



## DYNAMICS OF LACTOSE CHANGES DURING RIPENING OF EDAM CHEESE

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### ABSTRACT

The published data show that milk and dairy products are an important part of the diet in the European population and the population of North America, where they cover from 20 to 30% of protein, 15% of lipids and about 80% of calcium from food sources. The exclusion of milk and dairy products from human diet due to lactose intolerance (approximately 75% of the global population are lactose malabsorbers) can cause very serious health consequences. From a public health point of view, it is therefore extremely important for dairy products to capture all the facts about the fluctuation process or rather reduction of lactose content during dairy production technology. The aim of our study was to determine the lactose in various stages of Edam cheese ripening, to assess its suitability for consumption on the afflicted population. For the determination of lactose (day of production, first, second and sixth month of storage) the reference enzymatic method using the enzymatic set Megazyme International Ireland with a detection limit of 0.00296 g.100 g<sup>-1</sup> was applied. This set is intended for determination of lactose in samples presented as low-lactose or lactose-free products and is based on the hydrolysis of lactose to D-galactose and D-glucose by the enzyme  $\beta$ -galactosidase. After the subsequent oxidation of galactose, the amount of formed NADH (stoichiometrically related to the amount of lactose contained in the sample) is measured in a spectrophotometer at 340 nm. According to current legislation, the lactose-free product must contain less than 10 mg of lactose per 100 g or 100 mL of the consumed product, while a product that contains up to 1 g of lactose in 100 g or 100 mL of the product consumed is considered as a product with low lactose content. The study results showed that even after one month of storage Edam cheese can be, according to current national and EU legislation, designated as a lactose-free food. This means that the consumer receives a lactose-free product whenever buying this type of cheese.

**Keywords:** lactose; lactose intolerance; enzymatic method lactose determination; cheese Edam

### INTRODUCTION

The position of milk and dairy products as a legitimate part of human nutrition is the result of qualities such as filling ability, content of high quality essential amino acids, vitamins and minerals – calcium in particular. Despite these obvious benefits, in some social groups milk is a taboo subject (Meyer-Rochow, 2009), in other cultures and societies its consumption is influenced not only availability, but also a degree of popularity and preference, which are usually of population character.

Except for breast milk, which is the only natural food for a human during the first six months of their life due to which reason it is always accepted positively, in case of other foodstuffs, including milk of other mammals and products from it, this will not be so unambiguous. In the early childhood, an individual is protected a priori by a so-called food neophobia, which, as a certain evolutionary attitude, ensures that the individual does not consume new and unknown food that could endanger them. This neophobia is later carried through an individual's life and

applied on foods which are unknown or prepared in a new way. People in economically developed countries also respond to those foods they encounter without sufficient cultural and social adaptation, or consumption of which is traditionally considered undesirable in a particular social group or religious community, or within their immediate vicinity.

The published data show that milk and dairy products are an important part of the diet in the European population and the population of North America, where they cover from 20 to 30% of protein, 15% of lipids and about 80% of calcium from food sources. The nutritional importance of dairy products in the whole context of their individual types is still not completely described. But certainly a number of positive aspects of milk are enhanced by the used cultural microflora (Park and Haenlein, 2013).

Campaigns against the consumption of cow's milk and dairy products organised in economically developed countries often abuse the fact that part of the European and North American population is affected by the defect of

lactase enzyme production and suffers from lactose intolerance. This fact together with the low level of knowledge of the actual lactose content in various stages of dairy production, which covers cheese as well, is often fallaciously used for recommendation of total avoidance of consumption of any dairy products (Roginski, Foquay and Fox, 2002).

It should be mentioned that there exists a possibility of intolerance of some milk nutrients, which is often misused to the disadvantage of milk. However, it must be mentioned, that there may exist intolerance of any food (Roginski, Foquay and Fox, 2002; Park and Haenlein, 2013).

In principle we distinguish between allergy and intolerance. Unlike food intolerance, allergy is always the response of the body via the immune system. In some individuals, milk proteins, as well as the components of another 160 foodstuffs, may induce an inappropriate response of the immune system (Park and Haenlein, 2013).

