Trends in Food Science & Technology 109 (2021) 499–512

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review on factors affecting and control of post-acidification in yoghurt and related products

Gaurav Kr Deshwal [a], Swati Tiwari [b], Ajay Kumar [c], Rakesh Kumar Raman [a], Saurabh Kadyan [d,*]

[a] Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal, 132001, India
[b] Department of Food Science & Technology, Pondicherry University, Kalapet, 605014, India
[c] Nestle India Limited, Samalkha, Haryana, 132101, India
[d] Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, 132001, India

ARTICLE INFO

Keywords:
Post-acidification
Yoghurt
Fermented milk
High hydrostatic pressure
Strain mutagenesis

ABSTRACT

Background: Yoghurt and related products relish vast consumption pattern worldwide. The quality of such products depreciates during chilled-chain storage and transportation due to residual metabolic activity of viable starters leading to post-acidification, which in turn reduces product's shelf-life, decreases consumer acceptance and is even detrimental to stability of probiotics. This phenomenon is predominant in tropical and resource-poor nations, hence, there is growing interest to curb post-acidification without affecting product's quality.

Scope and approach: This review summarizes recent findings on various associated factors (starter type, milk composition, processing parameters, pre- and pro-biotics and packaging material), conventional and emerging approaches spanning the domains of food processing, microbiology and preservation to combat post-acidification in yoghurt and related products. Shortcomings and future scope of strategies have also been discussed which will provide new avenues for the researchers to work forward in improving the quality and shelf-life of fermented products.

Key findings and conclusions: Thermal treatment of fermented products indisputably controls post-acidification but also destroys heat labile beneficial peptides and microbes. Emerging techniques like HHP, PEF, ultrasound etc. could potentially be used for the development of mild flavor shelf-stable fermented products. However, cost-effectiveness, optimization of process parameters and specific legal requirement should be considered. Control via legally permitted preservatives or additives is feasible provided desirable sensorial attributes are not altered. Direct incorporation of bacteriocins in yoghurt is hindered by its high purification cost and stability in food matrix. Bioprotective cultures and strain improvement through random mutagenesis have been employed successfully for *in-situ* aversion of post-acidification.

1. Introduction

Fermented milk products like yoghurt are correlated with their positive influence on human health due to their rich pool of bioactive proteins, hydrolyzed carbohydrates, vitamins and minerals with improved bioavailability (Campos, Neves, Flach, Costa, & Sousa, 2017), and are widely consumed globally as highlighted by production and per capita consumption data in Table 1. Yoghurt and its counterparts (country-specific yoghurt alike traditional fermented milks as described in Table 2) are prepared by fermenting the milk base i.e. milk from any species or milk solids using food grade starter cultures, majorly lactic acid bacteria (Garrigues et al., 2017). Yoghurt is prepared by protocooperation of *Streptococcus salivarius* subsp. *thermophilus* (SST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LDB) added in 1:1 ratio as starters. Initially, SST, being oxygen tolerant, starts acidification and reduces pH to 5.2 while at pH 4.4 growth is dominated by LDB. At this pH, the fermentation is stopped by rapid cooling to 4 °C. The optimum acidic conditions of commercial yoghurt should be in the range of 7.0–9.0 mg/g of lactic acidity and pH 4.0–4.4 to avoid excessive acidic taste of the product (Oliveira, Florence, Perego, De Oliveira, & Converti, 2011). The acid tolerant LDB continues producing lactic acid at slow pace during refrigerated storage, transportation and marketing leading to well-known phenomenon of post-acidification.

Post-acidification or *post-fermentation acidification* is an undesired

* Corresponding author.
E-mail address: kadyan.saurabh3@gmail.com (S. Kadyan).

https://doi.org/10.1016/j.tifs.2021.01.057
Received 11 June 2020; Received in revised form 23 December 2020; Accepted 24 January 2021
Available online 30 January 2021
0924-2244/© 2021 Elsevier Ltd. All rights reserved.

Table 1
Production data of fermented milk products from 2005 to 2018 and per capita consumption during 2019.

Region/ Country	Fermented Milk Products Production (in '000 tonnes)*					Per Capita Consumption (kg/ person/year)#
	2005	2010	2015	2017	2018	2019
Asia						
China	1702	3600	5300	7629	8000	9.4
Japan	824	866	1087	1109	1095	12.3
India (A)	–	176	299	510	667	4.3
South Korea	482	503	597	561	556	9.5
Israel (B)	123	146	140	147	150	16.6
Europe						
Germany (C)	2956	3005	3066	3117	3109	17.4
France	1564	1656	1550	1430	1400	24.5
Spain	775	761	830	1022	1012	19.7
United Kingdom	368	410	403	448	457	12.2
Netherlands (D)	341	406	309	358	388	38.5
Belgium	326	319	265	288	326	19.9
Italy	170	210	215	325	285	10.9
Austria	252	277	254	262	262	11.4
Sweden	270	263	246	238	229	32.8
Finland (E)	203	203	215	200	204	29.7
Hungary	152	161	125	123	122	17.8
Denmark	104	105	112	112	106	19.5
Ireland	34	29	23	25	26	11.3
Russia	1856	2258	2636	2914	2914	19.3
Switzerland	229	262	266	256	263	14.6
Norway	67	88	88	87	86	26.1
Iceland	13	12	11	12	12	21.7
North and South America						
United States of America (F)	1387	1896	2105	2031	1990	6.9
Mexico	463	688	728	715	734	5.2
Canada	247	312	421	398	398	8.7
Argentina (F)	405	490	459	415	415	13.5
Africa						
South Africa (F)	–	–	231	438	450	14.4
Egypt	–	242	237	198	235	15.4
Zimbabwe	–	–	66	85	95	9.5

Production related remarks.
(A) Refers to cooperative dairies only. This may not reflect developments for the Indian dairy industry as a whole; dairy years ending March of the following year.
(B) Including dairy desserts.
(C) Including dairy desserts and mixed drinks.
(D) Excluding added ingredients.
(E) Including creme fraiche, smetana, sour milk, sour cream, "villi", pudding.
(F) Yoghurt only.
Source: *The World Dairy Situation 2019, IDF; #Yoghurt and Curdled Milk, Statista, 2019

process in fermented dairy products which refers to continued acidification beyond its optimal range due to persistent metabolic activity of product's microflora during its shelf-life. Apart from shortening the shelf-life, it results in numerous defects like severe acidity, whey syneresis, unclean flavor, decreased lactic acid bacteria count or sometimes gas production by contaminants mainly coliforms and yeasts, if any. It is also detrimental to the stability of probiotics incorporated in such products (Settachaimongkon et al., 2016). Post-acidification causes increased hydrophobic and electrostatic interactions among proteins leading to enlargement of casein particles, solubilization of colloidal calcium phosphate and partial restructuring of protein network (Guenard-Lampron, St-Gelais, Villeneuve, & Turgeon, 2020). This more dense and stable protein network diminishes rheological properties of fermented products by increasing the viscosity, firmness and whey syneresis which is directly linked to residual lactic activity of microbes coupled with the possible exopolysaccharide (EPS) production (Kumar, Hussain, Raju, Singh, & Singh, 2017; Saint-Eve, Levy, Le Moigne, Ducruet, & Souchon, 2008). The process of post-acidification in yoghurt and related defects are depicted in Fig. 1.

Even chilled-chain logistics are inefficient in controlling the post-acidification in fermented dairy products throughout its shelf-life and the situation is far worse in tropical and resource poor developing nations. Therefore, several researchers have attempted to minimize/prevent post-acidification using additives (Rajapaksha, Kodithuwakku, Silva, & Rupasinghe, 2013), thermization (Routray & Mishra, 2011), high hydrostatic pressure (Jankowska, Wisniewska, & Reps, 2005), pulse electric field (Chanos, Warncke, Ehrmann, & Hertel, 2020), bacteriocin (Rajapaksha et al., 2013), genetic engineering (Chuah & Mao, 2020) and ultrasound (Racioppo et al., 2017). More recently, Vieira et al. (2020) linked post-acidification with the enhanced accumulation of biogenic amines in fermented cow milk which further necessitates importance of post-acidification control in mitigation of biogenic amines formation in fermented milks. Moreover, due to increasing consumers' predilection for mild fermented products, the requirement of starters with weak post-acidification potential became an important criterion for starter suppliers and researchers who are looking for novel ways of mitigating post-acidification. Low post-acidification potential of starter at industrial level is generally assessed if pH drop is $\leq$ 0.3 units from initial pH of 4.5 during extended fermentation period of 24 h (Chuah & Mao, 2020). To the best of our knowledge and deep literature mining over several scientific databases, there is no review article addressing the holistic aspects of post-acidification in yoghurt and synonymous fermented dairy products. The present review will be informative not only to academicians but also to stakeholders of relevant food processing industries. Thus, keeping in view the above challenges, this review gathers information about factors affecting post-acidification and various strategies for its control (Fig. 2) to sustain the commercial value of product and fulfil consumer's acceptance.

2. Factors affecting post-acidification

The quality parameters of any fermented dairy product are affected by several factors including starter cultures itself; milk composition; temperature and pH; homogenization and stirring; pre- and pro-biotics; and packaging material.

2.1. Type of starter culture

Starter culture refers to the inoculum used to initiate milk fermentation. Starters are broadly classified on the basis of morphology (rod or cocci), fermentation pathway (homo-, facultative hetero- or hetero-fermentative), incubation temperature (mesophiles or thermophiles) and composition (mixed or defined). Most of the dairy starters applied during milk fermentation belongs to the genera of Lactococci, Streptococci, Leuconostoc and Lactobacilli (Garrigues et al., 2017). The growth characteristics of various dairy starters and their probable role in post-acidification are summarized in Table 3. To offset post-acidification, yoghurt manufacturing units exploit starters with high cocci/bacilli ratio, which sequentially lead to lower production of yoghurt's major flavouring component i.e. acetaldehyde (Pinto, Clemente, & De Abreu, 2009). Surprisingly, some commercial cultures such as Holdbac®YM-C plus and FreshQ®4 had been reported to increase post-acidification in fermented dairy products at room temperature (Nielsen, Hornbaek, Rasmussen, & Poulsen, 2018). Further, it had been postulated that the type of fermentation pathway recruited by starter can be used to deduce the post-acidification mechanism (Mishra & Mishra, 2013).

Strain-specific genomic variations within a culture leading to variation in enzyme activities also govern the post-acidification potential. A study based on co-culturing of SST with four strains of LDB namely IM1, IM2, IM3 and IM4, isolated from homemade cheese, in 1:1 ratio yielded yoghurt with fermentation time varying between 270 and 515 min (Xu et al., 2015). In terms of acidification kinetics, a faster acid production

and smooth texture was attainable using mixed starter culture. However, medium acidification rate was found to yield a product with more stable flavour and controlled acid production (Kristo, Biliaderis, & Tzanetakis, 2003). The co-culturing of exopolysaccharides (EPS) producing starter *L. mucosae* DPC6426 with yoghurt starter decreased the post-acidification, due to preferential utilization of sugars by *L. mucosae* for EPS rather than organic acids production (London et al., 2015).

2.2. Milk composition

The acidification and post-acidification kinetics are dependent upon milk of different ruminants, milk composition, total solids level and interaction among milk constituents. LDB exhibited enhanced growth, faster acidification and peptidase activity in goat milk (Tamime & Robinson, 2007). Similarly, goat milk showed faster acidification during yoghurt manufacturing and constant pH of 4.1 throughout 29 days storage contrary to pH 3.9 in yoghurt prepared from 50:50 ratio of cow and goat milk (Vargas, Chafer, Albors, Chiralt, & Gonzalez-Martinez, 2008). Higher post-acidification potential of SST in milk as compared to plant substrate (equal weight of 10% hydrolyzed oat powder and soy milk) was reported by Boufassa and Tourancheau (2004). Skim milk powder (SMP), milk protein concentrate (MPC) and casein hydrolysate (CH) milk bases revealed significant differences in fermentation time as compared to pure milk, and CH improved the fermentation rate and probiotic stability. Yoghurt supplemented with casein hydrolysate (CH) having varying degree of acidification (8.5%, 14.6% and 26.7%) exhibited higher pH (4.18–4.37) after 30th day of storage, signifying its post-acidification control potential. However, higher concentration of CH leads to bitterness and low viscosity in yoghurts (Zhao, Wang, Zhao, Jiang, & Chun, 2006).

