Food Chemistry 119 (2010) 829–833

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Rapid and accurate determination of D- and L-lactate, lactose and galactose by enzymatic reactions coupled to formation of a fluorochromophore: Applications in food quality control

Fira Shapiro, Nissim Silanikove *

Biology of Lactation Laboratory, Institute of Animal Science, Agricultural Research Organization, Bet Dagan 50250, Israel

ARTICLE INFO

Article history:
Received 17 February 2009
Received in revised form 24 May 2009
Accepted 15 July 2009

Keywords:
Lactate
Lactose
Galactose
Determination
Milk
Yogurt
Beer
Wine

ABSTRACT

A fluorometric-coupled reaction for the accurate and rapid determination D- and L-lactate and lactose, galactose in foods is presented. The method was found useful for an accurate determination of these metabolites in heterogeneous, opaque and colourful foods without pretreatments. Example for the determination of lactose, galactose, D- and L-lactate in milk, and yogurts and D- and L-lactate in milk, wine and beer is provided. Unexpectedly, we found that the composition of some commercial bio-yogurts produced in Israel is not consistent with the classical definition of yogurts. Thus, this method offers rapid and accurate methodology, which should be particularly valuable in food quality control.

1. Introduction

Lactic acid (lactate) is generated from glucose in eukaryote and prokaryotic organisms (Berg, Tymoczko, & Stryer, 2002). In mammals, L (+)-lactate (L-lactate) is the major lactate stereoisomer formed in intermediary metabolism and is present in blood. D (−)-Lactate (D-lactate) is also present but only at about 1–5% of the total lactate concentration. D-Lactate found in human physiologic fluids originates from bacterial production in the intestinal tract (Bongaerts et al., 1995; Ewaschuk, Naylor, & Gordon, 2005).

Bacteria can produce both D- and L-lactate and a group of bacteria commonly known as lactic acid bacteria (LAB) produce lactate as the major metabolic end product of carbohydrate fermentation (Salminen, von Wright, & Ouwehand, 2004). Yogurt is a dairy product produced by LAB fermentation of milk lactose to produce lactic acid. Classically, to be named yogurt, the product should be made with the bacterial species' *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* (Tamime & Deeth, 1980). These bacteria typically form a mixture of L-lactate and D-lactate, with significant contribution of D-lactate to total lactate production. Modern dairy products including yogurts frequently contain added live cultures of LAB such as *L. acidophilus*, *L. casei* and *Bifid bacterium* species, which are commonly known as probiotics (i.e. their consumption confers benefit to the host). The above-mentioned bacteria produce mostly L-lactate as the principal end product (Sarkar, 2008).

Lactose is a disaccharide, mainly found in milk and milk products. D-Galactose is a monosaccharide that can be found as a degradation product of carbohydrates such as lactose and β-galactosidic oligosaccharides in foods; thus both lactose and galactose are found in biological fluids (Berg et al., 2002).

Virtually, all varieties of the yogurts available nowadays in stores in Israel and other countries do not include in their labeling information on lactose, galactose and lactate contents as well as the distribution of lactate among the D- and L-subtypes. Thus, consumers cannot know to which extend yogurts bought in modern markets comply with its original definition. Fermentative products such as wine, pickled vegetables and cured meats and fish produce a mixture of D- and L-lactate. As D-lactate is a specific indicator of bacteria fermentation, it serves as a common indicator for the freshness quality of milk, meat and fruit juice (Soga & Ross, 1997). In the wine industry, the content of D-lactate can indicate wine spoilage by LAB.

Analysis of lactose galactose and lactate involves classical NADH-linked reactions (Berg et al., 2002; Shapiro, Shamay, & Silanikove, 2002). Thus, the oxidation of these metabolites may be estimated from the stoichiometrically linked increased absorption of NADH. A common alternative consists of measuring NADH disappearance by coupling its oxidation to NAD with enzyme such as diaphorase.

* Corresponding author. Tel.: +972 8 9484436; fax: +972 8 9475075.
E-mail address: nsilanik@agri.huji.ac.il (N. Silanikove).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.07.029

However, applying monochromatic absorbance photometry for analysis of foods is frequently difficult and problematic (Nielsen, 2003) because many of them are heterogeneous and compound substances: (i) that contain fat droplets of varying size that scatters light in an unpredictable way (e.g., milk and yogurt), (ii) that is opaque and colloidal solution of proteins (e.g., milk and yogurt) that scatter and absorb light, (iii) that contain intense colorant (e.g., red wine) that interfere with the monochromatic absorbance. Overcoming these problems necessitate the use of various pretreatments procedures of samples to minimize the above described problems. However, in many cases such pretreatments only partially resolve the problem, in addition to the fact that many of these procedures are cumbersome and time consuming.

