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ASSESSMENT OF FERMENTED DAIRY PRODUCTS ADEQUACY IN DIET OF LACTOSE INTOLERANT PERSONS

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Summary

Introduction and objective: Milk and dairy products are an important source of many nutrients that are known to have many beneficial effects on human health. On the other hand, milk and dairy products can cause allergies and intolerances. Allergy is caused by milk proteins that lead to immune reactions, while intolerance is caused by the milk sugar, lactose, due to reduced activity of enzyme lactase which digests it. To avoid the unpleasant symptoms, lactose intolerant persons can consume fermented milk products that are known to have reduced lactose content. The aim of this study was to determine the content of lactose in commercially available fermented dairy beverages by HPLC method and to assess their adequacy in diet of lactose intolerant persons.
Methods: Altogether 21commercially available type of fermented milk products was analysed of which 13 were plain yogurts, while remaining 8 belonged to the group of functional products. Lactose content was determined by HPLC method. Lactose detection was achieved by refraction index detector and its quantification by external standard method.
Results: Lactose content of analysed products ranged from 2.65 g/100 g up to 4.05 g/100 g in plain yogurts, and from 2.61 g/100 g up to 4.63 g/100 g in functional products.
Conclusions: Based on determined lactose content and a presumption that most of lactose intolerant persons can digest up to 6 g of lactose on a daily basis without obvious symptoms it is assessed that daily acceptable amount of the analysed products ranges from 130 to 230 g.

Keywords: lactose, lactose intolerance, fermented milk products, diet, HPLC method

Introduction

Milk is one of the most complete foods. For infants it represents the exclusive food which enables their normal growth and development, while in an adult diet plays an important role as a source of highly valued proteins (Mandić, 2007). Milk and dairy products are also an important source of vitamins, essential fatty acids and minerals, and the main source of calcium in human nutrition.
Milk and dairy products can cause health problems, and among them allergies and intolerances are the most common. Allergies on milk and dairy products are commonly caused by milk proteins, while sugar lactose causes intolerance. Namely, one third of the milk caloric value is contributed by the lactose, main dairy carbohydrate, commonly known as the milk sugar (HAH, 2009). Lactose intolerance implies incapability of lactose digestion caused by decreased activity of the enzyme lactase (Brown-Esters et al., 2012) which causes various gastrointestinal problems (Lomer et al., 2008). If lactase activity is decreased by 50% or more maldigestion of lactose occurs. Unabsorbed lactose furthermore raises the amount of water and electrolytes in the large bowel lumen accelerating bowel content movement and resulting in diarrhoea. Bacterial β-galaktosidase unchains glucose and galactose from the lactose and makes them accessible for the bacterial fermentation which occurs in the bowel and results in abdominal pain, bloating, flatulence and diarrhoea. On the other hand, some bacteria reduce carbon dioxide to methane and that may cause constipation (Lomer et al., 2008).
Hypolactasia, or lactase deficiency, exists in three distinct forms: congenital, primary and secondary (Lomer et al., 2008).
Congenital lactase deficiency is characterised by the lowest lactase activity. It is believed to be an autosomal recessive trait and the main symptom is infantile diarrhoea from the first exposure to breast milk (Madry i sur., 2010). Congenital lactase deficiency is extremely rare and a lifelong disorder (Lomer et al., 2008).
The most common type found in humans is primary lactose malabsorption or lactase non-persistence (adult type hypolactasia). It can be described as a genetically predetermined physiological condition inherited through an autosomal recessive mode. In this type, lactase activity decreases after weaning. Namely, healthy infants normally display an adequate expression of lactase which decreases during the weaning period. This might be an evolutionary adaptation since milk, in most adults, is not a main dietary product. The prevalence of this type of adult