Modification in individual milk components especially protein and lactose mediate the acidification kinetics during pre- and post-fermentation stages. Skim and whole milk treated with transglutaminase enzyme (40 °C/2 h) afore fermentation lowered the post-acidification in yoghurt during 25 days storage period due to reduced pore size of gel, higher water holding capacity of (γ-Glu)-Lys bonds, regular distribution of proteins and reduction in low molecular weight peptides required for microbial growth (Lorenzen, Neve, Mautner, & Schlimme, 2002). Reducing the lactose content in skim milk to <2% is an alternate strategy to avoid post-acidification but it produces a soft coagulum. Usually, a good coagulum is obtained with >2% lactose, and >8% protein leads to parallel increase in viscosity (Alvarez et al., 1998). From the industrial viewpoint, a patented process for producing stable fermented product by limiting fermentable carbohydrate claims no requirement of refrigeration with ΔpH of 0.3 units for 20 h at fermentation temperature and 0.2 units for 42 days at 6 °C (Garrigues et al., 2017).

2.3. Temperature and pH

Rapid cooling of yoghurt to refrigerated conditions (<10 °C) post-fermentation is most critical factor in controlling its final pH (4.0–4.4) by restricting the metabolic activity of starter. Storage at <1 °C is utilized to control yoghurt's acidity (Tamime & Robinson, 2007). Still, similar approaches are unable to prevent post-acidification due to microorganisms' residual activity. The cumulative effects of milk total solids (TS) (11.3–14.7%), incubation temperature (36.7–43.4 °C), and inoculum level (1.66–3.34%) on the acidification rate of yoghurt indicated reduced fermentation time as the TS and incubation temperature increased (Kristo et al., 2003). Fresh, refrigerated (7 °C/4 days) and frozen (−18 °C) for a month/thawed (7 °C) sheep milk reflected greater susceptibility of frozen/thawed milk's yoghurt to post-acidification due to higher buffering capacity of fresh milk yoghurt (Tribst, Falcade, Carvalho, Junior, & de Oliveira, 2020).

The termination pH of fermentation may also affect the starter activity, levels of organic acid, gel formation kinetics and probiotic survivability. Probiotic supplemented yoghurt had enhanced proteolysis which relies on termination pH of fermentation and strain. Enhanced proteolysis showed greater survivability of *Lactobacillus bulgaricus* and higher organic acid production during 28 days due to availability of free amino acids and peptides generated during proteolysis (Donkor, Henriksson, Vasiljevic, & Shah, 2006).

Table 2
Optimum pH, country of origin and characteristic attributes of products similar to yoghurt across the globe.

Fermented dairy product	Optimum pH	Country of origin	Description or starter culture used	Remarks
Yoghurt	4.0–4.4	Turkey	*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in 1:1	Acetaldehyde as major flavouring compound
Kefir	4.7–5.1	Caucasian region	Kefir grains containing bacteria and yeasts. *Lactobacilli* (*L. kefir*) and *Candida* (majorly)	Fermented milk drink made with yeast grains
Koumiss	4.1–4.5	Russia	*L. bulgaricus* and *Saccharomyces lactis*	Fermented mare milk
Dahi	4.5–4.7	India	*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* spp. *Lactis* biovar. *Diacetylactis;* mixed starter culture	Mild diacetyl/acetoin flavour
Misti Doi	4.3–4.8	India	*S. thermophiles, Lactococcus lactis ssp. lactis*	Caramel brown due to added sugar
Labneh	4.0–4.4	Iraq	*L. casei, L. plantarum* and *L. brevis*	Also termed as concentrated yoghurt
Zabady	3.6–4.0	Egypt	*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*	Yoghurt like consistency and cooked flavour, prepared from buffalo milk
Villi	5.0–5.5	Finland	*Lactococcus lactis* subsp. *lactis, L. lactis* subsp *cremoris* and *Geotrichum candidum*	*L. cremoris* produces a phosphate based heteropolysaccharide, named villian
Yakult	4.3–4.7	Japan	*Lactobacillus casei* spp. *shirota*	Probiotic strain
Skyr	<4.0	Iceland	*Streptococcus thermophilus*, *Lactobacillus spp.,* yeasts and moulds	Similar to Greek yoghurt but mild taste
Filmjolk	4.3–4.4	Sweden	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lac. lactis* subsp. *lactis biovar diacetylactis, Leuconostoc mesenteroides* subsp. *cremoris*	Villi milk or yogurt drink
Calpis	4.5–4.8	Japan	*Lactobacillus helveticus* and *Saccharomyces cerevisae*	Slightly acidic in taste and sold in concentrated form
Doogdh/Ayran	3.85–4.2	Iran/Turkey	National Drink in Iran	Drinking yoghurt in which salt is added after dilution and agitation
Langfil	4.5–5.0	Sweden	*Lactococcus lactis* var. *longi*	Thick buttermilk. "Long viili"-A characteristic long and elastic texture due to bacterial strain which converts carbohydrates into long chains of polysaccharides
Cultured Buttermilk	4.5	Scandinavia	*L. lactis* subsp *cremoris, Leuconostoc mesenteroides* subsp. *cremoris* and *Lac. lactis* subsp. *lactis* biovar. *diacetylactis*	Lightly salted, major flavouring compounds – diacetyl, acetic acid and lactic acid
Acidophilus milk	5.5–6.0	Sweden	*L. acidophilus*	Medium-acid therapeutic drink

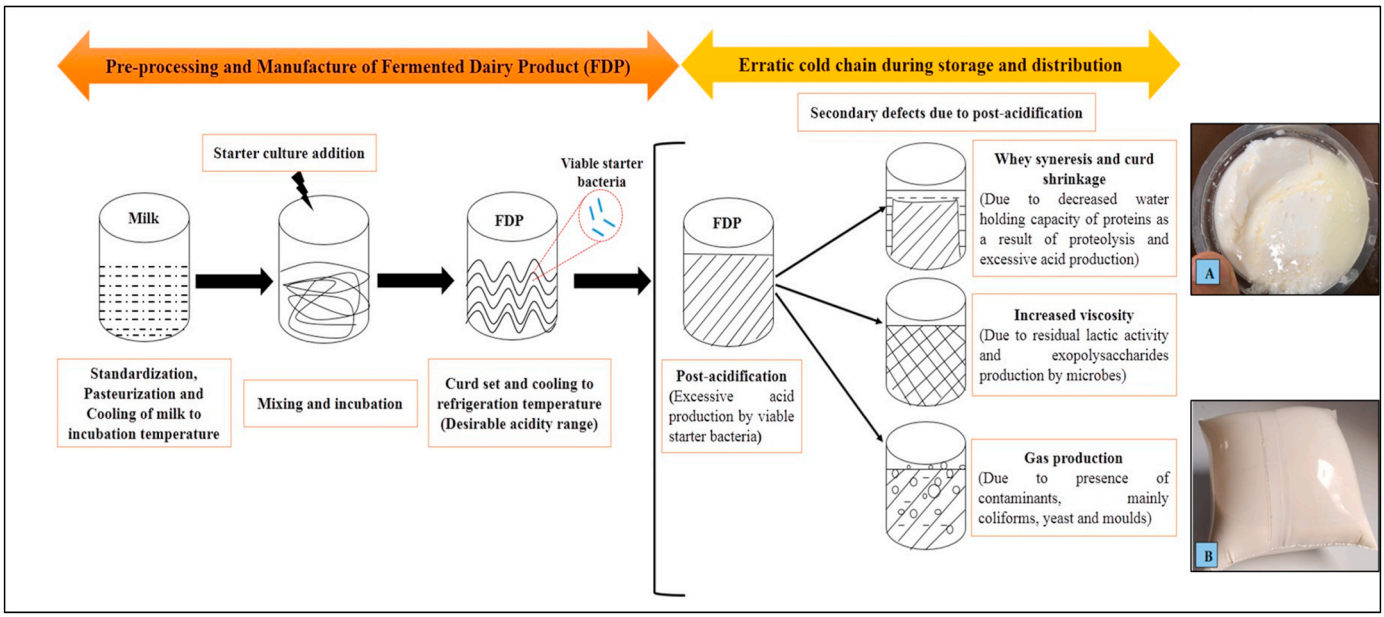

Fig. 1. Illustrative description of post-acidification process and its effect on quality of fermented dairy products. Images representing (A) whey syneresis in *Dahi* and (B) puffing of buttermilk pouch in market condition.

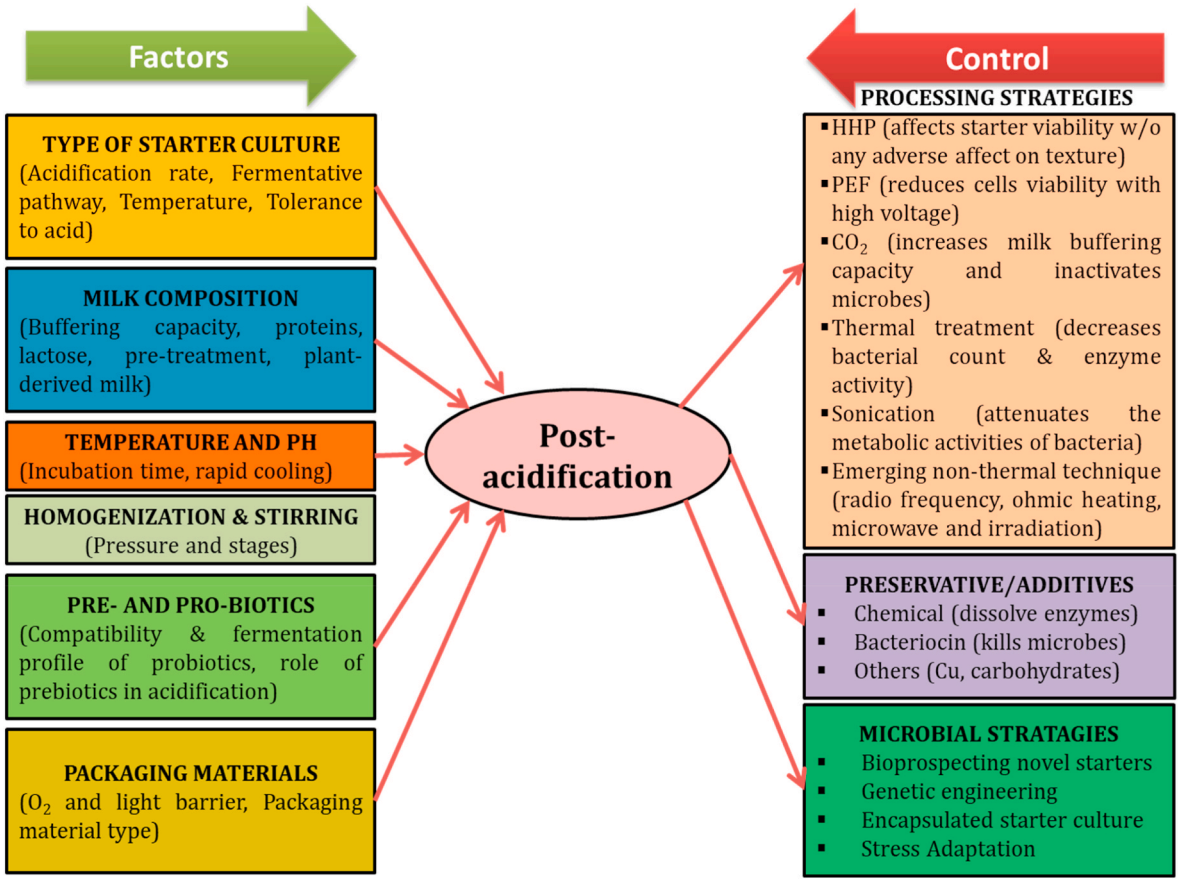

Fig. 2. Factors affecting post-acidification and control strategies applicable to yoghurt and related products.

2.4. Homogenization and stirring

During yoghurt manufacturing, milk is generally homogenized at 10–18 MPa at 55–65 °C to prevent fat separation and improve the stability, whiteness, viscosity and water holding capacity. Homogenization reduces the fat globule size (<1μ) which confers better incorporation of fat into protein network, thus fortifying the interaction of fat with casein and denatured whey protein during acidification subsequently improved gel characteristics (Ciron, Gee, Kelly, & Auty, 2010; Racioppo et al., 2017). A multiple pass homogenization at 50 MPa had significant effect

Table 3
Desirable growth temperature, pH and fermentable carbohydrates of starter cultures associated with yoghurt & related products.

