

Chapter 2

The Yoghurt: Chemical and Technological Profiles

Abstract The complex of fermentative reactions that are normally used in the industry of yoghurts shows extraordinary performances with relation to produced amounts and the qualitative composition of organic acids. The usual fermentative pathway in the yoghurt manufacture is coincident with the common homolactic fermentation. The synergic action of selected streptococci and lactobacilli in the fermentation of raw milks determines the increased production of lactic acid, acetaldehyde and polysaccharides. Other interesting variations of chemical profiles in yoghurts can be observed and explained with relation to the qualitative and quantitative distribution of different vitamins, benzoic and orotic acids, bacteriocins, enzymes, peptides and amino acids.

Keywords Acetaldehyde • Acetoin • Acetone • Bacteriocins • Diacetyl • Folic acid • Homolactic fermentation • Lactic acid • Pantothenic acid

List of Abbreviations

CO ₂	Carbon dioxide
CFU	Colony-forming unit
LAB	Lactic acid bacterium
LDB	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>
MW	Molecular weight
ST	<i>Streptococcus thermophilus</i>

2.1 The Yoghurt: Biochemical Variations

The complex of fermentative reactions that are normally used in the industry of yoghurts shows extraordinary performances with relation to produced amounts and the qualitative composition of organic acids. In fact, the usual fermentative pathway in the yoghurt manufacture is coincident with the common homolactic fermentation (Sect. 1.1). However, two distinct and important features have to be mentioned:

- The fermentative pathway is carried out by two different bacteria: *Lactobacillus delbruekii* subsp. *bulgaricus* (LDB) and *Streptococcus thermophilus* (ST)
- Above-mentioned lactic acid bacteria (LAB) are able to produce notable amounts of organic acids by converting the same substrate (lactose) without competition.

On the contrary, LDB and ST can act synergically. Differently from other opportunistic associations, the synergic interaction is extremely efficient because of the increased production of lactic acid, acetaldehyde and polysaccharides (Sect. 1.4).

The synergistic effect between above-mentioned LAB may be easily explained. ST is stimulated by the bioavailability of free amino acids and peptides in culture media. On the other side, LDB is able to attack and proteolyze milk proteins; consequently, needed amino acids and peptides can be easily found if lactobacilli can spread freely in milks.

In general, it can be affirmed that five amino acids—valine, glycine, histidine, leucine and isoleucine—and short peptides are preferred sources of nitrogen for ST. With reference to LDB, the microbial growth is stimulated by active compounds such as carbon dioxide and formic acid. It has to be highlighted that similar chemicals are massively produced by ST. As a clear result, the one bacterial species seems to act as a biochemical ‘growth engine’ for the other life forms: in other words, the synergic association can be considered such as a ‘binary feedback loop’. This situation has been repeatedly observed in different experiments with or without the addition of specified prebiotics (Oliveira et al. 2009; De Souza Oliveira et al. 2011).

It has been also noted that milk pre-treatments can reduce carbon dioxide to low values: this effect does not favour the rapid development of LDB because this bacterium would need 30 mg/Kg at least of carbon dioxide (CO₂). However, the release of CO₂ can be enhanced by decarboxylation of urea: this reaction, carried out by ST in yoghurts, has been reported to increase the initial level of CO₂ from 10 mg/kg at pH = 6.42 until more than 150 mg/Kg after only 60 min of incubation; pH may reach 5.7–5.8 at least. In addition, the higher the incubation period, the lower the pH and the higher the CO₂ release (Battistotti and Bottazzi 1998).

As a consequence, favourable conditions for the development of LDB can be obtained in milky mixtures (Sect. 1.5) after 37–40 min at 44.5 °C, in spite of the deficiency of CO₂ in original raw milks.

In addition, produced CO₂ can reach excessive values after 60 min. In fact, ST strains can easily hydrolyze dissolved urea in milk mixtures with the final conversion in CO₂ and ammonia, although the use of mutant streptococci without this ureasic activity has been recently reported (Corrieu et al. 2005).

