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Ocena przydatności jogurtów produkowanych w Polsce w diecie dzieci i młodzieży z hypolaktazją

Assessment of suitability of yoghurts produced in Poland for the diet of children and teenagers suffering from hypolactasia

Streszczenie

Wstęp. Istotnym elementem leczenia hypolaktazji i nietolerancji laktozy wśród dzieci i młodzieży jest ograniczenie spożycia laktozy. Jednakże w wielu przypadkach zamiast wyeliminowanego z diety mleka można wprowadzać produkty mleczne z całkowicie lub częściowo odfermentowaną laktozą.

Cel. Celem badań była ocena głównych determinantów podaży w diecie osób z hypolaktazją mlecznych napojów fermentowanych na przykładzie jogurtów. Ocenę dokonano w oparciu o stopień hydrolizy laktozy w jogurtach, aktywność enzymu laktazy oraz liczbę termofilnych bakterii fermentacji mlekowej jako źródła laktazy.

Materiał i metody. Materiałem do badań było 12 komercyjnych jogurtów naturalnych produkowanych w Polsce. Czas przydatności ich do spożycia wynosił zgodnie z deklaracjami producentów od 18 do 25 dni, a badano je 12, 7 i 0 dni przed upływem tego terminu.

Wyniki. Zawartość laktozy w badanych jogurtach wyniosła od 2,9 to 6,9g·100g^{-1}. Jej ilość w trakcie przechowywania zmniejszyła się o ponad 33%. Aktywność laktazy mieściła się w granicach od 0,23 to 0,92 μkat·100g^{-1}. Początkowa liczba bakterii z rodzaju *Lactobacillus* w jogurtach wyniosła od 7,8×10^5 to 6,9×10^8 CFU·g^{-1}, a z rodzaju *Streptococcus* 1,2×10^8 CFU·g^{-1}. Czas przechowywania spowodował ich obniżanie odpowiednio o 8,9% i 7,9%. Największą zależność uzyskano pomiędzy aktywnością laktazy a liczbą bakterii z rodzaju *Lactobacillus*. Liczba bakterii z rodzaju *Lactobacillus* w 50% przypadków była równa lub większa niż 10^7 CFU·g^{-1}, a w 41,6% przypadków mniejsza niż 10^5 CFU·g^{-1}, czemu odpowiadała bardzo niska aktywność laktazy (0,23-0,37 μkat·100g^{-1}).

Słowa kluczowe: jogurt, hypolaktazja, β-D-galaktozydaza, bakterie kwasu mlekowego.

Abstract

Introduction. A crucial element in the treatment of hypolactasia and lactose intolerance is to reduce its consumption. Other important aspects are high activity of endogenous lactase in yoghurt and a high count of lactic acid bacteria responsible for its presence in the consumed product.

Aim. The aim of the study was to assess suitability of yoghurts produced in Poland in the diet of patients with hypolactasia based on their contents of lactose, activity of lactase and the presence of viable lactic acid bacteria.

Material and methods. Material for analyses comprised 12 different commercially available yoghurts. Shelf-life of yoghurts was 18 to 25 days. Yoghurts were tested 12, 7 and 0 days before expiry date.

Results. The amount of lactose determined in yoghurts ranged from 2.9 to 6.9g·100g^{-1}. It decreased during storage by over 33%. Lactase activity fell within a range from 0.23 to 0.92 μkat·100g^{-1}. The initial count of bacteria from genus *Lactobacillus* in yoghurts ranged from 7.8×10^5 to 6.9×10^8 CFU·g^{-1}, while that from genus *Streptococcus* was 1.2×10^8 CFU·g^{-1}. Storage resulted in their reduction by 8.9 and 7.9%, respectively. The highest dependence was found between lactase activity and the count of bacteria from genus *Lactobacillus*. The count of *Lactobacillus* in 50% cases was at least 10^7 CFU·g^{-1}, while in 41.6% cases it was equal or lower than 10^5 CFU·g^{-1}, which corresponded to very low lactase activity (0.23-0.37 μkat·100g^{-1}).

Key words: yoghurt, hypolactasia, β-D-galactosidase, lactic acid bacteria.

