

Organic Milk as Medium for Lactic Acid Bacteria Growth: a Review

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Abstract. In recent years, the demand for organic fermented dairy products has been growing. Lactic acid bacteria (LAB) are of major interest in the food industry due to their ability to form the quality and functionality of fermented products. A significantly higher LAB cell count is presented in organic (ORG) fermented products such as yoghurt and kefir, than their conventional (CONV) counterparts. There is still a lack of information on the possible explanation and evidence of ORG milk as a better medium for LAB growth. The aim of the present review is to summarise the results of research articles on the milk compounds and their effect on LAB growth, and to identify differences between ORG and CONV milk that may provide a plausible explanation for their influence on LAB growth in ORG milk. The monographic method was used in this study. According to the previous research, significant differences were established in a content and range of carbohydrates, nitrogen sources, fats, macroelements and microelements, as well bioactive compounds in ORG and CONV milk. The composition of ORG milk may have potential benefits for the development of LAB due to the higher concentration of peptides and long-chain polyunsaturated fatty acids in it.

Key words: organic milk, yoghurt, lactic acid bacteria.

Introduction

Yoghurt is a traditional and popular dairy product, made from milk by lactic acid fermentation using *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*) as the most common starter cultures. In general, yoghurt is a source of probiotics and is considered a good functional food. Meanwhile, lactic acid bacteria are one of the most significantly important groups of bacteria in the food industry (Reuben *et al.*, 2020; Wu *et al.*, 2017).

Fermented dairy products should contain at least 10^8 colony forming units (CFU) of live starter microorganisms per gram of product to have a beneficial effect on host health (EFSA, 2010). The global demand for yoghurt has increased after COVID-19 regarding its effects: improvement of the barrier function of the gastrointestinal tract, improvement of immunity, maintenance of microbiota. Furthermore, the food industry is looking for new sources of bioactive compounds and the general direction of yoghurt development is to produce high-quality products enriched with compounds which provide functional properties (Bulca *et al.*, 2022; Gu *et al.*, 2020; Li *et al.*, 2021).

LAB require an appropriate biochemical habitat and are highly dependent on the nutrient availability in the environment to affect their growth, cell biosynthesis, and overall functionality (Ayivi & Ibrahim, 2022; Hayek *et al.*, 2019; Mohamad Zabidi *et al.*, 2020).

Recently, Breza-Boruta and co-authors have reported that LAB cell counts and their biochemical activity are significantly higher in organic (ORG) fermented products – yoghurt and kefir – than in their conventional counterparts. More intensive LAB growth may be attributed to the composition of the ORG milk used to produce fermented products, which appears to provide a more favourable medium for LAB growth (Breza-Boruta *et al.*, 2022).

Most of the world's dairy production is carried out in either an intensive or conventional (CONV) system. However, due to the growing environmental and nutritional awareness of society, the demand for ORG products shows a steady upward trend (Breza-Boruta *et al.*, 2022; Brodziak *et al.*, 2021; Pirlo *et al.*, 2019). The comparison of two production systems is extremely difficult due to the accompanying factors (breed, environment, feeding, lactation stage, health

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status) (Schwendel *et al.*, 2015). Nevertheless, there are studies reporting compositional differences between ORG and CONV milk. Organic dairy products may contain significantly higher whey proteins (Brodziak *et al.*, 2021), total PUFA and certain n-3 fatty acids (Liu *et al.*, 2020; Średnicka-Tober *et al.*, 2016). Meanwhile, there are compounds such as vitamins and minerals that have been shown to vary between the studies.

In order to promote the production of yoghurt enriched with functional compounds, reduce fermentation time and save production costs, the growth-promoting and growth-limiting factors of LAB should be studied in detail. However, the detailed interaction between the milk variables and the LAB cell counts in the final fermented product has not been fully investigated. Therefore, the aim of the present review is to summarise the results of research articles on the milk compounds and their effect on LAB growth, and to identify possible differences between ORG and CONV milk that may provide a plausible explanation for their influence on more intensive LAB growth in ORG milk.

Materials and Methods

The monographic method was used in the review. The type of review is semi-systematic. The review summarises the available articles on the chemical composition of ORG and CONV milk and the possible differences between them in the period from 1984 to 2022. Web of Science and Scopus databases were examined to analyse ORG and CONV milk as

a medium for lactic acid bacteria growth, covering a wide range of published research results on milk compounds that affect lactic acid bacteria growth during the milk fermentation process. To find relevant information as main key terms, chemical composition of organic milk and lactic acid bacteria growth in milk were used. In the review, 113 full text articles were examined. The authors used a wide range of synonyms to search for the maximum corresponding literature.

Results and Discussion

Milk is a complex of nutrients, including proteins, fats, carbohydrates, vitamins, and minerals. The differences in chemical composition between ORG and CONV milk may affect the growth of LAB during the fermentation process, quality, and functionality of the yoghurt (Abbasiliasi *et al.*, 2017; Breza-Boruta *et al.*, 2022). According to the previous research (Table 1), the differences exist in the content and range of carbohydrate sources (lactose, oligosaccharides), nitrogen sources (casein, whey protein, peptides and amino acids), fats (saturated and unsaturated fatty acids), macroelements and microelements (calcium, magnesium, manganese, etc.), bioactive compounds (lactoferrin, lysozyme, thiamin, riboflavin etc.).

Carbohydrates

The degradation of carbohydrates and related compounds to obtain energy and carbon molecules is the primary metabolic activity in LAB that is necessary for survival and growth (Hoefnagel *et al.*, 2002; Sanchez & Demain, 2008). Starting in the lag

Table 1

Differences in chemical composition of organic and conventional milk

Indicator	Results	Reference
Lactose	Similar content Lower in ORG	Roesch <i>et al.</i> , 2005; Bilik & Lopuszanska-Rusek., 2010 Tunick <i>et al.</i> , 2016; Kucevic <i>et al.</i> , 2016; Zagorska & Ciprovica, 2008.
Oligosaccharides	Higher in ORG	Schwendel <i>et al.</i> , 2017
Protein	Lower in ORG Higher in ORG Similar content	Manuelian <i>et al.</i> , 2022; Kouřimská <i>et al.</i> , 2014; Kuczyńska <i>et al.</i> , 2012 Kucevic <i>et al.</i> , 2016 Zagorska & Ciprovica, 2008; Butler <i>et al.</i> , 2008
Casein	Lower in ORG Similar total content α1-casein higher in ORG κ-casein higher in ORG β-casein higher in CONV	Manuelian <i>et al.</i> , 2022; Kuczyńska <i>et al.</i> , 2012 Schwendel <i>et al.</i> , 2017 Schwendel <i>et al.</i> , 2017 Schwendel <i>et al.</i> , 2017 Schwendel <i>et al.</i> , 2017
Whey protein	Similar total content Higher in ORG β-lactoglobulin higher in CONV	Brodziak <i>et al.</i> , 2021 Schwendel <i>et al.</i> , 2017 Kuczyńska <i>et al.</i> , 2012 Schwendel <i>et al.</i> , 2017