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hypolactasia in Europe increases toward the South and East and reaches 70% in southern Italy and Turkey (Madry et al., 2010).
Secondary lactase deficiency is acquired as a result of gastrointestinal disease which damages the brush border of the small intestine (giardiasis, coeliac disease, viral gastroenteritis, Cronhn´s disease, radiotherapy) (Madry et al., 2010; Lomer et al., 2008).
The most common management of lactose intolerance relies on reducing lactose exposure by avoiding milk, dairy products and all milk-containing products from the diet (Brown – Esters et al., 2012). Many persons who avoid milk and dairy products which constitute main source of calcium, vitamin D and other nutrients are not ingesting adequate amounts of these essential nutrients (Suchy et al., 2010). This rises their risk of health problems such as rickets, osteoporosis and osteomalacia (Rong et al., 2011), and therefore many studies suggest alternative approaches. These alternative approaches imply consumption of products developed for lactose-intolerant persons (lactose-reduced milk, probiotic yoghurts) and steadily increase of the dietary lactose load which allows the colon to adapt over a period of time (Shaukat et al., 2010). One of the recently studied strategies is a consumption of fermented dairy products (Shaukat et al., 2010), in which, due to the fermentation reactions, lactose content is reduced, which makes them more tolerable for consumption in lactose-intolerant persons (HAH, 2009; Tudor, 2009). Lately, lactase enzyme is commercially available in the form of tablets, what enables lactose digestion. Yet it should be considered that this is an option for cases of unintended lactose consumption like trips or out of home lunches and not preparation for daily use.
There are four different factors that may play a role in lactose digestion. One of them is dose of lactose consumed, where direct relationship between lactose consumed and the severity of maldigestion symptoms exists. In most lactose intolerant persons, lactose doses below 12 g causes no symptoms while higher doses (20-50 g) cause considerable symptoms (Brown-Esters et al., 2012).
The aim of this study was to determine the content of lactose in commercially available fermented dairy beverages by high-performance liquid chromatography (HPLC) method and assess their adequacy in diet of lactose intolerant persons.

Materials and methods

Samples

To conduct this study altogether 21 commercially available types of fermented milk products were purchased from the supermarkets in the area of city Osijek. Samples were divided into two groups. First group encompassed plain yogurts (13 samples), while second group (8 samples) included products declared as functional foods by the producer. Purchased samples were stored as indicated by the producer (refrigerated) and analysed within the "use by" date.

Lactose extraction from the samples

Carbohydrates were extracted from samples using the method described by Indyk et al. (1996).
Sample (5 g) was dissolved in warm demineralised water and transferred into a graduated 50 mL flask. Carrez reagents 1 and 2 were added (0.5 mL of each) sequentially with mixing and content was left to stand for about 20 minutes. Afterwards, extracts were made to volume with water and filtered. First few mL of filtrate were discharged.
Filtered sample solution was passed through a 0.45 μm nylon membrane filter.

High-performance liquid chromatography of the lactose content in samples

Analysis was performed on Varian instrument comprising of ternary Solvent Delivery Module (ProStar 230), Column Valve Module (ProStar 500) and Refractive Index Detector (ProStar 350). Instrument was supported with ProStar Chromatography Workstation 5.
To enable lactose quantification an external calibration method was applied. Lactose solutions for the calibration (D-lactose monohydrate for HPLC analyses, ≥99.5%, Sigma-Aldrich, Buchs, Switzerland) were prepared in range from 0.1 up to 5.0%. Each standard solution was analysed twice.
Samples were analysed using a Zorbax NH_2 (Agilent Technologies) column (4.6 x 250 mm, 5 μm particle size).
Separation of present carbohydrates was achieved applying an isocratic elution with acetonitrile/water (70/30 v/v). Acetonitrile (Merck, Darmstadt, Germany) of HPLC gradient grade purity and ultrapure water were used to prepare mobile phase solution.
Lactose identification was achieved based on the retention time and quantification based on the external calibration curve obtained based on the standard solutions prepared as described above. 10 μL loop was used for all analyses.
All analyses were conducted in two replicates, and each sample solution was injected twice.
Lactose content is expressed as g/100 g of the original sample.

Results and discussion

Lactose content of analysed plain yoghurts ranged from 2.65 g/100 g up to 4.05 g/100 g (Table 1). Vinko et al. (2011) studied changes of lactose content in dairy production and reported lactose content of 3.12 g/100 g of product after one day refrigerated storage and 2.80 g/100 of product after 28 days of refrigerated storage for plain yoghurt fermented using yoghurt culture of *Streptococcus thermophilus* and *Lactobacilus delbruckii* subsp. *bulgaricus*.

