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# Examination of the lactose content of 'Lactose Free' whey protein based sports nutrition products

# Laktózmentes, sportolóknak szánt termékek laktóztartalmának vizsgálata

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## 1.1. Abstract

Whey is the most popular protein source in the sports nutrition market but there is a shift towards plant based and lactose free options. Most lactose malabsorbers can tolerate at least 12g of lactose without symptoms. Five lactose free WPI sports nutrition products were tested, as well as one WPC product using HPLC and RI detection. All of the samples tested were produced by a popular sports nutrition brand. None of the WPI products contained detectable amounts of lactose. The WPC product was found to contain 1.6g/100g (±0.2) of lactose which is well below the threshold required to elicit symptoms in the vast majority of LI individuals when the product is consumed as indicated on the product label. The lactose content of all products tested was consistent with the product labels. Future studies should investigate a broader range of lactose free sports nutrition products from a variety of producers in order to obtain a more complete data set. Individuals particularly sensitive to lactose should consider using plant based alternatives to whey based products.

## 1.2. Absztrakt

A tejsavó a legnépszerűbb fehérjeforrás a sportolók táplálékkiegészítő piacán, de elmozdulás figyelhető meg a növényi alapú és laktózmentes lehetőségek felé, habár a legtöbb tejcukorérzékeny (LI) ember legalább napi 12 g laktózt tünetmentesen elvisel. Kísérleteink során öt laktózmentes tejsavófehérje izolátumot (WPI), valamint kontrollként egy (nem-laktózmentes) koncentrátumot (WPC) vizsgáltunk nagyteljesítményű folyadékkromatográfiás és refraktométeres kimutatással hatósági laboratóriumban. Az összes vizsgált mintát a népszerű sporttáplálkozási márka állította elő. A WPI termékek egyike sem tartalmazott kimutatható mennyiségű laktózt. A WPC termék 1,6 g/100 g (± 0,2) laktózt tartalmazott, ami jóval a tünetek kiváltásához szükséges küszöbérték alatt van a LI-személyek túlnyomó többségénél, ha a terméket a termék címkéjén feltüntetett módon fogyasztják. Az EFSA irányelvei szerint az ebben a kísérletben értékelt termékek mindegyike biztonságosnak tekinthető a LI egyének számára. A jövőben érdemes lesz a laktózmentes sporttáplálkozási termékek szélesebb körét vizsgálni, többféle gyártóktól, hogy statisztikailag releváns eredmények születhessenek. A tejcukorra különösen érzékeny egyéneknek fontolóra kell venniük a tejsavó alapú termékek helyett azok növényi alapú alternatíváinak használatát.

## Table of contents

## Abbreviations List

**CAGR - Compound Annual Growth Rate**

**CLD - Congenital Lactase Deficiency**

**EFSA - European Food Safety Authority**

**EU - European Union**

**FAO - Food and Agriculture Organisation**

**FLD - Fluorescence Detection**

**FTIR - Fourier Transform Infrared**

**G-FET - Graphene Field Effect Transistor**

**HPAEC - High-Performance Anion-Exchange Chromatography**

**HPLC - High Pressure Liquid Chromatography**

**HPTLC - High Performance Thin Layer Chromatography**

**LC MS/MS - Liquid Chromatography Tandem Mass Spectrometry**

**LHM - Lactose Hydrolysed Milk**

**LI - Lactose Intolerance**

**LM - Lactose Malabsorption**

**LNP - Lactase Non-Persistence**

**LOD - Limit of Detection**

**LOQ - Limit of Quantification**

**MIR - Mid-infrared**

**MWP - Milk Whey Powder**

**NAD - Nicotinamide Adenine Dinucleotide**

**NADH - Nicotinamide Adenine Dinucleotide + hydrogen**

**PAD - Pulsed Amperometric Detection**

**PDCAAS - Protein Digestibility Corrected Amino Acid Score**

**RI - Refractive Index**

**RTD - Ready to Drink**

**WHO - World Health Organisation**

**WPC - Whey Protein Concentrate**

**WPI - Whey Protein Isolate**

# 3. Introduction

## 3.1. The sports nutrition market

The sports nutrition industry is growing rapidly with an expected compound annual growth rate of 10.9% from 2021 to 2028 [1]. There are many reasons for this projected growth including a growing global population, a higher proportion of the public which consider themselves to be athletes and an increase in health consciousness among the public which has been accelerated due to the covid-19 pandemic. The sports nutrition market is typically divided into three categories; sports supplements, sports foods and sports drinks. By far the largest segment is that of sports supplements which is dominated by sports protein powder [2]. Despite the ongoing increase in market share of plant based protein powders in recent years, animal based protein sources most importantly whey protein still lead the way [3].

## 3.2. Label compliance in sports nutrition

There are numerous cases of sports food companies adulterating products or exaggerating nutrient content claims [4, 5]. Adulteration of these products is primarily economically motivated and is most likely to occur in competitive markets with poor regulation [6]. Powdered protein supplements are also an easy target for adulteration as they are not easily distinguished without employing experimental methods [7]. A significant example of this occurred in China in 2008, when almost 300,000 thousand infants were affected by melamine toxicosis from infant milk formula [8, 9, 10]. The adulteration of protein powders with melamine was also found to be widespread in South Africa. [11]. However, developed nations are not immune to food scandals, with multiple lawsuits filed against protein powder producers in the US in recent years. Protein spiking is a process whereby non protein nitrogens such as amino acids, urea and the aforementioned melamine are added to products which can falsely inflate the protein content of a product when measured using certain techniques such as the Dumas and Kjeldahl method. The Kjeldahl method is used as the standard for measuring the crude protein content of foods in the EU

(*Regulation (EU) No 1169/2011)*. The use of more selective methods for routine quality control to help reduce incidence of protein adulteration have been investigated in various studies [12, 13, 14].