Amino acids	Similar content Some are higher in CONV	Manuelian <i>et al.</i> , 2022 Chung <i>et al.</i> , 2019
Peptide	Higher in ORG	Brodziak <i>et al.</i> , 2021; Kuczyńska <i>et al.</i> , 2012
Lactoferrin	Higher in ORG	Kuczyńska <i>et al.</i> , 2012; Brodziak <i>et al.</i> , 2021
Lysozyme	Higher in ORG	Kuczyńska <i>et al.</i> , 2012; Zagorska & Ciprovica, 2008; Brodziak <i>et al.</i> , 2021
Urea	Lower in ORG	Toledo <i>et al.</i> , 2002
Fats	Higher in ORG	Zagorska & Ciprovica, 2008; Kouřimská <i>et al.</i> , 2014; Tunick <i>et al.</i> , 2016
Saturated FA	Higher in CONV	Delgadillo-Puga <i>et al.</i> , 2014
Unsaturated FA	MUFA higher in ORG MUFA higher in CONV PUFA higher in ORG PUFA higher in CONV PUFA similar content n-3 PUFA higher in ORG Linoleic acid higher in ORG Linoleic acid higher in CONV α -linolenic acid higher in ORG	Delgadillo-Puga <i>et al.</i> , 2014 Schwendel <i>et al.</i> , 2017 Bai <i>et al.</i> , 2022; Manuelian <i>et al.</i> , 2022 Delgadillo-Puga <i>et al.</i> , 2014 Schwendel <i>et al.</i> , 2017 Souza Tavares <i>et al.</i> , 2022; Manuelian <i>et al.</i> , 2022 Schwendel <i>et al.</i> , 2017 Tunick <i>et al.</i> , 2016 Schwendel <i>et al.</i> , 2017; Tunick <i>et al.</i> , 2016
Milk phospholipids	Higher in ORG	Ferreiro <i>et al.</i> , 2015
Total content of minerals	Similar content	Tunick <i>et al.</i> , 2016
Ca	Similar content Lower in ORG Higher in ORG	Florence <i>et al.</i> , 2009 Koperska <i>et al.</i> , 2013 Qin <i>et al.</i> , 2021
Fe	Higher in ORG Lower in ORG	Florence <i>et al.</i> , 2009 Qin <i>et al.</i> , 2021
Mg	Similar content Lower in ORG	Florence <i>et al.</i> , 2009 Koperska <i>et al.</i> , 2013
Mn	Lower in ORG	Koperska <i>et al.</i> , 2013; Qin <i>et al.</i> , 2021
Cu	Similar content Lower in ORG	Florence <i>et al.</i> , 2009 Qin <i>et al.</i> , 2021; Koperska <i>et al.</i> , 2013
Zn	Similar content Lower in ORG	Florence <i>et al.</i> , 2009 Koperska <i>et al.</i> , 2013; Qin <i>et al.</i> , 2021; Rodríguez-Bermúdez <i>et al.</i> , 2018
K	Higher in ORG	Qin <i>et al.</i> , 2021
P	Higher in ORG	Qin <i>et al.</i> , 2021
I	Lower in ORG	Rodríguez-Bermúdez <i>et al.</i> , 2018
Se	Lower in ORG	Rodríguez-Bermúdez <i>et al.</i> , 2018
Mo	Higher in ORG	Qin <i>et al.</i> , 2021
Al	Lower in ORG	Qin <i>et al.</i> , 2021
Thiamine	Lower in ORG	Zagorska & Ciprovica, 2008
Riboflavin	Lower in ORG	Zagorska & Ciprovica, 2008
β -carotene	Higher in ORG	Bergamo <i>et al.</i> , 2003; Brodziak <i>et al.</i> , 2018; Gortzi <i>et al.</i> , 2022
Retinol	Similar content	Bergamo <i>et al.</i> , 2003
α -tocopherol	Higher in ORG	Bergamo <i>et al.</i> , 2003; Brodziak <i>et al.</i> , 2018; Gortzi <i>et al.</i> , 2022

ORG - organic; CONV - conventional; PUFA - polyunsaturated fatty acids; MUFA - monounsaturated fatty acids

phase and during the exponential phase, limitations on bacterial growth occur due to the lack of nutrients, such as carbon sources, needed or preferred by LAB species. Most LAB are able to ferment monosaccharides such as glucose and galactose and disaccharides such as lactose and sucrose (Abbasiliasi *et al.*, 2017; Chervaux *et al.*, 2000). The ability of LAB to metabolise different carbon sources is based on the specific activities of the enzymes involved in carbohydrate degradation. Based on fermentation pathways, LAB can be divided into two physiological groups: homo-fermentative and hetero-fermentative bacteria. Both *Lb. bulgaricus* and *S. thermophilus* are *homo-fermentative* and produce energy by converting hexoses to lactic acid accordingly D (-) and L (+) via the Emden-Meyerhof pathway (Ayivi & Ibrahim, 2022; Hayek & Ibrahim, 2013; Hayek *et al.*, 2019).

It has been shown that LAB species and even strains have their preferences among sugars for optimal growth and metabolic activity. During fermentation, *S. thermophilus* and *Lb. bulgaricus* strains consume both glucose and lactose (Delgado-Fernández *et al.*, 2019). The enzymes responsible for glucose and lactose fermentation are present in *Lb. bulgaricus*, and it is likely that both sugars are consumed simultaneously. However, the growth rate of *Lb. bulgaricus* on glucose was shown to be less efficient than on lactose, suggesting that lactose is a more preferred carbohydrate for this bacterium (Chervaux *et al.*, 2000). It has also been shown that *Lb. bulgaricus* does not grow on galactose, and consumption of this monosaccharide was very low (Delgado-Fernández *et al.*, 2019). Oligosaccharides (N-acetylglucosamine, sialic acid) and polysaccharides can also be metabolised as carbon sources and can stimulate the growth of some LAB species (Borgonovi *et al.*, 2022; Belusko *et al.*, 2022).

Carbohydrate's content can also affect the growth and functionality of LAB. It has been shown that the growth of LAB is not only affected by the type of carbon sources, but also by their content and ratios in the medium (Abbasiliasi *et al.*, 2017; Hayek & Ibrahim, 2013; Özen & Özilgen, 1992). The presence of galactose (when content of galactose more than 1 g mL⁻¹ and content of lactose 1 g mL⁻¹) resulted in growth inhibition of *Lb. bulgaricus*, and the low content of lactose (below 0.4 g mL⁻¹) slowed down the growth of this bacterium (Chervaux *et al.*, 2000). This finding supports Cutrim *et al.* (2016) and Moreira *et al.* (2017) – the researchers found a variable fermentation time according to the lactose content in milk. An acceleration of the fermentation process was observed with milk containing 1.5 g·100 mL⁻¹ of lactose, while a delay of the fermentation process was observed in milk containing < 0.6 g·100 mL⁻¹ of lactose. The low lactose content in milk affected the metabolic activity

of the starter cultures, and a low lactic acid production was observed (Cutrim *et al.*, 2016; Vénica *et al.*, 2018).

With regard to lactose, the studies conducted showed the contradictory results in the content of ORG and CONV. Several publications reported no significant difference in lactose content between ORG and CONV milk (Bilik & Lopuszanska-Rusek, 2010; Roesch *et al.*, 2005), while several works indicated lower content of lactose in ORG milk (Tunick *et al.*, 2016; Kucevic *et al.*, 2016; Zagorska & Ciprovica, 2008). Schwendel *et al.* (2017) reported a higher concentration of oligosaccharides in ORG milk. Due to the higher number of LAB and their metabolic activity in ORG fermented products (Breza-Boruta *et al.*, 2022), it can also be assumed that ORG milk contains higher content of free glucose and galactose, which are available and readily utilised by starter cultures (Vénica *et al.*, 2018). Nevertheless, the possible differences in carbohydrate content between ORG and CONV milk may affect LAB growth during fermentation.

Proteins and other nitrogen sources

Carbon and nitrogen sources are essential for optimising the growth potential of LAB. Although simple sugars are the main sources of carbon and energy, it is also possible to obtain them from other organic compounds such as whey protein, casein, peptides, amino acids (Ayivi & Ibrahim, 2022; Hayek & Ibrahim, 2013; Hayek *et al.*, 2019). Nitrogen is required by LAB for all processes related to biological growth especially in terms of cellular protein synthesis and nucleic acid production (Abbasiliasi *et al.*, 2017). Protein sources enhance the growth of bacterial cells with a general effect on their functionality (Atilola *et al.*, 2015; Ayivi & Ibrahim, 2022).

LAB growth media usually contains more than one type of nitrogen source. Nitrogen sources used in LAB growth media, such as protease peptones, beef extract and yeast extract, are sources of amino acids, peptides, nucleic acid derivatives, minerals and vitamins (Aasen *et al.*, 2000; Aspmo *et al.*, 2005; Hayek *et al.*, 2019; Horn *et al.*, 2005; Lechiancole *et al.*, 2002). Purines and pyrimidines are also considered to be crucial for the growth and metabolic activity of LAB (Fan *et al.*, 2022a; Li *et al.*, 2022). After inoculation, cells begin to adapt in substrate during the lag phase and continue exponentially, presumably using the amino acids, dipeptides, tripeptides, and oligopeptides that are freely available in milk. Subsequently, amino acids become limiting, and the culture enters a post-exponential growth phase in which extracellular protease synthesis is required and initiated for the production of free amino acids (Juillard *et al.*, 1995;

Stulova *et al.*, 2011). Accordingly, although milk is a nutrient-rich growth medium, depending on lactation period it could contain low content of free amino acids and peptides required for the efficient growth of LAB (Shihata *et al.*, 2009; Vasiljevic *et al.*, 2005). To compensate for this and to be able to meet the nitrogen requirements to ensure the active and rapid growth in milk, LAB rely on a complex system of proteinases and peptidases called the proteolytic system (Ayivi & Ibrahim, 2022; Donkor *et al.*, 2007; Hayek *et al.*, 2019). In particular, each species has its own proteolytic system and level of activity (Rodríguez-Serrano *et al.*, 2018). For example, it has been shown that *S. thermophilus* primarily prefers different types of peptides than *Lb. bulgaricus* (Liu *et al.*, 2016).

With regard to the total protein content in ORG and CONV, the available literature shows the contradictory results. The majority of conducted studies have shown that CONV milk tends to have higher total protein content than ORG (Kouřimská *et al.*, 2014; Kuczyńska *et al.*, 2012; Manuelian *et al.*, 2022). Only some studies reported higher protein content in ORG (Kucevic *et al.*, 2016), or showed similar values in the total protein content (Butler *et al.*, 2008; Zagorska & Ciprovica, 2008).