Table 1 Basic characteristics and lactose content of analysed fermented milk products

Sample code	Yoghurt type	Fat content declared by producer (g/100 g)	Lactose (g/100 g)
Fermented products			
Y-7	Set	3.2	2.81 ± 0.24
Y-8	Set	3.2	3.50 ± 0.15
Y-9	Drinkable	2.8	4.05 ± 0.31
Y-10	Drinkable	0.1	3.35 ± 0.15
Y-11	Drinkable	3.2	3.05 ± 0.04
Y-12	Drinkable	0.9	3.93 ± 0.17
Y-13	Drinkable	0.9	3.14 ± 0.20
Y-14	Drinkable	2.8	2.65 ± 0.09
Y-17	Drinkable	0.5	2.88 ± 0.19
Y-18	Set	3.2	2.89 ± 0.06
Y-19	Drinkable	2.8	3.07 ± 0.33
Y-20	Drinkable	0.05	2.82 ± 0.22
Y-23	Drinkable	2.8	2.96 ± 0.11
Probiotic fermented products			
FY-2	Drinkable	3.2	2.87 ± 0.13
FY-3	Drinkable	1.5	3.33 ± 0.32
FY-4	Drinkable	3.5	3.49 ± 0.11
FY-5	Drinkable	1.0	2.77 ± 0.09
FY-6	Drinkable	0.5	2.61 ± 0.17
FY-21	Drinkable	0.9	4.63 ± 0.10
FY-22	Drinkable	3.5	4.33 ± 0.15
FY-24	Drinkable	3.1	3.17 ± 0.15

Lactose content of 8 functional dairy products varied from 2.61 g/100 g up to 4.63 g/100 g. Functional milk-based products available in the Italian market and provided from large stores were HPLC analysed by Manzi et al. (2007). They reported 2.32 up to 4.50 g of lactose per 100 g of product for probiotic fermented milk products. O'Brien (1999) determined lactose content of cultured dairy products available in the UK market by enzymatic method. He analysed 94 various products and reported average lactose content of 3.67% in live yoghurts (plain) and 2.54% in drinking yoghurts. O´Brien draw attention to the fact that sugar content of milk and dairy products published in food composition tables are lower from those which he obtained, and explained that by the wide range of products which were developed and broaden to the market recently. He concluded that fermented dairy products have significantly lower lactose contents than unfermented milk and as such might be appropriate for lactose-intolerant individuals (O´Brien, 1999).

Vinko et al. (2011) have reported that lactose content is influenced by the age of product, and drop in lactose content during the 28 days long refrigerated storage (within the „use by" date) reaches up to 38.73% of initial lactose content. Our study did not encompass the influence of storage time on lactose content since the exact time of inoculation and fermentation is not consumer available information, and durability of products varies from the few days up to few weeks. Proper storage, and the analysis within the „use by" date was the only criteria used.

Besides product age, lactose content is influenced by the microbial β-galaktosidase activity during the storage period. Vinko et al. (2011) reported that thermophilic cultures converted about 30% of lactose to lactic acid, while mesophilic culture converted 16-20% indicating that choice of culture can also play an important role in estimation of dairy product suitability for lactose intolerant persons.

Significance of a product in a person's diet depends not only on the content of the observed compound per 100 g of product but also on the frequency of consumption and the portion size. As a result, from the desirable compound point of view, important food source are even those food product which

contains small amount of the compound but it is consumed often and in large amounts. At the same time, from the undesirable compound point of view, product does not have to present a risk if it is consumed occasionally and in small amounts. Using that framework in the lactose intolerance perspective, although fermented milk products contain lactose, its content is lower due to fermentation, and they could be suitable for lactose intolerant persons. The amount of product should not exceed tolerable level of lactose. Namely, literature data purport the fact that most of the lactose-intolerant individuals can digest up to 6 g of lactose on a daily basis(HAH, 2009). Based on that value, acceptable daily amount of product is estimated for all samples analysed in our study. Results presented in Fig. 1 indicate that most of the lactose intolerant persons could ingest from 130 g up to 230 g of yoghurt daily (in dependence of the product type choice) without feeling discomfort caused by lactose digestion problems.