In the present report, we present a fluorometric-coupled reaction for the accurate and rapid determination of lactose, galactose, D- and L-lactate in foods. Thus, this method is useful for their accurate determination in heterogeneous, opaque and colourful foods without pretreatments. Example for the determination of lactose, galactose, D- and L- lactate in milk, and yogurts and D- and L-lactate in wine and beer is provided.

2. Materials and methods

2.1. Assay principle

D- and L-lactate were converted to D- and L- pyruvate with D-lactic dehydrogenase (D-LDH) and L-lactic dehydrogenase (L-LDH), respectively; thereby simultaneously reducing NAD^+ to NADH + H^+. The primary reaction in each case was coupled to conversion of NADH + H^+ to NAD^+ with diaphorase coupled with converting the non-fluorescent resazurin to the highly fluorescent substance resorufin (Dejong & Woodlief, 1977):

$$\text{L-lactate(or D-lactate)} + NAD^+ \xrightarrow{\text{L-LDH (or D-LDH)}} \text{L-(or D-) pyruvate} + NADH + H^+ \quad (1)$$

$$NADH + H^+ + \xrightarrow{\text{resazurin diaphorase}} NAD^+ + \text{resorufin} \quad (2)$$

Lactose and β-D-galactose determinations steps composed first the hydrolysis of lactose with β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) to β-D-gal and D-glucose followed by the conversion of gal to D-galactono-γ-lactone and NADH with β-galactose dehydrogenase. For the direct determination of galactose, the procedure started from Reaction (4), which enables the separate determination of lactose and galactose in given samples by Reaction (2):

$$\text{lactose} + H_2O \xrightarrow{\text{β-D-galactosidase}} \text{D-glucose} + \text{β-D-galactose} \quad (3)$$

$$\text{β-D-galactose} + NAD^+ \xrightarrow{\text{gal-DH}} \text{D-galactono-γ-lactone} + NADH + H \quad (4)$$

2.2. Foods

Milk was sampled from the commingled milk of cows with bacteriaL-free udders as described before (Leitner, Krifucks, Merin, Lavi, & Silanikove, 2006). The milk was stored on ice and analysed within the same day. An Israeli-produced cabernet sauvignon wine and white beer with an international trade name that was produced with a license in Israel were obtained from a local supermarket. Five types of white plain yogurts, four types of bio-yogurts (i.e. yogurts with added probiotic bacteria), three types of liquid bio-yogurts (for drink) were bought from local super market to represent three different batches (i.e., expiry days). The information regarding the producers and product labeling of the dairy products is given in supplementary information. To each of the foods, two levels of 1 and 10 μM of the tested compounds was added, for a recovery test.

2.3. Chemicals

The following chemicals were obtained from Sigma (Rehovot, Israel): D-lactic acid, D-lactic dehydrogenase from *Lactobacillus leichmannii* 1.5 ku/ml, L-lactic acid, L-lactic dehydrogenase from rabbit muscle 12 ku/ml, diaphorase from *Clostridium Kluyvera* 100 u/ml, D (+) galactose (), galactose dehydrogenase from *Pseudomonas fluorescens* 100 u/ml, β-D-galactosidase from *Escherichia coli* 1500 u/ml, β-D-lactose, β-NAD^+, potassium chloride, resazurin, Trisma base and Triton X-100.

2.4. Reagents

2.4.1. Reagents for the D- and L-lactate assay procedures

The following reaction mixtures were prepared once a week for D-, or L-lactate determination: All the components were dissolved in Tris/HCl buffer 75 mM pH 8.9, which included: KCl 100 mM, Triton X-100 – 0.0004%, β –NAD – 1 mM for L-lactic acid or 5 mM for D-lactic acid, Resazurin – 48 μM, diaphorase – 1 u/ml, L-lactic dehydrogenase 18 u/ml, or D-lactic dehydrogenase – 7 u/ml.

