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Production of selenium-enriched milk and dairy products

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Abstract. Until the middle of the last century, selenium was considered to be toxic, but recently it turned out to be a micronutrient with important physiological effects, whose lack impedes the functioning of several enzymes, while in the case of a prolonged deficiency, disease processes can also occur in the body. Hungary belongs to the selenium-deficient regions in Europe; therefore, our aim was to contribute to the improvement of selenium supply of the population through increasing the selenium content of milk and dairy products. A daily supplementation of 1-6 mg organic selenium to the feed of dairy cows increases the selenium content of milk from the value of 18 μg/kg to 94 μg/kg in 8 weeks, decreasing again to the initial value in 6 weeks after stopping the supplementation.

After producing various products from the control milk (18 μg/kg selenium content) and the selenium-enriched milk (53 μg/kg) obtained from dairy cattle fed on a feed supplemented with 2 mg selenium/day, we concluded that the selenium content of selenium-enriched milk compared to the products produced from the control milk increased from the value of 18.6 to 58.5 μg/kg in the case of yogurt, from 66.0 to 138.1 μg/kg in the case of *telemea*, from 80.8 to 163.7 μg/kg in the case of *orda* (urdă) and from 88.6 to 200.0 μg/kg in the case of semi-hard cheese obtained by mixed-coagulation. The selenium content of whey also increased significantly (from 8.8-9.7 μg/kg to 20.1-25.8 μg/kg), which could also be used as a food for people or feed for animals. According to our calculations, the selenium requirements of the developing organism could be satisfied by the consumption of 2-3 dl selenium-enriched milk until the age of 8 and with 4-6 dl selenium-enriched milk until the age of 20.

1 Introduction

Until recently, selenium was considered to be a toxic heavy metal because the consumption of larger amounts of it leads to the devastation of the living organism. Recently, however, with the improvement in sensitivity of analytical methods, it could be found that it has important physiological effects since the human body itself contains about 15 mg of selenium, which, together with some tocopherols, is involved in metabolic processes. The body's antioxidants play an important role in the defence against harmful free radicals; these natural protective mechanisms are involved in eliminating the harmful

effects of free radicals. Parts of these protective mechanisms, for example the glutathione peroxidase and thioredoxin reductase, are selenium-dependent enzymes, which, in the absence of selenium, are not able to neutralize the harmful and sometimes carcinogenic components. Glutathione peroxidase protects unsaturated lipids and helps preserve the integrity of cell membranes by catalyzing the peroxide degradation reaction. It was discovered in the recent decades that selenium is necessary for normal life activities and helps in treating certain forms of cancer and even in their prevention. Foods produced in Scandinavia and some other European countries are very selenium-deficient. The selenium amount entering into our bodies through our daily meals (0.05-0.10 mg) is not significant.

The relative atomic mass of selenium is 78.96 g. Among its isotopes, the isotope ^{80}Se occurs more frequently. Depending on the environmental influences, it occurs with the following oxidation states: -2, 0, +4, +6, (*Skinner*, 1999). In 1930, selenium was considered to be toxic and in 1943 its carcinogenic properties were also described (*Nelson et al.*, 1943). *Clayton & Baum* (1949) showed that the presence of selenium is essential in the living organism and selenium supplementation reduces the number of cancer diseases. The essential role of selenium was pointed out in 1957 (*Schwarz & Foltz*, 1957), when it was found that selenium added to the diet could prevent liver necrosis. In 1966 (*Shamberger & Rudolph*, 1966), the anticarcinogenic effect of selenium was also published, but at that time the total selenium content of the food was mentioned. In 1973 (*Turnder & Stadtman*, 1973), the catalytic activity of selenium-dependent enzyme proteins was studied, among which the glycine reductase plays a significant role in anaerobic bacteria, while the glutathione peroxidase (*Rotruck et al.*, 1973) plays a significant role in mammals. In 1976 (*Cone et al.*, 1976), the glycine reductase and the seleno-cysteine, the selenium analogue of cysteine, were discovered.

Researchers found that it is not possible to conclude the selenium supplementation suitability of a given biological system from its total selenium content as the different chemical modifications considerably differ from each other due to their toxicity, absorption and availability in the human body. Therefore, if we try to get information about their availability in the human body, each of the different chemical modifications must be determined (*Thomassen & Nieboer*, 1995). There is a need to consider the oxidation state of selenium, the distribution of metal complexes and the relationship with chelating agents, as these effects can significantly influence availability. It is therefore necessary to examine the distribution, quality and quantity of selenium modifications with the help of speciation analysis.

Foods of plant origin contain only selenomethionine, while those of animal origin contain both selenomethionine and seleno-cysteine. As methionine is an essential amino acid for humans as well as for animals, they cannot produce selenomethionine from inorganic sources, but the selenomethionine entered into the body can be converted into seleno-cysteine (*Beilstein & Whanger*, 1986). In addition to these seleno-amino acids, the derivatives of these, seleno-methyl-seleno-cysteine and γ-glutamil-seleno-methyl-seleno-cysteine, are also present. These two components occur in greater concentrations in the food of vegetable origin if the soil is treated with a significant amount of fertilizer.

Selenium is present in the human diet in the form of selenite and selenomethionine. After entering into the human organism, it reacts with thiols, in a reaction which is catalyzed by the enzyme glutathione. In the reduction process, selenate converts into selenite, and then hydrogen-selenide is formed. During the process of trans-sulphurization, seleno-cysteine will be formed from selenomethionine through seleno-cystationine. Due to the effect of a β-lyase enzyme, the seleno-cysteine will be broken down into hydrogen-selenide. In another pathway, it will be metabolized through trans-amination and decarboxylation, and about 90% of it gets incorporated through non-specific means into the organism (*Mitchell et al.*, 1978). During the reaction with S-adenosyl-methionine, methyl-selenol is formed from methyl hydrogen, which then converts into dimethyl selenide first, and then into trimethyl-selenonium ion (*Janghorbani et al.*, 1999). When treating plants with large amounts of selenium, γ-glutamyl-methyl-seleno-cysteine is formed primarily, whose availability is similar to that of methyl-seleno-cysteine. Due to the effect of β-lyase enzyme, the methyl-seleno-cysteine becomes converted into methyl selenol (*Dong et al.*, 2001). Assuming an average selenium intake, the excess selenium will be excreted through the urine in the form of amino sugars, but, if it enters into the organism in large amounts, it can also be excreted via the lung in the form of dimethyl-selenide, while through the urine in the form of trimethyl-selenonium ion (*Suzuki & Ogra*, 2002; *Kobayashi et al.*, 2002; *Bendhal & Gammelgaard*, 2004).

Selenium acts as an antioxidant through the binding to various enzymes. Skeletal muscle proteins integrate even the selenomethionine and seleno-cysteine from external sources (these are selenium-containing proteins), but those seleno-proteins which play an active role in metabolism can use only the seleno-cysteine formed in the body (*Levander & Burk*, 1996). These seleno-proteins are able to operate solely in the presence of selenium and their amount significantly decreases in the case of a selenium-deficient diet. The role of seleno-proteins in biochemical processes became well known already in the

last century; this way, seleno-protein P, iodotironin-deiodinase, thioredoxin reductase and seleno-phosphate synthase have also been identified (*Allan et al.*, 1999).

In 1975, *Awashti et al.* identified the antioxidant glutathione peroxidase, and it was found that it is present in all of the human tissues and that its function is primarily determined by the amount of reduced glutathione and the selenium supply of the body (*Meister & Anderson*, 1983). It was found about glutathione peroxidase that it protects the structure of the membranes, it inhibits DNA damage, reduces the formation of carcinogenic substances in the body through the removal of hydrogen peroxide as well as lipid and phospholipid hydroperoxides.

We know today that selenium integrates into the enzyme as seleno-cysteine, occupying the location of sulphur, and – as it gets reduced more easily than sulphur – it plays a very important role in the defence system of the body against oxidation (*Cser et al.*, 1998). In the last century, four selenium-containing glutathione peroxidases have been identified, of which all have antioxidant properties (*Holben & Smith*, 1999). The administration of selenium increases the activity of these enzymes, which, measured in platelets, serves as a good indicator of the body's supply of selenium. The selenium-containing iodotirozin-deiodinase enzyme plays an important role in the synthesis and activation of tri-iodide-iodothyronine and thyroxine hormone; therefore, the selenium is strictly necessary for normal development and growth (*Wilson et al.*, 1998; *Holben & Smith*, 1999). The thioredoxin reductase is involved in the regeneration of the organisms' antioxidant system, it converts dehydro-ascorbic acid back into ascorbic acid and – with the reduction of thioredoxin – plays an important role in the regulation of cell growth (*Holben & Smith*, 1999; *Mustacich & Powis*, 2000). Seleno-protein C has likely a role in the transmembrane transport processes and, due to its antioxidant effect, it protects the endothelial cells against the chemical attack of peroxy-nitrites.

From the literature data, it appears, therefore, that the availability and utilization by the body of metallic and inorganic state of selenium is very low, firstly because of limited absorption from the gastrointestinal tract and, secondly, because much of the absorbed selenium-containing compounds will be excreted in the urine, while the forms not excreted in the urine are of limited availability. The selenomethionine form of selenium, which is formed in plants due to the selenium content of the soil and which animals convert into seleno-cysteine, can be well utilized. In addition to these two forms of selenium, there exist some other selenium-containing compounds too, but, according to the literature, their use as selenium supplementation is very low. Due to the

above-mentioned facts, during the selenium supplementation of the Hungarian population, it is advisable to use cystine and methionine selenium analogues.

There are two possibilities to supplement the body with selenium: firstly, with food of plant and animal origin, and secondly with the consumption of foods with increased selenium content. Despite the fact that our foods contain selenium in very various amounts, in general, it can be stated that our most commonly consumed foods have very low selenium content. The richest source of selenium is considered to be animal organs (liver, kidney), marine fish, crustaceans and finally meat. There are certain foods that are distinguished by their high selenium content. Thus, Brazil nuts may contain even 100 μg of selenium (*Chang*, 1995, a, b). Since plants do not require selenium for the normal functioning of their organism, the selenium content of these can vary significantly.

The selenium content of foods of vegetable origin are affected by the selenium content of the soil and its oxidation forms, the pH of the soil, which affects even the different selenium forms, the organic compounds of the soil, the iron and aluminium content and rate of the soil, which can bind selenium, the content of sulphur-containing compounds, which, if present in large amounts, can inhibit the absorption of selenium from the soil, precipitation, which can wash out the selenium from the soil, and microorganisms which can convert insoluble selenium compounds into soluble forms.

Because of the fact that the selenium content of most of the foods is very low, with the increasing of their consumption, the selenium supply of the body cannot be increased, the selenium requirement of the body can be satisfied with dietary supplements, while, on the other hand, with selenium-enriched foods. Dietary supplements spread in the 80s of the past century and the well-controllable manufacturing technology of present day makes highly utilizable products available for the consumers in the form of encapsulated or tablet supplements (*Horacsek et al.*, 2005). These products contain mainly selenite, selenate, selenomethionine or selenium-enriched yeast.

Selenium-enriched foods with particular nutritional uses contain selenium in its natural or close-to-natural form. The production technology of such foods is extremely complicated as the selenium supplementation added to the feed or prepared for plants suffers several modifications and transformations until it reaches its natural form. During the transformation, the oxidation state of selenium may change; thus, it is important to monitor in what form the plant or animal nutrient contains selenium. The following selenium-enriched products are on the market in Hungary: selenium-enriched cereal, egg, margarine, bread, respectively bakery products. There appeared many data in

the literature regarding the selenium-enrichment of foods of animal and plant origin (*Gergely et al.*, 2004; *Shah et al.*, 2004; *Rayman*, 2000, 2002) and the supplementation of chicken meat, egg, broccoli, onion and wheat has also been reported.

According to *Schauzer* (2000), the most widely traded selenium supplementation is selenium-enriched yeast. The enrichment can be carried out with sodium selenite, sodium hydrogen selenite, sodium selenate and selenomethionine. The selenium content of selenium-enriched yeast may reach the value of 3,000 mg/kg, integrated mainly as selenomethionine into the yeast proteins (*Polatajko et al.*, 2004); on the other hand, methyl seleno-cysteine is present only in small amounts in the selenium-enriched yeast (*Kotrebai et al.*, 2000).

The selenium-enriched yeast is the result of the fermentation process of *Saccharomyces cerevisiae* in a medium with high selenium content, where the broth is usually a solution with high sugar content, containing usually sodium-selenite as selenium source. In order to assure an optimal growth of the yeast, the broth is supplemented with vitamins, minerals and nitrogen-containing compounds. After producing the selenium-enriched yeast paste, the yeast cells are killed through a heat treatment procedure and the paste is dried by spray-drying, obtaining this way a product which contains organically bonded selenium in high concentration. After the manufacturing process, the inorganic and organic selenium content has to be controlled as it affects primarily the quality of the end-product. The inorganically bonded selenium is present mainly in the form of selenomethionine, which is able to replace non-specifically the methionine of the organisms' proteins, acting as a selenium reserve for the consumer (*Thomson*, 2004, a, b).

The inorganic selenium content of the selenium-enriched yeast (sodium-selenite) is an excellent substrate for protein formation, but the organism cannot form a selenium stock from it (*Varo et al.*, 1988). Some countries approved to add selenium-enriched yeast even to the feed, where the aim is to produce foods of animal origin rich in selenium due to the different yeast strains used, the different manufacturing techniques applied and the different quality traits of the selenium forms used for enrichment. *Fox et al.* (2004) found that the selenium absorption may be of different quality. They also concluded that the selenium content of the selenium-enriched yeast is better utilizable than the inorganic forms and it remains longer in the organism after the selenium supplementation. This can be explained by the integration of selenomethionine into the tissue proteins, which then, after escaping from its bond due to protein degradation, adequately supplies the organism with selenium.

Levander et al. (1997) compared the utilization of inorganic selenium and organic selenium derived from selenium-enriched yeast and wheat, and they found that the supplementation with selenium-enriched yeast and/or wheat is similar in terms of uptake and retention, but it is much higher than that of the inorganic selenium. While studying the utilization of seleno-methionin by lactating and non-lactating mothers, *Mcguire et al.* (1993) found that the selenium content of blood plasma of lactating mothers who did not receive selenium supplementation was significantly lower than that of the non-lactating mothers. In the case of selenomethionine supplementation, the selenium content of blood plasma increased in both types, while selenium-enriched yeast increased the plasma selenium content only in the case of non-lactating mothers. Due to the supplementation with selenomethionine, the selenium content of breast milk increased significantly compared to those who consumed selenium-enriched yeast as supplement.

According to the data reported in the literature, it can be concluded that the seleno-enzyme activity increases due to selenium yeast supplementation and the selenomethionine content of yeast – being integrated into the organisms' proteins – allows the selenium to become only slowly eliminated from the organism, ensuring this way the selenium requirement of the organism. The utilization of selenium-enriched yeast is lower than that of selenomethionine; this can be explained by the fact that the selenium-containing yeast has to be broken down by the organism in order to make the selenomethionine become available. In addition to this, the lower utilization can also be explained by the fact that selenium is not only present in the form of selenomethionine, but a major part of it is in the form of inorganic selenium. According to the analysis of *Schrauzer* (2000), the half life of selenomethionine in our body is 252 days and that of selenite is 102 days, which also explains the better storability of the organic form of selenium. A similar result came out when the LD_{50} value of these two selenium forms was measured in a study made on rats. In the case of selenium-containing yeast, this value was 37.3 mg/kg, while in the case of sodium-selenite the value was 12.7 mg/kg, which proves that, in order to obtain toxicity levels, a higher amount is needed from the selenium-containing yeast than from inorganic selenium. However, there arises the problem that the long-term consumption of organic selenium-containing compounds can lead to selenosis as the body is capable to accumulate it. Therefore, it is very important to monitor the selenium accumulation of the body and to know what kind of selenium form the selenium-supplemented food or the selenium-enriched food contains.

1.1 Selenium enrichment possibilities of foods of plant origin

Plants are able to convert the inorganic selenium from the soil or that sprayed onto their leaves into organically bonded selenium compounds. This method presents a safety selenium supply for people compared to the initial selenite, as the risk to overdose the body with selenium by consuming selenium-containing food of plant origin is low (*Terry et al.*, 2000). Most plants contain up to 1-2 mg/kg selenium at 100% dry matter, and, even if the soil is rich in selenium, the selenium content does still not exceed the value of 10 mg/kg. These plants do not accumulate selenium, but there are some which are able to store large amounts of selenium in their organs if grown in selenium-rich soil.

Among the plants that accumulate selenium, the plants of the families of *papilionaceae* and *cruciferous* are considered to be primarily accumulating plants and are able to accumulate 1000 mg/kg selenium, while the plants considered to be secondary accumulating plants are able to take up only smaller amounts of selenium from the soil, and as a consequence their tissues contain only a few hundred mg/kg of selenium (*Bell et al.*, 1992; *Ellis & Salt*, 2003). The capability to take up high amounts of selenium can be explained by the fact that these plants synthesize seleno-amino acids primarily (90-95% methyl-selenocysteine), which the plant is capable to store for a prolonged period (*Brown & Shrift*, 2001). The plants called primarily accumulating plants do not integrate the seleno-amino acids into their proteins; therefore, they can be used for the detoxification of soils containing toxic levels of selenium (*Banuelos et al.*, 1996). In the case of plants grown on soils containing normal levels of selenium, the selenium gets integrated into the plant proteins and enters the food chain mainly in the form of seleno-cysteine and selenomethionine.

The main selenium-containing compound of plants growing on soils with higher selenium content is methyl-seleno-cysteine; selenium is stored in this form by e.g. broccoli and other bulb vegetables. The last ones contain sulphur compounds in high amount and, due to the fact that selenium is synergistic with sulphur, it displaces the sulphur from the amino acids; therefore, bulb vegetables are frequently chosen as host vegetables for the production of selenium-containing foods. While studying the supplementation of bulb vegetables with selenium (*Kotrebai et al.*, 2000), it was concluded that in the case of a low selenium concentration of the soil selenium is stored as γ-glutamyl-methyl-selenocysteine in garlic, whereas in the case when the selenium concentration of the soil becomes increased the main selenium-storing compound will be methyl selenocysteine, but selenomethionine also occurs in small amounts in the plants.

In an experiment where garlic was enriched with 3 mg/kg selenium, *Ip et al.* (1992, 1998) found that this amount could reduce the development of breast cancer from 83% to 33% in the case of rats. Experimenting with broccoli, *Finley et al.* (2001) concluded the same. *Dong et al.* (2001) concluded that selenium-enriched garlic was more effective in the reduction of breast cancer development than selenium-enriched yeast, which was explained with the fact that selenium is present in the form of selenomethionine in the case of yeast, while in the case of garlic it is present as methyl-seleno-cysteine or the glutamyl derivative of it. These latter two derivatives are assumed to provide a very effective protection against breast cancer.

In addition to the previously mentioned plants, selenium accumulation was also studied in the case of wheat, corn, rise and soy (*Olson et al.*, 1970; *Beilstein et al.*, 1991), whereby they found that selenium is present mainly in the form of selenomethionine in these plants. In contrast, the findings of the previous author, *Ip et al.* (2000), *Kápolna & Fodor* (2006) and *Cai et al.* (1995) found that the main selenium form in the garlic, green onion, chive and broccoli enriched with selenium is the form of methyl-seleno-cysteine.

After having examined the literature data, it becomes clear that the greatest threat to people is the consumption of selenium in inorganic form as the acute toxicity can develop in this case quickly and easily. It is less dangerous if the selenium is introduced into the body in its organic forms such as selenomethionine; however, in this case, the accumulation of selenium poses a risk as selenomethionine can be stored for a longer period by the body. It seems to be a better solution if selenium is consumed through selenium-enriched natural foods, but the solution to introduce the necessary amount of selenium into the body through products of animal origin enriched with selenium seems to be an even better solution as the animal executes such a conversion after which the food of animal origin does not pose any danger to the organism of the consumer-assuming normal eating habits.

The selenium absorbed from the soil occurs in some plants in the form of methyl-seleno-cysteine of glutamyl methyl selenocysteine; in the other parts of plants, the main selenium-containing organic compound is selenomethionine, while in foods of animal origin, the organic compound of selenium is present almost exclusively in the form of selenomethionine. Some plants are able to accumulate high amounts of selenium, while others contain selenium in an amount adequate for human consumption even when grown on soils with high selenium content. In the case of plants, their selenium content could be increased by various selenium-containing fertilizers; in the case of animals, inorganic selenite and selenate may be suitable for increasing the selenium

content of the animal tissues, but it is better if the animal feed also contains organically bonded selenium.

1.2 Possibilities of selenium enrichment of foods of animal origin

It is possible to produce selenium-enriched meat in the case of products of animal origin, but selenium-enriched poultry eggs and cattle milk could also be used as a selenium supplementation for the population with a very high efficiency. Since the selenium content of most of the foods is extremely low, by an increased consumption of these, the selenium intake cannot be increased. The selenium requirement of the organism can be satisfied with dietary supplements, on the one hand, and with selenium-enriched foods, on the other hand.

The preparation of selenium-enriched foods requires an extremely complicated technology because selenium is supplemented to the plants or to animals as a nutritional supplement, and subsequently the natural form of selenium will be reached through several transformations. During these transformations, the oxidation state of selenium can also change; therefore, it is important to monitor the selenium form contained in the foods of animal or of plant origin.

Through the supplementation of swine feed with inorganic (selenite, selenate) or organic (selenium-enriched yeast) selenium, it is possible to produce high selenium-containing pork. The similar supplementation of the feed of laying hens results in organic eggs with high selenium content, while the supplementation of cattle feed increases the selenium content in milk.

From the data reported in the literature, it can be concluded that the activity of seleno-enzymes increase due to selenium supplementation, and the selenomethionine – integrating into the organisms' proteins – allows the selenium to be slowly excreted from the body, ensuring this way the continuous selenium requirement of the organism. In the case of pigs and piglets, the consumption of a selenium-containing diet (sodium selenite and feed with selenium-enriched yeast) significantly increases the selenium content of organs and precious muscle parts.

Despite a decline in milk consumption, milk, as a basic food, has to be counted as a selenium source for humans; therefore, its selenium content should not be indifferent. It was also suggested that the selenium content of milk could be a suitable indicator to determine the selenium state of the herd. Knowing the concentration of selenium in milk, we could indicate the selenium supply

of the animal organism and the health state of the udder. By supplementing dairy cattle feed with selenium, it is possible to produce selenium-enriched milk.

1.3 The aims of the experiments

It is well known that Hungary belongs to the selenium-deficient regions within Europe. The selenium-deficient supply of the population may lead to various diseases which could seriously threaten the health of the population. To solve the selenium supplementation, a number of attempts were born – in which the authors were also involved (bread and egg with selenium content) – and even currently some research is being conducted (the analysis of selenium content in wheat, flour and bread, the possibilities of selenium enrichment of flour, the analysis of breast milk selenium content, the selenium content of breast milk and the selenium content of the mothers' diet as well as the link between these).

The investigation of dairy feed supplementation methods by which the selenium content of milk and dairy products could be increased and the analysis of the facts how the selenium content of milk passes into dairy products made from this milk fits well into the researches listed above. It would also be useful to examine the effect of inorganic and organic selenium added to raw milk on the growth of lactic acid bacteria and the kefir yeast applied in the dairy industry, as well as to measure how the inorganic selenium content converts into organic form during the production of dairy products.

The selenium supply of the population was also explored in Hungary, but relatively few have dealt with the selenium content of cow's milk and with the possibilities to increase it through the feed. Due to the above ones, we aimed at developing feeding methods which allow us to increase the selenium content of milk to the health protection level. We have studied and continuously examined the effect of various, commercially available selenium supplements and selenium supplements developed by us (selenite, selenate, selenium-enriched yeast and other organic forms of selenium) on the selenium content of milk. We have produced kefir and yogurt, cheese produced with different technologies, butter and butter creams from milk with high selenium content, and examined how the selenium passes from the milk to the various dairy products. The ultimate aim is to develop a technology through which it is possible to produce milk and dairy products with high selenium content in order to better supply the population with healthy selenium sources.

Since the selenium-containing compounds are not directly added to the food,

selenium overconsumption or poisoning can be safely avoided. In the present research, we supplemented the dairy cattle feed with selenium-enriched yeast, hoping that the selenium content increases significantly in the milk, and we may produce dairy products with high selenium content from this milk. We want to report in this paper about the results obtained due to the organic selenium supplementation, the increased selenium content of the milk and about the production of high selenium-containing dairy products.

2 Materials and methods

2.1 Experimental animals and selenium supplementation

We included in our experiments three Simmental dairy cows, which were four or five months pregnant. The milk production based on corn silage and hay meadows feed systems was of 4,000-5,000 litres of milk during their lactation period. The selenium supplementation was carried out by adding selenium-enriched yeast (Selplex-2300), which contained 2,300 mg of selenium in the form of selenomethionine and seleno-cysteine. We prepared a premix using ground corn grain in a way that 10 g of the premix contained 1 mg selenium in order to ease the administration. This premix was added to the daily feed of dairy cattle. The selenium content of the feed consumed by the animals was of 0.42 mg daily. Before beginning the experiment, we took milk samples from each cow (control group). In addition to the previous feed ratio (control group), the animals got 1 mg of selenium supplementation in the first two weeks, 2 mg in the 3^{rd} and 4^{th} week, 4 mg in the 5^{th} and 6^{th} week and 6 mg in the 7^{th} and 8^{th} week. After the 8^{th} week, samples were taken three more times every two week and the selenium content of the milk was measured. *Table 1* shows the selenium supplementation of dairy cows in the form of selenium-enriched yeast.

2.2 The production of different dairy products

According to the data from the literature, a 6 mg/kg selenium supplementation is considered to be fully safe; however, we consider to use a 2 mg selenium supplementation daily practice because of safety reasons, and we produce dairy products from the milk of the control group and that of cows obtaining a daily dose of 2 mg selenium supplementation, and examine what kind of selenium-containing dairy product can be produced from the milk of the control group and that containing selenium.