Culture	Minimum (Optimal) growth pH	Temperature (optimal) temp.	Fermentable carbohydrates	Fermentation by-products	Post-acidification Potential[b]
S. thermophiles	5.0 (6.5–7.5)	>15–52 °C (39 °C)	Lactose, sucrose, glucose, fructose	L(+) lactic acid, Formic acid, CO_2, Acetaldehyde (minor)	–
L. delbrueckii subsp. *bulgaricus*	4.0 (5.5–5.8)	>15–50 °C (45 °C)	Glucose, lactose, fructose, maltose[a], sucrose[a], cellulose[a]	D(−) lactic acid, Peptides, amino acids, Acetaldehyde (majorly)	+++
Lactococcus lactis spp. *lactis*	4.2–4.8 (6.3–6.9)	10–45 °C (30 °C)	Glucose, Lactose, galactose, maltose, ribose	L (+)-lactic acid	++
Lactococcus lactis spp. *cremoris*	5.0–5.5 (6.3–6.9)	10–45 °C (30 °C)	Glucose, Lactose, galactose, ribose	L (+)-lactic acid	–
Leuconostoc mesenteroides	5.4–5.7 (6.0–6.5)	20–30 °C (22 °C)	Lactose, sucrose, galactose, maltose	D (−)-lactic acid, diacetyl, CO_2, acetoin	–
L. acidophilus	4.4–4.5 (5.5–6.0)	>15–50 °C (40 °C)	Glucose, Maltose, Galatctose, Sucrose, Lactose, cellulose, fructose, mannose	DL-lactic acid	+
L. helveticus	4.0 (5.5–5.8)	15–50 °C (40–42 °C)	Glucose, Galactose, Lactose, Cellulose, Maltose[a], fructose[a]	DL-lactic acid	++
L. paracasei	<4.0 (5.5–6.0)	10–40 °C (37 °C)	Glucose, galactose, lactose[a], cellulose, fructose, maltose, sucrose, ribose	L (+)-lactic acid, acetic acid, ethanol and formic acid	+++
L. plantarum	4.0–4.7 (5.5–6.0)	12–40 °C (30 °C)	Glucose, Galactose, Maltose, Sucrose, Lactose, cellulose, fructose	DL-lactic acid, acetic acid, ethanol and formic acid	++

[a] Not fermented by all strains.
[b] Postacidifcation potential: No (−), Low (+), Moderate (++), High (+++).

on post-acidification characteristics of *Lactobacillus plantarum* as compared to single pass up to 100 MPa (Erkaya, Baslar, Sengul, & Ertugay, 2015). Homogenization (3.5 MPa) and stirring with hand blender for 2 min suppressed the adverse textural effect of refrigerated and frozen stored milk on yoghurt's characteristics but homogenized yoghurt exhibited non-significantly higher acidity on first and 28th day (Tribst et al., 2020). Microfluidization of milk at 150 MPa resulted in less uniform and larger particle size (D[v,0.9]- 73.36 μm) of yoghurt due to interconnected protein network with embedded fat globules without any significant effect on texture, syneresis and water holding capacity (Ciron et al., 2010). However, its impact on post-acidification characteristics needs further exploration.

Based on texture and method of preparation, yoghurt is of set type (fermented in their containers) and stirred (or strained or Greek) type. Stirred yoghurt are viscous, creamy and possess smooth texture as compared to continuous gel structure of set style yoghurt (Guenard-Lampron et al., 2020). The conversion of set yoghurt into their stirred version demonstrated better water holding capacity but objectionable coarse and grainy texture due to the ability of stirred gels to regain their structure during post-acidification at refrigerated storage (Serra, Trujillo, Guamis, & Ferragut, 2009).

2.5. Pre- and pro-biotics

Probiotics are live microorganisms which positively enhances the host health when administered in sufficient amounts. Fermented dairy products serve as an excellent platform for incorporating probiotics as these products promote probiotics growth during the fermentation phase in addition to providing excellent nutrient density (Fenster et al., 2019). The co-culturing of probiotics with lactic starters affects acidification kinetics and post-acidification. Yoghurt prepared with SST and probiotic *Bifidobacterium lactis* had lower post-acidification due to restricted ability of *Bifidobacterium* to produce acids at refrigerated temperatures (Damin, Minowa, Alcantara, & Oliveira, 2008). Synbiotic soy yoghurt prepared with different combination of probiotics (*Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus*) revealed maximum pH decrease for *L. plantarum* and *L. rhamnosus* combination as compared to other single or binary combinations during storage at 4 °C for 28 days. These single or diverse combinations of probiotics demonstrated non-conclusive pH variation during storage but it was in optimal range (Mishra & Mishra, 2013).

Prebiotics are the non-digestible food constituents that are specifically utilized by the group of beneficial microbes, present in gut microbiome, thereby promoting host health. Galacto-oligosaccharides (GOS) and fructo-oligosaccharides are the two commonly used prebiotics and are often utilized as food by probiotics (Davani-Davari et al., 2019). Several studies suggested the involvement of prebiotics in amplifying the fermentative behavior and post-acidification rate. Lactulose fortified skim milk (@4%) revealed increased acidification rate upon fermentation with LDB, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *Lactobacillus acidophilus* showed highest post-acidification (4%) rate after seventh and thirty-fifth day due to its superior metabolizing power of fructose moiety (Oliveira et al., 2011). Similarly, Oliveira et al. (2009) investigated the effect of co-cultures containing probiotics and prebiotics (maltodextrin, polydextrose and oligofructose) on fermentation kinetics of skim milk based fermented products. Oligofructose and polydextrose supplemented the growth of probiotics with highest positive effect on *B. lactis* and all three prebiotics stimulated the post-acidification. Based upon abovementioned studies, it is conclusive that prebiotics can undoubtedly amplify the post-acidification in fermented dairy products due to their selective utilization by specific strains of probiotics.

2.6. Packaging materials

Different combinations such as aluminium foil/plastic, paper/plastic laminate, thermoformed HIPS (high impact polystyrene), glass containers, high impact polystyrene, HDPE (high density polyethylene) bottles and LDPE (low density polyethylene) pouches are most commonly available options for both set and stirred type of fermented dairy products (Cruz et al., 2013). Kumar et al. (2017) reported higher post-acidification in ethylene vinyl alcohol (EVOH) and glass containers, but exhibited better flavor score due to improved retention of certain aroma compounds. These high barrier packaging materials resulted in negative redox potential due to oxygen consumption by microbes, thereby creating stressful conditions to aerobic microflora. Polypropylene, polystyrene and glass containers were found to affect post-acidification in 0 and 4% fat yoghurt with more significant effect in 4% fat yoghurt in polypropylene containers and least significant in 0% fat yoghurt in glass containers (Saint-Eve et al., 2008). The post-acidification analysis of probiotic yoghurt in plastic containers with different oxygen transmission rate (OTR) and fortified with glucose oxidase enzyme revealed higher post-acidification rate in containers with lower OTR. Since oxygen acts as toxic substance for

microaerophilic or anaerobic starter microbes and probiotics as reactive oxygen species are formed. Moreover, these containers also had lower dissolved oxygen and higher probiotic microflora count. Glucose oxidase enzyme was inefficient to prevent deteriorative effect of oxygen because of multi-directional oxygen entry and degradation of enzyme activity during storage (Cruz et al., 2013). The only reported literature on modified atmosphere packaging (MAP) of yoghurt cites nitrogen flushing in the headspace of yoghurt cups which reduced the residual oxygen in headspace to 0.1–0.2% and extended its shelf-life to 8 months at 4.4 °C (Blakistone, 1990). Jansson, Edsman, Gedde, and Hedenqvist (2001) reported increased CO_2 and decreased O_2 in headspace of packed yoghurt with increasing crystallinity and polarity of packaging material, which could be explored for modifying package barrier properties. Therefore, packaging material should be meticulously selected after considering the starter type and its metabolism, food composition, and storage conditions for controlling post-acidification.

3. Approaches for controlling post-acidification

Refrigerated temperature (2–10 °C) during storage and transportation is the foremost technique for preventing post-acidification and it's after effects. Since, refrigeration conditions are difficult to maintain in resource poor countries, alternative strategies for post-acidification control are elucidated which are sub-divided into three broad categories viz. processing, use of preservatives (or additives) and microbiological interventions.

3.1. Processing intervention

3.1.1. High hydrostatic pressure (HHP)

High hydrostatic pressure (HHP), a non-thermal process utilizing pressure between 100 and 1000 MPa, not only controls post-acidification by treating either milk or yoghurt but also gives thick texture and natural refreshing taste (Serra et al., 2009). The prevention of post-acidification in pressurized yoghurt during cold storage is attributed to inactivation of enzymes (especially lactose dehydrogenase and β-D-galactosidase) responsible for lactose metabolism and transportation. ATPase activity is also inhibited/reduced due to HHP, thereby averting proton gradient and impairment of acid efflux from microbial cells into yoghurt (de Ancos, Cano, & Gomez, 2000).

HHP treated yoghurt possessed constant acidity during four weeks storage mainly due to increase in absorbable calcium and phosphorus. HHP (400 MPa/15 min) could also inactivate yoghurt starters thus preventing over-acidification and product deterioration. Nevertheless, the degree of inactivation depends on strain e.g. SST is more immune to inactivation by HHP (Reps, Jankowska, & Wisniewska, 2009). HHP (200–300 MPa/10 min/10–20 °C) treatment of full-fat yoghurt didn't adversely impacted texture and viable count of lactic acid bacteria (LAB) but pressure above 300 MPa successfully prevented the post-fermentation acidification and reduced the viable LAB count unfavorably (Tanaka & Hatanaka, 1992). Similarly, low fat yoghurt (0.3% fat) pressurized above 200 MPa for 15 min had lower acidification and reduced viability of lactobacilli after 20 days of chilled storage. Also, the cells which remained viable after HHP had lower acidification potential due to sublethal injuries which prevented their replication (de Ancos et al., 2000). Negligible rise in acidity over 21 days storage period along with reduction in yeast and bacterial count of HHP (600–800 MPa/15 min) treated Kefir had also been observed by Trujillo, Capellas, Saldo, Gervilla, and Guamis (2002).

The control of post-acidification in probiotic yoghurt is highly challenging as probiotic microorganisms like *Bifidobacterium* are extremely sensitive to both acid stress and HHP. HHP treated yoghurt (550 MPa/15 min/18 °C) with probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium* in lyophilized form had lower acidifying potential but number of probiotic and starter microflora progressively decreased during 28 days storage (Jankowska et al., 2005). This daunting task of controlling post-acidification without affecting probiotic viability was accomplished by Fonterra, a multinational dairy company in New Zealand, by using HHP. The probiotic yoghurt was prepared using selected baro-tolerating probiotic strains and then different pressure intensity-time combination were applied that increased its shelf-life up to 90 days at refrigeration temperature. This process was later patented by Carroll, Chen, Harnett, and Harnett (2010).

3.1.2. Pulse electric field (PEF)

Pulse electric field (PEF) is another non-thermal method utilizing high electric field pulses of 15–50 kV/cm for microseconds, leading to cell membrane electroporation, loss of mechanical permeability and finally cell death. Even mild electric field (2 V/cm, 45 Hz, 30 °C) treatment led to maximum cell membrane permeabilization of *Lactobacillus acidophilus* OSU 133 during lag phase followed by exponential phase while no electroporation occurred during stationary phase, which can be used for the optimization of fermentation process (Loghavi, Sastry, & Yousef, 2009). Despite its huge potential, PEF is limited to liquid food without any bubbles, low electrical conductivity, and its performance is affected by electric field strength, food properties (pH, ionic strength, antimicrobials), pulse duration and number of pulses (Cueva, 2009). Reconstituted skim milk acidified with *Streptococcus thermophilus* DIL 5218 and *Lactobacillus bulgaricus* DSMZ 20081 revealed faster acidification of PEF treated samples by 12 min and drastic decrease of redox potential as compared to non-PEF treated samples. The enhanced fermentation rate in PEF samples was due to electro-permeabilization of culture cells, thus increasing nutrients uptake and decreasing lag phase (Chanos et al., 2020). Dunn and Pearlman (1987) reported shelf-life extension of PEF treated yoghurt by significantly reducing yeast viability and slightly less reduction of lactobacilli. A study on combined mild heat (60 °C/30 s) and PEF treated yoghurt-based pudding showed that aerobic bacteria, fungi can be killed using 30 kV/cm electric field for 32 μs in a semi-selective manner (related to cell size). Mild heat-PEF treated samples showed pH decrease after 70 days while untreated samples showed after 14 days at 22 °C, signifying post-acidification control potential of PEF. However, mildly heated non-PEF treated samples were having higher microbial count in yoghurt-based products (Yeom, Evrendilek, Jin, & Zhang, 2004). Swelling, a major adverse effect in Doogh (Iranian yoghurt drink), caused by yeast *Kluveromyces marxianus* was significantly reduced at electric field strength of 4 kV/cm and 100–250 pulses due to changes in morphology of yeast cell (Didar, 2020). Cueva (2009) also reported reduction in probiotic activity due to slower release of enzymes in PEF-treated products. Albeit, operational parameters should be optimized for successful application of PEF in yoghurt manufacturing and its effect of PEF on post-acidification should be undertaken.

