Foods 2021

MDPI

Proceeding Paper

Indigenous *Lactococcus lactis* with Probiotic Properties: Evaluation of Wet, Thermally- and Freeze-Dried Raisins as Supports for Cell Immobilization, Viability and Aromatic Profile in Fresh Curd Cheese †

Justina Mileriene [1,*], Loreta Serniene [1], Kristina Kondrotiene [1], Valentini Santarmaki [2], Yiannis Kourkoutas [2], Milda Kersiene [3], Daiva Leskauskaite [3], Lina Lauciene [1], Neringa Kasetiene [1] and Mindaugas Malakauskas [1]

[1] Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes st. 18, LT-47181 Kaunas, Lithuania; email1@gmail.com (L.S.); email2@gmail.com (K.K.); email3@gmail.com (L.L.); email4@gmail.com (N.K.); email5@gmail.com (M.M.)

[2] Laboratory of Applied Microbiology & Biotechnology, Department of Molecular Biology & Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece; email6@gmail.com (V.S.); email7@gmail.com (Y.K.)

[3] Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas, Lithuania; email8@gmail.com (M.K.); email9@gmail.com (D.L.)

* Correspondence: justina.mileriene@lsmu.lt

† Presented at the 2nd International Electronic Conference on Foods, 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Citation: Mileriene, J.; Serniene, L.; Kondrotiene, K.; Santarmaki, V.; Kourkoutas, Y.; Kersiene, M.; Leskauskaite, D.; Lauciene, L.; Kasetiene, N.; Malakauskas, M. Indigenous *Lactococcus lactis* with Probiotic Properties: Evaluation of Wet, Thermally- and Freeze-Dried Raisins as Supports for Cell Immobilization, Viability and Aromatic Profile in Fresh Curd Cheese. **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Received: date
Accepted: date
Published: date

Abstract: Indigenous *Lactococcus lactis* enriched raisins were incorporated in fresh curd cheese in wet, thermally dried, and freeze-dried form to produce a novel probiotic dairy product. Probiotic properties of *Lactococcus lactis* LL16 were evaluated in previous studies. Thus, in this study the viability of *L. lactis* cells was assessed in the cheeses during storage at 4 °C for 1, 7, and 14 days and the effect of the added enriched raisins on physicochemical parameters, microbiological characteristics, sugar content, aromatic profile and sensory acceptance of cheeses were evaluated. Results showed that the nature of *L. lactis* LL16 (free or immobilized cells), the support (raisins/immobilized cells on raisins) and the nature of immobilized cultures on raisins (wet or dried) had a significant ($p < 0.05$) effect on cheese pH, moisture, and lactococci counts. Immobilized *L. lactis* cells maintained viability at necessary levels (>6 cfu/g) during storage and significantly increased the acceptability of cheese. Control cheeses were the least acceptable, while cheese samples with *L. lactis* and raisins, which had the highest lactococci counts, expressed the highest scores in overall sensory acceptability among all samples. Only the addition of thermally dried raisins with immobilized *L. lactis* LL16 cells resulted in an increased cheese sensory acceptability by the end of the storage. This finding might me influenced by a significant increase in total sugars and volatile compounds in this sample. Overall, the addition of raisins enhanced the volatile profile of cheeses with 2-furanmethanol, 1-octanol, 3-methylbutanal, 2-methylbutanal, 2-furancarboxaldehyde, 1-(2-furanyl)-ethanone, 5-methyl-2-furancarboxaldehyde. The obtained results are encouraging for the production of novel fresh cheeses with improved sensorial and probiotic characteristics on industrial and/or small industrial scale.