Table 1: Selenium supplementation of dairy cows in the form of selenium-enriched yeast

Number of weeks	Selenium supplementation (mg) cow/day
1-2	1
3-4	2
5-6	4
7-8	6
9-10	0
11-12	0
13-14	0

The selenium content of the basic forage is 0.42 mg/cow/day.

We produced from both the control milk (18 μg/kg selenium content) and the selenium-enriched milk (53 μg/kg selenium content) yogurt, Telemea cheese, curd cheese, Orda cheese and cheese prepared with mixed coagulation. The selenium content of the product as well as that of the whey obtained as a by-product of the production process was measured in each case. The dairy products were produced according to the Romanian standards at the Department of Food Science of the Sapientia Hungarian University of Transylvania, Miercurea Ciuc. The selenium content of the produced dairy products was determined at Kaposvár University, Faculty of Animal Science, Department of Chemistry and Biochemistry and Analytical Laboratory as well as at the Sapientia Hungarian University of Transylvania, Department of Food Science.

Both the control and the selenium-containing milk was pasteurized at 78 °C for 50 seconds, and then, during the yogurt production, the pasteurized milk was inoculated with a pure culture mixture of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* after the milk was cooled down to 27 °C, and then the samples were incubated at 27 °C for 7 hours in a thermostat and frozen at -25 °C.

During the production of the semi-hard cheese with mixed coagulation, its fat content was adjusted to 3.9%, and we treated the milk with a 70 °C heat treatment for 50 seconds in a laboratory pasteurizer, after which it was cooled down to the inoculation temperature of 34-36 °C and inoculated with 0.001% pure culture of *Propioni* bacteria, letting it age until reaching 19 degrees acid. The rennet was dissolved in lukewarm water before using it, and it was added to the cheese milk in order to seed it uniformly. After the addition of the

rennet, the milk was stirred for two minutes and, after stopping the movement, the mixture was left to coagulate for 40 minutes until the curd was separated from the wall of the tub and crushed like porcelain, which meant the end of the coagulation. During the processing of the clot, this was chopped into pea-sized lumps, and it was rotated for 10 minutes in order to precipitate the whey, while we measured the degree of acidity of the whey, which increased by two degrees acid. Then it was poured into shapes and allowed to desiccate at room temperature for 24 hours, rotating it every 30 minutes and storing it in the refrigerator afterwards. During the storing at 8-10 °C with a 95% air humidity, we also rotated it several times a day.

During the production of Telemea cheese, the fat content of the cheese was adjusted to 3.8% and it was pasteurized using a laboratory pasteurizer at 70 °C for 50 seconds. After having cooled down the milk to the inoculation temperature of 35-37 °C, we added 0.0015% pure culture of *Propioni* bacteria, and then allowed to age for 20 minutes, adding to it the above-calculated volume of rennet, and then coagulating it for 35 minutes. During the processing of the clot, we chopped it into walnut-sized lumps, rotated it for 15 minutes in the whey, and then, in order to promote the drying of the curd, we continuously increased the temperature to 38-40 °C while stirring it slowly and continuously. After pouring it into shapes, we allowed it to dry for 2 hours at room temperature, and then put it into a saline solution of a concentration of 12% at 12-14 °C for 24 hours. As a result, the salinity of the Telemea cheese reached 2.5-3.0%. The cheese was allowed to age at 10-12 °C by 95% air humidity and it was repeatedly rotated.

During the production of fresh curd cheese, we heated the raw milk to the temperature of 45-50 °C and skimmed it and adjusted the fat content to 0.9%; after that, it was pasteurized with a laboratory pasteurizer at 70 °C for 50 seconds. Subsequently, the temperature was cooled down to 28-30 °C, after which 1.5% of pure *Propioni* bacteria culture and a 2% pure culture mixture of *Streptococcus termofilus*, *Lactococcus lactis lactis*, *Lactococcus lactis cremoris*, *Lactococcus acidophilus* was added. The milk was allowed to age for 45 minutes. After that, we added 0.001% of rennet in the way described earlier and let it age for 10 minutes. During the processing of the clot, we chopped it into walnut-sized lumps; the whey was removed by filtration, and then the clot was kneaded and packaged.

During the production of Orda (whey cheese), we used the whey left as by-product from the production of the mixed-coagulated semi-hard cheese and the Telemea cheese. The whey was heated to 95-96 °C for 1-2 hours, the precipitated whey proteins were removed and the product was kept in a

refrigerator until the analysis. The whey obtained during the production of Orda practically did not contain any proteins.

2.3 The determination of selenium content of milk and dairy products

We measured the selenium content of milk after digesting it with nitric acid and perchloric acid by applying fluorometric measurements from the piazselenol form obtained after the derivatization with diamino-naphthalene of the selenite, which was obtained from selenate reduced into selenite with hydrogen chloride. In a series of experiments, the selenium content was measured in the form of hydrogen-selenide both fluorometrically and by atomic absorption spectrophotometer, equipped with hydrogen generator. Since we did not observe significant differences between the two types of measurements, hereinafter we applied the more economical fluorometric method.

3 Results

The changes in the selenium content of the milk obtained as a result of selenium supplementation with Selplex-2300 are shown in *Table 2*.

Table 2: The effect of selenium supplementation on the selenium content of milk

Control/experimental groups	Average daily selenium intake (mg)	Selenium content of milk*** (mg/kg)
Control group (CG)	0.42*	0.018 ± 0.002 ****
CG+1 mg Se**/cow/day 1st and 2nd weeks	1.42	0.031 ± 0.002 (0.022)
CG+2 mg Se**/cow/day 3rd and 4th weeks	2.42	0.053 ± 0.003 (0.022)
CG+4 mg Se**/cow/day 5th and 6th weeks	4.42	0.081 ± 0.005 (0.001)
CG+6 mg Se**/cow/day 7th and 8th weeks	6.42	0.094 ± 0.006 (0.001)
CG+0 mg Se at the end of 10th week	0.42	0.062 ± 0.002 (0.001)
CG+0 mg Se at the end of 12th week	0.42	0.021 ± 0.002 (NS)
CG+0 mg Se at the end of 14th week	0.42	0.019 ± 0.002 (NS)

* Selenium content of the basic materials
** Selenium supplementation in the form of Sel-Plex-2300 (seleno-yeast)
*** Milk sampling at the end of the experimental periods
**** In parentheses, the level of significance is shown compared to the control.

Analysing the data in the table, we found that the selenium content of the milk increased by the increasing amount of the selenium supplementation com-

pared to the value of 0.0145 mg/kg measured in the control group to 0.031 mg/kg in the case of 1 mg/day selenium supplementation, 0.053 mg/kg when supplemented with 2 mg/day, 0.081 mg/kg in the case of 4 mg/day selenium supplementation and 0.094 mg/kg when 6 mg/day of selenium was added. After the completion of selenium supplementation, the selenium content decreased to 0.062 mg/kg after two weeks, 0.021 mg/kg after 4 weeks, 0.019 mg/kg after 6 weeks, which almost coincided with the value of 0.018 mg/kg measured in the control group.

The results of the statistical analysis indicate that the selenium content of milk originating from cows which obtained a varying amount of selenium supplementation was significantly higher at $P<0.001$ level than that of the control group, and the increase of selenium supplementation led to a significant increase in the selenium content of the milk. The greatest increase was measured in the 6^{th} week, when the degree of selenium supplementation was about 4 mg/day; although a further increase of the selenium supplementation to 6 mg/day increased the selenium content of milk significantly again, the degree of this increase was far more less than that of the previous periods. Two weeks after finishing the selenium supplementation, the selenium content of milk was significantly lower than the value measured in the 6^{th} and 8^{th} week, but it statistically exceeded the values measured in the case of animals obtaining only 2 mg/day selenium supplementation. A month after finishing the selenium supplementation (12^{th} week), the selenium content of milk was the same as that of the control group which did not receive any selenium supplementation.

From our experiments, it can be concluded that a daily selenium supplementation of 6 mg in the form of organic selenium increases the selenium content of milk about fivefold. However, the data in *Table 2* show also that in the case of the absence of selenium supplementation the selenium content of the milk decreases to 0.019 mg, which is practically the same value as the one measured at the beginning of the experiment. This draws the attention to the fact that in order to continuously obtain milk with increased selenium content it is necessary to add selenium supplementation to the dairy cattle feed in a continuous manner.

The question arises how the milk with increased selenium content satisfies the selenium requirements of the children and adult population, on the one hand, and, on the other hand, whether the milk with increased selenium content poses a health risk to the public or not. *Table 3* shows the daily selenium requirement of 1–19-year-old people and the milk quantity necessary to satisfy the reported need. If we compare the data shown in *Table 3* with the compo-

sition of the control and the selenium-enriched milk as well as with the milk consumption quantities of the population, then we come to the conclusions reported below.

Table 3: Recommended dietary allowance of selenium and the quantity of milk necessary to satisfy RDA with control and selenium-enriched milk

Age (years)	RDA (μg Se/day)	Quantity of milk (L)	
		Control milk	Selenium-enriched milk
1–3	20	1.00	0.21
4–8	30	1.50	0.32
9–13	40	2.00	0.43
14–18	55	2.75	0.59
19–	55	2.75	0.59

RDA = Recommended Dietary Allowance

The milk and dairy product consumption of Hungarian adult population is 0.26 litre in the case of women and 0.28 litre in the case of men. Comparing these data with the selenium content of milk, it can be concluded that milk contributes to the satisfaction of the selenium requirements in only 7% when it is not supplemented with selenium. If we consider the data of milk with the highest selenium content (0.094 mg/kg), we realize that this milk contributes to the satisfaction of the daily selenium requirement in 34%. As it is well known that a high selenium intake can lead to serious illnesses, it is necessary to examine whether the increased selenium content of milk poses a health risk for the various ages.

Various studies clearly report that the daily selenium intake of adults from food sources of animal origin, but without integrating the amount of dairy products, ranges from 73 to 126 μg selenium, whereas this value is 12 μg in the case of 1–3-year-old children; therefore, the consumption of selenium-enriched milk by the adult population can be evaluated as safe, as the maximal selenium intake by a consumption of one litre milk is 220 μg, while the acceptable daily intake is about 300 μg. In the case of children aged 1–3 years, a daily consumption of 0.5 litre of selenium-enriched milk results together with other foods in a value of 59 μg selenium intake, which is almost the same as the upper limit of selenium intake (60 μg/day). Due to the foregoing, it is recommended that 1–3-year-old children do not consume more than half a litre of selenium-enriched milk. The consumption of selenium-enriched milk should be limited to a maximum of half a litre/day for this age-group.

Table 4 shows the changes in the selenium content of milk and dairy products depending on the selenium content of the raw material. According to the results of the statistical analysis, each dairy product produced from selenium-enriched milk had a significantly higher selenium content at $P < 0.001$ level than the products derived from the control group. The selenium content of the yogurt produced from the control milk was measured to be 18.6 μg/kg, while that made from selenium-enriched milk was 58.5 μg/kg. The minimal growth observable in the value of selenium content of the yogurt produced from the control milk can be explained by the water loss occurred during the heating phases of the production, which increased the selenium content of the resulting fermented product.

Table 4: Selenium content of dairy products as a function of selenium supplementation

The name of the dairy product	Selenium content (μg/kg)	
	Control	Selenium-enriched milk
Whole milk	18.0 ± 1.39	53.0 ± 2.80 (0.001)*
Yogurt	18.6 ± 0.72	58.5 ± 0.40 (0.001)
Telemea	66.0 ± 5.60	138.1 ± 2.01 (0.001)
Telemea whey	9.7 ± 0.60	20.1 ± 0.31 (0.001)
Curd cheese	57.4 ± 0.21	154.8 ± 1.75 (0.001)
Curd cheese whey	8.8 ± 0.20	25.8 ± 0.56 (0.001)
Orda	80.8 ± 1.62	167.2 ± 1.59 (0.001)
Orda whey	4.6 ± 0.10	10.8 ± 0.26 (0.001)
Mixed curding cheese	88.6 ± 1.17	200.0 ± 2.10 (0.001)
Cheese whey	9.2 ± 0.23	21.4 ± 0.85 (0.001)

* In parentheses, the level of significance is shown compared to the control.

The selenium content of Telemea cheese made from the control milk was 66.0 μg/kg and the value of the Telemea cheese made from selenium-enriched milk was 138.1 μg/kg. The whey contained 9.7 μg/kg selenium in the case of the control and 20.1 μg/kg in the case of selenium-enriched milk. Comparing the data obtained for the products produced from the control milk with the data of the products produced from the selenium-enriched milk, it can be stated that the Telemea cheese produced from the selenium-enriched milk contains twice as much selenium as that produced from the control milk. The same is truth in the case of whey as the whey obtained by the manufacturing procedure of Telemea cheese also contains twice as much selenium as the control.

In the case of Orda, the product produced from the control milk contained 80.8 μg/kg selenium; that produced from selenium-enriched milk contained 167.2 μg/kg selenium, which is also double the amount of the control. The selenium content differences among the whey obtained from the production of Orda from control milk (4.6 μg/kg) and that of the whey obtained from Orda produced from selenium-enriched milk (10.8 μg/kg) were even greater. The selenium content of the mixed-coagulated cheese produced from control milk was 88.6 μg/kg, while that of the mixed-coagulated cheese produced from selenium-enriched milk was 200.0 μg/kg.

The selenium content of the whey obtained during the production process from the product produced from the control milk was 9.2 μg/kg, while the value in the case of whey obtained during the production process of the product produced from selenium-enriched milk was 21.4 μg/kg. Compared to the dairy products previously discussed, the increase in selenium content is again a two-fold for the benefit of selenium-enriched milk. The selenium content of the curd produced from the control milk was 57.4 μg/kg and that of the curd produced from the selenium-enriched milk was 154.8 μg/kg. These values are very similar to the values obtained in the case of Telemea cheese. The selenium content of the whey obtained during the production process of curd cheese produced from the control milk was 8.8 μg/kg, while the value in the case of whey obtained during the production process of curd cheese produced from selenium-enriched milk was 25.8 μg/kg.

We concluded, therefore, that the selenium content of Telemea, Orda and mixed-coagulated cheese produced from selenium-enriched milk was each time more than the double of the amount of dairy products produced from control milk. The highest amount of selenium content, 200.0 μg/kg, was measured in the mixed-coagulated cheese, followed by Orda with 167.2 μg/kg selenium content, curd cheese with 154.8 μg/kg selenium content and finally the Telemea with 138.1 μg/kg selenium content. We also concluded that the selenium content of the whey obtained during the manufacturing procedure of the products produced from selenium-enriched milk was more than double the selenium content of the whey obtained from the products produced from control milk. Therefore, it can be concluded that the whey of products produced from selenium-enriched milk can be a valuable source of selenium for people as well as for animals. This is also proved by the high selenium content of 167.2 μg/kg of the Orda cheese obtained from whey.

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Examination of the selenium content of wheat grasses produced in different soil types in Csik Basin

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Abstract. In the course of the research, we determined selenium and dry matter content of 35 wheat grass and 35 wheat seed samples. The selenium content of the preparation plant probes was measured by spectrofluorimetric determination ($\lambda_{excitation} = 380$ nm, $\lambda_{emission} = 519$ nm) of the resulted piazselenol complex. It was established that between the selenium content of the wheat grass and wheat seed the correlation coefficient was 0.36 at $p = 0.05$ level, which indicates a medium-close correlation. Similarly, there was a medium-close correlation between the selenium content of the wheat grass calculated on dry-matter basis and total selenium content of the wheat, with a correlation coefficient of 0.40 at $p = 0.02$ level. Afterwards, beside the selenium content, we measured the selenomethionine content by ion-exchange chromatography and high-performance liquid chromatography, and the organic selenium content

Keywords and phrases: inorganic, organic selenium content, selenomethionine, wheat grass, wheat seed, soil types.

was calculated. A very close correlation was established between the total selenium, selenomethionine and calculated organic selenium content of wheat (the correlation coefficients were between 0.92 and 0.99 at $p = 0.01$ level). The correlation between the selenomethionine content of wheat grass and wheat seed was very weak ($r = 0.23$).

1 Introduction

In the 1930s, selenium was considered to be a toxic element, but in 1943 its essential role in the living organisms was detected as it decreased the occurrence of cancer in certain conditions (*Nelson et al.*, 1943; *Clayton* and *Bauman*, 1949; *Schwarz* and *Foltz*, 1957).

In 1966, the discovery of the mechanism of the anticarcinogenic effect of selenium was published (*Shamberger* and *Rudolph*, 1966), but then only the total selenium content of the aliments was mentioned. Recently, due to the increasing sensitivity of the analytical instruments, the physiological importance of the selenium was revealed in details: as antioxidant, together with tocopherols, it is involved in the metabolism, it helps in the healing and even in the prevention of certain cancer diseases as well as in the preservation of cell membrane integrity. By catalysing the decomposition of peroxides, the enzyme glutathione peroxidase (GPx) protects the unsaturated lipids against oxidation in the organism, and the selenium has an important role in the regulation of the GPx (*Cser* and *Sziklainé*, 1998).

The foods produced in the European countries are highly deficient in selenium. The daily selenium intake by way of food (0.05–0.10 mg) is insignificant (*Cser* and *Sziklainé*, 1998). The Romanian (*Serdaru et al.*, 2003) and the Hungarian (*Combs*, 2005) soils also have an extremely low selenium content; therefore, by the intake of foods of vegetal origin, the selenium demand of the organism cannot be satisfied. In the opinion of modern nutrition science, the fortification of foods with selenium is almost indispensable (*Reilly*, 1998).

The selenium content of plants is determined mainly by the selenium content – not the total but only the bioavailable selenium content – of the soil (*Terry et al.*, 2000). The elemental and the selenid forms are almost unavailable by way of plants from the soil; nevertheless, the absorption of the selenite and selenate compounds is more effective. The absorption of the selenates in the human organism is almost quantitative, but before the incorporation of the selenium in the proteins the main fraction is eliminated by urine. In contrast, the selenite is absorbed only in a proportion of 50%, but their incorporation is higher (*Bendhal* and *Gammelgaard*, 2004).

In addition to the inorganic selenium compounds, seleno-amino acids or their derivatives occur in plants in significant quantities. Foods of plant origin contain selenomethionine, while foods of animal origin have selenomethionine and selenocysteine content too. The selenomethionine in plants is formed from the absorbed inorganic selenium content of the soil, and in animal organisms it is able to transform into selenocystine. In the organism, about 90% of the selenomethionine is able to convert in active form (*Dumont et al.*, 2004). In human food, selenium occurs preponderantly as selenite and selenomethionine.

To our best knowledge, in Romania, especially in the Szekler Region, the total selenium, selenomethionine and organic selenium content in wheat and wheat seed has not been studied; so, the aim of our research was the determination of selenium, selenomethionine and organic selenium content calculated from the selenomethionine content of wheat grass and wheat grown on different soil types, as well as finding correlations between these components.

Our ultimate goal is to find out the proportion of the recommended selenium intake (necessary for the human organism) which comes from the main public nutrition product, bread, obtained from wheat flour for the population of the Szekler Region. In this research article, we present the results of the study on total selenium, selenomethionine and organic selenium content of wheat grass and wheat, as well as the correlations between these selenium forms.

2 Materials and methods

2.1 Collection of the samples

During the research, at first, we determined the dry matter contents and the selenium contents of 35 different wheat grasses and wheat seed probes, while also the dry matter contents as well as the total selenium, selenomethionine and organic selenium contents of the 44 probes. The wheat plants were collected from the soil types in conformity with *Table 1.*

During sample collection, the geographic locus was marked by GPS, and we accorded attention to taking the wheat seed samples (at the beginning of harvesting) from the same place, from where the wheat grass probes had been taken in the autumn of the previous year. The plant samples were pulled out manually from the soil; the soil was washed out from the roots by flowing water. The root was cut from the rest of the plant just over 0.5 cm above the junction of the root–green part, and only the green part was used for the analysis. The green wheat grass was immediately transported to the laboratory and it was stored at a temperature of t = –25 °C until the preparation for analysis.

Table 1: The investigated soil types

Soil types			
No.	Sign of soil types	Type of soil	Soil characteristics
1	ASen	Young, immature, crude alluvial soil without diagnostical level	
2	ASen-gc	Crude alluvial soil – outwashed gluey soil	
3	ASgc	Crude alluvial soil – gluey soil – extremely wet, hydromorfic soil, persistently exposed to moisture	Slightly developed soils, without segmentation
4	RSka	Earthy barren soil – limestone furred	Sandy, pebbly, glacial deposit
5	KZti	Kastanozem soil (low organic matter production, few humus)	Chestnut-coloured prairie soil variant
6	KZmr	Maroon kastanozem soil	
7	$CZka_1$-kz	Chernozem with limestone accumulation – maroon-coloured soil	Prairie soil, rich in organic matter; their
8	$Czka_2$-kz	Chernozem with limestone-furred surface – maroon-coloured soil	characteristic is the occurrence of the
9	CZti	Typical chernozem soil (slight organic residuum – Ca-rich soil-forming ground, approx. 1% humus)	limestone for 30-70 cm deep. In the dry periods, the limestone
10	Czka-fru	Carbonated chernozem soil – groundwater-soaked	peels out on the surface of the soil crumbs,
11	Czka-e	Chernozem soil with limestone accumulation eroded by erosion	forming pseudomycelium.

The seeds were manually (with gloved hands) rolled out from the ears, then, after the removal of the chaff and awn parts, the seeds were stored in nylon package in refrigerator at a temperature of t = +5 °C until the preparation for analysis.

2.2 Determination of dry matter content

In the course of the determination of dry matter content, 10 g of sample was weighed in a measuring vial; then the sample was dried at a temperature of t = 60 °C in a drying oven until mass constancy was reached. The dried samples were left overnight in open vials, and then their weight was measured again. The air-dry samples were ground in a hammer mill to flour fineness, and the dry matter content of the resulting powder was determined in conformity with the Romanian Standard (STAS 9682-2-74) method: drying in oven at a temperature of t = 105 °C until mass constancy is reached. Then, from the determination results, the dry matter content was calculated.

The appropriately dried and flour-fine-milled wheat grass samples were sieved with a 200 μm mesh sieve and the retentate was milled repeatedly until the whole sample passed through the sieve holes.

The selenium analysis was carried out based on the samples prepared in the above-mentioned mode.

2.3 Fluorimetric determination of selenium content, preceded by wet digestion of the samples

The solution of the samples was obtained by wet digestion, and to the acidic solution 2,3-diaminonaphtalene (DAN) was added (*Bayfield* and *Romalis*, 1985). The obtained piazselenol-complex was determined by fluorimetry ($\lambda_{excitation}$ = 380 nm, $\lambda_{emission}$ = 519 nm).

Acidic digestion with Aqua Regia

From the pretreated sample, a 3-g-probe was taken (measured with ±1 mg precision) in a 250 cm^3 round-bottom flask with ground-glass joint, and then 0.5–1.0 cm^3 distilled water was added. After the wetting of the sample, 21 cm^3 hydrocloric acid (12 M) solution was added under continuous stirring, and then 7 cm^3 of nitric acid (15.8 M) solution was added dropwise, taking care to avoid the foaming of the mixture.

The round-bottom flask was connected to a water-cooler and the cooler, in its turn (trough the ground-glass joint), was connected to an absorption vessel filled with 15 cm^3 of nitric acid (0.5 M).

Having mounted the apparatus, the sample – with the mixture containing hydrochloric and nitric acid – was allowed to rest for 16 hours, waiting for the completion of the slow oxidation process.

After 16 hours of waiting (in general, the following morning), the mixture was heated until the reflux of the condensed solvent vapour occurred, and the temperature of the system was maintained for 2 hours. The content of the absorption vessel was poured into the content of the flask, and then the vessel and the reflux cooler were both washed with a volume of 10 cm^3 of nitric acid solution (0.5 M). Subsequently, the sedimentation of the insoluble particles in the reaction vessel was allowed, and then the supernatant (with relatively low solid content) was filtered through a filter paper into a volumetric flask with 100 cm^3 volume. After the whole solution had gone through the filter, the insoluble retentate on the filter was washed with a few millilitre of nitric acid solution (0.5 M). The solution thus obtained was suitable for the determination of selenium content.

The formation and quantitative measurement of the piazselenol-complex

To the digested sample, a volume of 5 cm^3 masking solution was added and the pH of the resulted mixture was adjusted to the value of pH = 2.0 by addition of ammonium hydroxide solution. Hereinafter, a volume of 5 cm^3 DAN-solution was added, and the mixture was left in dark for about 2 hours. After the formation of the complex, the solution was washed into a separation funnel, and then extracted with aliquots of 2×5 cm^3 cyclohexane with a 2-minute duration of each extraction. Finally, the resulted organic phases were unified. The organic phase and the blank probe were measured with fluorimetry within 20 minutes after the extraction ($\lambda_{excitation} = 380$ nm, $\lambda_{emision} = 519$ nm).

Calibration curve

Volumes of 0.2, 0.4, 0.6, 0.8 and 1.0 cm^3 selenium standard solutions were pipetted into 100-cm^3-volume Berzelius beakers, then the solution volumes were completed to 50 cm^3 with distilled water. (Hereinafter, the same procedure is followed as in the case of the samples). In the organic phase, with the total volume of 10 cm^3, the concentrations of selenium were 0.2, 0.4, 0.6, 0.8 and 1.0 $\mu g/cm^3$, respectively.

Calculation of the result

The calibration curve is linear in the 0.2-1.0 $\mu g/cm^3$ concentration domain. The selenium content of the sample is calculated with the following formula:

$$C = \frac{\text{quantity of the added sample}}{\text{quantity of the extract solvent}} \cdot C_M$$

Where: C_M the measured concentration, $\mu g/cm^3$,
C the selenium content of the probe, $\mu g/g$.