Casein, whey protein, peptides

Whey, which is an excellent source of nitrogen compounds, organic acids, lactose, vitamins, and minerals, has been reported to be used as a growth medium for several LAB species. A whey permeated medium can support LAB biomass production without external supplementation with nitrogen sources and has low costs (Burns *et al.*, 2008; Hayek *et al.*, 2019; Macedo *et al.*, 2002; Sarenkova *et al.*, 2022). Compared to casein, whey protein was reported to be used by *Lb. bulgaricus* 2038 as a more favourable nitrogen source (Liu *et al.*, 2016).

The proteolytic system also enables LAB bacteria to break down casein into peptides and amino acids, which are used as an additional source of organic nitrogen (Donkor *et al.*, 2007). There are differences in LAB preferences for casein fractions, for example, *Lactococcus* spp. use mainly β -casein for their growth. Also, the oligopeptides, regardless of their origin (i.e., initially present in milk or released from caseins), are necessary for early growth in milk and account for 98% of the growth of *L. lactis* (Juillard *et al.*, 1995). Increasing content of oligopeptides can be used to support exponential growth of *Lb. bulgaricus* and a second exponential growth phase of *S. thermophilus* (Ayivi & Ibrahim, 2022; Sieuwerts *et al.*, 2010).

Schwendels' study has shown that the total content of proteins in milk does not vary between farming systems. However, the composition of casein and whey proteins in milk varied between ORG and

CONV systems. According to Schwendel *et al.* (2017), α s1-casein and κ -casein were significantly higher in ORG milk, whereas β -casein and β -lactoglobulin A were higher in CONV milk. Brodziak *et al.* (2021) reported that the production system did not affect the whey protein content in milk ($0.72 \text{ g} \cdot \text{L}^{-1}$ in the organic system versus $0.70 \text{ g} \cdot \text{L}^{-1}$ in the intensive system), but significantly affected bioactive peptides, β -lactoglobulin, α -lactalbumin, bovine serum albumin, lactoferrin, and lysozyme, which were higher in ORG milk. While milk from ORG farms has significantly higher content of major whey proteins and some bioactive peptides, it showed lower content of caseins and bovine serum albumin (Kuczyńska *et al.*, 2012; Manuelian *et al.*, 2022). These existing differences in content and spectrum of protein between ORG and CONV milk may play a significant role in the LAB growth during fermentation.

Urea

Urea is a non-protein nitrogen compound that is present in milk and plays an important role in the growth and metabolism of LAB during milk fermentation. Urea is a source of nitrogen that can be metabolised by LAB, providing an additional nutrient source for bacterial growth easily digested by LAB (Pernoud *et al.*, 2004). The urea cycle is a metabolic pathway that converts urea to ammonia, which can be utilised by the bacteria for the biosynthesis of amino acids and other nitrogen-containing compounds. Some LAB, such as *S. thermophilus*, *Lactococcus lactis*, and *Lb. bulgaricus*, have been shown to possess urease activity, which allows them to convert urea into ammonia (Puniya *et al.*, 2016).

The utilisation of urea as a nitrogen source by LAB is advantageous, especially in spring when milk is deficient in nitrogen sources. During the fermentation process, the availability of nitrogen sources in milk is limited. This can result in a nitrogen-limited environment that slows down bacterial growth and reduces the quality of the fermented product. In addition, the presence of urea in milk can also contribute to the buffering capacity of the milk. Urea can act as a weak base that in a high dose can neutralise acids produced by LAB during the fermentation process, thus preventing the decrease in pH that could inhibit bacterial growth. Urea can serve as an important nitrogen source for LAB (Pernoud *et al.*, 2004).

The differences in urea content between organic and conventional milk may affect LAB growth in yoghurt production. Some studies have found that ORG milk has lower urea content than CONV milk (Toledo *et al.*, 2002).

Amino acids

Amino acids (AA) are essential nitrogen and carbon sources for the metabolism and growth of

lactic acid bacteria. During the fermentation process, free AA in milk are depleted. LAB produce several extracellular and intracellular proteases that hydrolyse milk proteins into peptides and AA. These nitrogen-containing compounds are metabolised and used for energy production and biomass synthesis. AA can be used for protein synthesis, enzyme activity, and energy metabolism of LAB (Pederson *et al.*, 1999).

The requirement for the amount and spectrum of AA in the growth medium appears to be species dependent. For example, some strains of *Lactobacillus* spp. require only four AA, whereas other LAB require from 14 to 20 AA. Overall, *S. thermophilus* appears to be much less demanding than other LAB, especially *Lactococcus lactis* and *Lactobacillus* spp. (Fan, 2022b; Pederson *et al.*, 1999; Rodríguez-Serrano *et al.*, 2018).

The role of several AA in the growth stimulation of LAB can be emphasised. Sulfur-containing amino acids: cysteine, methionine and branched-chain amino acids: leucine, isoleucine, valine are a quantitatively important group of AA for *S. thermophilus* strains (Ayivi & Ibrahim, 2022; Garault *et al.*, 2000). Most *S. thermophilus* strains also share requirements for glutamine and methionine (Letort & Juillard, 2001). Cysteine has been proposed in order to reduce fermentation time and improve the growth of *Lb. bulgaricus*, *S. thermophilus* and other LAB in milk (Shah, 2000). Glycine, serine, and threonine are most important in promoting the growth of *Lactobacillus brevis* 54 (Fan *et al.*, 2022a; Du *et al.*, 2022). Most *Lactococcus lactis* strains require branched chain AA as well as histidine and methionine for their growth (Chopin, 1993). Histidine increases the buffering capacity of the growth medium which promotes the growth of LAB (Ayivi *et al.*, 2022).

However, there is also evidence that free AA alone play an important role in the growth of LAB (Juillard *et al.*, 1995). Only when the medium was supplemented with 20 amino acids, vitamins (cobalamin, riboflavin, niacin, folic acid, biotin, thiamine and others), macroelements and microelements (magnesium, manganese, iron, copper, zinc and other compounds) was it able to effectively support the growth and viability of *Lb. bulgaricus* CNRZ397 during fermentation (Chervaux *et al.*, 2000). The proteolytic activity of *Lb. bulgaricus* was observed to be enhanced by trace elements such as Fe, Mn, Cu, and Se, which at certain concentrations, act as a co-factor during the fermentation process (Nour *et al.*, 2014).

Differences in the AA composition of ORG and CONV milk have not been sufficiently investigated. Available data from a detailed comparison of ORG and CONV milk showed that the profile did not differ between the farming systems (Manuelian *et al.*, 2022). Another recent comparison showed lower $\delta^{13}\text{C}$

amino acid content of isoleucine, leucine, methionine, phenylalanine, proline, serine and valine in ORG milk (Chung *et al.*, 2019), and authors emphasised more seasonal variation in AA composition.

Bioactive compounds

Lactoferrin

The presence of lactoferrin in milk and its influence on yoghurt production process can have both positive and negative effects on the LAB growth. Lactoferrin is a protein naturally present in milk and has been shown to have a number of health benefits, including immune system support, anti-inflammatory effects, and antimicrobial properties (Actor *et al.*, 2009). In recent years, there has been growing interest in adding lactoferrin to dairy products after fermentation, such as yoghurt, in order to improve their nutritional value and potential health benefits (Tomita *et al.*, 2009). Lactoferrin can promote the growth of certain LAB species, such as *Lb. acidophilus* (Kim *et al.*, 2004), but can also cause partial inhibition in the growth of *S. thermophilus*. The effect of lactoferrin on LAB growth in milk may depend on various factors, such as the type and concentration of lactoferrin, the species and count of LAB, and the pH and temperature conditions during the fermentation process (Franco *et al.*, 2010).

Several studies have reported the higher concentrations of lactoferrin in milk from certified ORG farms (Brodziak *et al.*, 2018; Kuczyńska *et al.*, 2012). Zagorska & Ciprovica obtained an almost twofold higher concentration of lactoferrin in ORG milk as compared to intensive milk production systems with a total mixed ration (Zagorska & Ciprovica, 2008). The existing potential differences in lactoferrin concentration between ORG and CONV milk may have an impact on the growth and development of LAB in yoghurt production.

Lysozyme

Lysozyme is an enzyme that is naturally presented in milk and has been shown to have potential benefits in yoghurt production. Several scientific papers have been published on the antibacterial properties of lysozyme-based enzyme preparations and their use in food processing for better fermentation control (Liburdi *et al.*, 2014). Lysozyme has a selective antimicrobial activity based on the hydrolysis of peptidoglycan, the component of the cell wall, increasing its permeability and bursting the susceptible gram-positive bacteria cell layer (Ragland, 2017). The efficacy of lysozyme in inhibiting LAB growth depends on species/strain (Blattel *et al.*, 2009; Delfini *et al.*, 2004), on the cell's physiological state (Cunningham *et al.*, 1991), the structure of lysozyme (Ibrahim *et al.*, 1996), and the presence of certain compounds in the medium (Guzzo *et al.*, 2011; Liburdi *et al.*, 2014). As reported by

Selamoglu *et al.* (2020), lysozyme reduced the growth of pathogenic bacteria in milk without affecting the desirable probiotic bacteria in fermented milk (Selamoglu, 2020).