Keywords: indigenous *Lactococcus lactis*; freeze-drying; thermal drying; immobilization; raisins; curd cheese; survival; aroma profile

1. Introduction

Concerned by the rising number of life-threatening chronic illnesses in western countries consumers are not only more careful about their food selections but also focusing their attention on healthy food (Petrescu, Vermeir, and Petrescu-Mag 2020). Responding

to the consumer needs, dairy producers are constantly searching for innovations, which has led to the wide use of probiotics in various dairy products, targeting fermented milk at first and invading various cheese productions now. The latest trend in this area is the usage of wild-type, indigenous novel presumptive probiotics isolated from various local sources (Yadav and Shukla 2020; Yerlikaya 2019). They are being extensively investigated as possible starters, protective cultures, nutraceuticals, probiotics, and flavor enhancers (Arena et al. 2018; Colombo, Nero, and Todorov 2020; Ribeiro, O'Connor, Ross, Stanton, and Silva 2016). In our previous study, we aimed to characterize indigenous *Lactococcus lactis* strains, previously isolated from food-grade samples, through the measure of their protective, technological, and probiotic properties among others (Kondrotiene et al. 2020). However, supplementation of curd cheese with LAB cultures can be problematic especially when high curd temperature is an essential part of the manufacturing process to form the uniform cheese body.

2. Results and Discussion

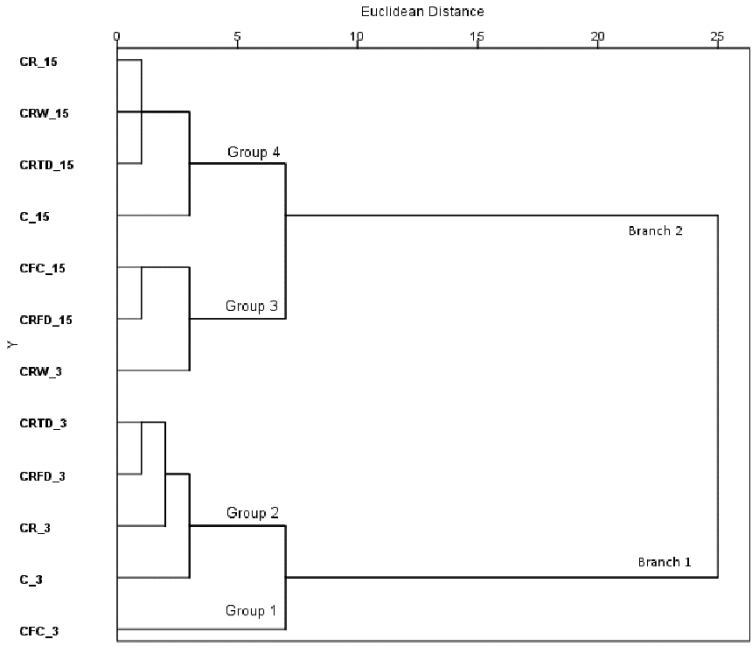


Figure 1. Dendrogram for the hierarchical cluster analysis (HCA) results of minor volatile compounds during storage days 3 and 15 of sweet curd cheese with *Lactococcus lactis* LL16 cells immobilized on raisins. Samples: control cheese (C); cheese with free cells (C+FC); cheese with raisins (C+R); cheese with wet immobilized cells on raisins (C+RW); cheese with freeze-dried immobilized cells on raisins (C+RFD); cheese with thermally dried immobilized cells on raisins (C+RTD).