2.4 Determination of the selenomethionine content

Determination of the selenomethionine with ion-exchange column chromatography (IEC) and with high-performance liquid chromatography (HPLC)

The selenomethionine content was determined with INGOS AAA (amino acid analyser) apparatus, based on the principle of ion-exchange chromatography and using the method described by *Mándoki et al.* (2007a, b). Parallel with the AAA measurements, the determination of the selenomethionine was performed also with HITACHI LaChrom HPLC apparatus, using precolumn derivatization with OPA-mercaptoethanol (*Mándoki et al.*, 2008).

Statistical analysis

For linear regression and statistical data analysis, MicroCal Origin software was used.

3 Results and discussion

The selenium contents of wheat grass and wheat seeds are presented in *Table 2*. The selenium content of the samples are given both wet-based and dry-based (100% dry weight). The average moisture content of wheat grass is 20%; the dry-based selenium content is about five times higher than the selenium content of the dry matter of the original sample (wet-based selenium content). In the case of the wheat seed samples, the differences between the two calculated values are minor, given by the low differences between the dry matter contents expressed in two modes.

First, we analysed the correlation between the total selenium content of wheat grass related to the original dry matter content and 100% dry matter. As the result of the measurement for the 35 samples, the correlation coefficient had the value of 0.92 (at the significance level of $P < 0.1$). This extremely close correlation is not surprising since we are dealing with the same data set, where the single source of error is carried by the precision of the determination of dry matter content. Secondly, the correlation between the total selenium content related to the original dry matter of the wheat grass and the total selenium content of the wheat was analysed. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.36 (at the significance level of $P < 0.1$), indicating a moderately strong relationship between the selenium content related to the original dry matter of the wheat grass and the total selenium content of the wheat. The relationship between the selenium content related to the 100% dry matter of the wheat grass related to the original dry matter and the total selenium content of the wheat is also moderately strong, as the correlation coefficient was 0.40 (at the significance level of $P < 0.1$).

We found that the dry matter content of the samples varied between the values of 16.3 and 22.6%. The selenium content of the original dry matter was about 7.7-25.0 μg/kg, on average 14.76 μg/kg. The lowest selenium content for sample P13 (7.7 μg/kg) and the highest for sample P3 were measured (25.0 μg/kg). Converting to 100% dry-matter, the highest selenium content was found to be 126.3 μg/kg (sample P3), while a very similar value, 122.4 μg/kg, was detected for sample P11 with very low dry matter content.

Table 2: Selenium content of wheat grass and wheat seeds

Code of the sample	Wheat grass			Wheat seeds	
	Dry matter, %	Selenium content (μg/kg)		Dry matter content, %	Se content, 100% dry matter (mg/kg)
		In original dry matter content	100% dry matter		
P1	21.9	16.7	76.3	90.3	0.142
P2	18.1	14.0	77.3	89.9	0.084
P3	19.8	25.0	126.3	90.2	0.007
P4	19.1	13.0	68.1	90.4	0.014
P5	19.3	13.4	69.4	91.2	0.149
P6	20.0	12.1	60.5	90.6	0.115
P7	18.3	17.4	95.1	90.6	0.129
P8	20.2	15.8	78.2	90.4	0.142
P9	17.1	13.1	76.6	91.1	0.122
P10	19.3	14.9	77.2	90.9	0.014
P11	15.6	19.1	122.4	90.6	0.068
P12	16.3	17.3	106.1	90.1	0.021
P13	20.6	7.7	37.4	90.4	0.139
P14	20.2	9.7	48.0	90.9	0.095
P15	22.6	16.2	71.7	90.0	0.047
P16	22.1	13.3	60.2	90.1	0.046
P17	19.7	13.7	69.5	90.6	0.120
P18	20.4	19.6	96.1	90.7	0.184
P19	18.9	19.8	104.8	90.0	0.065
P20	18.0	15.3	85.0	91.2	0.096
P21	17.0	14.4	84.7	90.6	0.047
P22	19.6	17.8	90.8	90.8	0.079
P23	20.4	14.0	68.6	91.0	0.079
P24	18.9	9.0	47.6	90.4	0.063
P25	16.8	17.4	103.6	90.0	0.055
P26	18.2	14.1	77.5	90.0	0.047
P27	15.9	14.3	89.9	91.0	0.096
P28	21.5	19.4	90.2	90.8	0.152
P29	20.9	23.0	110.0	90.1	0.104
P30	20.0	9.6	48.0	90.7	0.160
P31	16.6	8.7	52.4	90.0	0.031
P32	18.4	10.7	58.2	90.6	0.047
P33	21.0	11.1	52.9	90.5	0.037
P34	22.4	20.1	89.7	90.2	0.041
P35	17.6	18.4	104.5	90.0	0.037

The lowest selenium content, 37.4 μg/kg, was measured for sample P13 due to relatively high dry matter content (20.6%) and very low selenium content in the original dry matter. The next lowest value, 47.6 μg/kg, was found in sample P24, followed by 48.0 μg/kg (sample P30). Calculated on the 100%

dry matter content, the studied wheat grass samples had 77.65 μg/kg selenium content on average.

The results of measurements on selenomethionine content of wheat grass are summarized in *Table 3*. The results were given related to both the original dry matter and to 100% dry matter content. *Table 3* shows, however, the organic selenium content of the wheat grass (in μg/kg value), expressed from the selenomethionine content related to the original dry matter and the 100% dry weight content.

The selenomethionine content of wheat grass samples related to the original dry matter content (around 20%) was between 14 and 27 μg/kg. The lowest selenomethionine-containing sample was sample P13 (13.9 μg/kg) and the highest value was measured for sample P3 (35.5 μg/kg). Accordingly, related to the 100% dry-weight content, the highest selenomethionine content, 178.3 μg/kg, was measured for sample P3 likewise, while the lowest, 67.5 μg/kg, for sample P13. The organic selenium content of the samples related to the original dry matter contents was between 5.6 and 14.3 μg/kg, and between 27.2 and 72.2 μg/kg related to 100% dry matter content.

From the data in *Table 3*, the correlation between the total selenium content related to the original dry matter of the wheat grass and the total selenium content of the wheat, calculated from the selenomethionine content, was analysed. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.34 (at the significance level of $P < 0.1$), indicating a moderately strong relationship. Next, the correlation between the total selenium content of the wheat grass related to 100% dry-weight content and the total selenium content of the wheat was analysed. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.40 (at the significance level of $P < 0.1$), indicating a moderately strong relationship.

Hereinafter, the correlation between the total selenium content of the wheat grass related to 100% dry-weight content and the selenium content of the wheat calculated from the selenomethionine content was investigated. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.40 (at the significance level of $P < 0.1$), indicating likewise a moderately strong relationship.

In addition to analysing the wheat grass, the analysis of the selenomethionine contents of the supplemented number of 44 samples was carried out. The selenomethionine content of the wheat samples (reported in mg/kg) was summarized in *Table 4*, together with the selenium content of the wheat samples calculated from the selenomethionine content also reported in mg/kg.

Table 3: Selenomethionine and organic selenium content calculated from the selenomethionine content of wheat grass

Sample	Dry matter, %	Selenomethionine content in the original dry matter (μg/kg)	Selenomethionine content in the dry matter (μg/kg)	Organic Se content in the original dry matter (μg/kg)	Organic Se content in the dry matter (μg/kg)
P1	21.9	25.8	117.8	10.4	47.5
P2	18.1	22.1	122.1	8.9	47.3
P3	19.8	35.5	178.3	14.3	72.2
P4	19.1	17.1	89.5	6.9	36.1
P5	19.3	19.9	103.1	8.0	41.6
P6	20.0	18.1	90.5	7.3	36.5
P7	18.3	24.6	134.4	9.9	54.1
P8	20.2	23.9	118.3	9.6	47.5
P9	17.1	18.9	110.5	7.6	44.4
P10	19.3	22.1	114.5	8.9	46.1
P11	15.6	31.3	200.6	12.6	80.8
P12	16.3	23.6	144.8	9.5	58.3
P13	20.6	13.9	67.5	5.6	27.2
P14	20.2	16.9	83.7	6.8	33.7
P15	22.6	21.6	95.6	8.7	38.5
P16	22.1	19.9	90.0	8.0	36.2
P17	19.7	21.9	111.2	8.8	44.7
P18	20.4	26.3	128.9	10.6	52.0
P19	18.9	23.9	126.5	9.6	50.8
P20	18.0	20.4	113.3	8.2	45.6
P21	17.0	20.9	122.9	8.4	49.4
P22	19.6	22.9	116.8	9.2	46.9
P23	20.4	19.6	96.1	7.9	38.7
P24	18.9	15.9	84.1	6.4	33.9
P25	16.8	23.4	139.3	9.4	56.0
P26	18.2	21.1	115.9	8.5	46.7
P27	15.9	20.6	129.6	8.3	52.2
P28	21.5	23.9	111.2	9.6	44.7
P29	20.9	27.6	132.1	11.1	53.1
P30	20.0	18.9	94.5	7.6	38.0
P31	16.6	15.7	94.6	6.3	38.0
P32	18.4	18.4	100.0	7.4	40.2
P33	21.0	16.9	80.5	6.8	32.4
P34	22.4	26.3	117.4	10.6	47.3
P35	17.6	24.4	138.6	9.8	55.7

In the case of wheat seeds, the lowest selenomethionine content (0.0097 mg/kg) was measured for sample P43, while the highest, 0.306 mg/kg, was measured for sample P30. Accordingly, the lowest organic selenium content was 0.0039 mg/kg and the highest was 0.123 mg/kg.

From the data of *Table 4*, the correlation between the total selenium content of the wheat and the selenium content of the wheat calculated from the selenomethionine content was calculated.

Table 4: The selenomethionine and the organic selenium content of wheat calculated from the selenomethionine content

Sample	Dry matter, %	Selenomethionine content, mg/kg (3)	Organic selenium content, mg/kg (4)
1.	90.3	0.241	0.097
2.	89.9	0.147	0.059
3.	90.2	0.0122	0.0049
4.	90.4	0.030	0.012
5.	91.2	0.258	0.104
6.	90.6	0.221	0.089
7.	90.6	0.241	0.097
8.	90.4	0.253	0.102
9.	91.1	0.211	0.085
10.	90.9	0.0244	0.0098
11.	90.6	0.132	0.053
12.	90.1	0.042	0.017
13.	90.4	0.236	0.095
14.	90.9	0.176	0.071
15.	90.0	0.082	0.033
16.	90.1	0.092	0.037
17.	90.6	0.221	0.089
18.	90.7	0.268	0.108
19.	90.0	0.122	0.049
20.	91.2	0.181	0.073
21.	90.6	0.080	0.032
22.	90.8	0.144	0.058
23.	91.0	0.152	0.061
24.	90.4	0.122	0.049
25.	90.0	0.109	0.044
26.	90.0	0.089	0.036
27.	91.0	0.167	0.067
28.	90.8	0.276	0.111
29.	90.1	0.189	0.076
30.	90.7	0.306	0.123
31.	90.0	0.072	0.029
32.	90.6	0.082	0.033
33.	90.5	0.072	0.029
34.	90.0	0.067	0.027
35.	90.9	0.147	0.059
36.	90.2	0.236	0.095
37.	90.3	0.159	0.064
38.	90.5	0.065	0.026
39.	90.4	0.176	0.071
40.	91.0	0.114	0.046
41.	90.6	0.072	0.029
42.	90.4	0.169	0.068
43.	90.0	0.0097	0.0039
44.	90.5	0.166	0.067

The calculus was based on the known molecular mass of the selenomethionine. From the data analysis of the 44 measurements, the value of the corre-

lation coefficient was found to be 0.99 (at the significance level of $P < 0.001$), indicating a very strong relationship.

This very strong correlation was expectable since the selenium bounded in the selenomethionine represents an important part of the total selenium. As the wheat samples were collected in the same time (in the same developmental state), it was expectable that the correlation between the two values should be very tight. Next, the correlations between the total selenium content related to the original dry-matter of the wheat grass, the selenomethionine content of the wheat and the selenium content calculated from selenomethionine content, respectively, were investigated. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.92 (at the significance level of $P < 0.001$), indicating a very strong relationship. In the case of the selenium content calculated from the selenomethionine content, for the 35 measurements, the obtained correlation coefficient was likewise 0.92 (at the significance level of $P < 0.1$), indicating a very strong correlation as well.

Hereinafter, the correlation between the total selenium content of the wheat grass related to 100% dry-weight content and the selenomethionine, respectively the selenium content of the wheat calculated from selenomethionine content was calculated. From the data analysis of the 35 measurements in the case of selenomethionine content, the value of the correlation coefficient was found to be 0.92 (at the significance level of $P < 0.001$), indicating a very strong relationship. For the selenium content of the wheat, calculated from the selenomethionine content of the 35 analysed samples, the value of the correlation coefficient was the same, 0.92 (at the significance level of $P < 0.001$).

Next, the correlation between the selenomethionine content of the wheat and the selenomethionine content of the wheat grass related to 100% dry-weight and the selenomethionine, respectively, the selenium content of the wheat related to the original dry matter content, from selenomethionine content, was investigated. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.23 (at the significance level of $P < 0.5$), indicating a weak relationship. And, at last, the correlation between the selenomethionine content of the wheat and the selenomethionine content of the wheat grass related to 100% dry-weight was investigated. From the data analysis of the 35 measurements, the value of the correlation coefficient was found to be 0.27 (at the significance level of $P < 0.5$).

In *Table 5*, the correlations between the selenium, selenomethionine and organic selenium content calculated from the selenomethionine content of wheat and wheat grass samples are summarized.

Table 5: Correlations between the selenium, selenomethionine and organic selenium content calculated from the selenomethionine content of wheat and wheat grass samples (The summarizing table of the correlation coefficients)

Components	P%	r
Wheat grass Se content related to original dry matter - Wheat grass Se content related to 100% dry matter	0.001	0.92
Wheat grass Se content related to original dry matter - Wheat Se content related to original dry matter	0.1	0.36
Wheat grass Se content related to 100% dry matter - Wheat original dry matter content	0.1	0.40
Wheat grass Se content related to original dry matter content - Wheat Se content calculated from Se-Met content	0.1	0.34
Wheat grass Se content related to 100% dry matter - Wheat Se content calculated from Se-Met content	0.05	0.40
Wheat Se content related to original dry matter - Wheat Se content calculated from Se-Met content	0.001	0.99
Wheat grass Se content related to original dry matter- Wheat grass Se-Met-content	0.001	0.92
Wheat grass Se content related to original dry matter - Wheat grass Se content calculated from Se-Met content	0.001	0.92
Wheat grass Se content related to 100% dry matter - Wheat grass Se-Met content	0.001	0.92
Wheat grass Se content related to 100% dry matter - Wheat grass Se content calculated from Se-Met content	0.001	0.92
Wheat Se-Met content Wheat grass Se-Met content related to original dry matter	0.5	0.22
Wheat Se-Met content Wheat grass Se-Met content related to 100% dry matter	0.5	0.27

The strongest correlation was observed between the selenium content of the wheat grass samples related to the original and to 100% dry matter content, as well as between the selenium content and the selenium content calculated from the selenomethionine content of the wheat and wheat grass samples. In these cases, the correlation coefficient values are ranged between 0.92 and 0.99. A considerably weaker relationship between the analysed data obtained for wheat and wheat grass was observed when the values of the correlation coefficients were situated between 0.34 and 0.40. A very loose correlation was obtained when the wheat and the wheat grass selenomethionine with the selenium (both dry- and wet-based) content was compared since the correlation coefficient was between 0.22 and 0.27.

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Somatic cell count of milk from different goat breeds

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Abstract. There is no standard limit value for somatic cell count (SCC) of raw goat milk in the EU despite that excellent hygienic quality milk is needed for the manufacture of fermented milk products or cheese varieties. Mastitis often results such high SCC – besides the potential risk for humans – that the clotting of milk will not be perfect, resulting slack curd with higher whey releasing; furthermore, wrong structure, ripening, bad sensory properties of cheese can also be its consequences. In this paper, we report the SCC of milk samples from five different goat breeds bred in Hungary, measured with two fast methods compared with the results from the reference method. Furthermore, we investigated the applicability and the accuracy of the MT-02 (Agro Legato Ltd., Hungary) instrument. We determined that the White Side test and the instrument MT were suitable for the estimation of possible risks and consequences in the case of the use of high SCC milk before production. The general summarized average milk SCC was $6.64 \times 10^5\,\mathrm{ml}^{-1}$. The highest difference between the results from MT-02 and the fluorometric (reference) method was $5 \times 10^5\,\mathrm{ml}^{-1}$, but it was a singular, extreme value. The r^2 of the calculated linear calibration equation was 0.7819; consequently, this method seems to be applicable in the measurement of SCC with MT-02 instrument. Furthermore, the SCC of samples did not differ significantly by genotypes and by seasons (spring: $5.85 \times 10^5\,\mathrm{ml}^{-1}$, autumn: $6.22 \times 10^5\,\mathrm{ml}^{-1}$).

Keywords and phrases: SCC, goat milk, fast test.

1 Introduction

The popularity of milk products – mainly cheeses – from goat milk having high physiological value shows a rising tendency. Mainly soft cheeses are made from goat milk and they show a high variety of shape, size and flavouring. Usually, goat milk is processed in small creameries (farmer creameries) by hand on the base of the traditions regarding the consumers' demands. The fermentation ability of milk is a very important criterion of cheese making.

The fermentation ability of milk and the quality of cheese are also decisively influenced by the hygienic quality of raw milk (*Unger*, 2001). One of these hygienic properties is Somatic Cell Count (SCC), which has a strict regulation for limit values in many countries (e.g. 4×10^5 ml^{-1} for cow milk). However, in most of the countries, the SCC of raw goat milk is not regulated. The SCC of milk has been strongly investigated also by many Hungarian researchers in the past; so, nowadays, we already have knowledge related to the adverse effects of mastitis and subclinical mastitis on cheese making (*Merényi, Wágner*, 1985; *Gulyás*, 2002; *Varga*, 2008).

Several researchers reported a close relationship between the high SCC of milk and cheese yield and the losses of constituents in whey (*Barbano et al.*, 1991; *Politis & Ng-Kwai-Hang*, 1988; *Mitchel et al.*, 1986). Similar observations were also published by some researchers (*Kukovics et al.*, 1995; *Zeng & Escobar*, 1995; *Pajor et al.*, 2009; *Chen et al.*, 2010) in the case of goat milk, having proved the fast determination of SCC of raw goat milk, essentially for making fermented milk products and cheeses. So, there is a need for fast methods – due to the specialty of small-scale milk processing and the lack of regulation – in order to select the goat milk with very high SCC because this milk can be mentioned as unsuitable for cheese making.

Our goal was monitoring the SCC of raw milk samples from different goat breeds and from different lactation periods. White Side test and MT-02 instrument (Agro Legato, Budapest, Hungary) were used for SCC determination. Additionally, we evaluated the applicability and the precision of MT-02 instrument – a fast test for the SCC determination. For this aim, calibration samples data were used from official fluoro-optical method (Fossomatic instrument).

2 Materials and methods

Materials

The samples were collected from two farms located on the Hungarian Great Plain. Kidding was scheduled for spring (February-March) in both farms. Samples were collected from ten Alpine and ten Saanen goats in spring and in autumn on three occasions in the first farm. Samples were collected in the second farm from Alpine and from Alpine x Saanen cross-bred goats also in spring and autumn, but only in autumn from Native goats.

Goats were milked by hand twice a day. Samples were prepared by mixing of morning and evening individual milk and they were refrigerated at 5 °C until investigation. The samples were investigated in the laboratory of the Department of Food Engineering at the Faculty of Engineering, University of Szeged, Hungary. The calibration samples were investigated at the Hungarian Dairy Research Institute Ltd., Budapest, Hungary.

Methods

White Side test

The White Side test is based on the complex molecule formation between the sodium-hydroxide and the DNA of somatic cells, and then the denaturation phenomenon. Samples can be evaluated with naked eyes based on the ratio of denaturation (*Szakály*, 1966). The milk is accepted (the test is negative; ("-") if there is no change in any visible milk properties, including consistency. The result is positive ("+") if visible small (clumping) particles appear in the sample (approx. of 0.5 mm in diameter, like semolina). In this case, the SCC is between $2.5 \times 10^5\,\mathrm{ml}^{-1}$ and $1.0 \times 10^6\,\mathrm{ml}^{-1}$. The used samples enter only these two classification groups in the evaluation.

MT-02 instrument

The principle of this test is very similar to the White Side test. The SCC determination is based on the change of the viscosity of the milk sample. 10 ml of milk sample (37 °C) has to be mixed with 5 ml 20% reagent (diluted with distilled water) rapidly; then it has to be filled into the funnel and measuring has to start immediately. The structure of the instrument is very similar to a Höppler viscometer: the viscometer pipe is rotated to an adjusted angle after 20 sec. The results can be read from the scale built in the pipe. The measuring range is $10 \times 10^3 - 2 \times 10^6\,\mathrm{ml}^{-1}$. This method was developed for

cow milk measuring; so, we had to make a calibration for goat milk with known SCC goat milk samples. For this purpose, first of all, twenty Saanen goat milk samples were investigated both with MT-02 instrument and with official fluoro-optical method (Fossomatic).

3 Results and discussion

Estimation of the applicability of MT-02 instrument, calibration

In order to evaluate the accuracy of data from MT-02 instrument, we measured 20 raw milk samples from Saanen goats. The samples were measured first by MT-02 instrument, and then the selected 10 samples that seemed to be useable were sent to the official laboratory. After receiving the data, we looked for the correlation between the data groups obtained with different methods. Our hypothesis was that if the correlation is sufficiently close, by using this correlation equation and the data from the MT-02 instrument, we can create similarly acurate data as those obtained with the reference method. *Figure 1* shows the founded correlation between the official data and the data from the MT-02 instrument. The acceptable determination coefficient of this linear trend line gives us a chance to receive a more precise evaluation of the SCC of goat milk as compared to the White Side test. The presented correlation equation was used for SCC determination in the further investigation.

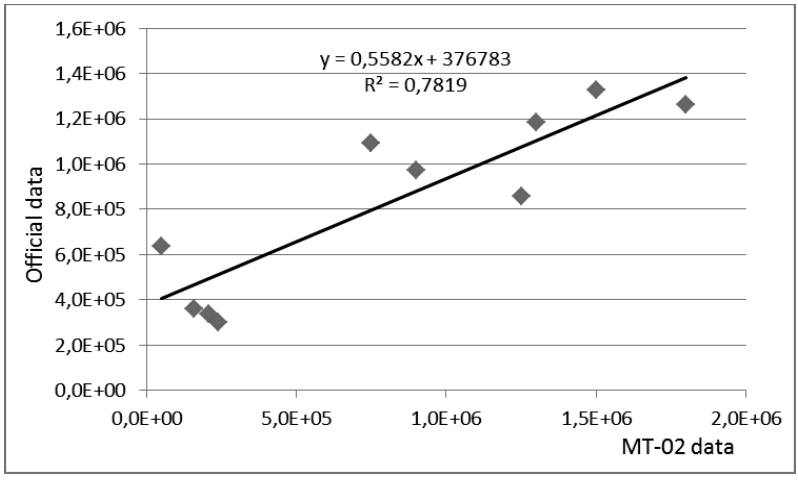

Figure 1: Correlation between official data and MT-02 data

Results from different goat breeds

The average SCC of all measured samples determined with MT-02 was 5.69× $10^3\,\mathrm{ml}^{-1}$, but the values varied within a very wide range. The summarized data of all measured samples are presented in *Table 1.*

Table 1: Comparison of the original MT-02 and the modified data, using explored calibration (n=116)

"Method"	Average ($10^3\,\mathrm{ml}^{-1}$)	Variation ($10^3\,\mathrm{ml}^{-1}$)	CV (%)
Original MT-02 data	569	669	117.5
Data from calibration	665	365	54.9

30% of samples did not fit in the measurement range – which was $1.0 \times 10^3\,\mathrm{ml}^{-1}$ $-2.0 \times 10^6\,\mathrm{ml}^{-1}$ – maybe due to the abnormal composition of milk samples, causing extreme low or extreme high milk viscosity. We did not investigate the reasons of this phenomenon; consequently, we have no proper explanation for this. According to our results, the SCC values from MT-02 were underestimated. The calibrated SCC average was "only" $9.5 \times 10^5\,\mathrm{ml}^{-1}$ higher than the original MT-02 value, but the difference between the data pairs from the different methods showed a very high variation ($5.0 \times 10^4 - 5.0 \times 10^5\,\mathrm{ml}^{-1}$).

Anisity (2008) investigated the precision of the MT-02 instrument, measuring cow milk samples, and determined a $1.18 \times 10^5\,\mathrm{ml}^{-1}$ average difference from the official data. Our calculated difference stands very close to his data, suggesting that MT-02 can be used also for measuring the SCC of goat milk, but mainly below the $1 \times 10^6\,\mathrm{ml}^{-1}$ SCC value. We explain this limited application with the special resolution of the scale of the instrument because its resolution is fine enough only below $1 \times 10^6\,\mathrm{ml}^{-1}$ value and – due to the limited number of samples – we were not able to use an optimal sample series for the calibration.

The smallest difference between the official and the MT-02 data was explored in the range of $5.0 \times 10^5\,\mathrm{ml}^{-1} - 8.0 \times 5 \times 10^5\,\mathrm{ml}^{-1}$. We strongly suggest taking these comments into consideration, reviewing our detailed results.

Alpine goats

The milk samples from the Alpine goats were measured in spring and autumn on both goat farms (*Table 2*). The first sampling was carried out during the suckling period in farm "A".

Table 2: SCC of milk samples from Alpine goats ($10^5\,ml^{-1}$) n = 120

	Farm A		Farm B	
	Autumn	Spring	Autumn	Spring
Min.	3.80	4.90	3.90	2.60
Max.	11.00	14.00	8.60	12.00
Average	**7.28**	**9.25**	**5.90**	**5.78**
Variation	3.50	4.76	2.69	3.02
CV%	48.08	51.46	45.59	52.25
WST (%)	**67.90**	**63.40**	**70.80**	**68.80**

Data represent the result of White Side tests and they show the summarized ratio (%) of negative and "+" samples: estimated SCC $< 1.0 \times 10^6\,ml^{-1}$.

