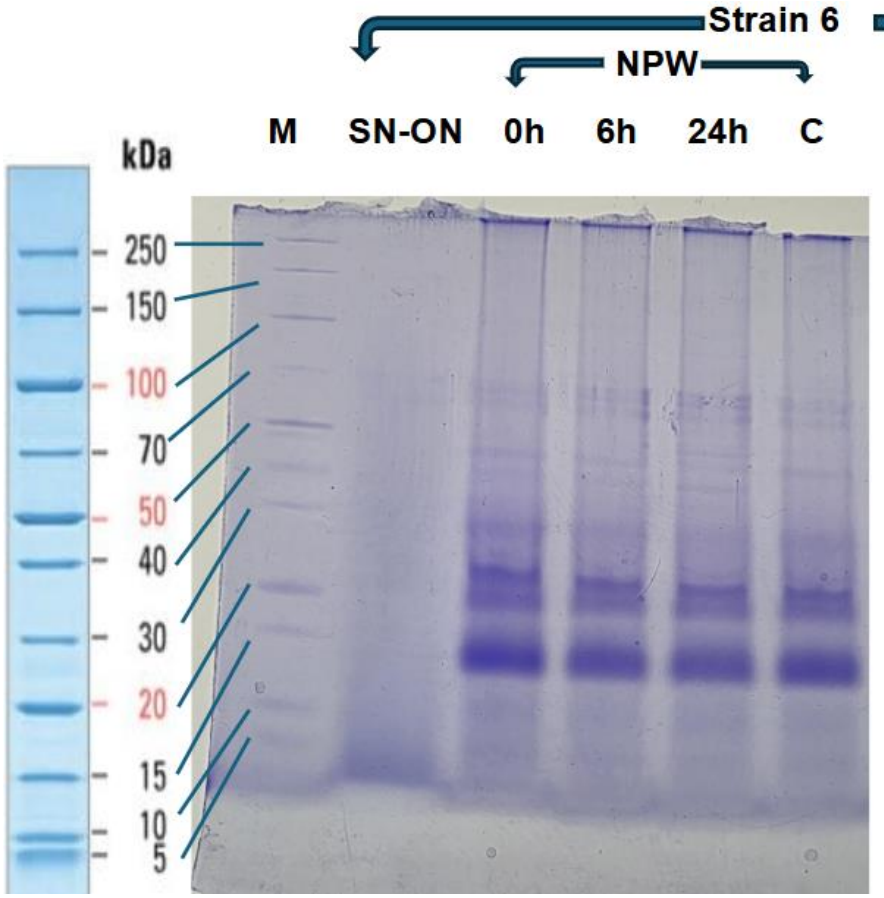
C = uninoculated whey 0, 6, 24 hours = incubation period MRS= MRS media uninoculated

Strain 3 = 176 strain *Lactococcus lactis subsp. lactis*

Strain 4 = 1277 strain *Lactobacillus johnsonii*

SŪKALU PROTEĪNS PĒC FERMENTĀCIJAS. AR CBB-R250 KRĀSOTS 10% dPAAG-TRICĪNA GĒLS





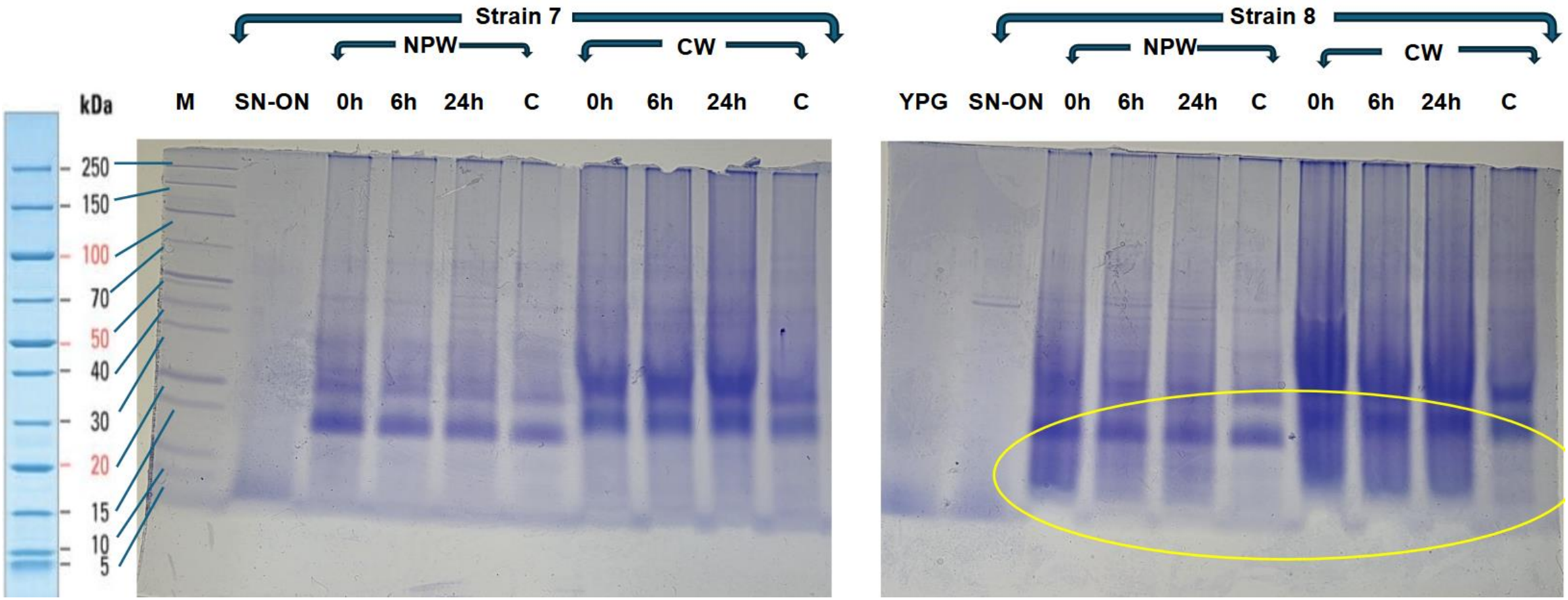
M = Marker NPW = Non-pasteurized autoclaved whey CW = Conc autoclaved whey SN-ON= Supernatant of overnight culture

C = uninoculated whey 0, 6, 24 hours = incubation period MRS= MRS media uninoculated

Strain 5 = 1479 strain *Limosilactobacillus fermentum*

Strain 6 = 1452 strain *Lacticaseibacillus paracasei*

SŪKALU PROTEĪNS PĒC FERMENTĀCIJAS. AR CBB-R250 KRĀSOTS 10% dPAAG-TRICĪNA GĒLS



M = Marker NPW = Non-pasteurized autoclaved whey CW = Conc autoclaved whey SN-ON= Supernatant of overnight culture

C = uninoculated whey 0, 6, 24 hours = incubation period YPG= YPG media uninoculated

Strain 7 = Industrial 150

Strain 8 (Yeast) = 286 strain *Kluyveromyces marxianus*

4. MIKROORGANISMU SKAITA DINAMIKA SŪKALU PREPARĀTU FERMENTĀCIJAS LAIKĀ

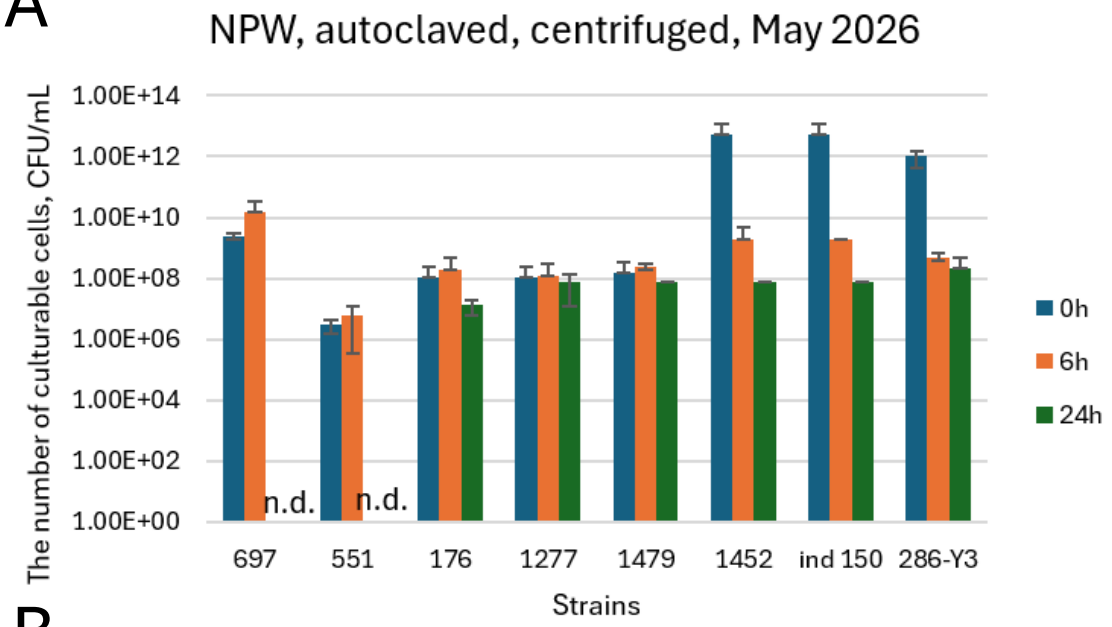
Dzīvotspējīgo šūnu koloniju veidojošo vienību (KVV) skaits uz agarizētas MRS barotnes atlasītajās LAB un LFY kultūrās 24 stundu ilgā inkubācijas laikā NPW un CW sūkalās vispārīgā veidā raksturo mikroorganismu spēju saglabāt fizioloģisko aktivitāti, kas nepieciešama arī BAS, AO un PF veidošanai.

Orientējošie KVV dinamikas rezultāti rāda (1) būtiskas atšķirības atlasīto baktēriju kultūru dzīvotspējā 24 stundas fermentējot sūkalu substrātus; (2) kopumā intensīvāku baktēriju skaita pieaugumu CW substrātā salīdzinājumā ar nelielu pieaugumu vai pat skaita samazināšanos, fermentējot NPW; (3) būtisku dzīvotspējīgu rauga šūnu samazinājumu kā NPW, tā arī CW fermentācijas laikā.