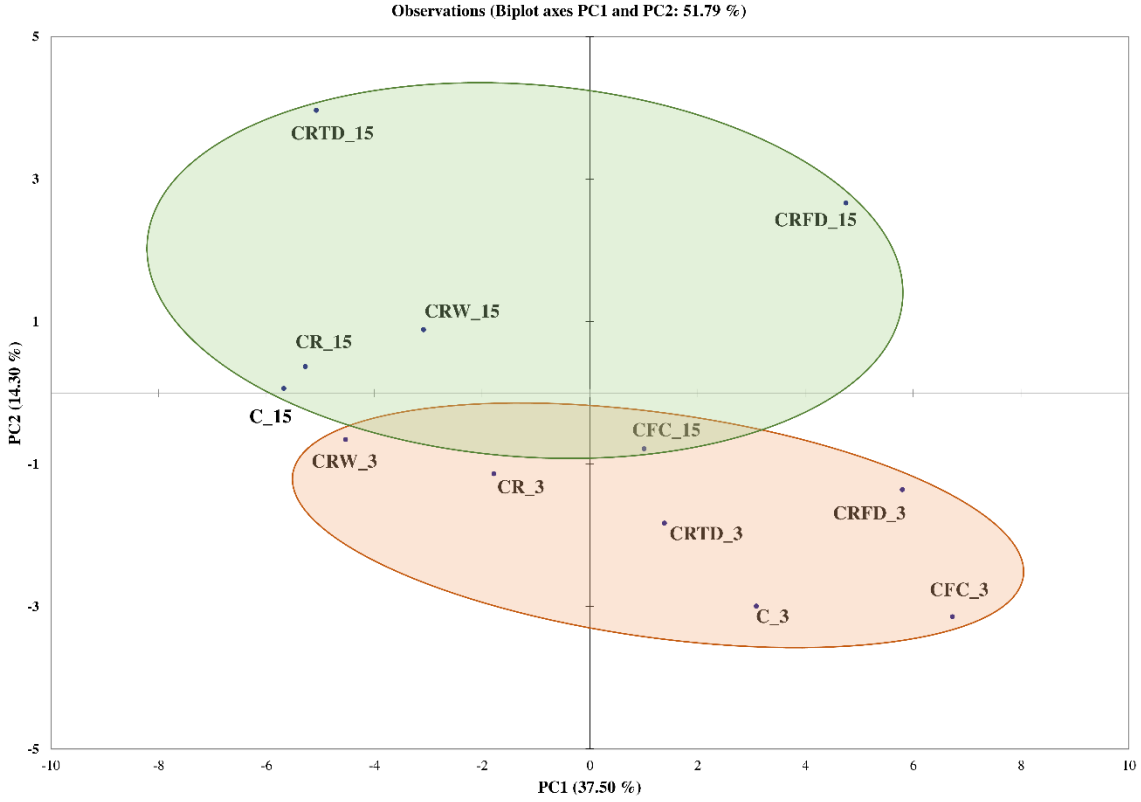
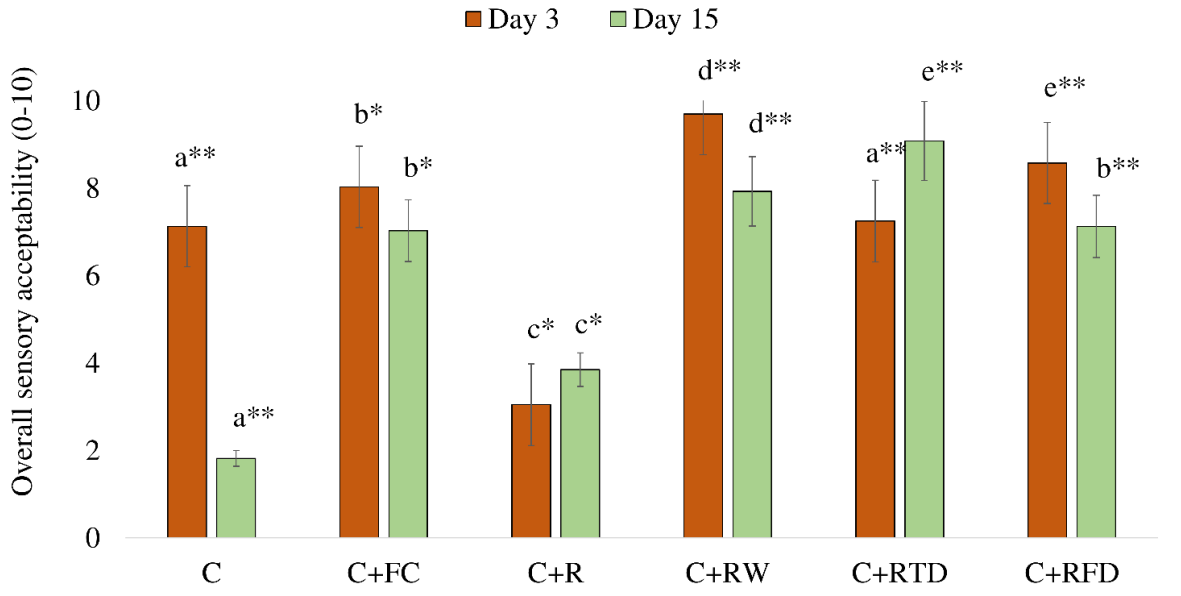