The SCC averages of Farm B from spring and autumn were very similar. We noticed that one of the samplings was carried out before the separation of the kids. Furthermore, the SCC average value of this sampling was lower than the summarized average from this farm; consequently, the suckling had no adverse effect on the SCC of milk; it did not cause SCC increase.

The average SCC values on Farm A were higher than on Farm B, but none of the averages exceeded the $1 \times 10^6\,ml^{-1}$ threshold. This result differs from *Varga's* (2008), who explored higher SCC than $1 \times 10^6\,ml^{-1}$ in the case of all investigated samples from refrigerated storage. In our investigation, only 28% of the Alpine goats' milk samples have reached this limit. This result can be mentioned as a good result regarding the large number of samples exceeding the measuring limit of the MT-02 instrument during our whole experiment.

Other breeds

The SCC averages of samples from Hungarian White goats presented much higher values (*Table 3*). Most frequently, mastitis was explored in the population of this breed. Higher SCC values were typical, and we found more samples displaying extremely high SCC at each sampling. Extreme viscosity increase and stickiness were explored. In the case of the extreme samples, adding the reagent to the milk made measuring impossible.

Results of Native, Saanen and Alpine x Saanen cross-bred goats are presented in *Table 4*. Native goats varied very much regarding the horn and colour varieties. There were black & white, fawn-coloured, grey and white goats as well. The results from Native goats showed the highest variation.

Table 3: SCC of milk samples from Hungarian white goats ($10^5\,\mathrm{ml}^{-1}$) n = 90

	July	August	September	Average
Min.	3.60	5.80	5.60	5.00
Max.	9.30	11.00	17.00	12.40
Average	**6.93**	**9.05**	**11.2**	**9.06**
Variation	3.54	2.94	5.07	3.85
CV%	51.08	32.49	45.27	42.49
WST	**71.80**	**63.20**	**57.40**	**64.10**

Data represent the result of White Side tests and they show the summarized ratio (%) of negative and "+" samples: estimated SCC $< 1.0 \times 10^6\,\mathrm{ml}^{-1}$.

Table 4: SCC of samples from Native, Saanen and Alpine x Saanen cross-bred goats ($10^5\,\mathrm{ml}^{-1}$) n = 150

	Native		Saanen		Alpine x Saanen cross-bred	
	Autumn	Spring	Autumn	Spring	Autumn	Spring
Min.	2.10	nd	4.90	1.80	1.60	5.90
Max.	8.20	nd	8.10	8.80	9.20	9.60
Average	**6.87**	**nd**	**6.22**	**5.85**	**5.91**	**8.87**
Variation	3.24	nd	2.95	3.10	3.17	2.63
CV%	47.16	nd	47.43	52.99	53.64	29.65
WST	48.20	nd	73.40	75.10	69.80	61.10

Data represent the result of White Side tests and they show the summarized ratio (%) of negative and "+" samples: estimated SCC $< 1.0 \times 10^6\,\mathrm{ml}^{-1}$.
nd= There is no data.

Evaluating our results, we can mention that these results are very similar to the results of certain cited Hungarian foreign authors (*Turin et al.*, 2004; *Gomes et al.*, 2006; *Stella et al.*, 2007). But our SCC averages do not reach the results published by *Garcia-Hernandez* et al. (2006) and *Delgado-Petrinez* et al. (2003). Furthermore, the different authors, including us, agree that the SCC of goat milk is higher than that of the cow milk, but goats may not suffer from mastitis. This observation also implies that there is not a very close relationship between the SCC of milk and the health condition of goats, contrary to the cows. The instrument seemed to be the most precise between the SCC range of $4.0 - 8.0 \times 10^5\,\mathrm{ml}^{-1}$.

The results of the White Side tests also proved that the SCC of goat milk (can be called as good, quality milk) can well exceed the SCC of cow milk. The action limit (threshold) for the SCC of goat milk is $1 \times 10^6\ \mathrm{ml}^{-1}$ in the USA. This can be explained by the different physiology and different milk secretion mechanisms of the goats and cows (*Mc Dougall &, Voermans*, 2002). Regarding the results of the White Side tests, it is presumable that the "negative" and "+" samples give 60-70% of all goat milk on a typical goat farm. But it is very important to mention that the milk having "++" or "+++" White Side test classification has a limited value, it exceeds the $1 \times 10^6\,\mathrm{ml}^{-1}$ SCC value in every case, it is not homogenous and it contains sticky and mucous precipitations very often. We can confirm that goat milk having very high SCC, as mentioned above, is not suitable for making fermented goat milk products. Furthermore, it is absolutely sure that very high SCC goat milk (with serious precipitations) is not suitable for making any milk product. Based on our result, we agree with the suggestion of *Zeng* (1996), who offered different standards (made from goat milk and not from cow milk) for the calibration of SCC measuring instruments. Additionally, the producer should create a new scale of MT-02 instrument for measuring goat milk samples. There is a need to investigate a huge number of samples in future research to refine the precision of this method, and we suggest the exclusive application of the range of $2.0 \times 10^5\,\mathrm{ml}^{-1} - 1.5 \times 10^6\,\mathrm{ml}^{-1}$ in order to attain the highest reliability.

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Milling technological experiments to reduce Fusarium toxin contamination in wheat

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Abstract. We examine 4 different DON-toxin-containing (0.74 – 1.15 – 1.19 – 2.14 mg/kg) winter wheat samples: they were debranned and undebranned, and we investigated the flour's and the by-products' (coarse, fine bran) toxin content changes. SATAKE lab-debranner was used for debranning and BRABENDER lab-mill for the milling process. Without debranning, two sample flours were above the DON toxin limit (0.75 mg/kg), which are waste. By minimum debranning (and minimum debranning mass loss; 6-8%), our experience with whole flour is that the multi-stage debranning measurement significantly reduces the content of the flour's DON toxin, while the milling by-products, only after careful consideration and DON toxin measurements, may be produced for public consumption and for feeding.

1 Introduction

One of the most important factors to improve the way of life of humans is ensuring healthy foods. Food safety deserves special attention: cereal grains

Keywords and phrases: wheat kernel, DON toxin, scalping, debranning.

and animal feed are infected by the mycotoxins of moulds at variable rate and in variable amounts. We eat grain-based products every day, so it is essential to minimize the material toxin contamination. The mould of the largest food and feed safety hazards caused by the Fusarium fungus species is called mycotoxicosis (*Raffaseder*, 2003; *Laczay*, 2004; *Reiss*, 1981). Grain processing requires that the mycotoxin content stay in the grist (flour, bran, germ etc.) under the regulation (EC 1881/2006) limits (*Weidenbörner*, 2001; *Mesterházy*, 2002).

One of the most important, most dangerous toxins is the deoxynivalenol (DON), which inhibits protein synthesis and the autoimmune system (*Mesterházy*, 2007; *Szeitzné et al.*, 2009), and in extreme cases it can cause cancer (*Marasas*, 1995). Most of the Fusarium toxin formation is stable, resistant to food treatments and responsive to heat (*Hopmans & Murphy*, 1993; *Scott*, 1990). Fusarium toxins are especially on the wheat grain surface and the outer layer of the hull; so, the surface cleaning, before the grinding – as a toxin reducing process – comes into view (*Bottega et al.*, 2009; *Brera et al.*, 2006).

During our experiments, we applied paring of the wheat grains, which is a new surface treatment (debranning) procedure in milling technology to investigate the effect of this technique on the ability of decreasing mycotoxin contamination. The most common and the most abundant toxin is the Fusarium toxin, the DON (deoxynivalenol), so we measured the amount of this toxin by using different debranning times and the wheat contamination was also different.

Tolerable daily intake (TDI) values of DON for adults and infants were found to be 3 and 1.5 μg/kg body weight, respectively (*Kuiper-Goodman*, 1985). Therefore, we measured the DON content of the milling fractions of wheat samples with different contamination level, using various degrees of paring by using liquid chromatography-mass spectrometry (HPLC-MS), which has become the most frequently used technique in mycotoxin analysis (*Zöllner & Mayer-Helm*, 2006).

2 Materials and methods

Materials

Experiments were performed at four different places, from different vintages and the wheat had different toxin contaminations.
Sample 1: 2008, naturally infected wheat (Gabonakutató Ltd, Szeged), DON content: 0.75 mg/kg.

Sample 2: 2008, Fusarium strains, artificially infected wheat (Gabonakutató Ltd, Szeged), DON content: 1.19 mg/kg.
Sample 3: 2010, naturally infected mill wheat (BÁF Ltd., Szeged); DON content: 1.15 mg/kg
Sample 4: 2010, naturally infected wheat, which was excluded from the milling process (Szatmári Malom Ltd., Jászberény), DON content: 2.14 mg/kg.

Debranning experiments

We modelled the PeriTec technology with a laboratory-size, batch-operating horizontal debranning machine by SATAKE. The main part of the equipment is a cylindrical working space delimited by a perforated plate. In this working area, there is a horizontal-spindle, corundum-covered grinding wheel rotating. The operation of the machine is batch-type; 200 g of wheat can be treated at a time. After tempering the samples to a moisture content of 15%, we leave the samples to rest for 12 hours. 2 kg of samples were used in the experiment, 10 repetitions were made for each sample. The debranning time was: 10, 20 (one time, 30 s) and 40 s.

Grinding

The undebranning and debranning samples were ground by BRABENDER Quadrumat Senior laboratory mill. The device has 2×2 rollers and sieve classification. The roll pairs are fixed; four rolls and three roll gaps grind the wheat. The grinding takes place in two stages and there are three fractions: flour, fine bran and coarse bran.

Determination of DON toxin content

Briefly, the DON toxin content of the samples was determined by using an Agilent 1100 HPLC system containing a well-plate autosampler, a quaternary pump, a column thermostat and an Agilent 1946A quadrupole mass spectrometer equipped with an electrospray ion source. The homogenized grist samples (1 g each) were extracted with 4 ml mixture of acetonitrile/water (84/16 v/v) in polypropylene centrifuge tubes (10 ml volume) at room temperature for 120 min, by using an overhead shaker, and centrifuged at 10,000 x g for 10 min. 750 µl volume of the supernatants were pipetted into the well plate of the HPLC autosampler and subsequently analysed. HPLC separations were performed on a Phenomenex, Kinetex C18 column (50×4.6 mm, 2.6 µm) at

a flow rate of 0.8 ml/min by using a binary gradient of acetonitrile and water. The solvents were supplemented with 0.1% formic acid.

The protonated DON molecules ($[M^+H]^+$) were detected at m/z value of 297, using the SIM mode of the mass spectrometer. Internal standard quantitation was performed by using a Romer Labs (Tulln, Austria) $^{13}C_{15}$DON standard.

3 Results and discussion

Table 1 shows the results. From the wheat samples, three were under the limit (1.25 mg/kg) and one sample (Sample 4) had DON toxin content above the limit (2.14 mg/kg). During the debranning, 6-8% of hull was removed, which caused the DON toxin content a significant reduction (reduced to half the initial toxin content) (Figure 1). As the result of the intense debranning, Sample 4, as "technological waste," was good (the DON content reduced under the toxin limit: 1.14 mg/kg) for the milling process. The debranning loss (10-13%) is not proportional to the weight loss values in the toxin content reduction; so, the toxin contamination is concentrated on the outer surface of the grain.

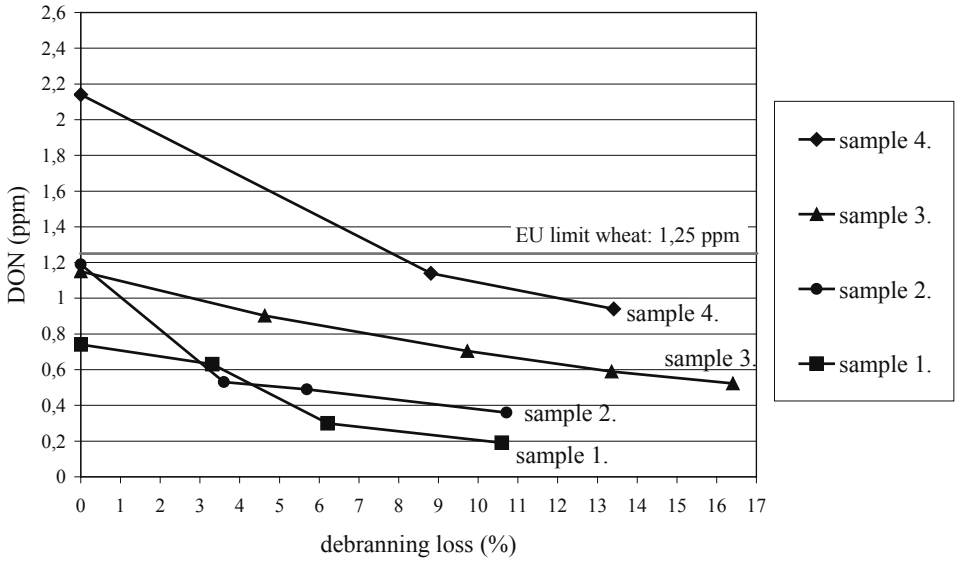

Figure 1: DON toxin contamination changes of wheat during debranning

Table 1: DON toxin content of wheat and grits samples after debranning and milling

	Debranning time (s)	Sample 1		Sample 2		Sample 3		Sample 4	
		Amount (%)	DON (mg/kg)	Amount (%)	DON (mg/kg)	Amount (%)	DON (mg/kg)	Amount (%)	DON (mg/kg)
Grain	0	100.00	0.74	100.00	1.19	100.00	1.15	100.00	2.14
	10	96.69	0.63	96.40	0.53	95.37	0.90		
	20	93.79	0.30	94.31	0.49	90.27	0.70	91.19	1.14
	30					86.64	0.59	86.59	0.94
	40	89.40	0.19	89.29	0.36	83.59	0.52		
Removed parts of the hull	10	3.31	4.20	3.60	5.04	4.63	8.33		
	20	6.21	4.07	5.69	4.66	9.73	6.85		
	40	10.60	3.83	10.71	2.74	16.41	5.03		
Flour	0	68.44	0.25	66.80	0.88	63.56	0.58	68.73	1.09
	10	68.60	0.20	70.97	0.40	63.25	0.42		
	20	70.09	0.14	71.28	0.38	65.43	0.37	66.63	0.60
	30							66.02	0.57
	40	69.37	0.11	71.77	0.29	60.98	0.31		
Coarse barn	0	27.79	2.25	26.38	1.48	26.52	2.46		
	10	20.93	1.67	18.96	1.30	23.31	2.39		
	20	16.38	1.41	17.30	1.05	17.10	2.22		
	40	15.96	1.31	14.23	1.01	14.18	2.01		
Fine bran	0	3.77	1.59	6.82	1.12	9.92	1.48		
	10	7.16	1.20	6.47	0.90	8.81	0.86		
	20	7.02	0.94	5.72	0.86	7.74	1.00		
	40	4.07	0.61	3.28	0.95	8.43	0.89		

We measured (0.75 mg/kg) DON content above the limit in Sample 2 (0.88 mg/kg) and in Sample 4 (1.09 mg/kg) without debranning. Close to the limit was Sample 2 (grains DON: 1.19 mg/kg), which will be "waste" flour without debranning, while with a minimum debranning loss (~3.6%) it will be consumed because the DON level of flour was reduced (0.4 mg/kg). The DON content of Sample 4, flour without debranning, is over the limit (1.09 mg/kg), but with the practical debranning (with 8.81% bran loss) the milling flour's DON toxin content (0.6 mg/kg) is under the health limit (*Figure 2*).

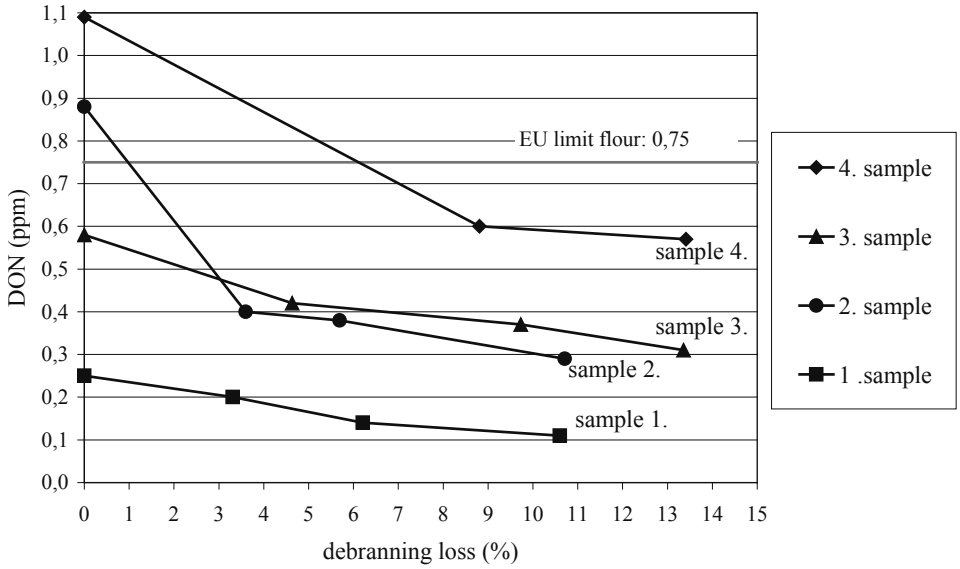

Figure 2: DON toxin contamination changes of flour during debranning

We examined 4 different DON-toxin-containing (0.74–1.15–1.19–2.14 mg/kg) winter-wheat samples: they were debranned and undebranned, and we investigated the flour's and the by-products' (coarse, fine bran) toxin content changes. The toxin content of the removed part of the hull was determined only for the first three samples: the amount was in connection with debranning time (10, 20, 40 sec.): at least, debranning: 3-4%, medium debranning: 6-9%, intensive debranning: 10-16%.

In the first stage, we received the highest DON content, 4-8 mg/kg; with further debranning, the DON contamination decreases. It shows that the mycotoxin is generally on the outer bran layer, where it is strongly adsorbed during debranning; the toxin content is smaller in the inner bran layers.

The same conclusion is reinforced in the DON toxin results of coarse and fine bran fractions: a minimal DON contamination (0.74 mg/kg) excludes the eating of bran. The by-product of milling (coarse, fine bran), which is close to the limit (1.19, 1.15 mg/kg DON), is "hazardous waste".

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Investigation of rheological properties of winter wheat varieties during storage

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Abstract. The paper shows the results of some experimental researches on the rheological characteristics of the dough obtained from the flour of three winter wheat varieties. We used valorigraph test to determine the rheological properties of wheat flour dough, because it determines the quality of the end-products. Winter wheat varieties (Lupus, Mv Toldi and GK Csillag) were produced and their samples were collected on Látókép Research Farm of the University of Debrecen in the crop year of 2011/2012. We have carried out a short-term storage experiment (from July to August, 2012). We analysed the changes in water absorption capacity, dough stability time and valorigraph quality number for 3 times (24.07.2012, 31.07.2012, 21.08.2012) during short-term storage. Our results showed that the baking quality of Lupus, Mv Toldi and GK Csillag improved during the storage period.

Keywords and phrases: winter wheat, rheological properties, storage, valorigraph test.

1 Introduction

Wheat is one of the most important bread crops in Hungary. Wheat flour has a unique property, which means if the flour is mixed with water it creates a viscoelastic dough capable of retaining the produced gas during fermentation and at the beginning of baking (*Walker* and *Hazelton*, 1996; *Khatkar*, 2004; *Véha et al.*, 2012).

Storage time has influence on the quality of wheat and milled flour parameters, but it also depends on wheat variety, environmental factors, the conditions of harvest and the milling technology. All the mentioned factors are important for the use of flour and the quality of the end-products (*Hrušková* and *Machová*, 2002; *Gyimes et al.*, 2011).

Cereal technologists used traditional dough testing instrument, such as valorigraph, which is an empirical rheological method to predict final product quality (*Miranda-Garcia*, 2013; *Berland* and *Launay*, 1995). The mixing characteristics of wheat dough are largely attributed to gluten proteins. During wheat storage, several biological and chemical interactions occur. *Balla et al.* (1993) found that a slight growth could be in the quantity of wet gluten content and that the quality of gluten (stability of gluten) improved significantly during after-ripening and storage period also.

Our aim was to determine the changes of rheological properties during the short-term storage of three winter wheat varieties (Lupus, MV Toldi and GK Csillag). We also measured how rheological properties (water absorption capacity, dough stability time and valorigraph quality number) change by the applied different fertilization levels.

2 Materials and method

Winter wheat samples were collected from Látókép Research Institute of the University of Debrecen in 2012. The long-term experiment was set up in 1983 and it had a split plot arrangement with three repetitions. *Table 1* shows the applied $N/P_2O_5/K_2O$ doses in this experiment. We analysed how the valorigraph properties change on the second and fourth NPK treatment levels.

After harvesting and cleaning (MSZ 6367/2:2001), the wheat samples were stored (MSZ 6383:1998) in the sample storage room of the Institute of Food Science, Quality Assurance and Microbiology of the University of Debrecen in polypropylene bags. Three winter wheat varieties were the basis of our study (Lupus, Mv Toldi and GK Csillag), and we examined the wheat samples for three times (24.07.2012, 31.07.2012, 21.08.2012).

Table 1: The applied NPK doses of the winter wheat field experiment (Látókép)

Treatments	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
Control	0	0	0
1.	30	22.5	26.5
2.	60	45	53
3.	90	67.5	79.5
4.	120	90	106
5.	150	112.5	132.5

We mixed 1 kg from each sample and, after homogenization, the wheat conditioning was performed. The moisture content of wheat varies around 14%, depending on the variety, and these will normally need to be conditioned to 16.5% prior to milling (25 °C, 24 hrs). Winter wheat samples were milled by a LABOR MIM FQC 109 (METEFÉM, Budapest, Hungary) laboratory mill, and we used a 250-µm sieve.

We have carried out a valorigraph test (MSZ ISO 5530-3:1995, METEFÉM FQA 205) to examine the influence of short-term storage on water absorption capacity, dough stability time and valorigraph quality number. The experimental results were analysed by One-Way analysis of variance (Post Hoc test – Duncan test) by the SPSS 13 (statistical programme package (SPSS Inc., USA)) for Windows.

3 Results and discussion

The application of 60/45/53 kg/ha $N/P_2O_5/K_2O$ doses in a field experiment, water absorption capacity (%), dough stability time (s) and valorigraph quality number were evaluated three times during the short-term storage (*Table 2*).

Table 2 shows that the water absorption capacity (%) and dough stability time (s) did not change significantly within one month after the harvest, but the valorigraph quality number increased during storage time. As the last column of *Table 2* shows, quality group increased (from B2 to B1) upon the second time (31.07.2012), but it did not change upon the last measuring time (B1). Significant difference was found in the valorigraph quality number 4 weeks after the first measurement (21.18.2012).

Table 2: Influence of short-term storage on the valorigraph parameters of Lupus (60/45/53 kg/ha $N/P_2O_5/K_2O$)

Lupus (60/45/53)	Water absorption capacity (%)	Dough stability time (s)	Valorigraph quality number	Quality group
24.07.2012	61.4 ± 0.4^a	620 ± 17.3^a	54.3 ± 1.3^a	B2
31.07.2012	61.3 ± 0.4^a	535 ± 56.7^a	60.2 ± 2.4^a	B1
21.08.2012	61.4 ± 0.3^a	580 ± 34.6^a	67.7 ± 5.4^b	B1

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Dough stability time (s) and valorigraph quality number changed significantly (*Table 3*) during storage on a higher fertilization level of Lupus (120/90/106 kg/ha $N/P_2O_5/K_2O$). The water absorption of Lupus was not influenced by the duration of storage.

Table 3: Influence of short-term storage on the valorigraph parameters of Lupus (120/90/106 kg/ha $N/P_2O_5/K_2O$)

Lupus (120/90/106)	Water absorption capacity (%)	Dough stability time (s)	Valorigraph quality number	Quality group
24.07.2012	63.13 ± 0.2^a	670 ± 45.8^b	69.4 ± 1.1^b	B1
31.07.2012	62.6 ± 1.0^a	530 ± 17.3^a	64.7 ± 2.4^a	B1
21.08.2012	62.87 ± 0.4^a	655 ± 8.6^b	69.6 ± 5.4^b	B1

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Dough stability time was the highest at the beginning of our evaluations and the strength of dough decreased upon the second time. Dough stability also increased to the last test, but it did not reach the first value. Valorigraph quality number was the lowest on 31.07.2012. Quality group did not change during the short-term storage.

Storage time did not influence the rheological properties of Mv Toldi on the 60/45/53 kg/ha $N/P_2O_5/K_2O$ fertilization level (*Table 4*). Water absorption capacity, stability of dough and valorigraph number showed a slight increase in relation to the advancement of storage time, but it was not statistically significant.

Table 4: Influence of short-term storage on the valorigraph parameters of MV Toldi (60/45/53 kg/ha $N/P_2O_5/K_2O$)

MV Toldi (60/45/53)	Water absorption capacity (%)	Dough stability time (s)	Valorigraph quality number	Quality group
24.07.2012	63.2 ± 3.3^a	480 ± 51.9^a	60.5 ± 2.6^a	B1
31.07.2012	64.6 ± 0.5^a	480 ± 51.9^a	61.3 ± 4.1^a	B1
21.08.2012	65.4 ± 0.3^a	530 ± 51.9^a	64.9 ± 3.1^a	B1

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Water absorption of MV Toldi was not changed on a higher fertilization level (*Table 5*). Rheological properties, such as dough strength, valorigraph quality number and quality group, increased significantly to the end of the storage period.

Table 5: Influence of short-term storage on the valorigraph parameters of MV Toldi (120/90/106 kg/ha $N/P_2O_5/K_2O$)

MV Toldi (120/90/106)	Water absorption capacity (%)	Dough stability time (s)	Valorigraph quality number	Quality group
24.07.2012	65.2 ± 0.4^a	462 ± 88.9^a	61.3 ± 2.7^a	B1
31.07.2012	65.4 ± 0.2^a	495 ± 51.9^a	62.3 ± 2.8^a	B1
21.08.2012	65.6 ± 0.2^a	670 ± 73.9^b	74.5 ± 1.0^b	A2

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

The structure of gluten improved during after-maturing because dough stability time increased significantly. Quality group has also changed (from B1 to A2). In the case of GK Csillag, storage time did not induce significant changes in dough stability, valorigraph quality number and quality group at lower fertilization level (*Table 6*).