Some studies have shown significantly higher concentrations of lysozyme in ORG compared to CONV milk. In the study conducted by Brodziak *et al.* (2018), a strong positive correlation between concentrations of lactoferrin and lysozyme was detected, and the highest concentrations of lactoferrin (123.8 mg·L⁻¹) and lysozyme (11.14 µg·L⁻¹) were found in ORG milk. Previously, Kuczynska *et al.* (2012) also reported higher lysozyme concentration in the late pasture ORG milk (15.68 µg·L⁻¹) compared to the early indoor feeding CONV milk (12.56 µg·L⁻¹).

Fats

Fats take a central place in the metabolic pathways of LAB and are crucial for optimising their growth potential (Hayek & Ibrahim, 2013; Ayivi & Ibrahim, 2022). Lipase is the main enzyme in LAB that is required to utilise milk fat as a substrate. The activity of the enzyme that allows LAB to hydrolyse mono-, di-, and triglycerols in milk has been reported to be highly genus and species and/or strain dependent. Furthermore, not all LAB are capable of metabolising lipids (Katz, 2002; Regula, 2007). Cultures of *Lb. helveticus*, *Lb. acidophilus*, and *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. delbrueckii* subsp. *lactis* showed high content of esterase activity compared to lipase activity (Hofi *et al.*, 2011).

Nevertheless, the presence of milk fat during fermentation is important to achieve a higher final cell density (Tan *et al.*, 2012; Zhang *et al.*, 2020). In the study conducted by Stulova *et al.*, the values of maximum growth rates depended significantly on the milk fat content, especially in the second exponential phase, decreased fat concentration (0.05%) has a positive effect on the bacteria growth (Stulova *et al.*, 2011).

Several studies have shown that CONV milk has a lower fat content than ORG milk (Kouřimská *et al.*, 2014; Tunick *et al.*, 2016; Zagorska & Ciprovica, 2008). These existing differences in total fat content in ORG and CONV milk may have an influence on LAB growth. However, the composition of milk fats must also be considered.

Long chain fatty acids

The presence of fatty acids (FA) in the medium serves as a necessary growth factor for LAB (Fan *et al.*, 2022b; Li *et al.*, 2022). The chemical and enzymatic reactions of LAB involved in the fermentation of milk first break down milk fat triglycerides into monoglycerides – potential substrates for bacterial growth (Regula, 2007).

The effect on LAB growth may depend on the spectrum and the concentration of FA. Previous studies have shown that low content of medium or long-chain unsaturated FA promotes LAB growth, whereas high content of long-chain saturated FA have an inhibitory effect on LAB growth (Partanen *et al.*, 2001; Zhang *et al.*, 2020). Long-chain unsaturated FA are considered to be essential and most beneficial for most LAB species, including *Lb. bulgaricus* (Ayivi & Ibrahim, 2022; Partanen *et al.*, 2001). Mono- and polyunsaturated long-chain fatty acids (MUFA, PUFA) can influence the growth and activity of LAB by providing an energy source and modulating the cell membrane structure. Oleic acid and linoleic acid can stimulate the growth and can improve LAB metabolic activity during fermentation (Abbasiliasi *et al.*, 2017; Hayek *et al.*, 2019). This can result in faster fermentation rates and improved sensory characteristics, such as flavour and aroma, in the final product (Farak *et al.*, 2020; Florence *et al.*, 2009). Authors have concluded that LAB produce a higher concentration of conjugated linolic acid, linolenic acid and ω-3 fatty acids in fermented organic dairy products (Florence *et al.*, 2012).

Compared to CONV milk, ORG milk had higher concentrations of MUFA, PUFA, including n-3 and n-6 FA (Bai *et al.*, 2022; Delgadillo-Puga *et al.*, 2014; Schwendel *et al.*, 2017; Souza Tavares *et al.*, 2022; Manuelian *et al.*, 2022; Tunick *et al.*, 2016). In contrast, a higher content of MUFA in CONV milk and a similar content of PUFA were reported by Schwendel *et al.* (2017).

It should be emphasised that the effect of long-chain FA on LAB growth during milk fermentation may also depend on several other factors, such as concentration of LAB species used, the presence of suboptimal content of other compounds in the media, such as vitamins (Hayek & Ibrahim, 2013), and the pH and temperature conditions during fermentation.

Phospholipids

Milk phospholipids (MPL) are valuable components of the milk fat globule membrane. MPL are associated with several health benefits, including promoting neurological development, protecting against inflammation, and reducing lipids as well as LDL cholesterol in blood plasma (Bhinder *et al.*, 2017; Castro-Gómez *et al.*, 2016; Snow *et al.*, 2011; Zhang *et al.*, 2020). Some studies have been focused on the effect of MPL on LAB growth. Zhang *et al.* reported that the addition of 0.5 and 1% milk phospholipids to the culture medium significantly promotes the growth of LAB, which is consistent with the suggestion that LAB may utilise MPL as a source of energy or growth factors. However, it still remains unclear which MPL are utilised by LAB (Rocha-Mendoza *et al.*, 2020;

Zhang *et al.*, 2020). The recent comparison of milk composition and milk fat showed that ORG milk had significantly higher content of phospholipids studied than CONV counterparts (Ferreiro *et al.*, 2015). The existing differences in MPL concentration between ORG and CONV milk may influence the LAB growth and the functional properties of yoghurt.

Vitamins

Vitamins are essential micronutrients that play an important role in the growth and metabolism of LAB during the lactic fermentation process. Vitamin requirements of LAB vary among species, and some vitamins can be substituted for others. The individual strains require one to four vitamins in certain concentrations for normal growth (Hayek & Ibrahim, 2013). The vitamin requirement of LAB includes B group vitamins such as thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, and cobalamin (Chervaux *et al.*, 2000; Letort & Juillard, 2001). These vitamins are essential regulatory and growth stimulating factors for LAB, serve as an important co-factor for many metabolic enzymes, act as electron carriers and acyl group carriers, and perform other essential functions of cell membrane metabolism (Tripuraneni, 2011; Yoo *et al.*, 1997;). Riboflavin, niacin, and pantothenic acid increase the metabolism of substrates such as glucose, lactate, and pyruvate, and have been shown to be a minimum requirement by both *Lb. bulgaricus* and *S. thermophilus* strains (Chervaux *et al.*, 2000; Letort & Juillard, 2001).

Cow's milk contains all the B group vitamins, but most comparative milk quality analysis studies have focused on the fat-soluble vitamins. Zagorska & Ciprovica reported lower concentration of thiamine and riboflavin in ORG milk (Zagorska & Ciprovica, 2008). The concentration of vitamins in milk is well attributed to the animal's diet (Schwendel *et al.*, 2015). Therefore, in some cases, milk may be deficient in certain vitamins, which may limit the growth and metabolic activities of LAB, leading to reduced fermentation efficiency and product quality.

Macroelements and microelements

Minerals found in milk are important compounds that support LAB growth. LAB cultivation media mainly contains manganese and magnesium as mineral sources that act as a growth co-factor (Hayek *et al.*, 2019; Rocha-Mendoza *et al.*, 2020). In addition, manganese, iron, and copper ions serve as a major metal co-factor for the enzymatic function of superoxide dismutases (SODs). SODs are among most important enzymes of LAB that provide protection against hydrogen peroxide toxicity, which is the growth inhibitory factor of LAB (Feng & Wang, 2020). Calcium, manganese, and magnesium are required for

proper growth and cell assembly of *Lb. bulgaricus*, *Lb. casei* and *S. thermophilus* (Ayivi & Ibrahim, 2022; Hayek *et al.*, 2019; Letort & Juillard, 2001).

Presumably, the sufficient availability of several essential minerals, rather than the presence of a single mineral, is crucial for supporting the growth of LAB. As reported by Wright & Klaenhammer, the presence of manganese in a broth medium alone could not support the optional growth of *Lb. bulgaricus* (Wright & Klaenhammer, 1984). This idea was supported by Florence *et al.* The chemical composition of CONV and ORG milk was similar (calcium, zinc, magnesium, and copper contents were not significantly different), with the exception of higher protein and iron concentrations in the ORG milk ($3.72 \pm 0.03 \text{ g} \cdot 100\text{g}^{-1}$ and $0.514 \pm 0.113 \text{ mg} \cdot 100\text{g}^{-1}$) than in CONV milk ($3.24 \pm 0.04 \text{ g} \cdot 100 \text{ g}^{-1}$ and $0.415 \pm 0.083 \text{ mg} \cdot 100\text{g}^{-1}$). The final counts of *S. thermophilus* ($9.0\text{--}10.1 \log_{10} \text{ CFU} \cdot \text{mL}^{-1}$) and *Lb. bulgaricus* ($8.2\text{--}8.5 \log_{10} \text{ CFU} \cdot \text{mL}^{-1}$) were not significantly different in the two types of milk (Florence *et al.*, 2009). Contrary to this opinion, it has been shown that magnesium is the only essential oligoelement required for the growth of *Lb. lactis* (Hayek *et al.*, 2019; Hébert *et al.*, 2004a, 2004b).