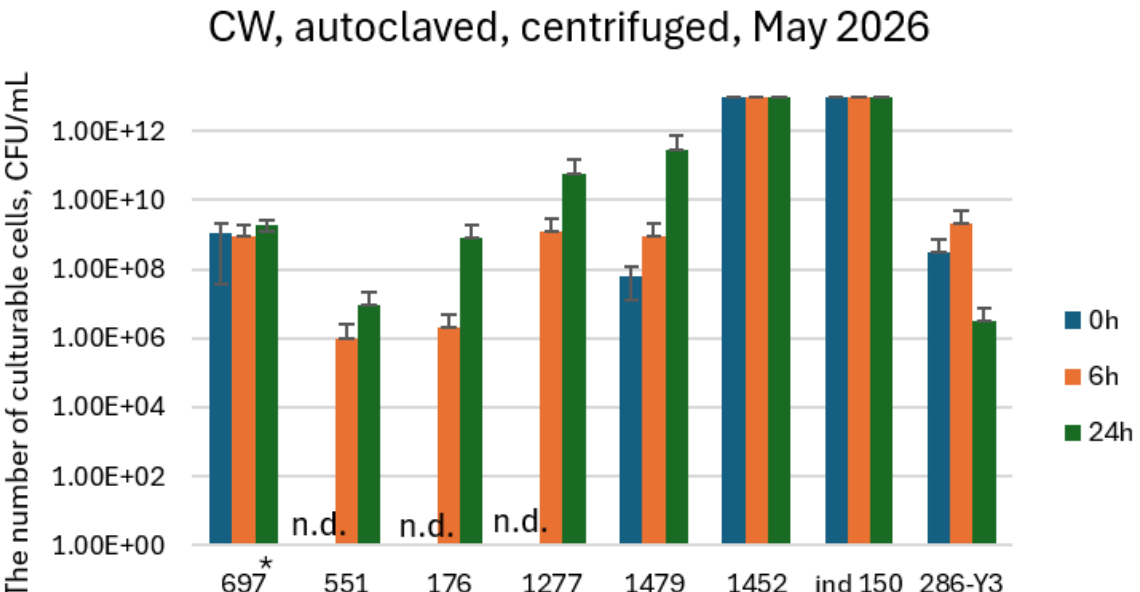
Lai arī KVV skaita noteikšana veikta trīs atkārtojumos katrā substrātā, eksperimentu nepieciešams atkārtot. Mikroorganismu dzīvotspējas faktors jāņem vērā, plānojot un vērtējot turpmākos fermentācijas eksperimentus.

BAKTĒRIJU UN RAUGU KVV SKAITA DINAMIKA FERMENTĀCIJAS SUBSTRĀTĀ 24 STUNDU LAIKĀ

A



B



The number of culturable cells in cultures of seven bacterial strains and one yeast strain during fermentation in autoclaved and centrifuged NPW (A) and CW (B). n.d. - not detected.
* - bacterial contamination of autoclaved CW was detected.

The number of culturable bacteria in NPW and CW after inoculation changed during fermentation from 0h to 24h. These changes were strain-specific and also depended on the type of whey (i.e., NPW or CW).
The highest initial CFU count was shown for strains “1452”, “ind150”, and 286-Y3, reaching up to 12-13 log after the resuspension of inoculum in NPW. After 6h and 24h of fermentation, the CFU counts have been reduced by 3 and 5 log.
The changes in the CFU count in CW showed another trend. Specifically, the strains “551”, “176”, and “1277” after resuspending in CW did not show a colony forming ability at 0h, while after 6h the CFU count of 6-9 LOG was recovered in these cultures, followed by further increase by 2 log values.

5. A/S “CESVAINES PIENS” IZMANTOTO SIERA IERAUGA KULTŪRU IETEKME UZ SŪKALU BIOĶĪMISKO SASTĀVU

Apkopoti un sistematizēti dati par galvenajiem industriālo ieraugu tipiem, kurus uzņēmums A/s «Cesvaines piens» izmanto siera ražošanā.

Apkopoti dati par 906 siera ražošanai izmantotajiem sūkalu paraugiem kopš 2025. gada 1, jūlija līdz 2026. gada 24.martam.

A/s «Cesvaines piens» lielākoties izmanto Chr. Hansen / Novonesis (Dānija) kontinentālo sieru ierauga kultūras. Ap 10% sieru izgatavošanai izmantoti arī *Mediterranea Biotech* (Francija) ieraugi.

Līdz šim veiktā analīze parāda, ka industriālās ierauga kultūras vairāk iespaido sūkalu tauku, mazāk proteīna vai laktozes saturu.
No pārējiem ieraugiem būtiski atšķirīgu sūkalu sastāvu iegūst, izmantojot ieraugus, kuru sastāvā iekļauts firmas Hansen DCC265 vai DCC260 kultūru maisījums.

CHR. HANSEN / NOVONESIS INDUSTRIĀLO IERAUGU KLASIFIKĀCIJA

O kultūras ir tikai skābi veidojošās baktērijas; **L** kultūras satur *Leuconostoc* aromāta veidošanai; **LD** kultūras satur gan *Leuconostoc*, gan *L. lactis biovar diacetylactis* un ir tradicionālās Gouda/Edam kultūras; **D** kultūras galvenokārt izmanto aromāta veidošanai.

Kultūras tips	Sastāvs	Raksturojums	Tipiski sieri
O	*Lactococcus lactis* subsp. *lactis* un *cremoris*	Homofermentatīvas, neveido CO_2	Cheddar, Feta, Cottage cheese
L	O + *Leuconostoc* spp.	Veido aromātu un nelielu CO_2 daudzumu	Edam, Gouda
D	O + *Lactococcus lactis* biovar *diacetylactis*	Izteikta diacetila (sviesta) aromāta veidošanās	Svaigie un krējuma sieri
LD	O + *Leuconostoc* + *diacetylactis*	Aromāts + CO_2 + acu veidošanās	Gouda, Edam, Havarti, Danbo
ST	*Streptococcus thermophilus*	Termofīlas kultūras	Mozzarella, Pasta filata
LH/LB	*Lacticaseibacillus* (agrāk *Lactobacillus*) spp.	Nogatavināšanas kultūras	Cietie sieri
Propionibacterium	Propionskābes baktērijas	Acu veidošanās, riekstu aromāts	Emmental, Maasdam

CHR. HANSEN / NOVONESIS INDUSTRIĀLO IERAUGU NOMENKLATŪRA

Kultūra	tips	Galvenie mikroorganismi	Raksturojums
Flora C150, 170, 190	O	*Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*	Homofermentatīva, neveido CO_2, maiga garša, slēgta siera struktūra.
BS20, 30, 40	O	*Lactococcus lactis*, *subsp. lactis*	Mērens aromāts, neliela CO_2 veidošanās, Gouda/Edam tipa sieri.
PS 80	N/A	*Propionibacterium freudenreichii*	Emmental, Maasdam
CSK L100	D	*Lactobacillus helveticus*	Intensīvāka garša un acu veidošanās.
DCC 280, 265	D vai LD	*Lactobacillus helveticus*. *Lactobacillus paracasei*. *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis, Lactococcus lactis subsp. lactis, Leuconostoc species, Streptococcus thermophilus*	Paredzēta diacetila (sviesta aromāta) veidošanai.

A/s «Cesvaines piens» lielākoties izmantotais *Mediterranea Biotech* (Francija) ieraugi pēc LAB sugu sastāva atbilst Hansen DCC ieraugiem

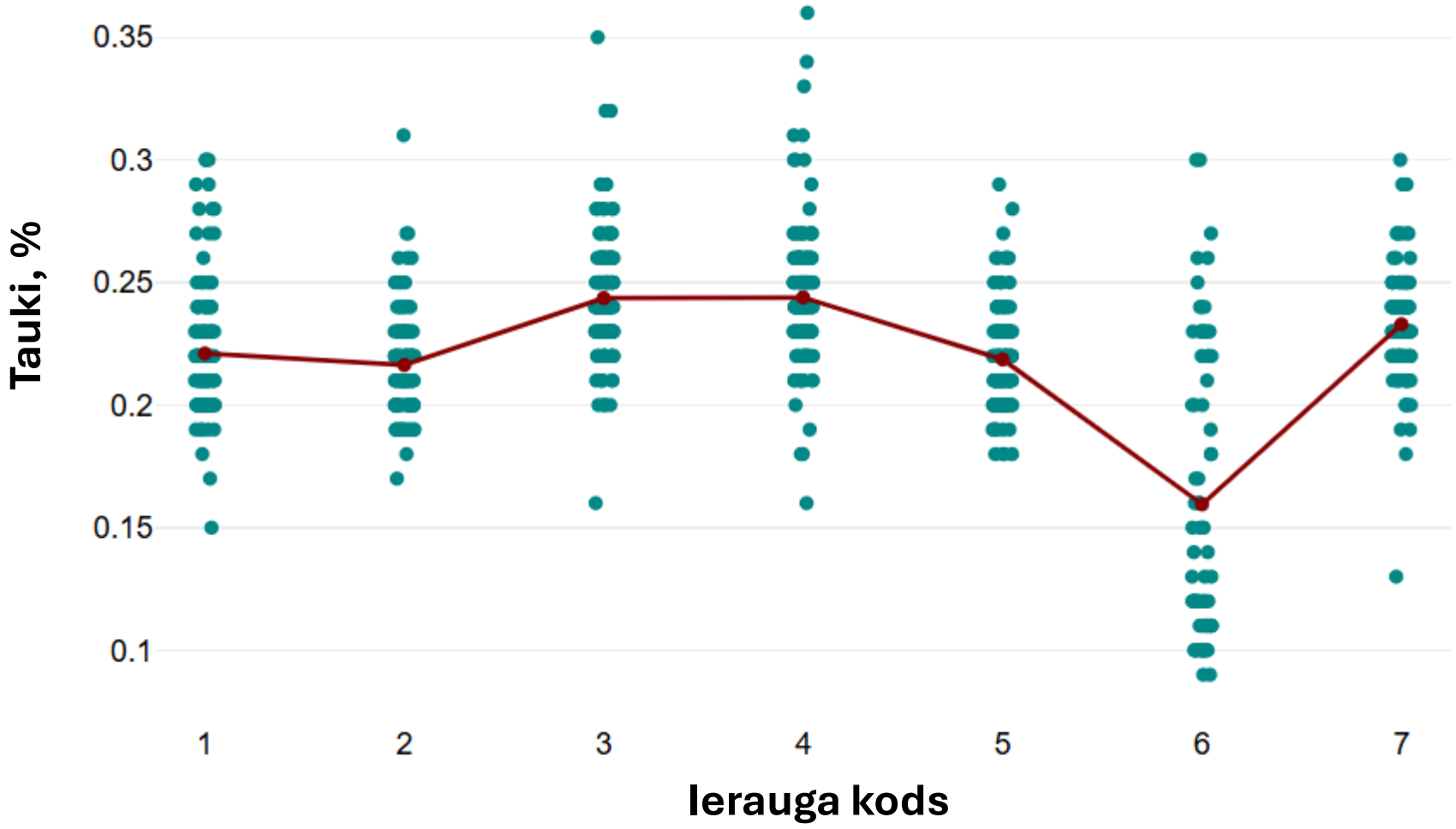
A/SČESVAINESPIENS»SIERA RAŽOŠANĀ IZMANTOTIE IERAUGI

Kods	Ierauga nos.	Ražotājs	LAB sugas
1	FloraC170, BSXX	H	*Lactococcus lactis subsp. cremoris*
			Lactococcus lactis subsp. lactis
2	Flora 190(150);BSXX	H	*Lactococcus lactis subsp. cremoris*
			Lactococcus lactis subsp. lactis
3	1 + CSK L100	H	*pap. Lactobacillus helveticus*
4	2 + CSK L100	H	*pap. Lactobacillus helveticus*
5	PS80 saturošs	H	*Propionibacterium freudenreichii*
6	DCC 265(260) bāze	H	*Lactobacillus helveticus*
			Lactobacillus paracasei
			Lactococcus lactis subsp. cremoris
			Lactococcus lactis subsp. lactis biovar. diacetylactis
			Lactococcus lactis subsp. lactis
			Leuconostoc species
			Streptococcus thermophilus
7	TV-G	MB	*Lactococcus lactis subsp. lactis*
			Lactococcus lactis subsp. lactis biovar diacetylactis
			Lactococcus lactis subsp. cremoris
			Leuconostoc mesenteroides subsp. mesenteroides
			Streptococcus themophilus