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INTRODUCTION

A crucial element in the treatment of hypolactasia and lactose intolerance is to reduce its consumption [1-4]. In the opinion of the American Academy of Pediatrics Committee on Nutrition [5], the diet of patients suffering from this disorder, except for individuals with innate lactase deficiency, may be based on products containing partly hydrolyzed lactose or added exogenous lactase. These requirements are met by fermented dairy drinks such as fermented milk, kefir or yoghurt. They are products with high nutritional and nutritive value, which is of special importance in the diet of children and teenagers [6-9]. It is estimated that in Poland hypolactasia may affect as much as even 40% individuals before the age of 30 years. When examining children aged 8 to 11 years in a city of Zabrze Kwiecień et al. [10] diagnosed hypolactasia in 17.4% of that population. A study by Szostak-Węgierek [11] showed that 19.4% children aged 7-15 years in Warszawa suffered from hypolactasia. In turn, Socha et al. [12] reported that hypolactasia affects 37.5% teenagers and young adults with a mean age of 21.9 years. At the same time it needs to be stressed that 37.2% children with hypolactasia and 31.9% children not suffering from hypolactasia do not drink milk, but instead drink kefir or yoghurt [10]. In Poland in 2010 the production of dairy drinks was 650 mln L, including 441 mln L yoghurts, and it was a figure similar to that of 2009 [13]. Mean monthly consumption of yoghurts in 2010 was 0.47 kg/per capita. However, for yoghurts to be recommended in the diet of individuals with hypolactasia they have to contain the lowest possible amounts of lactose [4]. Other important aspects are high activity of endogenous lactase in yoghurt and a high count of lactic acid bacteria responsible for its presence in the consumed product [5,14-17].

AIM

The aim of this study was to assess the suitability of yoghurts produced in Poland in the diet of patients suffering from hypolactasia, based on their lactose content, lactase activity and the presence of viable cells of lactic acid bacteria.

MATERIAL AND METHODS

Materials. Experimental material comprised natural yoghurts purchased in Poland in retail outlets over a period of 10 months 2010. These yoghurts were produced by Polish enterprises employing over 50 workers. Analyses were conducted on 12 different natural yoghurts. Yoghurts did not contain sucrose. Fat content ranged from 1.5 to 4.5 g per 100 g product. Yoghurts were packaged in PS (Polystyrene) tubs with unit weight from 150 to 400 g. Shelf-life declared by 9 producers was 21 days, by 2 producers - 18 days and that declared by 1 producer was 25 days. Yoghurts were tested at 12 days (time A), 7 days (time B) and 0 days (time C) before expiry date declared by the producer. Yoghurts in unit containers were cold stored at a temperature of 5±0.5°C. Yoghurts for analyses were collected from 5 different production batches.

Methods. Analysis of lactose content. Acid thermal hydrolysis was performed in yoghurt samples in order to eliminate protein. For this purpose 0.3 g sample were vortexed with 0.3 ml 0.01 M H_2SO_4. Hydrolysis was run at 80°C for 20 min, next samples were centrifuged (10 min, 3 000 rpm) and the produced supernatant was filtered using Millex – LCR filters by Millipore (Carrigtwohill, Ireland) Low Protein Binding Hydrophilic LRC PTFE 0.45 nm. Filtrate was injected over an HPX 87H column (BioRad - Life Science Group, Hercules, CA, USA) coupled with an RI detector. The volume of the injected sample was 20 µl, the mobile phase was a 5 mM H_2SO_4 solution, time of analysis 30 min, temperature 30°C and flow rate 0.6 ml×min^{-1} [18].

Determination of lactase activity. Lactase activity was determined by a method described by Passerat and Desmaison [19]. Results are expressed for 100g of dairy product and given in microkatal·100g^{-1}. One unit of enzyme was defined as the amount which hydrolyzed one micromole of lactose per second.

Determination of bacteria from genus *Lactobacillus*. Bacteria from genus *Lactobacillus* in the examined yoghurts were determined using agar MRS by De Man, Rogosa and Sharpe - medium 110660 Merck KgaA (Darmstadt, Germany). Ice-cold acetic acid was added to the media to ensure that its active acidity after sterilisation, expressed in pH units, was 5.4, as measured at 25±1°C. Inoculated upturned plates were incubated at 37±1°C for 72 h under anaerobic conditions in a microbiological thermostat WTB Binder (Tuttlingen, Germany) [20].

Determination of bacteria from genus *Streptococcus*. *Streptococcus* bacteria counts in the examined yoghurts were determined using the agar medium M_{17} according to Terzaghi- medium 115108, obtained from Merck KgaA (Darmstadt, Germany). In order to provide medium acidity after sterilisation ranging from 7.1 to 7.2 at 25±1°C, 0.1 M solution of sodium hydroxide or 0.1 M solution of hydrochloric acid was added to the dissolved components of the medium. Inoculated plates for the determination of the *Streptococcus* bacteria in the examined yoghurts were incubated at 37±1°C for 48 h [20].