2.4.2. Reagents for lactose and galactose determination

The following reaction mixtures were prepared once a week for lactose, or galactose determination: For reaction mixture A all the components were dissolved in Tris/HCl buffer 75 mM pH 8.9, which included: KCl 100 mM, Triton X-100 – 0.0004%, β-NAD – 1 mM, resazurin – 48 μM, diaphorase – 1 u/ml, Galactose dehydrogenase – 0.15 u/ml. For reaction mixture B to 1 ml of the reagent A was added 3 μL of beta-galactosidase – 4.5 u/ml.

2.5. Standard curve

Standards were prepared by serial dilutions of stock solution of the test substances in distilled water to yield concentrations of 1, 5, 10, 25, 50, 100, 250, 500 and 1000 μM.

The standard curves of either D-, or L-lactate were also assayed in the presence of 100 μM of the counter stereoisomer in all the standard points.

2.6. Sample preparation

Skim milk (Silanikove & Shapiro, 2007) was analysed without further processing and dilution. Beer was analysed without further processing and dilution. Wine was analysed without further processing and was diluted 1–5 or 10 folds with 75 mM Tris/HCl buffer, pH 8.9. Yogurts were processed as follows: 1 g of sample, or sample spiked with D-, or L-lactate were homogenised for about 10 min in 10 ml of 75 mM Tris/HCl buffer, pH 8.9, which was diluted 100 folds by mixing 100 μL of the homogenate with 900 of the 75 mM Tris/HCl buffer, pH 8.9.

2.7. Reaction procedure

2.7.1. Reaction procedures for D- and L-lactate Assays

The reaction was initiated in 96-well plate by the addition of 10 μL of standard or sample and 100 μL of the reaction mix to the wells. The mixture was incubated for 30 min at room temperature and the plate was red in a fluorimeter (BioTek Instruments, USA), under E_x/E_m = 540/590.

2.7.2. Reaction procedures for lactose and galactose assay

The reaction was initiated in 96-well by the addition of 10 μl of standard or sample and 100 μl of reaction mixture B for lactose assay or 100 μl of reaction mixture A for lactose and galactose assays. The mixtures were incubated for 4 h at room temperature and the plate was red in a fluorimeter (BioTek Instruments, USA) under E_x/E_m = 540/590. Lactose, corrected for galactose content and galactose concentration were determined from the respective standard curves.

2.8. Calculation, validation parameters and statistical treatment

Concentration of the analysed metabolites was derived from the linear regression analysis of the calibration curves. For the determination of linearity, regression lines were calculated as $y = a + bx$, where x was concentration, and y the response. Nine concentration points in triplicate were used to prepare the calibration curves as described above. For each compound, the coefficients of determination (R^2) were calculated and the linearity was analysed based on the relative standard deviation (R.S.D.) values for the corresponding response factors. Limit of detection for each metabolite were calculated as $y = a + 3$ S.D (of a) to concentration. Assay repeatability of the method was analysed by calculating the R.S.D. values of three replications of the standard curve analysis. Day-to-day repeatability was estimated by calculating the R.S.D. derived from analysis of standards curve over three consecutive days. Recovery was determined using an added external standard. The samples were spiked at two levels (1 and 10 μM), each in triplicate with known quantities of the test compounds and the percentage of recovery was calculated. The percentage of recovery rate for the tested compounds was established from the experimental response values [(blank + standard) − blank] obtained according to the calibration curves and the real concentration of the standard added. Each of the foods was analysed in triplicate and the repeatability was calculated from the R.S.D. With the fermented dairy products, the procedure was repeated for three different batches and the batch-to-batch variability was calculated from the R.S.D.

3. 3 Results and discussion

3.1. Performance of the standard curves

Linearity of the calibration curves (R^2 = 0.9996–0.9999), and estimation of the accuracy (R.S.D. of the estimate of 1–3%), repeatability (day-to-day R.S.D of 1–3%) and sensitivity (minimal detection limit of 4–5 μM) of the test substances are reported in Table 1. The within day R.S.D and day-to-day R.S.D. were essentially similar indicating that the described methods provided consistent results over time. The limit of detection of D- and L-lactate by the present analysis was ~40 folds more sensitive than that of currently available commercial enzymic colorimetric method for the determination of D-lactate or L-lactate (Bongaerts et al., 1995; Boeringer uv-kit) in foods, with typical limit of detection at the range of ~200 μM. The sensitivity of the present methods is also better than in the above-mentioned commercial kits. The limit of detection of lactose and galactose was similar to that found with colorimetric methods (Shapiro et al., 2002), but the recovery in fermented dairy products is expected to be superior for reasons discussed in the introduction.

