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Selenium-sulphur effects on the chemical composition of alfalfa (*Medicago sativa* L. cv. Verko)

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Abstract. Selenium (Se) is an essential micronutrient, and the ability of some crops to accumulate Se is crucial for human and animal nutrition and health. Se deficiency can cause white muscle disease characterized by muscle weakness, heart failure, unthriftiness, and death in livestock. This study was undertaken to investigate the effect of sulphur (S) on Se concentration in alfalfa (*Medicago sativa* L. cv. Verko) as a non-hyperaccumulator plant. Alfalfa plants grown in the field were treated foliarly with 3 g Se ha^{-1} solutions of selenate, 3 g S ha^{-1} sulphate, and both. The concentration of Se in both the leaves and stems of plants was similar in the control and S-treated plants. Se concentration in plants treated with S was undetectable, as expected. S was shown to enhance Se accumulation in alfalfa. Furthermore, although foliar Se + S spray increased biomass, photosynthetic pigments decreased peroxidase activity and malondialdehyde content. Overall, results suggested that foliar Se + S spray can be applied as a biofortification to improve alfalfa plants with appropriate amounts of Se and better nutritional as well as functional quality.

1   Introduction

Selenium (Se) is a metalloid that resembles sulphur (S). As an essential micronutrient and component of seleno-amino acids and seleno-proteins for animals, many prokaryotes and some algae, Se is needed for good health in small amounts. Se has an important place for antioxidant defences and is a cofactor for the antioxidant enzyme glutathione peroxidase (*Lu et al.*, 2010; *Fariduddin et al.*, 2014; *Lehotai et al.*, 2016). Selenium, a non-essential element for plants, plays many beneficial roles in them *(Hartikainen et al.*, 2000; *Cartes et al.*, 2005). Selenium enters the food chain through plants, and increasing evidence has shown that Se promoted the plant growth and, exogenous application of Se significantly increased chlorophyll a and b as well as carotenoid content in plant leaves (*Filek et al.*, 2009; *Malik et al.*, 2012). New findings have approved that in low concentrations Se can prevent plants from oxidative stress (*Barbara et al.*, 2014). In contrast, as extra-Se affects growth and chlorophyll synthesis, it could be toxic because it is important in the formation of reactive oxygen species (ROS) that cause oxidative stress (*Bañuelos et al.*, 2010; *Cabannes et al.*, 2011). To prevent extra-ROS in plant cells, non-enzymatic antioxidants, such as antioxidative enzymes, have important roles (*Dai et al.*, 2015). In higher plants, Se is taken up at the root level through the sulphate transporters. After uptake, Se may remain in inorganic form or get metabolized into seleno-cysteine or seleno-methionine through the sulphate assimilation pathway (*Terry & Zayed*, 2000). As Se and sulphur (S) are chemically similar, Se can affect S homeostasis, redox status, and protein folding (*De Kok & Kuiper*, 1986). Seleno-amino acids could be combined with protein instead of cysteine and methionine and then cause misfolding events that may trigger ubiquitination and subsequent degradation of protein (*Sabbagh & Van Hoewyk*, 2012).

Alfalfa is an important leguminous forage that has a wide distribution in the world, and it is an important component of the agroecosystem (*Deng et al.*, 2014; *Fan et al.*, 2015). The agronomic biofortification of different food crops with Se could lead to a suitable strategy for increasing the intake of Se by animals and humans and reveal Se malnutrition problems (*Poblaciones et al.*, 2014; *Malagoli et al.*, 2015). Se fertilizers would be one agronomic practice for Se biofortification to increase Se bioavailability in plants (*Poblaciones et al.*, 2014; *Smolen et al.*, 2016).

There is limited evidence to suggest increased growth or other beneficial effects of Se for non-accumulator plants. Also, there is no known physiological mechanism involved in Se accumulation, translocation, and the characteristics

of Se/S discrimination in alfalfa as a Se non-accumulator. In this regard, we have grown alfalfa to figure out the effect of foliarly applied sulphate and selenate as well in different parts of the plant, with their possible effects on the antioxidant mechanism and growth.

2   Materials and methods

Alfalfa (*Medicagosativa* L. cv. Verko) seeds were grown in acidic soil in a field in Braunschweig (N: 52° 17', E: 10° 26', 81 m above sea level). Seeds were sown on May 25 on four blocks, each with five 9 m×12 m distributed plots, randomly. Foliar spraying with sodium selenate (Se) at a concentration of 3 g Se ha^{-1}, sodium sulphate (S) at a concentration of 3 g S ha^{-1}, and sodium selenate + sodium sulphate (Se + S), each at a concentration of 3 g Se ha^{-1} and 3 g S ha^{-1}, was performed at the five-leaf stage on 19 June. Control plants were sprayed with water. Four treatments, Se, S, Se + S, and control were applied as follows. Spraying was performed in calm weather by spray truck. On all plot margins, plants from the outer 1.5 m were discarded for minimizing the possibility of effects of the treatment from a neighbouring plot. In each plot, the central parts were harvested and analysed. During the experiment, the average mean temperature was 16 °C, and during the same period the amount of precipitation was 268.5 mm: 135.7 mm before the foliar treatment and 132.9 mm after the treatment. The plants were harvested after 75 days at the late stage of flowering, and ten replicates were obtained for every treatment.

Photosynthetic pigment measurement

Intact and erect leaves from 10 plants in each block were sampled for the extraction and determination of the photosynthetic pigments. The instrument settings and parameters were the same as described previously (*Garousi et al.*, 2016).

Malondialdehyde content

The malondialdehyde (MDA) content was measured from leaves based on Zhang and Huang's method (*Zhang & Huang*, 2013).

Peroxidase (POX) activity

The peroxidase activity of leaves was assayed following Sanchez et al.'s method (*Sanchez et al.*, 1995).

Total soluble protein content

Total soluble protein content of leaves was determined using the method followed by Bradford (*Bradford*, 1976).

Dry mass measurement

Above-ground parts of samples were collected at the late stage of flowering and separated into different parts of the leaves and stems. All samples were freeze-dried (ALPHA 1-4, Osterode am Herz, Germany) and milled (Fritsch, Pulverisette 7, Idar-Oberstein, Germany). These two parts were weighed using an analytical scale with an accuracy of 0.001 g (OHAUS Explorer, Switzerland) to record their dry masses.

Quantification of total Se and sulphur

The instrument settings and parameters were the same as described previously (*Garousi et al.*, 2017).

Data analysis

Data were analysed statistically by SPSS 19.0 software (2010). Standard error was calculated, and analysis of variance (ANOVA) was done on the data to detect the minimum significance difference (Tukey) between treatment means with the level of significance at $P \leq 0.05$.

3   Results

Photosynthetic pigments

Among all treatments, the concentrations of photosynthetic pigments were the highest in Se + S and the lowest in S. The contents of photosynthetic pigments are shown in *Table 1*. Concentrations of chlorophyll a, b and carotenoids were significantly increased by about 15.7, 25.2, and 18.7%, respectively, in Se + S treatments, compared to those with S exposure. Se samples were gradually increased in comparison with those under control conditions.

Table 1: The contents of the photosynthetic pigments, MDA, protein, and activity of peroxidase in control leaves, Se – selenium-, S – sulphur-, and Se + S – selenium-and-sulphur-treated alfalfa for 75 days

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoid (mg g^{-1})	MDA (nmol MDA g^{-1}FM)	POX (Unit g^{-1} FM)	Protein (mg g^{-1} FM)
Control	1.2133±0.02[b]	0.3677±0.12[ab]	0.2897±0.01[ab]	3.5000±0.42[b]	0.1520±0.00[a]	1.3675±0.22[bc]
Se	1.2467±0.09[b]	0.3793±0.04[ab]	0.2953±0.02[ab]	2.9681±0.43[a]	0.1578±0.00[a]	1.4294±0.20[ab]
S	1.1233±0.05[b]	0.3227±0.02[b]	0.2620±0.01[b]	3.1707±0.19[a]	0.1393±0.00[ab]	1.4530±0.27[a]
Se+S	1.4400±0.10[a]	0.4317±0.03[a]	0.3223±0.01[a]	2.9752±0.20[a]	0.1234±0.01[b]	1.3574±0.17[c]

Significant differences in the mean value of each treatment group are indicated by different lower-case letters based on the Tukey test ($p < 0.05$, $n = 10 \pm$ s.e.)

Malondialdehyde content

Compared to the control plants, the concentrations of MDA decreased in the three treatments when exposed to Se (by 15.2), Se + S (by 15.0), and S (by 9.4 %) (*Table 1*).

Peroxidase (POX) activity

Under Se exposure, the activity of POX was enhanced by 3.7% in comparison with those under control conditions. In contrast, under S and Se + S exposure, POX was inhibited by 8.4 and 18.8%, respectively, as compared to those under control conditions (*Table 1*).

Total soluble protein content

Sulphur significantly increased the content of soluble protein in leaves by 5.9%, just as selenium, which increased the soluble protein by 4.3% compared to the control (*Table 1*). However, in plants which received both selenium and sulphur, the protein content dropped by 0.7%.

Dry mass

After the experimental period, the biomass increment of alfalfa plants was monitored (*Table 2*). Total leaves and stem biomass of all treated samples was markedly higher than in those grown under control. The biomass of stem and leaves formed during Se + S exposure was higher compared to the biomass of plants treated only with Se or S.

Quantification of total Se and sulphur

In all examined samples, alfalfa leaves accumulated significantly higher Se and S than stems, whereas sulphate supplementation did not change significantly the sulphur content in either leaves or stems. Selenium concentration became very low as undetectable, selenate treatment caused 5- and 3-fold higher Se in leaves and stems, respectively. Furthermore, the concentration of Se in both the leaves and stem parts of alfalfa was 7- and 6-fold higher, respectively, in plants treated with both S and Se. Treatment with both Se and S did not cause significant changes in the sulphur concentration of leaves although its amount in the stems was very low, of 1-fold, in comparison with those under control conditions (*Table 2*).

Table 2: Dry mass and concentrations of Se and S in leaves and stems of alfalfa in control, Se – selenium, S – sulphur, and Se + S – selenium and sulphur treatments

Treatment	Dry mass (g)	Se $(\mathrm{mg\,kg^{-1}})$	Se $(\mathrm{g\,kg^{-1}})$
	leaves		
Control	$0.7901 \pm 0.05^{\mathrm{b}}$	$0.102 \pm 0.00^{\mathrm{c}}$	$0.33 \pm 0.1^{\mathrm{a}}$
Se	$0.7932 \pm 0.03^{\mathrm{a}}$	$0.543 \pm 0.01^{\mathrm{b}}$	undetectable
S	$0.7923 \pm 0.08^{\mathrm{a}}$	undetectable	$0.33 \pm 0.2^{\mathrm{a}}$
Se + S	$0.7932 \pm 0.05^{\mathrm{a}}$	$0.703 \pm 0.01^{\mathrm{a}}$	$0.35 \pm 0.1^{\mathrm{a}}$
	stem		
Control	$0.7918 \pm 0.04^{\mathrm{b}}$	$0.030 \pm 0.00^{\mathrm{c}}$	$0.083 \pm 0.00^{\mathrm{b}}$
Se	$0.7941 \pm 0.01^{\mathrm{a}}$	$0.096 \pm 0.00^{\mathrm{b}}$	undetectable
S	$0.7939 \pm 0.03^{\mathrm{a}}$	undetectable	$0.085 \pm 0.00^{\mathrm{ab}}$
Se + S	$0.7944 \pm 0.06^{\mathrm{a}}$	$0.186 \pm 0.00^{\mathrm{a}}$	$0.094 \pm 0.00^{\mathrm{a}}$

Significant differences in the mean value of each treatment group are indicated by different lower-case letters based on the Tukey test ($p < 0.05, n = 10 \pm s.e.$ for dry mass measurement and $n = 3 \pm s.e.$ for Se and S concentrations).

4   Discussion

In every terrestrial plant species, the assimilation of selenium in plants occurs via the same metabolic pathway of sulphur (*Barak & Goldman*, 1997). It should be noted that the absorption of selenate competes with the uptake of sulphate (*Arnault & Auger*, 2006). Se contributions such as selenate cause the decline in the concentration of S metabolites even when there are high amounts of available sulphate (*Van Hoewyk et al.*, 2008).

Results show some pronounced properties of non-hyperaccumulator alfalfa (*Medicago sativa* L. cv. Verko) with regard to Se and S accumulation and movement within the plant.

Dry mass in both the leaves and stem was significantly higher in Se-, S-, and Se + S-treated plants than in controls (*Table 2*). In the present study, a 3 g Se ha^{-1} solution of selenate and 3 g S ha^{-1} sulphate as well as a combination of both elements clearly caused to stimulate the biomass of alfalfa plant. The application of Se in plants increases biomass accumulation (*Cheng et al.*, 2016) or yield (*Põldma et al.*, 2013). Growth-promoting Se effects have been reported in lettuce (*Xue et al.*, 2001), ryegrass (*Hartikainen et al.*, 1997; *Hartikainen & Xue*, 1999), and soybean (*Djanaguiraman et al.*, 2005). On the other hand,

Se application did not have any significant effect on the dry weight of other crops such as red cabbage that was treated with a 0.025 mM aqueous solution of selenate every second day for two months or a 6.3 mM solution used twice in the test period in fertilized soil (*Mechora et al.*, 2011), red chicory sprayed with an aqueous solution of sodium selenate (12.6 mM) foliarly twice (*Germ et al.*, 2007), and potato cultivated in quartz sand and fertilized once a week with sodium selenate, the amounts corresponding to 0.075 mg and 0.3 mg Se kg^{-1} (*Turakainen et al.*, 2004).

The application of Se + S significantly increased photosynthetic pigment content (chlorophyll a, chlorophyll b, and total carotenoids). Previous works have shown useful influences of Se on chlorophyll contents in various crops such as spinach (*Saffaryazdi et al.*, 2012) and wheat (*Yao et al.*, 2009). Due to the positive effects of Se and S, an increase in chlorophyll content could be seen in terms of chlorophyll synthesis, the degradation of chlorophyll could be prevented, or both (*Yao et al.*, 2010; *Wang*, 2011; *Iqbal et al.*, 2015). This kind of useful Se effects on the biosynthesis of photosynthetic pigments by protecting chloroplast enzymes have been seen in leaves of spinach (*Pennanen et al.*, 2002). In the work, the individual application of Se and S did not have any significant effect on the photosynthetic pigments, while foliar S applications reduced them (*Table 1*).

Foliar applications of Se and Se + S reduced leaf MDA content (*Table 1*). The decline of Se-mediated in MDA may be attributed to its useful effect and to activating the antioxidants in stressed plants (*Pukacka et al.*, 2011). Moreover, Se could act as an antioxidant and reduce the amount of ROS (*Filek et al.*, 2008), perhaps due to its metabolism regarding selenite and then regarding the volatile dimethylselenide (*Pilon-Smits et al.*, 1998). The findings suggested that foliar Se and Se + S acted as both a ROS quencher and an increaser of antioxidant activity, whereupon reducing lipid peroxidation.