3.1.3. Carbon-dioxide (CO_2) treatment

Carbon dioxide possess numerous interesting properties which makes it practical for various types of food applications. The incorporation of CO_2 in raw milk is very effective in its quality preservation for longer period in two ways: (i) CO_2 incorporated milk has two extreme peaks for buffering at pH 4.95 and 5.4 as compared to normal milk with single peak at pH 5.1 (ii) inactivation of wide range of vegetative and endospore forms of bacteria and fungi in milk. For instance, raw milk treated with 50 atm of CO_2 and subsequently, stored at 10 atm of CO_2 subsided the growth of indigenous milk microflora and extended its shelf-life up to 72 h while the milk without CO_2 treatment got curdled within 24 h (Bonnaillie & Tomasula, 2015). In particular to fermented milks, the shelf-life of yoghurt was extended up to 4 months using CO_2 treated milk while non-carbonated samples were spoiled within 30 days (Choi & Kosikowski, 1985). Also, CO_2 incorporation didn't adversely affect the growth of probiotics microflora (Gueimonde, Corzo, Vinderola, Reinheimer, & de los Reyes-Gavilan, 2002). Choi and Kosikowski (1985) demonstrated increase in fungi count of non-carbonated yoghurt

beverage in glass containers from 10 to 100 and 200 cfu/g at 4.4 °C and 10 °C respectively while, count remained same even after 80 days in carbonated samples irrespective of storage temperatures. They concluded that carbonation at the rate of 0.5 kg/cm^2 for yoghurt beverage can enhance the keeping quality up to 4 months due to retarded acid development. Similarly, lactic acid production in yoghurt by LDB and SST after CO_2 treatment was little lower (Calvo, Montilla, & Cobos, 1999). Moreover, higher levels of CO_2 can stimulate the growth of yoghurt starters and subsequently reduces the incubation time (Karagul-Yuceer, Wilson, & White, 2001).

3.1.4. Thermal treatment

Thermization is a mild heat treatment of milk between 62 and 65 °C for 15–20 s with the objective of controlling psychotropic microbes and reducing bacterial load (Poltronieri & Rossi, 2018), yet different time and temperature combinations can also be utilized based upon product's requirement. Thermization of fermented dairy products after fermentation not only diminishes post-acidification but also enhances their shelf-life without significantly affecting flavour and nutritional value (Routray & Mishra, 2011). The fermented products which are heated after fermentation should be prefixed with "heat treated" as per requirements of Food Safety and Standard Authority of India (FSSAI). Neirinckx (1972) also recommended thermization of fermented dairy products having pH 4.2–4.5. For instance, thermization treatment was successfully applied to make *Mishti Dahi* shelf-stable for 3 weeks due to inhibition of almost 99.99% acid producers as compared to only 48 h without thermization treatment (Sarkar, Dave, & Sannabhadti, 1992). Similarly, thermized cow milk yoghurt (75–80 °C for 60 s) had lower acidity (2.01%) as compared to unthermized samples (2.53%) after 35 days at room temperature (Alakali, Unwiyi, & Ejiga, 2009). Dagher and Ali (1985) concluded that heating of *Labneh* for 10–15 min at 60 °C destroyed 95% of the bacterial population thereby controlling post-acidification. Several energy efficient techniques like PEF or electrically induced heating systems etc. could also be employed to achieve thermization. Guerrero-Beltran, Sepulveda, Gongora-Nieto, Swanson, and Barbosa-Canovas (2010) reported energy consumption of 44 J/mL for thermization (20-72 °C) of whole milk using PEF equipped with heat regeneration as against 287.15 J/g for HTST pasteurization of milk. With the advent of new mechanization processes, innovations and emerging technologies, it would be feasible to achieve thermization of fermented milk products at low running and investment cost in near future. Although, heat treated yoghurt preserves well and also circumvents post-acidification but is limited by the consumers demand to have live beneficial microorganisms in product (Fenster et al., 2019).

3.1.5. Ultrasound

Sonication not only assists in food preservation, cell lysis, texture modification, enhanced mass transfer and denaturation of enzymes but also attenuates the metabolism of probiotics without adversely affecting their survivability and functional attributes (Racioppo et al., 2017). Ultrasound causes internal and/or external cavitation, leakage of intracellular components and lethal or sublethal injuries depending on the power and duration of treatment (Ciron et al., 2010). Ultrasound pressure of up to 40 kPa significantly shortened the fermentation time due to deaeration effect which favorably promoted the growth of anaerobes. However, at 80 kPa, the lactic bacteria were suppressed due to cavitation (Masuzawa & Ohdaira, 2002). Ultrasound attenuation of probiotics at 60% power for 6 min treatment time (2 s pulse) circumvented post-acidification caused by probiotic strains implying incorporation of desired (10^7-10^9 cfu/mL) or higher probiotic level in yoghurt without excess acidification (Racioppo et al., 2017). Similarly, single and multiple pass ultrasound (US) treatment for 4 min (2 s pulse) confirmed the attenuation of *Lactobacillus plantarum* without affecting their viability, thus controlling post-acidification for minimum 7 days in organic rice beverage even when if it underwent thermal abuse up to 4 h (Bevilacqua, Casanova, Petruzzi, Sinigaglia, & Corbo, 2016). Thermosonication (35 kHz for 1, 3 and 5 min at 60, 70 and 80 °C) of *Ayran* (diluted yoghurt) not only prevented acidity increase during its 30 days storage but also improved its viscosity and water holding capacity, implicating its potential as an alternative to heat treatment post-manufacture. Moreover, low frequency (20–100 kHz) high intensity (10–1000 W/cm^2) ultrasound has lower investment and ease of cleaning as compared to traditional homogenization (Erkaya et al., 2015).

3.1.6. Ohmic heating

Ohmic heating has not been studied for yoghurt shelf-life extension and post-acidification control, however a report indicated enhanced cell permeability and non-thermal injuries to SST after ohmic heating (titanium electrode, 7A current and 20 kHz frequency) (Sun et al., 2011). Similarly, ohmic treatment (alternating current of 60 Hz at 15 V) shortened the lag phase by enhancing the growth of *Lactobacillus acidophilus* OSU133 but inhibited its growth during later phase. Oscillating electric field would have dislodged the polar antimicrobials and macromolecules adhering to cell wall and membranes, thus increasing the absorption of nutrients during lag phase. Ohmic heating as compared to conventional heating also showed higher final pH of the fermented medium, which could be utilized beneficially in fermented product segment to inhibit post-acidification (Cho, Yousef, & Sastry, 1996). Further investigations are required to elucidate the effect of ohmic heating on post-acidification in yoghurt.

3.1.7. Irradiation

Gamma irradiation (1–10 kGy) exposure of yoghurt enhanced its shelf-life at 4, 20 and 35 °C without any adverse effect on sensory, amino acid content and reduced the allergenicity too (Ham et al., 2009). However, yoghurt samples irradiated at 3 kGy and above in plastic cups had lower sensorial acceptance (Ham et al., 2009). Ultraviolet-C treated (185 and 254 nm) *Ayran* had 0.20% lactic acidity after 60 days as compared to 0.83% for untreated samples, highlighting the potential of UV-C treatment to replace severe thermal treatments (Borcakli et al., 2013). Neodymium-doped yttrium aluminium garnet (Nd:YAG) laser pasteurization of milk followed by bacterial fermentation resulted in higher pH of yoghurt (4.54) as compared to thermally pasteurized sample (4.36) (Marouf & Siddiq, 2018). Low dose irradiation (Cobalt-60) (0.02–0.04 Mrad for 8 min) singly and in combination with refrigeration successfully extended the shelf-life of plain yoghurt by 3-fold and 5-fold, respectively. The effect on pH or acidity was not studied however microbial growth was retarded significantly in irradiated samples as compared to control (Yuceer & Gunduz, 1980). It is better to use irradiation as a supplement to other techniques for preventing post-acidification as doses above 0.15 Mrad generated off-flavors in yoghurt (Yuceer & Gunduz, 1980). Along with, the legal aspects of irradiated yoghurt should be considered as food laws pertaining to use of irradiation are quite diverse worldwide.

3.1.8. Microwave and radio frequency

A very few studies pertaining to use of emerging non-thermal technologies to avert post-acidification has been reported. Microwave treatment (720 W power) of yoghurt in plastic cups at 2450 MHz for 10, 20 and 30 s resulted in significantly lower acidity (0.82–0.92%) as compared to control (1.04%). Additionally, 30 s microwave exposure had higher inhibitory effect on SST as compared to LDB as indicated by higher count of latter after 28 days (Turgut, 2016). Radio frequency (RF) heating (27.12 MHz) of stirred yoghurt at 58, 65 and 72 °C for 60, 90 and 120 s averted post-acidification by maintaining constant pH of 4.3 for five weeks and inactivating yeasts and moulds, while LAB survived partially. Although, initial investment costs are high for RF but energy costs are comparable with electricity and fossil fuel-based heaters and expected to drop further with semi-conductor technology-based RF power generators (Siefarth, Tran, Mittermaier, Pfeiffer, & Buettner, 2014). Although, these pilot studies show future potential but, safety

aspects and approval from regulatory authorities remain strong opposition for the utilization of these emerging technologies.

3.2. Preservatives/additives intervention

3.2.1. Chemical preservatives

Preservation using commercially approved chemicals is universally adopted practice to improve the shelf-life of fermented products by restricting the growth of survivor cultures for controlling post-acidification. Examples include sodium and potassium salts of sorbate (E201/E202), benzoate (E211/E212) and propionate (E282/E283). Benzoic and sorbic acids, and their salts inhibit fungi and a wide range of bacteria. In human body ingested benzoic acid conjugates with glycine or glucuronic acid to form water soluble derivatives and is eliminated in urine while sorbic acid is converted to water and carbon dioxide via β-oxidation. The permitted levels of sorbic acid in yoghurt ranges from 50 mg/kg as per Lebanese standards to 600–1000 mg/kg as per Brazilian and Turkish standards. Contrarily, benzoic acid is also produced by LAB in yoghurt, so its permitted level should be assessed in relation to naturally available benzoic acid (Mroueh et al., 2008).

Potassium sorbate is frequently used as post-acidity controller with least allergenic potential and GRAS status for its use in food products as per USFDA Code of Federal Regulations 21CFR182.3640. It checks microbial growth by inhibiting key metabolic enzymes involved in carbohydrate and citrate utilization such as lactate dehydrogenase, malate dehydrogenase, fumerase etc. Sorbates at different concentrations (0.05%, 0.075% and 0.1%) in yogurt signified direct correlation between concentration of additive and its shelf-life. Potassium sorbate was able to control post-acidification most significantly at 0.1% level (Rajapaksha et al., 2013). But, owing to its “non-natural” image in consumer’s mind, its use for shelf-life enhancement and post-acidity regulation is limited.

Natural food grade preservatives namely nisin and vanillin not only inhibited microbial spoilage but also deterred post-acidification with shelf-life extension of minimally processed blueberry yoghurt (Penney, Henderson, Blum, & Johnson-Green, 2004). Myrrh, an USFDA permitted (21CFR172.510) essential oil with natural flavouring, antifungal and antibacterial properties, when added in yoghurt at 1% (v/v) level, possessed lower acidity after 5 weeks due to release of free hydroxyl ions and microbial inhibition during storage (Alhejaili et al., 2019). However, these natural additives could only be utilized in flavoured variants of yoghurt due to their intense inherent taste and aroma.

