Viability of *Lacticaseibacillus paracasei* in Ultrafiltered white brined cheese packaged in modified atmosphere and flexible multilayer films

DOI: 10.15567/mljekarstvo.2021.0402

Maryam Heidarvand¹, Vajiheh Fadaei¹*, Zohreh Khodaii²

¹Department of Food Science & Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran
²Dietary Supplements and Probiotic Research Centre and Department of Biochemistry, Genetic, and Nutrition, Alborz University of Medical Sciences, Karaj, Iran

*Corresponding author: vn.fadaei@gmail.com

Received: 01.02.2021. / Accepted: 01.09.2021.

Abstract

In this study, two packaging materials, modified polypropylene (MPP) and polyethylene terephthalate/aluminium/ low-density polyethylene (PETFA-Al-LDPE) were studied under various atmospheric conditions: 100 % CO₂, 70 % N₂ - 30 % CO₂, 80 % N₂ - 20 % CO₂ for packing probiotic ultrafiltered (UF) white brined cheese. pH, titratable acidity, moisture content, *Lacticaseibacillus paracasei* viability and overall acceptability were monitored within a 12-week period. The control samples were packaged in atmospheric air. Results revealed that samples packaged in PETFA-Al-LDPE with the combination of 70 % N₂ - 30 % CO₂ had the lowest pH, highest acidity and moisture content. The viability of *Lacticaseibacillus paracasei* was reported to be 4*¹⁰⁶ CFU/g within 12 weeks. The highest and lowest overall acceptability was for the cheese packaged in 70% N₂ - 30 % CO₂ and the control sample, respectively.

Key words: MAP; Multilayer films; white brined cheese; *Lacticaseibacillus paracasei*
Introduction

White brined cheese has an extraordinary position among all kinds of cheeses in Iran and its continuous industrial production has been extending lately. During ultrafiltration, milk becomes concentrated via the filtration process. The obtained concentrated milk interacts with the starter culture and coagulates. In comparison to other fermented dairy products such as yogurt and fermented milk, cheese is a better carrier for probiotics due to specific physicochemical properties like higher pH, lower titratable acidity, higher buffer capacity and denser structure as a result of which probiotics have a safer condition and maintain their biological activity while passing the gastrointestinal system (Karimi et al., 2011; Gomes da Cruz et al., 2009).

Probiotic viability depends on various factors including final pH of the product, \( \text{H}_2\text{O}_2 \) existence, other microorganisms, incubation time, thermal treatment, probiotic strain, added stimulators, microbial growth inhibitors and finally, the applied packaging systems as well as their nature (Karimi et al., 2011; Gionchetti et al., 2003b). Production of desirable taste and odour by specific microbial strains is of utmost importance in probiotic food manufacturing. Lactic acid bacteria are used in industry for improvement of odour, taste, and structure of fermented products. Due to their inhibition effects on undesirable bacteria, it has been widely studied to use them or their pure bacteriocins for food preservation purposes (Sreekumar et al., 1996; Alam et al., 2016; Chen and Hotchkiss, 1993; Mannheim et al., 2006; Gammariello et al., 2009; Mannheim and Soffer, 1996; Conte et al., 2009; Del Nobile et al., 2009; Garabal et al., 2010; Olarte et al., 2001; Rodríguez-Alonso et al., 2011; Pintado and Malcata, 2000, 2007; Jakobsen and Risbo, 2009; Govaris et al., 2011; Rodríguez-Aguilera et al., 2011; Conte et al., 2011; Temiz et al., 2009; Alves et al., 1996; Alam et al., 2016; Chen and Hotchkiss, 1993; Gammariello et al., 2009; Felfoul et al., 2017; Mortensen et al., 2003; Del Nobile et al., 2009; Barukčić et al., 2020). However, there has been no experiment conducted on the effect of MAP with multilayer packaging material on probiotic bacteria viability. UF white brined cheese has the highest production quantity in Iran and is very popular among Iranians. Within the production process, packaging is regarded as an inseparable part which has a huge influence on the quality of the probiotic dairy products. Since most fermented products are packaged in plastic material which lets oxygen in, serious problems occur for viability of probiotic bacteria. As a result, within the present study, viability of the probiotics in UF white brined cheese in modified atmosphere packaging system has been investigated.

Materials and methods

Materials

Raw milk was bought from Zarrin Toranj Sepehr (Iran), the starter culture was purchased from Mit Co. (Germany) and \( \text{CaCl}_2 \) was prepared from Jiozeuang (China). \textit{Lactobacillus lactis} (CHR Hansen R 704) was purchased from Christian Hansen (Denmark) and \textit{Lactocaseibacillus paracasei} (CHR Hansen A/S) from collection. Co. (Australia). PETFA-Al-LDPE and MPP materials were obtained from Pan Co, (Iran). All the chemicals were of analytical grade acquired from Merck Co, (Germany).
Producing UF probiotic cheese packaged in modified atmosphere and flexible multilayer films

