Journal of Elementology ISSN 1644-2296

Kawęcka A., Pasternak M. 2020.
Nutritional value of żentyca, traditional Polish sheep whey product – preliminary results.
J. Elem., 25(4): 1509-1516. DOI: 10.5601/jelem.2020.25.3.2039

RECEIVED: 14 July 2020
ACCEPTED: 4 September 2020

ORIGINAL PAPER

NUTRITIONAL VALUE OF *ŻENTYCA*, TRADITIONAL POLISH SHEEP WHEY PRODUCT – PRELIMINARY RESULTS

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Abstract

In Poland, whey from the production of traditional mountain cheeses is used to make *żentyca*, one of several traditional products made from milk from Polish mountain sheep raised in the Polish Carpathian Mountains. Żentyca is produced by heating whey to 90°C, that is the temperature at which the whey proteins coagulate and, by being lighter, they form a tender skin on the surface of liquid heated in the cheese vat. This skin is removed from the surface and with a little of remaining whey it is poured as żentyca into a wooden barrel, where it undergoes fermentation by own natural microflora. Research on this unique product is very rare, so the purpose of our study was to evaluate the nutritional and dietary value of *żentyca* depending on the production date. The samples were taken in shepherd's huts located along the "Oscypek Trail", during the sheep grazing period in the Carpathian Mountains, at the beginning (May) and the end (September) of the period. The tested traditional product was characterized by high mean dry matter content and low pH. The study proved that the month in which *żentyca* was made had an impact on the product quality. Żentyca made from milk produced at the onset of the grazing period contained more protein, lactose and phosphorus but less fat than the product made in the late grazing period. The production month also had influence on percentages of individual protein fractions, and over 60% of the analysed amino acids had varied shares in the protein depending on the żentyca production date. It was found that *żentyca* is a product of high nutritional and dietary value which depends on the period of production.

Keywords: sheep, traditional product, whey, żentyca, nutritional value.

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INTRODUCTION

Keeping mountain sheep in the area of the Polish Carpathians and processing sheep milk have remained unchanged for centuries, sustaining the pastoral tradition. Sheep are milked by hand on mountain pastures, and white milk is processed to cheese after milking. *Bundz, bryndza podhalańska, oscypek and redykołka. bryndza podhalańska, oscypek* and *redykołka* are protected by the European Union as Protected Designation of Origin (PDO), denoting a product created in a specific place, possessing the characteristics associated with a given region. Fresh, rennet *bundz* is very tasty, although less durable. Under the influence of characteristic strains of bacteria from the *Lactobacillus* group, the *bundz* matures for a few days, then it is crushed, salted and ground to make *bryndza,* which can be kept longer. When the whey formed during the cheese production is heated, the whey proteins coagulate and a very tasty and nutritional product is made, *żentyca* (Kawęcka et al. 2020). Slovak žinčica is similar; it is a beverage with kefir-like consistency made from whey with milk in a similar way as in the Polish Carpathians, (Laukowa et al. 2020).

Żentyca is a seasonal product, made only during the grazing season of mountain sheep in the Carpathian Mountains, from May to September. It enjoys tremendous popularity among tourists visiting the Polish mountains. It is treated as a curiosity, but its nutritional and health values have been known for a long time, and the production method has not changed for centuries. It is drunk warm, just after "cooking" or after a few days of fermentation, which gives it a typical sour-tart taste. It is served to tourists in shepherd's huts in wooden mugs. The amount of whey, coagulated whey proteins, and consequently the density and composition of *żentyca* depends on the maker and differs among individual shepherd's huts (Drożdż 2007).

Żentyca is made by heating the whey left from production of mountain cheeses. The whey protein coagulates at 90°C and, by being lighter, it forms a tender skin on the surface of liquid heated in the cheese vat. Unlike Italy's *Ricotta*, which is made by filtering the whey proteins left after coagulation on a strainer (Scatassa et al. 2018), fresh *żentyca* is poured every day, after each milk processing, to a wooden barrel, where thanks to own natural microflora it undergoes fermentation (acidification).

The purpose of the study was to evaluate the nutritional and dietary value of *żentyca*, a traditional whey product made from the milk of mountain sheep raised in the Polish Carpathian Mountains, depending on the production date.