Physiological solution with peptone - N° 112535 Merck KgaA (Darmstadt, Germany) was used for dilutions in microbiological assays [21].

Statistical analysis. Statistical calculations were performed using a data analysis software system STATISTICA (version 8.0) by StatSoft, Inc. (2008). Pearson's linear correlation coefficients were calculated in order to determine the degree of proportional correlations between values of the lactose content and those obtained by lactase activity. On this basis regression lines were plotted and the coefficient of determination was calculated, constituting the basis for the size of the correlation. Coefficients of determination express the size of common variance for the two analysed variables. The significance of the correlation coefficient was determined in order to evaluate correlations between variables. The significance test for correlation coefficients was based on the assumption of the normal distribution of remainder values of variable y and on the equality of variances of remainder values for all values of variable x. In order to eliminate the departures from linearity of Pearson's distribu-

tion, which might cause an increase in the sum squares of deviations from regression lines, scatter diagrams were analyzed for results recorded for each examined object.

RESULTS

Based on the conducted analyses in the first period (time A) it was found that yoghurts contained from 2.9 to 6.9 g·100g-1 lactose (Table I). With storage time the content of lactose decreased significantly, on average by 33.1%. On the last day of analysis (time C) mean lactose content in yoghurts was 3.31 g·100g-1.

Mean lactase activity determined in yoghurts on the first day of the analyses (time A) was 0.63 µkat·100g-1 (Table I). No statistically significant differences were found between lactase activity in yoghurts analysed in successive periods of time. Irrespective of storage time lactase activity in yoghurts fell within the range of 0.23 - 0.92 µkat·100g-1, yielding a mean of 0.58 µkat·100g-1. (Table 1)

TABLE 1. Contents of lactose and lactase activity in yoghurts produced in Poland, p=0.05, *df*=11.

	Storage time*		
	A	B	C
	Lactose content (g·100g^{-1})		
mean±SD	4.95±1.35	4.05±1.07	3.31±1.22
Test-t		5.73	5.57
P		<0.001	<0.001
	Lactase activity (µkat·100g^{-1})		
mean±SD	0.63±0.19	0.59±0.15	0.52±0.21
Test-t		0.91	1.70
P		0.376	0.112

*time A -12 days before expiry date
time B - 6 days before expiry date
time C - the last day of shelf life
df - degrees of freedom
SD - standard deviation
P - value statistically significant
Test-t - value of Bonferroni test

Analysis of relationships between lactase activity and lactose content in the same yoghurt samples produced a low coefficient of determination β=0.098 (Figure 1). Over 99% results were found within the ellipsis of covariance defined by the covariance matrix. However, the plotted regression line and the correlation coefficient (r=-0.314) are the basis for the low level of interrelation between analysed variables (Figure 1).

A confirmation of the significant correlation between results of analyses of lactase activity in tested yoghurts and LAB counts is the high level of interrelation between analysed variables. This procedure was performed for the entire sample storage period. Calculated correlation coefficients for bacteria from genus *Lactobacillus* amounted to r=0.712 and for bacteria from genus *Streptococcus* r=0.474. This was confirmed by the analysis of plot area smoothed using the least square method with weighted distances (Figure 2).

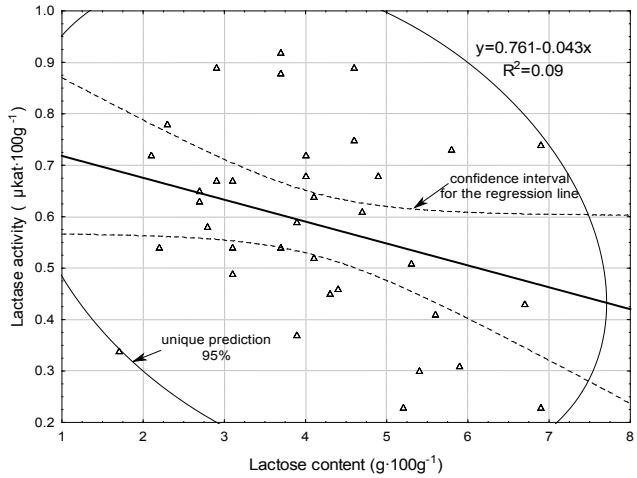

FIGURE 1. Relative probabilities of normal distribution for results concerning lactase activity and lactose content in yoghurts produced in Poland, n=36, R^2-coeficient of determination.