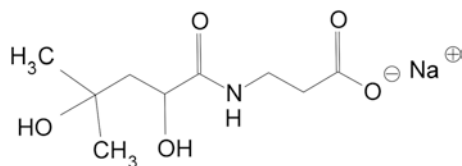
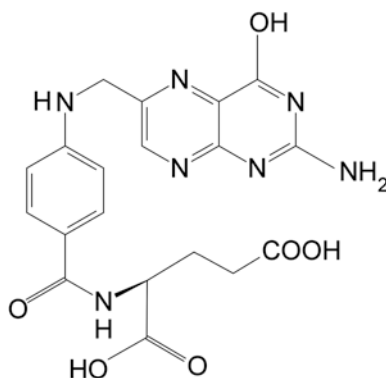


Fig. 2.1 Chemical structure of pantothenic acid, also named vitamin B₅, molecular formula: C₉H₁₆NNaO₅, MW: 241.216797 g mol⁻¹. BKchem version 0.13.0, 2009 (<http://bkchem.zirael.org/index.html>) has been used for drawing this structure

Fig. 2.2 Chemical structure of folic acid, also named vitamin B₉, molecular formula: C₁₉H₁₉N₇O₆, MW: 441.397491 g mol⁻¹. BKchem version 0.13.0, 2009 (<http://bkchem.zirael.org/index.html>) has been used for drawing this structure



Other interesting biochemical variations can occur with relation to the conversion of lactose in lactic acid—the main, declared and expected result of the homo-lactic fermentation—and other monosaccharides (Sect. 1.5). The amount of lactose in fresh milks is reported between 4.8 and 5.1 %; this content may be approximately 6 % when speaking of raw milk for yoghurt purposes. After evaporation and fermentation (Sect. 1.5), the chemical profile of detectable sugars should correspond to the following list: total carbohydrates, 4.8–5.2 %; unfermented lactose, 3.8–4.0 %; galactose, 1.0–1.2 %; glucose, negligible traces (Goodenough and Kleyn 1976).

LAB, homolactic fermentation and concomitant but ‘extraneous’ chemical reactions have other important effects on chemical profiles of vitamins, benzoic and orotic acids, bacteriocins, enzymes, peptides and amino acids.

It has been reported (Sect. 1.6) that several B vitamins—pantothenic acid, biotin, etc.—can decrease during the whole process of yoghurt productions. On the other side, the demolition of vitamins by microbial activity is not absolute but selective. For example, pantothenic acid—molecular formula: C₉H₁₆NNaO₅, molecular weight (MW): 241.216797 g mol⁻¹, Fig. 2.1—can diminish in yoghurts after three hours: 20 % of the original content in milks (Battistotti and Bottazzi 1998). Vitamin B₁₂ can also decrease in the same condition: the measured diminution appears approximately 16.9 %. On the other side, folic acid—molecular formula: C₁₉H₁₉N₇O₆, MW: 441.397491 g mol⁻¹, Fig. 2.2—appears to increase. The final amount of this chemical substance—3,805 µg/100 g—is 10.3 times higher than the original content in raw

milks (Battistotti and Bottazzi 1998). Other studies indicate 80 µg/L in comparison with the original content—40 µg/L—in the original milk (Chandan and Kilara 2013). Another vitamin, niacin, appears to slightly increase during time: +8.0 %.

The above-shown chemical profile of vitamins may be explained as follows: both LAB culture components require bioavailable pantothenic acid (Chandan and Kilara 2013). Additionally, LDB needs also a considerable amount of folic acid, but this molecule is abundantly synthesized by ST.

Actually, chemical profiles may always change during the storage of yoghurts. For example, the maximum production of folic acid is obtained at 42 °C during the incubation period. Subsequently, this molecule is reported to loss approximately 30 %, while vitamin B₁₂ continues to decrease: related contents appear undetectable after 5 days of storage (Chandan and Kilara 2013).

It should be noted also that there is a close relation between LAB and acetic acid bacteria with reference to the synthesis of vitamin B₁₂ in acid–alcoholic drinks such as *kefir*. In particular, the detection of this vitamin shows increased values when LAB and acetic acid bacteria are associated with the fluid food medium (Otles and Cagindi 2003).

With relation to organic acids, two of those normally present in the milk undergo significant transformations. Citric and uric acids do not seem normally metabolized by LAB-associated cultures. On the other side, orotic acid is reduced to 4.6 mg/100 mg ppm from the original amount of 8.2 mg/100 mg in the original milk, depending on productive conditions (Okonkwo and Kinsella 1969). Benzoic acid is present in traces in milk for yoghurt purposes: after the production, it can be found at 15–30 ppm and is associated with the metabolism of LAB cultures (Otles and Cagindi 2003). The precursor of benzoic acid, hippuric acid, can be converted depending on the activity of certain LAB strains (Tamime and Robinson 2007); in yoghurt, benzoic acid may be found between 35 and 60 ppm. Detectable contents appear approximately 30 ppm when LDB and *Lactobacillus acidophilus* are associated, while the same bacteriostatic substance is reported to be between 10 and 35 ppm when mesophilic cultures of *Lactococcus lactis* subsp. *lactis* are used (Battistotti and Bottazzi 1998).