3.2.2. Bacteriocin

LAB produces several proteinaceous compounds called bacteriocins e.g. nisin, acidocin, lacticin, pentocin, plantaricin, ε-polylysine etc., which possess antimicrobial activity against similar species (narrow spectrum) or across genera (broad spectrum). The mechanism of bactericidal activity of bacteriocin is ascribed to alteration in membrane permeability and destabilization of cytoplasmic membrane functionality in target microorganisms. Bacteriocin producing microorganisms are immune to their own bacteriocin(s) due to synthesis of certain neutralizing proteins (Martinez, Balciunas, Converti, Cotter, & de Souza Oliveira, 2013). Unlike chemical preservatives, bacteriocins produced by food grade strains of LAB are non-toxic, biocompatible, easily digested by native proteases (trypsin, pepsin and chymotrypsin), and hence do not adversely impact the gut microbiota (Fahim, Khairalla, & El-Gendy, 2016). The incorporation of bacteriocins in purified or semi-purified form as food additive, can be exploited for post-acidification regulation. For instance, Poly-L-lysine produced by *Streptomyces albulus* subsp. *lysinopolymerus* got electrostatically adsorbed onto the target cell membrane leading to cytoplasmic disruption. It inhibited post-acidification at a concentration of <100 μg/mL under aerobic conditions (Rajapaksha et al., 2013). Oh et al. (2006) listed significant differences in pH of yoghurt containing microencapsulated crude bacteriocin against control as 4.37 and 3.92, respectively, after 24 h fermentation at 42 °C with negligible pH rise after 20 days storage at room temperature, highlighting its role in preventing post-acidification under ambient conditions.

Owing to bacteriocins’ adsorption on food matrices and high purification cost, their commercial applicability is restricted to some extent. Moreover, new bacteriocins has to pass through stringent regulations of regulatory authorities for their use as food preservative (Silva, Silva, & Ribeiro, 2018). To subdue the above limitations, bioprotective cultures from LAB genera with proven safe historical use in traditional fermentations as adjunct cultures can be employed since they are capable of producing bacteriocin *in-situ* during the course of fermentation to specifically inhibit dominant acid producing and tolerating starter cultures. Plantaricin C (3.5 KDa) produced by *L. plantarum* LL 441 under aerobic incubation at 30 °C/20 h in MRS broth having alteast 0.6% glucose, showed inhibition spectrum against *L. bulgaricus, L. fermentum*, *L. helveticus* and *L. sake* (Gonzalez, Arca, Mayo, & Suarez, 1994). Likewise, heat and pH stable bacteriocin (3.1 KDa) produced by *L. acidophilus* ATCC 4356 after incubation at 37 °C for 18 h in MRS broth, was found inhibitory to fast acid producing *Lactobacilli* (Han, Imm, Oh, Jeon, & Kim, 2002).

Potential cytotoxicity of added bacteriocin should be taken into consideration as recently, reports underpinning reuterin synthesis by *Lactobacillus reuteri* via glycerol metabolism as an endogenous source of acrolein (a cytotoxic electrophile) accumulation in gut had raised concerns (Zhang, Sturla, Lacroix, & Schwab, 2018). Further, broad spectrum antimicrobial may have tendency to cause dysbiosis in well-balanced gut of a healthy individual (Walsh, Guinane, O’Toole, & Cotter, 2014), which demands critical impact assessment of bacteriocin producing strains on intestinal ecology and putative adverse effects. Wang, Zhang, and Zhu (2019) observed reduced water and food intake along with significant compositional differences of faecal microbiota in healthy mice, when treated with wild type (bacteriocin producer) and mutant (bacteriocin non-producer) *L. acidophilus* strains. Considering the potential of bacteriocins in curtailing the undesirable acidity, bioprospection of new bioprotective strains or their antimicrobial metabolites from different environmental niches, and their safety assessment should be given more focus.

3.2.3. Miscellaneous additives

Many ingredients are added to modify the flavour, functionality, shelf-life and textural properties during manufacture of fermented products. FSSAI delineated various permitted additives along with microbiological requirement for sale of yoghurt in India which are listed in Table 4. Very few ingredients have been tested to control post-acidification in yoghurt. The inhibitory effect of copper (1.25 ppm) addition in whole milk fermented by SST revealed reduced post-acidification (Han et al., 2012). However, at >1.25 ppm concentration, it showed lipid oxidation and longer incubation time, which is non-economical from production viewpoint. Rajapaksha and Kodithuwakku (2014) reported significantly lower acidity of yoghurt added with 0.25% (w/v) chitosan after 20 days of cold storage.

On the contrary, acai fruit pulp addition to fermented milk and yoghurt promoted post-acidification with more pronounced effect during first 7 days of storage (Campos et al., 2017). Passion fruit peel powder addition to skim and whole milk yoghurt fermented using yoghurt cultures and *Bifidobacterium animalis* subsp. *lactis* publicized the positive effect of said ingredient on increasing and decreasing the pH in whole and skim milk yoghurt, respectively. It was believed that pectin fibers present in passion fruit converted to uronic acid in fermented skim milk variants, whilst, in whole milk yoghurts, production of uronic acid and consumption of fatty acid as a source of carbon after the sugar depletion were occurring concurrently with domination of latter one (doEspirito Santo, Perego, Converti, & Oliveira, 2012). Vacuum infusion of pear fruits with cryostabilizer and subsequent incorporation in fruit yoghurt resulted in lower acidity ($P < 0.05$) after 30 days of cold storage against plain and non-infused pear yoghurt due to buffering action of

Table 4
FSSAI regulation on permitted additives and microbiological specifications of yoghurt.

Permitted additives		Microbiological requirements as per FSSAI		
Name/Category of the additive	Maximum level FSSAI	Parameter	m*	M#
Sugar (For sweetened flavoured and fruit yoghurt)	Not less than 6%	Total plate count	–	–
Aspartame (methyl ester)	600 ppm	Coliform count	10/g	50/g
Sucralose	300 ppm	*E. coli*	–	Absent/g
Isomalt	GMP	Salmonella	–	Absent/25g
Erythritol	GMP	Staphylococcus aureus	50/g	100/g
Polydextrose	GMP	Yeast and moulds	50/g	100/g
Gelatine	10 g/kg	Anaerobic spore count (*Clostridium perfringens*)	10/g	
Pectin	10 g/kg	*Listeria monocytogens*	–	Absent/g
Carrageenan, Agar, Guargum, Sodium carboxy methyl cellulose, Xantan gum, Tragacanth, Karaya gum, Furcellaran	Singly 5 g/kg	Specific lactic acid bacterial count	NLT 10,00,000/g	
Natural flavours and natural flavouring substances/Nature identical flavouring substances/ Artificial flavouring	GMP subject to declaration	Titratable acidity	0.85–1.2% lactic acid	
Colors (Natural: singly or in combination)		Storage & transport	0–4 °C	
Curcumin	100 ppm	*m	Satisfactory Limit	
Riboflavin	50 ppm	#M	Unsatisfactory Limit	
Beta carotene	100 ppm	m < X < M	Marginally Acceptable Limit	
Annatto extract (Bixin:Norbixin = 50:50)	100 ppm			
Methyl ester or Beta apo-8-carotenoic acid	100 ppm			
Canthaxanthin	100 ppm			
Caramel colors (Plain)	GMP			
Caramel colors (Ammonium sulphate process)	3 ppm			
Colors (Synthetic: Singly or in combination)				
Ponceau 4R, Carmoisine, Erthyrosine, Tartrazine, Sunset yellow FCF, Indigo carmine, Brilliant blue FCF, Fast green FCF	100 ppm maximum (only in flavoured and fruit yoghurt)			

cryoprotectants infused in porous fractions of pear fruits, highlighting the significance of vacuum infusion of fruits prior to their incorporation in fruit yoghurt (Cattaneo, Leva, Maraboli, Saurel, & Torreggiani, 2003).

Oligosaccharides of raffinose family (RFOs) obtained from *Lupinus albus* var. *multolupa* seeds, not only stimulated probiotics growth but also resulted in lower acidity of skim milk fermented with *Bifidobacterium lactis* and *L. acidophilus* after 21 days storage against sample without RFOs (Martinez-Villaluenga, Frias, Gomez, & Vidal-Valverde, 2006). The addition of α-lactalbumin calcium complex at 20mg/100 mL level stimulated starter growth and attenuated post-acidification, suggesting its future role for stabilizing yoghurt (Zhao et al., 2018). This reveals that food ecology developed as a resultant of raw material, ingredients and cultures finally govern post-acidification process and warrants careful ingredient selection procedures.

3.3. Microbiological interventions

3.3.1. Modifications in starter culture

LDB is mainly responsible for post-acidification in yoghurt, prompting researchers to employ strategies such as variation in composition of starter culture i.e. from complete absence of LDB to flexible ratios of yoghurt starter culture or searching for novel strains with lower post-acidification potential from different ecological niches. Yoghurt Mild, originated in Germany, with weak sourness and pleasant yoghurt taste is prepared by substituting LDB with *L. acidophilus* (El Demerdash, Oxmann, Heller, & Geis, 2006). Lowering the inoculum of LDB in preparation of probiotic fat-free yoghurt supplemented with different ratio of SMP and whey protein concentrate (WPC) resulted in lower post-acidification, and WPC improved survival of probiotics with non-significant differences in sensory score (Antunes, Cazetto, & Bolini, 2005). *Streptococcus thermophilus* MN-BM-A02 isolated from *Dairy Fan* (a traditional fermented product of China), showed high fermentation rate and low post-acidification potential (Shi et al., 2015).

Various know-how has been generated over years by the combined efforts of academia and industries to produce cultures, enzymes or combination thereof, with negligible post-acidification potential. Commercial cultures with low post-acidification activity are available under the brand name of Mild 2.0® by Chr. Hansen® and YO-MIX® by DuPont®. Examples of some industries supplying specific strains as post-acidity regulators, tailor made for different products are listed in Table 5. Patented process of strained fermented dairy products using lactase enzyme with only thermophilic lactic acid bacteria demonstrated reduced post-acidification, good texture and stability (McCormick, 2019). Similarly, another patent revealed *Lactobacillus fermentum* species capable of maintaining pH > 4 for 14 days at 25 °C with low diacetyl level (0–2 ppm) (Nielsen et al., 2018). Production of fermented milk without post-acidification using lactase enzyme to reduce lactose content along with modified starter composition was reported (Riis, Vojinovic, & Gilleladen, 2019).

Dan et al. (2019) demonstrated the supplementation of probiotic strain *L. plantarum* P-8 with yoghurt starters in ratio of 1:100 resulted in better flavor and delayed post-acidification of yoghurt during 14 days storage period. Another study investigated the role of cell enveloped protease PrtS in post-acidification (Tian et al., 2018). Yoghurt prepared by co-culturing of two SST strains (PrtS– and PrtS+), with LDB, exhibited increased post-acidification during cold storage in PrtS + variant, but pH remained optimum during 14–28 days storage. Hence, such strains could be applied for development of mild yoghurt in combination with weak strains of LDB to regulate post-acidification.

3.3.2. Genetic engineering

Genetic engineering is frequently applied for biotechnological upgradation of LAB with desired functions. Natural strain improvement methods (e.g. random mutagenesis, transformation, conjugation, and transduction) are mainly used due to resistance on use of genetically modified LAB. These natural spontaneous strategies aimed at modification of starter metabolism exploiting biosynthesis (transcription/translation) or alteration of bacterial enzymes involved in acid production like lactose permease, β-galactosidase, H^+-ATPase, urease, lactate dehydrogenase, transglutaminase at genetic level, which also amends post-acidification (Lan & Liao, 2011).

Table 5
Commercially available starter cultures used in yoghurt and related products along with their characteristic properties, post-acidification control potential and their suppliers.