In CONV milk, mineral content is related to sanitary-management practices and mineral supplementation added to the concentrate feed, which are limited in ORG systems (Zagorska & Ciprovica, 2005; Rodríguez-Bermúdez *et al.*, 2018). Nevertheless, in most cases, the mineral content did not differ significantly between the dairy management systems (Tunick *et al.*, 2016). On the other hand, the available research has also shown the possible differences in several minerals in ORG and CONV milk, such as calcium, magnesium, manganese, and iron (Breza-Boruta *et al.*, 2022; Koperska *et al.*, 2013; Qin *et al.*, 2021). The differences in the content, spectrum, and ratios of carbohydrates (lactose), nitrogen sources (peptides and amino acids), fats (unsaturated long-chain fatty acids), macroelements and microelements (calcium, magnesium, manganese, iron, etc.), and bioactive compounds (lactoferrin and vitamins), may provide a plausible explanation for the more intensive growth of LAB in ORG milk.

Conclusions

This review provides a better understanding of organic milk as a medium for the growth of lactic acid bacteria and highlights the key factors that promote or limit the growth of lactic acid bacteria. The composition of organic milk may have potential benefits for the development of lactic acid bacteria due to its higher concentration of peptides and long-chain polyunsaturated fatty acids. In addition, the quality of yoghurt produced from organic and conventional milk should be given the greatest attention.

References

- Aasen, I. M., Møretør, T., Katla, T., Axelsson, L., & Storrø, I. (2000). Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. *Appl. Microbiol. Biotechnol.*, 53, 159-166. DOI: 10.1007/s002530050003.
- Abbasiliasi, S., Tan, J. S., Ibrahim, T. A. T., Bashokouh, F., Ramakrishnan, N. R., Mustafa, S., ... Ariff, A. B. (2017). Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: A review. *Rsc. Advances*, 7(47), 29395-29420. DOI: 10.1039/C6RA24579J.
- Actor, J. K., Hwang, S. A., & Kruzel, M. L. (2009). Lactoferrin as a natural immune modulator. *Curr. Pharm. Des.*, 15(17), 1956-1973. DOI: 10.2174/138161209788453202.
- Ayivi, R. D., & Ibrahim, S. A. (2022). Lactic acid bacteria: an essential probiotic and starter culture for the production of yoghurt. *Int. J. Food Sci. Technol.*, 57(11), 7008-7025. DOI: 10.1111/ijfs.16076.
- Ayivi, R. D., Ibrahim, S. A., Krastanov, A., Somani, A., & Siddiqui, S. A. (2022). The impact of alternative nitrogen sources on the growth and viability of *Lactobacillus delbrueckii* ssp. *bulgaricus*. *J. Dairy Sci.*, 105(10), 7986-7997. DOI: 10.3168/jds.2022-21971.
- Aspmo, S. I., Horn, S. J., & Eijsink, V. G. (2005). Hydrolysates from Atlantic cod (*Gadus morhua* L.) viscera as components of microbial growth media. *Process Biochem.*, 40(12), 3714-3722. DOI: 10.1016/j.procbio.2005.05.004.
- Atilola, O. A., Gyawali, R., Aljaloud, S. O., & Ibrahim, S. A. (2015). Use of phytone peptone to optimize growth and cell density of *Lactobacillus reuteri*. *Foods*, 4(3), 318-327. DOI: 10.3390/foods4030318.
- Bai, Y., Zhang, B., Zhang, X., Zhao, S., Qie, M., Wang, Q., ... Guo, J. (2022). Discrimination between organic and conventional raw and UHT milk by fatty acid profile in Inner Mongolia, China. *Int. J. of Dairy Technol.*, 75(1), 94-105. DOI: 10.1111/1471-0307.12811.
- Belusko, A., Aumeistere, L., & Ciprovica, I. (2022). Oligosaccharides in human milk, achievements in analysis: a review. *Res. Rural Dev.*, 37, 100-105. DOI: 10.22616/rrd.28.2022.015.
- Bergamo, P., Fedele, E., Iannibelli, L., & Marzillo, G. (2003). Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. *Food Chem.*, 82(4), 625-631. DOI: 10.1016/S0308-8146(03)00036-0.
- Bhinder, G., Allaire, J. M., Garcia, C., Lau, J. T., Chan, J. M., Ryz, N. R., ... Vallance, B. A. (2017). Milk fat globule membrane supplementation in formula modulates the neonatal gut microbiome and normalizes intestinal development. *Sc. Rep.*, 7(1), 1-15. DOI: 10.1038/srep45274.
- Bilik, K., & Łopuszańska-Rusek, M. (2010). Effect of organic and conventional feeding of Red-and-White cows on productivity and milk composition. *Ann. Anim. Sci.*, 10(4), 441-458.
- Blattel, V., Wirth, K., Claus, H., Schlott, B., Pfeiffer, P., & König, H. (2009). A lytic enzyme cocktail from *Streptomyces* sp. B578 for the control of lactic and acetic acid bacteria in wine. *Appl. Microbiol. Biotechnol.*, 83, 839-848. DOI: 10.1007/s00253-009-1926-7.
- Borgonovi, T. F., Virgolin, L. B., Janzantti, N. S., Casarotti, S. N., & Penna, A. L. B. (2022). Fruit bioactive compounds: Effect on lactic acid bacteria and on intestinal microbiota. *Food Res. Int.*, 161. Elsevier Ltd. DOI: 10.1016/j.foodres.2022.111809.
- Breza-Boruta, B., Ligocka, A., & Bauza-Kaszewska, J. (2022). Natural bioactive compounds in organic and conventional fermented food. *Mol.*, 27(13). DOI: 10.3390/molecules27134084.
- Brodziak, A., Król, J., Litwińczuk, Z., & Barłowska, J. (2018). Differences in bioactive protein and vitamin status of milk from certified organic and conventional farms. *Int. J. Dairy Technol.*, 71(2), 321-332. DOI: 10.1111/1471-0307.12462.
- Brodziak, A., Wajs, J., Zuba-Ciszewska, M., Król, J., Stobiecka, M., & Jańczuk, A. (2021). Organic versus conventional raw cow milk as material for processing. In *Animals* 11(10). MDPI. DOI: 10.3390/ani11102760.
- Bulca, S., Umut, F., & Koç, A. (2022). The influence of microbial transglutaminase on camel milk yogurt. *LWT*, 160, 113339. DOI: 10.1016/j.lwt.2022.113339.
- Burns, P., Vinderola, G., Molinari, F., & Reinheimer, J. (2008). Suitability of whey and buttermilk for the growth and frozen storage of probiotic lactobacilli. *Int. J. Dairy Technol.*, 61(2), 156-164. DOI: 10.1111/j.1471-0307.2008.00393.x.
- Butler, G., Nielsen, J. H., Slots, T., Seal, C., Eyre, M. D., Sanderson, R., ... Leifert, C. (2008). Fatty acid and fat-soluble antioxidant concentrations in milk from high-and low-input conventional and organic systems: seasonal variation. *J. Sci. Food Agric.*, 88, 1431-1441. DOI: 10.1002/jsfa.3235.
- Castro-Gómez, P., Rodríguez-Alcalá, L. M., Monteiro, K. M., Ruiz, A. L., Carvalho, J. E., ... Fontecha, J. (2016). Antiproliferative activity of buttermilk lipid fractions isolated using food grade and non-food grade solvents on human cancer cell lines. *Food Chem.*, 212, 695-702. DOI: 10.1016/j.foodchem.2016.06.030.