MATERIALS AND METHODS

The material for the study was collected in shepherd's huts along the so-called "Oscypek Trail", which brings together over 30 huts producing traditional sheep milk products, where tourists can learn the production methods. Participation in this cultural trail offers an opportunity to try the genuine products. The *żentyca* samples for studies were taken during the grazing period of mountain sheep in the Polish Carpathian Mountains. The samples were taken twice during the grazing season: at beginning (May) and near the end (September) of the season. The samples were taken in three shepherd's huts, from wooden barrels in which żentyca is stored to test tubes. The samples from each shepherd's huts were taken for three consecutive weeks and then transported to the laboratory for analysis. A total of 18 samples were evaluated.

The density was determined using a lactodensimeter; the *żentyca* pH was measured using a CP-411 Elmetron digital pH-meter (Poland). The *żentyca* chemical composition, that is dry solids, protein, fat, lactose, ash and phosphorus and titratable acidity, was determined according to AOAC (2006). The protein fractions were separated using the SDS-PAGE gel electrophoresis. The separation was made in a Mini Protein 3Cell from BIORAD and the analysis was performed using the software for gel electrophoresis Gelscanversion 2.0 from Kucharczyk. The amino acid profile was determined using the reverse-phase liquid chromatography and the ACCQ Tag kit from Waters. The chromatographic separation was performed with the use of a Dionex Ultimate3000 liquid chromatograph from ThermoScientific, equipped with an LPG – 3400 SD gradient four-channel lamp, WPS 3000 TSL autosampler, and an FLD-3400RS detector–Termo Scientific fluorescence detector. The results were processed with the use of the Chromeleon 7.0 software.

The results were developed statistically with the use of Statisica package, ver. 10., applying a one-way analysis of variance. The Tukey's test was used to locate significant differences between the means. The testing was performed at the significance level $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

First and foremost, it should be noted that the tested food product is not whey. Żentyca is a product formed from whey, or more precisely only this portion of whey which separates during the heating process and is collected as its upper layer. Research into this unique product is very rare, so in our considerations we had to refer to the properties of sheep's milk sheep's milk products. Table 1 presents the chemical composition and selected parameters of the examined product.

Table 1

Chemical composition, acidity and pH of *żentyca*

Item	May	September
Dry matter (g kg^{-1})	15.08±1.98	14.18±0.51
Fat (g kg^{-1})	5.65±1.18^{A}	6.84±0.53^{B}
Protein (g kg^{-1})	3.37±0.46^{A}	2.71±0.09^{B}
Ash (g kg^{-1})	0.59±0.02	0.53±0.03
Lactose (g kg^{-1})	5.47±0.57^{A}	4.09±0.11^{B}
Fosfor (mg g^{-1})	64.21±3.4^{A}	50.38±1.87^{B}
Acidity (°SH)	11.98±0.53^{A}	15.11±1.87^{B}
pH	4.67±0.11	4.73±0.46

Means (± SD) with different letters in a row are significantly different: *A*, *B* – $p \leq 0.01$

The mean dry matter content of this regional Polish product was 14.63%, which was double the result obtained by Paciorek and Bonczar (2001). According to Laukova et al. (2020), two types of Slovak *žinčica* differ in chemical composition. Acidic *žinčica* contains 11.8% DM, 4.9% fat, 2.4% protein, 2.6% lactose, 0.40% ash, whereas sweet žinčica has a DM content of 12.2%, a fat content of 5.1%, protein 2.6%, lactose 3.8% and ash 0.41%.