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 7 demonstrates the valorigraph parameters of GK Csillag (120/90/106 kg/ha $N/P_2O_5/K_2O$). In the storage experiment, there were significant differences in dough stability and valorigraph quality number.

At the end of the storage period, these two parameters showed increase; so, the baking quality of GK Csillag improved by higher doses of N/P/K fertilizer.

Table 6: Influence of short-term storage on the valorigraph parameters of GK Csillag (60/45/53 kg/ha $N/P_2O_5/K_2O$)

GK Csillag (60/45/53)	Water absorption capacity (%)	Dough stability time (s)	Valorigraph quality number	Quality group
24.07.2012	66.7 ± 0.8^a	270 ± 30.0^a	48.5 ± 4.2^a	B2
31.07.2012	65.4 ± 0.3^a	300 ± 0.0^a	47.1 ± 1.6^a	B2
21.08.2012	65.3 ± 0.6^a	300 ± 25.9^a	50.4 ± 4.0^a	B2

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 7: Influence of short-term storage on the valorigraph parameters of GK Csillag (120/90/106 kg/ha $N/P_2O_5/K_2O$)

GK Csillag (120/90/106)	Water absorption capacity (%)	Dough stability time (s)	Valorigraph quality number	Quality group
24.07.2012	67.5 ± 0.4^a	280 ± 45.8^a	49.7 ± 3.3^a	B2
31.07.2012	66.2 ± 0.5^a	335 ± 31.22^{ab}	53.3 ± 3.1^a	B2
21.08.2012	65.6 ± 1.5^a	375 ± 39.6^b	62.2 ± 0.7^b	B1

Means followed by the same letter within columns were not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

We analysed the influence of storage time on the valorigraph properties of winter wheat varieties on two N/P/K fertilization levels. In our study, we analysed wheat samples from a lower, 60/45/53 kg/ha $N/P_2O_5/K_2O$, and a higher, 120/90/106 kg/ha $N/P_2O_5/K_2O$, fertilization level. Based on our research, the water absorption capacity of the three winter wheat varieties (Lupus, MV Toldi and GK Csillag) did not change during storage on the applied levels of fertilizer treatments.

Dough stability was not changed on the level of 60/45/53 kg/ha $N/P_2O_5/K_2O$ treatment in the case of all the measured wheat varieties, but on the higher level of fertilization (120/90/106 kg/ha $N/P_2O_5/K_2O$) significant differences were found one month after the harvest as dough stability improved from 31.07.2012 to 21.08.2012 in the case of Lupus, MV Toldi and GK Csillag.

Valorigraph quality number and quality group increased by the end of the storage time by the use of 120/90/106 kg/ha $N/P_2O_5/K_2O$ in the case of Mv Toldi, Lupus and GK Csillag. We concluded that the changes in the valorigraph properties of the three wheat varieties were significant in the case of a higher level of fertilization.

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Colour characteristics of winter wheat grits of different grain size

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Abstract. Nowadays, wheat has spread all over the world due to its extensive usability. The colour of wheat grits is very important for the milling and baking industry because it determines the colour of the products made from it. The instrumental colour measuring is used, first of all, for durum wheat. We investigated the relationship between colour characteristics and grain size in the case of different hard aestivum wheats. We determined the colour using the CIE (Commission Internationale de l'Eclairage) 1976 L*, a*, b* colour system measured by MINOLTA CR-300 tristimulus colorimeter. After screening the colour of the wheat fractions of different grain size, grits was measured wet and dry. We determined the L*, a*, b* colour co-ordinates and the whiteness index, too. To evaluate the values we had obtained, we used analysis of variance and regression analysis. We pointed out that the colour of wheat grits of different grain size is dependent on the hardness index of wheat. The lightness co-ordinate (L*) of grits of the harder wheat is smaller, while a* and b* co-ordinates are higher. We also found that while grain size rises, the L* co-ordinate decreases and a*, b* values increase in the case of every type of wheat. The colour of grits is determined by the colour of fractions of 250-400 μm in size, independently from the average grain size. The whiteness index and the L* colour co-ordinate have a linear relation ($R^2 = 0.9151$); so, the determination of whiteness index is not necessary. The L* value right characterizes the whiteness of grits.

Keywords and phrases: colour measurement, wheat grits, colour of wheat.

1 Introduction

Wheat grindings are one of the most important and most frequently used raw materials. As for every alimentary product, colour is an important parameter for wheat grindings as well, giving them a primary image, especially for the durum wheat pasta since they do not contain eggs. This explains the fact why instrumental colour measurements are applied on durum semolinas in industrial practices as well. In literature, various research results report on the colour measurements of wheat grindings. Already in 1993, *Oliver et al.* (1993) showed, during the qualification, that ash content influences the colour of flours. *Halászné et al.* (1995) proposed a qualification system based on the colour measurements of durum semolina. *D'egido & Pagani* (1997) compared the colour characteristics of pasta made of durum flours obtained by different grinding procedures.

During the product manufacturing, the colour characteristics were mainly used to determine the appropriate roastedness (*Hotti et al.*, 2000). *Humphries et al.* (2004) found a correlation between CIE b* and the lutein concentration of wheat. *Konopka et al.* (2004) established a relation between the colour characteristics of the flours and their lipid and colorant content. *Gökmen & Senyuva* (2006) investigated the effect of heating on the colour parameters of wheat flour. *László et al.* (2008) examined the effects of ozone, UV and combined ozone/UV treatment on the colour of wheat flour. *Lamsal & Faubion* (2009) studied the effect of an enzyme preparation on wheat flour and dough colour and suggested that enzyme preparation did not improve the lightness (L*) and yellowness (b*) of the flour system, but benzoyl-peroxide sharply reduced b*.

We investigated how the colour characteristics depend on the grain size and hardness index of wheat. The relationship between the lightness co-ordinate and whiteness index was analysed too.

2 Materials and methods

Materials

In the course of our investigation, we used three different hard wheats. We can see their genus and hardness indexes in *Table 1.* At first, the samples were milled in drawn and air-dry condition, and then their colour characteristics were measured.

Table 1: Wheat genus and their hardness index

Wheat genus	Hardness index
GK-Jubilejnaja-50 (J-50)	80.44
GK-Öthalom (Öthalom)	71.66
GK-Mérő (Mérő)	20.78

Methods

The 250 g of grits made of different wheat types was screened for 10 minutes to separate particles of different grain size. *Table 2* shows the grading limits of fractions. Then we measured the mass and colour co-ordinates of the parts.

Table 2: Grading limits of classes

Grading limits of classes
0 μm – 100 μm
100 μm – 250 μm
250 μm – 400 μm
400 μm – 600 μm
600 μm – 800 μm
800 μm – 1,000 μm
1,000 μm – 1,250 μm

We determined the colour using the CIE 1976 L*, a*, b* colour system measured by MINOLTA CR-300 tristimulus colorimeter. In the CIE 1976 colour space, the colour points are characterized by three colour co-ordinates. L* is the lightness co-ordinate, ranging from no reflection for black ($L^* = 0$) to perfect diffuse reflection for white ($L^* = 100$). a* is 'the redness' co-ordinate, ranging from negative values for green to positive values for red. b* is 'the yellowness' co-ordinate, ranging from negative values for blue and positive values for yellow. The total colour change is given by the colour difference (ΔE^*_{ab}) in terms of the spatial distance between two colour points interpreted in the colour space (*Park*, 1993).

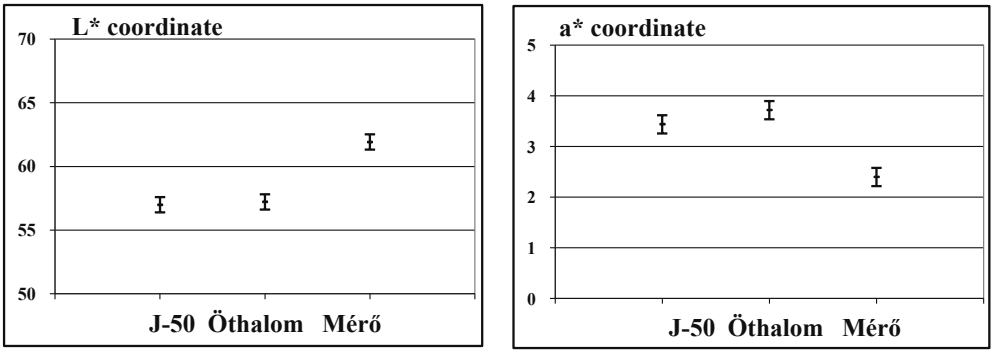
$$\Delta E^*_{ab} = \sqrt{\left(L^*_1 - L^*_2\right)^2 + \left(a^*_1 - a^*_2\right)^2 + \left(b^*_1 - b^*_2\right)^2}$$

If $\Delta E^*_{ab} < 3$, then the colour difference between two grists cannot be visually distinguished. The L* (lightness), a* (redness) and b*(yellowness) parameters as well as whiteness index (WI) were measured. The measurements were made on 5 parallel samples and the colour co-ordinates were measured dry and wet. We estimated the measured values using analysis of variance and regression analysis (*Rice*, 1995).

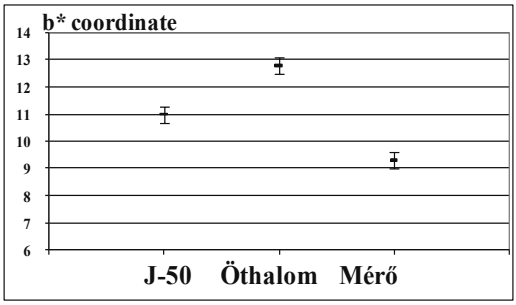
3 Results and discussion

Effect of the hardness index of wheat on colour co-ordinates

As the result of the analysis of variance, we made a comparison for the colour co-ordinates of different hard wheats as you can see in Figure 1 a) and Figure 1 b). We could say that the L* and a* co-ordinates of hard wheats J-50 and Öthalom do not differ significantly, but they significantly differ from Mérő, having a hardness index of 20.78.



a)



b)

Figure 1: Effect of the hardness of wheat on colour co-ordinates – results of the analysis of variance (averages $\pm^1/_2\,\mathrm{SD}_{95\%}$)

The b* yellowness co-ordinates of hard wheat are higher. Therefore, we can say that the colour of wheat with high hardness index was darker, redder and yellower.

Relationship between whiteness index and lightness co-ordinate

The relationship of whiteness index and L* lightness co-ordinate was examined. In *Figure 2*, we plotted the whiteness indexes as a function of lightness co-ordinates measured on wet samples. The equation of regression line and the determination coefficient are represented in the figure.

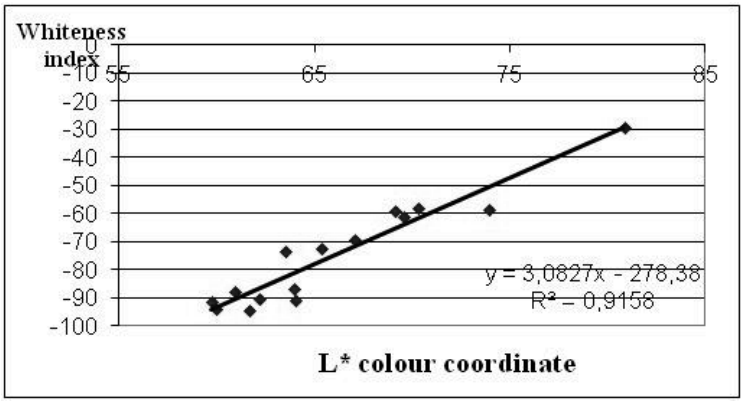


Figure 2: The relationship between whiteness index and lightness co-ordinate was measured wet.

We can see that they have a significant linear relation ($r = 0.9151$), so the determination of whiteness index is not necessary. The L* value characterizes the whiteness of grits.

Effect of grain size on colour co-ordinates

The results of the analysis of variance were calculated on colour parameters of fractions of different grain size, which in the case of J-50, Öthalom and Mérő wheats were similar. The L* lightness co-ordinate, a* redness co-ordinate and b* yellowness co-ordinate were significantly influenced by grain size. We only present the results obtained in the case of J-50 wheat.

In *Figure 3*, we marked the average values with significant differences that appertain to the 95% probability level (SD95%). It seems that while grain size increases, the L* co-ordinate decreases. Consequently, the grains smaller than 100 μm are the lightest and whitest ones, while the bigger grains are the darkest ones. a* co-ordinate and b* co-ordinate increase, while grain size increases; thus, the bigger grains are redder and yellower.

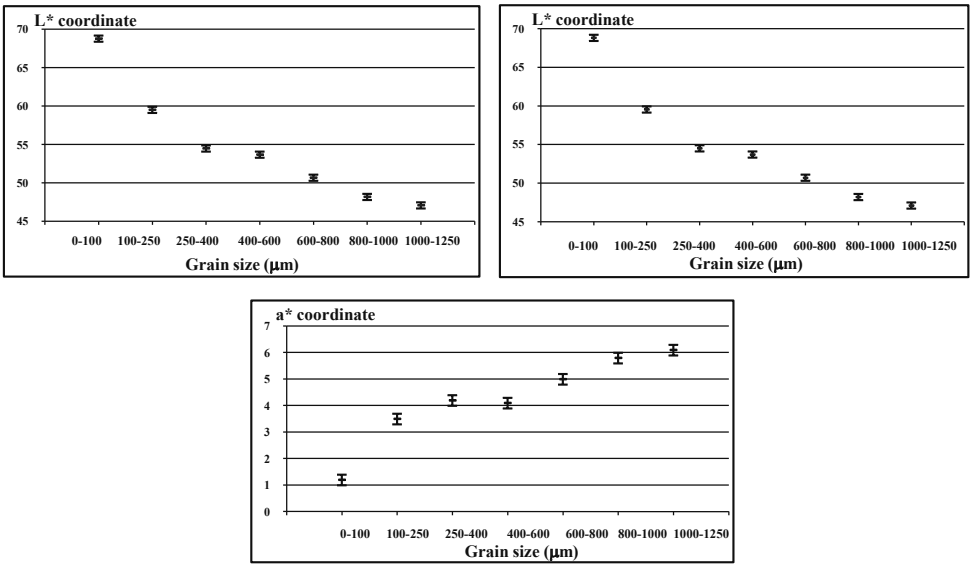


Figure 3: Effect of grain size on colour co-ordinates. Results of the analysis of variance (averages $\pm^1/_2\,\mathrm{SD}_{95\%}$)

We analysed what grain sizes determined the colour of grits. Therefore, we calculated the colour difference values between grits and their fractions of different grain size. We can show the results in *Table 3*. It was found that the smallest ΔE^*_{ab} values were obtained in the case of the 250-400-µm fractions at all times.

Table 3: Colour difference between wheaten grits and their fractions of different grain size

Grain size (µm)	Öthalom	J-50	Mérő
0 – 100 µm	12.427	12.147	11.050
100 – 250 µm	3.055	2.602	5.292
250 – 400 µm	1.542	2.849	1.934
400 – 600 µm	2.777	3.698	3.587
600 – 800 µm	5.466	6.722	10.832
800 – 1,000 µm	8.134	9.315	10.081
1,000 – 1,250 µm	8.699	10.327	14.244

So, they determine the colour of grits despite that these grain size fractions – shown in Table 4 – are only 11.80 – 16.67% of the whole grits.

Table 4: Grain size distribution of samples (%)

Grain size	JK-50	Öthalom	Mérő
0 μm – 100 μm	3.75	3.15	4.85
100 μm – 250 μm	11.38	12.20	18.90
250 μm – 400 μm	11.80	11.80	16.47
400 μm – 600 μm	32.60	31.99	31.01
600 μm – 800 μm	32.99	34.15	20.91
800 μm – 1,000 μm	6.42	6.02	5.52
1,000 μm – 1,250 μm	1.06	0.69	2.34

In conclusion, we can say that the colour parameters of wheat grits are significantly dependent on grain size; upon increasing, the grits become darker and redder. The colour of wheat grits is determined by the colour of grains between 250 and 400 μm. The colour of wheaten grits of different grain size is dependent on the hardness index of wheat. The lightness co-ordinate (L*) of the grits of the harder wheat is smaller, while a* and b* co-ordinates are higher. The whiteness index and the L* colour co-ordinate have a linear relation ($r = 0.9151$); so, the determination of whiteness index is not necessary.

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Investigation of colour agent content of paprika powders with added oleoresin

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Abstract. The paprika oleoresin, that is an oil soluble extract from the fruits of Capsicum Annum Linn or Capsicum Frutescens, is used often to raise the colour agent content of paprika powders. We investigated how the colour agent content of paprika powder samples with added oleoresin change in the course of storage. The colour agent content of 7 different quality powders was increased with 7-75% using two types of oleoresin. The initial colour agent content of the samples changed between 41 and 169 ASTA units. The powders were made from Chinese, Peruvian and Hungarian paprika. The colour agent content of the samples was measured throughout 10 months. The measured values were analysed using ANOVA. The decrease of colour agent content varied between 22 and 51 percent, while the average reduction was 33 percent. The initial colour agent content of the paprika powder samples did not influence the colour agent content decrease significantly. The effect of the quantity of added oleoresin did not influence either the colour agent content decrease significantly. The decrease of the colour agent content of the Hungarian paprika samples significantly differs from the Chinese and Peruvian paprika samples colour agent content decrease.

Keywords and phrases: paprika powder, colour agent content, storage.

1 Introduction

The use of natural food colours is preferred to that of artificial dyestuffs for modern alimentary purposes. Paprika is a spice plant grown and consumed in considerable quantities world-wide, and also used as a natural food colour. The colouring power of paprika powders is directly determined by the quality and quantity of the colouring agent of paprika. The colour agent content of powders decreases during storage time and is influenced by the steps of the processing. Dehydration is the most critical step of the processing. The effect of the heat impairs the colour agent, aroma and flavour substratum of paprika. Several researchers investigated the optimal parameters of dehydration (*Minguez-Mosquera et al.*, 2000; *Ramesh et al.*, 2001; *Shin et al.*, 2001; *Doymaz* and *Pala*, 2002; *Kim et al.*, 2004; *Perez-Gamez et al.*, 2005; *Simal et al.*, 2005). *Topuz et al.* (2011) compared the Refractance Window (RWD) method to dry paprika with freeze-drying, hot-air oven drying and natural convective drying methods. It was depicted that the least colour agent content decrease was in the case of natural convective drying method. The colour agent content reduction is affected by the condition of storage. There are many papers on the changes in the colour agent content of the paprika storage processes (*Park et al.*, 2007, *Banout et al.*, 2011, *Topuz et al.*, 2011, *Chetti et al.*, 2012).

The paprika oleoresin, which is an oil soluble extract from the fruits of Capsicum Annum Linn or Capsicum Frutescens, is used often to raise the colour agent content of paprika powders. We investigated how the colour agent content of paprika powder samples with added oleoresin changes in the course of storage.

2 Materials and methods

Materials

The colour agent content of 7 different quality powders was increased. The initial colour agent content of samples changed between 41 and 169 ASTA units.

The powders were made from Chinese, Peruvian and Hungarian paprika. The colour agent content was increased using 0.5-3.0 g oleoresin added to 100 g paprika powder. *Table 1* shows the investigated powder samples, their initial colour agent content and the quantity of added oleoresin.

Table 1: The parameters of stored paprika samples

Samples	Initial colour agent content (ASTA unit)	Added oleoresin (g)				
Hungarian paprika	169	0.5	1.0	1.5	2.0	
Hungarian paprika	65	0.5	1.0	1.5	2.0	
Hungarian paprika	129	0.5	1.0	1.5	2.0	
Peruvian paprika	61	1.0	1.5	2.0	2.5	3.0
Peruvian paprika	41	1.0	1.5	2.0	2.5	3.0
Chinese paprika	106	1.0	1.5	2.0	2.5	
Chinese paprika	109	1.0	1.5	2.0	2.5	

Methods

After the homogenization of powders, the colour agent content of samples was measured. The ASTA unit was used to mark the colour agent content of the paprika powders according to MSZ EN ISO 7541. The samples were stored at room-temperature, protected from light. The colour agent content was measured monthly for 5 months, and after 8 months and then 10 months. The measured values were analysed using analysis of variance (ANOVA). To control for the homogeneity of variances, the Hartley, Cochran and Bartlett tests were applied.

3 Results and discussion

To evaluate the change of the colour agent content, we calculated the value of the decrease of the colour agent content, measured different times, correlate to the initial value. The values were given in percentage. First, we analysed how the colour agent content decrease during 10 months was influenced by the initial paprika samples and the quantity of added oleoresin. The ANOVA was applied. In *Table 2*, we can see the results of the tests for homogeneity of variances. The values show that the homogeneity was realized; so, the ANOVA was applicable.

Table 2: Results of tests for homogeneity of variances in case of colour agent content decrease during 10 months

Factor	Hartley F-max	Cochran test	Bartlett ξ^2	Significance level
Quantity of added oleoresin	8.31	0.28	6.76	0.34
Initial paprika powder	2.61	0.36	2.65	0.44

The result of ANOVA is shown in *Table 3*. It can be established that the quantity of added oleoresin did not influence the colour agent content decrease significantly, but the initial paprika powder affected it significantly.

Table 3: Variance table in case of colour agent content decrease during 10 months

Factor	F value	Significance level
Quantity of added oleoresin	0.72	0.54
Initial paprika powder	31.99	0.00

In *Figure 1*, we can see the averages decrease with confidence interval at a level of 95%.

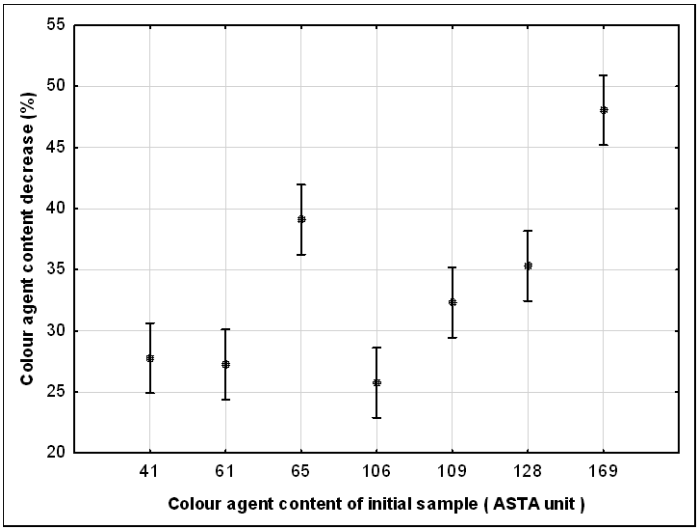

Figure 1: Results of ANOVA for colour agent content decrease during 10 months (average with confidence interval at a level of 95%)

The decrease of colour agent content varied between 22 and 51 percent. It seems good that the reduction was most significant in the case of Hungarian paprika powders (65 ASTA, 128 ASTA and 169 ASTA) and the loss was small for Peruvian powders. So, the change of colour agent during storage was investigated separately for Hungarian, Peruvian and Chinese paprika powders. In Table 4, we can see the results of the tests for homogeneity of variances.

Table 4: Results of tests for homogeneity of variances in case of different paprika powder samples

Samples	Factor	Hartley F-max	Cochran test	Bartlett ξ^2	Significance level
Hungarian paprika	Quantity of added oleoresin	2.21	0.73	1.84	0.76
	Storage time	5.32	0.28	10.96	0.09
Peruvian paprika	Quantity of added oleoresin	1.74	0.21	2.53	0.77
	Storage time	2.27	0.24	5.82	0.44
Chinese paprika	Quantity of added oleoresin	2.87	0.31	5.57	0.23
	Storage time	6.84	0.39	10.44	0.10

The values show that the homogeneity was realized; so, the ANOVA was applicable. The results of ANOVA are shown in *Table 5*. It can be established that the quantity of added oleoresin did not influence the colour agent content decrease significantly during storage.

Table 5: Results of analysis of variances in case of different paprika powder samples

Samples	Factor	F value	Significance level
Hungarian paprika	Quantity of added oleoresin	2.30	0.071
	Storage time	54.1	0.001
Peruvian paprika	Quantity of added oleoresin	2.13	0.061
	Storage time	75.73	0.000
Chinese paprika	Quantity of added oleoresin	2.21	0.055
	Storage time	13.79	0.010

Storage time affected colour agent reduction significantly. In Figure 2 and Figure 4, we can see the averages decrease with confidence interval at a level of 95% in the case of Hungarian, Peruvian and Chinese paprika powders.

The colour agent content of Hungarian paprika powders decreased with 40% on average after a period of 10 months. The rate of decrease was 20% during the first month, while, after the reduction, it was slower. In the case of Peruvian paprika powders, the loss of colour agent was smaller during the storage: it was only 27% on average. It was 20% after 4 months.

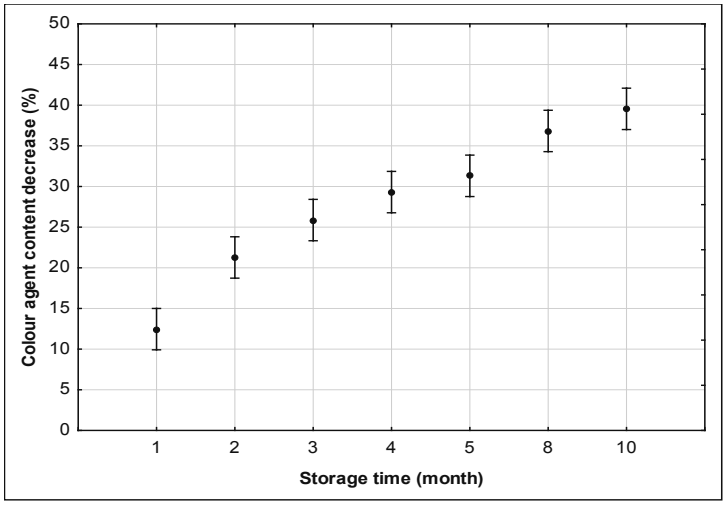

Figure 2: Results of ANOVA for colour agent content decrease in case of Hungarian paprika (averages with confidence interval at a level of 95%)

The colour agent of Chinese paprika powders dropped 30% on average after 10 months. The rate of the loss was the most in the first month.