Company (Head Office)	Culture Name	Type of Microorganisms	Desirable properties	Suitable for product type	Post-acidification control potential	Contact Point
Chr Hansen™ (Paris)	DAC 03	Mesophilic	Homogeneous curd body with firm texture	Dahi	NA	incss@chr-hansen.com
	Yoflex®	Thermophilic	Gives characteristics yoghurt quality	Mainly for yoghurt	Controls acid production; reduces post-acidification	
	Exact®	Mesophilic	Texture, flavour and reduces gas formation	Buttermilk, Kefir, Sour cream, quark and Cream cheese	Affects acidification speed thereby post-acidification	
	NU-TRISH®	Single strains/ convenient culture blends	Optimized probiotic cell count with good texture and flavor	Probiotic dairy products.	NA	
	NOLA® FIT	Not disclosed	Gives clean taste to product; totally free from lactose	Lactose free; imparts sweetness without adding sugar	NA	
	FRESHQ®	Bio-protective cultures	Reduces risk of spoilage caused by yeast and moulds	Yoghurt with extended shelf life	Indirectly reduces post-acidification	
DuPont™ Danisko® (Copenhagen, Denmark)	YO-MIX® Greek	Multiple species blends	Mild and creamy flavor development	Especially for Greek yogurt	NA	www.food.dupont.com
	YO-MIX® Multi 100 & 200 series	Multiple species blends	Multiple species blends	Yogurt and fresh fermented milks	NA	
	YO-MIX® PRIME 800 & 900 series	NA	Ultimate mildness with premium texture		NA	
	YO-MIX® Real 300 & 400 series	Blends of *St. thermophilus* & *Lb. bulgaricus*	Mild flavour profile		NA	
	YO-MIX® Real 500 & 600 series		Traditional flavour profile		NA	
	YO-MIX® Quick 700 & 800 series		Contain highly texturizing strains		Reduced fermentation time and limited post-acidification	
DSM Food Specialities™ (Heerlen, Netherlands)	Delvo® Fresh YS-140	Not disclosed	Premium, creamy yogurts with a mild taste and velvety mouthfeel	Used for different yoghurt types	Fast fermentation time and limited post-acidification	stephen.hufton@dsm.com
	Maxilact®		Lactose-free and sugar-reduced dairy products		NA	
	Delvo® Guard	*Lactobacillus rhamnosus* & *Lactobacillus sakei* strains	Reduces risk of spoilage caused by yeast and moulds, Extends shelf-life	Dairy products and dairy snacks with inclusion of fruits, cereals and chocolate	Reduces post-acidification in indirect way	
CSK food enrichment (Wageningen, Netherlands)	Flavor wheel™ Dairy safe™	Not disclosed	Gives signature taste cheese Ensured bio-protection; avoids late blowing defects in cheese	Mainly used for cheeses	NA	www.cskfood.com
	Ceska® Star		Distinct flavour profile in cheese			

3.3.2.1. Mutation in H^+-ATPase. The pH homeostasis by LAB in acidic environments like yoghurt is often governed by membrane bound proton-translocating pump (H^+-ATPase) catalyzing the extrusion of H^+ ions from cell and maintaining the neutral pH inside. Controlling the acidifying potential of LDB and making it more pH sensitive can be accomplished by reducing the activity of H^+-ATPase pump (Ongol et al., 2007). H^+-ATPase deficit mutants of LDB showed reduced post-acidification defect in yoghurt concomitantly maintaining the viability of *Bifidobacterium breve* for 21 days at 10 °C. Wang et al. (2013) reported reduced H^+-ATPase activity of two LDB mutants by 51.3% and 34.3%, respectively from parent strain, resulting in delayed post-acidification by atleast 10 days without hampering sensory of yoghurt. Similarly, Jaichumjai, Valyasevi, Assavanig, and Kurdi (2010) used strain mutagenesis to isolate *Lactobacillus plantarum* strains unable to grow below pH 4.6, thereby enhancing the shelf-life of *Nham* (fermented pork sausage of Thailand) at ambient temperature.

This strategy provides economic and simpler avenues of inhibiting post-acidification in yoghurt and prolonging the shelf-life using naturally improved starters. However, use of neomycin sulphate as selective antibiotic marker for selection of such mutants restricts its application considering the emerging concern and dissemination of antibiotic resistance. This concerned can be overcome by using FDA approved 'nisin' as selection markers as demonstrated by Druesne, Garault, and Faurie (2014), wherein nisin resistant strains of Lactobacilli reduced post-acidification by 0.1–0.2 pH units in contrast to 0.3 units for mother strains.

Another technological constraint faced during spontaneous mutagenesis is degeneration or reversal of desired mutation to original state by successive passages of mutant strain. Hence, stability of desired mutation is another utmost criterion for future strain improvement programmes. Lately, Chuah and Mao (2020) isolated LDB mutant showing 0.35 units higher pH after 48 h fermentation in MRS broth, with said trait attributed to point mutation from GGT to GAT at positions 505 to 507 in F_0F_1-ATPase α-subunit. The degeneration of this desired mutation was successfully prevented even after 10 passages by supplementing MRS with 0.1 M potassium phosphate buffer and reducing the

inoculation rate from 10% to 2%. Similarly, random mutagenesis had also been applied to adjunct starter *L. helveticus* SH2-1 to generate a lower post-acidifying mutant with 0.57 higher pH units and 57.1 °T lower acidity than parent strain (Guan et al., 2020). The desired mutation was found genetically stable for 100 sub-passaging and fermented milk showed improvement in its textural, rheological and flavor attributes.

3.3.2.2. Others (mutations in other genes, use of promoters). Several researchers also attempted different mutation/engineering strategies to combat post-acidification. SST mutants devoid of AmiA and PrtS oligopeptide transport systems exhibited retarded growth and thus reduced post-acidification in milk (Garault, Le Bars, Besset, & Monnet, 2002). LDB continued acid production is attributed to its constitutive expression of *lacSZ* gene due to lost regulatory function of *lacR* gene in lac operon (Lapierre, Mollet, & Germond, 2002). Liu et al. (2014) proposed a model of pH induced promoter which acts as an on/off switch at reduced pH environments to regulate a repressor gene, thereby controlling the expression of lacSZ operon to restrain lactic acid development by LDB. Another study reported generation of galactose fermenting and glucose secreting spontaneous mutants of industrial strains of SST and LDB utilizing lactose as carbon source (Sorensen, Curic-Bawden, Junge, Janzen, & Johansen, 2016). These specific traits can be exploited for development of sweet yoghurt with high glucose levels and can help in suppression of excessive sour taste. The strain improvement by natural selection seems efficient way but it requires extensive screening and precise knowledge of microbial physiology. Only drawback associated is untargeted mutations which accounts for further characterization and selection for target variants.

3.3.3. Encapsulated starter culture

Encapsulation of dairy starters including probiotics provides dual benefits including reduced cell injury against adverse environments (organic acids, hydrogen peroxide, reactive oxygen etc.) and decreased proliferation kinetics of encapsulated cell thereby restricting post-acidification to a certain extent. Microencapsulation of yoghurt cultures retarded milk acidification (end pH 4.6) by 10 h as opposed to 6 h by free cells while maintaining higher cell viability during storage and simulated gastrointestinal tract (De Prisco, van Valenberg, Fogliano, & Mauriello, 2017). Kia, Ghasempour, Ghanbari, Pirmohammadi, and Ehsani (2018) found reduced post-acidification in probiotic yoghurt due to combined action of high buffering capacity of added MPC and encapsulation of *L. paracasei* in sodium caseinate-gellan gum material. Probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) encapsulated in calcium induced alginate beads and starch as filler polymer, maintained the final pH at 4.25 against 3.95 for free culture containing yoghurt after six weeks (Kailasapathy, 2006). Microencapsulation of *L. acidophilus* LA-5 by ionic gelation and coacervation reduced post-acidification in probiotic yoghurts, and conferred cell protection and safe transit through simulated gastrointestinal tract during 35 days of refrigerated storage (Ribeiro et al., 2014). Other studies conducted on similar concept includes encapsulation of *Bifidobacterium breve* in whey protein microcapsules (Picot & Lacroix, 2004) and alginate-goats's milk inulin probiotic encapsulate for goat's milk yoghurt (Pradeep Prasanna & Charalampopoulos, 2019).

3.3.4. Stress adaptation

Adaptive stress response is evolutionary strategy to improve the starter performance by modifying its physiological features. Stress adaptation involves sublethal doses of stress (heat, salt, pH or pressure) to weaken the metabolic activity of starters. Among the various stresses, heat stress is additive free and can be implemented easily during continuous processing. Stress exposure of *Lactobacillus plantarum* WCFS1 to elevated levels of salt and low pH followed by co-culturing with yoghurt starters reduced post-acidification during refrigerated storage of yoghurt by impairing survival of LDB, possibly due to induced plantaricin production (Settachaimongkon et al., 2016). Mild heat treatment (46 °C/1 min) of fermented milk diminished acidification rate during first three days and minimized the post-acidification caused by *L. rhamnosus* by 70% via decreasing its metabolism and proliferation (Zhang et al., 2019).

Alternatively, use of strains expressing heat-shock proteins at elevated incubation temperature is another approach to address post-acidification. For instance, plasmid pSt04 encoding heat shock protein in *S. thermophilus* S4 can carry out fermentation at 50 °C in combination with LDB yielding mild yoghurt with low post-acidification (not below pH 4.2) (El Demerdash et al., 2006). Further, incorporation of this specific plasmid into commercial SST strain using two-plasmid system provided a food-grade non-genetically modified strain.

Stress adaptive mechanisms of LAB considerably change the technological and functional attributes of product and is a promising approach to control post-acidification, however, induction of correct stress and stability of desired attributes during sub-culturing followed by inspection for any undesirable changes to product's quality is an area of research.

4. Future prospects

Post-acidification can certainly be attenuated by maintenance of chilled-chain storage and transportation; however, this demands extensive infrastructural requirements and may not be well suited for nations where refrigeration systems are not popularized. Futuristically, control strategies like i) development of designer milk for manufacturing mild flavour fermented products by inducing compositional changes to enhance buffering capacity viz. adjustment of protein/lactose ratio in milk via membrane filtration ii) use of pH sensitive gels encapsulated with GRAS antimicrobial agents for allowing sustained release of antimicrobial after reaching desired pH iii) untargeted mutations in conventional approaches demand investigation of novel genome editing tools like 'clustered regularly interspaced short palindromic repeats' (CRISPR)-Cas9 which allows implementation of straight-forward and precise mutations in targeted sequences to increase regulatory acceptance as some of Cas9 edited plants (tomato/fruit crops) are approved in US (Wang et al., 2019) iv) integration of intelligent packaging with yoghurt packs for indicating ΔpH during storage without opening the container along with mechanisms of controlling pH using active ingredients in packaging material itself (Eker & Eker, 2011) v) effect of permitted/natural additives on post-acidification in conjugation with cost-effective processing approaches could be attempted vi) Up-scaling and commercialization of emerging non-thermal techniques should also be undertaken for controlling post-acidification.

5. Conclusion

Various strategies discussed in this review can be applied either alone or in unison to combat post-acidification without affecting the rheological, organoleptic and shelf-life of fermented products. However, each strategy has its own merits and demerits. Thermal treatment of fermented milks indisputably extends their shelf life and reduces post-acidification but are not suitable for functional fermented products due to loss of heat-labile bioactive peptides and beneficial microbes. CO_2 addition was found useful in prolonging shelf-life and retarding acid development in fermented dairy products, however, its parallel effect on organoleptic properties and post-acidification has not been studied. Given the potential of HHP in controlling post-acidification, studies pertaining to commercial feasibility as well as its application in probiotic yoghurts are required. Similarly, emerging technologies (PEF, ultrasound, irradiation, MF, RF) need further explorations and regulatory approval for application in such products. Nevertheless, these emerging processing techniques being additive-free, environment friendly, sustainable and innovative continues to gain momentum in

preventing post-acidification. The encapsulation of probiotics is useful in curtailing the post-acidification, but use of expensive encapsulant material will add cost on one end and might affect the sensory attributes of product. Similarly, direct incorporation of bacteriocins in yoghurt is hindered by its high purification cost and stability in food matrix.

Bioprotective cultures provide an edge over direct bacteriocin addition, being a cheaper alternative and simple *in-situ* post-acidification controller, provided they have been critically evaluated from safety viewpoint. Although, the antibiotic based strain selection through random mutagenesis requires rigorous screening, but if used in conjugation with non-antibiotic selection markers (like nisin or heavy metals) and high-throughput selection techniques, seems a safe and efficient approach, and economically feasible to implement on existing production lines as direct vat starters (DVS). Bioprospecting for novel strains with improved functional characteristics is another encouraging way along with their evolutionary adaption, provided the desired traits are not lost during subsequent passaging. In brief, further integrated efforts utilizing the discussed techniques are required for the development of mild flavor shelf-stable fermented products followed by their translation to industrial level worldwide. The analysis of literature sources will motivate the academicians and industrial stakeholders to develop novel cost-effective strategies for mitigating post-acidification in yoghurt having higher starter viability, longer shelf-life, superior sensorial acceptance and nutritional value.

References

Alakali, J. S., Unwiyi, I., & Ejiga, O. (2009). Effect of milk blends and temperature on the quality of thermized yoghurt. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 8*, 2647–2654.

Alhejaili, M., Olson, D. W., Velazquez, C., Janes, M., Boeneke, C., & Aryana, K. J. (2019). Influence of an aqueous myrrh suspension on yogurt culture bacteria over yogurt shelf-life. *Journal of Dairy Science, 102*, 2011–2016.

Alvarez, F., Arguello, M., Cabero, M., Riera, F. A., Alvarez, R., Iglesias, J. R., et al. (1998). Fermentation of concentrated skim-milk. Effects of different protein/lactose ratios obtained by ultrafiltration–diafiltration. *Journal of the Science of Food and Agriculture, 76*, 10–16.

de Ancos, B., Cano, M. P., & Gomez, R. (2000). Characteristics of stirred low-fat yoghurt as affected by high pressure. *International Dairy Journal, 10*, 105–111.