- Chervaux, C., Ehrlich, S. D., & Maguin, E. (2000). Physiological study of *Lactobacillus delbrueckii* subsp. *bulgaricus* strains in a novel chemically defined medium. *Appl. Environ. Microbiol.*, 66(12), 5306-5311. DOI: 10.1128/AEM.66.12.5306-5311.2000.
- Chopin, A. (1993). Organization and regulation of genes for amino acid biosynthesis in lactic acid bacteria. *FEMS Microbiol. Reviews*, 12(1-3), 21-37. DOI: 10.1111/j.1574-6976.1993.tb00011.
- Chung, I. M., Kim, J. K., Yarnes, C. T., An, Y. J., Kwon, C., Kim, S. Y., ... Kim, S. H. (2019). Fatty acid- and amino acid-specific isotope analysis for accurate authentication and traceability in organic milk. *J. Agric. Food Chem.*, 67(2), 711-722. DOI: 10.1021/acs.jafc.8b05063.
- Cunningham, F. E., Proctor, V. A., & Goetsch, S. J. (1991). Egg-white lysozyme as a food preservative: an overview. *Worlds Poultr. Sci. J.*, 47(2), 141-163. DOI: 10.1079/WPS19910015.
- Cutrim, C. S., de Barros, R. F., da Costa, M. P., Franco, R. M., Conte-Junior, C. A., & Cortez, M. A. S. (2016). Survival of *Escherichia coli* O157:H7 during manufacture and storage of traditional and low lactose yogurt. *LWT*, 70, 178-184. DOI: 10.1016/j.lwt.2016.02.047.
- Delfini, C., Cersosimo, M., Del Prete, V., Strano, M., Gaetano, G., Pagliara, A., & Ambrò, S. (2004). Resistance screening essay of wine lactic acid bacteria on lysozyme: efficacy of lysozyme in unclarified grape musts. *J. Agric. Food Chem.*, 52(7), 1861-1866. DOI: 10.1021/jf034824m.
- Delgadillo-Puga, C., Sánchez-Muñoz, B., Nahed-Toral, J., Cuchillo-Hilario, M., Díaz-Martínez, M., Solís-Zabaleta, R., ... Castillo-Domínguez, R. M. (2014). Fatty acid content, health and risk indices, physicochemical composition, and somatic cell counts of milk from organic and conventional farming systems in tropical south-eastern Mexico. *Trop. Anim. Health Prod.*, 46, 883-888. DOI: 10.1007/s11250-014-0581-x.
- Delgado-Fernández, P., Corzo, N., Olano, A., Hernández-Hernández, O., & Moreno, F. J. (2019). Effect of selected prebiotics on the growth of lactic acid bacteria and physicochemical properties of yoghurts. *Int. Dairy J.*, 89, 77-85. DOI: 10.1016/j.idairyj.2018.09.003.
- Donkor, O. N., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2007). Proteolytic activity of dairy lactic acid bacteria and probiotics as determinant of growth and in vitro angiotensin-converting enzyme inhibitory activity in fermented milk. *Lait*, 87(1), 21-38. DOI: 10.1051/lait:2006023.
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2010). Scientific opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006. *EFSA J.*, 8(10), 1763.
- Evers, J. M. (2004). The milk fat globule membrane – compositional and structural changes post secretion by the mammary secretory cell. *Int. Dairy J.*, 14(8), 661-674. DOI: 10.1016/j.idairyj.2004.01.005.
- Fan, X., Du, L., Xu, J., Shi, Z., Zhang, T., Jiang, X., Zeng, X., Wu, Z., & Pan, D. (2022a). Effect of single probiotics *Lactocaseibacillus casei* CGMCC1.5956 and *Levilactobacillus brevis* CGMCC1.5954 and their combination on the quality of yogurt as fermented milk. *LWT – Food Sci. Technol.*, 163. DOI: 10.1016/j.lwt.2022.113530.
- Fan, X., Li, X., Du, L., Li, J., Xu, J., Shi, Z., Li, C., Tu, M., Zeng, X., Wu, Z., & Pan, D. (2022b). The effect of natural plant-based homogenates as additives on the quality of yogurt: A review. In *Food Biosci.*, 49. Elsevier Ltd. DOI: 10.1016/j.fbio.2022.101953.
- Farag, M. A., Jomaa, S. A., El-wahed, A. A., & El-seedi, H. R. (2020). The many faces of kefir fermented dairy products: quality characteristics, flavour chemistry, nutritional value, health benefits, and safety. *Nutrients*, 12(2). DOI: 10.3390/NU12020346.
- Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes*, 12. DOI: 10.1080/19490976.2020.1801944.
- Ferreiro, T., Gayoso, L., & Rodríguez-Otero, J. L. (2015). Milk phospholipids: Organic milk and milk rich in conjugated linoleic acid compared with conventional milk. *J. Dairy Sci.*, 98(1), 9-14. DOI: 10.3168/jds.2014-8244.
- Florence, A. C. R., Da Silva, R. C., do Espírito Santo, A. P., Gioielli, L. A., Tamime, A. Y., & de Oliveira, M. N. (2009). Increased CLA content in organic milk fermented by bifidobacteria or yoghurt cultures. *Dairy Sci. Technol.*, 89(6), 541-553. DOI: 10.1051/dst/2009030.
- Florence, A.C., Béal, C., Sliva, R. C., Bogsan, C. S. B., Pilleggi, A. L., Gioielli, L. A., Oliviera, M.N. (2012) Fatty acid profile trans-ocatadecenoic, α -linolenic and conjugated linoleic acid contents differing in certified organic and conventional probiotic fermente milks. *Food Chem.*, 135(4), 2207-2214. <https://doi.org/10.1016/j.foodchem.2012.07.026>.

- Franco, I., Castillo, E., Pérez, M. D., Calvo, M., & Sánchez, L. (2010). Effect of bovine lactoferrin addition to milk in yogurt manufacturing. *J. Dairy Sci.*, 93(10), 4480–4489. DOI: 10.3168/jds.2009-3006.
- Garault, P., Letort, C., Juillard, V., & Monnet, V. (2000). Branched-chain amino acid biosynthesis is essential for optimal growth of *Streptococcus thermophilus* in milk. *Appl. Environ. Microbiol.*, 66(12), 5128–5133. DOI: 10.1128/AEM.66.12.5128-5133.2000.
- Gorissen, L., Taes, K., Weckx, S., Dannenberger, D., Leroy, F., De Vuyst, L., De Smet, S. (2010). Production of conjugated linolic acid and conjugated linolenic acid isomers by *Bifidobacterium* species. *Appl. Microbiol. Biotechnol.*, 87(6), 2257–2266. DOI: 10.1007/s00253-010-2713-1.
- Gu, Y., Li, X., Xiao, R., Dudu, O. E., Yang, L., & Ma, Y. (2020). Impact of *Lactobacillus paracasei* IMC502 in coculture with traditional starters on volatile and non-volatile metabolite profiles in yogurt. *Process Biochem.*, 99, 6169. DOI: 10.1016/j.procbio.2020.07.003.
- Guzzo, F., Cappello, M. S., Azzolini, M., Tosi, E., & Zapparoli, G. (2011). The inhibitory effects of wine phenolics on lysozyme activity against lactic acid bacteria. *Int. J. Food Microbiol.*, 148(3), 184–190. DOI: 10.1016/j.ijfoodmicro.2011.05.023.
- Hayek, S. A., & Ibrahim, S. A. (2013). Current limitations and challenges with lactic acid bacteria: A Review. *Food Nutr. Sci.*, 04(11), 73–87. DOI: 10.4236/fns.2013.411a010.
- Hayek, S. A., Gyawali, R., Aljaloud, S. O., Krastanov, A., & Ibrahim, S. A. (2019). Cultivation media for lactic acid bacteria used in dairy products. *J. Dairy Res.*, 86(4), 490–502. DOI: 10.1017/S002202991900075X.
- Hébert, E. M., Raya, R. R., & Giori, G. S. D. (2004a). Nutritional requirements of *Lactobacillus delbrueckii* subsp. *lactis* in a chemically defined medium. *Curr. Microbiol.*, 49, 341–345. DOI: 10.1007/s00284-004-4357-9.
- Hébert, E. M., Raya, R. R., & Savoy de Giori, G. (2004b). Evaluation of minimal nutritional requirements of lactic acid bacteria used in functional foods. *Environ. Microbiol. Methods Protoc.*, 139–148. DOI: 10.1385/1-59259-765-3:139.
- Hoefnagel, M. H., Starrenburg, M. J., Martens, D. E., Hugenholtz, J., Kleerebezem, M., Van Swam, I. I., ... Snoep, J. L. (2002). Metabolic engineering of lactic acid bacteria, the combined approach: kinetic modelling, metabolic control and experimental analysis. *Microbiol.*, 148(4), 1003–1013. DOI: 10.1099/00221287-148-4-1003.
- Hof, M., Tanboly, E. S., & Abd-Rabou, N. S. (2011). Industrial application of lipases in cheese making: a review. *Int. J. Food Saf.*, 13, 293–302.
- Horn, S. J., Aspmo, S. I., & Eijsink, V. G. H. (2005). Growth of *Lactobacillus plantarum* in media containing hydrolysates of fish viscera. *J. Appl. Microbiol.*, 99(5), 1082–1089. DOI: 10.1111/j.1365-2672.2005.02702.x
- Ibrahim, H. R., Higashiguchi, S., Juneja, L. R., Kim, M., & Yamamoto, T. (1996). A structural phase of heat-denatured lysozyme with novel antimicrobial action. *J. Agric. Food Chem.*, 44(6), 1416–1423. DOI: 10.1021/jf9507147.
- Yoo, I. K., Chang, H. N., Lee, E. G., Chang, Y. K., & Moon, S. H. (1997). Effect of B vitamin supplementation on lactic acid production by *Lactobacillus casei*. *J. Ferment. Bioeng.*, 84(2), 172–175. DOI: 10.1016/S0922-338X(97)82551-2.
- Juillard, V., Le Bars, D., Kunji, E. R., Konings, W. N., Gripon, J.-C., Richard, J., ... Biol, J. (1995). Oligopeptides are the main source of nitrogen for *Lactococcus lactis* during growth in milk. *Appl. Environ. Microbiol.*, 61(8), 3024–3030. DOI: 10.1128/aem.61.8.3024-3030.1995.
- Katz, M., Medina, R., Gonzalez, S., & Oliver, G. (2002). Esterolytic and lipolytic activities of lactic acid bacteria isolated from ewe's milk and cheese. *J. Food Protect.*, 65(12), 1997–2001. DOI: 10.4315/0362-028X-65.12.1997
- Kim, W. S., Ohashi, M., Tanaka, T., Kumura, H., Kim, G. Y., Kwon, I. K., ... Shimazaki, K. I. (2004). Growth-promoting effects of lactoferrin on *L. acidophilus* and *Bifidobacterium* spp. *Biomaterials*, 17, 279–283. DOI: 10.1023/B:BIOM.0000027705.57430.f1.
- Koperska, N., Kędzierska-Matysek, M., Litwińczuk, Z., & Wójcik-Saganek, A. (2013). Correlation between the content of macro- and microelements in milk obtained from organic and conventional farms. In *Proceed. Conf. Mater. XVI Lublin Sci. Magn. Conf.— Chem. Elements Health*, Lublin, Poland 64.
- Kouřimská, L., Legarová, V., Panovská, Z., & Pánek, J. (2014). Quality of cows' milk from organic and conventional farming. *Czech J. Food Sci.*, 32(4), 398–405. DOI: 10.17221/510/2012-CJFS.
- Kucevic, D., Trivunovic, S., Bogdanovic, V., Cobanovic, K., Jankovic, D., & Stanojevic, D. (2016). Composition of raw milk from conventional and organic dairy farming. *Biotechnol. Anim. Husb.*, 32(2), 133–143. DOI: 10.2298/bah1602133k.