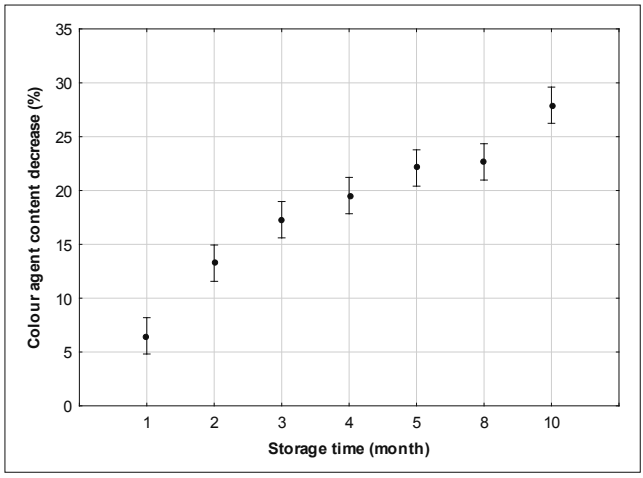

Figure 3: Results of ANOVA for colour agent content decrease in the case of Peruvian paprika (average with confidence interval at a level of 95%)

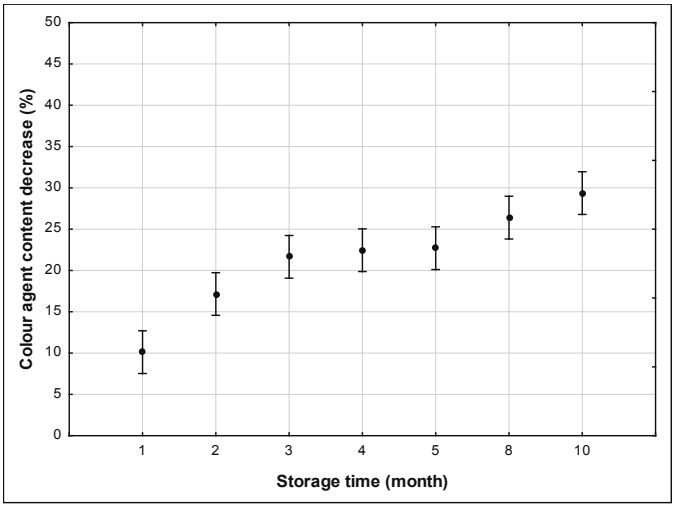

Figure 4: Results of ANOVA for colour agent content decrease in the case of Chinese paprika (average with confidence interval at a level of 95%)

In summary, we can state that the colour agent content decrease was the same in the case of samples with and without added oleoresin. The decrease of the colour agent content varied between 22 and 51 percent, while the average reduction was 33 percent. The colour agent content loss was most in the case of Hungarian paprika powders.

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High hydrostatic pressure: Can we trust published data?

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Keywords and phrases: high hydrostatic pressure, data comparison.

Abstract. There are numerous new technologies whose implementation in food industry is hampered by the fact that people hesitate to invest in expensive systems which they cannot be sure will work or at least are questionable in terms of a given product. Until recently, preservation by HHP, high hydrostatic pressure, was such a technology, and still is today in some branches of the food industry. Investigations were conducted to answer the question of whether the literature, the laboratory, and the industrial (or at least pilot plant) measurements and results agree with one another. We compared the literature data with two HHP systems which were significantly different in terms of treatment capacity, but their efficiency in killing microbes was studied under the same treatment parameters. Our results show that in nearly all cases only minimal differences exist between the data in the literature and the measurements taken on the two appliances.

1 Introduction

HHP, or high hydrostatic pressure, is one of the "gentle" food preservation methods, as it does not use heat. In high hydrostatic pressure technology, food products are preserved by being subjected to between 100 and 1000 Mpa hydrostatic pressure. In HHP treatment, food products are vacuum-sealed in flexible packages and placed in liquid. Hydrostatic pressure is applied quickly and evenly (isostatically) according to the Pascal method (*Dalmadi & Farkas*, 2006); thus, neither the size nor the shape of the container play a role in the effects of the pressure treatment (*San Martin et al.*, 2002; *Farr*, 1990; *Lechowich*, 1993).

The advantage of this method, in contrast to traditional heat treatment, is killing microbes and inactivating enzymes without the undesirable effects of high temperature. This method does not change the product's taste, texture or colour and is less harmful to its nutritional value. Product freshness is better preserved as well. Additives may be reduced or eliminated completely. Moreover, with this method, new technological and functional properties may be developed (*Rico et al.*, 2007; *Oey et al.*, 2008).

Even in the area of liquid products, HHP technology is applied with increasing frequency (*Table 1*, *Knorr et al.*, 2011). Until now, rather small-capacity laboratory equipment has been used for testing the many advantageous properties of high hydrostatic pressure treatment of foods. The aim of our experiments was to determine the laboratory and pilot plant systems' degree of ability to kill microbes and, further, to discover how those results are comparable with the data in the literature.

Table 1: Examples of high-pressure processed products commercially available in different countries

Country	Year	Product
Japan	1993	Rice wine
France	1994	Citrus juice
Mexico	2000	Citrus juice and concentrate
Lebanon	2001	Fruit juices
Portugal	2001	Apple and citrus flavour fruit juices
Czech Republic	2004	Broccoli, apple, beet and carrot juices
USA	2005	Fibre fruit juices
Northern Ireland	2006	Apple, wild strawberry and ginger juices
New Zealand	2009	Colostrum

2 Materials and methods

HHP treatment

Our experiments were carried out according to the parameters in published articles (matrix, treatment time, pressure during treatment and microbes). The Budapest Corvinus University Department of Refrigeration Technology and Animal Products owns two HHP systems: one laboratory type (Stansted Food Lab 900, plunger press system) and one pilot plant type (RESATO FPU 100-2000). Thus, it was possible to apply measurements derived from the literature, using similar parameters, on two separate machines.

Microbiological tests

We intended to examine each change in total viable cell count and the death of each important microbe specific to a given product. For our tests, we prepared inoculate with microbes grown on slant agar, after which we infected the samples with amounts which produced measurements similar to those found in the literature.

Preparation of *Salmonella Enteritidis*, inoculation of samples and colony counting

The *Salmonella enterica* subsp. enterica, serotype Enteritidis NCAIM B2052 species used in the experiments was obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM). We prepared inoculate from microbes grown throughout 24 hours on meat broth slant agar, after which we added two streaks of preparation to each 10 ml of sterile peptone

water (the diluting liquid), and then this was administered in various amounts to the samples. Following HHP treatment, dilution plating was completed with selective XLD and Harlequin Salmonella agar. Plates were incubated for 48 hours at 37 °C, after which the growth of colonies was determined with a colony counter.

Preparation of *Listeria monocytogenes*, inoculation of samples and colony counting

The inoculation and colony counting of the samples with *L. monocytogenes* was carried out similarly to *Salmonella Enteritidis*, except that Brain Heart agar was used for the slant growth and plate pouring was done with PALCAM agar.

Preparation of *Escherichia coli*, inoculation of samples and colony counting

Inoculation and colony counting of samples with *E. coli* was similar to that of *Salmonella Enteritidis*, but plate pouring was carried out with ChromoCult Coliform agar.

Preparation of *Staphylococcus aureus*, inoculation of samples and colony counting

Inoculation and colony counting of samples with *S. aureus* was completed in a similar way to *Salmonella Enteritidis*, but here plate pouring was done with Baird Parker agar.

Preparation of *Pseudomonas aeruginosa*, inoculation of samples and colony counting

The inoculation and colony counting of *P. aeruginosa* samples happened in a similar way to that of Salmonella with the exception that Cetrimide agar was used for plate pouring.

Determination of aerobic viable cell count

A series was prepared from both the treated and untreated (uninfected) samples. They were diluted at a ratio of 10^{-1} with sterile water, after which we used Nutrient agar plate pouring to determine the microbe levels in the samples. Plates were incubated for 48 hours at 37 °C and a colony counter was used to determine how many colonies had appeared.

3 Results and discussion

It can be seen from our results that the levels of microbe death in certain foods varied only slightly between the laboratory and the (pilot) plant, while they agreed with the data in the literature (*Figure 1*). Slight variations in the total viable cell count test could be attributed to deviations in the microflora composition; variations in the test of given phyla may derive from differences of serotype; moreover, it can be said that the homogeneity of the food used as matrix was imperfect (water and fat content).

It is apparent from the data that results taken from the literature and laboratory systems allow us to make conclusions about how the material's microbiological condition will change during processing in a plant.

Figure 1 shows literature data (the measurements which we tried to reproduce) and correlation to results from pilot plant systems. It is clear that there is a close correlation, ($r^2 = 0.96$). The r^2 value was 0.99 for *Salmonella*, 0.92 for *Listeria*, 0.98 for *E. coli*, 0.77 for *S. aureus*, 0.96 for *Pseudomonas aeruginosa* and 0.87 for total viable cell count.

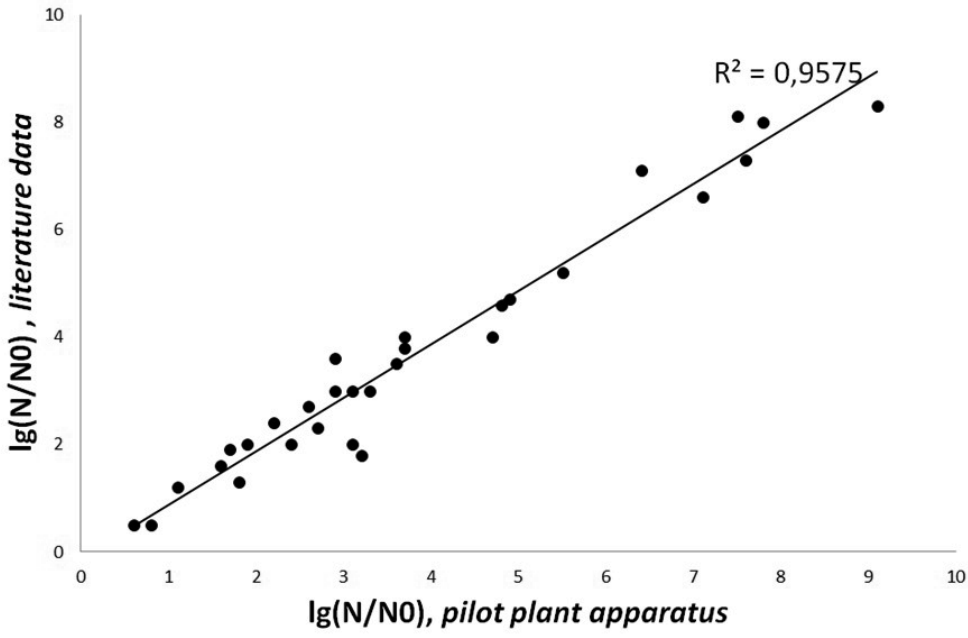

Figure 1: Correlation between the literature and the industrial results

Table 2: Comparison of literature data and our results

	p	t	LAB	PPA	LIT.	REF.
Salmonella						
(Enteritidis) meat broth	345	600	3.3	3.1	3.0	*Alpas et al., 2000*
phosphate buffer	350	600	0.9	1.1	1.2	*Tholosan et al., 2000*
boiled ham	400	600	2.0	1.7	1.9	*Aymerich et al., 2005*
(Enteritidis) ham	500	600	6.5	6.4	7.1	*Yuste et al., 2000*
broth	500	600	8.2	7.8	8.0	*Mackey et al., 1994*
Listeria monocytogenes						
phosphate buffer	375	900	2.2	2.4	2.0	*Patterson et al., 1995*
boiled ham	400	600	2.5	2.2	2.4	*Aymerich et al., 2005*
turkey breast	400	60	0.7	0.6	0.5	*Chen, 2007*
ham	450	600	3.0	2.9	3.6	*Morales et al., 2006*
turkey breast	500	60	4.1	3.7	3.8	*Chen, 2007*
Escherichia coli						
liquid whole egg	300	600	0.8	0.8	0.5	*Ponce et al., 1998*
(O157-H7) meat broth	345	600	7.7	7.5	8.1	*Alpas et al., 2000*
liquid whole egg	450	600	3.3	3.6	3.5	*Ponce et al., 1998*
phosphate buffer	500	300	7.5	7.6	7.3	*Hauben et al., 1997*
phosphate buffer	600	300	8.7	9.1	8.3	*Hauben et al., 1997*
Staphylococcus aureus						
sheep's milk	345	600	4.2	3.7	4	*Alpas et al., 2000*
water	400	1800	5.0	5.5	5.2	*Arroyo et al., 1999*
sheep's milk	450	1800	3.0	3.2	1.8	*Arroyo et al., 1999*
milk	600	900	2.2	1.9	2.0	*Mackey et al., 1994*
turkey meat	600	900	3.2	2.9	3.0	*Mackey et al., 1994*
Pseudomonas						
sheep's milk	250	1800	7.0	7.1	6.6	*Trujillo et al., 2002*
(aeruginosa) water	300	1800	5.1	4.8	4.6	*Arroyo et al., 1999*
(fluorescens) water	300	1800	4.5	4.9	4.7	*Arroyo et al., 1999*
broth	345	600	4.5	4.7	4.0	*Alpas et al., 2000*
corned beef	500	300	3.4	3.1	2.0	*Rubio et al., 2007*
Viable cell count						
chicken liver	200	1200	1.5	1.8	1.3	*Tuboly, 2009*
sliced pineapple	270	900	1.5	1.6	1.6	*Aleman et al., 1997*
liquid egg	300	600	2.4	2.7	2.3	*Németh et al., 2012*
sliced pineapple	340	900	2.9	3.3	3.0	*Aleman et al., 1997*
ham in brine	600	360	3.0	2.6	2.7	*Garriga et al., 2004*

p: Mpa treatment pressure, Mpa
t: treatment time
LAB: lg(N/N0) value in a laboratory apparatus
P.P.A.: lg(N/N0) value in a pilot plant apparatus
LIT.: lg(N/N0) value from literature data
REF.: literature which was used and which belongs to the LIT

4 Conclusions

In summary, we can say that the microbe-reducing effect of the HHP treatment – for the purposes of a processing plant – can well be calculated from literature data and from laboratory measurements. This very advantageous characteristic is due to the fact that with HHP technology the same pressure

bears on every point of the treated material simultaneously, regardless of the volume or size of the product.

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Aluminium toxicity in winter wheat

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Abstract. Aluminium is the most frequent metal of the earth crust; it occurs mainly as biologically inactive, insoluble deposit. Environmental problems, industrial contaminations and acid rains increase the soil acidity, leading to the mobilization of Al. Half of the world's potential arable lands are acidic; therefore, Al-toxicity decreases crop productivity. Wheat is a staple food for 35% of the world population. The effects of Al-stress (0.1 mM) were studied on winter wheat; seedlings were grown hydroponically, at acidic pH. After two weeks, the root weight was decreased; a significant difference was found in the P- and Ca-content. The shoot weight and element content changed slightly; Al-content in the root was one magnitude higher than in the shoot, while Al-translocation was limited. The root plasma membrane H^+-ATPase has central role in the uptake processes; Al-stress increased the Mg^{2+}-ATPase activity of the microsomal fraction.

1 Introduction

Aluminum is a light metal, ubiquitous throughout the environment; it occurs mainly as insoluble deposit, which form is biologically inactive. Environmental factors increase the acidity of the soils, leading to the mobilization of the

Keywords and phrases: aluminium stress, plasma membrane H^+-ATPase, phosphorus, Tricitum aestivum.

bound form of Al. Food packaging and post-use disposal cause environmental pollution; recently, aluminium foils are priority toxins in the United States and Germany. Al is generally harmless to plant growth in pH-neutral soils, but in acid soils the concentration of toxic Al^{3+} cations increases (*Bedő et al.*, 1992; *Bóna* & *Carver*, 1992; *Zsoldos et al.*, 1998; *Yang et al.*, 2013.). Approximately over 50% of the world's potential arable lands are acidic; therefore, Al toxicity is a major factor reducing plant growth and limiting crop productivity. The trivalent Al^{3+} ion is toxic to all living cells (*Driscoll*, 1985). In humans, Al^{3+} ion may cause a variety of neurological disorders and it has a significant role in Alzheimer's disease (AD). In plants, ionic Al rapidly inhibits root elongation and subsequently the uptake of nutrients and water (*Ligaba et al.*, 2004.), resulting in poor growth. Al toxicity is a major limiting factor of crop production on acid soils (*Kochian et al.*, 2004). Some plants have evolved mechanisms to detoxify Al^{3+}, both externally and internally (*Ma* & *Furukawa*, 2003).

In higher plants, the Al-induced secretion of organic acid (OA) from the roots and the role of phosphorus (binding to the aluminium) is a known mechanism of Al tolerance (*Ma et al.*, 2001). The Al-induced secretion of OA anions may be related to several factors, including anion channels, transporters and the root plasma membrane (PM) H^+-ATPase. In spite of the accumulated data, little is known about the avoidance of the toxic effects caused by aluminium stress on plants and about the changes in physiological and enzymatic properties of the root plasma membrane.

In this study, the in vivo effect of soluble Al^{3+} form on winter wheat was followed for two weeks. The changes caused by Al-stress in the growth and in some important element content were measured. Root microsomal fractions enriched in PM were prepared from the Al-treated and the control plants, and the Mg^{2+} ATPase activity of the fractions was compared.

2 Materials and Methods

Plant growth and element analysis

A nutrient solution (modified Hoagland) at pH = 4.5 containing 0.1 mM $AlCl_3$ was used to examine the effect of Al on wheat (*Triticum aestivum* L. cv. Martonvásári-8). Control plants were grown under Al-free condition and P-deficient plants were grown without phosphorus. Fifty seeds were then placed on plastic nets over plastic beakers, each containing 4 dm^3 of nutrient solution, and 4 replicate beakers were cultivated in the case of each treatment.

The seedlings were grown hydroponically under controlled condition for two weeks with a 12-h day-time illumination of $60\,W \cdot m^{-2}$; day/night temperatures were 23/18 °C and the relative humidity of the air was 85%. The fresh and the dry weight of the root and shoot were measured, and N, P, K, Ca and Al content were determined after wet digestion. Nitrogen content was analysed with the Kjedahl method, phosphorus was determined colorimetrically and the content of K, Ca and Al was measured by atomic absorption spectrophotometry described by *Szabó-Nagy et al.*, 1987.

Isolation of plasma membrane vesicles and enzyme assay

Young roots were harvested, washed in cold distilled water and homogenized in 3 × volumes of homogenization buffer containing 250 mM sucrose, 5 mM EDTA and 50 mM Tris-MES buffer (pH 7.5). The 10,000-30,000 g microsomal fraction was prepared and Mg^{2+}-ATPase activity was measured as described earlier (*Szabó-Nagy et al.* 1987, 1992). Background ATPase activity was measured in the presence of 0.1 mM EDTA, 0.1 mM $(NH_4)_6Mo_7O_{24}$, 1 mM NaN_3, 3 mM ATP, 25 mM Tris-MES buffer (pH 6.0) with 0.015 mg protein-containing fraction.

The fresh and the dry weight of the root and shoot were measured as averages of 5 plants. The element concentrations were given as the mean of three replicate determination; replicates differed by less than 10%. Microsomal ATPase activities were determined for three independent series of plants, and they gave similar results.

Statistical analysis

For statistical analysis, the ANOVA method was used.

3 Results and discussion

In this study, the in vivo effects of the ionic form of Al (0.1 mM, at pH 4.5) on wheat growth and on the element contents were followed (*Table 1*); control plants were grown under Al free condition. The weight of the roots was decreased (82% of the control) by Al stress, but in the case of the shoots no significant changes were found after two weeks. The decreased root growth could be a consequence of the Al-induced inhibition of the root elongation. The Al content of the roots was nearly one magnitude higher than that of the

shoots in the Al-treated plants, suggesting that the translocation of Al was hindered.

No significant differences were found in the potassium and nitrogen content of the control and Al-treated plants: it varied between 95 and 104% as regards the control, both in the case of the roots and the shoots. In the P and Ca content of the roots, significant differences were found caused by 0.1 mM Al-addition to the nutrient solution. The P content was increased by 35% and the Ca content was decreased by 26%. The precipitation of aluminium by phosphorus in the apoplast and the accumulation of Al-P complex in the root can explain the measured increased P level. The Al-induced inhibition of Ca^{2+} uptake was found by *Huang et al.* (1992) in the Al-sensitive wheat. Changes in ionic composition of wheat root indicate that the ionic uptake processes were affected by Al-stress.

Table 1: Comparison of the growth and some important element content of the control and Al-treated wheat plants. Similar results were obtained in three independent series of plants

	Changes caused by Al treatment as % of the control plants
weight of roots	$82\% \pm 5\%$
weight of shoots	$98\% \pm 6\%$
P content of roots	$135\% \pm 5\%$
P content of shoots	$108\% \pm 7\%$
Ca content of roots	$74\% \pm 7\%$
Ca content of shoots	$102\% \pm 4\%$
	Al content, umol/g dry weight
in the roots	79 ± 5
in the shoots	8 ± 2

The root plasma membrane H^+ATPase plays a central role in the ionic uptake processes. The microsomal fractions enriched in PM vesicles were prepared from the roots and the Mg^{2+}-ATPase activity was measured in the case of Al-treated and control plants (*Figure 1*). In vivo Al stress caused an increase of the Mg^{2+}-ATPase activity in the microsomal fraction: the Mg^{2+}ATPase activity was more than two times higher in the case of Al-treated plants than

that of the control. The Al-induced changes may be related to different factors, such as phosphorus metabolism, as it was suggested by increased P content and the increased Mg^{2+}-ATPase enzyme activity. Similarly, increased Mg^{2+}-ATPase enzyme activity was observed in P-deficient plants, where the measured increase connected to an increased acid phosphatase activity (*Szabó-Nagy et al.*, 1987).

The microsomal fraction may contain other enzymes attached to the vesicles, for example acid phosphatases from cytoplasm or from cell wall. Acid phosphatases are widely distributed in plants. These enzymes have intra- and extracellular activity, and their roles are very important in phosphorus metabolism: they increase the amount of the available inorganic phosphate or they play a role in signal transduction. However, acid phosphatase activity is increased by other stress factors, for example by salt and osmotic stress (*Szabó-Nagy et al.*, 1992; *Szabó-Nagy* & *Erdei*, 1995; *Ehsanpour* & *Amini*, 2013).

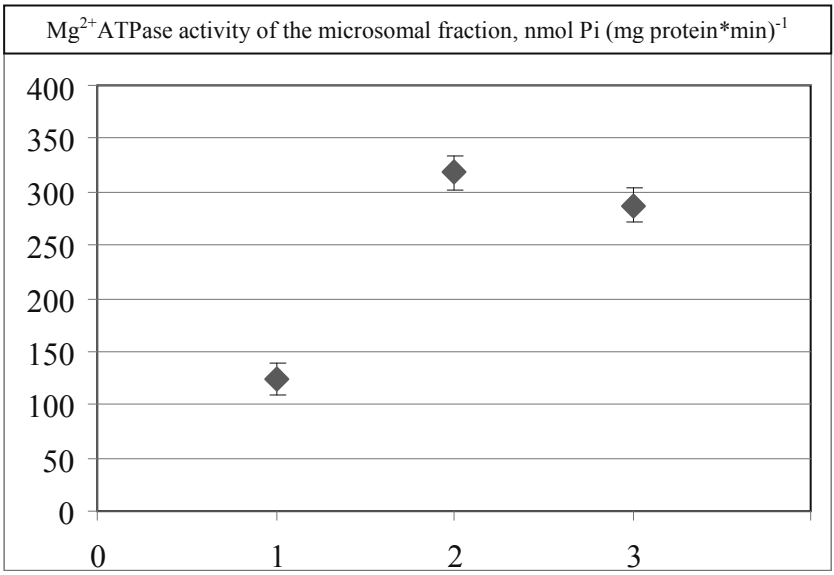

Figure 1: In vivo effect of Aluminium stress (2) and P deficiency (3) on the Mg^{2+}ATPase activity of the microsomal fraction in 14-day-old plants grown hydroponically with or without 0.1 mM $AlCl_3$ in the nutrient solution. 1 stands for the control.

Al-induced reduction in growth can be the result of a depletion of organic P pool in the plants. On the contrary, higher concentrations will just be the result of impaired growth. Effects of Al stress and P deficiency on nutrient uptake, proton efflux and root respiration were similar (*Keltjens & Loenen*, 1990). Suppressed ionic (Ca^{2+}) net uptake might be the result of the enhanced leakage of plasma membranes due to a shortage of phospholipids as induced by deficient P supply or the malfunctioning of Al-saturated phospholipids in the presence of Al^{3+}. Reduced root respiration and deteriorated root energy supply might also disturb the nutrient uptake. Aluminum (Al) toxicity and phosphorus (P) deficiency often coexist in acid soils and severely limit crop growth and production. P addition significantly increased Al tolerance in four soybean genotypes differing in P efficiency. Some genotypes may be able to enhance Al tolerance not only through direct Al-P interactions but also through indirect interactions (*Liao et al.*, 2006). Low P availability is another important limiting factor to plant growth in acid soils. Generally, Al toxicity and P deficiency are studied separately as independent factors. Al toxicity and P deficiency often coexist in acid soils, and these two factors may strongly interact through chemical and biochemical reactions. It is necessary to think of the interactions between Al and other factors in the soil, particularly the P status.