The production date affected the content of the remaining ingredients: the fat content was higher ($p \leq 0.01$) in *żentyca* produced in September, and the content of protein, ash and lactose was lower ($p \leq 0.01$) in September. Similar values of basic ingredients were found by Rzepka et al. (2013). The *żentyca* acidity was higher ($p \leq 0.01$) in September, but the production month did not affect the pH – Table 1. According to Skryplonek and Jasińska (2017), the rennin whey (sweet) obtained by enzymatic coagulation of milk has acidity of about pH 6-7; the sour whey, a by-product from milk solidification by acidification, has the pH below 5. The traditional product tested in our study had low pH (4.6) (Table 1). Rzepka et al. (2013) found differences in both the pH and acidity between *żentyca* produced in June and in September, a result of different fermentation conditions in shepherd's huts in various seasons. Kawęcka et al. (2020) claimed that the month of milking had an impact on the content of most milk ingredients. The content of dry solids, fat, protein and casein increased with lactation, and the content of lactose was the lowest in September. The acidity of mountain sheep's milk, and also of the tested traditional product, increased in September. However, the mountain sheep grazing season did not affect the basic composition of traditional cheeses made from milk (Kawęcka, Sosin-Bzducha 2014). The month of production had an impact on the content of phosphorus in the tested product, being the highest in May (Table 1). Phosphorus is a mineral which in general is not deficient. In fact, the phosphorus oversupply is a more serious nutritional problem. The products which are a good source of calcium

usually supply significant amounts of phosphorus, and phosphate additives are generally used in dairy products, particularly in processed cheeses (Kaczkan et al. 2018). The phosphorus content in the natural sheep whey product was low, hence the product can be consumed in larger quantities, but the seasonality and regional occurrence limit the access to this nutritious product.

During the cheese making, more than 50% of solids go to whey: lactose, most minerals and about 20% of milk proteins (Skryplonek, Jasińska 2017). The proteins are composed of soluble whey proteins and contain only trace amounts of casein, which forms the cheese curd. The casein proteins were from 10.42 to 15.54% of all proteins in the tested product (Table 2).

Table 2

Fractions of protein (% of total protein)

Item	May	September
Immunoglobulin	7.46 ± 0.18^{A}	3.89 ± 0.51^{B}
Lactoferrin	4.62±0.78	4.04±1.29
Serum albumina	9.17±1.51	10.67±1.77
Casein protein	10.42 ± 2.42^{A}	15.54 ± 0.63^{B}
β-lactoglobulin	56.65 ± 4.75^{A}	49.70 ± 3.58^{B}
α-lactoalbumin	11.67 ± 1.05^{A}	16.13 ± 0.77^{B}

Means (± SD) with different letters within a row are significantly different: A, B – $p\leq0.01$.

For the pro-health aspect of food, the presence of functional whey proteins is particularly important. They constitute 20-25% of milk proteins, of which 75% are albumins, i.e. α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and serum albumin (Król, Brodziak 2015). The whey proteins include anti-microbial proteins, such lactoferrin, lactoperoxidase and lysozyme, which show multidirectional actions on the human organism, preventing many civilization diseases (Hernandez-Ledesma et al. 2011). A few protein fractions were identified in the tested product (Table 2). Analysing seasonal changes of whey protein composition in the goat and sheep milk, Hejtmánková et al. (2012) stated that the main ingredient of the sheep whey was β-lactoglobulin, whereas α-lactalbumin prevailed in the goat whey. At the end of the lactation period, the content of β-lactoglobulin increased in both the sheep and goat milk. Analysing the composition of whey from production of sheep and goat cheeses, Casper et al. (1998) found differences in proportions of individual fractions between the raw material obtained from these ruminants. The whey from production of the Manchego cheese contained 74% of β-lactoglobulin, and the goat whey only 59%. In our study, β-LG made up more than a half of proteins (52.8%) – Table 2. The aforementioned authors found that the amounts of α-LA reduced during the season, but the amounts of β-LG grew during the lactation, and then gradually reduced at the end

of the lactation period. The relative proportions of serum albumin stayed rather stable during the year, and the content of immunoglobulin was reduced at the end of the season. The changes in these two fractions in *żentyca* were similar: the immunoglobulin content was reduced by half, and the serum albumin content practically did not change (Table 2). In terms of biological activity, lactoferrin is the most important whey protein (KUCZYŃSKA et al. 2013), characterized by many properties such as forming a bacteriostatic environment or performing the immune-modulation functions, preventing for instance the formation of β-amyloid deposits during the Alzheimer's disease. It also has anticancerogenic, antiviral, opioid, antifungal and probiotic properties. The lactoferrin content in *żentyca* did not change depending on the production date and amounted to 4% of total proteins (Table 2).