Based on clinical symptoms, it is possible to define two forms of allergic reaction in IgE-mediated food allergy. Food allergic reactions may be manifested differently with respect to localisation, time horizon, and the severity of the symptoms, the last mentioned ranging from the simplest to the anaphylactic shock. One form of allergy is manifested shortly after birth and in early childhood. Sensitization is triggered by a reaction in the gastrointestinal tract and is most often manifested as atopic dermatitis. Other possible forms of allergy are inflammation of the intestinal mucosa (accompanied by vomiting, nausea, diarrhoea and abdominal pain) and/or affection of the respiratory tract, namely bronchoconstriction, rhinitis, rhinoconjunctivitis (Park and Haenlein, 2013).

Allergens represent a wide range of substances (antigenic molecules) which are present in foodstuffs. Most allergens are proteins. Antigenic determinants of food allergens have different tertiary and quaternary structures. However, their definition is not easy because their conformation may be modified during the food technology (Park and Haenlein, 2013).

The main allergens of milk are also proteins, for example casein (in most cases  $\alpha$ -casein) or whey proteins ( $\beta$ -lactoglobulin, but also  $\alpha$ -lactalbumin).

Another possible type of milk intolerance is caused by lactose intolerance. Lactose intolerance is not an answer of the immune system, even though some of its manifestations may be identical with the manifestations of allergy. On the contrary, it is based on varied degrees of lactose malabsorption. This disease is also referred to as e.g. lactase deficiency, alactasia or hypolactasia.

On physiological conditions, lactose received in milk is hydrolysed in the jejunum (but also in the duodenum and ileum) into glucose and galactose by the enzyme lactase ( $\beta$ -galactosidase). Reduced or absent activity of this enzyme will induce manifestations of intolerance very similar to the gastrointestinal manifestations of allergy (Roginski, Foquay and Fox, 2002).

According to a number of factors, it is possible to classify various types of lactose intolerance. The first type is congenital lactase deficiency which is characterised by a rare genetic abnormality of lactase activity in which the

activity of the enzyme at birth is low or none at all (Roginski, Foquay and Fox, 2002).

Another type of this disease is also a very rare disease, referred to as congenital toxic lactase deficiency. This disease is characterised by the absorption of intact lactose which has a toxic effect on the liver and kidneys (potentially causing the death of the patient). The third type is so-called primary lactose intolerance. There is also secondary lactose intolerance in which lactase activity is low as a result of diseases such as tropical sprue, regional ileitis, various parasitoses, infections and/or gastrointestinal surgery (Roginski, Foquay and Fox, 2002).

Primary lactose intolerance has the highest prevalence in the world population. Besides other benefits, dairy products offer solution for a number of cases of lactose intolerance. Azcarate-Peril, Ritter and Savaiano (2017) indicate that approximately 75% of the global human population are lactose malabsorbers. The principle of this metabolic disease is a lactose malabsorption of various degrees. The extent of symptoms of lactose intolerance is dependent on the amount of lactose ingested. Lactose can be ingested both consciously and unconsciously in foods that have not been adequately labelled, and the complete composition was not stated. Lactose intolerance has been described 400 years ago, but its clinical symptoms have been described and recognised only in the last sixty years (Matthews et al., 2005). The symptoms occur within two hours after the ingestion of the food containing lactose; they include abdominal pain, flatulence, diarrhoea, nausea, vomiting, and stomach discomfort. The formation of short chains of fatty acids, hydrogen, methane and carbon dioxide during the fermentation of unabsorbed lactose by bacterial microflora increases the time of food passage through the intestinal tract. This slowdown causes abdominal pain and flatulence. There are several types of lactose intolerance (LI). The most common cause of LI is primary lactase deficiency. The inducing dose is variable, most individuals tolerate one glass of milk (12 – 18 g of lactose) (Arola, 1994; Roginski, Foquay and Fox., 2002; Park and Haenlein, 2013; Rosado, 2016).