P deficiency and Al stress have some similar effects on plants, for example significantly reduced root weight. However, the interaction of Al and the metabolism of other important elements, such as P, needs more studies. A general mechanism of Al tolerance in plants is the release of organic acid anions from the roots in response to Al stress (*Kochian et al.*, 2005); these anions chelate Al and form non-phytotoxic Al forms (*Ma*, 2007). *Xia et al.* (2013) found the gene of a plasma membrane-localized transporter for Al in rice. The changes caused by Al stress in transporters and in root plasma membrane H^+-ATPase need further studies.

4 Conclusion

The aluminium stress is a very serious and growing problem, strengthening crop production in the last decades since the acidity of the soil is increasing. Some plant species and cultivars have evolved some mechanisms for detoxifying Al both internally and externally. There is some evidence that aluminium detoxification and phosphorus metabolism have some connections. The Al stress caused retardation of root growth parallel to the increased Al and in-

creased P content. Against the increased P level, there could be a shortage of the accessible phosphorus. This increased P level could be the part of the strategy against Al stress since the insoluble Al-P complexes could reduce the toxic form of aluminium. The available P level could be decreased, leading to P deficiency symptoms. The decreased Ca^{2+} content of the roots could be the result of the changes of the ionic uptake processes caused by Al stress. ATPase activity of the root microsomal fraction, enriched in PM, was increased in Al-stressed 14-day-old plants, which increase was similar to that of the roots grown under P deficiency. The increase in the microsomal Mg^{2+}ATPase activity due to Al stress needs further studies, since the measured increase could be originated from acid phosphatase induction, as it was found in P deficient wheat roots.

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Role of the farinograph test in the wheat flour quality determination

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Abstract. Most objectively, the dough rheological methods can characterize the quality of winter wheat. The Farinograph test is the traditionally used one in Hungary, but the importance of other methods, such as Alveograph and Extensograph tests, are getting more widely known due to the interest and requirements of the industry and export markets. The Hungarian Standard on wheat quality follows the changes in the global markets; this is why the falling number appeared in the MSZ (Hungarian Standard) 6383 in 1998. As the interest in the results of other rheological test increased from our accession to the European Union, the evaluation of these parameters on different varieties has become an important issue of qualification and in 2012 limit values for Alveograph and Extensograph parameters appear in the Hungarian wheat quality standard. Additionally, while the baking value was the only evaluated parameter of the Farinograph test earlier, the standard was supplemented with limit values for water absorption capacity and stability too.

In this study, we revised different diagrams of Farinograph tests again from the previous years to reveal whether the new limit values for these Farinograph parameters change the valuation of wheat flour samples, therefore whether the quality groups in which the samples were ranked change considering the new requirements.

Keywords and phrases: wheat, farinograph, Hungarian Standard, absorption capacity, stability.

1 Introduction

Maybe the most informative quality parameter of the Hungarian Standard on wheat quality was the baking quality determined by Farinograph or Valorigraph and its classification (A, B and C) was the basis for the determination on use. The standard was changed in 1998, the falling number and sedimentation volume appeared in the Standard, following the international requirements (MSZ 6383:1998). After sixteen years, the Hungarian Standard was changed again: limit values for Alveograph and Extensograph parameters appeared and the valuation of Farinograph or Valorigraph test enlarged two new test analysis parameters. The standard was supplemented with parameters with limit values: water absorption capacity and stability measured by Farinograph test (MSZ 6383:2012).

With the special instruments, such as Farinograph, Extensograph and mixograph, the comparison of different dough rheological parameters can be performed (*Liu et al.*, 2005). The Farinograph test is described by ISO 5530, the method is applied to winter wheat. The resistance of dough is evaluated by the Farinograph test, which means the evaluation of behaviour of dough against mixing at a specified constant speed with specified water addition (ISO 5530-1:2013).

Parameters determined by 5530 are consistency, farinograph unit (FU), water absorption capacity of flour, dough development time, stability, mixing tolerance index and farinograph quality number (FQN) (*D'Appolonia & Kunerth*, 1984). The different baking products require wheat flours with different quality.

In the study, we revaluated different Farinograph diagrams analysed in the previous years. We would like to know whether the introduction of new limit values causes change in the classification of wheat samples.

2 Materials and methods

2.1 Materials

In 2009 and 2010, several wheat samples were evaluated in the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management Institute of Food Science. We revalued the results of 58 Farinograph tests of winter wheat samples.

The baking value was the only evaluated parameter of the Farinograph test earlier; it corresponded to the MSZ 6383:1998 standard for wheat. Nowa-

days, the MSZ 6368:2012 standards consider limit values for water absorption capacity and stability. We revalued these results with the new standard.

2.2 Methods

The Farinograph test characterizes the rheological behaviour of dough, therefore the quality of winter wheat. Brabender units are used in the Farinograph test. Units are arbitrary units that incorporate torque or dough resistance to mixing.

Figure 1 shows part of the diagram of the Farinograph test:

- Arrival time: time to develop to 500 BU consistencies.
- Peak time: when the dough reaches the maximum strength.
- Departure time: when the top of the curve leaves the 500 BU line.
- Stability: the difference of arrival and departure time.
- Water absorption capacity (*Reese et al.*, 2007).

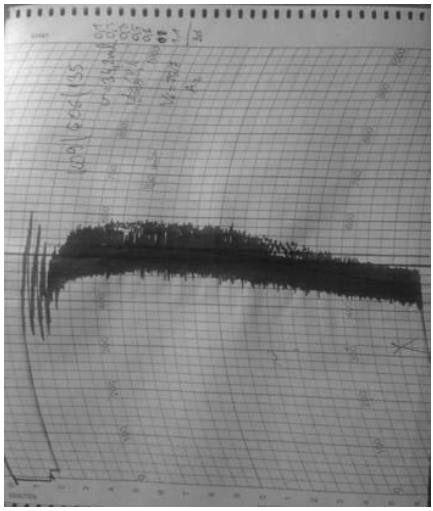

Figure 1: Representative Farinograph diagram

We determined the water absorption capacity and stability (*Table 1*). The "A" quality group shows a minimum of 60.0% water absorption and the "B" quality group has a minimum of 55.0% water absorption (14% moisture content). The other parameter is stability: the limit value of stability is 10 minutes at least

for the "A" quality group, while the limit of the "B1" baking value is minimum 6 minutes and the "B2" quality group has minimum 4 minutes.

Table 1: Quality groups and required water absorption and stability

Quality group	A	B	
		B1	B2
Water absorption % (14% moisture content)	60.0	55.0	55.0
Stability (minimum minutes)	10.0	6.0	4.0

The results were perfomed by boxplots using IBM SPSS Statistics 19 programme. The dark line in the middle of the boxes is the median. The lower and upper parts of the boxes represent the quartiles and the farthest (T-bars) line the 95 per cent interval (*Hruzsvai & Vincze*, 2012).

3 Results

Figure 2 shows the baking value. The selection of samples used in our tests was performed by their baking value.

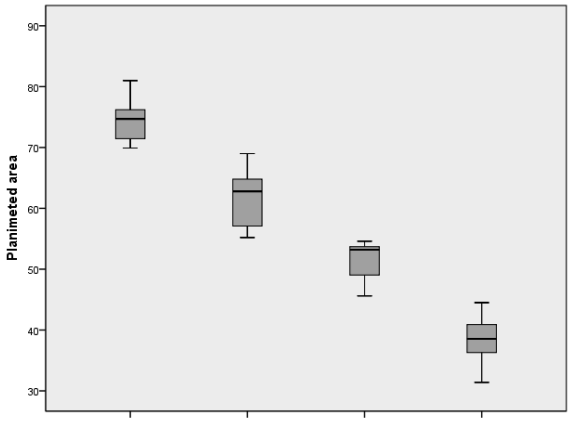

Figure 2: Baking values of evaluated samples by quality groups

We had 58 samples, what is near the limit of each baking value. 12 samples were selected from A2 quality group, 15 samples from B1 quality group, 15

samples from B2 and 16 samples from C1 quality group classified by the MSZ 6383:1998. Those samples were chosen which had their baking value close to the limit value of two quality groups, as the probability of the change in quality groups is higher for these ones. In the revaluation by MSZ 6383:2012, the new classification had the same results.

We revalued 58 samples according to MSZ6383:2012 Hungarian Standard. The results were identical with the previous values of MSZ 6380:1998 Hungarian Standard: A2 12 pieces, B1 15 pieces, B2 15 pieces and C1 16 pieces.

The readings of water absorption capacity of selected samples by quality groups can be seen in *Figure 3*. The inclusion of water absorption capacity values did not result any change between the B1 and B2 quality categories, while the samples of C1 category could have been classified into a higher group. According to their water absorption capacity, the samples in the last category could have been ranked 2 levels higher.

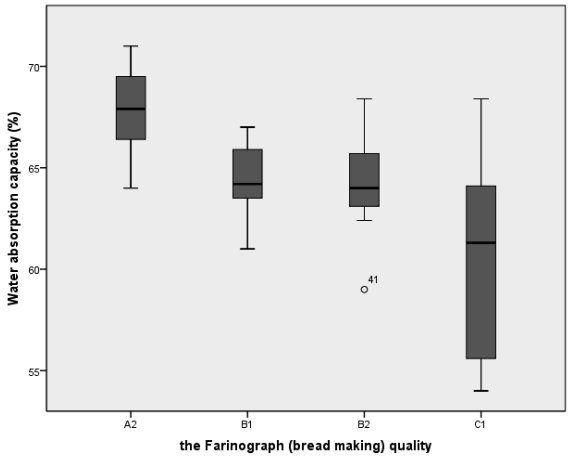

Figure 3: Water absorption capacity values of evaluated samples by quality groups (%)

In *Figure 4*, the readings for stability, the second new parameter of MSZ 6383:2012, can be seen. After the revaluation, we have 11 samples A2, 16 samples B1, 15 samples B2 and 16 samples C1 baking value. Therefore, with the use of the new Hungarian Standard, one sample went from A2 quality group to B1 quality group.

From the A2 quality group, the stability of 1 sample is lower than the limit value for this group; thus, it was classified to the B1 quality group. The

stability time of the sample is 9 minutes; therefore, it does not reach the A1 category's minimum (10 minutes) value. We distinguish 12 samples A1, 16 samples B1, 15 samples B2 and 16 C1 samples according to the requirements of the new standard.

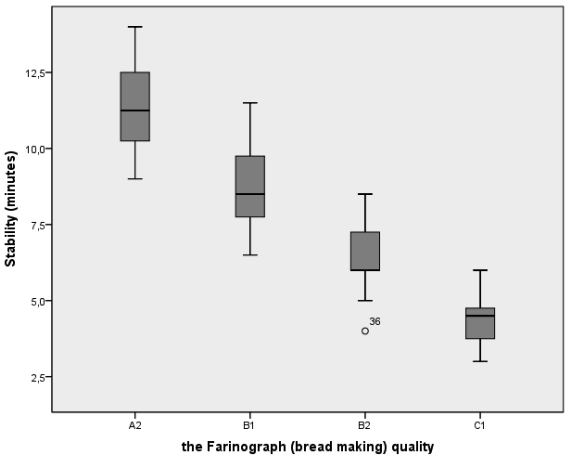

Figure 4: Stability values of evaluated samples by quality groups (minutes)

Based on the evaluations, we concluded that the new Hungarian Standard (MSZ 6383:2012) did not change the baking values significantly. We evaluated 58 winter wheat samples and only one of them stepped back one quality group due to its stability time.

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Lycopene and flesh colour differences in grafted and non-grafted watermelon

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Abstract. The experiment was carried out in three regions in Hungary (Jászszentandrás, Cece, Újkígyós) in 2013 to determine the fruit quality of grafted watermelon (*Citrullus lanatus Thunb.*). The "RX 467" seedless watermelon variety was grafted on two commercial rootstocks "FR STRONG" [*Lagenaria siceraria* (Mol.) *Standl.*] and "RS 841" (*Cucurbita maxima Duchesne* × *Cucurbita moschata Duchesne*). The lycopene and flesh colours are important quality characteristics even of the self-rooted and grafted watermelon. Some differences can be attributed to different environments, technological methods and to the type of rootstock-scion combination. Lycopene is a strong antioxidant; therefore, we considered to examine the content change. Regardless of growing location, the lycopene concentration and the chroma (C*) showed the best result in the case of interspecific rootstock. The result also showed that in two regions (Jászszentandrás, Cece) we can find negative correlation between the lycopene concentration and the L* value of the flesh colour.

Keywords and phrases: grafting, watermelon, lycopene, flesh colour.

1 Introduction

The aim of the study was the determination of the lycopene amount and the colour sense in grafted and self-rooted watermelon as well as to find a positive or negative relationship between them. The other aim of the experiment was to see the lycopene influence on the intensity and "deepness" of the red colour. The sample collection was set in three places with the same varieties and grafting combinations but with different technological backgrounds. Beside the goals mentioned above, we were interested whether the result of the different technologies and regions would be the same.

We can find a high amount of lycopene in red-fleshed watermelons (*Perkins-Veazie et al.*, 2001). This carotenoid pigment can behave like an antioxidant by quenching free radicals formed in normal metabolism (*Sies* & *Stahl*, 1998). We can see the lycopene content as another quality attribute of watermelon (*Perkins-Veazie* & *Collins*, 2004). Watermelon is a well-known refreshing fruit in the Mediterranean basin and also in Northern Europe. We can find different varieties of antioxidants such as lycopene and β-carotene, vitamins, phenols and amino acids (*Perkins-Veazie*, 2002; *Perkins-Veazie et al.*, 2007).

The low caloric value and health benefits of eating watermelon make it a popular fruit. The antioxidant and bioactive compound identification and quantification of many vegetables and fruits are well-defined, but the antioxidant and phytotechnical properties quantification and characterization of watermelon are limited. It has been reported that the external factors and genotype differences strongly influence the fruits' and vegetables' antioxidant and health-promoting bioactive compound activity (*Waterman* & *Mole*, 1994; *Abushita et al.*, 2000; *Dumas et al.*, 2003; *Lenucci et al.*, 2009).

One of the most important quality traits is the watermelon's flesh colour. The flesh coloration is attributable to the carotenoid content and composition. Carotenoids are very important antioxidants which can reduce the risk of cardiovascular diseases and certain cancers (*Bramley*, 2000; *Gerster*, 1997; *Giovannucci et al.*, 2002). The flesh colour can be categorized as coral red, scarlet red, salmon yellow, canary yellow, white and orange flesh (*Gusmini* & *Wehner*, 2006).

2 Materials and methods

2.1 Materials

The experiment was carried out in 2013 in three different regions of Hungary (Jászszentandrás, Cece and Újkígyós). The scion was "RX 467" seedless watermelon. This variety was grafted on two well-known rootstocks: "FR STRONG" [*Lagenaria siceraria* (Mol.) Standl.] and "RS 841" (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne). We used chroma meter (Konika Minolta CR 300) to measure the colour of the fresh-cut watermelons' flesh surface. The lycopene content was measured from the cut and squeezed watermelon flesh.

2.2 Methods

We tried to harvest and measure those watermelon fruits which were fully ripe. The methodology of the colour measurement: The harvested watermelons were cut in four parts and the chroma meter was put on the surface of the fruit flesh to measure the colour. The methodology of lycopene measurement: After the harvest, the watermelons were cut in four parts and the fruit flesh was squeezed and put in a 50-ml falcon pipe. The amount of lycopene, after hexane extraction, was evaluated by spectrophotometric process (*Sadler et al.*, 1990). The measurements were carried out at 502 nm. In order to calculate the lycopene content, we used molar extinction coefficient ($M \cdot cm^{-1}$) in hexane (158500, Merck & Co, 1989). The sample lycopene content was given in mg/100g fresh weight and we normalized it in 6 Brix° dimensions (*Barrett-Anthon*, 2001). An SPSS-programme-based analysis was executed on the statistical data with the level of 95% confidentiality.

3 Results and discussion

In *Figure 1*, we can state that in the case of lycopene the plants grafted on interspecific rootstock showed the best results and the plants grafted on the Lagenaria rootstock had the lowest amount of lycopene. If we compare the regions, we can see that the lowest lycopene concentration (4.85 mg/100g) was measured in the Újkígyós Region on *Lagenaria* rootstock and the highest in the Jászszentandrás Region on interspecific rootstock. Differences among cultivars were also detected in other studies. *Tlili et al.* (2011) measured the lowest amount of lycopene (4.45 mg/100g fresh weight) in Crimson sweet cultivar,

followed by P403 (4.48 mg/100g fw), Dumara (4.71 mg/100g fw), Giza (6.26 mg/100g fw) and finally P503 (6.45 mg/100g fw) cultivars at the red-ripe stage. The β-carotene, lipophilic antioxidant activities (LAA) and lycopene concentration markedly increased with ripening. The data showed that the antioxidant potential of watermelons is determined by genetic background and ripening stage (*Tlili et al.*, 2011).

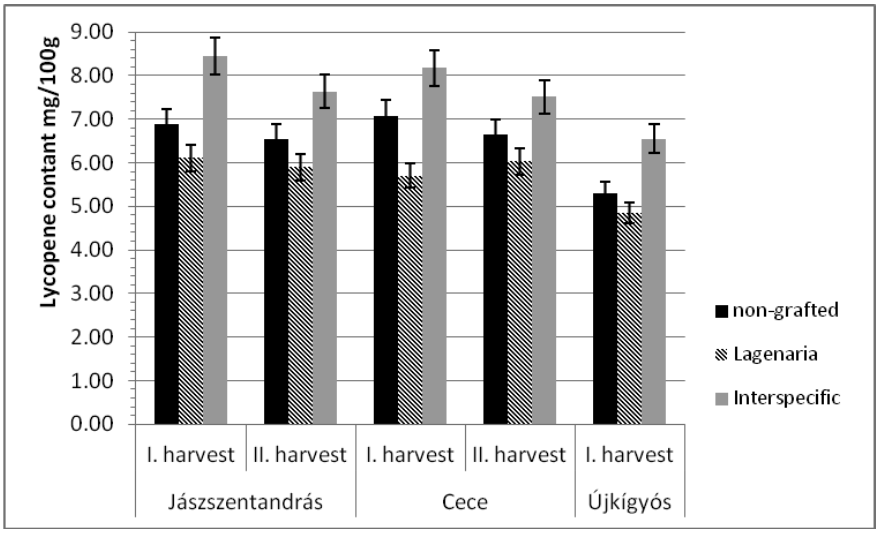

Figure 1: Lycopene average results

The added nutrient quantities were very different in the three regions (*Figure 2*), but in the case of lycopene we can see in *Figure 1* that in two regions (Jászszentandrás, Cece) the lycopene concentrations are very similar. The highest (double the amount compared to other regions) amount of potassium was given in Újkígyós nevertheless; the result was lower in the case of lycopene compared to the other two regions because the harvest was early and the watermelons were not fully ripe.

If we compare the non-grafted and grafted plant's fruits to each other (*Table 1*), then we can see that the *Interspecific x scion* grafting combination fruits' colour value L* (lightness) and b* (yellow colour) increased in two regions (Cece, Jászszentandrás) and decreased in the Újkígyós Region. From the harvest data of Újkígyós, we can find positive correlation, but in the case of the other two regions the four harvest data results showed the opposite because there is a negative correlation between the L* and lycopene concentration.

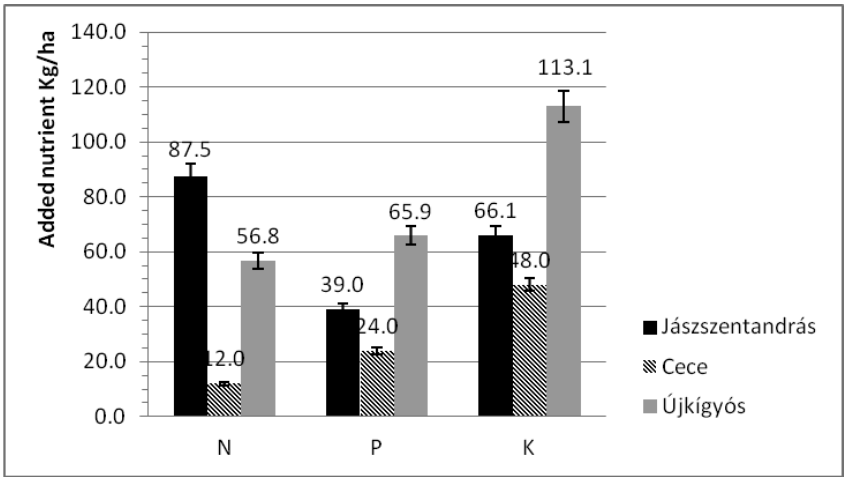

Figure 2: Added nutrient quantities in 2013

Table 1: Measured and calculated colour data according to region and harvest

Region	Harvest	Combination	L*	a*	b*	C*	h*
Cece	I.	non-grafted	35.48	26.13	20.65	33.32	0.67
		Lagenaria	33.21	25.14	19.97	32.12	0.67
		Interspecific	35.17	27.80	22.03	35.48	0.67
	II.	non-grafted	37.32	24.15	22.09	33.00	0.73
		Lagenaria	37.31	22.71	20.95	30.92	0.75
		Interspecific	38.39	25.90	22.83	34.55	0.72
Jászszentandrás	I.	non-grafted	35.41	24.83	19.52	31.59	0.67
		Lagenaria	36.06	26.34	20.08	33.13	0.65
		Interspecific	37.62	29.62	22.29	37.08	0.65
	II.	non-grafted	36.63	25.25	21.82	33.40	0.71
		Lagenaria	38.58	26.12	22.15	34.27	0.70
		Interspecific	42.71	29.49	24.57	38.41	0.69
Újkígyós	I.	non-grafted	40.35	25.87	22.44	34.25	0.72
		Lagenaria	41.28	25.27	21.75	33.37	0.71
		Interspecific	37.48	27.53	21.27	34.80	0.66

Negative correlation between L* and lycopene concentration was also reported by *Perkins-Veazie & Collins* (2004). Independently from region or harvest,

the interspecific rootstock combination fruits had the highest score by a* (red colour); this means that the fruit flesh was redder than the other combinations. The chroma (C*) value also changed by the grafting. The colour values L*, a*, b* and chroma in our study showed similar data to another report (*Perkins-Veazie & Collins*, 2004). The hue (ratio of a* and b*) mostly decreased by the grafting, but just a few percents. Based on this experiment, we can make the conclusion that it is worth to graft on the interspecific rootstock in the case of this watermelon type because the lycopene concentration and the colour sense have been both influenced in a positive way. The *Lagenaria x scion* grafting combination produced lower quality in the case of lycopene and colour than the ungrafted plants. Experiments showed that there is a correlation between the lycopene amount and colour sense.

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Effect of oil-seed pressing residue on bread colour and texture

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Abstract. Cold-pressing residue of walnut kernel (WKR) and brown linseed (BLR) was applied in wheat flour blends at 100:0, 95:5 and 90:10 ratios, of which enriched breads were baked, then stored for 3 days at ambient temperature. Colour parameters and firmness of bread crumb were measured daily. Bavarian rye-bread (BR) and wholemeal multigrain bread (WMMG) were used as competitive, marketable breads for comparing tests.

Keywords and phrases: bread, walnut, linseed, pressing residue, by-product, colour, hardness.

The studied oil-seed pressing residues (OSRs) resulted brown colour with different characteristics, depending both on the type of OSR and in comparison with marketed breads, too. The type and the ratio of OSR applied had no influence on the varying of crumb texture (P = 0.107). WKR and BLR enrichment provided stable texture for breads with a 3-day shelf-life, independently from their addition ratio. BLR resulted in softer crumb than WKR; however, this difference was considered to be negligible (P = 0.128). The WKR- and BLR-enriched breads stayed significantly softer at the end of storage time than the marketed breads (P = 0.000). Our results indicate that competitive bakery goods can be produced using oil-seed pressing residue/wheat flour blends.

1 Introduction

Oil-seed pressing residues (OSRs) as by-products of vegetable oil industry are obtained in lower quantity typically in the technologies of SMEs (small and middle enterprises). In addition, OSRs are valuable, macronutrient-rich raw materials (*Mueller et al.*, 2010; *Vanhanen & Savage*, 2006); so, they could be a cheap and novel type of food components. Although the OSRs have found wide application in feed rations, it has already been demonstrated that these materials can be safe even for food application. From the manufacturing point of view, OSRs can have greater potential in bakeries because bread fortification is increasing in world-wide research (*Preedy et al.*, 2011). Due to the high oil and dietary fibre content of OSRs, improved nutrients, colour, texture and the sensory profile of OSR-fortified breads are expected.

Marketability of foods like bread is influenced by consumer acceptation, basically by the texture of bread crumb. Besides, colourful baked products are rare. Different hues of white and brown colours are normally or traditionally accepted. Consumer demand toward multicoloured baked products is increasing regardless of artificial colouring additives. Black, blue, purple wheat grains are produced in small amounts for making specialty food (*Abdel-Al et al.*, 2006). *Li et al.* (2007) reported purple-wheat-bran-fortified muffin and demonstrated that such kind of bakery products have higher antioxidant capacity than whole-meal bread. In the 1990s, chemically extracted oil-seed meals were used and showed some colouring effect in breads, as *Mansour et al.* (1999) demonstrated the firming of pumpkin seed meal added breads. Nowadays, the application of oil-seed pressing residues in baked products, as recycled wastes, has novelty. The development of eco-innovative products is financed in the EU in the frame of Horizon 2020 strategy.

Our research has been focused on developing nutritious, acceptable brown-type bread products using pressing residue of walnut kernel and brown linseed. This objective was addressed by a comparative study of brown-type breads including similar breads commercially available with a 3-day shelf-life, using instrumental determination of crumb texture and colour. We have shown that the application of WKR and BLR can have great potential in the field of bakery innovation.

2 Materials and method

2.1 Materials

Cold-pressing residues of walnut kernel (moisture: 3.6%, protein: 39.4%, fat: 15.5% ash: 4.9%) were purchased from Tarpai Manufaktúra Kft. (Tarpa). Cold-pressing residues of brown linseed (moisture: 5.8%, protein 29.9%, fat: 18.9%, ash: 7.1) were purchased from Solio Kft. (Szekszárd). The pressing residue was milled in laboratory, then passed through a sieve with apertures of 500 μm. Wheat flour (type: 0.8 m/m% ash) was acquired from the local market. Bavarian rye-bread and whole-meal multi-grain bread were purchased from the local market and were used for comparative study.