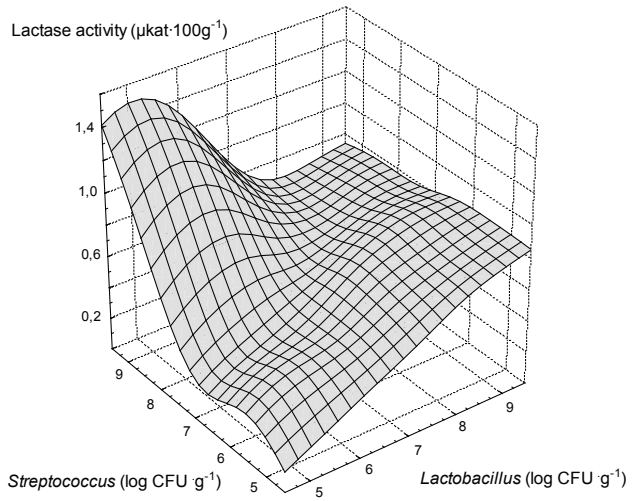

FIGURE 2. The effect of counts of bacteria from genera *Lactobacillus* and *Streptococcus* on lactase activity in yoghurts produced in Poland, n=36.

Assays of LAB showed that the initial count (time A) of bacteria from genus *Lactobacillus* in yoghurts ranged from 7.8×10^5 to 6.9×10^8 CFU·g^{-1} (Table 2). This count was statistically the same also after 6 days (time B). However, after 12 days (time C) the count of bacteria from genus *Lactobacillus* decreased significantly by 8.9% to a mean value of 3.0×10^7 CFU·g^{-1} (Table 2).

TABLE 2. Contents of lactic acid bacteria in yoghurts produced in Poland, p=0.05, *df*=11.

	Storage time*		
	A	B	C
	Bacteria from genus *Lactobacillus* (log CFU·g^{-1})		
mean±SD	7.19±1.28	6.89±1.19	6.55±1.10
Test-t		1.51	3.48
P		0.157	0.005
	Bacteria from genus *Streptococcus* (log CFU·g^{-1})		
mean±SD	7.23±1.05	6.66±1.22	6.37±1.21
Test-t		2.89	2.26
P		0.014	0.044

*denotation as in Table 1

The highest count of bacteria from genus *Streptococcus* was recorded to be 7.2×10^8 CFU·g^{-1} (time A), while the lowest value was 2.3×10^4 CFU·g^{-1} (time C) (Table II). With the passage of storage time in analysed yoghurts fewer and fewer bacteria from genus *Streptococcus* were found. In comparison to the mean initial value of 1.2×10^8 CFU·g^{-1} (7.23 log CFU·g^{-1}) the count of assayed bacteria was by 7.9% lower after 6 days of storage and by 11.9% on the last day of their shelf-life.

No deviations from linearity were shown, as measured by the dependence between log count of bacteria from genus *Lactobacillus* and those from genus *Streptococcus* in 1 g yoghurt (Figure 3). For each type of bacteria the plotted regression line was described by a very similar initial ordinate of 7.5 and 7.6. In turn, the value of line slope was -0.3 (bacteria from genus *Lactobacillus*) and -0.4 (bacteria from genus *Streptococcus*).

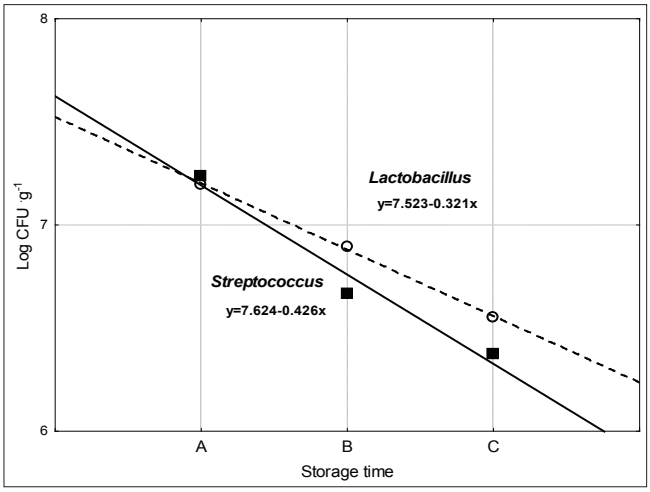

FIGURE 3. Changes in viable counts of Lactobacillus and Streptococcus in yoghurts produced in Poland depending on storage time, n=12.