The prevalence of lactose intolerance is characterized by binding to ethnic groups. In the Scandinavian countries 1 – 5% of population is affected; 10-20% in countries such as England and Russia; 15 – 50% in Central Europe. In the Mediterranean countries such as Greece, the incidence is 46 – 70% in children within 5 – 12 years of age, 75 – 80% in children more than 12 years, just as in adults; 70 – 100% in Japan, China, Thailand. The prevalence is extremely high in Asian population, 60 – 80% or higher. The situation in Africa is completely different. In North Africa it is within 70 – 80%, in both nomadic and black cattle-breeding population it is lower than 40%. The prevalence in Bedouins in Jordan and Saudi Arabia is lower than 25%. In the northern parts of America, Canada and Australia, the prevalence is typically lower (under 30%) than in Native Americans and aboriginal populations of Australia and Oceania, in which the prevalence usually exceeds 60% (in some cases it reaches 100%) (Roginski, Foquay and Fox, 2002).

Another factor influencing the prevalence of lactose intolerance is the age, since the activity of lactase physiologically decreases from the thirtieth year of life,

and its activity is dependent on the frequency of milk and dairy products intake.

The inducing dose appears to be of a great variability. In case of not a complete absence of the enzyme lactase, 250 mL of milk a day is accepted (Roginski at al., 2002; Hegar and Widodo, 2015, Buzas, 2015; Silanikove et al., 2015). Most dairy products (not considering lactose-free milk), contain a low or even no level of lactose and thus might be the solution for people with this disease. In addition, some strains of the added Lactobacilli (*L. acidophilus*) have the ability to adapt themselves in the digestive tract and then to product the required enzyme – lactase. Some studies show that the lactose coming from dairy products is better tolerated than lactose from milk (Roginski, Foquay and Fox, 2002). In case of fermented milk products, the lactose content is naturally reduced by 20 – 50% (Fox and McSweeney, 2008; Roginski, Foquay and Fox, 2002). The lactose content in cheese, however, shows quite great variability, depending on the type of cheese and used technology.

**Scientific hypothesis**

The aim of this study was to determine the lactose in various stages of ripening of Edam cheese of Czech provenance to assess the possibility of its consumption as a dietary option for those with lactose intolerance.

**MATERIAL AND METHODOLOGY**

The study was carried out in the dairy company Moravia Lacto Ltd. Jihlava, which is traditionally an important producer of Dutch type Edam cheese, the cheese is produced using the classical technology used in the production of this type of cheese (Düsterhöft, 2002). Four blocks (each weighing 15 kg) out of one batch of Edam cheese were collected, which were already during production divided into 10 smaller blocks (Figure1) and stored in the dairy at 8 – 12 °C and 80% relative humidity, and were successively analyzed: on the day of production, and then after one, two and six months of ripening.

On the day of sample analysis, the pads of Edam cheese were always transferred to the university laboratory in cooling boxes (0 – 5 °C) . From each cheese pad such a sample was taken to enable covering the process of ripening around the whole 15 kg block.

During each sampling (block of cheese pick up),

altogether 10 analyses were carried out; each time from the same ten locations. Each sample of cheese was grated and 10 g ±0.001 g of cheese was weighed out. Then the cheese was heated with distilled water at 50 °C under constant stirring and Carres rippling was performed. After subsequent alkalization and filtration, the filtrate was taken for determination.

For the detection of lactose content the reference enzymatic method was used. For the determination The enzymatic set Megazyme Assay Kit Lactose & D-Galactose Patent Pending [PCT/IE2004/00170] © 2005 (Ireland) was applied. This set is intended for determination of lactose in samples presented as low-lactose or lactose-free products and is based on the hydrolysis of lactose to D-galactose and D-glucose by the enzyme β-galactosidase. After the subsequent oxidation of galactose, the amount of formed NADH (stoichiometrically related to the amount of lactose contained in the sample) is measured in a spectrophotometer HALIOS β UNICAM (UK) at 340 nm. The enzymatic set, works with a detection limit of 0.00296 g.100 g<sup>-1</sup>. Methods based on this principle have been accepted by AOAC 2006.06, NBN, DIN, GOST and IDF