- Kuczyńska, B., Puppel, K., Gołębiewski, M., Metera, E., Sakowski, T., & Słoniewski, K. (2012). Differences in whey protein content between cow's milk collected in late pasture and early indoor feeding season from conventional and organic farms in Poland. *J. Sci. Food Agric.*, 92(14), 2899–2904. DOI: 10.1002/jsfa.5663.
- Lechiancole, T., Ricciardi, A., & Parente, E. (2002). Optimization of media and fermentation conditions for the growth of *Lactobacillus sakei*. *Annals of microbiology*, 52(3), 257-274.
- Letort, C., & Juillard, V. (2001). Development of a minimal chemically-defined medium for the exponential growth of *Streptococcus thermophilus*. *J. Appl. Microbiol.*, 91(6), 1023-1029. DOI: 10.1046/j.1365-2672.2001.01469.x.
- Li, H., Song, W., Liu, T., Xu, S., Zhang, S., Zhang, Y., ... Yu, J. (2022). Developing novel synbiotic yoghurt with *Lactobacillus paracasei* and lactitol: Investigation of the microbiology, textural and rheological properties. *Int. Dairy J.*, 135, 105475. DOI: 10.1016/j.idairyj.2022.105475.
- Li, S., Tian, Y., Jiang, P., Lin, Y., Liu, X., & Yang, H. (2021). Recent advances in the application of metabolomics for food safety control and food quality analyses. *Crit. Rev. Food Sci. Nutr.*, 61(9), 1448-1469. DOI: 10.1080/10408398.2020.1761287.
- Liburdi, K., Benucci, I., & Esti, M. (2014). Lysozyme in wine: An overview of current and future applications. *Compr. Rev. Food Sci. Food Saf.*, 13(5), 1062–1073. DOI: 10.1111/1541-4337.12102.
- Liu, E., Zheng, H., Shi, T., Ye, L., Konno, T., Oda, M., ... Ji, Z. S. (2016). Relationship between *Lactobacillus bulgaricus* and *Streptococcus thermophilus* under whey conditions: Focus on amino acid formation. *Int. Dairy J.*, 56, 141–150. DOI: 10.1016/j.idairyj.2016.01.019.
- Liu, N., Pustjens, A. M., Erasmus, S. W., Yang, Y., Hettinga, K., & van Ruth, S. M. (2020). Dairy farming system markers: The correlation of forage and milk fatty acid profiles from organic, pasture and conventional systems in the Netherlands. *Food Chem.*, 314. DOI: 10.1016/j.foodchem.2019.126153.
- Macedo, M. G., Lacroix, C., Gardner, N. J., & Champagne, C. P. (2002). Effect of medium supplementation on exopolysaccharide production by *Lactobacillus rhamnosus* RW-9595M in whey permeate. *Int. Dairy J.*, 12(5), 419-426. DOI: 10.1016/S0958-6946(01)00173-X.
- Manuelian, C. L., Vigolo, V., Burbi, S., Righi, F., Simoni, M., & De Marchi, M. (2022). Detailed comparison between organic and conventional milk from Holstein-Friesian dairy herds in Italy. *J. Dairy Sci.*, 105(7), 5561–5572. DOI: 10.3168/jds.2021-21465.
- Miller-Catchpole, R., Kot, E., Haloftis, G., Furmanov, S., & Bezkorovainy, A. (1997). Lactoferrin can supply iron for the growth of *Bifidobacterium breve*. *Nut. Res.*, 17(2), 205-213. DOI: 10.1016/S0271-5317(96)00252-7.
- Mohamad Zabidi, N. A., Foo, H. L., Loh, T. C., Mohamad, R., & Abdul Rahim, R. (2020). Enhancement of versatile extracellular cellulolytic and hemicellulolytic enzyme productions by *Lactobacillus plantarum* RI 11 isolated from Malaysian food using renewable natural polymers. *Molecules*, 25(11), 2607. DOI: 10.3390/molecules25112607.
- Moreira, T. C., da Silva, A. T., Fagundes, C., Ferreira, S. M. R., Cândido, L. M. B., Passos, M., & Krüger, C. C. H. (2017). Elaboration of yogurt with reduced level of lactose added of carob (*Ceratonia siliqua* L.). *LWT-Food Sci. Technol.*, 76, 326-329. DOI: 10.1016/j.lwt.2016.08.033.
- Nour, I., Fattouh, F., & El-Adawi, H. (2014). Chemically defined medium for optimization of proteolytic activity of *Lactobacillus bulgaricus* 761N. *Int. J. Life Sci. Med. Res.*, 4(4), 46–56. DOI: 10.5963/lsmr0404002.
- Özen, S., & Özilgen, M. (1992). Effects of substrate concentration on growth and lactic acid production by mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *J. Chem. Technol. Biotechnol.*, 54(1), 57-61. DOI: 10.1002/jctb.280540111.
- Partanen, L., Martinen, N., & Alatossava, T. (2001). Fats and fatty acids as growth factors for *Lactobacillus delbrueckii*. *Syst. Appl. Microbiol.*, 24(4), 500-506. DOI: 10.1078/0723-2020-00078.
- Paz, D., Aleman, R. S., Cedillos, R., Olson, D. W., Aryana, K., Marcia, J., & Boeneke, C. (2022). Probiotic characteristics of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* as influenced by Carao (*Cassia grandis*). *Fermentation*, 8(10), 499. DOI: 10.3390/fermentation8100499.
- Pederson, J.A., Steele, J.L., Christensen, J.E., Dudley, E.G. (1999). Peptidases and amino acid catabolism in lactic acid bacteria. Konings, W.N., Kuipers, O.P., In 't Veld, J.H.J.H. (eds) *Lactic Acid Bacteria: Genetics, Metabolism and Applications*. Springer, Dordrecht. DOI: 10.1007/978-94-017-2027-4_11.
- Pernoud, S., Fremaux, C., Sepulchre, A., Corrieu, G., & Monnet, C. (2004). Effect of the metabolism of urea on the acidifying activity of *Streptococcus*