H Hansem

MB Mediterranea Biotech

AR DAŽĀDIEM IERAUGIU TIPIEM IEGŪTO S\UKALU TAUKU SATURS (vienvirziena ANOVA)

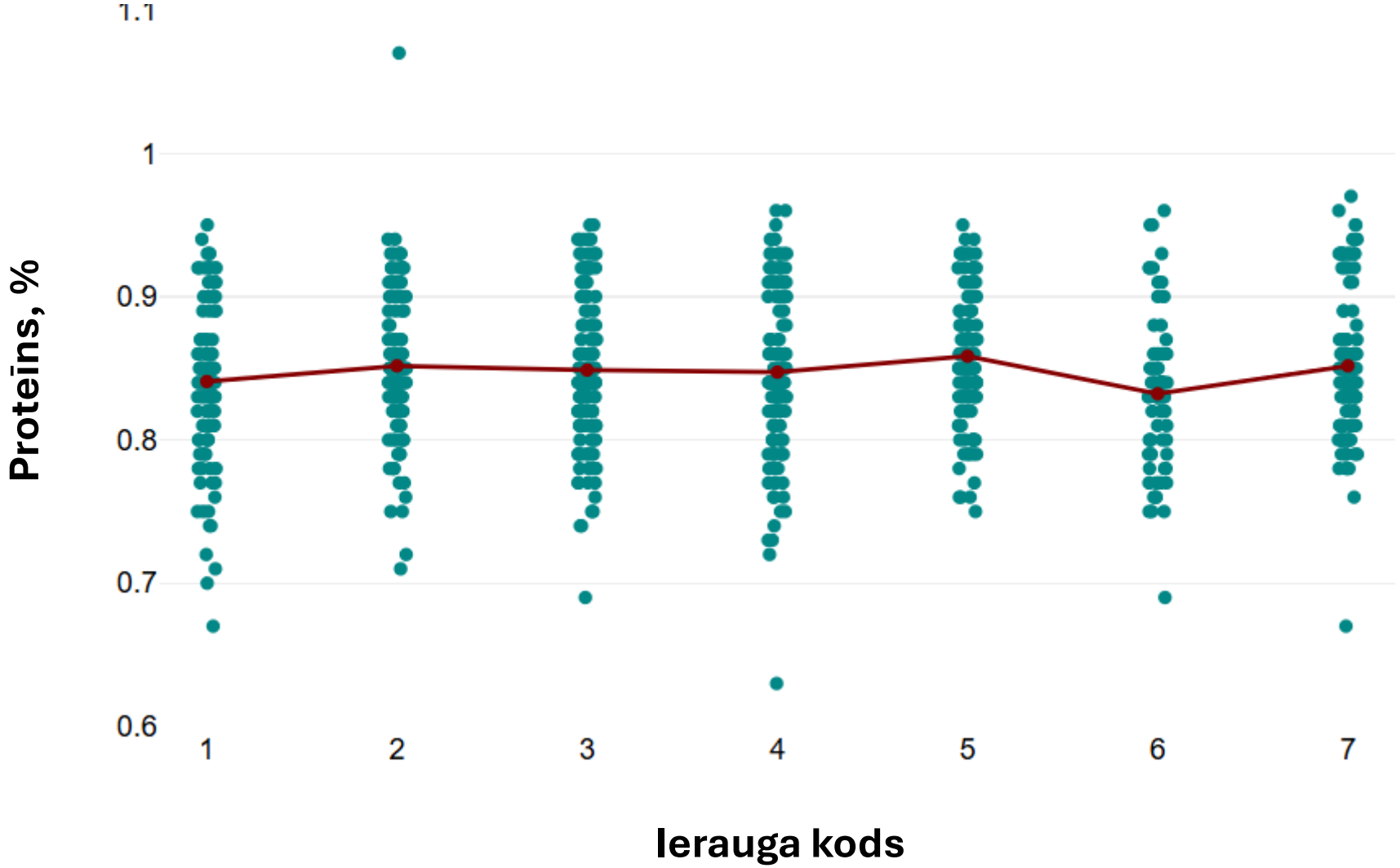


Fisher's Least Significant difference

Copy Explain this table

Variables	Mean Difference	t	p
5 - 2	0	0.67	.501
5 - 1	0	-0.7	.486
5 - 4	-0.03	-7.67	<.001
5 - 7	-0.01	-4.14	<.001
5 - 3	-0.02	-7.62	<.001
5 - 6	0.06	14.96	<.001
2 - 1	0	-1.34	.18
2 - 4	-0.03	-8.24	<.001
2 - 7	-0.02	-4.74	<.001
2 - 3	-0.03	-8.19	<.001
2 - 6	0.06	14.22	<.001
1 - 4	-0.02	-6.73	<.001
1 - 7	-0.01	-3.35	.001
1 - 3	-0.02	-6.68	<.001
1 - 6	0.06	15.24	<.001
4 - 7	0.01	3.24	.001
4 - 3	0	0.08	.933
4 - 6	0.08	21.69	<.001

AR DAŽĀDIEM IERAUGIU TIPIEM IEGŪTO S\UKALU PROTEĪNA SATURS (vienvirziena ANOVA)



Fisher's Least Significant difference

Copy Explain this table

Variables	Mean Difference	t	p
5 - 2	0.01	1.09	.275
5 - 1	0.02	2.83	.005
5 - 4	0.01	1.87	.062
5 - 7	0.01	1.08	.279
5 - 3	0.01	1.64	.102
5 - 6	0.03	3.68	<.001
2 - 1	0.01	1.72	.085
2 - 4	0	0.72	.469
2 - 7	0	0	.998
2 - 3	0	0.49	.622
2 - 6	0.02	2.7	.007
1 - 4	-0.01	-1.07	.284
1 - 7	-0.01	-1.71	.088
1 - 3	-0.01	-1.31	.192
1 - 6	0.01	1.17	.24
4 - 7	0	-0.71	.475
4 - 3	0	-0.24	.808
4 - 6	0.02	2.15	.031

6. SŪKALU PĒCFERMENTIJA IZMANTOJOT INDUSTRIĀLO IERAUGU UN PAPILDU BARĪBOTNES KOMPONENTU PIEVIENOŠANU

Siera sūkalas pēc siera graudu atdalīšanas satur pietiekami lielu daudzumu dzīvu ierauga kultūras mikroorganismu, kas dod iespēju izmantot to klātbūtni fermentācijas procesa turpināšanai, jeb pēcfermentācijai.

Sāda pieeja ir tehnoloģiski vienkārši īstenojama, taču tās efektivitātes paugstināšanai nepieciešams bagātināt sūkalu substrātu ar barības komponentiem,kas (1) papildina sastāvu ar oglekļa, slāpekļa un enerģijas avotiem; (2) padara s;ukalās esošos savienojumus vieglāk izmantojumus vai pieejamus tur saglabājušamies mikroorganismiem un stimulē to bioķīmisko aktivitāti; (3) regu’’e substrāta pH; (4) novērš nejaušas, kontaminējošas mikrofloras attīstību.

Salīdzināta (1) rauga ekstrakta, (2) melases, (3) itakonskābes, (4) spirta, (5) amonija sulfāta (6) MRS barotnes un deterģenta Tween-80 pievienošanas ietekme termiski neapstrādātā NPW substrātā uz tur jau klātesoša industriālā ierauga spēju producēt BAS un AO, kā arī, antimikrobiālo aktivitāti 6 un 24 stundu pēcfermentācijā.