**Statistic analysis**

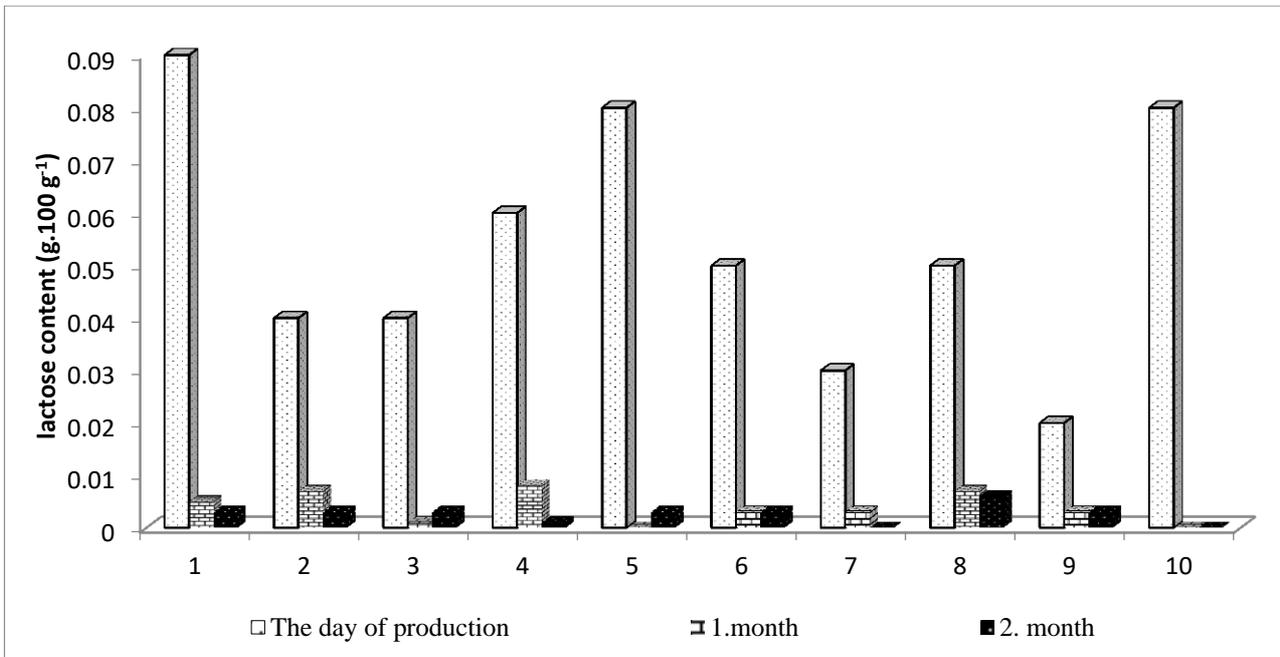
The obtained results were statistically evaluated using software STATISTIKA CZ (CR), version No. 10. The graphs were processed in Microsoft Office Excel and Word with respect to data obtained from STATISTIKA CZ, version No. 10.