A similar limit of detection and sensitivity was found with a method in which the quantification of D-lactate is based on direct recording of NADH fluorescent emission (Haschke-Becher, Baumgartner, & Bachmann, 2000). This method was developed to detect D-lactate in human urine for diagnosing metabolic acidosis caused by pathogenic conditions such as the short bowel syndrome. However, with this method, the presence of 1 mM L-lactate was associated with a bias ~ + 8% in D-lactate concentration and the presence of 10 mM L-lactate with a bias of + 57%. Thus, the present methods in which the cross interference between the two lactate isomers was practically zero offers significant advantages in defining reliable reference values for metabolic acidosis.

3.2. Concentration of the tested substances in milk, wine and beer

The concentrations of the test substances in milk wine and beer are reported in Table 2.

The recovery of the metabolites added to milk, beer and wine at the indicated two levels ranged 97–102%. The concentration of the tested substance is consistent with previous reports in milk (Davis et al., 2004; Lindmark-Mansson, Branning, Alden, & Paulsson, 2006), wine (Palleschi, Volpe, Compagnone, La Notte, & Esti, 1994) and beer (Mancini, Miniati, & Montanari, 2000). Milk D-lactate probably diffuses from the systemic fluids, and as in humans, it originates from microbial activity in the digestive tract. L-lactate is the main stereoisomer in milk as in the systemic fluids; its concentration has been shown to increase under inflammatory conditions in the mammary gland (Davis et al., 2004; Lindmark-Mansson et al., 2006), though the reason for that is not clear.

An increase in the concentration of D-lactate to above 5.5 mM in wine (Palleschi et al., 1994) and 1 mM in beer (Mancini et al., 2000) causes an irreversible transformation to bade sour taste, which give reasons for developing reliable methodology for determination D-lactate during food processing (Soga & Ross, 1997). The present data show that the sensitivity of the test is sufficient to detect

Table 2
Concentration, and pooled intra-day and day-to-day repeatability of the test substances in milk, wine and beer.

Substance	Concentration	R.S.D (%)
Milk		
L-Lactate (μM)	260	16
D-Lactate (μM)	7.71	4
Lactose (mM)	161	7
Galactose (mM)	1.01	5
Wine[a]		
L-Lactate (mM)	7.02	0.3
D-Lactate (mM)	4.03	0.5
Beer[a]		
L-Lactate (mM)	1.04	3
D-Lactate (mM)	0.75	1

[a] Single commercial source, see materials and methods.

Table 1
Regression equations of the calibration curves and analysis of linearity, accuracy, repeatability and sensitivity.

Substance	Range (μM)	Regression equation	R^2	R.S.D. of the estimate (%)	Day-to-day R.S.D (%)	Limit of detection (μM)
L-Lactate	1–1000	$Y = 19.544x + 1085.3$	0.9996	3	3	5
D-Lactate	1–1000	$Y = 10.689x + 1050.6$	0.9997	2	2	4
Lactose	1–1000	$Y = 74.376x + 1895$	0.9999	1	1	5
Galactose	1–1000	$Y = 251.34x + 17{,}796$	0.9996	2	2	5

R.S.D. – relative standard deviation.

such an undesirable changes; these and the high precision, as reflected in the high recovery and low R.S.D., indicate that this method is suitable for quality control of various foods.

3.3. Concentration of the tested substances in commercial yogurts produced in Israel

The recovery D- and L-lactate added to the tested yogurts at the two indicated levels ranged 96–103%. The range of concentration of D- and L-lactate in the plain yogurts (Table 3) is consisted with previous reports (Tamime & Deeth, 1980; Sarkar, 2008). The reduction in lactose from a typical concentration of 161 mM (5.5%) to 86–100 mM (3–3.5%) in the plain yogurts, and 105 mM (3.6%) in liquid bio-yogurt J is typical to yogurts products and reflects its fermentation by the manufacturing cultures (Tamime & Deeth, 1980).