The synthesis of above-shown acids is always considered with the concomitant production of substances with antibacterial activities: organic acids or hydrogen peroxide. With exclusive reference to yoghurts, the ‘bulgarican’ bacteriocin is synthesized by LDB. This substance is thermostable, active at low pH and is reported to show a wide spectrum of action towards both Gram-positive and Gram-negative micro-organisms (Otles and Cagindi 2003). ST can synthesize ‘thermophilin 13’, a peptide with MW of approximately 4,000 g mol⁻¹: it is thermostable and active at different pH values (Marciset et al. 1997). This LAB species produces also active bacteriocins towards moulds such as *Aspergillus* and *Rhizopus* genera.

The accumulation of beta-galactosidase, a significant probiotic, in fermented milks has to be remembered (Friend et al. 1983). The same thing can be affirmed when speaking of proteases and peptidases of microbial origin: the release of peptides and amino acids by hydrolysis is often correlated with nutritional and physiological benefits on the human health (Beshkova and Frengova 2012; Vasilijevic and Shah 2007).

On the basis of mentioned chemical profiles, it can be highlighted that the microbial metabolism of LAB cultures in yoghurts and other fermented milks determines the synthesis of following chemicals:

1. Compounds derived from the hydrolysis and the metabolism of lactose: CO₂, lactic acid, acetic acid, ethyl alcohol, formic acid, succinic acid, acetone, diacetyl, acetoin, galactose and polysaccharides
2. Compounds derived from the hydrolysis of proteins: peptides, amino acids, acetic aldehyde
3. Compounds derived from the utilization of urea: ammonia, formic acid, CO₂
4. Compounds derived from the use of organic acids: benzoic acid from hippuric acid.

Most detectable variations concerns:

- Modifications in the chemical profile of vitamins: increase in folic acid and niacin, decrease of pantothenic acid and B₁₂
- Changes in the amount of detectable nucleotides: increase of adenosine monophosphate, uridin monophosphate, guanine monophosphate and adenine dinucleotide
- Variations in the distribution of minerals: increase in ionic forms, destabilization of calcium caseinate–phosphate complexes
- Synthesis of proteins with recognized antibacterial activity: various bacteriocins
- Accumulation of enzymes: beta-galactosidase, proteases and peptidases.

Naturally, the strong increase of LAB microbial population should be also remembered: the presence of hundreds of millions per gram of LAB and other probiotic micro-organisms can notably determine the protection of the produced yoghurt (and intermediate milky mixtures during the fermentative process) against undesired life forms.

2.2 Compositional Features of Yoghurts

The qualitative and quantitative profiles of nutrients in the milk mixture are partially modified by fermentative processes: negligible modifications may be observed during storage at 4 °C because the enzymatic activity of the active microflora is slowed down or even inhibited. Actually, only a significant reduction of vitamin B₁₂ and pantothenic acid amounts—60 and 30 %, respectively—and a negligible decrease of lactose and galactose have been observed. By a general viewpoint, it can be affirmed that yoghurts show a compositional situation with more abundant nutrients in comparison with the original milk, excluding lactose. Anyway, the addition of milk proteins is crucial.

Table 2.1 shows the approximate composition of commercial yoghurts in the modern industry and original data for several milks. The composition of dessert or enriched yoghurts is determined also by the nature and the quantity of non-milk ingredients. For example, glucose is particularly variable—0.0 % in white products, 0.7–3.2 % in fruit yoghurts—depending on the type of product and the peculiar manufacturer. The composition of carbohydrates concerns (De Noni et al. 1998):

Table 2.1 Average composition and energetic percentage value of cow's milk and some types of yoghurt (Agroscope Composition 2007; Agricultural Research Service 2013)

	Milk		Yoghurt		
	Full cream	Skimmed	Full cream white	Skimmed white	Full cream fruit
Dry residual, %	12.5	9.5	15	14	22
Protein, %	3.3	3.4	4.3	6.7	4.3
Fat, %	3.4	0.2	4.1	0.2	4.5
Total sugar, %	4.8	5.0	4.6	4.9	15.7
Lactic acid, %	0.003	0.003	0.5	0.5	0.5
Energetic value, kcal/100 g	62	35	66	47	102

- Glucose
- Lactose (2.6–3.6 % in white products, 2.6–3.8 % in fruit yoghurts)
- Added sugars (sucrose and fructose: up to 8.9 and 6.7 %, respectively, in sugary white products)
- Lactulose, a disaccharide consisting of galactose and fructose, in form of concentrated syrups or high-purity crystals, obtained by means of the epimerization of lactose during the pasteurization process (Andrews 1984).