Whereas the foliar application of Se increased the effect of POX activity in alfalfa plants, Se + S treatment caused reduction in its activity (*Table 1*). Previous works showed that the application of Se did not change antioxidant activities (*Tan et al.*, 2012), while *Khattab* (2004) found that Se-mediated changes caused significant decline in antioxidant enzymatic activities. On the other hand, applications of Se caused an increase of enzymatic antioxidant capacity in plants exposed to various stresses (*Hu et al.*, 2013a, b; *Iqbal et al.*, 2015). As several results suggest, we may conclude that Se effects are based on concentration (*Hu et al.*, 2013a, b), type of stress, and crop species (*Iqbal et al.*, 2015).

The foliar application of Se causes sulphur replacement in amino acids, with

further changes in the protein three-dimensional structure and also causing the impairment of enzymatic function (*Amweg et al.*, 2003). The findings of this work therefore showed that Se + S concentration reduced protein content, whereas S treatment significantly improved protein content compared to those of the non-treated control plants (*Table 1*).

Se concentration was greater both in the stem and the leaves of plants treated foliarly with Se (*Table 2*). Se application has been reported to result in a significant increase of Se content in several plant species (*Drahoňovský et al.*, 2016). Se and S metabolism in plants are highly related to each other (*Terry et al.*, 2000), and therefore it was recommended to investigate their possible interactions with regard to Se and S concentration in Se, S, and Se + S-treated plants. In non-hyperaccumulator species, Se is regarded to be accumulated non-specifically by the sulphate transport system (*Persans & Salt*, 2000; *White et al.*, 2004). There is plenty of evidence that sulphate transporters also transport selenate: mutants with non-functional sulphate transporter genes show selenate resistance (*Shibagaki et al.*, 2002; *White et al.*, 2004), and the overexpression of a sulphate transporter led to an increase in selenate uptake (*Terry et al.*, 2000). In non-hyperaccumulator species, sulphate transporters were not reported to have a preference for sulphate over selenate. Therefore, their activity affects the uptake of Se and S alike, showing the observed positive correlation between tissue Se and S levels (*Pence et al.*, 2000; *Papoyan & Kochian*, 2004; *Weber et al.*, 2004). The concentration of Se in alfalfa was higher in Se + S-treated plants than in leaves as well as in stems of plants treated with Se alone. This finding shows that S did not have any negative effect on Se accumulation in alfalfa plants. Also, selenate can compete with sulphate in growth media, stimulate the sulphate starvation pathway, and activate sulphate transporters, thus causing a higher selenate accumulation (*Sors et al.*, 2005). This could be adjusted for foliarly fertilized plants, and, if so, it may be the reason for a higher Se concentration in Se + S-treated alfalfa plants mentioned in the present work than in plants treated with just Se.

On a global scale, Se shortage in livestock diets is very usual, and natural supplements provide a very good source of this element. According to the concentration of Se in alfalfa, an adequate amount of Se + S-treated plants can provide an Se supplement dose that has been associated with reduced risk of white muscle disease, reproductive and production losses, and immune system dysfunction (*Filley et al.*, 2007).

5   Conclusions

The present work has shown that the foliar spray of Se, S, and Se + S causes a higher concentration of Se in plants treated with Se + S than in plants treated with Se or S alone. Furthermore, Se + S treatment increases biomass and photosynthetic pigments, whereas it decreases malondialdehyde content and peroxidase activity in these plants as non-hyperaccumulators. The effect of S on Se accumulation in plants can be regarded as a strategy for the safe development of Se-rich crops.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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Effect of UV light on food quality and safety

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Abstract. The recent years have seen a great number of instances when ultraviolet (UV) radiation was used in the preservation process of all sorts of foods. Since the purine and pyrimidine bases of DNA and RNA absorb well the 254 nm radiation, its application with the use of a correct dosage can result in disinfections of various orders of magnitude. It can be particularly effective in cases where technology does not allow a more intensive heat treatment. When used properly, UV treatment can be a competitive procedure in the case of foodstuffs where the large surface area allows for UV rays to penetrate the entire volume of the substance. Incorrectly applied UV treatment may change the composition of foods. Free-radical as well as photochemical reactions can digest the proteins, damage the antioxidants, oxidize the lipids, make changes to the colour

and substance, and produce undesirable flavourings and odorous substances. Some vitamins are particularly sensitive to UV irradiation in the course of which losses could reach even 50%. Photosensitive water-soluble vitamins are vitamin C, B_{12}, B_6, B_2 and folic acid, while vitamins A, K and E are the fat soluble sensitive to light, carotene being the only provitamin with such properties. On the other hand, UV treatment can be a useful tool of food safety because of the photosensitivity of fungal toxins.

1   Introduction

The internationally accepted definition of pasteurization is as follows: "All methods and procedures, or the combination of these, applied on foodstuffs to reduce the number of pathogenic microorganisms relevant to human health to a level where, under normal conditions of production, transport, and storage, they cannot constitute a danger to humans." Treatment with UV rays is also in correspondence with the above definition provided it complies with the conditions outlined. There is broad consensus that both traditional and novel pasteurization procedures need to be validated, and it must be made certain that these methods will indeed lead to the destruction of the pathogenic microorganisms most relevant in terms of human health, which are followed by the authorities' (in the USA: *NACMCF – National Advisory Committee on Microbiological Criteria for Foods*, 2005) licensing procedures for the different foodstuffs.

UV radiation is a non-ionizing radiation from whose spectrum (140–400 nm) the wavelengths between 250 and 280 nm can be utilized as a germicide since the light of this wavelength can be absorbed by both nucleic acids and most proteins containing aromatic amino acids as well, the subsequent transformation having the potential to destroy microorganisms. UV lamps have been widely used before for purposes of air sterilization as well as for late-winter skin treatment of infants and young children because exposure to UV light leads to the synthetization of vitamin D, a necessary circumstance for optimal development. UV radiation can be used for the sterilization of air spaces and surfaces, its applicability being, however, limited by the fact that its energy decreases quadratically as distance from the light source grows and that it has a low penetrating capability. Caution is recommended during its application as it is harmful to the eyes and may cause conjunctivitis or even skin cancer when used in large doses (*Koutchma et al.*, 2009).

In food production, UV light is used to increase the shelf life of foods and

reduce the number of pathogenic microorganisms. UV treatment was applied with good results in increasing the shelf life of fruit juices, various drinks, vegetables, fruits, meat, poultry and seafood products, destroying the pathogenic microorganisms found therein and reducing maturation intensity. The question arises, on the other hand, as to what changes can occur in the composition of foods upon UV treatment. How does photodegradation affect organic molecules? What changes can photochemical reactions trigger that may ultimately have a negative impact on quality and nutritional value? Vitamins with a high structural diversity are of particular interest, most of them being potentially sensitive to UV light by virtue of their structure (*Koutchma et al.*, 2009).

2   Effects of UV-treated milk on microorganisms

Fruit juices and milk are perhaps the two food categories that permit the most efficient studying of UV treatment effects on microorganisms as both of these categories make laminar as well as turbulent flow possible, allowing for the entire volume of the liquid to be exposed to UV treatment.

Upon exposure of goat's milk to 15.8 mJ/cm^2 cumulative UV radiation, the amount of Listeria *monocytogenes* decreased by 5-log$_{10}$. Applying an UV treatment of 15 kJ/litre on cow's milk reduced the number of coliform bacteria by three orders of magnitude, but there was no significant decrease in the case of spores (*Matak et al.*, 2005). Under laboratory conditions, when using tubes permeable to UV light and applying static mixing, UV treatment was not efficient enough against *Mycobacterium avium subsp. paratuberculosis* as the rate of decrease was only half an order–one order of magnitude upon treatment with a dose of 1 J/ml. Using a special mixing device and the same level of irradiation improved the rate of decrease to 2.5–3.3-log$_{10}$ (*Altic et al.*, 2007).

This latter finding also draws attention to the fact that the dosage of UV light is not the sole determining factor in reducing the number of germs, but apparatus design is of crucial importance as well. Recently, UV reactors operating in continuous current mode have been developed primarily for the pasteurization of fruit juices, thus avoiding the formation of turbidity during treatment. According to one technological procedure, laminar flow is applied forming an extremely thin layer of film that reduces the path of UV light in the substance, thus allowing the light to permeate the entire volume of the substance (*Koutchma et al.*, 2004). Another solution is the application of a turbulence that allows the total amount of the substance to get in the im-

mediate proximity of the light source, likewise enabling the light to permeate all particles of the substance. These devices are currently being tested, in the course which flow rates, turbulence, or the level of UV irradiation are optimized.

In order for the conditions to be normalized during UV treatment, experimenters must succeed in exposing all areas of the liquid – whether it is a laminar or a turbulent flow – to a sufficient dose of UV light that is capable of destroying the microorganisms. A spiral tubular reactor could offer such a solution (*Koutchma et al.*, 2007), making possible that all of the treated liquid gets the optimal UV dose (*Forney & Pierson*, 2004; *Forney et al.*, 2004).

In the May 2011 issue of New Scientist, heat pasteurization was considered an alternative method (*Gupta*, 2011). According to the report, introducing pasteurization has significantly cut down the number of foodborne diseases despite not destroying all bacteria. At the same time, however, it reduces the nutritional value of milk, which is most significant in proteins and vitamins. Since this is especially the case with colostrum, it has been tested whether the UV treatment of colostrum would lead to the desired microbe-destroying effect without the drastic decrease of its immunological value. The question has been raised as to whether or not UV treatment can serve as an alternative for pasteurization in the case of colostrum.

In their experiments, they attempted to pasteurize colostrum with UV light on a farm keeping dairy cows, as it is widely known that immunoglobulins in colostrum condense due to heat and become immunologically worthless to the calf. A similar situation prevails during the pasteurization of mother's milk by heat treatment for the composition of mother's milk, considering its protein fractions, is similar to that of the bovine colostrum. In carrying out the procedure, the basic assumption was that although the applied dose of UV light would not destroy the bacteria completely, it would render them unable to reproduce due to the damage caused in their DNA, while the applied energy would not damage the immunoglobulins, which would preserve their ability to provide passive immunity to the calf.

To serve the purposes of the experiment, a device was constructed in which threaded tubes encircled the UV lamps, allowing the total amount of the turbulently flowing milk to receive the UV treatment. Upon treatment, part of the microorganisms was destroyed, but the proteins were not significantly damaged. Nonetheless, supervisory bodies contend that there are still plenty of experiments to be carried out in order to prove the applicability of this procedure for the preservation of mother's milk (*Gupta*, 2011).

Pereira et al. (2014) treated colostrum and milk with UV light in an at-

tempt to find out the degree to which bacteria would be destroyed and what changes would occur in the nutritional value of colostrum and milk, particularly in its immunoglobulin G content. Their experiments were driven by a USDA statement that 58% of the calves in the US are given unpasteurized colostrum and milk to drink, which carries the risk of infection. In the course of the experiment, both the milk samples and the colostrum were exposed to a continuous UV radiation of 45 J/cm^2. Prior to UV treatment, the colostrum as well as the sterile milk samples were inoculated with *Listeria innocua*, *Mycobacterium smegmatis*, *Salmonella serovar typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Acinetobacter baumannii* microorganisms. The IgG content of the treated and untreated samples was continuously determined with the ELISA method.

It has been established that UV treatment significantly reduced microbial count in milk (log CFU/ml) in the case of *Listeria monocytogenes* (a decrease of 3.2 ± 0.3 log CFU/ml), *Salmonella* spp. (3.7 ± 0.2 log CFU/ml), *Escherichia coli* (2.8 ± 0.2 log CFU/ml), *Staph. aureus* (3.4 ± 0.3 log CFU/ml), *Streptococcus* spp. (3.4 ± 0.4 log CFU/ml), and *A. baumannii* (2.8 ± 0.2 log CFU/ml). UV treatment did not result in a significant decrease in the case of *M. smegmatis* (1.8 ± 0.5 log CFU/ml), whereas with colostrum significant decrease was observed for *Listeria* spp. (1.4 ± 0.3 log CFU/ml), *Salmonella* spp. (1.0 ± 0.2 log CFU/ml), and *Acinetobacter* spp. (1.1 ± 0.3 log CFU/ml), but for *E. coli* (0.5 ± 0.3 log CFU/ml), *Strep. agalactiae* (0.8 ± 0.2 log CFU/ml), and *Staph. aureus* (0.4 ± 0.2 log CFU/ml) the decrease did not reach one order of magnitude. The UV treatment of colostrum resulted in an average of 50% decrease in IgG content.

Donaghy et al. (2009) studied the destruction of the various strains of *Mycobacterium avium ssp. paratuberculosis* (Map) in milk as an effect of UV treatment. Milk treated at ultrahigh temperature was inoculated with various strains of *Mycobacterium avium ssp. Paratuberculosis* and then treated with UV light of 0–1836 mJ/ml in a 20 litre reactor. Following treatment, the microorganisms were grown in an appropriate culture medium, and then their number was determined. It has been established that destruction took place at an order of magnitude of 0.1–0.6 log$_{10}$. They concluded that UV radiation treatment alone is not suitable for the destruction of pathogenic microorganisms, so it is advisable to be combined with other procedures. They take the view that milk is an inappropriate medium because UV rays can find their way and take effect with difficulty through the opaque liquid. In one of their studies, *Donahue et al.* (2012) ascertain that heat treatment significantly reduces total germ count in colostrum, while its IgG content barely undergoes

any change at all. First-milking colostrum was heated to 60 °C and kept in a pasteurizer tank for 60 minutes, and then the total germ count and immunoglobulin G content of colostrum were determined. After analysing 266 unique colostrum samples, they pointed out that due to heat treatment the total germ count of colostrum and the number of coliforms decreased by 2.25 $\log_{10}$ and 2.49 $\log_{10}$ respectively, but this heat treatment had only a slight impact on the IgG concentration of colostrum. They found that heat treatment of colostrum at 60 °C for a period of 60 minutes makes it possible to reduce the number of microorganisms by two orders of magnitude, while the IgG concentration of the colostrum suffers only minimal changes, and such a heat-treated colostrum can be applied safely to provide passive immunity to the calf.

Teixeira et al. (2013) studied the effects of heat and ultraviolet light on colostrum and hospital milk and analysed the impact of these treatments on calves' health and the growth parameters. The declared aim of the study was to examine the effects of heat and UV treatment on the total microbial count of milk, on the immunoglobulin G and lactoferrin concentration, and on calves' health, growth, and blood serum IgG level. Part of the colostrum samples was heat treated at 63 °C for 60 minutes, whereas another part of them was exposed to UV radiation of 45 J/cm^2, and test results were then compared to untreated milk samples. One part of hospital milk samples was heat treated at high temperature (72 °C) and for a short time (15 seconds), while the other part was exposed to UV radiation of 45 J/cm^2, and test results were then compared to untreated milk. They showed that heat treatment reduced the number of microorganisms more efficiently than UV treatment and that both IgG and lactoferrin concentration were significantly lower in treated milk when compared to raw milk. A comparison of hospital milk samples demonstrated that high-temperature heat treatment reduced the concentration of lactoferrin as compared to raw or UV-treated milk. An analysis of the IgG concentration of calves' blood serum showed that none of the treatment types had a significant effect thereon.