The preponderance of L-lactate over D-lactate in most of the plain yogurts is also consistent with previous reports and may be related to the predominance of *S. thermophilus* (an L-lactate producing LAB) over *L. delbrueckii* subsp. *Bulgaricus* (a D-lactate producing LAB) activity for most of the yogurt culturing process (Sarkar, 2008). The predominance of L-lactate is nutritionally advantageous, because D-lactate has no physiological significance in cell metabolism; hence its nutritional value is limited to improvement of casein digestion in the intestine. Concentration of D-lactate in yogurts could be reduced by increasing the proportion of *S. thermophilus* and introduction *Lactobacillus casei* subsp. *casei* into the culturing process (Sarkar, 2008). The low concentration of D-lactate in the liquid bio-yogurt J (Table 3) is likely an example for such a manipulation. This product has also lower level of L-lactate in comparison to plain yogurts.

In the other bio-yogurts, except product G, lactose concentration was approximately 130 mM (4.4%), which is lower than in the plain yogurts and bio-yogurt J. D-lactate levels in the bio- yogurts products (except product J) was on the low mM range, which is reflected by markedly lower D-lactate to L-lactate ratio in comparison to the plain yogurts (Table 3). Lactose serves as the source for the lactate, which explains the markedly lower D-lactate to lactose ratio in these products in comparison to plain yogurts and bio-yogurt J. In product G, the manufacturer either used lactose as a source of carbohydrate, or no fermentation of lactose occurred (Table 3), which is reflected by lactose concentration that is higher than in milk; thus, this product appear to be particularly unsuitable to lactose-intolerant consumers. High levels of lactose were also reported in some yogurts produced in Brazil (Batista et al., 2008). Thus, finding high lactose levels in a product produced by an international brand (product G) and in more than one country, suggests that marketing yogurts that are unsuitable for lactose-intolerant consumers might be a problem of worldwide concern.

In the course of yogurt culturing, the concentration of galactose increase considerably from approximately 0.2% in milk to 0.9–3.1% in the final product (Toba, Watanabe, & Adachi, 1982; Lamoureux, Roy, & Gauthier, 2002; Sarkar, 2008), which is consistent with values in the range of 1% found in the plain yogurts in the present study (Table 3). This reflects mainly the formation of oligosaccharides, including, galactooligosaccharides, during culturing. Thus, galactose can serve as a raw indirect criterion for the mass of LAB. In the bio-yogurts, expect product J, galactose concentration was in the range of 30–40 mM (0.5–0.7%; Table 3). The lower concentration of galactose in the bio-yogurts in comparison to the plain yogurts, suggest therefore that the contribution of fermentative process either from yogurts bacteria or from the added probiotics cultures to microbial mass in the final yogurts was up to 50% lower than in the plain yogurts. Oligosaccharides are considered as part of the prebiotic that are produced by LAB; thus, most of the present tested bio-yogurts (except product J) appear to be inferior in comparison to the plain yogurts in this respect.

Fermentation is inherently a variable phenomenon. Thus, the typically lower batch-to-batch variability in L-lactate concentration in the bio-yogurts products in comparison to the plain yogurts (6–12% vs. 10–40%; Table 3), suggest that natural fermentation was much lower in these products in comparison to plain yogurts.

Adding of modern probiotics cultures into yogurt cultures, such as those added to the bio-yogurts analysed in this study, may affect negatively the natural fermentation process of yogurts (Sarkar, 2008). Though we are not claiming it, or can prove it, one cannot ignore the possibility that products with similar composition to the tested bio-yogurts (except product J) can be to a large extent be simply made by adding commercially available L-lactate and probiotic cultures to milk-based delicates. This is particularly so with product G in which no evidence for the use of lactose for fermentation exist. Based on the present results, in order that consumers will be sure that commercially available yogurts products comply with the original definition of yogurt, it would be advantageous if more definitive criteria for qualification of yogurts, particularly to modern bio-yogurts, would be set. Based on the present result, criteria that would include minimal D to L-lactate ratio, minimal D-lactate or total lactate to lactose ratio, and minimal galactose concentration would increase the confidence of consumers regarding the probiotics and prebiotic qualities of yogurt products. The methodology presented in this manuscript allows for fast and accurate determination of these criteria and thus, may be found useful for researchers, producers and quality control agencies.

Table 3
Concentration (Avg, mM) intra-day (I, %) and batch-to-batch (B, %) repeatability of the test substances in commercial yogurts.