In these cases the primary focus has generally been on the misreporting of the protein content of the protein powders in question, however in this thesis we wish to explore whether the lactose content of whey protein powders is also misreported.

Whey protein is derived from whey; a byproduct of the cheese making industry. After milk is coagulated, either by rennet or an acid, the resulting liquid whey is collected. The liquid whey is then filtered by various processes in order to remove unwanted elements, predominantly lactose, fats and minerals [15].

Whey protein concentrate (WPC) is most commonly produced by a process known as cross flow ultrafiltration. In this process a membrane which is permeable to lactose but impermeable to protein is used to concentrate the protein and remove impurities. Using this technique a maximum purity of WPC of 70% can be achieved. Diafiltration can then be used to 'wash' the concentrated protein which will remove additional lactose and minerals [16].

Then in order to obtain the highest quality WPC and whey protein isolate (WPI) the additional process of microfiltration or ion exchange membranes must be used [17]. In this process fat and cheese fines are removed as well as bacteria and spores. The resulting low fat and high quality whey can then go through the ultrafiltration process again to reduce the lactose content even further.

As a result of the extra steps required to make WPI, it is therefore a more expensive and premium product. One of it's main benefits being the absence or negligible amounts of lactose it contains. Many formulations of WPI are therefore marketed as lactose free. WPI must have a minimum protein content of 90% whereas WPC products are usually found to be between 65-80% protein ( Table 1) [16, 18, 19].

**Table 1:**

**Protein and lactose content of standard WPC and WPI powders**

**(Onwulata and Huth, 2009)**

| | Protein g/100g powder | Lactose g/100g powder |
|---|---|---|
| WPC | 65-80 | 4-21 |
| WPI | 88-92 | <1 |

This table displays typical values for both protein and lactose content in commercial WPC and WPI formulations found on the sports nutrition market.

## 3.3. Lactose intolerance

Lactose is a disaccharide which is composed of glucose and galactose. It is the main carbohydrate source found in the milk of all mammals. The absorption of lactose occurs in the small intestine and is facilitated by the lactase enzyme found in the microvilli membranes of enterocytes. Lactase hydrolyzes lactose into its component parts, glucose and galactose which can then be absorbed by the gut [20]. When undigested lactose reaches the colon it becomes subject to colonic fermentation. It is this process which is responsible for the symptoms of lactose intolerance. Bacterial fermentation results in the production of methane, carbon dioxide and hydrogen gas, responsible for bloating. The production of lactic acid and reducing sugars by these bacteria can change the osmotic potential of the colon therefore drawing water into the lumen which can result in an increase of motility and diarrhoea [21].

Some 70% of the global population suffer from some level of lactose intolerance. In fact Lactase non persistence (LNP) is an ancestral condition. Only in Europe is LNP found in a minority of the population, with only 4% of northern europeans affected. The low incidence of LNP among Europeans can be traced back to a genetic mutation which coincided with the spread of milk farming. There is however a significant diversity of LNP

incidence throughout Europe for example only 4% of Irish people are affected compared to 40% of Hungarians [22]. LNP was found in 99% of the population in China [23].

Lactose intolerance or lactase deficiency can arise from primary or secondary processes. Primary lactose deficiency includes the very rare congenital lactase deficiency (CLD) whereby lactase activity is extremely low or totally absent from birth. The more common form of primary lactose intolerance is the previously mentioned LNP. LNP is in fact a normal developmental phenomenon where lactase activity drops to about one tenth of its peak level subsequent to weaning. Both CLD and LNP are autosomally inherited traits. Secondary lactose intolerance occurs due to damage of the small intestine through various processes including acute gastroenteritis, chronic intestinal inflammation, coeliac disease or chemotherapy [24]. This condition is not permanent and will resolve when the intestinal epithelium heals [25]. Therefore generally when we are discussing lactose intolerance we are referring to the phenomenon of LNP.

### 3.4. Thresholds of Lactose Intolerance

Lactose intolerance is not to be confused with a milk protein allergy, a condition where even tiny amounts of milk protein can elicit symptoms in an affected individual. Lactose can be tolerated at varying degrees by individuals with lactose intolerance and symptoms are dose dependent [26]. Symptoms of lactose intolerance most commonly include abdominal pain, gut distension, borborygmi and flatulence [27]. Less common but significant symptoms include nausea, vomiting, diarrhoea and constipation. Symptoms may be triggered by the consumption of as little as 3g of lactose in highly sensitive people tested, however the majority of individuals affected by lactose intolerance or maldigestion can consume as much as 12g of lactose with little or no effects [28]. A 24g serving of lactose was found to lead to symptoms in the vast majority of individuals with lactose intolerance, but not all. Interestingly the placebo effect was found to play a considerable role in the perception of symptoms related to lactose ingestion [29].