**RESULTS AND DISCUSSION**

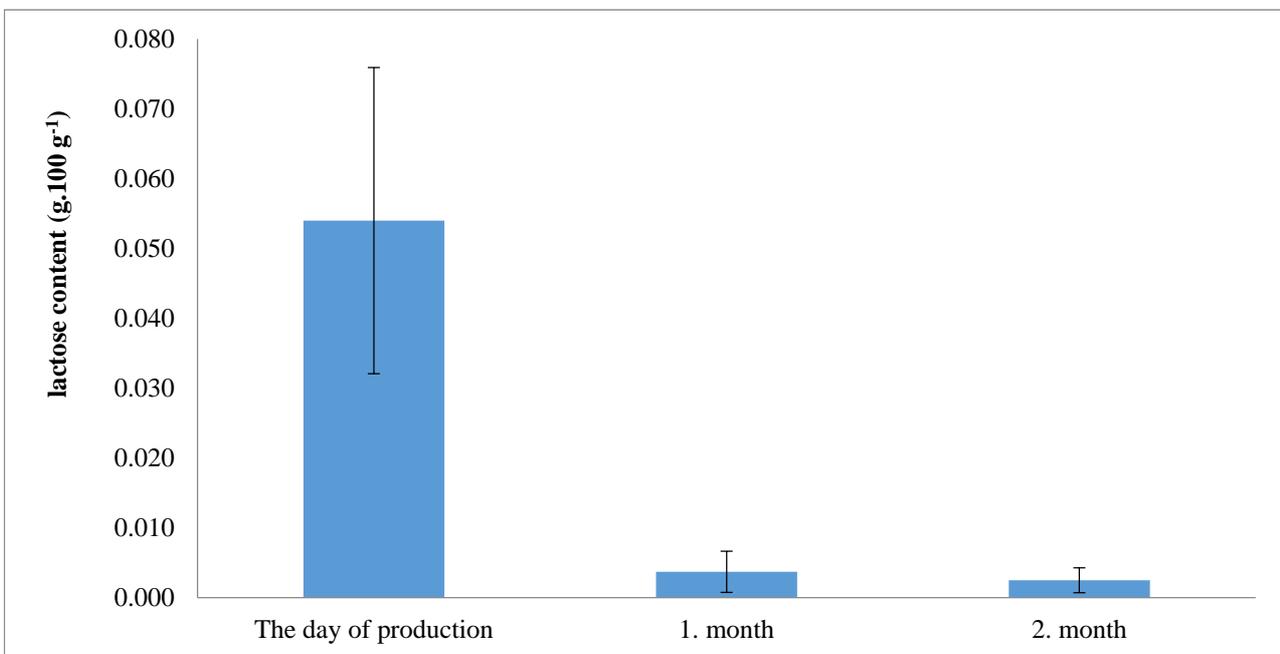
The usual period of ripening of Edam cheese prior to shipment to the market network in Czech dairies ranges from 4 to 8 weeks. In some cases, the cheese may be distributed into market network later, possibly up to six months after production. **Decree No. 46/2014 Coll. of Czech Republic, Amending Decree no. 54/2004 Coll. of Czech Republic**, On foodstuffs intended for particular nutritional uses and how to use them, as amended, addresses the definition of lactose-free foods and foods low in lactose. Lactose-free product contains up to 10 mg of lactose in 100 g or 100 mL of the product, while a product with low lactose content is such one that contains up to 1 g of lactose in 100 g or 100 mL of the product

<b>1</b>	<b>10</b>
<b>2</b>	<b>9</b>
<b>3</b>	<b>8</b>
<b>4</b>	<b>7</b>
<b>5</b>	<b>6</b>

**Figure 1** Each block of cheese (15 kg) was already during production divided into 10 smaller blocks, and each block sector was indicated by the same index.



**Figure 2** The lactose content (g.100 g<sup>-1</sup>) for each of the block sectors (1 to 10) on the day of production and after one and two months of storage.



**Figure 3** The lactose content in cheese including variability ( $x \pm SD$ ) in individual samplings.

consumed. Lactose content (g.100 g<sup>-1</sup>) for each of the block sectors (1 to 10) analyzed at the day of production, after one and two months of storage are shown in the Figure 2. In our study we found out the concentration of lactose in cheese at the day of production  $0.0540 \pm 0.0232$  g.100 g<sup>-1</sup>. Already after one month of ripening statistically significantly lower ( $p < 0.01$ ) concentration of lactose  $0.0037 \pm 0.0029$  g.100 g<sup>-1</sup> was determined. After another (second month of ripening), the concentration of lactose ( $0.0025 \pm 0.0018$  g.100 g<sup>-1</sup>), was very close to the detection limit of the method. The planned offtake of the sample in the 6th month of storage was superfluous regarding to the low lactose content being close to the detection limit of the method used, and therefore was not implemented.

From the results obtained, it is obvious that the residual lactose content in cheese is already very low on the day of manufacture and the main metabolic bacterial degradation of lactose takes place within the first month of ripening process. The results correspond with **Park and Haenlein (2013)**, who state that fresh cheese curd (rennet curd) contains approximately 1% of lactose. However, other types of cheese, technology of which also involve washing the curd with water, e.g. cottage cheese, the curd does not contain any lactose.