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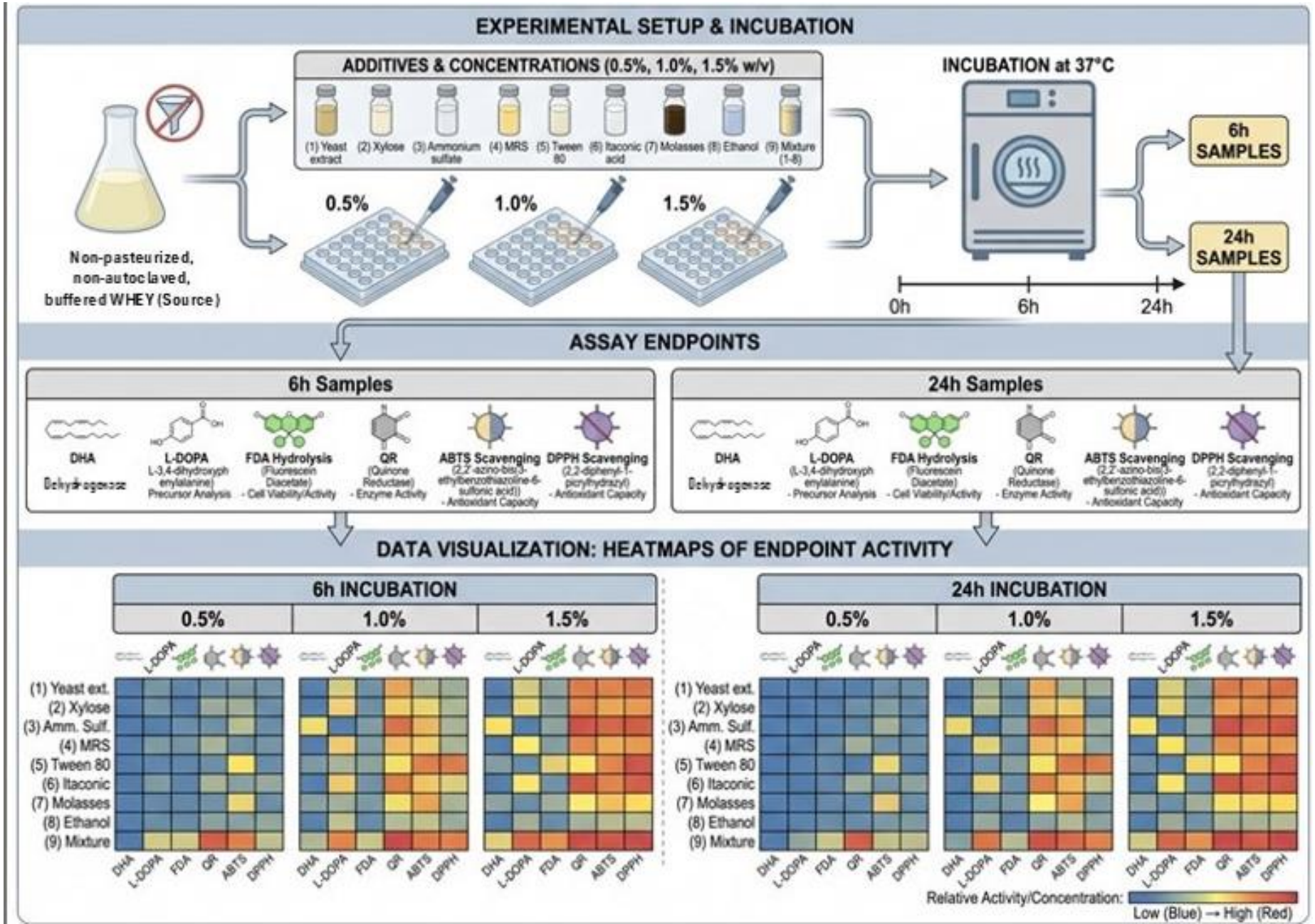
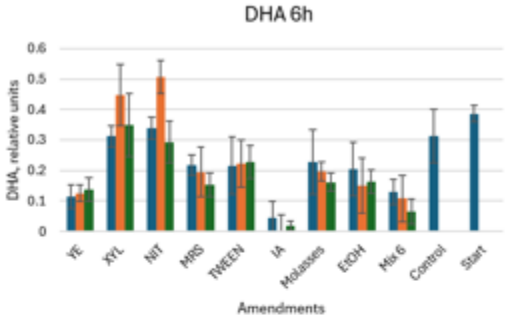
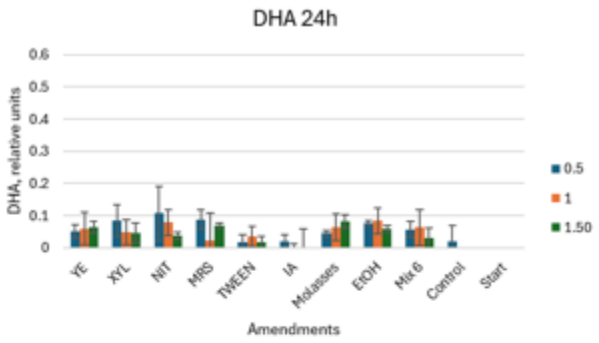
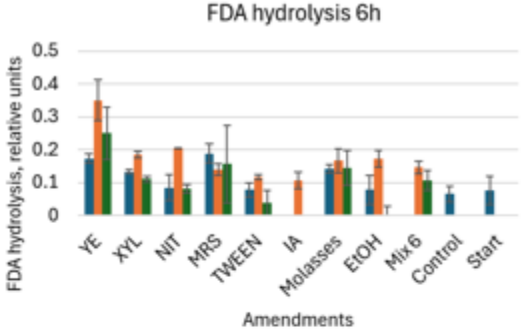
EKSPERIMENTA SHĒMA PĒCFERMENTĀCIJAS BARONES SASTĀVA OPTIMIZĒŠANAI

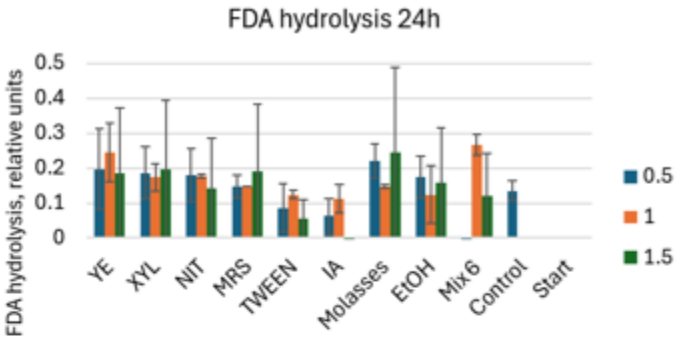


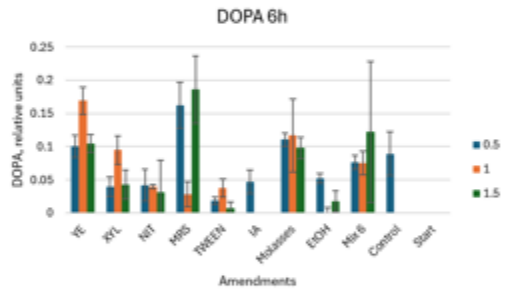
INDUSTRIĀLĀ IERAUGA BAS UN AO SINTĒZES AKTIVITĀTE NPW SUBSTRĀTĀ 6 UN 24 STUNDU LAIKĀ DAŽĀDU PIEDEVU KLĀTBŪTNĒ.