The whey proteins have high nutritional value due to a high content of essential amino acids (EAA). The content of amino acids in the product's protein is given in Table 3.

Table 3

Amino acid composition of *żentyca* (% of total amino acid)

Amino acid	May	September
Alanine	6.52 ± 0.22^{A}	3.67 ± 1.01^{B}
Arginine	3.74 ± 0.48^{A}	4.27 ± 0.43^{B}
Asparagine	10.27±0.62	9.66±0.85
Cysteine	2.92±1.12	3.22±1.32
Serine	4.52 ± 0.19^{A}	4.88 ± 0.26^{B}
Glutamine	15.50 ± 0.80^{A}	16.82 ± 0.63^{B}
Glycine	2.89 ± 0.32^{A}	2.48 ± 0.26^{B}
Histydine	2.29±0.19	3.24±0.55
Isoleucine	5.46 ± 0.13^{A}	6.04 ± 0.21^{B}
Leucine	10.58±0.36	10.74±0.28
Lysine	9.56 ± 0.61^{A}	13.62 ± 1.99^{B}
Methionine	6,91±0,75	6.82±0.16
Phenylalanine	3.89 ± 0.37^{a}	3.47 ± 0.41^{b}
Proline	5.06 ± 0.82^{A}	1.95 ± 0.58^{B}
Threonine	6.09±0.56	6.76±0.17
Tyrosine	1.83±1.37	1.92±1.39
Valine	6.91±0.31	6.82±0.17

Means (± SD) with different letters within row are significantly different: *a*, *b* – $p\leq0.05$, *A*, *B* – $p\leq0.01$.

The largest share in the *żentyca* protein was composed of the endogenous glutamic acid (16.16%) and lysine (11.41%) was the most prevalent essential protein. The production month influenced the product's amino acid profile.

Over 60% of the analysed amino acids had a varied share in the protein, depending on the *żentyca* production date. The EAA constituted from 37.5 to 39.5% of all amino acids in the tested product (Table 3). Hejtmánková et al. (2012) stated that essential amino acids constituted 37.61% of sheep milk's whey proteins. Analysing the impact of the applied technology on the nutritional value of soft sheep cheeses, Pakulski and Bagnicka (2017) determined the EAA content at 41.6 - 45.3%.

In recent years, when active lifestyle and intensive exercise have become popular, dietary supplements and nutrients are increasingly often taken by people who want to be fit and healthy. Such products contain Branched--Chain Amino Acids (BCCA), including three indispensable amino acids: leucine, isoleucine and valine. Branched-chain amino acids are one of the most important materials of which our muscles are built. They play a key part as metabolism regulators for lipids, protein and glucose, and are directly metabolized by the skeleton muscles, promoting the protein synthesis, maintaining the muscle mass and generating energy during prolonged effort (Yasmin et al. 2013). The BCAA can be an excellent training support because in addition to the building role, they perform the regulatory function in secretion of hormones and catecholamines, formation of neurotransmitters or simulation of protein synthesis. Leucine is particularly important in this regard, as it can stimulate the synthesis of muscle proteins. An increased leucine concentration stimulates the synthesis of muscle proteins by increasing their sensitivity to insulin Garlik (2005). The leucine content in the tested product did not depend on the production date, and on average corresponded to 10.66% of proteins (Table 3). The leucine content in the sheep and goat milk reached from 9.2 to 9.9%, and in sheep cheeses was 8.55 - 9.16% of milk proteins (Hejtmánková et al. 2012, Pakulski, Bagnicka 2017). This means that *żentyca* tested during this experiment had a high content of this ingredient.

CONCLUSIONS

1. The month of production had influence on the quality of the regional product made from mountain sheep milk – *żentyca*.

2. The product made from milk obtained at the beginning of the grazing season (May) contained more proteins, lactose and minerals but less fat than the product made at the end of the season (September).

3. The month of production had varied influence on the share of individual protein fractions, amino acids profile, but had no influence on the share of bioactive whey protein components, i.e. lactoferrin and serum albumin, and essential amino acids – valine and leucine.

4. Summarizing these preliminary results, it can be said that *żentyca*

is a product of high nutritional and dietary value, which depends on the time when it is produced.

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