Singh & Ghalya (2006) studied the efficiency of cheese whey sterilization by applying 5–70 ml/min flow time in a traditional and a spiral UV reactor. Test results in the traditional reactor failed to get close to a 100% efficiency, but attempts made in the spiral reactor approximated this level when flow rate ranged between 35 and 40 ml/min. In the case of both reactors, they managed to balance the lowering of treatment temperature by increasing the flow rate. 100% efficiency could only be achieved with an extremely long (45 and 240 minutes) flow time.

Further developing their methodology (*Singh & Ghalya*, 2007), they designed an UV spiral reactor for the sterilization of cheese whey, and then compared its antimicrobial effect with that of a traditional UV reactor. Both reactors were tested at equal volumes and at different (5, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70 ml/min) flow rates. It was found that despite the turbid nature of whey both reactors could be used with great efficiency for sterilization. Technical problems occurring in the spiral reactor were much fewer in number than in its traditional variant.

During the sterilization process of cheese whey, *Mahmoud & Ghalya* (2005) studied – at different values of fluid thickness and after different retention times – the obstructions formed in an UV tubular reactor as well as the composition of the substance responsible for the clogging. Substances precipitated on UV lamps significantly reduced sterilization efficiency. A close correlation was found between the degree of obstruction and the applied temperature. 63.5–77.2% of protein, 12.6–16.5% of fat, and 6.5–9.5% of minerals were measured in the dry matter content of the substance causing the blockage, which values were about 1%, 0.5%, and 0.4%, respectively, in the case of whey. Upon reducing the layer thickness of whey, the amount of precipitated matter increased. High temperature and low pH were favourable to precipitation, whose mechanism was explained with adsorption and direct exchange. It was established that contact between the flowing substance and the quartz wall must be reduced as that may also be the agent responsible for precipitation during the UV sterilization process.

3   Effects of UV irradiation on fungal toxins

Toxins are secondary metabolites of microscopic fungi, representing serious food and feed safety hazard for both humans and animals (*Beardall & Miller*, 1994) and resulting in a disease called mycotoxicosis. Although these compounds are generally heat-stable molecules, for example, the aflatoxins are decomposed only above 268–269 °C (*Peng et al.*, 2018), a specific wavelength of light sources can result degradation. Even the sunlight is useful for decreasing their concentration – direct solar irradiation applied on poultry feed for 3–30 hours resulted in a 25–60% decrease of aflatoxin B_1 (*Herzallah et al.*, 2008), but the UV light has a stronger decomposing effect of mycotoxins due their photosensitivity. *Murata et al.* (2008) evaluated the effect of mild and strong (0.1 and 24 mW/cm^2) UV irradiation at a 254 nm wavelength on toxin-contaminated feed samples and found that even a low dose of

irradiation totally decomposed the initial 30 mg/kg zearalenone (ZEN) and deoxynivalenol (DON) concentration after 60 minutes, while a higher dose resulted in a more rapid elimination. *Murata et al.* (2011) also evaluated the 1.5 mW/cm^2 intensity UV-C treatment at the same wavelength on artificially DON-contaminated corn silage and found a 21–22% decrease after a 30- and 60-min. treatment. *Jajic et al.* (2016) confirmed the DON-decreasing effect of both UV-A and UV-C radiation on naturally contaminated maize but underlined that the change is not consistent, maybe due to the uneven toxin distribution; therefore, the results of the evaluation on artificially contaminated solid samples and contaminated homogeneous solutions can be taken into account only to a limited degree on natural solid samples. They found the UV-A treatment much more effective than UV-C. Aflatoxins have been referred to as UV-resistant toxins for a long time because the 254-nm wavelength irradiation was found to have no effect, but *Patras et al.* (2017) applied medium-pressure UV lamp light in the 200 to 360 nm wavelength region in different doses (from 0 to 4.88 J/cm^2) on aflatoxins dissolved in pure water and found that the highest dose resulted 67%, 30%, and 98% reduction for AFG_1, AFB_2 and AFB_1, respectively, and noted that aflatoxins had an absorption maximum at 320 nm. *Dong et al.* (2010) made similar examinations on patulin-contaminated apple cider with 14.2 to 99.4 mJ/cm^2 UV-C treatment, found rapid decrease in toxin content (9.4 to 43.4% decrease within 15 s), and observed that the dose–effect connection is strongly linear.

4 Effects of UV irradiation on the quality of milk and dairy products

It is common knowledge that milk and dairy products are highly sensitive to UV irradiation since quality deterioration occurs very quickly if kept for a longer period of time in a glass recipient or in a translucent polycarbonate packaging, whereas opaque multilayer packaging protects them against deterioration. Milk can very easily produce foul-smelling compounds that remind us of burning protein, but a cabbage-like taste can also be easily formed as well as the oxidation of fats and unsaturated phospholipids, which occurs during the photochemical reaction and leads to the development of oxidized flavour (*Spikes*, 1981).

With milk, it is well known that upon UV treatment its vitamin D content increases in the transformation of 7-dehydrocholesterol into vitamin D. In addition to this useful transformation, a great number of valuable compo-

nents can deteriorate on this wavelength and foul-smelling products can be generated. It has also been found, however, that the treatment increasing the vitamin D content of milk does not reduce the concentration of carotene, vitamin A, thiamine, and riboflavin.

Matak et al. (2005) attempted to reduce the population of *Listeria monocytogenes* in goat's milk by applying UV treatment. Certain types of goat's cheese are made of raw milk, which increases the products' food safety risk. Gourmets continuously look for these products in the supermarkets, which makes risk reduction a significant issue. As the U.S. Code of Federal Regulations and the Pasteurized Milk Ordinance strictly sets out the rules for pasteurization, UV treatment could serve as an alternative to heat treatment, while those substances responsible for the gourmets' preference for goat's cheese would not be damaged. In the course of the experiment, fresh goat's milk was inoculated with 10^7 CFU/ml of *Listeria monocytogenes* (L-2289) and then treated with UV light using a dosage ranging between 0 and 20 mJ/cm^2. They managed to achieve a decrease of more than 5 $\log_{10}$ when the cumulative UV dose reached 15.8 mJ/cm^2. Their experiment clearly indicated that UV treatment is appropriate for reducing the number of *Listeria monocytogenes* by several orders of magnitude.

Exposing goat's milk to UV treatment of 15.8 mJ/cm^2, thiobarbituric acid test was used to measure the oxidative and hydrolytic degradation processes. They found that UV treatment increased the amount of thiobarbituric acid– active compounds and the degree of acidity. It has been shown that not only lipase activity but UV treatment was also responsible for the increased amount of free fatty acids and that the amounts of pentane, hexane, and heptane also increased owing to UV treatment; what is more, treatment performed at 254 nm caused the milk to smell like cabbage (*Matak et al.*, 2007).

Lu et al. (2011) developed a new technology for reducing the bacterial count of milk. In the process, milk was transferred through a quartz spiral helical tube while being blasted with UV rays and radio frequency radiation of 2.65 MHz. It was found that decrease in microbial count is significantly affected by flow rate, the internal diameter of the quartz tube, the UV light sources of various quality, and the different types of bacteria. According to them, the apparatus functioning with electrodeless UV lamp was more efficient in destroying microorganisms compared to the traditional, low-pressure, high-intensity mercury-vapour lamp. When the UV dose reached 21.3 mJ/cm^2 at a 28.8 litre/hour flow rate and at 1.5 mm diameter, total bacteria count decreased by 6 $\log_{10}$. Upon repeating their experiment with milk and applying a dose of 21.3 mJ/cm^2, total bacteria count decreased by 3–4 $\log_{10}$ orders of

magnitude in the case of microorganisms such as *Salmonella* and *Shigella* spp., *Listeria monocytogenes*, *Staphylococcus* spp., *Enterobacteriaceae*, lactic acid bacteria, or pseudomonades. It has been found that electrodeless UV source is less energy consuming, requires less space, and its operation is much simpler too than with heat treatment. They came to the conclusion that this new technology is viable and can replace heat treatment methods.

5   Effects of UV irradiation on food composition

In summary, it can be said that properly applied UV treatment can be a competitive method for decreasing the number of harmful microbes in foods if we do not wish to use thermal processes. At the same time, UV treatment may adversely affect food composition since a number of harmful photochemical reactions may be activated following the formation of free radicals, which may reduce the number of valuable food components (*Lu et al.*, 2011). Undesirable reactions reduce vitamin content, digest proteins, destroy antioxidants, oxidize lipids, cause changes to substance and colour, and leave undesirable smells (*Koutchma et al.*, 2002; *Adhikari & Koutchma*, 2002).

UV treatment of citrus fruits can modify their taste and reduces β-carotene as well as vitamin A and C content in all fruit and vegetable juices (*Guerrero-Beltrán & Barbosa-Cánovas*, 2006). The aforementioned call our attention to the fact that UV radiation treatment adopted for microbial inactivation must have an intensity that will cause minimal changes to the nutritional value and palatability of foods (*Noci et al.*, 2008).

Photochemical reactions have the greatest impact on the components that are capable to absorb UV light such as vitamin A, riboflavin, vitamin C, and a few food colourings. Others have reported that vitamin A and β-carotene decreased only when food was irradiated with visible light. With liquid milk, the oxidation of ascorbic acid in the presence of riboflavin was activated by superoxide anion (*Koutchma & Shmats*, 2002).

UV treatment may have significant effects in the case of foodstuffs containing large amounts of unsaturated fatty acids when these, affected by free radicals, oxidize, thus producing a rotten smell and reducing antioxidant effect. We have no data as to whether any toxic substances are produced upon UV treatment that may present a risk to human health (*Koutchma et al.*, 2009).

6   Measuring the effects of UV treatment

Effects of UV treatment to food quality can be measured by drawing conclusions from the organoleptic properties (appearance, colour, fragrance, smell, texture, and flavour) and by determining the colour, pH, and chemical composition, including first of all vitamins, which are perhaps the most sensitive to UV treatment. Our examination must also comply with the sample type we intend to evaluate as different foods are not equally sensitive to UV light (*Heiss & Radtke*, 1968). Sour cream, whipped cream, dried vegetable soup powder, butter, margarine, and mayonnaise proved to be the most sensitive to sunlight and fluorescent light, and so these gave off an unpleasant smell after a short period of UV treatment. 1–3 days of irradiation was necessary for sugar, chocolate, cheese, bacon, raw sausage, green beans, and salty peanuts to undergo visible changes. Rice and potato chips making up the third group showed significant changes only after 4–7 days, while 10–30 days were necessary for pastries, almond, and split peas to show such changes. These changes are, of course, affected by the wavelength of UV light to which the given food shows sensitivity, the transparency of packaging, and treatment temperature (*Koutchma et al.*, 2002).

7   The photodegradation of organic molecules

In direct photochemical reactions, light energy gets directly absorbed, after which the chemical reaction as well as the changes in food composition take place. The chemical reaction is dependent on the photon's energy and light exposure time. The energy of a 254 nm UV photon corresponds to 472 Joules, which can be suitable both for loosening the bond between the O-H, C-C, C-H, C-N, H-N and S-S and for the activation of various chemical reactions. In the case of indirect photochemical reactions, there are one or two components in the system that are sensitive to light exposure, which then launch a series of reactions that can yield several kinds of products.

Direct photochemical reactions depend on the wavelength of the adopted light for that will determine the photon's energy and the wavelength of light which the molecule in question will be able to absorb. Once the photon has been absorbed, the molecule enters an excited state and undergoes a photochemical change during which it may dissociate into radicals, may isomerize, dimerize, or form ions. Free radicals and ions are particularly reactive intermediates that can enter into fast, additional reactions with other food components, in which end-products are created.

Photo-oxidation is one of the most sensitive reactions to light. In the course of this, photosensitive intermediate products get from ground state to a short-lived excited state and then transform into long-lived intermediates. In the following, these will transform into end-products either in free-radical reactions by way of hydrogen/electron transfer or through energy transfer. In this process, hydrogen peroxide or superoxide anion is produced, for instance, which are able to react with many kinds of food components (*Koutchma et al.*, 2009).

Nucleic acids are especially great absorbers of 254 nm UV light. Only purine and pyrimidine bases absorb in DNA and RNA, while the structural framework of nucleic acids, the phosphodiester bonds, do not absorb at this wavelength. Those components are sensitive to 254 nm that contain conjugated bonds such as compounds containing aromatic and double rings, while disulphide bridges are also sensitive absorbents. Proteins are sensitive to this wavelength only if they contain amino acids with aromatic rings (phenylalanine, tyrosine, tryptophan) or if they have a disulphide (S-S) bridge. Vitamins A, B_{12} (cyanocobalamin) and D, folic acid, vitamins B_2 (riboflavin or lactoflavin) and E (tocopherols), the aforementioned tryptophan, unsaturated fatty acids in oils and fats, and the unsaturated fatty acids of phospholipids are all extremely sensitive to UV light. Literature data suggest that the structure of vitamin D can change upon exposure to UV light, and intermediate superoxide radicals can also enter into reaction with vitamin K (*Spikes*, 1981). Visible light does not affect ascorbic acid, but it strongly absorbs UV light at 254 nm; at this wavelength, plant pigments too are highly light absorbent.

In assessing the effects of UV light, a problematic issue may be that the absorption of the various light-sensitive compounds was studied in clear solutions, but there are very limited data on transformations in complex matrices such as foods. Generally speaking, organic molecules containing unsaturated bonds are strong UV absorbents. The longer the system of conjugated bonds is, the higher the wavelength will be at which maximum absorption takes place. Heterocyclic aromatic compounds such as purine and pyrimidine bases and, e.g., aromatic side-chain amino acids show strong absorption at 254 nm, with maximum absorption values sometimes reaching above 300 nm (*Spikes*, 1981).

Carbohydrates are not particularly sensitive to light, but some carbohydrate derivatives, such as sugar alcohols or saccharic acids, can be sensitive to light, and upon its absorption the fragmentation of polysaccharides can take place, thus changing, for instance, the properties of fruits and vegetables. Research indicate that UV light accelerated the oxidation of fats and oils.

Of the essential amino acids, histidine, phenylalanine and tryptophan showed significant decomposition levels when exposed to UV light as a result of which their protein structures underwent certain changes that modified the solubility, thermal sensitivity, mechanic properties and enzymatic digestibility of the protein; what is more, in the process of treating milk, for instance, unwanted odorous substances appeared in significant amount.

Thanks to the special pigments, foods take on a characteristic colour which can substantially change upon UV treatment, although it is precisely UV light that promotes the formation of certain pigments.