Having conducted the regular tests, raw milk (2 % fat) was precooled and went through clarifier and bacteriophage filters. Then, it was pasteurized at 72 °C for 15 sec. Pasteurized milk enters the preheater and reaches a temperature of 50 °C; it then enters ultrafiltration, with a concentration factor of 5X. Ultrafiltration had three loops, and the final dry matter of retentate reached 35 % after leaving the third loop. The acquired retentate was homogenized and pasteurized at 77 °C for 1 min, then cooled to 35 °C. 0.00055 percent mesophilic starter culture, Lactococcus lactis and probiotic Lactocaseibacillus paracasei (10^8-10^9 cfu/mL) was added to retentate. 0.5 hr. later, 0.02 % (w/v) CaCl_2 was added. Having reached the pH of 5.6, the started culture was added (0.001% w/v) in sterilized distilled water. To form a clot in the second phase of enzymatic coagulation, the temperature remained the same at 35 °C and the retentate was filled in a 300-g package without any delay (Table 1). When the curd was formed, 3 % NaCl was poured on the samples and the lead was placed by MAP (VAC-Star S-225) (Italy). The produced cheese was kept and incubated at 30 °C (to reach pH 4.7) for 24 h and then moved to a refrigerator at 4 °C. Within 12 weeks, the products were examined for pH, acidity, moisture, Lactocaseibacillus paracasei viability and sensory properties.

Probiotic UF cheese samples analysis

The pH of samples was measured using a digital pH metre (Metrohm, Swiss, model 713). Titratable acidity was measured based on the titration method with NaOH and expressed in terms of % lactic acid (AOAC, 1995). Moisture content was determined based on the oven method and reported on weight percent basis (AOAC, 1995).

In order to investigate the viability of the mentioned bacteria, Rogosa agar medium culture (Merck Co, Germany) modified by acetic acid at 96 % (v/v) was implemented and pH was adjusted to 5.2 and finally incubated at 37 °C for 72 h. Viability measurement was done fortnightly according to Madureira et al. (2008).

Cheese samples were randomly coded and panellists evaluated the odour, colour, taste, texture and overall acceptability based on a hedonic scale (1= least favourable, 5=most favourable) every fortnight. 10 trained evaluators aged from 21 to 35 participated in the experiment. Prior to the test, participants were asked to fill in a form containing questions about their age and their cheese consumption history (none, less than once a month, 4-5 times a month, and more than 6 times a month). Evaluators who normally consume cheese 2-4 times a month were not selected for final analysis. Cheese slices were cut into a standard dimension ready to be bitten and were kept at a plastic chamber to reach ambient temperature 2 h before the analysis. Evaluators used distilled water to wash their mouth between samples.

All analyses were performed once a week within 12 weeks.

Statistical analysis

All treatments were conducted in completely randomized design and each treatment was performed for 3 times. Average values were compared with Duncan test and the level of significance was set at 0.05. Data was analysed by SPSS (Ver. 21).

Results and discussion

Titratable acidity and pH of probiotic UF cheese samples during cold storage

The comparison between pH and acidity of cheese samples (figs 1 and 2) showed that there is no significant difference between cheeses with various films and atmospheric composition (p<0.05). pH and acidity ranged from 4.78±0.96 - 5.01±0.14 and 1.38±0.01 - 1.17±0.08, respectively at the end of the 12 weeks of experiment. In almost all test intervals, cheese samples packaged in PETFA-Al-LDPE film composed of 30 % CO2-70 % N2 and aired PETFA-Al-LDPE films had the lowest and the highest pH, respectively. The reduction of pH at MAP may be a result of CO2 solubility in water. Furthermore, lactose transformation to lactic acid is effective in pH reduction

| Table 1. Introduction of treatments used in the research |
|-------------------------------|-----------------|
| Abbreviation                  | Description                                             |
| PP+CO2                        | Modified polypropylene film + 100 % CO2                |
| PP+CO2+N2 (30:70)             | Modified polypropylene film + 70 % N2 - 30 % CO2       |
| PP+CO2+N2 (20:80)             | Modified polypropylene film + 80 % N2 - 20 % CO2       |
| PP                            | Modified polypropylene film + air (Control 1)          |
| PETFA-PE+CO2                  | Polyethylene terephthalate/aluminium/ low-density polyethylene film + 100 % CO2 |
| PETFA-PE+CO2+N2 (30:70)       | Polyethylene terephthalate/aluminium/ low-density polyethylene film + 70 % N2 - 30 % CO2 |
| PETFA-PE+CO2+N2 (20:80)       | Polyethylene terephthalate/aluminium/ low-density polyethylene film + 80 % N2 - 20 % CO2 |
| PETFA-PE                      | Polyethylene terephthalate/aluminium/ low-density polyethylene film + air (Control 2) |
(Dermiki et al., 2008; Felfoul et al., 2017), pH reduction was lower in samples with 100 % CO₂ compared to 30% CO₂/70 % N₂ samples which may be a consequence of atmospheric pressure reduction after CO₂ absorbance and moisture loss in cheese samples. In 30 % CO₂/70 % N₂ samples, the inert N₂ gas prohibits atmospheric pressure drop, hence moisture loss too. Generally, with the increase in in CO₂, pH reduces and acidity increases. In this study, CO₂ effect alone led to a 0.4-unit reduction in pH. The results are in agreement with similar studies (Dermiki et al., 2008; Temiz et al., 2009; Felfoul et al., 2017).