The European Food Safety Authority Panel also concluded that lactose maldigesters can consume higher doses of lactose without symptoms when taken in multiple doses over the course of a day rather than a one off 12g dose [20]. It was also found that lactose ingestion may also lead to an increase in tolerance due to the adaptation of gut microbiota [30]. Consuming lactose with food also seemed to mitigate symptoms when compared to consuming it with water alone [31, 32].

Clinical diagnosis of lactose intolerance is not clear cut because the level of lactase activity does not correlate neatly with reported abdominal symptoms. This may be due to the impact of gut flora which differs greatly between individuals [33]. Self diagnosis based on reported symptoms has been found to be very inaccurate also [34]. Therefore in order to accurately measure an individual's level of lactose intolerance it may be necessary to perform multiple tests. Methods for diagnosing lactose intolerance include the hydrogen breath test, where the amount of hydrogen in a person's breath is measured after the consumption of lactose. Lactose intolerant individuals will exhale greater amounts of hydrogen after lactose ingestion as lactose will be fermented in the colon by bacteria which produce hydrogen gas, whereas in lactose absorbers lactose will not reach the colon and so is not fermented [35]. Other methods of diagnosis include the lactose tolerance test which measures the level of glucose in the blood after lactose consumption (which should not rise appreciably in individuals with lactose intolerance) [36], intestinal bowel biopsy where the lactase activity of a piece of intestine is measured [37], and finally by genetic testing for the C/T-13910 genotype which is most accurately detected using sequencing [38].

### 3.5. Lactose Intolerance and athletic performance

Although at the time of writing no studies have been performed specifically regarding the effect of LNP and LM on athletic performance post lactose ingestion, there have been studies which reflect this indirectly. Studies have been performed which demonstrate the negative effects of gastrointestinal symptoms on exercise performance [39, 40]. Another study performed in Myanmar made the finding that there was a vast overrepresentation of

lactose tolerant individuals found among the country's top athletes. Out of 324 athletes tested for lactose intolerance using the hydrogen breath test, over 70% were found to be lactose tolerant, in a country where up to 93% of the population has been found to be affected by LM. This translates to a greater than 30 fold increased likelihood for a lactose tolerant Burmese person to become a top athlete compared to that of one with LI or LM. [41].

### 3.6. Galactosaemia

While individuals with LI can suffer significant discomfort, pain and inconvenience post lactose ingestion, for individuals suffering from galactosaemia it can be a matter of life and death. Galactosaemia is a relatively rare condition caused by three separate congenital enzyme defects which influence the metabolism of galactose. It can result in both liver and kidney failure, as well as cataract formation in young infants. It can only be reversed by the removal of galactose from the diet. Due to the seriousness of this condition the EU has created harmonized limit values for the labels of infant formula, regulation (EU) 2016/127 states that 'The statement 'lactose free' may be used for infant formula and follow-on formula provided that the lactose content in the product is not greater than 2,5 mg/100 kJ (10 mg/100 kcal)'. These limits ensure that infant formula labelled as 'lactose free' can be safely consumed by patients suffering from galactosaemia. It must be noted however that milk products in which lactose has been hydrolyzed to its component parts glucose and galactose without removing the galactose are not safe for patients with galactosaemia irrespective of the residual lactose concentration. In the case of LI individuals lactose hydrolyzed dairy products they can be tolerated without any negative effects [20].

### 3.7. Lactose Free Market

The global demand for lactose free dairy products is on the increase with a projected CAGR of 8.7% from 2020 to 2025, resulting in an increase in global market value from

12.1 to 18.4 billion USD. This market segment faces stiff competition from the fast growing sector of plant based alternatives [42]. While estimates vary according to various market research companies, projected growth in the global sports supplements market is expected to be very strong among both the plant based and whey protein market sectors [43, 45].

### 3.8. Why is whey still popular?

Whey protein remains the most popular protein source in the global supplement market. This sustained popularity despite a fiercely competitive market can be explained by many factors such as: the superior biological value of whey protein when compared to other protein sources, especially those of plant origin [45]. This disparity in protein quality when measured by biological value can be mitigated by blending different plant proteins and some plant proteins such as pea protein compare well when using this method of protein assessment. Though there is a trend towards plant based products in general, there are undeniable benefits to the consumption of dairy products which are high in calcium, vitaminD and riboflavin amongst other beneficial nutrients and adequate supplementation should be sourced by individuals hoping to remove dairy from their diet completely [20].

Other scales for protein quality assessment differ and interestingly according to the Protein Digestibility Corrected Amino Acid Score (PDCAAS) system which is the gold standard for human nutrition according to the Food and Agriculture Organisation and the World Health Organisation [46], soy protein has a perfect score of 1, along with animal protein sources, whey, casein, and egg, and superior to beef which comes in at 0.92 [47]. This truncated PDCAAS system does not truly reflect the ability of high quality proteins such as whey to compensate for amino acid imbalances of inferior proteins. This is because it examines each protein source individually as a sole source of protein and does not take into account additional protein sources in a diet. For example only 1g of beef protein or 1.6g of milk protein is required to compensate for the low lysine level in 1g of gluten to bring it up to the requirements of pre-school age children of 58mg/g of mixed crude protein, however 6.2g of soy protein would be required for the same effect (Table 2) [48].