8   Effects of UV light on vitamin content

UV treatment of vitamins gives us cause for concern since many of the vitamins are sensitive to light, especially to UV radiation. Photosensitive water-soluble vitamins include vitamins C, B_{12}, B_6, B_2 and folic acid, while vitamins A, K and E are among the photosensitive fat-soluble vitamins, carotene being the only provitamin with such properties. Most experiments were performed in the 290–700 nm wavelength range, and very few were carried out in the 240–260 nm range so crucial for disinfection (*Ye*, 2007). Upon examination of the vitamin C content of apple juice before and after UV treatment (254 nm, 25W), they found that UV treatment caused 50% of the original vitamin C content to decompose at the slowest flow rate (*Ye et al.*, 2007). Vitamin C shows maximum absorption at around 260 nm, which is why vitamin C content has significant influence on the absorption of UV rays at this wavelength. Therefore, higher-energy UV rays must be applied in the pasteurization of products enriched with vitamin C. Another experiment showed that vitamin C decomposition takes place according to zero-order reaction and that the death rate for *E. coli* was two and a half times bigger with samples receiving vitamin C supplementation. In the case of apple juice, a correlation was found between vitamin C decomposition, the applied energy and the adopted technology (e.g. flow rate).

In fresh fruit juices, vitamin A plays an important role as well, contributing with 25% to the daily vitamin A requirement. Irradiating apple juice with an UV dose of 200 mJ/cm^2 caused its vitamin A content to decrease to around 50% of the original value, which calls attention to the vitamin-A-damaging effect of UV treatment (*Adhikari & Koutchma*, 2002). Considering that vitamins A and C are the two most essential vitamins in fruit juices, this raises awareness of the fact that UV treatment may cause substantial vitamin loss.

Besides these two vitamins, a 50% decrease was observed with riboflavin and β-carotene content as well, while others reported on a much slighter decrease of 11–16% upon the UV treatment of vitamins C, B_6 and A. The irradiation of a similar dose caused vitamin C to undergo a more significant decomposition than β-carotene (*California Day-Fresh Food Inc.*, 1999).

Summarizing the data obtained, it can be established that UV treatment resulted a decrease of about 30–40% and 18–25% in the vitamin C content of apple juice and carrot juice respectively. In the above cases, the applied irradiation dose was 600 mJ/cm^2 and 1450 J/s respectively (*Koutchma & Shmalts*, 2002).

9   Shelf life and changes in quality due to UV treatment

Effects of UV treatment have been primarily studied in the nowadays very popular fruit juices. The bulk of the dry matter content of fruit juices is carbohydrate, wherefore UV light has no particular effect on these types of foods. UV treatment is first of all applied to extend shelf life – in this process, they analysed how UV light affects aroma, colour and nutrient content (*Tandon et al.*, 2003). Applying UV light of various energy and wavelengths for various durations and at varying temperatures yielded no significant changes in the organoleptic properties of the treated and untreated fruit juices and led to no significant differences between pasteurization with UV light and heat. Following the treatment of orange juice with UV light of 100 mJ/cm^2, the loss of vitamin C was around 17%, just as if pasteurization were performed with heat treatment (*Tran & Farid*, 2004). The amount of total phenolic components in the apple juice significantly decreased due to UV treatment, but this was a slighter decrease compared to a heat treatment of similar efficiency (*Tran & Farid*, 2004).

UV treatment makes changes to enzymatic activity as well. In mango nectar, polyphenol oxidase activity decreased to 19%, and the product kept its bright fresh colour over a long period of time (*Guerrero-Beltrán & Barbosa-Cánovas*, 2006). Another experiment focused around the UV treatment of apple juice and found that total polyphenol amount was significantly reduced, but this decrease was smaller than in the case of heat treatment. UV light did not affect total antioxidant capacity and did not reduce either polyphenol oxidase or peroxidase activity as compared to the corresponding heat treatment. They concluded that UV treatment in no respect caused more negative changes in

composition than the equally efficient mild heat treatment (*Noci et al.*, 2008).

10 Application of continuous and pulsed UV light during food production

Application of UV treatment aimed at extending shelf life and destroying pathogenic microorganisms does not have an unequivocal reception due to the component-damaging and -changing effects of ultraviolet light. In 2000, the U.S. Food and Drug Administration claimed that UV treatment was completely safe and destroyed human pathogenic microorganisms in fruit juices (*US, FDA*, 2000a). It has also been established that UV irradiation may cause the decomposition of some components and the creation of others, but these are not dangerous to human health. Fruit juices treated with UV rays were claimed to be at least as safe as the commercially available products not treated with UV rays. However, it has also been made clear that UV treatment may be considered as microbiologically safe only if the number of human pathogenic microorganisms decreases by five orders of magnitude when compared to the control sample. This degree of reduction must be ensured and verified at all times whenever it comes to applications for human use (*US, FDA*, 2000b).

In most cases, UV rays are created with low-pressure mercury-vapour lamps, and the liquid is transferred through tubes that permit the full passage of UV rays. As most fruit juices absorb UV rays to the maximum, the greater part of the energy emitted gets absorbed within a few millimetres from the radiation source and does not reach other parts of the food, wherefore the light energy inside the tube will not be enough to destroy the human pathogenic microorganisms. Therefore, it is recommended that there be a turbulent flow in the light-transmitting tube allowing the bulk of the liquid to be in contact with the tube wall, where it can get the radiation dose necessary for the destruction of microorganisms. Compliance with the above conditions helps in destroying pathogenic microorganisms (*US, FDA*, 2001).

The intensity of UV radiation necessary for the destruction of human pathogenic microorganisms varies according to the type of liquid and fruit juice, the initial microbial count, the design of the applied apparatus, flow rate, number of lamps and time of irradiation. In view of the aforementioned, authorities do not prescribe a maximum and minimum radiation dose but recommend that maximum safety be achieved in the various applications, i.e. pathogenic microorganisms be destroyed in sufficient amount at all times. It must also be

taken into consideration that the production of UV radiation is also capital intensive, wherefore using a dose that is higher than the optimum can be uneconomical and may contribute to the adverse transformation of certain components.

In 2005, authorities approved the use of pulsed UV treatment in food production, processing and treatment (*US, FDA*, 2005). Pulsed UV treatment is safe in the producing, processing and treating of foods if xenon lamps emitting radiation in the range of 200–1.000 nm are used with a pulsing frequency not greater than 2 milliseconds, if the treatment aims at the destruction of the microorganisms on the surface, if the effects of the pulsed UV-light treatment are appropriate, and if total radiation dosage does not exceed 12.0 J/cm^2 (*US, FDA*, 2005).

Krishnamurthy et al. (2004) used pulsed UV radiation for the inactivation of *Staphylococcus aureus*. The energy of the pulsed light was 5.6 J/cm^2, and pulsing duration increased up to 30 seconds. This technology yielded a decrease of 7–8 log$_{10}$ when UV radiation time reached at least five seconds. Sample thickness, exposure time, and treatment method significantly influenced the bactericidal effect. Pulsed UV radiation is considered a potential solution in the destruction of pathogenic microorganisms.

11   How safe is treatment with UV light?

A Canadian institute specialized in the safety of new food products examined how efficient an apparatus suitable for UV treatment is in reducing the microbial count of apple juice as well as of cider. They studied the changes in the composition and organoleptic properties of apple juice and cider upon UV treatment as compared to the control sample and if there was a possibility for the formation of toxic substances during UV treatment (*Health Canada*, 2004). They found that UV treatment had no harmful effect whatsoever on human organism, and it could be used efficiently for reducing the number of microorganisms in both cider and apple juice. Nevertheless, they have also established that UV treatment was not sufficient to completely destroy the microorganisms, particularly when the initial total microbial count was extremely high.

12 Regulating the use of UV light

In the European Union, there is no specific legislation regarding the usage of UV light in food production but only decisions with reference to the irradiation of foods, varying between Member States. Food products for which radiation can be used during their production or storage are now under discussion in the Member States. Radiation is allowed only if it is absolutely necessary in the technological process, if it is useful for the consumers, and if it does not aim at replacing either hygiene and health protection rules or the best practices used in production and agriculture (*Koutchma et al.*, 2009).

Acknowledgement

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Colostrum composition of cows after twin- and triplet-calving

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Abstract. Earlier we determined the colostrum and milk composition of cows after single- and twin-calving as well as the changes in the composition as a function of postpartum time. It was established that the dry matter, protein, whey protein, and immunoglobulin-G (IgG) content

of the first-milked colostrum immediately after calving was significantly higher with twin-calving cows than with single-calving animals. As regards the other components, there were no significant differences among the animals. During the last years, we managed to collect the first-milked colostrum from five cattle after triplet-calving. The composition of these samples were determined by the methods we used earlier at twin-calving animals, and the results were compared to the colostrum composition of single- and twin-calving animals. It was found that although as an effect of triplet-calving the protein and IgG content of colostrum increased, the difference was not significant between twin- and triplet-calving animals. We are aware that others have not reported data from the point of view of the colostrum composition of twin-calving, and in the case of triplet-calving our results are unique in the world. In our publication, we report on the results of our investigations.

1 Introduction

Between 1980 and 1990, in connection with the cattle breed restructuring and new cattle breed producing programmes, we determined the colostrum and milk composition of hundreds of cows resulted from crosses between Holstein-Friesian and Jersey cows (*Csapó et al.*, 1982a, 1982b, 1982c; *Csapó & Csapó*, 1984). Our investigations were performed at the Hajdúnánás State Farm and at Szigetvár State Farm. During the examination of the composition of first-milked colostrum, it was found that the colostrum of twin-calving animals had more dry matter, total protein, whey protein, and in particular more immunoglobulin G (IgG) than the single-calving ones (*Csapó*, 1995). Then, we systematically collected the first milk colostrum of twin-calving animals, and the colostrum composition was compared to the composition of single-calving animals. At the Hajdúnánás State Farm, we investigated the first-milked colostrum of 17 single-calving and 17 twin-calving Holstein-Friesian paternity cows, five of which calved bull calf, five heifer calf, seven calved one bull, and one heifer calf. At Szigetvár State Farm, we investigated the first milk colostrum of 15 twin-calving cows, four of which calved bull calf, three heifer calf, and eight calved one bull and one heifer calf. Whereas in all cases the gender of the calves was known, we could investigate the impact of the gender of the calves on the composition of the first-milked colostrum.

Comparing the colostrum composition of the single- and twin-calving cows, it was established that the colostrum of the twin-calving animals contained significantly more dry matter, total protein, true protein, whey protein, true whey protein, and IgG than that of cows with single calving. In contrast, there

were no significant differences in the casein and non-protein nitrogen (NPN) content between the two groups.

According to the results of our investigations, there are no differences in the colostrum composition between the cows that calved different calves. It seems therefore that the gender of the calves at twin-calving has no effect on the colostrum composition. The biological value of the colostrum from twin-calving animals calculated on the amino acid composition was higher with 9.4 in both state farms than that of single-calving animals. The higher biological value can be explained with the higher whey protein content of the colostrum because the biological value of the whey protein is significantly higher than that of the casein. We were unable to confirm the differences by mathematical statistical analysis. Significant differences were not found in the macro- and microelement content of the colostrum between the twin- and single-calving animals (*Csapó et al.*, 1991a; 1991b).

The protein fractions, amino acid composition, and biological value are indicative of the differences between the colostrum composition of single- and twin-calving animals, whereas the IgG is part of the whey protein, the whey protein is part of the total protein, primarily due to IgG or whey protein surplus (*Csapó*, 2013).

In the national and foreign literature dealing with animal breeding and milk production, we did not find any information with regard to twin pregnancy having any effect on the composition of the colostrum, and therefore we could not evaluate our data in the light of the scientific literature. In addition, based on this, we assumed that our investigations are unique in this area. After this, we received the first milk colostrum of three Holstein-Friesian paternity cows from the Hajdúnánás State Farm, of one Holstein-Friesian cow from Bos-Frucht Agricultural Cooperative, Kazsok, and of one from Gödöllő Experimental Farm Ltd., Kartal, after triplet-calving. In our publication, we report on the composition of the first milk colostrum of triplet-calving animals, and we compare the colostrum composition of twin- and triplet-calving animals.

2   Literature review

A lot of information has been published in the literature on the passive immunity of the calves (*Ehrlich*, 1892), the own immunoglobulin synthesis of the calf during intrauterine life (*Mielke*, 1979a), calves born with agammaglobulinaemia (*Mc Guireet al.*, 1976; *Losonczy et al.*, 1979), production and synthesis

of the colostrum (*Mielke*, 1979b), dependence of the colostrum composition on breed, duration of the lactation, and drying period (*Mielke*, 1979a). The IgG content of the colostrum was measured between 50 mg/ml (*Butler*, 1969; *Logan et al.*, 1981) and 144 mg/ml (*Senft et al.*, 1976), and the average values ranged between 100 and 110 mg/ml. According to our investigations (*Csapó*, 1995), the Jersey gene ratio did not change significantly the IgG content of the first-milked colostrum; the IgG content of the first-milked colostrum was the lowest in a Jersey paternity cow (61.4 mg/ml) and the highest in a Holstein-Friesian paternity cow (142.4 mg/ml).

The average dry matter content of the first-milked colostrum of Holstein-Friesian and Jersey paternity genotypes was 24.74% (*Csapó et al.*, 1982b, 1982c), which was 2–3% lower than the result published by *Sztarodubcev et al.* (1974). Considering the average of the two genotypes, the total protein content of the first-milked colostrum was 14.94%, whey protein content was 10.07%, casein content was 4.87%, and non-protein nitrogen (NPN × 6.38, calculated protein content) content was 0.43%. According to our investigations (*Csapó*, 1995), the average total protein content of the two genotypes was 1.0–1.5% lower than in the results of *Sztarodubcev et al.* (1974), was more by the same amount than in the results of *Grieb* (1968), and 1.5–2.5% higher than *Voigtländer & Glässer* (1974) determined for German black-spotted. The whey protein content of the first milk colostrum was 10.02–10.12%, while the average of the first three samplings was around 7.6%. This last value, well approaching the 8.26% value, was measured by *Kvapilik et al.* (1975) and the one approaching the 8.35% value was measured by *Grieb* (1968) for colostrum sampled after five and a half hours after calving.

Many investigations were published about the dry matter content and protein fractions of colostrum (*Grieb*, 1968; *Sztarodubcev et al.*, 1974; *Voigtländer & Glässer*, 1974; *Kvapiliket al.*, 1975). In examining the changes of the composition of the colostrum after calving as a function of time, it was concluded that significant changes in the dry matter, total protein, and whey protein content took place in the 24-hour period after calving, after which there was only a slight change in the composition of colostrum and transitional milk.

According to our investigations (*Csapó et al.*, 1991a), the dry matter content of the colostrum was the lowest in the Jersey cow (19.4%) and the highest in a Holstein-Friesian cow (31.2%). The total protein content of the colostrum was the lowest in a Jersey cow (10.23%) and the highest in a Holstein-Friesian cow (21.7%). The whey protein content was the lowest in a Jersey cow (7.32%) and the highest in a Holstein-Friesian cow (13.21%). The lowest (2.91%) and the highest (7.12%) casein content were measured in two Holstein-Friesian cows.

The NPN content was the lowest in a Jersey cow (0.29%) and the highest (0.53%) in a Holstein-Friesian cow.