2.2 Bread baking process and storage experiment

Pressing residue was applied in wheat flour blends at 100:0, 95:5 and 90:10 ratios. To prepare dough, 1.5 kg flour blend, 2.0% salt, 1.5% active yeast and, depending on the type of OSR, variable quantities of water, necessary to comply with dough consistency, were mixed. The kneading was followed by leavening for 25 minutes at 28 °C. The dough was punched down; individual pieces of 580 g dough were cut and fermented in dough basket for 45 minutes at 30 °C and 85% relative humidity. Round loaves were loosely baked at 230 °C for 35 minutes. The breads were left for cooling, then packed into plastic bags individually and stored at ambient temperature for 3 days.

2.3 Instrumental determination of the physical properties of bread crumb

Firmness and colour were measured daily and within 24 hours after baking for initial data. Bread slices with 1.25 cm width were cut right before measurement with fixed electric bread slicer. Firmness was determined with LFRA

CT3 texture analyser (Brookfield, USA) according to AACCI 74-10.02 Compression Test. The colour was determined on a 5-cm-diameter piece of bread crumb in Lab and LCh system with Colorite sph860 (CL150 model Z) spectrophotometer (Colorlite Gmbh, Germany) applying D65 illuminant and 10° observer.

2.4 Statistical evaluation of results

Data were analysed by standard analysis of variance analysis. Tukey's HSD procedure was used for testing significant differences between means at the probability level $p < 0.05$. Harmonic mean sample size was 5.14 for texture and 3.79 for colour statistical analysis.

3 Results and discussion

3.1 Effect of OSR addition on crumb colour and texture

The OSR addition resulted complex changes in crumb colour. The results of colorimetric measurement can be seen in *Table 1.* The lightness parameter varied by the type of OSR and negatively correlated with the ratio of enrichment. The BLR addition positively correlated with hue and the redness value, while negatively with the yellowness. The WKR addition positively correlated with the redness and yellowness values, while the hue value did not change. Regarding total colour difference (ΔE values), the crumb colour of any studied OSR-enriched bread was a significantly different type of brown colour ($P = 0.000$). OSR enrichment resulted in similarly dark breads with different hue and chroma from the studied marketed breads. However, WKR5 bread and BR bread are considered to be equivalent by total colour difference.

WKR- and BLR-enriched breads had soft crumb; the values varied between 2.27 ± 0.2 N and 3.11 ± 0.3 N. Besides, the type and the ratio of the applied OSR had no influence on the varying of crumb texture ($P = 0.107$). The crumb of BR bread was significantly firmer (6.15 ± 0.3 N; $P = 0.000$) than the other ones. The initial firmness of WMMG bread and WKR-10, BLR-5 was 3.42 ± 0.3 N and in the range of 2.54 ± 0.2 N, which was considered nearly identical ($P = 0.08$).

Table 1: Colour parameters of different brown-type breads after baking

Bread type	L*	C*	h	ΔE	a*	b*
WKR-5	58.51 ± 1.33^{b}	15.57 ± 0.21^{b}	57.50 ± 0.63^{a}	55.59 ± 0.34^{c}	8.37 ± 0.16^{d}	$13.13 \pm 0.23^{a,b}$
WKR-10	51.57 ± 0.64^{a}	17.18 ± 0.48^{c}	57.38 ± 0.52^{a}	53.70 ± 0.40^{a}	9.26 ± 0.27^{e}	14.47 ± 0.42^{c}
BLR-5	64.38 ± 1.79^{c}	14.03 ± 0.14^{a}	72.04 ± 0.32^{c}	60.31 ± 0.30^{e}	4.33 ± 0.11^{a}	13.35 ± 0.10^{b}
BLR-10	60.07 ± 2.29^{b}	13.36 ± 0.52^{a}	65.48 ± 0.23^{b}	58.80 ± 0.48^{d}	5.54 ± 0.21^{b}	12.16 ± 0.48^{a}
WMMG	49.31 ± 0.84^{a}	20.91 ± 0.56^{d}	71.50 ± 0.18^{c}	54.56 ± 0.32^{b}	6.63 ± 0.21^{c}	19.83 ± 0.52^{d}
BR	60.33 ± 0.28^{b}	23.50 ± 0.80^{e}	74.10 ± 0.08^{d}	$54.96 \pm 0.31^{b,c}$	6.44 ± 0.20^{c}	22.60 ± 0.77^{e}

Mean ± SD. The number in the name of bread type means the addition ratio of the selected OSR. **L***: lightness; **C***: Chroma; **h**: hue; **ΔE**: colour difference; **a***: red (+), green (-); **b***: yellow (+), blue (-). Various letters in the same column mean significant difference between the averages (Tukey HSD test; $P < 0.05$).

3.2 Effect of storage on the quality of OSR-enriched breads

During the storage term, either the colour or the firmness of OSR-enriched breads did not change significantly. WKR and BLR enrichment provided stable texture for breads with a 3-day shelf-life, independently from their addition ratio (see Fig. 1).

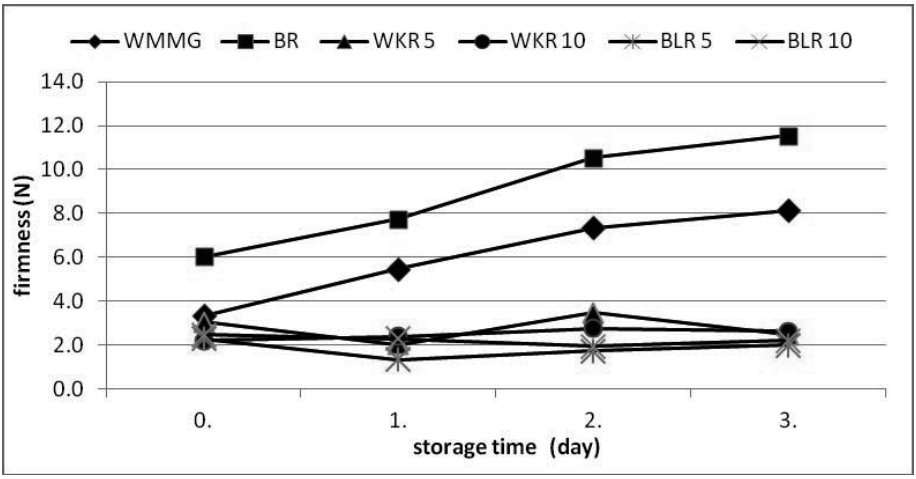

Figure 1: Bread crumb staling during a 3-day storage (average firmness in Newton)

The 3-day average firmness of different OSR-enriched breads was in the range of 1.8 ± 0.3 N and 2.8 ± 0.7 N. BLR resulted in softer crumb than WKR; however, this difference considered to be negligible ($P = 0.128$). Regarding the marketed breads, intensive staling of WMMG and BR breads was found; the 3-day average firmness was 6.1 ± 0.5 N for WMMG bread and 9.0 ± 0.5 N for BR bread.

The WKR- and BLR-enriched breads stayed significantly softer at the end of storage than the marketed breads ($P = 0.000$). According to *Ronda et al.* (2014), enrichment of bread results in firmer crumb was stated and an increase in water retention during storage would be expected to decrease firmness. Linseed pressing residue, through its water-holding capacity (*Mueller et al.*, 2010), can play the role of a stale-decreasing component in bread formulas.

We demonstrated in this experiment that the studied OSRs can be used as natural ingredients for double function as natural colouring and as texture

improvers of enriched breads. Our result can be applied in the field of bakery product innovation and for expanding the assortment of breads.

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Experiences of using the fruit waste of the modern Hungarian pálinka fermentation technology for the foraging of extensively kept grey cattle

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Abstract. In this article, the authors report on the experiences of six years of foraging, describing how the fruit wastes generated in the Pannonhalmi Pálinkárium are utilized for foraging Hungarian grey cattle. The goal is not the control or improvement of the cattle's growth indices but the problem-free, continuous and eco-friendly disposal of the fruit waste. They have found that the fruit waste or pomace is virtually nothing else than protein-enriched sugar-free fruit, and that during the utilization of this they have to maximally adapt to the cattle's life-cycle, biological nature and environmental factors, and they will repay you by eating the pomace. They conclude that the grey cattle are a skin-and-hairs-covered bioreactor, which provides an economical service for the distillery through the utilization of the fruit waste. Nowadays, 150,000-200,000 tons of fruit waste is produced every year, and only a few percent of this is utilized in ruminant forage. By writing this article, the authors would like to expand our very scarce knowledge on this topic.

Keywords and phrases: fruit wastes, Hungarian pálinka, Hungarian grey cattle.

1 Introduction

Formerly, the presence of a rural distillery could always be determined by the distinctive odour surrounding it. There was always the odour of the residue gathering in the distillation waste container. These had a storage capacity from 20 up to 100 cubic meters. It was only emptied once or twice per season. The fruit waste has gone through a strong parching phase, when its initial dry matter content of 6-8% increased to 12-16%. This was characterized by acid fermentation, decomposition of carbohydrates and protein putrefaction processes, as well as by high nitrogen losses. (The mineral and micronutrient content remain unchanged.) Its nutritional values and organoleptic properties were heavily damaged and its value as forage decreased. However, there are written records from Pannonhalma and its surroundings, dating back to 1800–1825, about using fresh pomace as forage (Benedictine Abbey of Pannonhalma, Archives, Economic archives. 1800–1825). Positive feeding experiences in subsequent times are not available.

In the last decade (2002–2013), the raw materials, the technology and the quality of the end-products of Hungarian Pálinka manufacturing changed drastically. Hungarian Pálinkas (fruit distillate) with fruity flavour and fragrance came to the fore. The quality, nature and composition of the fruit waste and pomace, which are the by-products of the production process, also changed significantly. Nowadays, the disposal or utilization of the generated wastes is a key issue of Hungarian Pálinka distillation. The following practical possibilities open up for the utilization of these by-products: soil strengthening, composting, disposal at wastewater treatment plants, bio-gas production and foraging.

In 2007, after the change of ownership and modernization, the commercial distillation of Hungarian Pálinka has begun again. During and after the remodification and restart of manufacturing, the owners considered the environment-friendly operation of the distillery as a key element. One of the cornerstones of this was the environment-friendly use of the fruit waste. The history of the distillery can be traced back to 1818 (Benedictine Abbey of Pannonhalma, Archives, Contract, 1818). Written records were found from 1833 and 1845 (Benedictine Abbey of Pannonhalma, Archives, Economic archives. 1825–1835), and, according to these, pomace and fruit waste were “used to feed the cattle by the hostelry and butchery of Tényő”. In the winter, primarily pomace was used to feed the beef cows. This also contributed to the decision to utilize the by-product for feeding the grey cattle.

In the fruit waste area, significant changes have occurred in the past 10 years. In the past, Hungarian Pálinka has been produced from only a few plant species, but nowadays the following types can also be used for foraging: apple-, pear-, plum-, peach, mixed-, pomace wastes, and other minor types in smaller quantities.

With the development of the spirits industry, to meet the consumer needs, more and more types of plants were used to produce Hungarian Pálinka, and, obviously, the fruit wastes of these were also created. Instead of the previous 5-6 types of fruit, now there are about 30 species which we prepare distillate from. Mashes of elder-berry, black-currant, sloe, hip, holly, hawthorn, strawberries, raspberries etc. have appeared. Today, an average distillery can easily produces 15 types of distillates.

2 Material and method

The Hungarian grey herd

The distillery entered into an agreement with the owners of the herd consisting of about 100 grey cattle, held on the slopes of the hills of Pannonhalma, to utilize the fruit waste for foraging. The herd consists of one bull, which is replaced every 3 years, 30-35 mature cows, and 65-70 young calves, which are 1-2 years old.

The feeding of the herd during the grazing period included the pomace as an addition, and even outside this period, from October till April, they were given the pomace besides the hay and corn stalk as main forage. The feeding occurred from 300-400-litre troughs, of which there were 5-6 pieces placed in the pens or on the pastures. Taking into account the seasonality of the Hungarian Pálinka distillation, the pomace can be utilized mainly in the winter season.

Production of fruit wastes

In the case of rapidly utilized fruit wastes, the temperature of the fruit waste is initially 92-95 C°. The heat amount is nowadays utilized in preheating the next cooking batch with heat exchangers. The fresh fruit waste CFU count is extremely low, but even so you have to try to use it up within 48 hours to avoid spoilage processes; the flavours are also better preserved this way. During the winter period, especially in harsh cold outdoor environments, the using of 30-40 C° warm forage is extremely beneficial, especially with calves

and juveniles. Effectively, it is an additional energy input method. The fresh fruit waste has a more pleasant aroma of fresh fruit; so, it is more willingly consumed by the cattle.

During the fermentation, partially pectinolytic enzymes and partially aroma-amplifier enzymes (e.g. beta-glucosidase) are used to open up cell walls and release the fruit esters. It enhances not only the Hungarian Pálinka's flavour but also that of the fruit waste. It contributes a lot to the fact that the grey cattle consume this type of fruit waste with great appetite. Fruit wastes today are much tastier than before due to the use of these enzymes.

One of the cornerstones of the controlled fermentation is that the fermentation is not left to the natural yeasts spontaneously, but consciously selected yeast strains are used, which are adequate for our purpose; and the fermentation is immediate in lieu of the aerobe growth phase. The target CFU is reached by adding the required amount of yeast, not by growing. The yeast concentration shows 10^6-10^8 CFU. This cell mass is constantly increasing during the fermentation, and, especially in relation to the fruit, the protein content of the slop also increases. In fact, a major cellular protein production takes place during fermentation. When inducing alcoholic fermentation, and on the 3^{rd}-5^{th} days of the fermentation, we have to add feeding salt to the mash. This is a yeast autolysate supplemented with macro- and microelements. At the end of fermentation, this remains in the slop ingrained in the yeast cells, increasing its value by minerals and proteins.

During the controlled fermentation, pH is kept between 3.0 and 3.5 to avoid infections. Fruit production is around 5.5 pH. The pH required for fermentation can be set with a mixture of lactic and phosphoric acids (90+10%), and then repeated once in the course of the fermentation. The use of other organic or mineral acids is no longer applicable. The yeast incorporates some of the phosphoric acid in its living cells, so pH is constantly increasing. Fresh fruit waste has a pH of approximately 4.2-4.5. This is a very stable value and it has great significance in terms of the rumen flora, while using it as fodder. The acidic pH of the fruit waste during distillation allows even larger molecules to hydrolyse.

Consistency of fruit wastes

Fundamental changes have taken place in this area in recent times, which have significantly increased the foraging value of the fruit wastes. One feature of the former fruit wastes was that fruits had only undergone severe mechanical shredding; so, the waste often contained large pieces of fruit and, in most

cases, parts of cores and seeds in the mash. The homogenization of the mash did not happen either.

In modern fruit processing mill, the cracking grinder, stemmer and crusher machines practically form a uniform quality mash. The seeds of the hard-shell fruits are completely removed from the pulp and are separately, fully utilized mainly for energy production, partly as forage for wild animals. Only the grape and black-currant wastes can contain seeds.

Added pectin enzyme (1-2 g per 100 kg of fruit) during the processing of the mash increases homogenization (virtually, a biochemical homogenization). The moving of the liquid-like mash is easy and simple with pumps.

3 Results

Results of our studies on the composition of fruit wastes

Comparing today's knowledge about the fruit texture of the mash with earlier ones, the following observations can be made: The dry matter content nowadays is lower than before. This is due to the fact that a while ago mash was parched in pits, where often half of the moisture content evaporated or drained. Nowadays, this is unimaginable. In the past, mash solids content was 12-15%, whereas today it is between 6% and 9% (wild fruits have higher values). The fibre content and the flesh pieces of the mash are highly homogenized. Despite this, the mash begins to get layered after 48 hours of storage. Typically, the fibres and flesh pieces settle at the bottom of the tank, except for the quince and apple, where the solid phase floats on the top of the slop and often forms a one metre thick cap. Knowing this difference, you can save yourself a lot of trouble during the treatment of the mash. Hence, the seeds cannot be found anymore in today's mash: they are unsuitable for feeding pigs. (Formerly, Mangalitsas were fed with this). Now the seeds are mainly used to feed wild boars.

The fruit pulp consistency has a significant impact on the amount of slop as well. A significant amount of water must be added during the processing of many fruits (e.g. quince). There are factors in the Hungarian Pálinka production which reduce the amount of slop. These are: alcohol removed by distillation: 3-10% reduction; carbon dioxide departing during fermentation: 1-3% reduction; seeds and stalks removed during processing: 10-30% reduction.

Technological processes which increase the amount of slop: addition of nutrient salts, acids and yeast: 1-2% volume growth during fermentation; water

added for processing can result in a 30-40% growth. In extreme cases, even a 2-3-fold increase in the amount of slop can be observed (e.g. chestnut).

Loss and gain factors add up to give the final quantity of the slop. The amount of fruit waste generated during the processing of some fruit species is shown in *Table 1.*

Table 1: The amount of fruit waste generated during the processing of some fruit species in the Pannonhalmi Pálinkárium between 2009 and 2013

Fruit species (100 kg)	Quantity of waste (kg)
Apple	90-92
Pear	90-92
Rasp- / Strawberry	95-98
Plum	75-80
Peach	77-82
Grape	85
Pomace	95-110
Blackthorn, Hawthorn	90-110
Quince	110-130
Chestnut	200-250

Nutritional values of fruit wastes

Perhaps the most significant change has taken place in this field because of the development of fermentation and distillation technologies. Under fruit waste (f.w.) nutritional values, not only the chemical parameters can be examined but also the classic organoleptic properties. The cattle's willingness to consume fruit wastes is greatly affected by this. A few new technological steps may significantly affect the nutritional values of the wastes.

Below, we present five fruit waste feed sample test results in *tables 2* and *3*, which show that the Williams pear represents well the waste of apple-like fruits; 40% added water was used when processing the quince, the elderberry waste has one of the highest dry matter contents, peach represents the cultivated stone fruit type and sloe represents the wild stone fruit type.

Table 2: Nutrient content of 1 kg fruit waste

Parameter (g/kg forage)	Quince	Williams Pear	Elder-berry	Peach	Sloe
Original solids	67	75	172	79	90
Crude protein	4	5	25	10	5
Crude fat	1	1	27	1	1
Crude fibre	11	12	38	10	4
Crude ash	4	7	17	9	9
N-free extract	47	50	65	49	71

Table 3: Specifics of 1 kg fruit waste

Parameter (g/kg forage)	Quince	Williams Pear	Elder-berry	Peach	Sloe
Crude protein	56	66	146	125	56
Crude fat	12	10	158	13	8
Crude fibre	160	161	219	119	43
Crude ash	62	92	99	112	101
N-free extract	710	671	378	631	792

Special content formulas of fruit wastes

Based on our measurements, the alcohol content of the fruit waste did never reach 0.1% by volume during the six years examined. By complying to standard distillation parameters, it may be excluded that the fruit waste contains ethyl alcohol. The presence of higher alcohols and polyhydric alcohols is natural.

Theoretically, the yeasts reduce the fermentable sugars in the mash to below 0.1%. Normally, sugar-like compounds may become 3-4 carbon atom carbohydrates or oligosaccharides. If you encounter fermentation after distillation (so, there is sugar in the mash), that indicates a huge technological error, which can be one of the following: Alcoholic fermentation has stuck, hence sugar remained in the mash. This can happen because of the high alcohol content (e.g. chestnut waste) or because of a too cold environment. The latter one

can occur in late maturing plants (blackthorn). Or, the degradation of starch or oligosaccharides did not happen in some fruits which would have required post-maturation; instead, it happens during or after the distillation. In this case, the fruit waste given as forage may fizz after a few days. Distillers seek the perfect fermentation; so, the presence of residual sugar is very rare.

Fruit wastes can contain hydrogen cyanide, but only when the fruit seeds are in the mash during the alcoholic fermentation and distillation. In case of stoning, this cannot happen. Stoning is the guarantee to avoid the formation of ethyl carbamate as well. However, a not properly configured stoning machine can break the seeds, which can contaminate the slop. This is a real danger when working with early cherry and sour cherry (it has weak cores). Interesting, as it should be mentioned, that according to literature (*Szigeti*, 2010), the non-seeded mash of sloe has the highest content of hydrogen cyanide.

High copper content is a real danger, but it can be easily avoided. Mash with a low pH can dissolve some copper from the walls of the distiller. However, to have a high enough content to be able to measure by quick tests, you have to keep the warm mash in the distiller for more than 50 hours. Observing these three rules, the increased level of copper can be ruled out by following a few simple rules: the mash has to be in the distiller only for the time of the distillation, the distilling equipment, which has not been used for several months, should be always washed with water first, and never spill the predistillate into the mash (this is nowadays impossible because of the excise labels).

Experiences of the consumption of fruit wastes by grey cattle

Our first feeding experiences were that the grey cattle liked these various by-products very differently. There are types which we could hardly get the cattle to consume and types which cause real fights among the herd. Based on our experiences, the fruity, juicy, sweet-smelling fruit wastes were the majority; the ones with strong smell or taste of vegetable alkaloids were the less popular. Thus, elder-berry and black-currant definitely belong to the latter category. Experience shows that cattle do not like plant wastes, which they do not consume on the pasture (e.g. elder-berries, rose-hips).

Based on this experience, the popularity and consumability of fruit waste can be classified into three groups, which we have to take into account when developing a feeding strategy. The groups are: most liked and always gladly consumed: strawberries, raspberries, cherries, peaches and pear; consumed after a few days of getting accustomed to: plum, apple, pomace and mixed

mash; hardly ever consumed: elder-berry, black-currant, hawthorn, sloes, rosehips and quinces.

According to our experiences, the propensity of the grey cattle for the consumption of fruit wastes is also affected by the seasons and the feeding environment. The main points here are: In the early autumn, the herd gets less used to eat fruit waste – because of the shortage of it; so, they have to get used to it again. During the winter period, especially the extremely cold temperature, the propensity for consumption increases. The February calving period and the start of lactation also increase consumption. From the end of April, the availability of fresh pastures drastically reduces the propensity for consumption. The herd is in a "green intoxication". These foraging experiences determine the order of distilling in order to move the grey cattle in the direction of the largest possible quantity of fruit waste consumed.

The habituation to consume the fruit waste of the autumn season is achieved by the most popular types: raspberries, cherries and strawberries. The consumption begins virtually immediately, and the consumption patterns develop. With winter approaching, the reduction of green fodder makes the moderately popular fruit wastes more and more accepted; so, these are preferred (plum, apple). During the winter, the more difficult types can be given, purely or mixed with other types. Elder-berry can be fed only mixed with other fruits because grey cattle never eat it in pure form. The consumption of the less popular types can also be improved by reducing the quantity of drinking water.

When the pastures become green, the propensity to consume fruit waste is greatly reduced; in this case, the feeding with peach and pear wastes is effective. Not only the plant species affect the edibility of a certain type of fruit waste but, in many cases, fruit varieties within the species as well. As an example, among pears, the type of "helmet of Bosk" is far less popular than the Williams pear, or "Oliver Irsai" is more popular than any red grape waste. The explanation of the differences is in fruit succulence and flavour.

Eating sequence, quantities eaten

Grey cattle can be accustomed to good quality fruit waste in only 3-4 days, even if they never have eaten something like this before. In a mixed herd structure, we can count about 25-35 kg/head consumption/day. Standard deviation is depending on the size of the cattle and can be anywhere between 1 and 40 kg. Among the categorized types of grey cattle (primitive, yoke, dairy, manorial), the primitive consumes the least and the dairy the most. The Hungarian grey cattle is not a homogeneous breed, several types have

developed:

- Primitive, small type: Undeveloped, low-shoulder-height model. It features an unattractive horn evolution and a faulty foot structure.
- Rough yoke type: Large shoulder height, deep-chested. The legs are long, their movements are lively.
- Fine dairy type: Sleek, small, precious, delicate constitution grey type cattle. Thin horns, udders more advanced.
- Large-scale type: Noble, good-looking, often exceptionally beautiful horns; the most common type today.

In case of changing the type of the fruit waste, the change-over in 2-3 days, with mixed dosages, is problem-free. Changing to a more popular type (peach) is immediate, changing to a less popular one (black-currant) takes more days. We have never experienced any problems in life functions or digestive functions during change-overs. We had no cases of any diseases either. These are personal experiences.

The eating sequence is based on the rank in the herd. The sequence is: bull, old cow, young cow and calf. The heads in the top of the ranks are usually guarding the more popular types of fruit wastes (peach, raspberry and strawberry); they do not let others near them. In the case of extremely popular types (Irsai pomace), we also have to be aware of occasional overconsumption. The best method to resolve the problems posed by the eating sequence is to create more feeding points. One feeding point can accommodate 10-20 heads. The young calves begin to eat the slop after 1 month of age. According to our observations, the calves like the warm fruit waste best in the February-March period.

After that, the massive appearance of pasture grass causes a temporary reduction in the fruit waste consumption. This is the main concern to pay attention to because fruit waste consumption can completely cease in a few days and the quality of the waste degrades over time; so, the cattle will likely eat it even less near the fresh pasture. We can say that this time is the most critical period of the foraging with fruit wastes. However, with several small tricks, we can overcome this problem.

Economic implications of fruit waste consumption

Strive to place the distillery as close to the forage production site as possible: the best is in a 5-km radius from the herd. Transportation is not economi-

cal due to the low nutrient content and high water content. In spite of the pasturage, the logistics of the continuously generated fresh fruit waste is to be resolved. The distillery's production quantity should follow the size of the herd, never vice versa. Based on the low performance data of the grey cattle, under extensive keeping, the fruit waste becomes a main feed. For grey cattle, 30-40% of the nutrient needs can be covered by it. Vitamin B content of this forage is significant, but this is still not really taken advantage of (*Hegóczki*, 1997).

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