Antunes, A. E., Cazetto, T. F., & Bolini, H. M. (2005). Viability of probiotic micro-organisms during storage, postacidification and sensory analysis of fat-free yogurts with added whey protein concentrate. *International Journal of Dairy Technology, 58*, 169–173.

Bevilacqua, A., Casanova, F. P., Petruzzi, L., Sinigaglia, M., & Corbo, M. R. (2016). Using physical approaches for the attenuation of lactic acid bacteria in an organic rice beverage. *Food Microbiology, 53*, 1–8.

Blakistone, B. A. (1990). Extending the shelf-life of refrigerated yogurt. In *Proceedings of pack alimentaire '90, 4th annual food and packaging expo & conference, may 1990*. San Francisco: Innovative Expositions, Inc.

Bonnaillie, L. M., & Tomasula, P. M. (2015). Carbon dioxide: An alternative processing method for milk. In *Emerging dairy processing technologies: Opportunities for the dairy industry* (pp. 205–220). Oxford: John Wiley and Sons.

Borcakli, M., Lucas, J., Caputo, L., Ozturk, T., Baruzzi, F., Fusco, V., et al. (2013). Effect of UV-C light in the preservation of raw fermented beverages. *Italian Journal of Food Science, 25*, 213–220.

Boufassa, C., & Tourancheau, M. (2004). *Method for preparing food products by fermenting soy milk with Streptococcus thermophilus*. Washington, DC: U.S. Patent and Trademark Office.. U.S. Patent No. 6,699,517.

Calvo, M. M., Montilla, A., & Cobos, A. (1999). Lactic acid production and rheological properties of yogurt made from milk acidified with carbon dioxide. *Journal of the Science of Food and Agriculture, 79*, 1208–1212.

Campos, D. C. D. S., Neves, L. T. B. C., Flach, A., Costa, L. A. M. A., & Sousa, B. O. D. (2017). *Post-Acidification and evaluation of anthocyanins stability and antioxidant activity in acai fermented milk and yogurts (Euterpe Oleracea Mart.)* (p. 39). RevistaBrasileira De Fruticultura.

Carroll, T., Chen, P., Harnett, M., & Harnett, J. (2010). *Pressure treating food to reduce spoilage*. Washington, DC: U. S. Patent and Trademark Office.. U.S. Patent No. 7,854,950.

Cattaneo, T. M. P., Leva, A. A., Maraboli, A., Saurel, R., & Torreggiani, D. (2003). Improvement of pear yoghurt ingredients using vacuum infusion. *Italian Journal of Food Science, 15*, 269–276.

Chanos, P., Warncke, M. C., Ehrmann, M. A., & Hertel, C. (2020). Application of mild pulsed electric fields on starter culture accelerates yogurt fermentation. *European Food Research and Technology, 246*, 621–630.

Choi, H. S., & Kosikowski, F. V. (1985). Sweetened plain and flavored carbonated yogurt beverages. *Journal of Dairy Science, 68*, 613–619.

Cho, H. Y., Yousef, A. E., & Sastry, S. K. (1996). Growth kinetics of Lactobacillus acidophilus under ohmic heating. *Biotechnology and Bioengineering, 49*(3), 334–340.

Chuah, L. O., & Mao, Y. (2020). Stability assessment and improvement of a Lactobacillus plantarum mutant with low post-fermentation acidification characteristics. *Journal of Dairy Science, 103*(9), 7898–7907.

Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2010). Comparison of the effects of high-pressure microfluidization and conventional homogenization of milk on particle size, water retention and texture of non-fat and low-fat yoghurts. *International Dairy Journal, 20*, 314–320.

Cruz, A. G., Castro, W. F., Faria, J. A. F., Bolini, H. M. A., Celeghini, R. M. S., Raices, R. S. L., et al. (2013). Stability of probiotic yogurt added with glucose oxidase in plastic materials with different permeability oxygen rates during the refrigerated storage. *Food Research International, 51*, 723–728.

Cueva, O. A. (2009). *Pulsed electric field influences on acid tolerance, bile tolerance, protease activity and growth characteristics of Lactobacillus acidophilus LA-K*. Louisiana State University. Master's Thesis.

Dagher, S., & Ali, A. (1985). Effect of pasteurization, centrifugation and additives on quality of concentrated yogurt (Labneh). *Journal of Food Protection, 48*, 300–302.

Damin, M. R., Minowa, E., Alcantara, M. R., & Oliveira, M. N. (2008). Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yogurt and probiotic bacteria. *Journal of Texture Studies, 39*, 40–55.

Dan, T., Chen, H., Li, T., Tian, J., Ren, W., Zhang, H., et al. (2019). Influence of Lactobacillus plantarum P-8 on fermented milk flavor and storage stability. *Frontiers in Microbiology, 9*, 3133.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., et al. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods, 8*, 92.

De Prisco, A., van Valenberg, H. J., Fogliano, V., & Mauriello, G. (2017). Microencapsulated starter culture during yoghurt manufacturing, effect on technological features. *Food and Bioprocess Technology, 10*, 1767–1777.

Didar, Z. (2020). Investigation the effect of pulsed electric field treatment on Kluyveromyces marxianus of Doogh. *Journal of Food Research, 30*, 29–40.

Donkor, O. N., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal, 16*, 1181–1189.

Druesne, A., Garault, P., & Faurie, J. M. (2014). *Use of nisin resistant mutant strains of Lactobacilli for reducing the post acidification in food products*. Washington, DC: US Patent and Trademark Office. U.S. Patent Application No. 14/114,279.

Dunn, J. E., & Pearlman, J. S. (1987). *Method and apparatus for extending the shelf-life of fluid food products*. Washington, DC: US Patent and Trademark Office. U.S. Patent No. 4,695,472.

Eker, B., & Eker, A. A. (2011). Developing packaging by using smart material which helps to realize spoilage in yoghurt. *Key Engineering Materials, 471*, 185–190.

El Demerdash, H. A., Oxmann, J., Heller, K. J., & Geis, A. (2006). Yoghurt fermentation at elevated temperatures by strains of Streptococcus thermophilus expressing a small heat-shock protein: Application of a two-plasmid system for constructing food-grade strains of Streptococcus thermophilus. *Biotechnology Journal: Healthcare Nutrition Technology, 1*, 398–404.

Erkaya, T., Baslar, M., Sengul, M., & Ertugay, M. F. (2015). Effect of thermosonication on physicochemical, microbiological and sensorial characteristics of ayran during storage. *Ultrasonics Sonochemistry, 23*, 406–412.

Fahim, H. A., Khairalla, A. S., & El-Gendy, A. O. (2016). Nanotechnology: A valuable strategy to improve bacteriocin formulations. *Frontiers in Microbiology, 7*, 1385.

Fenster, K., Freeburg, B., Hollard, C., Wong, C., Ronhave Laursen, R., & Ouwehand, A. C. (2019). The production and delivery of probiotics: A review of a practical approach. *Microorganisms, 7*, 83.

Garault, P., Le Bars, D., Besset, C., & Monnet, V. (2002). Three oligopeptide-binding proteins are involved in the oligopeptide transport of Streptococcus thermophilus. *Journal of Biological Chemistry, 277*, 32–39.

Garrigues, C., Gilleladen, C., Curic-Bawden, M., Janzen, T., Birkelund, M., Buchhorn, G. L., et al. (2017). *Method of producing a fermented milk product with improved control of post-acidification*. Washington, DC: US Patent and Trademark Office. U.S. Patent Application No. 15/317,682.

Gonzalez, B., Arca, P., Mayo, B., & Suárez, J. E. (1994). Detection, purification, and partial characterization of plantaricin C, a bacteriocin produced by a Lactobacillus plantarum strain of dairy origin. *Applied and Environmental Microbiology, 60*, 2158–2163.

Guan, C., Chen, X., Zhao, R., Yuan, Y., Huang, X., Su, J., et al. (2020). A weak post-acidification Lactobacillus helveticus UV mutant with improved textural properties. *Food Sciences and Nutrition, 9*, 469–479.

Gueimonde, M., Corzo, N., Vinderola, G., Reinheimer, J., & de los Reyes-Gavilan, C. G. (2002). Evolution of carbohydrate fraction in carbonated fermented milks as affected by β-galactosidase activity of starter strains. *Journal of Dairy Research, 69*, 125–137.

Guenard-Lampron, V., St-Gelais, D., Villeneuve, S., & Turgeon, S. L. (2020). Effect of stirring operations on changes in physical and rheological properties of nonfat yogurts during storage. *Journal of Dairy Science, 103*, 210–214.

Guerrero-Beltran, J. A., Sepulveda, D. R., Gongora-Nieto, M. M., Swanson, B., & Barbosa-Canovas, G. V. (2010). Milk thermization by pulsed electric fields (PEF) and electrically induced heat. *Journal of Food Engineering, 100*, 56–60.

Ham, J. S., Jeong, S. G., Lee, S. G., Han, G. S., Jang, A., Yoo, Y. M., et al. (2009). Quality of irradiated plain yogurt during storage at different temperatures. *Asian-Australasian Journal of Animal Sciences, 22*, 289–295.

Han, K. S., Imm, J. Y., Oh, S., Jeon, W. M., & Kim, S. H. (2002). Bacteriocin produced by Lactobacillus acidophilus ATCC 4356. *Food Science and Biotechnology, 11*, 531–536.

Han, X., Zhang, L., Du, M., Yi, H., Li, J., & Zhang, L. (2012). Effects of copper on the post acidification of fermented milk by St. thermophilus. *Journal of Food Science, 77*, 25–28.