We did not find literature data on the colostrum composition of the cows after twin-and triplet-calving.

3   Materials and methods

3.1   The examined breeds, feeding and keeping conditions, sampling of colostrum and milk

The genotype of the triplet-calving cows from Hajdúnánás was sired by Holstein-Friesian bulls (62.5% Holstein-Friesian + 25% Jersey + 12.5% Hungarian red-spotted). The population of single-calving, twin-calving, and triplet-calving cows alike was under summer-feeding conditions, based chiefly on grass. Of the three cows calving triplet-calves, two were in the second and one in the third lactation.

Of the 17 twin-calving control cows, eight were in the 2nd lactation and nine in the 3rd lactation. Of the 17 single-calving cows, there were 10 in the second and seven in the third lactation. From each of the cattle stables of the Bos-Frucht Agricultural Cooperative, Kazsok and Gödöllő Experimental Farm Ltd., Kartal, we obtained the first colostrum of one triplet-calving Holstein-Friesian cow. Both cows started their 2nd lactation and were mainly fed in a corn-based silage feeding system.

During the previous experiments, when the colostrum composition of the single- and twin-calving cows were compared, we sampled the colostrum, transient milk, and milk immediately after calving (within half an hour) and repeated it in the 12th and 24th hours as well as on the 2nd, 3rd, and 5th days after calving. However, as results showed that there are significant differences only in the first-milked colostrum between single- and twin-calving cows, when examining the influence of the number of offspring on colostrum composition, we focused only on the first-milked colostrum. In all cases, milk sampling took place from the mixed milk of the totally-milked udder. After homogenization, samples were stored in a deep freezer at -25 °C.

3.2   Analytical methods

The frozen colostrum and milk samples were warmed up in 38–40 °C water, homogenized, and then dry matter content was determined by drying the sample to constant weight according to the Hungarian Standard MSZ-6830-6.

The protein content and protein fractions of the samples were determined using Kjel-Foss 16200 nitrogen analyser. During the determination of the protein fractions, the whole milk (N% × 6.38 = total protein) was defatted by a T30-type laboratory centrifuge at 8,000 rev/minute for 10 minutes, and then the pH of the skim milk was set to pH = 4.55 with the help of an Op-264-type pH meter. The precipitated casein was removed by centrifugation at 8,000 rev/minute for 10 minutes from the whey (N% × 6.36 = whey protein). The whey protein was removed from the whey by 12% trichloroacetic acid precipitation, and the nitrogen content of the permeate was determined (non-protein nitrogen, NPN). By subtracting the NPN from the whole milk nitrogen, the milk true protein nitrogen was received, through the subtraction of the NPN from the whey nitrogen the true whey nitrogen was received, and through the subtraction of the whey nitrogen from the total nitrogen the casein nitrogen was received. The protein content of the different fractions was received after multiplying the nitrogen content of the fraction by 6.38 (conversion factor). Between 1980 and 2000, the nitrogen content of the milk samples and the different fractions were determined by Kjel-Foss 16200-type fast nitrogen analyser, while after 2000 Kjel-Tec nitrogen analyser was used for the samples of the first three triplet-calving cows at the Chemical Institute of Kaposvár University, Faculty of Animal Science, and the nitrogen content of milk samples from the last two cows was determined at the Food Chemistry Department of Sapientia Hungarian University of Transylvania, Department of Food Science.

The IgG content of the colostrum was determined by the single radial immunodiffusion method described by *Mancini et al.* (1965). The anti-cattle IgG rabbit serum and the cattle IgG standard were obtained from the Human Vaccine and Research Institute (Budapest, Gödöllő, Hungary).

3.3   Statistical analysis of the results

The mean value and standard deviation of the results were calculated, and the mean values were compared with the help of Student t-test.

4   Results and conclusions

The dry matter content, protein content, and protein fractions of the first-milked colostrum of the triplet-calving cows are shown in *Table 1* and the concentration of the IgG in *Table 2*. In *Table 3*, the dry matter content and protein fractions and in *Table 4* the IgG concentration of the first-milked colostrum of single-, twin-, and triplet-calving cows are compared.

From the data of the first and second tables, it can be concluded that in the case of triplet-calving the concentrations of the dry matter, protein fractions, and immunoglobulin-G in first-milked colostrum are higher than the data published earlier in the literature. On the other hand, they are very similar to the data of twin-calving cows published by us earlier. There are very few data on the composition of first-milked colostrum in the literature because the sampling was not taken immediately after calving, for which reason the calves sometimes sucked before sampling, and the sucking stimulus caused attenuation in the colostrum. We can compare the 31.80% dry matter content value only to our earlier investigations or to the dry matter content of the first milk colostrum of twin-calving cows, which was 29.58%. The 18% value for total protein content is also extremely high because total protein content in the literature is in most cases between 13 and 15%, and this finding is valid for true protein (17.26%), whey protein (13.79%), and true whey protein (13.24%) as well. However, this is not surprising because the protein fractions listed here are all part of the total protein; accordingly, the substantially higher concentration found there is reflected in the protein fractions.

Table 1: Dry matter content, protein content, and protein fractions of the first colostrum of triplet-calving cows

Components g/100g	1. cow[1]	2. cow[1]	3. cow[1]	4. cow[2]	5. cow[3]	Average ± SD
Dry matter	31.43	30.89	31.12	32.61	32.93	31.80 ± 0.92
Total protein	17.84	18.11	17.92	18.01	18.13	18.00 ± 0.12
True protein	17.37	17.60	17.37	16.45	17.51	17.26 ± 0.46
Whey protein	13.19	13.42	14.11	13.98	14.23	13.79 ± 0.46
True whey protein	12.72	12.91	13.56	13.42	13.61	13.24 ± 0.40
Casein	4.65	4.69	3.81	4.03	3.90	4.22 ± 0.42
NPN × 6.38	0.47	0.51	0.55	0.56	0.62	0.54 ± 0.06

[1]Hajdúnánás State Farm
[2]Bos-Frucht Agricultural Cooperative, Kazsok
[3]Gödöllő Experimental Farm Ltd., Kartal

The 136.42 mg/ml for IgG is also one of the outstanding data in the literature. Only *Senft et al.* (1976) published in the literature a higher concentration than this one for IgG (144 mg/ml), which can be explained perhaps by the special keeping and feeding conditions, special environment, which made the animals produce such a high concentration of IgG in the colostrum. As for other researchers, they reported 100–120 mg/ml of IgG content of the colostrum at best and 50–70 at worst.

Table 2: Immunoglobulin-G content of the first-milked colostrum of triplet-calving cows

Components mg/cm^3	1. cow[1]	2. cow[1]	3. cow[1]	4. cow[2]	5. cow[3]	Average $\pm$ SD
IgG	132.8	133.1	139.7	135.3	141.2	136.42$\pm$3.84

[1]Hajdúnánás State Farm
[2]Bos-Frucht Agricultural Cooperative, Kazsok
[3]Gödöllő Experimental Farm Ltd., Kartal

Table 3: Dry matter and protein content as well as protein fractions of first-milked colostrum from single-, twin-, and triplet-calving cows

Component g/100g	Single-calving (n = 17)	Twin-calving (n = 17)	Triplet-calving (n = 5)
Dry matter	24.73 $\pm$ 2.31	29.58 $\pm$ 1.93	31.80 $\pm$ 0.92
Total protein	14.71 $\pm$ 1.62	17.32 $\pm$ 1.39	18.00 $\pm$ 0.12
True protein	14.19 $\pm$ 1.44	16.89 $\pm$ 1.40	17.26 $\pm$ 0.46
Whey protein	10.22 $\pm$ 1.43	12.99 $\pm$ 1.32	13.79 $\pm$ 0.46
True whey protein	9.71 $\pm$ 1.28	12.56 $\pm$ 1.34	13.24 $\pm$ 0.40
Casein	4.50 $\pm$ 0.91	4.33 $\pm$ 0.69	4.22 $\pm$ 0.42
NPN$\times$6.38	0.52 $\pm$ 0.094	0.43 $\pm$ 0.137	0.54 $\pm$ 0.06

Table 4: Immunoglobulin-G content of the first-milked colostrum of single-, twin-, and triplet-calving cows

Component mg/cm^3	Single-calving (n = 17)	Twin-calving (n = 17)	Triplet-calving (n = 5)
IgG	104.51 $\pm$ 14.5	128.15 $\pm$ 18.81	136.42 $\pm$ 3.84

The purpose of this study was not to compare our results to those published in the literature but to compare the composition of the first-milked colostrum of twin-calving and triplet-calving cows living in the same keeping and feeding conditions, in the same environment and to focus on how to influence the composition of the first-milked colostrum in twin- and triplet-calving cows. The results of these investigations are shown in tables 3 and 4. Earlier we discovered (*Csapó et al.*, 1991a; *Csapó*, 1995) that the sex of the offspring at twin-calving had no influence on the composition of the first milk colostrum; for some reason, this aspect – among others, due to the low number of triplet-calving cows – was not investigated.

It has also turned out from our previous investigations that the first colostrum of the twin-calving animals has significantly more dry matter, total protein, true protein, whey protein, true whey protein, and IgG content than that of single-calving animals. In addition, we found no significant differences in the casein and NPN content of the two groups. We assumed that these differences also existed between single- and triplet-calving animals, wherefore we wished to examine in particular that triplet-calving can lead to further increase in the concentration of the dry matter content, protein fractions, and especially IgG content of first-milked colostrum.

From the results of the statistical analysis, it can be seen that the first colostrum of the triplet-calving animals has significantly ($P < 0.001$) more dry matter, total protein, true protein, whey protein, and true whey protein content than that of single-calving cows. In the case of casein and NPN content, there were no significant differences between the three groups. It was not found any significant differences between the twin-calving cows and triplet-calving cows. The first-milked colostrum of the triplet-calving cows had 2.22% more dry matter, 0.68% more total protein, 0.37% more true protein, 0.80% more whey protein, 0.68% more true whey protein, 0.11% less casein, and 0.11% more NPN $\times$ 6.38 than that of twin-calving animals.

The IgG content of the first-milked colostrum was measured at single-calving: 104.51, twin-calving: 128.15, and triplet-calving: 136.42 mg/ml, which reveal significant differences between the single- and triplet-calving animals. Although the first-milked colostrum of triplet-calving animals had 8.27 mg/ml more IgG, the difference was not significant between the two groups.

Based on the foregoing, the first-milked colostrum of the triplet-calving animals has more protein, and in it more whey protein, than approximately the same amount of casein as the single- and twin-calving animals, as proved to be the case, but significant differences between the twin- and triplet-calving animals could not be detected.

Finally, there is a single question to be answered: what causes the difference in the composition of the first-milked colostrum of single-, twin-, and triplet-calving animals? There is a clear response to it: IgG is part of the whey protein, the whey protein is part of total protein, and the received differences are primarily due to IgG or whey protein surplus. But what cause the differences between the groups during pregnancy?

Of the hormones that control the function of the mammary gland, there is only one whose concentration is influenced by twin pregnancy – that is the placental lactogen (PL), which is produced by the placenta and which enhances cell protein synthesis and cell division in the body. Besides the oestrogens,

it is crucial to mammogenesis. As in humans there is a close correlation between the size of the placenta and the production of PL, our premise is that two and three placentas will produce more placental lactogen than only one, which leads to increased protein production and increased concentration of immunoglobulin-G and of different protein fractions in the first-milked colostrum.

Acknowledgement

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Meat starter cultures: Isolation and characterization of lactic acid bacteria from traditional sausages

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Abstract. Fermented meat products represent an important segment of our alimentation. Obtaining these products is based on beneficial microorganism activity. In the case of traditional food products, these are commercial starters or autochthonous microflora. Fermentation of raw materials is mainly done by sugar metabolization of lactic acid bacteria (LAB). In addition, these microorganisms can have other beneficial properties too such as probiotic properties, antimicrobial compound production abilities, etc.

In order to meet consumer demands, starter cultures are continuously developed to produce high-quality, healthy, and tasty products, thus contributing to guaranteeing microbiological safety and to improving one or

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more sensory characteristics, technological, nutritional, or health properties of the fermented products. The aim of our research is to determine the technological properties of autochthonous lactic acid bacteria originated from commercial fresh sausages in order to select and use them as potential starter cultures in the meat industry. In our work, we determined the relevant characteristics (such as salt tolerance, proteolytic activity, antimicrobial activity, and antibiotic resistance) of bacteria isolated from 16 fresh sausages. Based on our results, the studied bacterial isolates originated from sausages could be potentially used as autochthonous meat starter cultures.

1   Introduction

Some of the important preservation methods of meat products are fermentation and drying. Traditional sausage production has a long history, originating from the Mediterranean region. Worldwide, Europeans are the main consumers and producers of this type of fermented meat products. Over centuries, the environmental conditions, certain intrinsic factors, and tradition had an impact on the production of sausage and fermented meat (*Zeuthen*, 1995).

Traditional sausages are defined (*Leroy et al.*, 2015) as meat products containing different meat mixtures with added spices and nitrite/nitrate salts, stuffed in casing, eventually smoked. The final product is obtained by a ripening process including fermentation and drying.

Depending on the sausage type, the fermentation is initiated by commercial starters or autochthonous microflora. Fermented meat products have several benefits (*Singh et al.*, 2012; *Bourdichon et al.*, 2012) such as enhanced nutritional and quality characteristics, are rich in essential amino and fatty acids, and are less perishable with an extended shelf life.

In fermentation, two groups of microorganisms play a key role, namely lactic acid bacteria (LAB) (counts of 10^3–10^9 CFU/g) and coagulase-negative Gram-positive staphylococci (counts up to 10^8 CFU/g). In addition to these, less significant are eukaryotic microorganisms, yeasts, and moulds. Microbes and their enzymes are responsible for the complex biochemical changes in the meat matrix. LAB have an impact on the technological characteristics of the product and also influence microbiological stability. In sausages, bacterial starter culture strains determine the functional properties and safety aspects of the product. Acidification, flavour development, proteolytic activity, amino acid metabolism, antioxidant enzymes (catalase and superoxide dismutase), and ni-

trate reduction all contribute to the formation of the final product. Regarding the safety of the starter culture, it should not possess biogenic amine production and acquired antimicrobial resistance. It also has to be free of enterotoxin determinants (*Cocconcelli & Fontana*, 2014). During the metabolic activities, lactic acid (resulted from the fermentation of carbohydrates by LAB) reduces pH-level and thus contributes to safety, texture transformation, and acid taste development. Depending on the microflora, raw materials, and additives, the following metabolites are released in varying amounts: acetoin, pyruvic and acetic acid, ethanol, and carbon dioxide. Safety aspects result also from the bioprotective character of some of the LAB (*Garriga & Aymerich*, 2015) as different antimicrobial natural peptides (bacteriocins) are produced by them. Furthermore, owing to probiotic properties, LAB may enhance the functional value of the fermented food.