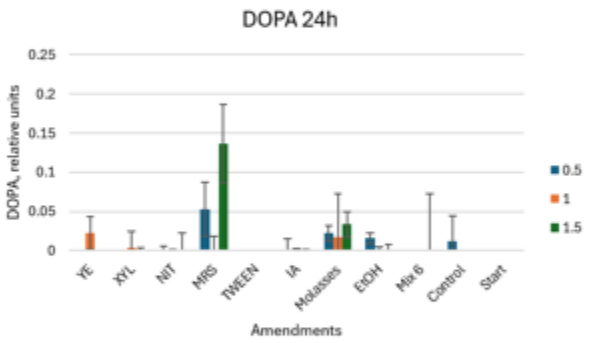


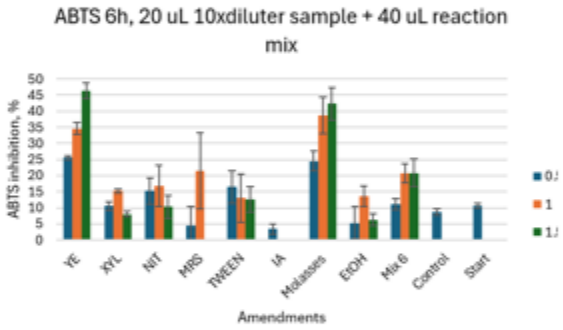


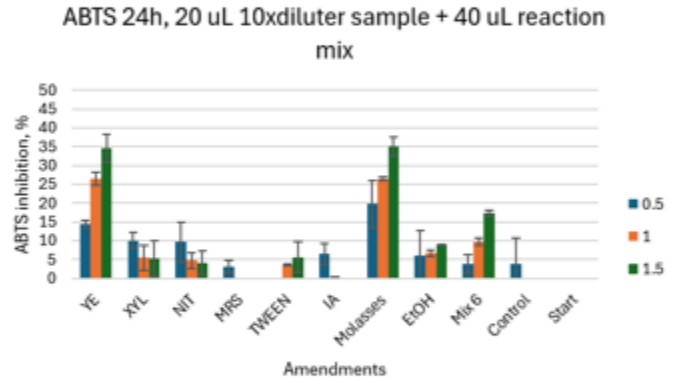


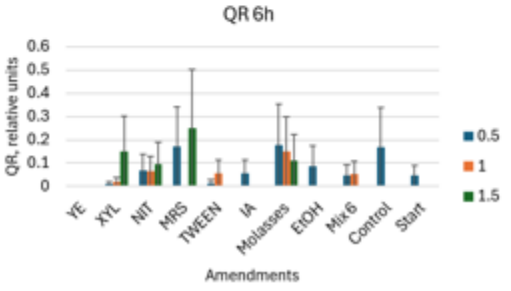


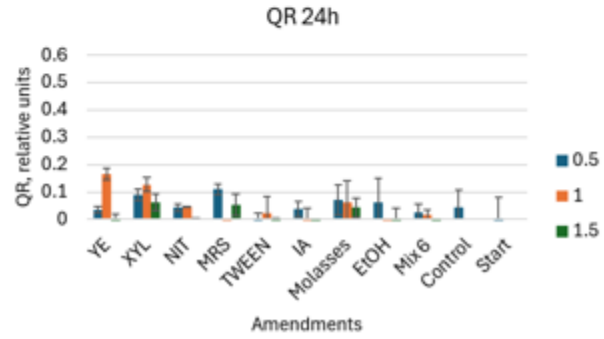


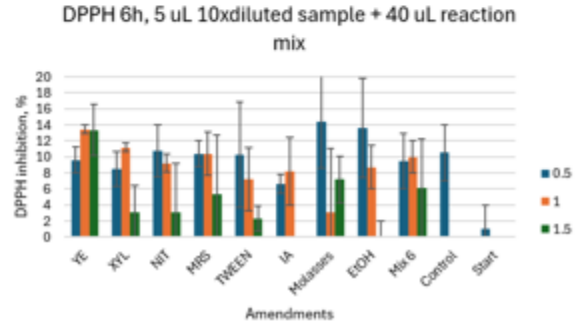


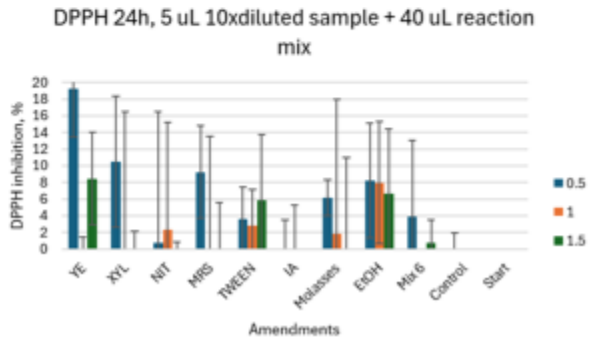








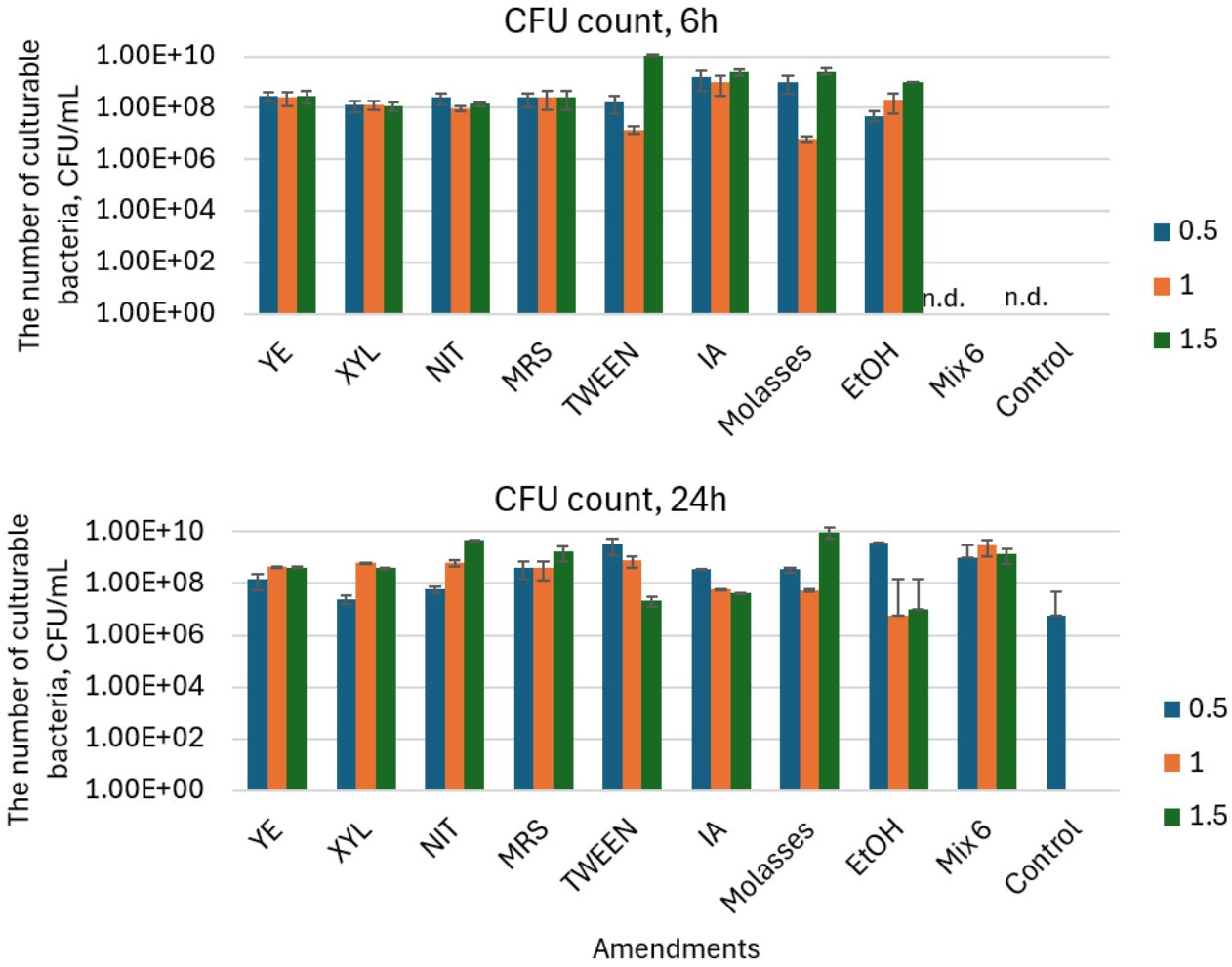




INDUSTRIĀLĀ IERAUGA BAS UN AO SINTĒZES AKTIVITĀTE NPW SUBSTRĀTĀ 6 UN 24 STUNDU LAIKĀ DAŽĀDU PIEDEVU KLĀTBŪTNĒ.

	DHA			DOPA			QR			FDA			ABTS			DPPH		
6h (24h)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
YE	0.177667	0.349333	0.188667	0.100333	0.169	0.104667	-0.0575	0.0005	-0.063	0.174	0.351667	0.250667	25.53333	34.6	46.33333	9.615385	13.46154	13.33333
XYL	0.403	0.622333	0.454333	0.04	0.095	0.042667	0.0095	0.0195	0.151	0.1335	0.1865	0.112	10.53333	15.2	8	8.461538	11.15385	3.076923
NIT	0.46	0.61	0.460333	0.042	0.04	0.031667	0.0685	0.065	0.094333	0.0855	0.205	0.082333	15.26667	16.8	10.33333	10.76923	9.230769	3.076923
MRS	0.298667	0.044667	0.237	0.162	0.028	0.185667	0.171	-0.043	0.251667	0.189	0.14	0.156667	4.533333	21.46667	-3.46667	10.38462	10.38462	5.384615
TWEEN	0.424333	0.519	0.205	0.018667	0.037667	0.007667	0.013667	0.057333	-0.07033	0.078667	0.117667	0.039667	16.46667	13	12.66667	10.25641	7.179487	2.307692
IA	0.034	0.004333	-0.08733	0.047333	-0.08667	-0.18933	0.057333	-0.03933	-0.132	-0.05233	0.107	-0.22133	3.4	-6.33333	-8.26667	6.666667	8.205128	-2.05128
Molasses	0.415	0.421667	0.266	0.110667	0.116667	0.098	0.176333	0.149333	0.111667	0.142	0.167	0.145333	24.46667	38.6	42.33333	14.35897	3.076923	7.179487
EtOH	0.557	0.468	0.142	0.052	-0.00767	0.017667	0.087667	-0.044	-0.021	0.078667	0.173	-0.00733	5.333333	13.6	6.266667	13.58974	8.717949	-0.51282
Mix6	0.325333	0.336	0.298	0.076667	0.075	0.122	0.046	0.053333	-0.00967	-0.05167	0.147	0.106333	11.13333	20.66667	20.8	9.487179	10	6.153846
Control	0.493333			0.089333			0.169333			0.065667			8.733333			10.51282		
Start	0.272667			-0.00667			0.045333			0.077333			10.66667			1.02		
24h (24h)																		
YE	-0.25867	0.144333	0.285333	-0.06267	0.022333	-0.07567	0.034	0.1665	-0.03567	0.1965	0.245667	0.186	14.46667	26.53333	34.6	19.23077	-5.64103	8.461538
XYL	0.229333	0.147333	0.282333	-0.022	0.003333	-0.019	0.091	0.1275	0.061333	0.186	0.174	0.196333	10	5.466667	5.4	10.51282	-1.53846	-3.84615
NIT	0.262667	0.198	0.200667	-0.01867	-0.00133	-0.02467	0.043	0.046	-0.00267	0.179333	0.177	0.142333	9.666667	4.8	4.133333	0.769231	2.307692	-10
MRS	0.249333	0.277	0.269	0.052333	-0.00033	0.136667	0.1095	-0.0425	0.054333	0.1475	0.147	0.191	3.2	-3.26667	-10.3333	9.230769	-9.23077	-0.25641
TWEEN	0.163667	0.218	0.091333	-0.017	-0.02067	-0.01467	-0.02367	0.024	-0.027	0.086	0.124	0.054333		3.6	5.666667	3.589744	2.820513	5.897436
IA	-0.01633	0.435	-0.12867	-0.00267	-0.013	-0.03733	0.038667	-0.02967	-0.156	0.063	0.111333	-0.09067	6.466667	-6.53333	-18.8	-4.35897	-0.51282	-2.5641
Molasses	0.143667	0.504667	0.130667	0.022	0.017333	0.033667	0.072	0.062667	0.043667	0.219667	0.148	0.243667	19.8	26.46667	35.06667	6.153846	1.794872	-7.69231
EtOH	0.215333	0.299	0.054	0.015667	-0.011	-0.00833	0.061667	-0.10333	-0.03267	0.173667	0.123333	0.157667	6	6.733333	8.933333	8.205128	7.948718	6.666667
Mix6	-0.027	0.222333	0.272667	-0.01633	-0.047	-0.03367	0.024333	0.016	-0.02733	-0.038	0.265667	0.121333	3.866667	9.733333	17.33333	3.846154	-4.61538	0.769231
Control	0.301333			0.011667			0.045333			0.134333			3.933333			-1.53846		
Start																		

INDUSTRIĀLĀ IERAUGA KVV SKAITA DINAMIKA PAPILDINĀTAJĀ NPW SUBSTRĀTĀ



The number of culturable bacteria in the industrial starter culture after its fermentation in non-pasterized whey during 6h (A) and 24h (B) in the presence of different amendments. n.d. -not determined.

The number of CFU in ISC after 6h and 24h varied in the range from 6 to 9 LOG. No considerable changes were found in sets with different amendments and their concentrations, as well as fermentation time. The number of CFU in the non-amended ISC after 24 fermentation in NPW was 6.00E+06 CFU/mL.

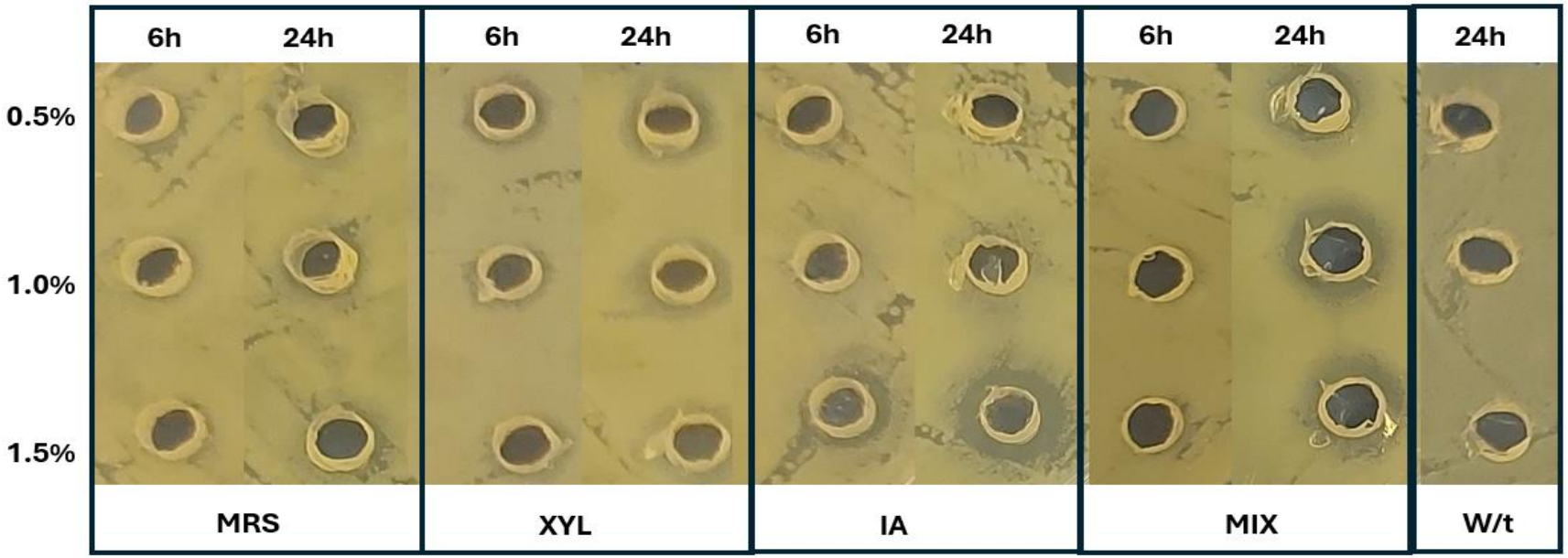
A comparatively higher CFU count was detected after 6h fermentation in the sets with Tween, IA, and MOL, while after 24h - with ammonium sulfate, Tween, and MOL, respectively. Additional testing on the changes in the nubmer of culturable bacteria, as a response to specific treatment, is needed.