Figure 2. Score plot of the principal components (PC) of minor volatile compounds during storage days 3 and 15 of sweet curd cheese with *Lactococcus lactis* LL16 cells immobilized on raisins. Samples: control cheese (C); cheese with free cells (C+FC); cheese with raisins (C+R); cheese with wet immobilized cells on raisins (C+RW); cheese with freeze-dried immobilized cells on raisins (C+RFD); cheese with thermally dried immobilized cells on raisins (C+RTD).



	P		*P*		*P*
Free/immob. cells	0.043	Raisins_immob. raisins	0.0001	Immob. wet/dry	0.009
Day (D)	0.047	Day	ns	Day	0.009
Dxfree/immob.cells	ns	DxRaisins_immob. raisins	ns	DxImmob. wet/dry	0.003

Figure 3. Overall sensory acceptability of sweet curd cheese with *Lactococcus lactis* LL16 cells immobilized on raisins on days 3 and 15 of storage. Samples: control cheese (C); cheese with free cells (C+FC); cheese with raisins (C+R); cheese with

wet immobilized cells on raisins (C+RW); cheese with freeze-dried immobilized cells on raisins (C+RFD); cheese with thermally dried immobilized cells on raisins (C+RTD). No significant differences were found among samples with the same letters within the same day. Differences between storage days for the same sample were significant when $p < 0.001$ (*), $p < 0.0001$ (**). ns: not significant.

3. Conclusions

The latest trend in the development of probiotic enriched food product is the usage of wild-type, indigenous novel potential probiotics isolated from various local sources. Moreover, the use of local and natural prebiotics as a support for the viability of such bacteria is highly encouraged by the consumers. Therefore, this study evaluated the possibility of enriching fresh sweet curd cheese with indigenous *Lactococcus lactis* strain immobilized on raisins and subsequently dried by various methods (wet, thermal drying, freeze-drying).

A total of 54 volatile compounds were identified from cheese samples during storage, using HS-SPME extraction method and GC–MS analysis: 5 esters, 8 acids, 8 alcohols, 18 carbonyl compounds and 15 miscellaneous compounds were found. The addition of raisins enhanced the volatile profile of cheeses with 2-furanmethanol, 1-octanol, 3-methylbutanal, 2-methylbutanal, 2-furancarboxaldehyde, 1-(2-furanyl)-ethanone, 5-methyl-2-furancarboxaldehyde.

Microbiological analysis revealed that all immobilized cells demonstrated steady gradual growth in lactococci counts throughout the storage of cheese with thermally dried cells expressing the lowest counts, compared to wet and freeze-dried cells. Obtained results were ambiguous: even though the growth of lactococci was slower in cheese samples with thermally dried immobilized cells on raisins, preliminary overall sensory evaluation results indicated that this supplementation was the most acceptable by the end of the cheese storage. Therefore, in future studies, lower thermal drying temperature should be considered in order to increase the viability of probiotic cells.