Jaichumjai, P., Valyasevi, R., Assavanig, A., & Kurdi, P. (2010). Isolation and characterization of acid-sensitive Lactobacillus plantarum with application as starter culture for Nham production. *Food Microbiology, 27*, 741–748.
Jankowska, A., Wisniewska, K., & Reps, A. (2005). Application of probiotic bacteria in production of yoghurt preserved under high pressure. *High Pressure Research, 25*, 57–62.
Jansson, S. E. A., Edsman, C. J., Gedde, U. W., & Hedenqvist, M. S. (2001). Packaging materials for fermented milk: Effects of material crystallinity and polarity on food quality. *Packaging Technology and Science: International Journal, 14*, 119–127.
Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT-Food Science and Technology, 39*, 1221–1227.
Karagul-Yuceer, Y., Wilson, J. C., & White, C. H. (2001). Formulations and processing of yogurt affect the microbial quality of carbonated yogurt1. *Journal of Dairy Science, 84*, 543–550.
Kia, E. M., Ghasempour, Z., Ghanbari, S., Pirmohammadi, R., & Ehsani, A. (2018). Development of probiotic yogurt by incorporation of milk protein concentrate (MPC) and microencapsulated Lactobacillus paracasei in gellan-caseinate mixture. *British Food Journal, 120*, 1516–1528.
Kristo, E., Biliaderis, C. G., & Tzanetakis, N. (2003). Modelling of the acidification process and rheological properties of milk fermented with a yogurt starter culture using response surface methodology. *Food Chemistry, 83*, 437–446.
Kumar, A., Hussain, S. A., Raju, P. N., Singh, A. K., & Singh, R. R. B. (2017). Packaging material type affects the quality characteristics of Aloe-probiotic lassi during storage. *Food Bioscience, 19*, 34–41.
Lan, E. I., & Liao, J. C. (2011). Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. *Metabolic Engineering, 13*, 353–363.
Lapierre, L., Mollet, B., & Germond, J. E. (2002). Regulation and adaptive evolution of lactose operon expression in Lactobacillus delbrueckii. *Journal of Bacteriology, 184*, 928–935.
Liu, K., Zeng, X., Qiao, L., Li, X., Yang, Y., Dai, C., et al. (2014). The sensitivity and significance analysis of parameters in the model of pH regulation on lactic acid production by Lactobacillus bulgaricus. *BMC Bioinformatics, 15*, S5.
Loghavi, L., Sastry, S. K., & Yousef, A. E. (2009). Effect of moderate electric field frequency and growth stage on the cell membrane permeability of Lactobacillus acidophilus. *Biotechnology Progress, 25*, 85–94.
London, L. E., Chaurin, V., Auty, M. A., Fenelon, M. A., Fitzgerald, G. F., Ross, R. P., et al. (2015). Use of Lactobacillus mucosae DPC 6426, an exopolysaccharide-producing strain, positively influences the techno-functional properties of yoghurt. *International Dairy Journal, 40*, 33–38.
Lorenzen, P. C., Neve, H., Mautner, A., & Schlimme, E. (2002). Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt. *International Journal of Dairy Technology, 55*, 152–157.
Marouf, A. A. S., & Siddiq, E. O. H. (2018). Evaluation of laser pasteurization on production of yoghurt. *International Journal of Advanced Research in Physical Science, 5*, 15–23.
Martinez-Villaluenga, C., Frias, J., Gomez, R., & Vidal-Valverde, C. (2006). Influence of addition of raffinose family oligosaccharides on probiotic survival in fermented milk during refrigerated storage. *International Dairy Journal, 16*, 768–774.
Martinez, F. A. C., Balciunas, E. M., Converti, A., Cotter, P. D., & de Souza Oliveira, R. P. (2013). Bacteriocin production by Bifidobacterium spp. A review. *Biotechnology Advances, 31*, 482–488.
Masuzawa, N., & Ohdaira, E. (2002). Attempts to shorten the time of lactic fermentation by ultrasonic irradiation. *Japanese Journal of Applied Physics, 41*, 3277–3278.
McCormick, C. (2019). *Use of lactase in the preparation of a strained fermented dairy product.* Washington, DC: US Patent and Trademark Office. U.S. Patent Application No. 16/064,718.
Mishra, S., & Mishra, H. N. (2013). Effect of synbiotic interaction of fructooligosaccharide and probiotics on the acidification profile, textural and rheological characteristics of fermented soy milk. *Food and Bioprocess Technology, 6*, 3166–3176.
Mroueh, M., Issa, D., Khawand, J., Haraty, B., Malek, A., Kassaify, Z., et al. (2008). Levels of benzoic and sorbic acid preservatives in commercially produced yoghurt in Lebanon. *Journal of Food Agriculture and Environment, 6*, 62–66.
Neirinckx, J. (1972). Thermization of yoghurt and French cheese. *Rev ue Lait-fr., 29*, 465–467.
Nielsen, C. L. M., Hornbaek, T., Rasmussen, P., & Poulsen, L. (2018). *Lactobacillus fermentum bacteria inhibiting post-acidification.* Washington, DC: US Patent and Trademark Office. U.S. Patent Application No. 15/755,026.
Oh, S., Heo, H. J., Park, D. J., Kim, S. H., Lee, S. J., & Imm, J. Y. (2006). Effect of encapsulated bacteriocin on acid production and growth of starter cultures in yoghurt. *Food Science and Biotechnology, 15*, 78–83.
Oliveira, R. P. D. S., Florence, A. C. R., Perego, P., De Oliveira, M. N., & Converti, A. (2011). Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. *International Journal of Food Microbiology, 145*, 22–27.
Oliveira, R. P., Florence, A. C., Silva, R. C., Perego, P., Converti, A., Gioielli, L. A., et al. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. *International Journal of Food Microbiology, 128*, 467–472.
Ongol, M. P., Sawatari, Y., Ebina, Y., Sone, T., Tanaka, M., Tomita, F., et al. (2007). Yoghurt fermented by Lactobacillus delbrueckii subsp. bulgaricus H+-ATPase-defective mutants exhibits enhanced viability of Bifidobacterium breve during storage. *International Journal of Food Microbiology, 116*, 358–366.
Penney, V., Henderson, G., Blum, C., & Johnson-Green, P. (2004). The potential of phytopreservatives and nisin to control microbial spoilage of minimally processed fruit yogurts. *Innovative Food Science & Emerging Technologies, 5*, 369–375.
Picot, A., & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal, 14*, 505–515.
Pinto, S. M., Clemente, M. D. G., & De Abreu, L. R. (2009). Behaviour of volatile compounds during the shelf-life of yoghurt. *International Journal of Dairy Technology, 62*, 215–223.
Poltronieri, P., & Rossi, F. (2018). Stabilization of milk quality by heat treatments. In *Microbiology in dairy processing: Challenges and opportunities* (pp. 63–68). New Jersey, USA: John Wiley & Sons.
Pradeep Prasanna, P. H., & Charalampopoulos, D. (2019). Encapsulation in an alginate–goats' milk–inulin matrix improves survival of probiotic Bifidobacterium in simulated gastrointestinal conditions and goats' milk yoghurt. *International Journal of Dairy Technology, 72*, 132–141.
Racioppo, A., Corbo, M. R., Piccoli, C., Sinigaglia, M., Speranza, B., & Bevilacqua, A. (2017). Ultrasound attenuation of lactobacilli and bifidobacteria: Effect on some technological and probiotic properties. *International Journal of Food Microbiology, 243*, 78–83.
Rajapaksha, D. S. W., & Kodithuwakku, K. A. H. T. (2014). Evaluation of chitosan for its inhibitory activity on post-acidification of set yoghurt under cold storage for 20 days. *Journal of Chitin and Chitosan Science, 2*, 16–20.
Rajapaksha, D. S. W., Kodithuwakku, K. A. H. T., Silva, K. F. S. T., & Rupasinghe, R. A. J. N. L. (2013). Evaluation of Potassium sorbate and E-polylysine for their inhibitory activity on post-acidification of set yoghurt under cold storage for 20 days. *International Journal of Scientific and Research Publications, 3*.
Reps, A., Jankowska, A., & Wisniewska, K. (2009). The effect of high pressure on selected properties of yoghurt. *High Pressure Research, 29*, 33–37.
Ribeiro, M. C. E., Chaves, K. S., Gebara, C., Infante, F. N., Grosso, C. R., & Gigante, M. L. (2014). Effect of microencapsulation of Lactobacillus acidophilus LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. *Food Research International, 66*, 424–431.
Riis, S. N., Vojinovic, V., & Gilleladen, C. (2019). *Fermented milk product with a reduced content of lactose*. Washington, DC: US Patent and Trademark Office. U.S. Patent Application No. 16/309,892.
Routray, W., & Mishra, H. N. (2011). Scientific and technical aspects of yogurt aroma and taste: A review. *Comprehensive Reviews in Food Science and Food Safety, 10*, 208–220.
Saint-Eve, A., Levy, C., Le Moigne, M., Ducruet, V., & Souchon, I. (2008). Quality changes in yogurt during storage in different packaging materials. *Food Chemistry, 110*, 285–293.
doEspirito Santo, A. P., Perego, P., Converti, A., & Oliveira, M. N. (2012). Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts. *LWT-Food Science and Technology, 47*, 393–399.
Sarkar, S. P., Dave, J. M., & Sannabhadti, S. S. (1992). Effect of thermization of mistidahi on shelf-life and beta-D-galactosidase activity. *Indian Journal of Dairy Science, 45*, 135–139.
Serra, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009). Evaluation of physical properties during storage of set and stirred yogurts made from ultra-high pressure homogenization-treated milk. *Food Hydrocolloids, 23*, 82–91.
Settachaimongkon, S., van Valenberg, H. J., Gazi, I., Nout, M. R., van Hooijdonk, T. C., Zwietering, M. H., et al. (2016). Influence of Lactobacillus plantarum WCFS1 on post-acidification, metabolite formation and survival of starter bacteria in set-yoghurt. *Food Microbiology, 59*, 14–22.
Shi, Y., Chen, Y., Li, Z., Yang, L., Chen, W., & Mu, Z. (2015). Complete genome sequence of Streptococcus thermophilus MN-BM-A02, a rare strain with a high acid-producing rate and low post-acidification ability. *Genome Announcements, 3*, 979, 15.
Siefarth, C., Tran, T. B. T., Mittermaier, P., Pfeiffer, T., & Buettner, A. (2014). Effect of radio frequency heating on yoghurt, I: Technological applicability, shelf-life and sensorial quality. *Foods, 3*, 318–335.
Silva, C. C., Silva, S. P., & Ribeiro, S. C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. *Frontiers in Microbiology, 9*, 594.
Sorensen, K. I., Curic-Bawden, M., Junge, M. P., Janzen, T., & Johansen, E. (2016). Enhancing the sweetness of yoghurt through metabolic remodeling of carbohydrate metabolism in Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. *Applied and Environmental Microbiology, 82*, 3683–3692.
Sun, H., Masuda, F., Kawamura, S., Himoto, J. I., Asano, K., & Kimura, T. (2011). Effect of electric current of ohmic heating on nonthermal injury to Streptococcus thermophilus in milk. *Journal of Food Process Engineering, 34*, 878–892.
Tamime, A. Y., & Robinson, R. K. (2007). Microbiology of yoghurt and related starter cultures. In *Tamime and robinson's yogurt: Science and technology* (pp. 468–511). Boca Raton, Florida, USA: CRC Press.
Tanaka, T., & Hatanaka, K. (1992). Application of hydrostatic pressure to yoghurt to prevent its after-acidification. *Journal of the Japanese Society for Food Science and Technology, 39*, 173–177.
Tian, H., Li, B., Evivie, S. E., Sarker, S. K., Chowdhury, S., Lu, J., et al. (2018). Technological and genomic analysis of roles of the cell-envelope protease PrtS in yoghurt starter development. *International Journal of Molecular Sciences, 19*, 1068.
Tribst, A. A. L., Falcade, L. T. P., Carvalho, N. S., Junior, B. R. D. C. L., & de Oliveira, M. M. (2020). Are stirring and homogenisation processes capable of improving physicochemical and sensory characteristics of stirred yoghurt produced with fresh, refrigerated and frozen/thawed sheep milk? *International Dairy Journal*, 104778.

Trujillo, A. J., Capellas, M., Saldo, J., Gervilla, R., & Guamis, B. (2002). Applications of high-hydrostatic pressure on milk and dairy products: A review. *Innovative Food Science & Emerging Technologies, 3*, 295–307.
Turgut, T. (2016). The effect of microwave heating on the some quality properties and shelf-life of yoghurt. *Kafkas Univ Vet Fak Derg, 22*, 809–814.
Vargas, M., Chafer, M., Albors, A., Chiralt, A., & Gonzalez-Martinez, C. (2008). Physicochemical and sensory characteristics of yoghurt produced from mixtures of cows' and goats' milk. *International Dairy Journal, 18*, 1146–1152.
Vieira, C. P., da Costa, M. P., de Melo Silva, V. L., Delgado, K. F., da Silva Frasão, B., Elias, T. A., et al. (2020). Interactive effect of physicochemical and microbial variables on bioactive amines content during storage of probiotic fermented milk. *LWT-Food Science and Technology*, 110700.
Walsh, C. J., Guinane, C. M., O'Toole, P. W., & Cotter, P. D. (2014). Beneficial modulation of the gut microbiota. *FEBS Letters, 588*, 4120–4130.
Wang, X., Ren, H., Liu, D., Wang, B., Zhu, W., & Wang, W. (2013). H+-ATPase-defective variants of Lactobacillus delbrueckii subsp. bulgaricus contribute to inhibition of postacidification of yogurt during chilled storage. *Journal of Food Science, 78*, M297–M302.
Wang, T., Zhang, H., & Zhu, H. (2019). CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. *Horticulture Research, 6*, 1–13.
Xu, Z., Li, S., Gong, G., Liu, Z., Wu, Z., & Ma, C. (2015). Influence of different acidifying strains of Lactobacillus delbrueckii subsp. bulgaricus on the quality of yoghurt. *Food Science and Technology Research, 21*, 263–269.
Yeom, H. W., Evrendilek, G. A., Jin, Z. T., & Zhang, Q. H. (2004). Processing of yogurt-based products with pulsed electric fields: Microbial, sensory and physical evaluations. *Journal of Food Processing and Preservation, 28*, 161–178.
Yuceer, S., & Gunduz, G. (1980). Preservation of cheese and plain yogurt by low-dose irradiation. *Journal of Food Protection, 43*, 114–118.
Zhang, J., Sturla, S., Lacroix, C., & Schwab, C. (2018). Gut microbial glycerol metabolism as an endogenous acrolein source. *mBio, 9*, e01947, 17.
Zhang, C., Yang, L., Gu, R., Ding, Z., Guan, C., Lu, M., et al. (2019). Mild heat stress limited the post-acidification caused by Lactobacillus rhamnosus hsryfm 1301 in fermented milk. *Biotechnology Letters, 41*, 633–639.
Zhao, L. L., Wang, X. L., Liu, Z. P., Sun, W. H., Dai, Z. Y., Ren, F. Z., et al. (2018). Effect of α-lactalbumin hydrolysate-calcium complexes on the fermentation process and storage properties of yogurt. *LWT-Food Science and Technology, 88*, 35–42.
Zhao, Q. Z., Wang, J. S., Zhao, M. M., Jiang, Y. M., & Chun, C. (2006). Effect of casein hydrolysates on yogurt fermentation and texture properties during storage. *Food Technology and Biotechnology, 44*, 429–434.