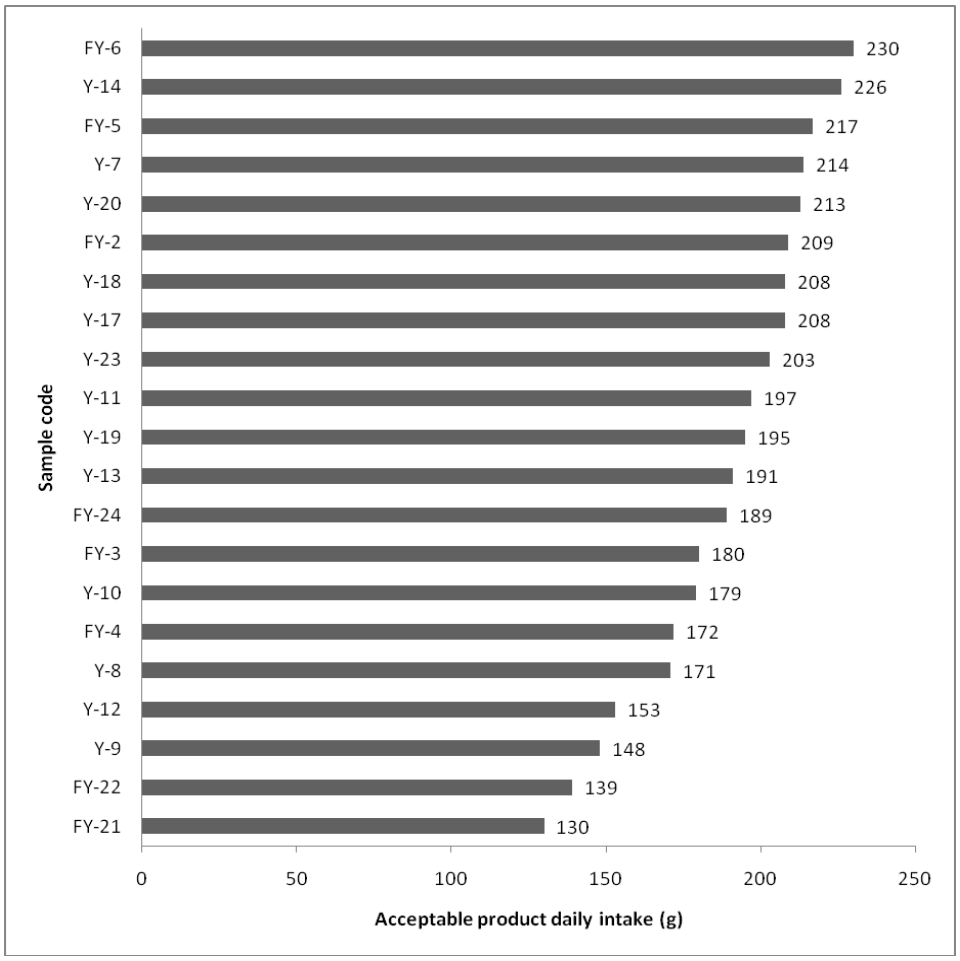

Fig. 1. The individually assessed acceptable amount of daily intake of analysed fermented dairy products in lactose-intolerant persons for plain yoghurts (Y) and functional yoghurts (FY) under the presumption that person can digest 6 g of lactose daily with no or minor symptoms

Suchy et al. (2010) reported that adults and adolescents with diagnosed lactose malabsorption could ingest at least 12 g of lactose when administered in a single dose with no or minor symptoms, and even larger amounts if ingested with meals and distributed throughout the day. The intake of up to 12 g was supported by Brown-Esters et al. (2012) based on the results of studies which indicate how colonic bacteria develop an increased ability to ferment lactose over prolonged period of time with lactose ingestion. Dairy consumption should therefore not be restricted but managed, and the useful approach is to ingest fermented milk products throughout the day in small portions.

Individual differences in sensitivity are influenced by many factors among which ethnicity and age are studied most intensively. Lomer et al. (2008) reported that incidence is lower in adults from northern

European countries and Australia, while higher in South America, Africa and Asia. Acceptable tolerable daily amount is assumed to vary according to the same pattern. Croatian Food Agency (2009) reports higher lactose intolerance incidence in elderly adult due to diminished lactase production with age. Vonk et al. (2003) concluded that the major difference in intolerance symptoms is caused by differences in the colonic processing of maldigested lactose.

Altogether, determined patterns and mechanisms, as well as the individual differences, show that ingestion of lactose containing products should be personalised.

Conclusions

Lactose content of analysed fermented dairy products ranged from 2.65 g/100 g up to 4.05 g/100 g in plain yogurts, and from 2.61 g/100 g up to 4.63 g/100 g in functional products. Values are in line with those found in literature for the same product type.

Based on determined lactose content and a presumption that most of lactose intolerant persons can digest up to 6 g of lactose on a daily basis without obvious symptoms it is assessed that acceptable amount of analysed products range from 130 to 230 g. Nevertheless, individual differences should be considered prior to ingestion.

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