**Table 2:**

**The Biological value and PDCAAS for some common protein sources (Falvo and Hoffman., 2004).**

| Protein source | Biological value | PDCAAS |
|---|---|---|
| Whey | 104 | 1.0 |
| Egg | 100 | 1.00 |
| Casein | 77 | 1.00 |
| Beef | 80 | 0.92 |
| Soy | 74 | 1.0 |
| Black beans | 58 | 0.75 |
| Wheat gluten | 64 | 0.25 |

This table serves to compare the average measurements for both the biological value and the PDCAAS scores of common protein sources. Note the superior biological value of whey proteins.

Market analysis company Lumina intelligence have found that while whey protein still dominates the sports nutrition market where performance and muscle gain are paramount but plant based options have their own niche. For consumers focused on health and wellbeing as well as ethical and sustainable produce, plant based protein supplements have an advantage. One finding worth noting is that consumer satisfaction with plant protein powders is significantly less, frequently due to inferior texture and flavour [49].

The aim of this thesis is to investigate the lactose content of some ‘Lactose Free’ WPI products from popular sports nutrition companies on the Hungarian market using High-Performance Liquid Chromatography and a refractive index detector. With our findings we hope to highlight the importance of label compliance in the sports nutrition industry along with the need for effective testing and authentication of products on the market. These steps could effectively improve consumer confidence and would ensure the availability of safe WPI sports nutrition products for LI individuals.

### 3.9. Objectives/Questions

The objective of this study is to determine the lactose content of some popular ‘lactose free’ sports nutrition products and one WPC powder. We then intend to compare our findings with the existing labelling laws in Hungary regarding ‘lactose free’ limits to decide whether the products tested are compliant.

We hypothesise that lactose contamination will not be detected in the ‘lactose free’ WPI sports nutrition products tested as they are produced by a well reputed and popular brand, however we believe that lactose contamination may be prevalent in ‘lactose free’ whey based sports nutrition products from poorly regulated markets.

# 4. Literature review

## 4.1. Methods for lactose detection in dairy products

Various methods have been developed for quantifying the lactose content of dairy products through the years, however amongst these methods there is a large range of specificity and sensitivity. Older less sensitive methods include;

Polarimetry: a technique used to determine the concentration of chiral substances such as lactose in solutions using its optical rotation [50, 51].

Gravimetric analysis: which uses the conversion of copper sulphate to cuprous oxide and empirical tables to quantify the lactose content of a given sample [51, 52].

Enzymatic methods: Many different methods using enzymes to quantify lactose have been developed, the most common of which being the NAD enzymatic method. In this method lactose is split into its component parts glucose and galactose. Galactose is then oxidised to galacturonic acid using the enzyme β-galactose dehydrogenase in the presence of NAD which is reduced to NADH. The results of this interaction can then be quantified by measuring the absorbance of NADH at 340 nm using UV spectrophotometry. [50, 53]

Mid-infrared (MIR) analysis: Using this method we calculate the absorbance of infrared energy by hydroxyl groups in lactose to determine the quantity of lactose present in a sample. Older methods employed the use of optical filters to select the correct wavelength for lactose [54]. Newer methods use an interferometer which can obtain information on the complete spectrum within the MIR range by using Fourier Transform Infrared Spectroscopy (FTIR), which allows for in depth computational analysis of data collected [55].

High Performance Liquid Chromatography with Refractive Index detector (HPLC RI): This method was used in this study and it is among the most widely used techniques for the quantification of lactose and a variety of other carbohydrates in foods. HPLC boasts many advantages including the speed and simplicity of sample preparation. Carbohydrates, in this case lactose, are detected based on their absorbance of UV light at wavelengths below 200 nm. The disadvantages of HPLC in this case are that detection in this area of the spectrum has a relatively low sensitivity and specificity and expensive reagents are required. Refractive index detection allows for very straightforward measurement of results however it has been found to be non-specific and sensitive to various factors including solvent composition, temperature and pressure [50]. The Limit of Detection (LOD) has been found to be 250 mg/l and the Limit of Quantification (LOQ) 380 mg/l for RI [57]. The most widely used of these HPLC methods are based on reverse phase systems or cation exchange [58].

High-Performance Anion-Exchange Chromatography (HPAEC) with Pulsed Amperometric Detection (PAD): This is a fast and sensitive technique that uses the different pKa values of various carbohydrates as the basis for their separation. The LOD for lactose using this technique is less than 1 mg/l and so is a highly sensitive and effective method for quantifying lactose in lactose free products [50, 59].

### 4.2. Adulteration of whey products

Interestingly there is a dearth of studies pertaining to the adulteration of whey protein powders with lactose containing substances such as spray dried milk whey powder (MWP). The literature generally tends to focus on the adulteration of WPI and WPC with nitrogenous compounds such as urea, ammonium sulphate or melamine which can be used to artificially inflate the protein content of these powders when their protein value is determined with the commonly used Kjeldahl method [60]. Since whey protein has become a valuable commodity due to its increased popularity in recent years it has become

more of a candidate for adulteration with inferior products. Lactose has commonly been found in adulterated powders and so is worthy of investigation [55, 61].