Along with the progress of storage time, pH reduced and acidity increased. The pH of cheese samples is an indicator of the activity of starter and non-starter lactic acid bacteria. However, pH reduction rate is varying in different cheeses (McSweeney et al., 1993). Decline in pH and increase in acidity were mainly due to lactic acid formation from lactose via lactic acid bacteria (LAB), acidic amino acids and free fatty acid production through proteolysis and lipolysis by the starter culture. It is also likely that short chain fatty acids, which are released as final metabolites as probiotic bacteria, are influential in this matter (Yerlikaya and Ozer, 2014; Chevanan and Muthkumarppan, 2007; Dermiki et al., 2008; Osman et al., 2008; Rotaro and Clementi, 2008).

Cheese pH is not solely dependent on lactic acid produced by bacteria and the curd’s buffer capacity, which is related to casein content, citrate and phosphate levels, is also important in this regard. The increase in moisture content leads to a decrease in H⁺ ion concentration which ends in higher values of pH. Based on the production process, pH change ranges from 4.38-5.94 in the ripening period. Similarly, the pH reduction was reported in Turkish white cheese and Armada by Öner et al. (2006) and Lemya et al. (2010) which was attributed to metabolic reactions in LAB and organic acid such as lactic acid. Maniar et al. (2006), Dermiki et al. (2008) and Felfoul et al. (2017) also reported an increase in acidity level of the cheese packaged in MAP.

Moisture of probiotic UF cheese samples during cold storage

The effect of packaging and atmospheric condition on the moisture content was not significant (P>0.05). Results revealed that PETFA-Al-LDPE film with the composition of 30 % CO₂/70 % N₂ had the highest moisture content (Fig. 3). Having compared the moisture content of samples, it can be concluded that the largest moisture loss occurred in the control samples which is related to moisture evaporation. In comparison to PETFA-Al-LDPE, MPP maintained higher moisture content, most probably due to lower permeability of PETFA-Al-LDPE. 30 % CO₂/70 % N₂ also preserved higher moisture content in comparison to the other compositions. As mentioned earlier, N₂ gas prohibits pressure loss restricting thereby moisture reduction. Dermiki et al. (2008) and Felfoul et al. (2017) also reported the lack of MAP effect on the moisture content of cheese samples.

Moisture content reduced during storage which is due to surface evaporation and the permeable film. Acid production by LAB helps the pH reduction trend within storage time which consequently increases whey and moisture loss (Caridi et al., 2003). Therefore, during the storage period, moisture content decreased and reached the minimum after 12 weeks. This moisture loss during ripening was also observed in hard cheeses such as Parmigiano Reggiano (D’Incecco et al., 2020).

Lactobacillus paracasei viability of probiotic UF cheese samples during cold storage

The effect of packaging and atmospheric composition on viability of Lactocaseibacillus paracasei was not significant (p>0.05). Results revealed that with the increase in CO₂ concentration in films, Lactocaseibacillus paracasei viability showed a greater reduction (Fig. 4) since CO₂ inhibits microbial growth because of its bacteriostatic
properties. Not only does MAP retain the quality properties of cheese, but it also enhances its shelf life (Temiz, 2010; Dermiki et al., 2008; Gammiariello et al., 2009; Conte et al., 2009; Khoshgozar et al., 2012; Del Nobile et al., 2009; Conte et al., 2011; Temiz et al., 2009; Mannheim and Soffer, 1996). Papaoianou et al. (2007) reported mesophilic bacteria growth in cheese samples at 30 % CO\textsubscript{2}/70 % N\textsubscript{2} composition which subsequently resulted in longer shelf life. In addition, packaging type affected Lactocaseibacillus paracasei viability. The lowest and highest viability was observed in MPP with 100 % CO\textsubscript{2} and PETFA-Al-LDPE with 30 % CO\textsubscript{2}/70 % N\textsubscript{2}. On the other hand, high moisture content is crucial in microorganism growth and higher moisture in PETFA-Al-LDPE packages helped Lactocaseibacillus paracasei growth in 30 % CO\textsubscript{2}/70 % N\textsubscript{2} packages. Dermiki et al. (2008) also reported an increase in the total number of LAB in cheese samples during a 50-day period in modified CO\textsubscript{2}-N\textsubscript{2} system. However, Papaoianou et al. (2007) reported a decrease in mesophilic bacterial growth in Greek cheese in CO\textsubscript{2}-N\textsubscript{2} packages during 40 days. Maniar et al. (2006) also observed an ascending pattern in LAB in cottage cheese samples packaged in air and a stable pattern in MAP.