Product	L-Lactate			D-Lactate			D/L ratio	Lactose			D-l/L ratio	Galactose		
	Avg.	I	B	Avg.	I	B		Avg.	I	B		Avg.	I	B
Plain yogurts														
A	75.1	1.1	10.3	122.2	1.0	4.8	1.64	89.1	5.6	6.7	0.84	55.1	2.3	4.6
B	116.1	1.3	28.2	73.0	1.8	6.7	0.63	92.1	6.1	6.9	0.79	60.1	3.2	7.7
C	83.1	2.4	25.1	54.2	2.6	23.1	0.65	98.1	6.3	7.1	0.55	47.3	3.5	10.1
D	79.2	1.3	34.0	29.0	1.5	27.2	0.37	100.2	7.7	8.1	0.29	45.5	3.1	17.2
E	87.1	1.3	26.4	90.1	1.7	15.4	1.01	85.5	5.9	6.5	1.05	60.3	3.3	19.6
Bio-yogurts														
F	96.2	2.7	8.5	2.0	11.5	47.2	0.02	128.5	3.1	5.4	0.02	33.2	3.8	6.6
G	123.2	4.1	7.5	5.1	6.8	7.4	0.04	173.0	4.4	7.7	0.03	29.2	2.7	8.8
H	101.5	2.2	6.6	4.0	1.8	15.1	0.04	129.3	4.5	6.6	0.03	37.0	4/2	5.5
I	123.0	4.5	12.0	3.2	7.0	24.1	0.03	130.3	5.2	6.6	0.02	41.0	3.6	5.9
Bio-yogurts in drinking form														
J	25.0	4.1	6.2	30.1	1.5	7.7	1.20	105.2	2.8	4.4	0.29	48.0	4.2	5.6
K	84.1	3.6	5.6	2.0	6.5	9.2	0.02	125.0	2.7	4.4	0.02	29.3	3.8	4.5
L	66.2	2.1	4.9	3.1	7.0	8.8	0.05	128.2	3.1	5.2	0.02	32.1	3.0	5.9

Avg. = average; I = inter assay R.S.D in%, B = batch-to-batch R.S.D in%; D/L ratio = D-lactate/L-lactate ratio; D-l/l ratio = D-lactate/lactose ratio.

Appendix

Information appearing on the labels of the tested commercial yogurts.

Trade name	Int. trade name	Producer	Code	Energy (cal)	Protein (g)	Carbohydrates (g)	Fat (g)	Calcium (mg)	Sodium	Specific editions	Bacterial edition
Tnuva	–	Tnuva	A	68	3.3	3.5	4.5	120	50	Milk proteins	Not mentioned
Danoba	–	Tnuva	B	71	3.6	5.1	4	100	50	Milk proteins, modified starch	Unspecified acidophilus
Natural yogurt	–	Tara	C	58	4.3	6.7	1.5	150	70	Milk powder	Unspecified Bio
Prili strawberry flavoured	–	Tnuva	D	96	2.4	18.2	1.5	81	60	Carbohydrate	Not mentioned
Yogurt of old time	–	Shomron	E	64	4.3	2.6	4.3	157	40	Made of Sheep milk	Not mentioned
Natural bio	DANONE	Strauss	F	63	4.6	4.3	3	130	90	Milk protein, Ca (E341iii)	Unspecified Bifidus+acidophilus
Bio	Muller	Tara	G	108	4	18	2.2	170	60	Ca (E341iii), sugar	Bifidobacterium, (BB-12)
Activia	DANONE	Strauss	H	44	3.2	4.4	1.5	130	80	Milk protein, dietary fibre, Ca (E341iii)	Bifidus Essensis
Diet bio white	Yoplait	Tnuva	I	37	5.5	3.7	0.1	130	55	Dietary fibre, Ca, (E341iii) Pectin (E406)	*Lactobacillus rhamnosus* GG (LGG)
Actimel (for drink)	DANONE	Strauss	J	90	3	15.5	1.8	100	55	Sugar, dextrose	Unspecified yogurt bacteria + *L. casei*
Activia (for drink)	DANONE	Strauss	K	44	3.2	4.4	1.5	130	80	Milk protein, dietary fibre, Ca(E341iii)	Bifidus Essensis
You (for drink)	–	Tara	L	93	3.1	16.9	1.5	110	45	Sugar, other editions	Unspecified Bio

Tnuva – Head office: Rav Mecher Bldg., Glilot Junction 47210 Ramat Hasharon, Israel.
Strauss Group Ltd. – Arlozorov 84, Ramat Gan 52505, Israel.
Tara Ltd – Yigal Alon St 115, Tel Aviv, Israel.
Shomron Dairy Ltd – Dagan St 19, Binyamina, Israel.