DISCUSSION

Whether an enzyme may run effective catalysis depends on many factors. Enzymes found in products differed in their molecular mass, the number of native molecule subunits, optimal temperature and pH of their action. The supply of metal ions required for enzyme activity and/or its stabilization in the medium also varies. Such a variation results from the presence of different micro-organisms in the product [22]. Moreover, certain micro-organisms are capable of synthesizing several different β-D-galactosidases, e.g. *Bifidobacterium adolescentis* DSM 20083 produces its 2 types. It was also shown that certain β-D-galactosidases do not degrade lactose, but hydrolyse glycoside bonds in oligosaccharides formed from lactose via transglycosylation [23].

Lactase activity in yoghurt tested by Onwulata et al. [24] was 1.0 U·g^{-1}, where 1 U=1 µmol *O*-nitrophenol formed·min^{-1}. In turn, Martini et al. [25] when investigating 7 commercially available natural and flavoured yoghurts recorded in them from 3.88 to 6.89 units of lactase activity, with U=µmol ortho-nitro-β-D-galaktopyranoside (ONPG) hydrolyzed·min^{-1}·g^{-1}. Passerat and Desmaison [19] when analysing fermented milk containing *Streptococcus thermophillus, Lactobacillus delbrueckii subsp.bulgaricus* and *Bifidobacterium bifidum* showed lactase activity at 0.60-0.63 µkat·100g^{-1}. Lactase activity determined in yoghurt analysed by Savaiano et al. [26] was 0.64 mg·h^{-1}·g^{-1}, while in pasteurised yoghurt it was only 0.07 mg·h^{-1}·g^{-1}.

Microflora intrinsic to yoghurt comprises thermophilous strains of lactic acid bacteria (LAB) represented by *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [27]. These bacteria introduced as *inoculum* at the stage of yoghurt production constitute a source of endogenous lactase. Thus the concentration of used bacteria and the conditions for their development determine the rate of lactose attenuation both in milk processed during yoghurt production and in the ready-to-eat yoghurt during its further storage.

Vinderola et al. [28] when analysing viability of microflora in commercial yoghurts showed total LAB count to be 10^7 CFU·g^{-1}. *Streptococcus* counts were higher – by at least 1 log order – than those for *Lactobacillus*. Those authors also stated that during 4 weeks of yoghurt storage at 5°C the smallest fluctuations were found for the number of rods in comparison to streptococci. Birollo et al. [29] when analysing 25 different yoghurts recorded on MRS agar (anaerobiosis) 7.38 log CFU g^{-1} and bacteria from genus *Streptococcus* on M_{17} agar at 8.54 log CFU g^{-1}. Numbers of *Streptococcus* were higher than those of *Lactobacillus* irrespective of storage time and temperature. The level of *Lactobacillus* bacteria determined by the authors after 60 days of sample storage at 6°C was 10^5, while for *Streptococcus* it was 10^7 CFU g^{-1}. Bacteria from genus *Lactobacillus* exhibited lower viability than streptococci also in a study by Giraffa [30], who applied different combinations of starter cultures. Shihata and Shah [31] when analysing growth of *Lactobacillus* in different yoghurts reported 6.88 and 7.71 log CFU g^{-1}, while after 4-week storage it was 4.63 and 5.14 log CFU g^{-1}, respectively. In the same yoghurt samples the count of *Streptococcus* bacteria exhibited small fluctuations from 9.31 and 9.27 to 9.25 and 9.21 log CFU g^{-1}. Kumar and Mishra [32] in produced control yoghurts found counts of bacteria from genus *Lactobacillus* at 2.23×10^8 CFU·ml^{-1}, while for *Streptococcus* it was 1.58×10^8 CFU·ml^{-1}. The same authors showed very similar counts of LAB in yoghurts containing gelatine, pectin and sodium algineate in amounts ranging from 0.2 to 0.6 % as those in the control samples. They amounted to $1.57\text{-}1.60\times10^8$ CFU·ml^{-1} (*Lactobacillus*) and $2.22\text{-}2.24\times10^8$ CFU·ml^{-1} (*Streptococcus*), respectively.

CONCLUSION

1. The amount of lactose determined in yoghurts ranged from 2.9 to 6.9g·100g^{-1}. It decreased during storage by over 33%.
2. Lactase activity fell within a range from 0.23 to 0.92 µkat·100g^{-1}.
3. The highest dependence was found between lactase activity and the count of bacteria from genus *Lactobacillus*. The count of *Lactobacillus* in 50% cases was at least 10^7 CFU·g^{-1}, while in 41.6% cases it was equal or lower than 10^5 CFU·g^{-1}, which corresponded to very low lactase activity (0.23-0.37 µkat·100g^{-1}).

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