Lactocaseibacillus paracasei viability decreased in cheese samples during storage (p<0.05). LAB are natural residents of cheese microflora which play a key role in cheese ripening (Tornadijo et al., 1995) and increase the amount of short-chain peptides, free amino acids and free fatty acids (Arenas et al., 2004). LAB reduction in ripening period is dependent on factors such as salt, sensitivity of starter cultures, water activity and autolysis ability of breeds (Vassiliadis et al., 2009). Slower metabolism rate of Lactobacillus sp. and their ability to cope with harsh conditions (acidity, low water activity, and high sanity) is a major reason for their survival until the end of storage time in comparison with the other LAB (Arenas et al., 2004). During the fermentation period of cheese, various metabolites like lactate, citrate, glycerol, and amino acids are produced and are efficiently used by Lactobacillus sp. (Öner et al., 2006). Lactococcus bacteria rapidly ferment lactose and are numerous within the first days, so they create high acidity (Arenas et al., 2004). According to some previous studies, health effects of probiotics occur only if at least 10\textsuperscript{6}-10\textsuperscript{7} bacteria per g or mL of food sample enter the intestine (Thamaraj and Shah, 2004). These results confirm the viability of probiotics till 56 days of storage in all samples in 8x10\textsuperscript{1}-3x10\textsuperscript{7} cfu/g level as in 30 % CO\textsubscript{2}/70 % N\textsubscript{2} samples, the number of bacteria was still in probiotic range after 84 days. Generally, probiotic viability in fermented products is rather low due to low pH and high acidity as these factors are the major restricting elements for their viability (Kitazawa et al., 2001; Dinakar and Mistry, 1994). So, the reduction in their viability is natural. Xing-Xin et al. (2015) and Abd EL.Salam et al. (2012) also reported Lactobacillus casei reduction in Cheddar and Domiati during storage. Bifidobacterium animalis subsp. lactis BB-12 viability in the Mascarpone-type cheese declined throughout the whole storage period (de Almeida et al., 2018). The results of Cárdenas et al. (2014) also confirmed Lactobacillus salivarius reduction after 28 days. Also, viability of encapsulated L. acidophilus reduced in Requeijão cremoso processed cheese formulations during 45 days (Povetta et al., 2020). On the contrary, Barros and De Carvalho Delfino (2014) reported no meaningful difference in Lactobacillus casei viability during 30 days in petit-Suisse cheese as the pH did not pass 4.2 during this time due to buffer capacity of nutrients in milk (phosphates, citrate, peptones). They also reported that lactobacillus casei BGP 93 was resistant to acid (up to pH=3), consequently, it was not affected by acidity. Yerlikaya and Özer (2014) reported no reduction or increase in Lactocaseibacillus paracasei viability during 28 days in white cheese. Madureira et al. (2008) observed that Lactobacillus casei in cheese sample made of sweet whey protein had a slight increase after 21 days. In Cheddar cheese, Lactococcus casei was sustained in its initial level and then decreased slightly during the following 32 weeks. Lactocaseibacillus paracasei reduced up to week 8, increased in week 12 and then decreased again up to week 32 (Phillips et al., 2006). The results of Bodzen et al. (2019) also confirmed this important fact that atmosphere air in Lactobacillus casei preparation triplicates final viability in intestine simulated condition with N\textsubscript{2}/H\textsubscript{2}/CO\textsubscript{2}.

**Overall acceptance of probiotic UF cheese samples during cold storage**

Packaging and atmospheric composition had a significant effect on overall acceptance of the cheese samples (p<0.05). In both films, after 12 weeks of storage, cheese packaged in 30 % CO\textsubscript{2}/70 % N\textsubscript{2} and the control sample showed the highest and the lowest overall acceptance (Fig 5). Higher viability and moisture content were the reason of higher acceptance in 30 % CO\textsubscript{2}/70 % N\textsubscript{2} samples. Samples with PETFA-Al-LDPE system showed higher acceptance in comparison to MPP samples. At week 6, the score for this test was 4.7±0.01 to 4.61±0.02 for PETFA-Al-LDPE with 30 % CO\textsubscript{2}/70 % N\textsubscript{2} and MPP film with air, respectively. This pattern in modified atmosphere can be explained by different variations in structure, colour, taste and odour of cheese.

The influence of storage time on the acceptance of the samples was meaningful (p<0.05). This score initially...
increased and then decreased and the highest point was observed in weeks 5 and 6, and the minimum was attributed to week 12. After 12 weeks, PETFA-Al-LDPE with 30% CO₂/70% N₂ and MPP with air content exhibited the highest and the lowest scores, respectively. Improved odour and taste as result of microorganism activity and their proteolysis and lipolysis, reduced moisture content and textural properties led to the best scores for weeks 5 and 6.

The best atmospheric composition was different in various studies based on the cheese type. Temiz (2010) reported that 40% CO₂/60% N₂ and 100% CO₂ had the same acceptability score at the end of the experiment. Maniar et al. (2006) claimed satisfactory acceptance for cottage cheese with 100% CO₂. Dermiki et al. (2008) reported that 60% CO₂/40% N₂, 40% CO₂/60% N₂ saved the sensorial quality of Myzithra Kalathaki cheese for 30 days. After 14 days of storage, fresh Cameros cheese of goat milk packaged on 40% CO₂/60% N₂ and 50% CO₂/50% N₂ had a satisfactory odour and taste although their appearance and texture were not favourable (Olarte et al., 2001). Rodríguez-Alonso et al. (2011) declared that 80% N₂/20% CO₂ and 100% N₂ compositions are not suitable for preserving the sensorial properties of semi-hard “Arzu-ulloa” cheese. Temiz et al. (2009) concluded that the taste and odour of Turkish whey cheese (Lor) packaged in modified atmosphere (40% N₂, 60% CO₂, and 30% N₂/70% CO₂) was sustained after 45 days. Mozzarella cheese overall acceptability after 43 days of storage in 50% CO₂/50% N₂ was lower in comparison with 100% CO₂ (Alves et al., 1996). Gammariello et al. (2009) proposed 75% CO₂/25% N₂ composition for maintenance of sensorial properties and prolonged shelf life of Italian cheese Giuncata. Felfoul et al. (2017) reported that 40% CO₂/60% N₂ was successful for saving Mozzarella cheese sensorial features.