### 4.3. Residual lactose determination techniques in recent studies

There are many studies regarding the detection of lactose in food products, but in recent times due to the increased awareness of LI and the growing 'Lactose Free' market, more studies have been focused on the detection of low levels of lactose. Researching the literature it is evident that there is debate within the scientific community regarding the relative efficacy of these different methods of detection. Trani *et al*. concluded that enzymatic methods as well as HPLC RI techniques are insufficient to detect residual lactose in 'Lactose Free' milk. They found Liquid Chromatography Tandem Mass Spectrometry (LC MS/MS) to be an extremely sensitive method with highly reproducible results, suitable for the detection of tiny quantities of lactose. Gille *et al*. have developed an enzymatic method with high sensitivity, suitable for the detection of lactose levels below 0.1%. They found that older enzyme based methods of detection were less accurate due to the interference of monosaccharides liberated by lactase during the manufacturing process. The removed the interfering glucose by oxidising it to gluconate with the use of glucose oxidase.

Contrastingly, scientists at PerkinElmer found that the HPLC RI method allowed for lactose detection at levels as low as 0.005%, only 50 ppm. These results would suggest that HPLC RI is suitable for the detection of residual lactose in lactose free products, but these results are not in line with most of our sources. One study used reverse phase HPLC and compared the sensitivity of UV detection against fluorescence detection for the determination of residual lactose concentration in skimmed milk samples in which the lactose had been enzymatically hydrolyzed. The two detection methods had a LOD of 0.2 mg per 100 ml, for UV detection and a LOD 0.013 mg per 100 ml for fluorescence detection from which they determined that both of these techniques are suitable for lactose detection in skimmed milk containing less than 1mg per 100ml of lactose [64].

A study from 2014 determined that a modified form of traditional HPLC called High Performance Thin Layer Chromatography (HPTLC) coupled with Fluorescence Detection (FLD) was the most streamlined of all methods on offer, boasting high matrix robustness, as well as excellent efficiency in terms of cost and time. This method was found to have simple sample preparation, took as little as 3 minutes and cost only 0.3 euro per analysis [65]. They argued that while some methods of lactose detection techniques such as capillary electrophoresis with electrochemical detection have been reported to have an LOD of as little as 0.1 mg/l, or HPAEC PAD which has reported LOD values for lactose as little as 0.12mg/l, they can also be time consuming with separation times taking 24 and 65 minutes respectively [66, 67]. One study using a Graphene Field Effect Transistor (G-FET) biosensor found they could detect lactose at concentrations as low as 200 attomoles (aM). They acknowledge however that the application of this incredibly sensitive method will more likely be found in the field of medicine (cancer research) as opposed to the food industry [68].

### 4.4. Review of current lactose determination technologies

A recent review published in November 2021 by Rao *et al*. examined the merits and drawbacks of the various traditional analytical approaches for the detection of residual lactose in lactose hydrolysed milks. Reviewing the literature they concluded that spectrophotometric techniques, while being fast and cost effective, were not suitably sensitive or accurate for detecting lactose in low lactose products. They found that the HPLC RI, while being the most commonly used (for reasons including widespread availability and ease of operation, along with the relatively low cost of RI detectors when compared to more specific MS or PAD detectors), lacked resolution during the separation process.

In the case of biosensors and enzymatic methods, overestimation of lactose concentration was found to be the main issue. In both cases, high concentrations of monosaccharides were found to interfere with the readings obtained. They recognised that HPAEC-PAD had

the greatest sensitivity, accuracy and reliability with detection of lactose levels as low as 100 mg/l (0.01%). However they did not find this method to be suitable for widespread adoption by the food industry due to the high cost of establishment and the greater technical expertise required by operators when compared to biosensors for example.

### 4.5. Lactose detection going forward

Ultimately they concluded that none of the techniques reviewed fully satisfy all the requirements for the detection of residual lactose in lactose hydrolysed milks. They believe that for the detection of residual lactose on an industrial scale there is a need to develop a new technology which couples the simplicity and rapid response achieved by biosensors with the accuracy and sensitivity of the HPAEC-PAD technique [69].

Most sources seem to agree that due to the growth of the lactose free market there is a great need to find fast and cost effective methods of lactose detection which are adaptable with regard to varying sample matrices presented by the large variety of ‘lactose free’ products [61-69].

## 5. Materials and Methods

### 5.1. Samples used

Six samples were collected for this experiment, two of which were ‘lactose free’ whey protein bars. The remaining four samples were composed of three ‘lactose free’ WPI powders and one WPC powder. All of the samples examined were produced by a popular sports nutrition brand, products are pictured and listed below:

**Protein bars tested:**

WPI bar cappuccino flavour

WPI  bar hazelnut flavour

**Whey protein powders tested:**

WPI powder pistachio flavour

WPI powder berry brownie flavour

WPI clear tropical fruit flavour

WPC powder

### 5.2. Brief Overview of the HPLC RI Method

HPLC analysis is a process whereby the components of a solution are separated in a column. The column contains the ‘stationary phase’ and a ‘mobile phase’ or the solvent is pumped through the column. When a sample is added to the HPLC system the various analytes in the sample are separated in the column based on their level of interaction with the stationary phase. As each of the analytes reach the end of the column they are registered by a detector, in our case a refractive index detector. The data registered by the detector is then sent to a computer system which represents the passing of these analytes through the column on a chromatogram.. On the chromatogram each analyte will be seen as an individual peak which occurs along the x-axis based on how long it took them to

travel through the column. The size of the peaks are representative of the concentration of that particular constituent of the sample (Figure 1).