ISC – Industrial Starter Culture
NPW – Non-Pasterized Whey
AST - Antimicrobial Sesceptibility Testing
YE - yeast extract
XYL - xylose
NIT - $(NH_4)_2SO_4$

IA - itaconic acid
MOL - molasses
W/t - without amendments

AR INDUSTRIĀLO IERAUGU PĒCFERMETĒTA NPW SUBSTRĀTA ANTIBAKTERIĀLĀ AKTIVITĀTE

Antimicrobial activity of ISC after 6h and 24h fermentation towards *Escherichia coli* MSCL 536* was tested in a 24h antimicrobial susceptibility testing (AST) in (i) Mueller-Hinton agar, which is primarily used as a standard microbiological growth medium for AST, and (ii) Plate Count Agar, which is typically used for general bacterial enumeration and can be adapted for antimicrobial testing to determine viability. The results showed that PCA had higher sensitivity in revealing the antimicrobial properties of ISC cultures. The effect of amendments on the antimicrobial activity of ISC towards E. coli MSCL 536 varied as follows: IA ≥ MIX > XYL ≥ MRS (comparison is based on a 1.5% amendment and 24h fermentation).



Antimicrobial activity of ISC after 6h and 24h incubation at 37 °C in NPW amended with MRS, xylose (XYL), itaconic acid (IA), mixture of six substrates (YE, XYL, NIT, MRS, Tween-80, IA, MOL) in 0.5%, in concentrations of 0.5%, 1.0%, and 1.5%.

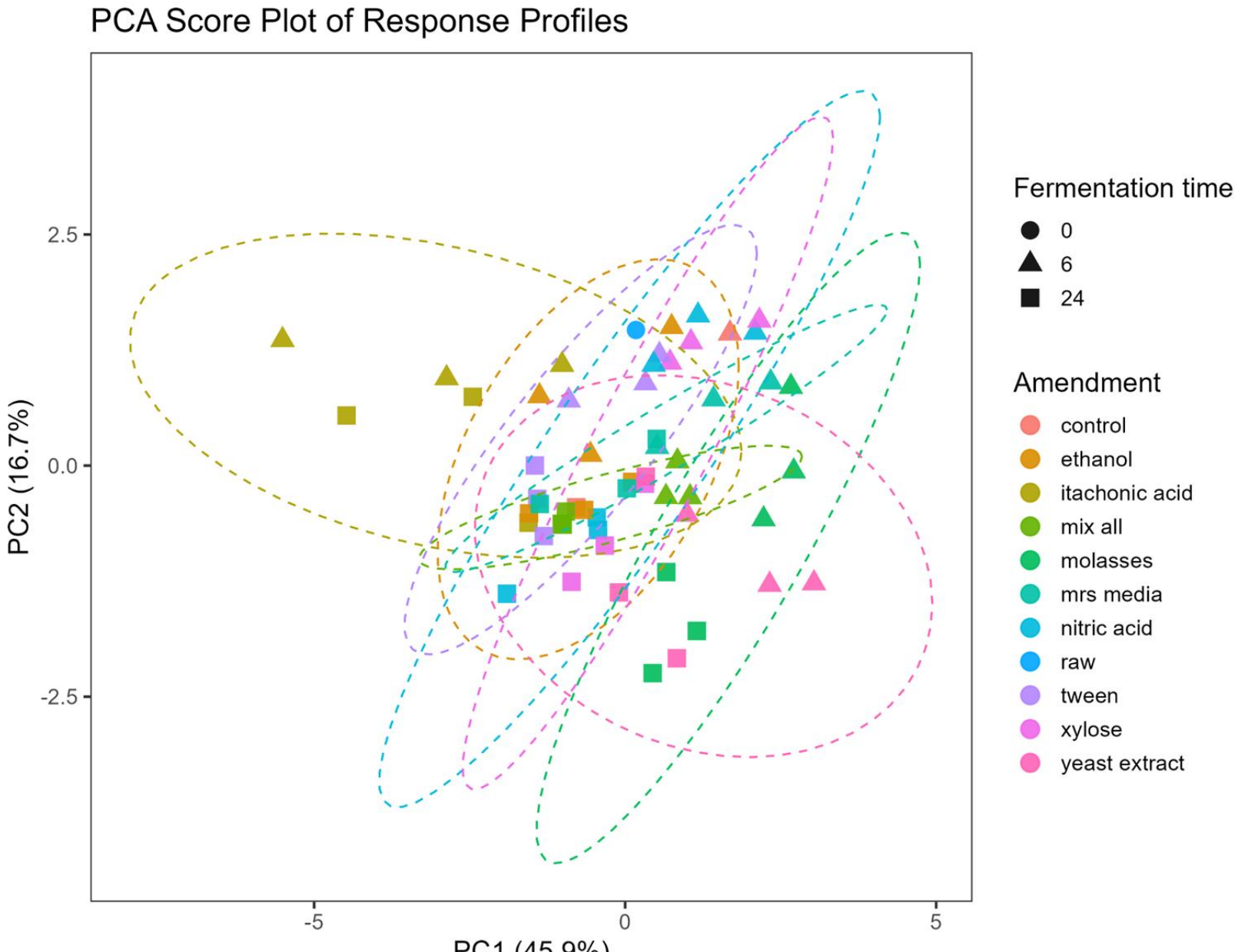
7. BŪTISKĀKIE PĒCFERMENTĀCIJAS AKTIVITĀTI IESPAIDOJOŠIE FAKTORI

Pēcfermentāacijas substrāta papildināšanas rezultātu statistiskais izvētyējums veikts ar Galveno komponentu analīzes (PCA) pieejas palīdzību.

PCA (Principal Component Analysis) ir multivariāta datu dimensiju novērtēšanas metode, kas ļauj vizualizēt paraugu līdzības un atšķirības.
PCA score plot:

1. novērtē datu dispersiju veidojošo elementu īpatsvaru;
2. identificē iedarbību klasterus;
3. atrod kopā vai klasterī neiederīgo datu punktus;
4. pārbauda grupu salīdzinājuma statistikas rezultātus.

UZ SUBSTRĀTA PIEDEVĀM REAKCIJU VEIDOJOŠO GALVENO KOMPONENTU STATISTISKĀ ANALĪZE PĒCPERMENTĀCIJAS EKSPERIMENTĀ

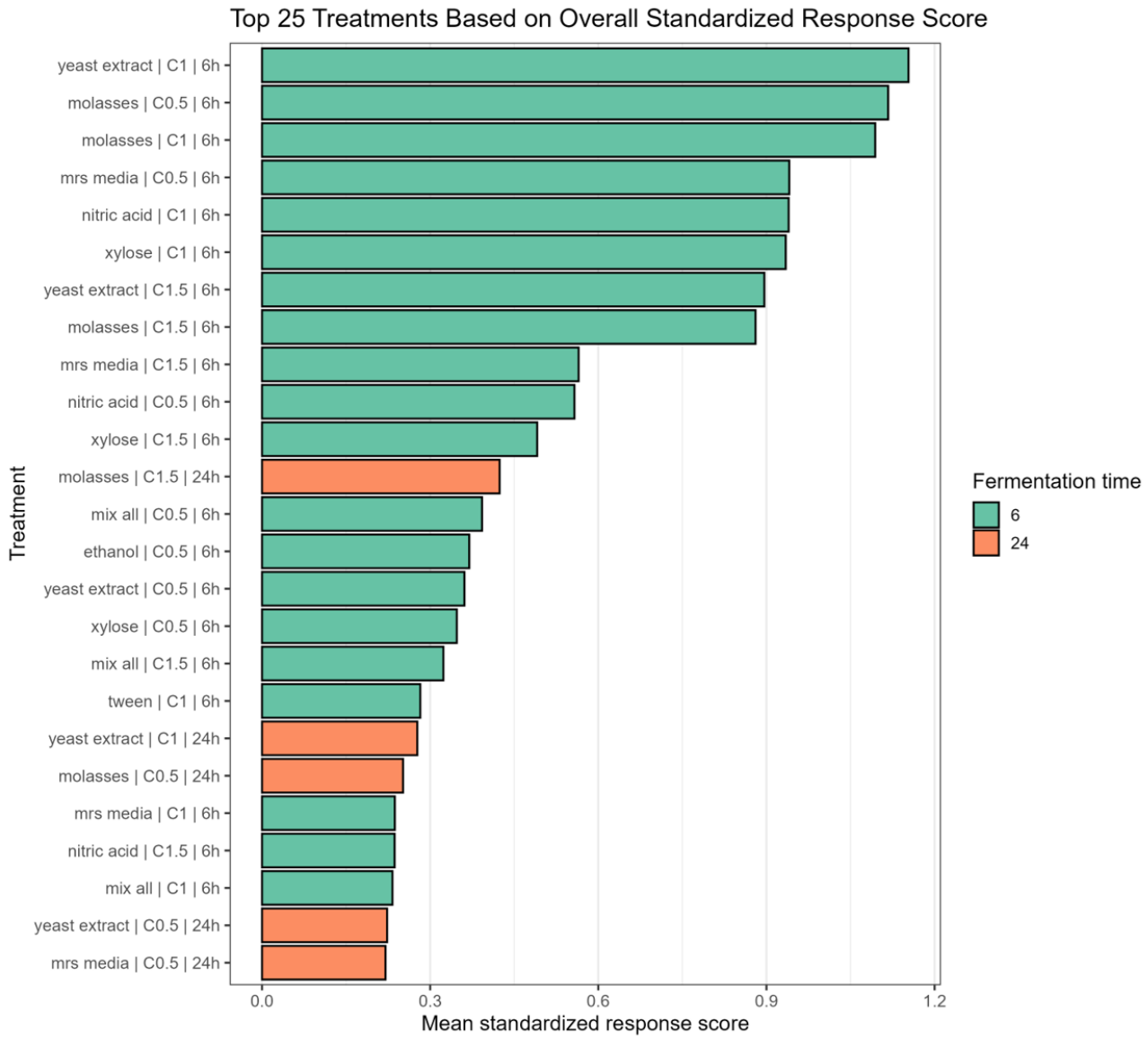


The first two principal components explained 62.6% of the total variation, with PC1 accounting for 45.9% and PC2 accounting for 16.7%.