Funding: This project has received funding from European Regional Development Fund (project No 01.2.2-LMT-K-718-01-0032) under grant agreement with the Research Council of Lithuania (LMTLT).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

References

1. Arena, M.P.; Capozzi, V.; Russo, P.; Drider, D.; Spano, G.; Fiocco, D. Immunobiosis and probiosis: antimicrobial activity of lactic acid bacteria with a focus on their antiviral and antifungal properties. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 9949–9958. https://doi.org/10.1007/s00253-018-9403-9
2. Bosnea, L.A.; Kopsahelis, N.; Kokkali, V.; Terpou, A.; Kanellaki, M. Production of a novel probiotic yogurt by incorporation of L. casei enriched fresh apple pieces, dried raisins and wheat grains. *Food Bioprod. Process.* **2017**, *102*, 62–71. https://doi.org/10.1016/j.fbp.2016.11.010
3. Colombo, M.; Nero, L.A.; Todorov, S.D. Safety profiles of beneficial lactic acid bacteria isolated from dairy systems. *Braz. J. Microbiol.* **2020**, *51*, 787–795. https://doi.org/10.1007/s42770-020-00227-y
4. International Organization for Standardization. *ISO 13299:2016 Sensory Analysis—Methodology—General Guidance for Establishing a Sensory Profile*; International Organization for Standardization: Geneva, Switzerland, 2016.
5. Kondrotiene, K.; Kasnauskyte, N.; Serniene, L.; Gölz, G.; Alter, T.; Kaskoniene, V.; Maruska, A.S.; Malakauskas, M. Characterization and application of newly isolated nisin producing Lactococcus lactis strains for control of Listeria monocytogenes growth in fresh cheese. *LWT Food Sci. Technol.* **2018**, *87*, 507–514. https://doi.org/10.1016/j.lwt.2017.09.021
6. Kondrotiene, K.; Lauciene, L.; Andruleviciute, V.; Kasetiene, N.; Serniene, L.; Sekmokiene, D.; Malakauskas, M. Safety Assessment and Preliminary In Vitro Evaluation of Probiotic Potential of Lactococcus lactis Strains Naturally Present in Raw and Fermented Milk. *Curr. Microbiol.* **2020**, *1*, 3. https://doi.org/10.1007/s00284-020-02119-8
7. Mileriene, J.; Serniene, L.; Henriques, M.; Gomes, D.; Pereira, C.; Kondrotiene, K.; Kasetiene, N.; Lauciene, L.; Sekmokiene, D.; Malakauskas, M. Effect of liquid whey protein concentrate–based edible coating enriched with cinnamon carbon dioxide extract on the quality and shelf life of Eastern European curd cheese. *J. Dairy Sci.* **2021**, *104*, 1504–1517. https://doi.org/10.3168/jds.2020-

18732
8. Petrescu, D.C.; Vermeir, I.; Petrescu-Mag, R.M. Consumer understanding of food quality, healthiness, and environmental impact: A cross-national perspective. *Int. J. Environ. Res. Public Health* **2020**, *17*, 169. https://doi.org/10.3390/ijerph17010169
9. Ribeiro, S.C.; O'Connor, P.M.; Ross, R.P.; Stanton, C.; Silva, C.C.G. An anti-listerial Lactococcus lactis strain isolated from Azorean Pico cheese produces lacticin 481. *Int. Dairy J.* **2016**, *63*, 18–28. https://doi.org/10.1016/j.idairyj.2016.07.017
10. Yadav, M.; Shukla, P. Efficient engineered probiotics using synthetic biology approaches: A review. *Biotechnol. Appl. Biochem.* **2020**, *67*, 22–29. https://doi.org/10.1002/bab.1822
11. Yerlikaya, O. Probiotic potential and biochemical and technological properties of Lactococcus lactis ssp. lactis strains isolated from raw milk and kefir grains. *J. Dairy Sci.* **2019**, *102*, 124–134. https://doi.org/10.3168/jds.2018-14983