As stated in study of **Hettinga et al. (1970)**, who for the determination also used the enzymatic method, the presence of lactose in cheese of Cheddar type can be detected even during long storage, up to the level of detection of 0.004% lactose. These and lower values can

be then detected by thin layer chromatography. **Shakeel-Ur-Rehman, Waldron and Fox (2004)** in his study compared the breakdown of lactose in cheese of Cheddar type with a modified amount of lactose. For his tests he produced Cheddar cheese of standard lactose content, which is commercially available, the second variant of the cheese was produced with a reduced content of lactose and the third type was produced by enriching the cheese with 8.4% of lactose. For Cheddar cheese with the reduced content of lactose, a value close to 0 was found within 60 days of production. For cheese designated as standard, therefore with the usual lactose content, the values were close to zero after about 150 days of ripening and in cheese with added lactose there was even after 180 days of ripening the amount of 1.4% lactose detected in laboratory findings. Later degradation of lactose in cheese of Cheddar type than in cheese of Edam type could be due to a different technology of production, when the Cheddar curd could have been more heated by a couple of degrees. In both types of cheese, the technology of low-boiled curd with the heating range of 36 – 40°C is used, and the range of used temperature could influence the viability of the fermentation culture and the depth of subsequent fermentation. The concentration of the salt bath used, the size of the produced block, the temperature of the cellars and many other factors could have affected the lactose content of these (similar, yet different) cheeses (**Shakeel-Ur-Rehman, Waldron and Fox, 2004**).

A study by the **Izco (2002)** team is dedicated to the determination of lactose and other substances in cheese by capillary electrophoresis. By this method his team determined not only cheeses as such but also yogurt. Two variants of farmer cheese, which is an unripened cheese, were analyzed by capillary electrophoresis. The one variant was stored at a standard temperature of 8 – 10°C, the amount of the detected lactose was 2.3 g.100 g<sup>-1</sup> of dry matter. The other variant of the same cheese was stored for two days at 32 °C and the detected lactose content was 1.9 g.100 g<sup>-1</sup> of dry matter. From this study can be concluded that the lactose content is influenced by many aspects, not only by the amount of the fermentation culture, the salt bath concentration, but also by storage temperature. The **Izco study (2002)** has shown that even two days can affect the lactose content of the cheese, because at higher temperatures, the surviving microorganisms in the cheese are much more active in decomposing lactose into final products. **Silanikove, Leitner and Merin (2015)** in their study state that both hard and fresh cheeses always contain residual amounts of lactose since the separation of curd from whey is never absolute, at least when the conventional process of production is used. The portion of fresh cheese (according to the authors this is 43 g) contains lactose but within the tolerated limit of 12 g. The lactose content of hard-matured (long-ripened) cheeses is very low and according to **Van Calcar et al. (2014)** it is also well tolerated by those who suffer from congenital defect of lactase production (or even galactosemia). Nonetheless, **Silanikove, Leitner and Merin (2015)** take it for useful that these types of cheese, which are expected to contain low or tolerable lactose content, bear specific information about their content as well, so as to form a safe part of the regular diet of people suffering from the defect of lactase formation.

The results obtained in this Edam ripening study (the cheese is in many ways the optimal choice for consumption) are also significant in the context of prevention of osteoporosis or rather bone mineral density (BMD). For example a study done by **Makbul et al. (2016)** proved a relationship between lactose intolerance and BMD ( $p = 0.031$ ). Hence, lactose intolerance consequently affects decreasing of an individual's BMD.

All the studies that have been conducted on cheeses suggest that nearly all ripened cheeses can be described as low-lactose and those ripened longer with higher dry matter, even as lactose-free dairy products.

## CONCLUSION

In our study, using the reference enzymatic method we verified that the content of lactose in Edam cheese is already the first day after the production (concentration of lactose 0.0540 ± 0.0232 g.100 g<sup>-1</sup>) as low as the product may be marked, in accordance to current legislation, as low lactose food. One month after date of production, the Edam cheese may be declared as lactose-free food (concentration of lactose 0.0037 ± 0.0029 g.100 g<sup>-1</sup>).

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