The PCA score plot showed that **itaconic acid-treated samples were clearly separated from most other treatments along PC1**, indicating a distinct response profile. In contrast, the majority of the amendments exhibited considerable overlap, suggesting similar overall responses. **Molasses-treated samples tended to cluster toward the positive end of PC1, indicating a response pattern distinct from that of** the control and several other treatments.

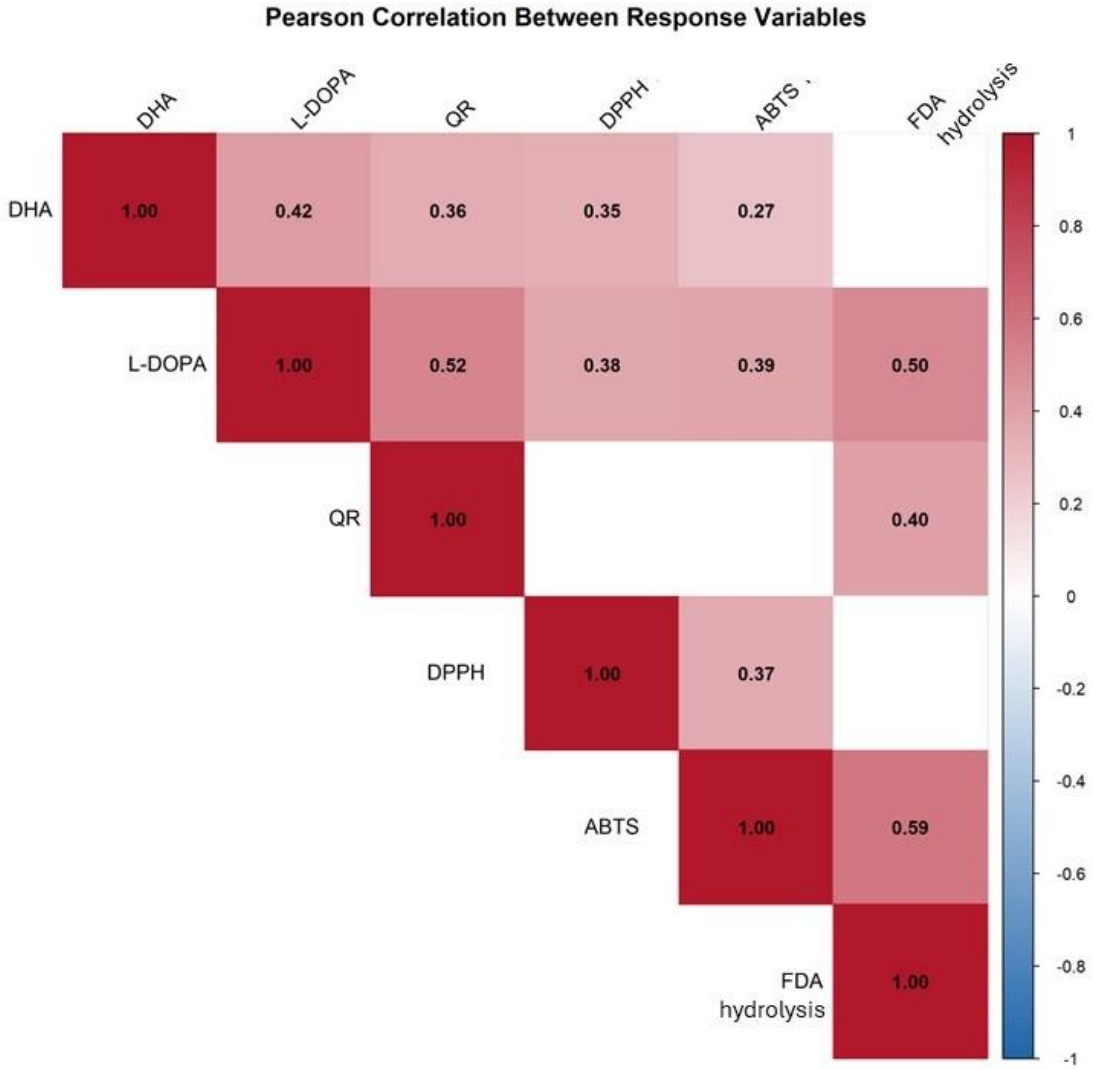
The distribution of sample shapes (representing fermentation times of 0, 6, and 24 h) showed substantial overlap within treatment groups, suggesting that **amendment type had a stronger influence on the response profiles than fermentation time.**

PĒCFERMENTĀCIJAS SUBSTRĀTA PIEDEVU KOPĒJĀS IEDARBĪBAS STANDARTIZ;ETAIS SADALĪJUMS



The highest overal standardized response was detected after 6h fermentation in sets with 1.0% yeast extract, as well as 0.5% and 1.0% molasses.

PĪRSONA NEPARAMETRISKĀ KORELĀCIJA STARP INDUSTRIĀLĀ IERAUGA KULTŪRAS SINTEZĒTAJIEM BAS UN AO PĒC NPW SUBSTRĀTA SASTĀVA PAPILDINĀŠANAS



The highest correlation between activities of the tested enzyme groups has been revealed in the following pairs:

- ABTS vs FDA hydrolysis (0.59)
- L-DOPA vs QR (0.52)
- L-DOPA vs FDA hydrolysis (0.50)

8. PUBLICITĀTES PASĀKUMI: ZIŅOJUMI STARPTAUTISKAJĀS KONFERENCĒS

Dzīvības zinātņu konference "The COINS 2026“ Viļņas universitātē, Lietuvā

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VALORISATION OF CHEESE WHEY BY PROLONGING FERMENTATION WITH INDUSTRIAL STARTER CULTURES

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Introduction

Around 70-90% of milk used in cheese production is converted into whey. Cheese whey is rich in organic and mineral substances, yet it is only partially used to manufacture high-value-added products [1]. Instead, nearly half of the cheese whey produced in Latvia is discarded, straining wastewater treatment facilities because of its high BOD and increasing waste disposal costs for industry [2, 3]. The capital expenditure (CAPEX) required to establish modern whey-processing lines for permeate, protein, or concentrate production, along with the resulting energy costs, is too high for many SMEs in the dairy business [4].

During cheesemaking, lactic acid bacteria (LAB) are added to milk as a starter culture, along with calcium chloride and a rennet complex [5]. Here, we report an approach to leverage the potential of LAB retained in whey after curd separation, without a substantial increase in processing cost or the need for sophisticated equipment. The approach can be developed to produce new fermented, whey-based beverages.

Table 1. Physicochemical characteristics of NPCW.

Dry weight, %	Lipids %	Proteins, %	Lactose, %	pH
6.51	0.15	0.83	4.62	5.8

Table 2. Experiment setup.

Amendments	NPCW, mL	MRS 10x stock, mL	Phosphate buffer 500 mM, pH 7.52, mL	Sterile water, mL
N	8.5	0	0	1.5
MRS	8.5	0.5	0	1
B	8.5	0	0.5	1
B+MRS	8.5	0.5	0.5	0.5

Materials and methods

Non-pasteurized whey was obtained from the first milk coagulation phase of cheese production (JSC "Cesvaines piens"), physicochemical characteristics were read by using Milcoscan™ (**Table 1**). It contained starter cultures BS-30 and DCC-265 (Chr. Hansen): blends of Lactococcus, Lactobacillus, Leuconostoc, and Streptococcus strains. After the coagulation, a considerable amount of starter culture (4.64×10^9) remains in the NPCW. We used a complex LAB growth medium, De Man-Rogosa-Sharpe agar (MRS), and K-Na-phosphate buffer to increase the pH of the acidified NPCW, to restart the initial bacterial culture (**Table 2**) and initiate bacterial growth and enzyme production. Use of fluorescein diacetate (FDA) hydrolysis, quinone reductase (QR) activity assay were used to ascertain enzymatic activity (**Figure 1**). Effect of carbon based substrates were also assessed by the use of Ecoplate™, and enzymatic activity of the NPCW bacteria were noted after three hours using FDA hydrolysis, QR activity and Levodopa (L-DOPA) accumulation assays (**Figure 2**).

Results

Supplementing acidified NPCWas with 50% MRS and raising the pH to 6.5 reinitiated the growth of the starter bacterial culture as shown by the FDA hydrolysis and OD changes. EcoPlate™ (Biolog, USA) demonstrated the capacity of bacteria in NPCW to utilize D-xylose, Itaconic acid, Tween 40, Tween 80, and alpha-cyclodextrin as the sole carbon source. QR activity and L-DOPA conversion indicated antioxidant properties, and the synthesis of biologically active compounds within three hours of NPCW supplementation. The temperature- and time-dependence of these features was characterized.

Overall, this first step into the topic suggests a practical and affordable way for dairy producers to make better use of cheese whey. With some optimization, it could lead to simple fermented whey-based beverages and help cut down on waste - all without the need for major investments or complicated equipment.

Figure 1. Culture growth (A,B,C,D), FDA hydrolysis activity (F,G,H,I), and QR activity (E) during NPCW fermentation at 37 °C and 42 °C. Measurements were performed with a rinsed biomass. Supplements: N – non-supplemented; MRS – MRS broth; B – buffer; MRS+B – buffer and MRS. Concentrations of supplements as indicated in Table 2.

Figure 2. Enzymatic activity of NPCW bacteria after 3h fermentation at 30 °C in EcoPlate™. QR – quinone reductase; FDA hydrolysis – fluorescein diacetate hydrolysis; L-DOPA – levodopa. Period of incubation of reaction mixture 19h at 30 °C. B+ - in the presence of 50 mM phosphate buffer; B- - without buffer addition.

Conclusions

1. A practical, affordable approach to valorizing cheese whey into whey-based beverages and reducing waste is proposed, without significant investment or complex equipment. The use of a complex LAB growth medium and phosphate buffer supplementation re-initiated the growth of starter bacteria and enzyme production in NPCW.
2. QR activity and L-DOPA conversion indicated antioxidant properties and the synthesis of biologically active compounds within three hours of NPCW supplementation.
3. With further optimization, a simple, cheese-whey-based beverage technology could be developed to reduce waste.