**Conclusion**

Packaging systems and their related materials influence probiotic bacteria. In this study, the effect of modified atmosphere and multilayer films on pH, acidity, moisture, overall acceptability and viability of *Lacticaseibacillus paracasei* in ultrafiltration cheese was analysed based on the collected data, UF Feta cheese with PETFA-Al-LDPE and 30% CO₂/70% N₂ was the best option due to better moisture preservation, higher probiotic viability and the higher overall acceptance.

**Preživljavanje soja Lacticaseibacillus paracasei u ultrafiltiriranom siru u salamuri pakiranom u modificiranoj atmosferi i fleksibilnim višeslojnim filmovima**

**Sažetak**

U ovom istraživanju je ispitivana mogućnost primjene dvaju ambalažnih materijala - modificiranog polipropilena (MPP) i polietilen tereftalata/aluminij/polietilen niske gustoće (PETFA-Al-LDPE) u različitim uvjetima modificirane atmosfere (100% CO₂, 70% N₂ – 30% CO₂, 80% N₂ – 20% CO₂) za pakiranje probiotičkog sira iz salamure proizведенog iz ultrafiltriranog (UF) mlijeka. Tijekom 12 tjedana čuvanja uzorcima sira su određivani pH, titracijska kiselost, sadržaj vlage, preživljavanje soja *Lacticaseibacillus paracasei* i prihvatljivost. Kontrolni uzorci su pakirani u normalnoj atmosferi (zrak). Dobiveni rezultati ukazuju kako uzorci pakirani u PETFA-Al-LDPE i modificiranoj atmosferi 70% N₂ – 30% CO₂ imaju najniži pH, te najveću kiselost i sadržaj vlage. Preživljavanje soja *Lacticaseibacillus paracasei* je bilo oko 4x10⁶ CFU/g tijekom 12 tjedana. Najnižu i najvišu prihvatljivost su imali uzorak pakiran u atmosferi 70% N₂ – 30% CO₂ te kontrolni uzorak.

**Ključne riječi:** MAP, višeslojni film; sir u salamuri; *Lacticaseibacillus paracasei*
References

1. Abd El-Salam, M.H., Hippen, A.R., Salem, M.M., Assem, F.M., El-Aassar, M. (2012): Survival of probiotic Lactobacillus casei and Enterococcus fecium in Domiati cheese of high conjugated linoleic acid content. Emirates Journal of Food and Agriculture 24 (2), 98-104.

2. Alam, T., Saha, N.C., Bhardwaj, A., Goyal, G.K. (2016): Influence of Modified Atmosphere Packaging (MAP) on textural properties of Mozzarella cheese during refrigeration storage. Asian Journal of Dairy and Food Research 35 (2), 103-110. https://doi.org/10.18805/ajdfr.v35i2.10720

3. Alves, R.M.V., De Luca Sarantopoulos, C.I.G., Van Dender, A.G.F., De Assis Fonseca Faria, J. (1996): Stability of sliced Mozzarella cheese in modified-atmosphere packaging. Journal of Food Protection 59 (8), 838-844. https://doi.org/10.4315/0362-028X-59.8.838

4. AOAC. (1995): Official Methods of Analysis, vol. 1: Agricultural Chemicals, Contaminates, Drugs. 16th ed. Washington D.C.

5. Arenas, R., González, L., Bernardo, A., Fresno, J., Tornadijo, M. (2004): Microbiological and physico-chemical changes in Genestoso cheese, a Spanish acid curd variety, throughout ripening. Food Control 15, 271-279. https://doi.org/10.1016/S0956-7135(03)00067-7

6. Barros, L.S.S., De Carvalho Delfino, N. (2014): Petit-Suisse Cheese Production with Addition of Probiotic Lactobacillus casei. Food and Nutr Sciences 5, 1756-1764. https://doi.org/10.4236/fns.2014.518189

7. Barukčić, I., Ščetar, M., Marasović, I., Jakopović, L.K., Galić, K., Božanić, R. (2020): Evaluation of quality parameters and shelf life of fresh cheese packed under modified atmosphere. Journal of Food Science and Technology 57, 2722-2731. https://doi.org/10.1007/s13197-020-04308-6

8. Bodzen, A., Iaconelli, C., Charriau, A., Dupuy, S., Beney, L., Patrick Gervais, P. (2020): Specific gaseous conditions significantly improve Lactobacillus casei and Escherichia coli survival to freeze drying and rehydration. Applied Food Biotechnology 7 (1), 201-208. https://doi.org/10.22037/afb.v7i1.26343

9. Cárdenas, N., Javier Calzada, J., Peiróten, A., Jiménez, E., Escudero, R., Rodríguez, J. M., Medina, M., Fernández, L. (2014): Development of a potential probiotic fresh cheese using two Lactobacillus salivarius strains isolated from Human Milk. BioMed Research International Article ID 801918, 1-12. https://doi.org/10.1155/2014/801918 . ISBN: 1110-7251