Microbial ecology of fermented sausages could be considered as a complex and rich microbiological niche. Bacterial strains involved in fermentation and biochemical transformation could be characterized by robustness and flexibility. Acidification is a functional characteristic of the most frequently present species of the *Lactobacillus* genus. *Lactobacillus sakei* was the predominant species among the LAB isolated from different sausages prepared with altered technologies. Different genotypes of this bacteria were isolated, and they represent 55% of all LAB (*Garriga & Aymerich*, 2015). The main explanation of the fact that these bacteria harboured and adapted to the conditions is that they are able to use as energy source both carbohydrates and amino acids (*Cocconcelli & Fontana*, 2015). Other species of the *Lactobacillus* genus were also detected: *Lactobacillus plantarum*, *Lb. curvatus*, and *Lb. rhamnosus*. Unlike in Europe, *Pediococcus* was a relevant member of the microbial ecology of sausages in the United States as it is mostly added as starter culture. *Pediococcus acidilactici* and *P. pentosaceus* are responsible also for acidification and are able to produce bacteriocins (*Cocconcelli*, 2007; *Cocconcelli & Fontana*, 2014). The predominant Gram-positive, catalase-positive cocci are *Staphylococcus saprophyticus*, *S. xylosus*, *S. succinus*, and *S. equorum*. Different technological functions are related to this species due to the involvement of the fatty acid metabolism and amino acid catabolism in flavour development. Nitrate reduction leads to the red colour formation – for example, *Kocuria varians* is added for nitrate reduction (*Selgas & García*, 2014) –, and catalase activity prevents lipid oxidation (*Macedo et al.*, 2017).

Safer and functional foods are the current trends in food consumption. To satisfy consumer demands, there are certain innovative attempts, for example, in starter culture development. Besides product processing, environmen-

tal factors also influence the metabolic activity of starter cultures. A current strategy in meat industry is the application of wild strains for standardized sausage fermentation. To meet this demand, selection criteria focus on naturally occurring strains from meat ecology with desired technological aspects and stress resistance (*Corbo et al.*, 2017; *Pereira et al.*, 2019).

The aim of the present study was to determine the technological properties of autochthonous lactic acid bacteria originated from commercial fresh sausages in order to select and use as potential starter cultures in the meat industry.

2 Materials and methods

Isolation of lactic acid bacteria

In the course of our work, more than 60 lactic acid bacteria were isolated on de Man, Rogosa, and Sharpe (MRS) agar from 16 different commercially available, traditionally processed, fresh sausages. Surface-sterilized (2 min in 72% ethanol) 10 g sausages were smashed in a sterile mortar and homogenized with 90 ml physiological solution. From these samples, dilution series were prepared and an amount of 0.1 ml from the homogenized mixture was spread on the surface of MRS agar medium and incubated at 37 °C for 24 hrs in aerobic conditions. The isolated bacteria were analysed for the most representative technological characteristics of LAB; the experiments were done in two replicates.

Bacterial growth at different salt concentrations

LAB isolates 0.1% (v/v) were inoculated in MRS broth with different salt concentrations: 6.5%, 8%, and 10% (*Papamanoli et al.*, 2010). Bacterial growth was evaluated after incubation at 30 °C for 48 hrs as absorbance values at 630 nm. Salt tolerance was expressed as growth index (G_i) (*Speranza et al.*, 2014), calculated from the formula:

$$G_i = 100 \cdot \frac{A_s}{A_c},$$

where: A_s – sample absorbance in the present of salt and A_c – absorbance of the control.

Bacterial growth at different temperatures

LAB isolates 0.1% (v/v) were inoculated in MRS broth and incubated at three different temperatures: 20 °C, 30 °C, and 37 °C. Bacterial growth was evaluated after incubation as absorbance values at 630 nm.

Bacterial growth at different pH values

LAB isolates 0.1% (v/v) were inoculated in MRS broth adjusted to different pH values: 3, 4, 5 (*Papamanoli et al.*, 2010). Bacterial growth was evaluated after incubation at 30 °C for 48 hrs as absorbance values at 630 nm.

Gas production

Gas production of LAB isolates was detected in MRS broth containing inverted Durham tubes (*Patil et al.*, 2010).

Proteolytic activity

Proteolytic activity of LAB isolates was determined with well diffusion method on modified skimmed milk agar medium (0.5% casein, 0.25% yeast extract, 0.1% dextrose, skim milk powder 2.8%, and agar 1.5%). 50 μl of supernatant LAB isolates were inoculated in the 8-mm-diameter hole in the modified skimmed milk agar medium and incubated at 37 °C for 48 hrs. Proteolytic activity was determined in accordance with the measured clear zone diameter surrounding each culture.

Antibacterial activity

The antibacterial activity of LAB isolates was determined with agar diffusion method on different pathogenic and spoilage indicator strains (from the microbiological laboratory of the University) as *Micrococcus luteus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Proteus vulgaris*. These bacterial cultures were grown for 24 hrs at 28 °C and 37 °C on Nutrient agar. 0.1 ml bacterial suspension (with OD = 1) was spread in the case of each bacteria with surface streaking on Nutrient agar (meat extract 1 g, yeast extract 2 g, peptone 5 g, NaCl 5 g, agar 15 g, distilled water 1000 ml), and 50 μl of the cell-free supernatant of LAB isolates (centrifuged at 14,000 rpm, for 5 min) was dropped in the 8-mm-diameter hole cut with the help of a sterile test-tube. The inoculated Petri dishes were incubated at 28 °C

and 37 °C. The antibacterial effect of the tested LAB isolates was expressed in accordance with the diameter of the inhibition zone.

Antibiotic susceptibility

Determination of antimicrobial susceptibilities of LAB isolates was realized according to the guidelines reported in EFSA (2012). For the assessment of the susceptibility to ampicillin, gentamicin, streptomycin, erythromycin, chloramphenicol, and kanamycin, two-fold serial dilutions were realized ranging from 0 up to 128 μg/mL, with the exception of erythromycin (1–2 μg/mL) in MRS broth (*Laslo et al.*, 2015).

3   Results and discussions

Enumeration of viable LAB counts were done from 16 different, commercially available, traditionally processed sausage samples. The viable count of LAB in tested samples ranged between $3.3 \cdot 10^1$ and $5.8 \cdot 10^5$ CFU/g. In three samples, the viable count of LAB was very low, perhaps because these samples underwent heat treatment during the production process. Based on colony size and morphology, 35 colonies were picked from MRS agar for further characterization.

Salt content is one of the major environmental factors that has an impact on bacterial strains involved in fermentation. Salt is used in processed meat to develop the taste and extend shelf life. Almost all of the tested LAB isolates were able to grow in the presence of 6.5% salt. According to *Cruxen et al.* (2019), salt tolerance is a selection criterion in the case of native isolates. Our results regarding the tolerance of 6.5% NaCl are in concordance with those reported by *Almeida et al.* (2015). A higher salt concentration decreased the growth of the isolates, with an exception, as found also by *Aina* (2019). *Lb. plantarum* originated from artisanal sausage could survive the presence of 7% NaCl, whereas *Staphylococcus* spp. tolerated salt concentration up to 15% (*Cruxen et al.*, 2019).

The growth index (*Fig. 1*) varied in the range of 4–99%, and 74% of the isolates showed excellent growth. With the exception of 11% of LAB isolates, all of them had a higher growth index. In the presence of 8% salt, 26% of the isolated LAB were able to grow well, 37% of the isolated bacteria were inhibited, and 37% of bacterial isolates showed medium growth. The growth index ranged between 2.17% and 92.83%. In the presence of 10% salt, most

of the isolates were inhibited. Only 20% of the isolates showed mild growth, with a growth index between 2 and 22.64%.
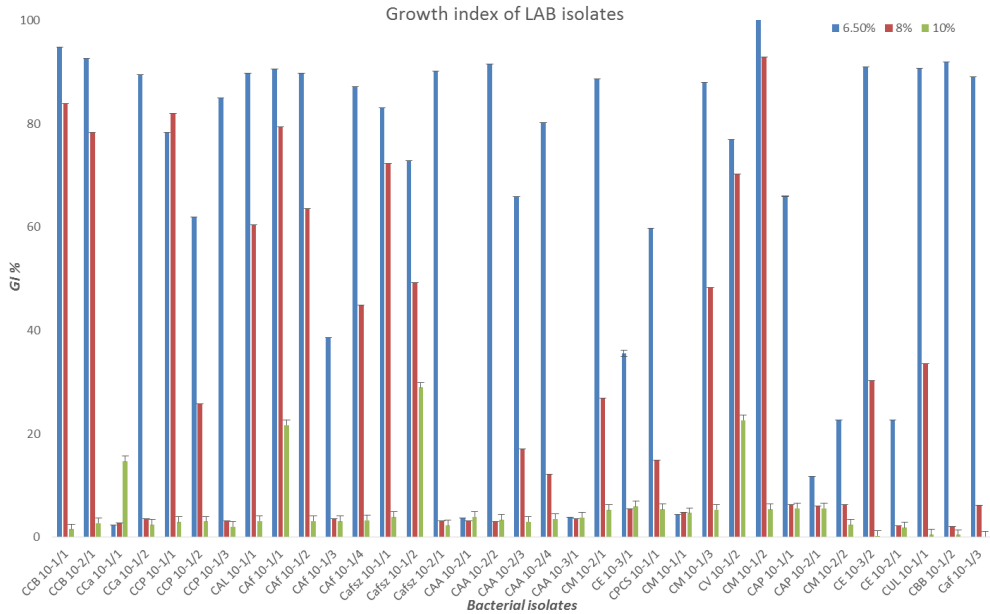

Figure 1: Growth index of LAB isolates in the presence of different salt concentrations

Further, the most beneficial strains were selected. The results of proteolytic activity for the most representative strains are presented in *Table 1*. All selected LAB showed proteolytic activity on the assayed medium agar. The clear zone diameter ranged in the interval of 13–17.33 mm. The benefits of proteolytic activity consist in texture and flavour development, contribution to water release through enhancing the drying process, and that via hydrolysation different peptides and volatile compounds are released, which serves as aroma precursor. It was demonstrated that different LAB, such as *Pediococcus pentosaceus* or *Lactobacillus curvatus*, originated from sausages hydrolysed sarcoplasmic proteins, generating different volatile compounds (*Cruxen et al.*, 2019). Different bioactive peptides derived from the peptidase and aminopeptidase activity of starter cultures are involved in functional food development (*Arihara*, 2014).

Table 1: Proteolytic activity of the isolates

Bacterial isolates	Clear zone mm
CCB 10-1/1	13.33 ± 1.5
CCB 10-2/1	14.33 ± 0.6
CCP 10-1/1	14.33 ± 1.5
CAL 10-1/1	13.2 ± 1
CAf 10-1/1	15.67 ± 3.2
CAf 10-1/2	16.33 ± 2.1
CAf 10-1/4	15 ± 1
Cafsz 10-1/1	15 ± 1
Cafsz 10-1/2	16 ± 2
CM 10-2/1	16.07 ± 2.1
CM 10-1/3	17.33 ± 3.06
CV 10-1/2	14.33 ± 1.53
CM 10-1/2	16.67 ± 3.1
CE 10-3/2	13 ± 1
CUL 10-1/1	14 ± 2

Table 2: Antimicrobial effect of bacterial isolates on Gram-positive bacteria

	LAB isolates	Inhibition diameter (mm)			
		M. luteus	B. cereus	B. subtillis	S. aureus
1	CCB 10-1/1	-	11.33±0.58	12.53±0.5	11.67±0.58
2	CCB 10-2/1	13.53±0.5	13.20±0.2	14.00±0.2	13.73±0.64
3	CCP 10-1/1	-	14.33±1.53	-	16.33±1.53
4	CAL 10-1/1	-	13.00±1	-	14.33±1.53
5	CAf 10-1/1	-	13.07±0.9	11.67±0.58	12.40±0.53
6	CAf 10-1/2	12.33±0.58	14.33±1.53	14.53±0.5	15.60±0.53
7	CAf 10-1/4	-	14.00±2	8.80±0.2	11.67±0.58
8	Cafsz 10-1/1	-	13.07±1.01	8.60±0.2	11.67±0.58
9	Cafsz 10-1/2	-	-	10.60±0.53	10.60±0.53
10	CM 10-2/1	-	-	13.87±0.31	13.53±0.5
11	CM 10-1/3	13.00±1	14.40±0.87	12.67±3.06	14.00±2
12	CV 10-1/2	-	11.00±1.0	-	10.07±0.31
13	CM 10-1/2	-	-	-	13.93±2.10
14	CE 10-3/2	-	11.60±0.53	11.67±0.58	15.00±1.0
15	CUL 10-1/1	20.13±1.28	13.00±1.0	13.33±1.15	16.00±2.0

The tested LAB isolates displayed different levels of bacteriostatic effects against the studied bacterial strains. Concerning Gram-positive bacteria, the most suppressive effect was detected in the case of *Staphylococcus aureus*, with a large inhibition zone of 16.63 mm in the case of the CCP 10-1/1 iso-

late. The characterized LAB isolates from sausages exerted greater inhibitory effect against *S. aureus* in contrast with bacterial isolates from home-made fermented foods (*Ren et al.*, 2018). In the case of the two *Bacillus* strains, the inhibition zone ranged between 11.40 and 14.40 (*B. cereus*) and between 8.6 and 14.53 (*B. subtilis*). The cell-free supernatant of CAf 10-1/2 inhibited with a larger inhibition zone both *Bacillus* bacterial strains. The most effective bacteriostatic effect was observed in the case of CAf 10-1/2. That isolate suppressed all the four tested Gram-positive bacteria with a large inhibition zone. LAB isolates exerted weak or no antibacterial activity against *Micrococcus luteus*. In concordance with our results, it has been shown that *Micrococcus luteus* was the most resistant bacteria (*Evurani et al.*, 2018) and was not inhibited by *Lactobacillus casei* and *Lb. brevis*.

The antibacterial effect against the tested Gram-negative bacteria was not promising. The LAB isolates exerted weak suppressive effect against *P. vulgaris*. Only five isolates displayed antibacterial effect against these tested bacteria. According to *Aruna et al.* (2016), *P. vulgaris* – tested by them – is resistant to bacteriocins. The largest inhibitory zones were observed in the case of CM 10-2/1. The inhibition zone was 13.00 mm in the case of *E. coli* and 14.33 in the case of *P. vulgaris*. The largest inhibition zone appears for *E.coli*: it was 14.47 mm.