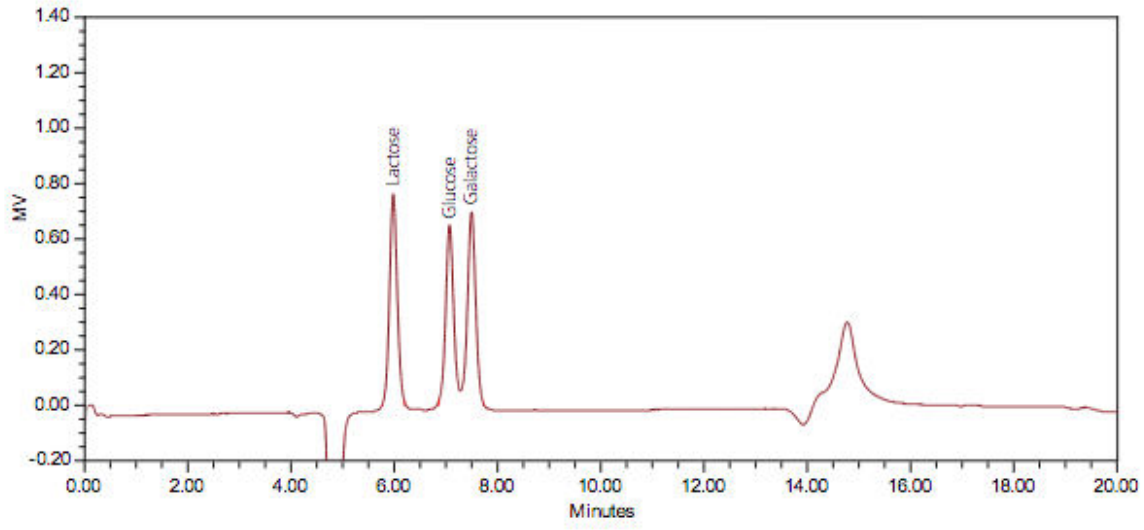


**Figure 1. Example of a chromatogram.**

This chromatogram was sourced from an Application Note by PerkinElmer [63]. The first peak on this chromatogram represents lactose which shows that it was eluted from the column first, followed by glucose and then galactose. These results are from the analysis of a standard solution of these three sugars at a concentration of only 50ppm.

### 5.3. Lactose Determination of Samples

In this experiment lactose determination was carried out in a Hungarian state laboratory and was performed using a High Performance Liquid Chromatography system and a refractive index detector (HPLC RI). In order to perform this analysis a number of calibration steps must be performed and HPLC parameters must be set which remain consistent throughout the sample analysis. These parameters include the flow rate, pressure and temperature of the column and the injection volume of the sample.

To calibrate the HPLC system a solution containing purified water and a known amount of lactose was prepared and from this a serial dilution was made. Each dilution in the

calibration series was prepared to range from our lower limit of measurement to the expected lactose content of non-lactose-free products. All of this, and the dilution ratio of the samples, was done by optimizing the linear measurement range of the refractive index detector to achieve reliable results. This is achieved by making a calibration curve, the linear fit of which ($R^2$) must be as close to one as possible.

The examination of the lactose-free products was achieved by preparing the most concentrated solution possible so that it could be easily filtered to the appropriate purity for the HPLC system. This allows for the detection if the product contains a minimal amount of lactose. Carrez I-II reagents were used for the preparation of the samples. The use of these two reagents helps to precipitate compounds which interfere with the analysis such as proteins and removes high colloidal turbidity, thus they function as a good clarifier. After the Carrez I and II solutions are added to the sample they form a precipitate which consists of Zn2[Fe(CN)6] which binds high molecular weight compounds by adsorption which are precipitated too. After the addition of the Carrez solutions the samples were filtered to remove the precipitate.

The sugar compounds dissolved in the sample were then separated by a HPLC system and detected by a refractive index detector. The chromatogram thus obtained was evaluated by means of a computer program.

## 6. Results

The lactose content of the collected samples as measured by High Performance Liquid Chromatography and refractive index detector are expressed in the table below.

**Table 3:**

**Results of residual lactose determination by HPLC RI**

| Sample | Lactose content |
|---|---|
| WPC powder | 1.6 g/100 g (±0.2) **or** 1600 mg/100 g |
| WPI powder - Tropical fruit flavour | <0.1g/100g **or** <100 mg/100 g |
| WPI powder - Pistachio flavour | <0.1g/100g **or** <100 mg/100 g |
| WPI powder- Berry brownie flavour | <0.1g/100g **or** <100 mg/100 g |
| WPI Bar cappuccino flavour | <0.1g/100g **or** <100 mg/100 g |
| WPI Bar chocolate hazelnut flavour | <0.1g/100g **or** <100 mg/100 g |

This table displays our findings regarding the residual lactose in each of our samples. Note that all of the products labeled 'lactose free' were found to contain less than 100 mg per 100 g which is in line with Hungarian law regarding the use of the 'lactose free' label. The WPC sample '100% pure whey' contained 1.6 g of lactose per 100 g with a margin of error of ±0.2 which was in line with the 1.4 g stated on the product label.

The lactose content of all five of the 'lactose free' products tested were in compliance with the 'lactose free' labeling standards set by Hungarian law which requires that these products contain <100 mg/100 g of lactose. The WPC '100% Pure Whey' was found to

contain 1.6g/100g with a margin of error of ±0.2g of lactose which was consistent with the values expressed on the label of the product (Table 3).