References

Batista, A. L. D., Silva, R., Cruz, A. G., Faria, J. A. F., Moura, M. R. L., & Carvalho, L. M. J. (2008). Lactose intolerance possibility of ingesting fermented dairy products. *Milchwissenschaft/Milk Science International, 63*, 364–366.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistry* (pp. 465–484, 498–501) (5th ed.). WH Freeman and Company.

Bongaerts, G., Tolboom, J., Naber, T., Bakkeren, J., Severijnen, R., & Willems, H. (1995). D-Lactic acidemia and aciduria in pediatric and adult patient with short bowel. *Clinical Chemistry, 41*, 107–110.

Davis, S. R., Farr, V. C., Prosser, C. G., Nichols, G. D., Turner, S-A., Lee, J., et al. (2004). Milk L-lactate concentration is increased during mastitis. *Journal of Dairy Research, 71*, 175–181.

Dejong, D. W., & Woodlief, W. G. (1977). Fluorometric assay of tobacco leaf dehydrogenases with resazurin. *Biochimica et Biophysica Acta, 484*, 249–259.

Ewaschuk, J. B., Naylor, J. M., & Gordon, A. Z. (2005). D-Lactate in human and ruminant metabolism. *Journal of Nutrition, 135*, 1619–1625.

Haschke-Becher, E., Baumgartner, M., & Bachmann, C. (2000). Assay of D-lactate in urine of infants and children with reference values taking into account data below detection limit. *Clinica Chimica Acta, 298*, 99–109.

Lamoureux, L., Roy, D., & Gauthier, S. F. (2002). Production of oligosaccharides in yogurt containing bifidobacteria and yogurt cultures. *Journal of Dairy Science, 85*, 1058–1069.

Leitner, G., Krifucks, O., Merin, U., Lavi, Y., & Silanikove, N. (2006). Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *International Dairy Journal, 16*, 648–654.

Lindmark-Mansson, H., Branning, C., Alden, G., & Paulsson, M. (2006). Relationship between somatic cell count, individual leukocyte populations and milk components in bovine udder quarter milk. *International Dairy Journal, 16*, 717–727.

Mancini, F., Miniati, E., & Montanari, L. (2000). Determination of organic acid anions in Italian beers by a new HPLC method. *Italian Journal of Food Science, 12*, 443–450.

Nielsen, S. S. (2003). *Food analysis*. Birkhäuser. ISBN 0306474956, 9780306474958.

Palleschi, G., Volpe, G., Compagnone, D., La Notte, E., & Esti, M. (1994). Bioelectrochemical determination of lactic and malic acids in wine. *Talanta, 41*, 917–923.

Salminen, S., von Wright, A., & Ouwehand, A. C. (2004). *Lactic acid bacteria: Microbiological and functional aspects* (3rd ed.). New York: Marcel Dekker, Inc.. ISBN 0-8247-5332-1.

Sarkar, S. (2008). Effect of probiotics on biotechnological characteristics of yoghurt. *British Food Journal, 110*, 717–740.

Shapiro, F., Shamay, A., & Silanikove, N. (2002). Determination of lactose and -galactose using thio-NAD+ instead of NAD+. *International Dairy Journal, 12*, 667–669.

Silanikove, N., & Shapiro, F. (2007). Distribution of xanthine oxidase and xanthine dehydrogenase activity in bovine milk: Physiological and technological implications. *International Dairy Journal, 17*, 1184–1194.

Soga, T., & Ross, G. A. (1997). Capillary electrophoretic determination of inorganic and organic anions using 2, 6-pyrydinedicarboxylic acid: Effect of electrolyte's complexing ability. *Journal of Chromatography A, 767*, 223–230.

Tamime, A. Y., & Deeth, H. C. (1980). Yogurt: Technology and biochemistry. *Journal of Food Protection, 44*, 78–86.

Toba, T., Watanabe, A., & Adachi, S. (1982). Allolactose and 6-*O*-/3-D-alactopyranosyl-D-Galactose in Commercial Yogurt. *Journal of Dairy Science, 65*, 702–706.