Table 3: Antimicrobial effect of bacterial isolates on Gram-negative bacteria

	LAB isolates	Inhibition diameter (mm)	
		E. coli	P. vulgaris
1	CCB 10-1/1	12.33 ± 0.58	-
2	CCB 10-2/1	13.67 ± 0.42	-
3	CCP 10-1/1	13.33 ± 0.58	-
4	CAL 10-1/1	13.67 ± 1.15	-
5	CAf 10-1/1	10.00 ± 1.00	-
6	CAf 10-1/2	11.73 ± 0.64	11.67 ± 0.58
7	CAf 10-1/4	-	-
8	Cafsz 10-1/1	-	-
9	Cafsz 10-1/2	-	11.00 ± 1.0
10	CM 10-2/1	13.00 ± 1.00	14.33 ± 1.53
11	CM 10-1/3	-	-
12	CV 10-1/2	-	-
13	CM 10-1/2	10.60 ± 0.4	-
14	CE 10-3/2	14.47 ± 0.5	10.53 ± 0.64
15	CUL 10-1/1	11.00 ± 1.0	13.67 ± 0.58

Fermented meat microbiota is made up of bacterial strains of *Lactobacillus* and *Lactococcus* genus with bacteriocinogenic features and biopreservative role (*Castilho et al.*, 2019). Different strains from fermented meat are called bacteriocin producers: various *L. sakei* and *L. curvatus* strains, *Pediococcus acidilactici* strain MCH14, and *S. xylosus* strain SX S03/1 M/1/2 (*Laranjo et al.*, 2017).

LAB exhibits a broad-spectrum antimicrobial activity due to various mechanisms. The antagonistic mechanisms of LAB are diverse due to the altered gene expression and molecular structure of bacterial strains. The inhibitory activity of LAB strains is associated with their primary metabolites such as organic acids, alcohol, and carbon dioxide. LAB also produce different compounds with antagonistic effect. It was shown that formic and benzoic acids, hydrogen peroxide, diacetyl, acetoin, and bacteriocins have a role in the suppression of different microorganisms. For the suppression of Gram-negative bacteria, the organic acids and hydrogen peroxide are presumed to be responsible, while in the case of Gram-positive bacteria proteinaceous compounds are the potential causes. Reduced pH contributes to the inactivation of bacterial cell as well. The weak acids entering the cell may cause the acidification of the cytoplasm, leading to different disorders in the metabolism, structure, and function of the bacterial cell (*Olorunjuwon et al.*, 2013; *Gao et al.*, 2019). The mode of action of the nisin is dual, consisting of pore formation in the membrane or disrupting cell wall synthesis, leading to bacterial cell death (*Jozala et al.*, 2015).

From the isolated bacteria, 6 isolates showed CO_2 production. In the manufacture of different meat products, homofermentative strains are used (*Cocconcelli*, 2007).

Almost all tested LAB isolates showed varying levels of growth at pH 4 and pH 5 (data not shown). None of the tested bacterial strains showed activity at pH 3. According to *Erkkilä* and *Petäjä* (1999), most of the LAB isolated from meats has shown no decrease at pH 4. Our results also showed that the isolated strain was not able to grow at 3 pH. In the case of pH 4 and 5, isolates showed the highest growth. The highest growth was detected at pH 5, with the exception of two bacterial isolates: Cafsz 10-1/1 and CCB 10-1/1.

Based on the results of the growth assay carried out at different temperatures, it can be said that almost all isolates grew at 20 °C (data not shown), with an OD value of 1.92. As for 30 and 37 °C, the same results were obtained. At these two temperatures, the highest OD value was 2.02. From the assayed bacterial isolates, two LAB isolates (CAf 10-1/3, CCa 10-1/1) did not show growth at 20 °C. In the case of one isolate, the growth was observed at 30 °C.

It was shown that pH and temperature influence the growth of lactic acid bacteria in different ways (*Adamberg et al.*, 2003).

Table 4: Minimal inhibitory concentration in the selected LAB isolates in the case of the tested antibiotics

Bacterial isolates	Ampicillin	Gentamicin	Streptomycin	Kanamycin	Chloramphenicol	Erythromycin
CCB 10-2/1	4	8	64	>128	8	2
CCP 10-1/1	4	8	32	>128	8	2
CAL 10-1/1	4	4	32	>128	8	2
CAf 10-1/2	4	64	>128	>128	8	>2
CAf 10-1/4	4	8	64	>128	8	2
Cafsz 10-1/2	4	64	>128	>128	16	>2
CM 10-2/1	4	16	64	>128	8	>2
CV 10-1/2	8	8	64	>128	8	2
CE 10-3/2	2	16	128	>128	8	2
CUL 10-1/1	4	16	64	>128	8	2

LAB resistance towards antibiotics represents an emerging and serious food safety concern. The World Health Organization suggests that LAB used in food industry should be free of antibiotic resistance (*Álvarez-Cisneros* & *Ponce-Alquicira*, 2018).

In the case of the most beneficial ten LAB isolates, the MICs of six antibiotics were determined. Based on the results, the differences in MIC values did not exceed one or two orders of dilution.

In the case of ampicillin, the MIC was 4 mg/L with the exception of two isolates, where it was 2 and 8 mg/L. In the case of gentamicin, the MIC values ranged between 4 and 64 mg/L, whereas the majority was equal with 8 mg/L. The MIC values for streptomycin ranged between 32 and 128 mg/L with two exceptions, where the MIC was higher than the tested concentration. Also, with kanamycin, the tested isolates showed growth at the maximum tested concentration (128 mg/L). With the exception of one isolate, MIC was 8 mg/L for chloramphenicol. In the case of erythromycin, the MIC was 2 mg/L for seven LAB isolates, while the other isolates were growing at this concentration. Based on the results, the majority of the tested bacterial isolates showed susceptibility to ampicillin with the exception of one isolate. In general, it

is considered that bacterial strains belonging to the *Lactobacillus* genus show sensitivity to the cell wall synthesis inhibiting penicillin and β-lactamase antibiotics (*Gueimonde et al.*, 2013).

In the case of gentamycin, two isolates were resistant. Three of the tested isolates were found resistant to streptomycin. All tested isolates showed resistance to kanamycin and chloramphenicol. In LAB, the resistance type towards kanamycin, gentamycin, and streptomycin is intrinsic. The absence of cytochrome-mediated electron transport contributes to the formation of resistance to the aminoglycosidic antibiotics such as kanamycin and streptomycin (*Narayanan & Raghavan*, 2019). Enzymatic deficiency or disabled enzymatic transport of aminoglycoside-modifying enzymes, such as N-acetyltransferases, O-phosphotransferases, and O-nucleotidyltransferases, results the above mentioned resistance (*Álvarez-Cisneros & Ponce-Alquicira*, 2018).

All ten selected isolates showed resistance to erythromycin.

In concordance with our results, previous studies reported different LAB strains isolated from fermented sausages with multiple antibiotic resistance. *L. plantarum*, *L. fermentum*, and *L. helveticus* showed resistance to kanamycin, tetracycline, erythromycin, chloramphenicol, and to other antibacterial compounds (*Patel et al.*, 2012). LAB strains originated from Portuguese and Italian sausages showed resistance towards streptomycin, gentamycin, chloramphenicol, erythromycin, etc. (*Álvarez-Cisneros & Ponce-Alquicira*, 2018). N*arayanan & Raghavan* (2019) suggested that the LAB of starter cultures show higher resistance against antibiotics and could be the store of resistance genes. In some cases, these genes are not expressed but can be transferred to other bacterial strains. The safety characterization of LAB is essential to avoid any risk of infection by using them (*Borriello et al.*, 2003, *Doron* and *Snydman*, 2015).

4   Conclusions

Based on the results, it can be concluded that the lactic acid bacterial isolates originating from the autochthonous microbial ecology of fermented sausages showed positive technological properties. Regarding the assayed technological characteristics of isolates, these strains can be involved in the fermentation of sausages, playing an important role in the development of sensorial characteristics, texture, aroma formation, and the inhibition of different pathogenic microorganisms. For a successful and safe application of these isolates, further studies are needed to assess the safety of the strains to avoid bacteremia. Also,

it is necessary to identify the isolates, determine their virulence factors, and the biogenic amine production capacity.

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Determination of drying parameters of carrot pomace

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Abstract. Carrot is one of the most important root vegetables rich in bioactive compounds such as carotenoids and dietary fibres, with appreciable levels of several other functional components and having significant health-promoting properties. Therefore, it is cultivated on a large scale throughout the world. The by-product (pomace) resulted during carrot juice production is used mainly as animal feed although it contains many valuable components and could therefore be used profitably in the food industry, too. Carrot pomace needs to be preserved by drying as otherwise it deteriorates rapidly. In our research, we studied the infrared drying kinetics of carrot pomace at various temperatures, the obtained data being very important in the drying practice.

1 Introduction

Carrots (*Daucus carota* L.) are grown and consumed in large quantities worldwide, this being also the case of our region as it is the second most consumed vegetable after potato (*Bogdan*, 2017). Carrot roots are traditionally used in salads and soups and could be commercially converted into nutritionally rich

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processed products such as juice, concentrate, dried powder, canned products, etc. (*Sharma et al.*, 2012; *Barzee et al.*, 2019). Carrot juice and its blends are the most popular non-alcoholic beverages, and a steady increase in carrot juice consumption has been reported from various countries (*Sharma et al.*, 2012).

Unfortunately, the yield associated with carrot juice production is quite low, wherefore up to 50% of the raw material remains as pomace, which is generally disposed of as feed or manure (*Surbhi et al.*, 2018).

Carrot has high levels of α- and β-carotene (*Kumar et al.*, 2012), which are precursors of vitamin A for the human organism, the latter being essential for vision. Carrot is also rich in fibres, having many beneficial effects on children's and adults' health (*Alam et al.*, 2013; *Surbhi et al.*, 2018). The benefits of carrots cannot be overestimated, especially when it comes to food and baby food.

Carrot pomace containing high amount of β-carotene could be profitably used for the supplementation of products such as cake, bread, biscuits, and extruded products and the preparation of several types of functional products (*Kumar et al.*, 2010; *Gayas et al.*, 2012; *Sharma et al.*, 2012; *Ahmad et al.*, 2016).

In Romania, the area cultivated with carrot is over 8,000 ha (according to Eurostat) with an average of 198,939 tons of carrot being grown between 2014 and 2016 (*MADR*, 2017). In recent years, consumers' demand for natural fruit and vegetable juices has increased, and as a result more and more businesses have started to process fruit and vegetable juices. At the same time, carrot residue is being produced in increasing quantities, and the further processing of this may bring economic benefits.

Since carrot pomace is perishable as it contains about 85% (wet basis) moisture (*Upadhyay et al.*, 2008; *Kumar et al.*, 2012), its shelf life could be increased by drying or dehydration for further use (*Alam et al.*, 2013) – for example, to make extrudate, flavoured breads or cakes. Fresh carrot pomace – when properly dried, packed, and stored – stays available for later use in fibre-rich products (*Alam et al.*, 2013; *Sahni & Shere*, 2018).

Drying of materials having high moisture content is a complicated process, involving simultaneous heat and mass transfer (*Kumar et al.*, 2012; *Ahmed*, 2018).

The most conventional dying method is the convective hot air drying (*Sarkar & Sharma*, 2011). *Upadhyay et al.* (2008) already reported results referring to the hot air oven drying of carrot pomace, the moisture content, ascorbic acid, β-carotene, crude fibre and ash content of the analysed samples (*Upadhyay et al.*, 2008).

In order to improve the drying efficiency and product quality, some other techniques, such as microwave, infrared, etc., have been combined with the hot air drying of vegetables (*Ly & Zhang*, 2017). The infrared (IR) radiation heating technology is a new environment-friendly heating technology with its intrinsic advantages such as high heat transfer and drying rate, uniform temperature distribution, nutrient loss reduction, and significant energy saving (*Krishnamurthy et al.*, 2008; *Riadh et al.*, 2015). Further development and application of IR-related processing technology and equipment will contribute to achieving sustainable agricultural and food processing and obtaining high-quality, high-value, and healthy vegetable food products (*Wu et al.*, 2017; *Ly & Zhang*, 2017).

In general, the moisture content of the materials does not refer only to water, but it includes all substances (i.e. fats, oils, alcohol, solvents, etc.) that evaporate when the sample is heated. In carrot pomace used for the experiments, moisture content could be assumed as being mainly water since carrot contains a minimal amount of fats (0.2–0.3 g/100 g) (*Chen & Mujumdar*, 2008; *Butt & Sultan*, 2011; *Surbhi et al.*, 2018), and samples have undergone only physical operations. The conventional drying chamber method for moisture content analysis (*Molnár*, 2015) requires a longer measuring period, while the sample is heated from the outside to the inside by a hot air current so as to remove the moisture convectively. In contrast, the moisture content determination by thermogravimetric method (as used by the Kern moisture meter) is associated with sample warming from the inside to the outside since the radiation applied penetrates mainly the sample in order to be transformed inside of it into heat energy (*Anonymus*, 2010). The thermogravimetric method follows the same principle as a conventional method, i.e. measuring the weight of the sample before and after heating and determining the moisture content from the weight difference. Due to the automatic operation, without manipulation and cooling of the probes, this method requires a shorter measuring time.

The purpose of this study is to characterize the infrared drying of carrot pomace at different temperatures and to compare drying kinetics for future practical use. Temperature values have been selected taking into account the recommendations of several studies (*Sharma et al.*, 2012; *Alam et al.*, 2013) to preserve the quality of all valuable components of carrot pomace.

2   Materials and methods

For the research, industrial carrot juice production by-product (carrot pomace) was used. After harvesting, the carrot was transported to the company's warehouse. After being washed with tap water, it was grinded (approximately 3–5 mm in size, pieces of different shapes) and pressed by a belt filter press (Voran EBP650). The resulting carrot pomace was frozen until the laboratory experiments were performed.

For laboratory drying measurements, a KERN MLB MB 10 moisture balance with 400 W mercury vapour lamp and digital scale with three decimal places was used. Pomace drying was studied at five different temperatures, i.e. 50, 55, 60, 65, and 70 °C. For each measurement, 5 g of carrot pomace was used (more precisely, between 5.004 and 5.012 g) and spread evenly on the 70.85 cm^2 surface sample plate ($d_{plate} = 95$ mm). Weight data was recorded every 2 minutes until a constant weight was reached. The collected data was processed with Microsoft Excel and Statistica 8.0 (StatSoft, Inc.) programs.

Mathematical modelling

The following mathematical models were used to characterize the drying rate and to calculate the drying constants (*Barbosa-Cánovas & Vega-Mercado*, 1996; *Szép et al.*, 2017):
- the moisture content (wet basis) of carrot pomace at time τ:

$$w_i = \frac{m_i - m_{edp}}{m_i}, \; \frac{kg \; moisture}{kg \; wet \; basis}, \tag{1}$$

where: m_i – the sample weight at time τ, g; m_{edp} – the final weight of the sample (when no further moisture removal was recorded at a particular drying temperature), g;
- the moisture content (dry basis) of carrot pomace at time τ:

$$W_i = \frac{m_i - m_{edp}}{m_i}, \; \frac{kg \; moisture}{kg \; dry \; basis}, \tag{2}$$

- drying rate:

$$r_{drying} = -\frac{\Delta w}{\Delta \tau} = \frac{w_{i+1} - w_i}{\tau_{i+1} - \tau_i} \; \min^{-1} \; or \; s^{-1}; \tag{3}$$

- drying time:

$$\tau = \frac{m}{S \cdot k} \left(\frac{w_1 - w_{cr}}{w_{cr} - w_{eq}} + \ln \frac{w_{cr} - w_{eq}}{w_f - w_{eq}} \right), \; s, \tag{4}$$

where: m – the mass of the wet material subjected to drying, g; S – transfer surface, m^2; w_1, w_f, w_{cr}, w_{eq} – the initial, final, critical, and equilibrium (corresponding to ambient conditions) moisture content, kg moisture/kg wet basis; k – rate constant, kg/(m^2·s).