- thermophilus*. *J. Dairy Sci.*, 87(3), 550–555. DOI: 10.3168/JDS.S0022-0302(04)73196-3.
- Pirlo, G., & Lolli, S. (2019). Environmental impact of milk production from samples of organic and conventional farms in Lombardy (Italy). *J. Clean. Prod.*, 211, 962–971. DOI: 10.1016/j.jclepro.2018.11.070.
- Poolman, B., Knol, J., Van Der Does, C., Henderson, P. J., Liang, W. J., Leblanc, G., ... Mus-Veteau, I. (1996). Cation and sugar selectivity determinants in a novel family of transport proteins. *Mol. Microbiol.*, 19(5), 911–922. DOI: 10.1046/j.1365-2958.1996.397949.x.
- Puniya, A., Kumar, S., Puniya, M., & Malik, R. (2017). Fermented milk and dairy products: an overview, *Fermented milk and dairy products*, 1(3-24), CRC Press, Boca Raton, FL.
- Qin, N., Faludi, G., Beauclercq, S., Pitt, J., Desnica, N., Pétursdóttir, Á., ... Stergiadis, S. (2021). Macromineral and trace element concentrations and their seasonal variation in milk from organic and conventional dairy herds. *Food Chem.*, 359, 129865. DOI: 10.1016/j.foodchem.2021.129865.
- Ragland, S. A., & Criss, A. K. (2017). From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS pathogens*, 13(9), e1006512. DOI: 10.1371/journal.ppat.1006512.
- Regula, A. (2007). Free fatty acid profiles of fermented beverages made from ewe's milk. *Lait*, 87(1), 71–77. DOI: 10.1051/lait:2006024.
- Reuben, R. C., Roy, P. C., Sarkar, S. L., Rubayet Ul Alam, A. S. M., & Jahid, I. K. (2020). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *J. Dairy Sci.*, 103(2), 1223–1237. DOI: 10.3168/JDS.2019-17092.
- Rocha-Mendoza, D., Kosmerl, E., Miyagusuku-Cruzado, G., Giusti, M. M., Jiménez-Flores, R., & García-Cano, I. (2020). Growth of lactic acid bacteria in milk phospholipids enhances their adhesion to Caco-2 cells. *J. Dairy Sci.*, 103(9), 7707–7718. DOI: 10.3168/jds.2020-18271.
- Rodríguez-Bermúdez, R., López-Alonso, M., Miranda, M., Fouz, R., Orjales, I., & Herrero-Latorre, C. (2018). Chemometric authentication of the organic status of milk on the basis of trace element content. *Food Chem.*, 240, 686–693. DOI: 10.1016/j.foodchem.2017.08.011.
- Rodríguez-Serrano, G. M., García-Garibay, J. M., Cruz-Guerrero, A. E., Gómez-Ruiz, L. D. C., Ayala-Niño, A., Castañeda-Ovando, A., & González-Olivares, L. G. (2018). Proteolytic system of streptococcus thermophilus. *J. Microbiol. Biotechnol.*, 28(10), 1581–1588. DOI: 10.4014/jmb.1807.07017.
- Roesch, M., Doherr, M. G., & Blum, J. W. (2005). Performance of dairy cows on swiss farms with organic and integrated production. *J. Dairy Sci.*, 88(7), 2462–2475. DOI: 10.3168/jds.S0022-0302(05)72924-6.
- Sanchez, S., & Demain, A. L. (2008). Metabolic regulation and overproduction of primary metabolites. *Microb. Biotechnol.*, 1(4), 283–319. DOI: 10.1111/j.1751-7915.2007.00015.x.
- Sarenkova, I., Sáez-Orviz, S., Rendueles, M., Ciprovica, I., Zagorska, J., & Díaz, M. (2022). Downstream approach routes for the purification and recovery of Lactobionic acid. *Foods*, 11(4), 583. DOI: 10.3390/foods11040583.
- Schwendel, B. H., Wester, T. J., Morel, P. C. H., Fong, B., Tavendale, M. H., Deadman, C., Shadbolt, N. M., ... Otter, D. E. (2017). Pasture feeding conventional cows removes differences between organic and conventionally produced milk. *Food Chem.* 229, 805–813. DOI: 10.1016/j.foodchem.2017.02.104.
- Schwendel, B. H., Wester, T. J., Morel, P. C. H., Tavendale, M. H., Deadman, C., Shadbolt, N. M., ... Otter, D. E. (2015). Invited review: Organic and conventionally produced milk-An evaluation of factors influencing milk composition. *J. Dairy Sci.*, 98(2), 721–746. DOI: 10.3168/jds.2014-8389.
- Selamoglu, Z. (2020). Use of enzymes in dairy industry: a review of current progress. *Arch. Razi Inst.*, 75(1), 131. DOI: 10.22092%2FARI.2019.126286.1341.
- Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *J. Dairy Sci.*, 83(4), 894–907. DOI: 10.3168/jds.S0022-0302(00)74953-8.
- Shihata, A., & Shah, N. P. (2000). Proteolytic profiles of yogurt and probiotic bacteria. *Int. Dairy J.*, 10(5-6), 401–408. DOI: 10.1016/S0958-6946(00)00072-8.
- Sieuwerts, S., Molenaar, D., van Hijum, S. A., Beerthuyzen, M., Stevens, M. J., Janssen, P. W., ... van Hylckama Vlieg, J. E. (2010). Mixed-culture transcriptome analysis reveals the molecular basis of mixed-culture growth in *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Appl. Environ. Microbiol.*, 76(23), 7775–7784. DOI: 10.1128/AEM.01122-10.
- Snow, D. R., Ward, R. E., Olsen, A., Jimenez-Flores, R., & Hintze, K. J. (2011). Membrane-rich milk fat diet provides protection against gastrointestinal leakiness in mice treated with lipopolysaccharide. *J. Dairy Sci.*, 94(5), 2201–2212. DOI: 10.3168/jds.2010-3886.

- Średnicka-Tober, D., Barański, M., Seal, C. J., Sanderson, R., Benbrook, C., Steinshamn, H., ... Leifert, C. (2016). Higher PUFA and n-3 PUFA, conjugated linoleic acid, α -tocopherol and iron, but lower iodine and selenium concentrations in organic milk: a systematic literature review and meta-and redundancy analyses. *Br. J. Nutr.*, 57001, 1043–1060. DOI: 10.1017/S0007114516000349.
- Stulova, I., Kabanova, N., Kriščiunaite, T., Laht, T.-M., & Vilu, R. (2011). The effect of milk heat treatment on the growth characteristics of lactic acid bacteria. *Agron. Res.*, 9, 473–478.
- Tan, W. S., Budinich, M. F., Ward, R., Broadbent, J. R., & Steele, J. L. (2012). Optimal growth of *Lactobacillus casei* in a Cheddar cheese ripening model system requires exogenous fatty acids. *J. Dairy Sci.*, 95(4), 1680–1689. DOI: 10.3168/jds.2011-4847.
- Toledo, P., Andren, A., & Björck, L. (2002). Composition of raw milk from sustainable production systems. *Int. Dairy J.*, 12(1), 75–80. DOI: 10.1016/S0958-6946(01)00148-0.
- Tomita, M., Wakabayashi, H., Shin, K., Yamauchi, K., Yaeshima, T., & Iwatsuki, K. (2009). Twenty-five years of research on bovine lactoferrin applications. *Biochim.*, 91(1), 52–57. DOI: 10.1016/j.biochi.2008.05.021.
- Tripuraneni, S. (2011). *Effect of nutrient supplements on cucumber fermentation by lactic acid bacteria*. Graduate Theses and Dissertations, University of Arkansas.
- Tunick, M. H., Van Hekken, D. L., Paul, M., Ingham, E. R., & Karreman, H. J. (2016). Case study: Comparison of milk composition from adjacent organic and conventional farms in the USA. *Int. J. Dairy Technol.*, 69(1), 137–142. DOI: 10.1111/1471-0307.12284.
- Utama, C. S., Sulistiyanto, B., & Yolansa, A. B. A. (2020). Quality improvement of fermented wheat pollard with addition of vitamin minerals seen from potential hydrogen content, total lactic acid bacteria and total yeast. In IOP conference series: Earth and environmental science, 518(1), 012–017. <https://doi.org/10.1088/1755-1315/518/1/012017>.
- Vasiljevic, T., Shah, N. P., & Jelen, P. (2005). Growth characteristics of *Lactobacillus delbrueckii subsp. bulgaricus* ATCC 11842 as affected by different neutralisers. *Aust. J. Dairy Technol.*, 60(1), 3.
- Vénica, C. I., Wolf, I. V., Suárez, V. B., Bergamini, C. V., & Perotti, M. C. (2018). Effect of the carbohydrates composition on physicochemical parameters and metabolic activity of starter culture in yogurts. *LWT - Food Sci. Technol.*, 94, 163–171. DOI: 10.1016/j.lwt.2018.04.034.
- Wright, C. T., & Klaenhammer, T. R. (1984). Phosphated milk adversely affects growth, cellular morphology, and fermentative ability of *Lactobacillus bulgaricus*. *J. Dairy Sci.*, 67(1), 44–51. DOI: 10.3168/jds.S0022-0302(84)81264-3.
- Wu, Z., Wu, J., Cao, P., Jin, Y., Pan, D., Zeng, X., ... Guo, Y. (2017). Characterization of probiotic bacteria involved in fermented milk processing enriched with folic acid. *J. Dairy Sci.*, 100(6), 4223–4229. DOI: 10.3168/jds.2017-12640.
- Zagorska, J., & Ciprovica, I. (2008). The chemical composition of organic and conventional milk in Latvia. In Proceedings 3rd Baltic Conference on Food Science and Technology Foodbalt-2008, 17–18 April (pp. 10–14), Jelgava, Latvia: Latvia University of Agriculture.
- Zagorska, J., & Ciprovica, I. (2005). The comparison of chemical pollution between organic and conventional milk. In Research for rural development: International scientific conference proceedings, 19–22 May 2005 (pp. 196–198). Jelgava, Latvia: Latvia University of Agriculture.
- Zhang, L., García-Cano, I., & Jiménez-Flores, R. (2020). Effect of milk phospholipids on the growth and cryotolerance of lactic acid bacteria cultured and stored in acid whey-based media. *J. Dairy Sci. Commun.* 1(2), 36–40. DOI: 10.3168/jdsc.2020-0007.