Normally, lactulose—also named 4-O- β -D-galactopyranosyl-D-fructose—is found between 0.02 and 0.07 % in sterilized milks depending on applied time/temperature cycles. On the other hand, lactulose is well known as a good sweetener: different synthetic processes are available at present with relation to the production of this substance (Aider and Halleux 2007). Theoretically, lactulose should be obtained by means of the isomerization reaction of lactose in alkaline media. One of favourite mechanisms, the reaction of Lobry de Bruyn–van Ekenstein, converts the glucose part of the original lactose in fructose by means of the formation of the enolic intermediate shape of lactose and epilactose in alkaline media such as calcium hydroxide and potassium hydroxide (Aider and Halleux 2007). However, several methods have been developed with the aim of enhancing the production: different catalyzers and technological solutions, such as the purification and the demineralization by anion and cation exchange resins have been recently proposed (Aider and Halleux 2007; Carobbi and Innocenti 1990; Carobbi et al. 1985).

The chemical profile of organic acids should be studied and discussed. With relation to lactic acid, the related content in all commercially available yoghurts appears greater than 0.8 %. This amount corresponds to the minimum quantity for this type of fermented milk. Normally, the maximum content should not exceed 1.3 %: otherwise, products might exhibit particularly acidic tastes with some risk of consumeristic unacceptability. Other milk-related constituents may be considered important on the nutritional level even if their amount is detected in limited quantities.

For instance, formic acid—produced by ST—is recognized capable of stimulating the multiplication of lactobacilli. Small amounts of this acid—less than 40 mg/kg—are also produced after the caramelization of lactose because of thermal treatments such as pasteurization (De Noni et al. 1998; Kroh 1994).

Flavours can be very important when speaking of yoghurts and the correlated commercial success. Basically, aroma is affected by the compulsory presence of minor constituents without fermentative origin: actually, these chemicals seem to be produced by reactions promoted by thermal processes in the same way of formic acid.

Generally, flavours are determined by the qualitative and quantitative presence of carboxylic acids. These substances may be obtained from fats lactones, and their concentration is crucial with concern to more or less pleasant and intense tastes (Cheng 2010; De Noni et al. 1998; Gaafar 1992; Laye et al. 1993):

- Acetaldehyde (2–40 mg/kg)
- Acetoin (2–6 mg/kg)
- Acetone (1–4 mg/kg)
- Diacetyl (0.5–1.0 mg/kg).

Finally, other minor substances may be cited: total free fatty acids, octanoic acid, 9-decenoic acid, tetradecanoic acid, octanol, ethyl acetate, etc., depending on the peculiar aroma and the desired result. On the other hand, off-flavours with unacceptable or questionable results appear to be correlated with the presence of tyrosine, acetic acid, 3-methyl butanol, ethyl acetate, etc. (Cheng 2010).

In fact, the process of thermal caramelization and the so-called Maillard reaction—in which lactose is involved—can be cause of the production of these compounds. In addition, the overall chemical composition remains unmodified during storage while microbiological profiles appear to be subjected to major modifications. Actually, the total number of LAB in yoghurts is comprised between 10^8 and 10^9 colony-forming units (CFU)/g during the production. Subsequently, a notable reduction is observed after 30 days at 4 °C until a ‘final’ 10^7 CFU/g microbial count, although some discordance may be found in the scientific literature depending on storage temperatures and other factors (Birolo et al. 2000).

Higher storage temperatures cause generally more rapid decreases of microbial counts until to 5×10^6 CFU/g before of the end of shelf life. It may be supposed that this level is critical for LAB counts in yoghurts. Anyway, the presence of relatively high numbers of vital LAB cultures depends also on the respect of low storage conditions during the declared shelf life. The same thing can be easily affirmed when speaking of healthy properties and sensory—or chemical—profiles of yoghurts.

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