## 7. Discussion

### 7.1 Implications of our findings

Our results suggest that there were no irregularities regarding the lactose content found in the products tested in relation to what was stated on the labels. Each of the five WPI 'lactose free' products were found to contain <100 mg/100 g which is in compliance with Hungarian regulation regarding the use of 'lactose free' labels. The WPC powder that we tested contained measurable amounts of lactose which were in line with the contents as stated on the product label. However, our results covered only a very narrow product range and cannot be said to represent the broad range of 'lactose free' sports nutrition products available on the Hungarian market. As mentioned in the literature review section, there is a significant lack of studies on this topic, and we contend that much more research needs to be done in this field as we believe that it could contribute significantly to food safety. In fact only one study was found which directly investigated lactose detection in adulterated whey protein powders [61]. This study used time resolved and stationary fluorescence spectroscopic techniques to detect lactose, creatine and caffeine in WPC powders at concentrations of 10%, 20% and 30% w/w. This experiment was not successful regarding the detection of lactose adulteration in WPC samples however they suggest that a detector covering higher wavelengths would perhaps yield positive results. As previously mentioned, spectroscopic techniques are not suitable for detection of lactose at the lower limits suggested for 'lactose free' products and would instead be a method more suited to the detection of adulteration with large amounts of lactose.

Our choice of method for lactose determination was largely based on availability. The HPLC RI equipment used in our experiment, while sufficiently sensitive for the detection of residual lactose for the standards found in Hungary (the analysis was performed in a

Hungarian state laboratory after all), would not satisfy the 'lactose free' standards in many other European countries as we will discuss in greater detail below. Many, more sensitive methods are available for lactose detection in dairy products, however, as Rao et al. found in their report that there is need for lactose detection methods which are more suited to large scale development in the food industry. Effective food control in this area could be greatly bolstered by techniques which allow for sensitive, accurate and rapid analysis while remaining cost effective and simple to use.

### 7.2 Risk of economically motivated adulteration

This study hopes to highlight the risk of adulteration in products found in the sports nutrition industry. While we did not find any cases of lactose contamination in our study, we believe that it is wise for governing food control bodies to push for more stringent and harmonised regulation as the growing popularity of 'lactose free' products presents an opportunity for unscrupulous enterprises to profit by adulterating whey protein powders with cheaper ingredients of inferior quality. Common adulterants include non protein nitrogen compounds including urea, ammonium sulphate, melamine and creatine which artificially boost the protein content when analysis is performed by the popular Kjeldahl method. Milk whey powder containing a high proportion of lactose is another strong candidate as an adulterant due to its relatively low cost, and easy availability [55, 61].

This economically motivated adulteration is especially rife in poorly developed nations with limited food control and poor food safety standards. It must be noted that the risk of adulteration is not only an issue in developing nations as the competitive market places of developed nations could provide a profitable opportunity for producers of WPC and WPI who wish to cut costs and increase profits. This is one reason why it would be wise for the EU to adopt fixed standards across the bloc relating to lactose content in food. While whey protein powders are the focus of this study, protein powders derived from plants are also at risk of adulteration for the same reasons, and high lactose milk whey powder is a candidate as an adulterant for these products too [7]. As there is a significant risk of economically motivated adulteration in the sports nutrition market there is a need for more studies on a

wide range of products to determine the prevalence of lactose contamination in ‘lactose free’ sports products.

### 7.3 Limitations of our findings

This experiment was a small scale and low budget operation and was insufficient to offer any real insight into lactose contamination in sport nutrition products available in Hungary, however these shortcomings shed light on improvements that could be made in future studies.

#### 7.3.1 Sample size:

only six samples were examined for their residual lactose content in this experiment and only five ‘lactose free’ products were tested. A much larger sample size would be required to get an overview of the prevalence of lactose contamination among ‘lactose free’ sports nutrition products.

#### 7.3.2 Sample diversity:

All of the samples used in this experiment were produced by a single sports nutrition company. <0.1g/100g was found in all of the ‘lactose free’ products tested suggesting that there was no lactose contamination in the products tested. Perhaps this experiment would have yielded more interesting results had a greater variety of samples from different brands been tested. Four protein powder products and two protein bars were tested, however no ready to drink products (RTD) were investigated which would also be worth investigating.

### 7.3.3 Lactose detection equipment:

For our experiment HPLC RI techniques were performed by a Hungarian state laboratory to quantify residual lactose in the samples. There is no harmonised set of rules to regulate the claims of reduced or absent lactose in food products across the EU [70]. This means that ultimately it has been left for member states to individually determine limit values for lactose content in 'lactose free' produce.

The detection limit achievable in our experiment was 0.1g lactose per 100g which translates to a sensitivity of 100mg/100g. This detection limit satisfies the criteria set by Hungary's national food safety standards for the definition of 'lactose free', however it would not be sensitive enough to satisfy the food safety standards of several European countries as shown in the table below (Table 4).