10. Caridi, A., Micari, P., Caparra, P., Cufari, A., Sarullo, V. (2003): Ripening and seasonal changes in microbial groups and in physico-chemical properties of the ewes’ cheese Pecorino del Poro. International Dairy Journal 13, 191-200. https://doi.org/10.1016/S0958-6946(02)00157-7
11. Chen, J.H., Hotchkiss, J.H. (1993): Growth of Listeria monocytogenes and Clostridium sporogenes in Cottage cheese in modified atmosphere packaging. Journal of Dairy Science 76, 972-977. https://doi.org/10.3168/jds.S0022-0302(93)77424-X

12. Chevanan, A., Muthkumarppan, N. (2007): Effect of calcium and phosphorus, residual lactose, and salt-tomooisture ratio on the melting characteristics and hardness of cheddar cheese during ripening. Journal of Food Science 72, 168-76. https://doi.org/10.1111/j.1750-3841.2007.00330.x

13. Conte, A., Gammariello, D., Di Giulio, S., Attanasio, M., Del Nobile, M. A. (2009): Active coating and modified-atmosphere packaging to extend the shelf life of Fior di Latte cheese. Journal of Dairy Science 92 (3), 887-894. https://doi.org/10.3168/jds.2008-1500

14. Conte, A., Brescia, I. Del Nobile, M. A. (2011): Lysozyme/EDTA disodium salt and modified-atmosphere packaging to prolong the shelf life of burrata cheese. Journal of Dairy Science, 94 (11), 5289-5297. https://doi.org/10.3168/jds.2010-3961

15. de Almeida, J.D.S.O., Dias, C.O., Pinto, S.S., Pereira, L.C., Verruck, S., Fritzen-Freire, C.B., Amboni, R.D.M.C. (2017): Probiotic Mascarpone-type cheese: Characterisation and cell viability during storage and simulated gastrointestinal conditions. International Journal of Dairy Technology 71, 195-203. https://doi.org/10.1111/1471-0307.12457

16. Del Nobile, M. A., Gammariello, D., Conte, A., Attanasio, M.A. (2009a): Combination of chitosan, coating and modified atmosphere packaging for prolonging Fior di latte cheese shelf life. Carbohydrate Polymers 78 (1), 2151-156. https://doi.org/10.1016/j.carbpol.2009.03.017

17. Del Nobile, M.A., Conte, A., Incoronato, A.L., Panza, O. (2009b): Modified atmosphere packaging to improve the microbial stability of Ricotta. African Journal of Microbiology Research 3 (4), 137-142. https://doi.org/10.5897/AJMR.9000503

18. Dermiki, M., Ntzimani, A., Badeka, A., Savvaidis, I. N., Kontominas, M. G. (2008): Shelf-life extension and quality attributes of the whey cheese “Myzithra Kalathaki” using modified atmosphere packaging. LWT - Food Science Technology 41 (2), 284-294. https://doi.org/10.1016/j.lwt.2007.02.014

19. D’Incecco, P., Limbo, S., Hogenboom, J., Rosi, V., Gobbi, S. Pellegrino, L. (2020): Impact of extending hard-cheese ripening: A multiparameter characterisation of Parmigiano Reggiano cheese ripened up to 50 months. Foods 9 (3), 263. https://doi.org/10.3390/foods9030268

20. Dinakar, P., Mistry, V.V. (1994): Growth and viability of Bifidobacterium bifidum in cheddar cheese. Journal of Dairy Science 77, 2854-2864. https://doi.org/10.3168/jds.50022-0302(94)77225-8

21. Felfoul, I., Attia, H., Bornaz, S. (2017): Shelf life determination of fresh cheese subjected to different modified atmospheres packaging. Journal of Agricultural Science and Technology 19 (4), 847-860.

22. Floros, J.D., Nielsen, P., Farkas, J. K. (2000): Advances in modified atmosphere and active packaging with applications in the dairy industry. Journal Bulletin of the International Dairy Federation 346.

23. Floros, J. D., Matsos, K. (2005): I. Introduction to modified atmosphere packaging. Innovations in Food Packaging 159-172. https://doi.org/10.1016/B978-012311632-1/50042-5 .

24. Gammariello, D., Conte, A., Attanasio, M., Del Nobile, M.A. (2009): Effect of modified atmospheres on microbiological and sensorial properties of Apulian fresh cheeses. African Journal of Microbiology Research 3(7), 370-378. https://doi.org/10.5897/AJMR.0000255

25. Gammariello, D., Conte, A. Di Giulio, S., Attanasio, M., Del Nobile, M.A. (2009b): Shelf life of Stracciatella cheese under modified-atmosphere packaging. Journal of Dairy Science 92 (2), 483-490. https://doi.org/10.3168/jds.2008-1571
26. Garabal, J.I., Rodríguez-Alonso, P., Franco, D.J.A., Centeno, J.A. (2010): Chemical and biochemical study of industrially produced San Simón da Costa smoked semi-hard cow’s milk cheeses: Effects of storage under vacuum and different modified atmospheres. *Journal of Dairy Science* 93 (5), 1868-1881. https://doi.org/10.3168/jds.2009-2749

27. Gionchetti, P., Rizzell, F., Helwig, U., Venturi, A., Lammers, K.M., Brigidi, P., Vitali, B., Poggioli, G., Miglioli, M., Campieri, M. (2003b): Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 124, 1202-1209. https://doi.org/10.1016/S0016-5085(00)82835-8