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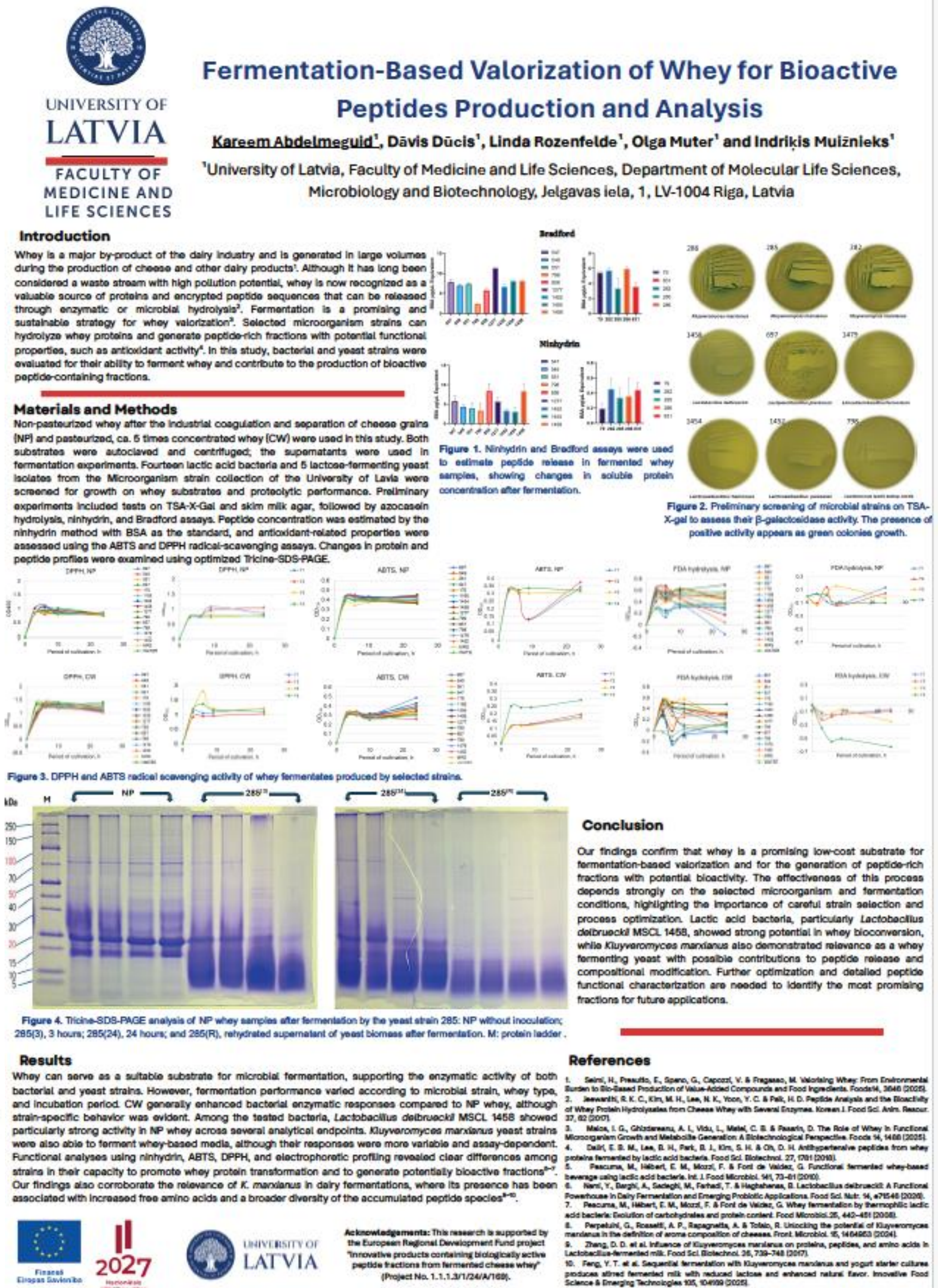
8. PUBLICITĀTES PASĀKUMI: ZIŅOJUMI STARPTAUTISKAJĀS KONFERENCĒS

Konferencē *Biosystems Engineering* 2026 Tartu Dzīvibas zinātņu universitātē, Igaunijā.

Biosystems Engineering 2026

The Biosystems Engineering conference is organised by Estonian University of Life Sciences. It aims to become the leading annual conference in Baltic region in fields related to traditional and modern engineering techniques and technical solutions applied to biological systems. The goal of BSE 2026 is to gather scholars from all over the world to present advances in the fields of biosystems engineering and to foster an environment conducive to exchanging ideas and information. This conference will also provide an ideal environment to develop new collaborations and meet experts on the fundamentals, applications, and products of the mentioned fields.


UNIVERSITY OF LATVIA
FACULTY OF MEDICINE AND LIFE SCIENCES
Fermentation-Based Valorization of Whey for Bioactive Peptides Production and Analysis
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Introduction
Whey is a major by-product of the dairy industry and is generated in large volumes during the production of cheese and other dairy products[1]. Although it has long been considered a waste stream with high pollution potential, whey is now recognized as a valuable source of proteins and encrypted peptide sequences that can be released through enzymatic or microbial hydrolysis[2]. Fermentation is a promising and sustainable strategy for whey valorization[3]. Selected microorganism strains can hydrolyze whey proteins and generate peptide-rich fractions with potential functional properties, such as antioxidant activity[4]. In this study, bacterial and yeast strains were evaluated for their ability to ferment whey and contribute to the production of bioactive peptide-containing fractions.
Materials and Methods
Non-pasteurized whey after the industrial coagulation and separation of cheese grains (NP) and pasteurized, ca. 5 times concentrated whey (CW) were used in this study. Both substrates were autoclaved and centrifuged; the supernatants were used in fermentation experiments. Fourteen lactic acid bacteria and 5 lactose-fermenting yeast isolates from the Microorganism strain collection of the University of Lavia were screened for growth on whey substrates and proteolytic performance. Preliminary experiments included tests on TSA-X-Gal and skim milk agar, followed by azocasein hydrolysis, ninhydrin, and Bradford assays. Peptide concentration was estimated by the ninhydrin method with BSA as the standard, and antioxidant-related properties were assessed using the ABTS and DPPH radical-scavenging assays. Changes in protein and peptide profiles were examined using optimized Tricine-SDS-PAGE.
Bradford
Ninhydrin
Figure 1. Ninhydrin and Bradford assays were used to estimate peptide release in fermented whey samples, showing changes in soluble protein concentration after fermentation.
Figure 2. Preliminary screening of microbial strains on TSA-X-gal to assess their β-galactosidase activity. The presence of positive activity appears as green colonies growth.
DPPH, NP
ABTS, NP
DPPH, CW
ABTS, CW
Figure 3. DPPH and ABTS radical scavenging activity of whey fermentates produced by selected strains.
Figure 4. Tricine-SDS-PAGE analysis of NP whey samples after fermentation by the yeast strain 285: NP without inoculation; 285(3), 3 hours; 285(24), 24 hours; and 285(R), rehydrated supernatant of yeast biomass after fermentation. M: protein ladder.
Conclusion
Our findings confirm that whey is a promising low-cost substrate for fermentation-based valorization and for the generation of peptide-rich fractions with potential bioactivity. The effectiveness of this process depends strongly on the selected microorganism and fermentation conditions, highlighting the importance of careful strain selection and process optimization. Lactic acid bacteria, particularly Lactobacillus delbrueckii MSCL 1458, showed strong potential in whey bioconversion, while Kluyveromyces marxianus also demonstrated relevance as a whey fermenting yeast with possible contributions to peptide release and compositional modification. Further optimization and detailed peptide functional characterization are needed to identify the most promising fractions for future applications.
Results
Whey can serve as a suitable substrate for microbial fermentation, supporting the enzymatic activity of both bacterial and yeast strains. However, fermentation performance varied according to microbial strain, whey type, and incubation period. CW generally enhanced bacterial enzymatic responses compared to NP whey, although strain-specific behavior was evident. Among the tested bacteria, Lactobacillus delbrueckii MSCL 1458 showed particularly strong activity in NP whey across several analytical endpoints. Kluyveromyces marxianus yeast strains were also able to ferment whey-based media, although their responses were more variable and assay-dependent. Functional analyses using ninhydrin, ABTS, DPPH, and electrophoretic profiling revealed clear differences among strains in their capacity to promote whey protein transformation and to generate potentially bioactive fractions[5-7]. Our findings also corroborate the relevance of K. marxianus in dairy fermentations, where its presence has been associated with increased free amino acids and a broader diversity of the accumulated peptide species[8-10].
References
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9. GALVENIE REZULTĀTI: APKOPOJUMS

9.1. No sākotnēji LU MKK identificētajiām 19 LAB un KFY kulūrām pēc BAS un PF sintēzes intensitātes kritēriju analīzes tālākajiem pētījumiem atlasītas sešās LAB un viena LFY kultūra.

9.2. Apkopoti dati par 906 siera ražošanai izmantotajiem sūkalu paraugiem kopš 2025. gada 1, jūlija līdz 2026. gada 24.martam. Datu analīze parāda, ka industriālās ierauga kultūras vairāk iespaido sūkalu tauku, mazāk proteīna vai laktozes saturu. Līdz ar to izmatotais siera ieraugs nevarētu atstāt būtisju iespaidu uz peptīdu frakcijas sastāvu tālākš apstrādes gaitā.

9.3. Izstrādāta standartizēta eksperimenta shēma un iegūti salīdzinoši dati par septiņu atlasīto mikroorganismu kultūru BAS, AO un PF sintēzes potenciālu, izmantojot 6-24 stundu kultivēšanas režīmu un augstu ierauga izsējas intensitāti. Kā references materiāls izmantota industriālā ierauga LAB kultūra

9. GALVENIE REZULTĀTI: APKOPOJUMS

9.4. Analīzes dati apliecina LAB dominanci BAS sintēzē arī koncentrētu sūkalu substrātā, bet LFY – PF veidošanā. Attīstot LAB un LFY kokultivēšanas metodes, jāņem vērā LFY kultūras salīdzinoši ar LAB zemāka dzīvotsēja izmantotajos sūkalu substrātos.

9.5. Izstrādāta siera sūkalu pēcfermentācijas apstrādes koncepcija, kas paredz industriālo ieraugu potenciāla pilnīgāku izmantošanu pēc siera graudu masas atdalīšanas.Izpētītas iespējas ar dažādu barības vielu, to pieejamību veicinošu vai pH stabilizējošu savienojumu piedevām stimulēt industriālo ieraugu aktivitāti pēcfermentācijā, termiski neapstrādātās NPW sūkalās. Parādīts, ka pēcfermentācijas efektivitāti veicina rauga ekstrakta vai melases pievienošana 0,5-1,5% koncentrācijā nepasterizētām sūkalām. Tādejādi parādīta iespēja citu pārtikas produktu ražošanas blakusproduktu iesaistīšanai sūkalu valorizācijas procesā, izmantojot aprites ekonomikas principus pārtikas ražošanā.

9.6. Projekta rezultāti izplatīti, komunicējot tos ar divu stenda ziņojumu palīdzību starptautiskās konferencēs Tartu un Viļņā.