**Table 4:**

**Threshold levels for the use of the term 'lactose free' in some European states**

| Country | 'Lactose free' threshold |
|---|---|
| Finland | 10 mg/100 g or 0.01% |
| Sweden | 10 mg/100 g or 0.01% |
| Norway | 10 mg/100 g or 0.01% |
| Iceland | 10 mg/100 g or 0.01% |
| Estonia | 10 mg/100 g or 0.01% |
| Germany | 100 mg/100 g or 0.1% |
| Slovakia | 100 mg/100 g or 0.1% |
| Hungary | 100 mg/100 g or ml or 0.1% |
| Ireland | No lactose present<br>No galactose present |

This table details the requirements enforced in different European nations in order for the term 'lactose free' to be used on normal food labels. As is mentioned in the text, different requirements exist for infant formula which has the standardised limit across all EU nations of 10 mg/100 kcal. All data in this table was found in the EFSA published paper - Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia.

As can be seen in the table above, the detection limit of the HPLC RI system used in this experiment does not have the sensitivity required to yield results which could determine the ‘lactose free; limit in the nordic nations of Sweden, Finland, Norway and Iceland. The Food Safety Authority of Ireland has the requirement that no lactose or galactose is to be contained in products labeled as ‘lactose free’. However in some EU nations the regulation is not so clear cut. In Italy for example ‘lactose-free’ labels can be applied to products if they contain residual lactose levels below 0.1g per 100g or 100ml, however other products with the same labeling use a lower threshold of 0.01g per 100g or 100ml. To complicate things further there is a ‘lactose-reduced’ label which is only applicable to milk or fermented milk products which require a lactose residue of less than 0.5 g per 100g or 100ml [70]. Not only is there no official EU requirements regarding lactose free labelling, there is also an absence of official and standardised methods for the determination of lactose as we have discussed in the literature review section. This lack of clarity at the EU level has led to the proliferation of many dairy products which claim low, reduced or lactose-free contents but with different limits [71, 72].

### 7.4 Need for EU action

We hope that the findings of this study clarify many aspects of this controversial topic which needs to be addressed by the EU in the near future. We have highlighted the need for the development of EU wide standards for the use of the terms ‘lactose free’, ‘low lactose’ and ‘reduced lactose’ both in relation to labeling and the adoption of appropriate technologies for residual lactose detection. If the EU ultimately adopts limits in the lower range for ‘lactose free’ products such as those found across nordic nations, much of the technology used for lactose detection in nations with a higher limit such as Germany and Hungary would be outmoded (such as the technology used for our experiment!) the consequence of which could compel them to invest heavily in more accurate lactose detection technology.

## Conclusion

Our study did not detect lactose contamination in any of the ‘lactose free’ sport nutrition products tested using the HPLC RI method. We believe that a larger and more diverse sample size may have yielded different results. However, the risk of lactose adulteration in whey protein based sport nutrition products is significant which poses a threat to the safety of individuals with LI or galactosaemia. Consumers should be advised to source their sports nutrition from trusted outlets and brands as products from poorly regulated markets are at greater risk of adulteration. We believe that the adoption of harmonised laws for the labeling of ‘lactose free’ products and of the methods used to determine their status as such would make a significant contribution to food safety across the EU. This study has identified a number areas which require further scientific investigation such as:

The link between lactose intolerance and athletic performance.

The prevalence of lactose contamination in whey protein based sports nutrition products across the EU and how this relates to national regulation.

Determination of the most appropriate limit to set for ‘lactose free’ labeling with regards to food safety and the technology available for lactose determination.

## Summary

For this experiment we determined the residual lactose content of five lactose free WPI based sports nutrition products as well as one WPC product. Analysis was performed using the HPLC RI method by a Hungarian state laboratory. The results obtained from this study suggest that all products tested contained concentrations of lactose which were consistent with those stated on the label. All of the lactose free products tested contained <100 mg per 100 g which complies with Hungarian law regarding the labeling of 'lactose free' products. The WPC product tested contains 1600 mg per 100 g of lactose with a margin of error of 200mg which was also in line with the 1.4 g of sugars (primarily lactose) stated on the label. The HPLC RI technique employed was sufficient to accurately quantify concentrations of lactose as low as 100 mg per 100 g. However this equipment was not sensitive enough to perform residual lactose determination in countries with stricter 'lactose free' criteria e.g the <10 mg/100g limit found in nordic countries. We believe that the EU wide adoption of standardised labeling laws regarding 'lactose free' products could make a significant contribution to food safety and facilitate trade across the region. There is also a need for the standardisation of the methodology employed for lactose determination in food products at the EU level. For future studies we would suggest analysing the lactose content of 'lactose free' products from a much broader range of sports nutrition brands and product types such as RTD formulas as well as traditional whey protein powders and bars. While we did not detect any traces of lactose contamination in our samples, the literature does indicate that there is a significant risk of the economically motivated adulteration of whey protein based sports nutrition products and for this reason we believe that this area merits further research.

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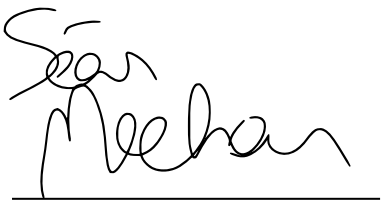

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- *facilitate professional relations and collaboration;*
- *support open access.*

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I hereby confirm that I am familiar with the content of the thesis entitled ***Examination of lactose content of lactose free products intended for athletes*** written by **Seán Robert Meehan**, which I deem suitable for submission and defence.

Date: Budapest, 16th of November 2021

…….
Pleva Dániel
Supervisor name and signature

…….
Department of Food Hygiene
University of Veterinary Medicine Budapest