28. Gomes da Cruz, A., Buriti, F.C.A., Sta de Souza, C.H., Fonseca Faria, J.A., Isay Saad, S.M. (2009): Probiotic cheese health benefits, technological and stability aspects. *Trends in Food Science and Technology* 20, 344e354. https://doi.org/10.1016/j.tifs.2009.05.001

29. Govaris, A., Botsoglou, E., Sergelidis, D., Chatzopoulou, P.S. (2011): Antibacterial activity of oregano and thyme essential oils against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in feta cheese packaged under modified atmosphere. *LWT - Food Science and Technology* 44 (4), 1240-1244. https://doi.org/10.1016/j.lwt.2010.09.022

30. Hong-Xin, J., Mi-Ya, S., Guang-Yu, G. (2015): Influence of *Lactobacillus casei* LC2W on the proteolysis and aroma compounds of Cheddar cheese during ripening period. *CyTA - Journal of Food Science and Technology* 13 (3), 464-471. https://doi.org/10.1080/19476337.2014.1003099

31. Jakobsen, M., Risbo, J. (2009): Carbon dioxide equilibrium between product and gas phase of modified atmosphere packaging systems: Exemplified by semihard cheese. *Journal of Food Engineering* 92 (3), 285–290. https://doi.org/10.1016/j.jfoodeng.2008.11.007

32. Karimi, R., Mortazavian, A.M., Gomes da Cruz, A. (2011): Viability of probiotic microorganisms in cheese during production and storage: a review. *Dairy Science and Technology* 91, 283-308. https://doi.org/10.1007/s13594-011-0005-x

33. Khoshgozaran, S., Azizi, M.H., BagheriPooFallah, N. (2012): Evaluating the effect of modified atmosphere packaging on cheese characteristics: a review. *Dairy Science and Technology* 92, 1-24. https://doi.org/10.1007/s13594-011-0044-3

34. Kitazawa, H., Ueha, S., Itoh, S., Watanabe, H., KeiKono, K., Kawai, Y., TadaoSaito, T., Itoh, T., Yamaguchi, T. (2001): At oligonucleotides inducing B lymphocyte activation exist in probiotic *Lactobacillus gasseri*. *International Journal of Food Microbiology* 65, 149-162. https://doi.org/10.1016/S0168-1605(00)00500-6

35. Lemya, M.W., El Zubeir, I.E., El Owni, O. (2006): Composition and hygienic quality of Sudanese white soft cheese in Khartoum North markets (Sudan). *International Journal of Dairy Science* 5, 177-184. https://doi.org/10.3923/ijds.2006.36.43

36. Madureira, A.R., Soares J.C., Pintado, M.E., Gomes, A.M.P., Freitas, A.C., Malcata, F.X. (2008): Sweet whey cheese matrices inoculated with the probiotic strain *Lactobacillus paracasei* LAFTI L26. *Dairy Science and Technology* 88, 649-665. https://doi.org/10.1051/dst:2008026 .

37. Maniar, A.B., Marcy, J.E., Bishop, J.R., Duncan, S.E. (2006): Modified atmosphere packaging to maintain direct-set cottage cheese quality. *Journal of Food Science* 59 (6), 1305-1308. https://doi.org/10.1111/j.1365-2621.1994.tb14701.x

38. Mannheim, C.H., Soffer, T. (1996): Shelf-life Extension of Cottage Cheese by Modified Atmosphere Packaging. *LWT - Food Science and Technology* 29 (8), 767-771. https://doi.org/10.1006/fstl.1996.0120

39. McSweeney, P., Fox, P., Lucey, J., Jordan, K., Cogan, T. (1993): Contribution of the indigenous microflora to the maturation of Cheddar cheese. *International Dairy Journal*, 3, 613-634. https://doi.org/10.1016/0958-6946(93)90104-8
40. Olarte, C., Gonzalez-Fandos, E., Sanz, S.A (2001): proposed methodology to determine the sensory quality of a fresh goat’s cheese (Cameros cheese): application to cheeses packaged under modified atmospheres. Food Quality and Preference 12 (3), 163–170. https://doi.org/10.1016/S0950-3293(00)00041-0

41. Öner, Z., Karahan, A.G., Aloğlu, H. (2006): Changes in the microbiological and chemical characteristics of an artisanal Turkish white cheese during ripening. LWT-Food Science and Technology 39, 449-454. https://doi.org/10.1016/j.lwt.2005.03.015

42. Osman, N.F ., Thiboutot, H.E., Dako, M. (2008): The impact of lactic acid bacteria on cheese flavor. FEMS Microbiology Reviews 87, 131-148.

43. Papaioannou, G., Chouliara, I., Karatapanis, A.E., Kontominas, M.G., Savvaidis, I.N. (2007): Shelf-life of a Greek whey cheese under modified atmosphere packaging. International Dairy Journal 17 (4), 358-364. https://doi.org/10.1016/j.idairyj.2006.04.001

44. Phillips, M., Kailasapathy, K., Tran, L. (2006): Viability of commercial probiotic cultures (L. acidophilus, Bifidobacterium sp., L. casei, L. paracasei and L. rhamnosus) in cheddar cheese. International Journal of Food Microbiology 108 (2), 276-80. https://doi.org/10.1016/j.ijfoodmicro.2005.12.009

45. Pintado, M.E., Malcata, F.X. (2000): Optimization of modified atmosphere packaging with respect to physicochemical characteristics of Requeijão. Food Reserch International 33 (10), 821-832. https://doi.org/10.1016/S0963-9969(00)00078-8

46. Pintado, M.E., Malcata, F.X. (2007): The effect of modified atmosphere packaging on the microbial ecology in Requeijao, a Portuguese whey cheese. Journal of Food processing and preservation 24 (2), 107-124. https://doi.org/10.1111/j.1745-4549.2000.tb00408.x

47. Povetta, F.P ., da Solva, M.N., Tagloapoetra, B.L., Rochards, N.S.S. (2019): Addition of green banana biomass as partial substitute for fat and encapsulated Lactobacillus acidophilus in requeijao cremoso processed cheese. Food Science and Technology Campinas 40(2), 451-457. https://doi.org/10.1590/fst.03919

48. Rodríguez-Aguilera, R., Jorge, C., Oliveira, J.C., Montanez, J.C., Mahajan, P.V. (2011): Effect of modified atmosphere packaging on quality factors and shelf-life of mold surface-ripened cheese: Part II varying storage temperature. LWT - Food Science and Technology 44 (1), 337-342. https://doi.org/10.1016/j.lwt.2010.06.014

49. Rodríguez-Alonso, P., Centeno, J.A., Garabal, J.I. (2011): Biochemical study of industrially produced Arzúa-Ulloa semi-soft cows’ milk cheese: Effects of storage under vacuum and modified atmospheres with high-nitrogen contents. International Dairy Journal 21 (4), 261–271. https://doi.org/10.1016/j.idairyj.2010.11.008

50. Rotaro, A., Clementi, F. (2008): Exopolysaccharides from lactic acid bacteria: Structure, production and technological applications. Italian Journal of Food Science 1, 23-45.

51. Ščetar, M., Barukčić, I., Kurek, M., Jakopović, K.L., Božanić, R., Galić, K. (2019): Packaging perspective of milk and dairy products. Mljekarstvo 69 (1), 3-20. https://doi.org/10.15567/mljekarstvo.2019.0101

52. Sreekumar, O., Mosono, A. (2000): Immediated effect of lactobacillus on the intestinal flora and fecal enzyme of rats and in vitro inhibition of E.coli in coculture. Journal of Dairy Science 93, 931-939. https://doi.org/10.3168/jds.S0022-0302(00)74956-3

53. Subramaniam, P.J. (1989): Dairy Foods, multi-component products, dried foods and beverages. In B. A. Blakistone, M.D. Gaithersburg (Ed.), Principles and Applications of Modified Atmosphere Packaging of Foods (pp. 158–193). Aspen Publishers Inc. https://doi.org/10.1007/978-1-4757-6252-5_8

54. Temiz, H., Aylut, U., Hursit, A.K. (2009): Shelf life of Turkish whey cheese (Lor) under modified atmosphere packaging. International Journal of Dairy Technology 62 (3), 378-386. https://doi.org/10.1111/j.1471-0307.2009.00511.x
55. Temiz, H. (2010): Effect of modified atmosphere packaging on characteristics of sliced Kashar cheese. *Journal of Food Processing and Preservation* 34 (5), 926-943. https://doi.org/10.1111/j.1745-4549.2009.00431.x

56. Terpou, A., Mantzourani, I., Galanis, A., Kanellaki, M., Bezirtzoglou, E., Bekatorou, A., Koutinas, A.A., Plessas, S. (2018): Employment of *L. paracasei* K5 as a novel potentially probiotic freeze-dried starter for Feta-type cheese production. *Microorganisms* 7, 3. https://doi.org/10.3390/microorganisms7010003

57. Tharmaraj, N., Shah, N.P. (2004): Survival of *Lactobacillus acidophilus, Lactobacillus paracasei* subsp. *paracasei, Lactobacillus rhamnosus, Bifidobacterium animalis* and *Propionibacterium* in cheese-based dips and the suitability of dips as effective carriers of probiotic bacteria. *International Dairy Journal* 14, 1055-1066. https://doi.org/10.1016/j.idairyj.2004.04.011.

58. Tornadijo, M., Fresno, J., Bernardo, A., Sarmiento, R.M., Carballo, J. (1995): Microbiological changes throughout the manufacturing and ripening of a Spanish goat’s raw milk cheese (Armada variety). *Lait* 75, 551-570. https://doi.org/10.1051/lait:1995643

59. Vassiliadis, A., Psoni, L., Nikolaou, S., Arvanitis, L., Tzanetakis, N., Litopoulou-Tzanetaki, E. (2009): Changes in microbial populations, kinds of lactic acid bacteria and biochemical characteristics of Greek traditional feta cheese during ripening. *International Journal of Dairy Technology* 62, 39-47. https://doi.org/10.1111/j.1471-0307.2008.00453.x

60. Yerlikaya, O., Ozer, E. (2014): Production of probiotic fresh white cheese using co-culture with *Streptococcus thermophilus*. *Food Science and Technology Campinas* 34(3), 471-477. https://doi.org/10.1590/1678-457X.6365