3 Results and discussions

Infrared drying of the carrot pomace was carried out at five different temperatures (i.e. 50–70 °C with 5 °C steps) in three successive runs. The data collected for each series is shown in *Fig. 1a*. There was no significant difference in the data values, so the standard deviation is not plotted.
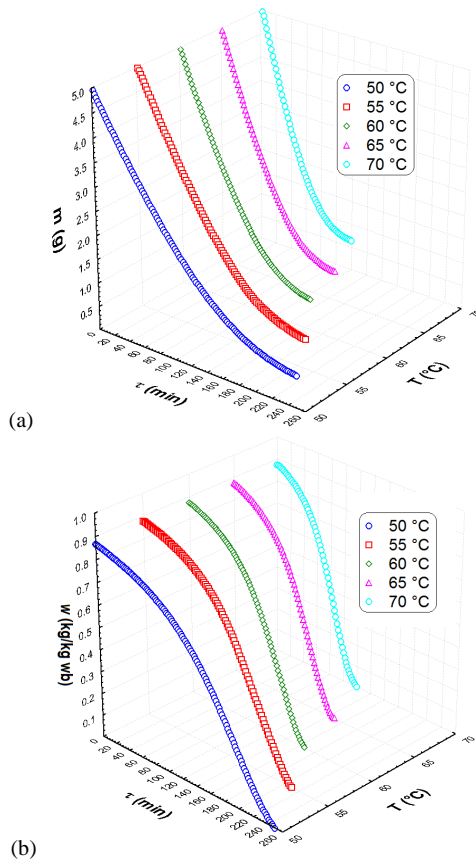
Figure 1: Measured weights (a) and drying curves (b) as functions of drying time and temperature

Drying time decreased from 250 min to 120 min as temperature rose; the higher temperature favoured the faster removal of moisture. The same observation was made by *Upadhyay et al.* (2008), but drying time was much longer (decreased from 7.5 to 5 hours) due to the different drying method used (*Upadhyay et al.*, 2008). As for the initial moisture content of the carrot pomace samples (calculated with equation (1)), there is no such difference between the two experiments. In our case, the average initial moisture content of the samples was $87.25 \pm 0.37\%$ (wet basis), while before the hot air convective drying it was 85.62% (wet basis) (*Upadhyay et al.*, 2008).

Drying curves (moisture content versus time) recorded at different temperatures are shown in *Fig. 1b*. The trend of the drying curves is typical for vegetables and is similar to other agricultural products reported as the moisture ratio decreased exponentially (*Ahmed*, 2018).

The drying curves are useful to plot the change in drying rate (eq. (3)) as function of moisture content, too (*Fig.* 2).
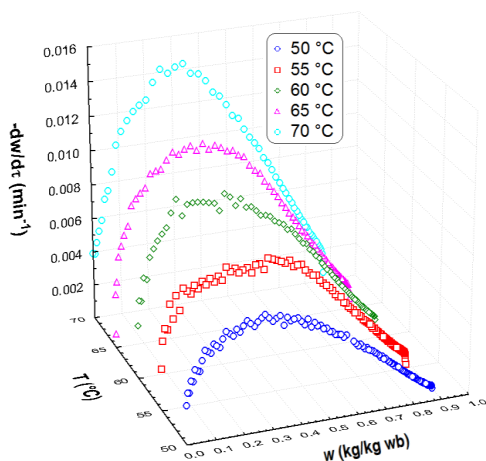
Figure 2: Drying rate changes as function of moisture content at different temperatures

As we can see from the curves, there are two different drying periods: the first, the so-called constant drying rate period, and the descending rate drying period. The value of moisture corresponding to the point of inflection is described in the literature as critical moisture content (w_{cr}). By the end of the second period, the moisture in the sample will run out, i.e. it will reach the equilibrium moisture (w_{eq}) level. In order to express the value of the dry-

ing rate constant (k) from the drying time equation (eq. (4)), these values must first be determined using the drying curves (*Fig. 2*). These values are summarized in *Table 1*. It has to be noted that during the experiments, the carrot pomace was carefully distributed evenly over the sample plate of the balance; therefore, the value of the drying surface was initially equal with the plate surface, numerically: $S = 0.00708$ m^2. Despite the varying shrinkage of the carrot pomace during the measurements, it was not taken into account in our calculations. The calculated rate constant values from equation (4) are listed in *Table 1*.

Table 1: Calculated parameters of infrared drying at various temperatures

Drying temperature, °C	Drying time, τ		Moisture content, kg/kg wb				Rate constant, k, 10^{-3} kg/(m^2·s)
	min	s	w_1	w_{cr}	w_{eq}	w_f	
50	250	15,000	0.8663	0.185	0.02	0.004	0.306
55	210	12,600	0.8775	0.186	0.02	0.005	0.368
60	166	9,960	0.8709	0.181	0.02	0.006	0.478
65	148	8,880	0.8749	0.195	0.02	0.002	0.489
70	120	7,200	0.8730	0.231	0.02	0.008	0.579

In the studied drying temperature interval, the rate constant variation is linear in function of temperature. As the value of coefficient of determination is close to 1 ($R^2 = 0.9627$), this suggests that the equation (5) is appropriate for drying rate constant estimation at various drying temperatures:

$$k = 1.33 \cdot 10^{-5} \cdot T - 0,0004 \tag{5}$$

4   Conclusions

Based on the study, we concluded that the carrot pomace drying with infrared radiation method would be a better choice compared to hot air convective drying due to significantly shorter drying time. The calculated rate constants of infrared drying at various temperatures could serve as a good starting point for industrial research and developments that need to be carried out to scale up this drying method. Dried carrot pomace can be easily grinded to powder and can be incorporated in bakery products as a cheap source of dietary fibres. Furthermore, this processing alternative represents a good opportunity for the vegetable juice manufacturing industry for efficient waste management and additional profit.

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Evaluation of the microbiological quality of some fresh dairy products with Soleris® Automated System

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Abstract. The manufacture of dairy products is an important sector of the food industry. From milking to processing, a number of hygiene rules must be strictly followed. During processing, dairy products can be contaminated with different microorganisms, causing spoilage, infectious diseases, and alterations in the sensory characteristics. There are strict requirements for the quality assurance of milk products. In spite of this, there occur infections linked to milk and dairy product consumption. The

analysis of the microbiological quality of these products is a health concern, and it also has an economic impact. The increase and development of the global market of processing technologies require rapid monitoring and controlling systems for food products. In our study, we investigated the microbiological quality of some fresh dairy products with the Soleris® test system. For instrument setting, calibration curves were realized with test bacterial strains. With known initial microbial load of the samples, the microbial growth versus time was measured by the above-mentioned system. The occurrence of *Escherichia coli*, *Pseudomonas aeruginosa*, and S*taphylococcus aureus* was evaluated in ten dairy products. Results obtained by the Soleris system showed that the system is efficient for this purpose. Calibration curves with high correlation coefficients permitted the quantitative determination of the aimed bacteria in the dairy product samples.

1 Introduction

Dairy industry is one of the most important sectors of the food industry, characterized by a variety of technologies producing a wide range of food products. In human nutrition, dairy products represent a major energy and nutrient source due to the high-value nutrition components.

The poor hygiene practice in the farm industry or the lack of regulations and supervision results in the contamination of the dairy products with pathogenic microorganisms and/or the occurrence of antibiotic residues. Milk is an ideal environment for bacteria; therefore, when microorganisms from the farm environment contaminate the milk supply, they will multiply rapidly (*Garcia et al.*, 2019). Worldwide several outbreaks were associated with milk-foodborne pathogens (*Landgraf & Destro*, 2013; *Desai & Trimble*, 2019). Food spoilage is a serious global problem, especially in developing countries, due to inadequate processing and refrigeration equipment. The high nutritional value, high water activity, and near-neutral pH of milk are very favourable for microbial growth. Different foodborne pathogens and food spoilage bacteria were detected in milk products. Diverse species of *Streptococcus* spp. (*Streptococcus agalactiae*, *S. pyogenes*, *S. zooepidemicus*) and *S. aureus* are linked to the health status of the mammary gland and cow herd. The working medium can also be a source of *Listeria monocytogenes*, *Salmonella* spp., *E. coli* O 157:H7, *E. coli* (STEC), *Yersinia enterocolitica*, *Enterobacter sakazakii*, *Campylobacter jejuni*, *Enterococcus faecalis*, *Citrobacter freundii*, and *Bacillus cereus*. Other waterborne microorganisms may appear in milk and milk products as well such as *Leptospira* spp., *Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas*

aeruginosa, *Clostridium disporicum*, *Aspergillus* spp., etc. (*Velázquez-Ordoñez et al.*, 2019).

The presence of coliforms in dairy products reflects the poor hygienic conditions during the production process. Coliforms could be eliminated by compliance with personal hygiene requirements. Coliforms are a wide variety of bacteria such as *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., etc. This group of microbes causes undesirable changes due to various enzyme activities and metabolic by-products (*Laslo & György*, 2018). They are responsible for organoleptic defects in cheese. *Escherichia coli* is a hygienic indicator organism of faecal contamination in dairy products (*Baranceli et al.*, 2014). Based on phenotypic characteristics and virulence factors, different serotypes of *E. coli* are known. These are the enterohaemorrhagic (EHEC), or enteropathogenic (EPEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive (EIEC) *E. coli* strains (*Batt*, 2014).

Staphylococcus aureus represents the third most important foodborne pathogen with enterotoxin-producing capacity and is characterized by multidrug resistance. Several outbreaks are attributed to enterotoxigenic strains. The main sources of contamination of dairy products are humans and poor hygiene. Also, raw milk can be contaminated, which is derived from cow mastitis (*Gillaspy & Iandolo*, 2014).

Major spoilage psychotropic bacteria are the *Pseudomonas* spp. due to their enzymes, such as protease, lipase, and lecithinase, that cause different alterations. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* are believed to be responsible for the spoilage of raw milk and dairy products (*Arslan et al.*, 2011; *Sharika & Balamurugan*, 2019).

For microbial analysis, different alternative rapid methods with high capacity are developed (*Jasson et al.*, 2010). On the market, different instruments are available that use electrical techniques able to detect different pathogens and food spoilage bacteria besides total viable count in food samples. These methods represent an alternative approach to standard plate counting methods. The systems consist of an incubator and computer with dedicated software. This lets users track impedance curves, create reports, or evaluate calibration curves. Such a system is Soleris® System for Rapid Microbial Detection (*Mozola et al.*, 2013; *Pereault et al.*, 2014). This technique checks the alteration of the chemical characteristics of the growth medium used for microbial detection. The result comes from the changes that appear due to the metabolic activity of microorganisms, which is detected optically. For quantitative determination, a correlation between the microorganism and detection time can be established (*Limberg et al.*, 2016).

In the case of positive *E. coli* detection, the Soleris® instrument tracks the alteration of the medium colour resulted from the pH change. It can be applied for the indication of zero tolerance (*Foti et al.*, 2012).

The advantages of these growth-based automatic measurements in contrast to conventional plate-counting methods are: precision, accuracy, reproducibility, time saving, and cost (*Curda & Sviráková*, 2014; *Blivet*, 2014; Soleris® *Operation Manual*). In the development of an adequate rapid method, several facts are included. *Jasson et al.* (2010) suggested several selection criteria. First at all, the target microbial analysis is the main decision factor. Furthermore, the time and cost are included, referring to them as managerial criteria, and there exist technological and sustainability criteria, too.

Our aim was to calibrate the Soleris® System for the quantitative determination of some pathogenic and food spoilage bacteria as well as to investigate the microbiological quality of some fresh dairy products.

2   Materials and methods

Quantitative detection of Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa with Soleris® System for Rapid Microbial Detection

For the quantitative detection of the targeted bacteria, calibration curves of the tested bacteria were realized. The vials with chromogen-selective mediums (E. coli Medium (EC-104), Staph Medium (SM-118), Pseudomonas medium (VIV-125)) were inoculated with *E. coli, Staphylococcus aureus*, and *Pseudomonas aeruginos*a and incubated at 37 °C for 12 hrs. In every three hours, one ml of each sample was aseptically transferred into 9 ml of physiological solution. From this, serial dilutions to 10^{-1}–10^{-12} were prepared, and a volume of 0.1 ml was spread on the selective agar mediums. After a 24-h incubation at 37 °C, the number of colony-forming units was estimated and used to realize calibration curves with the software of Soleris® Automated System software. Different dairy products were inoculated with *E. coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and un-inoculated control samples were used for the validity of quantitative detection based on the calibration.

Determination of the occurrence of Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa in some fresh dairy products

During our work, 10 different dairy products (fresh cheese 1, fresh cheese 2, fresh cheese 3, fresh cheese 4, fresh cheese 5, feta-like cheese, salty type

soft cheese, cottage cheese 1, cottage cheese 2, cottage cheese 3) were studied microbiologically with the Soleris Automated System for the detection of the mentioned bacteria. The dairy products sold by weight were obtained from local stores and open-air public market.

11 grams of each sample was aseptically transferred into 99-ml Butterfield's buffer (34 g KH_2PO_4, 175 ml 1 N NaOH, 1,000 ml distilled H_2O, pH=7.2, and diluted 1.25 ml/1,000 ml) and mixed well for 10 minutes. From this homogenate, the adequate volume based on the instruction protocol was added to the Soleris vials (E. coli Medium (EC-104), Staph Medium (SM-118), Pseudomonas medium (VIV-125)) for each bacterial detection. The inoculated Soleris vials were placed into the selected drawer location. After proper adjustments, determinations were initiated. The Soleris software indicated positive test results in less than 24 hrs. Determinations producing no detection within 24 hrs were considered negative. In the case of positive results, the growth curves were evaluated, and the visual validation of medium colour change was also carried out. In the case of positive results, confirmation was done using conventional methods.

3   Results and discussions

Different alternative microbiological methods are used successfully for the detection of different pathogenic and food spoilage microorganisms. According to *Moldenhaue* (2008), alternative methods use bacterial growth that results from the biochemical and physiological changes or the viability of the microorganisms or the detection of the cell components. Also, there are combined methods. *Elegado et al.* (2016) reported several demands for rapid detection methods. These types of methods must be characterized by time saving, selectivity for the desired specific microorganisms, and must be capable to detect more than one microbe simultaneously.

During this study, we calibrated the Soleris® Automated System for the quantitative determination of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. We used this growth-based method for the evaluation of the microbiological quality of some fresh dairy products.

The calibration points were obtained by the plot of mean of the log(10) CFU for each targeted bacteria in function of detection time. In the case of *E. coli*, calibration points are shown in *Fig. 1*. The correlation coefficient was 0.99 with detection time ranging from 0 to 10 hrs.
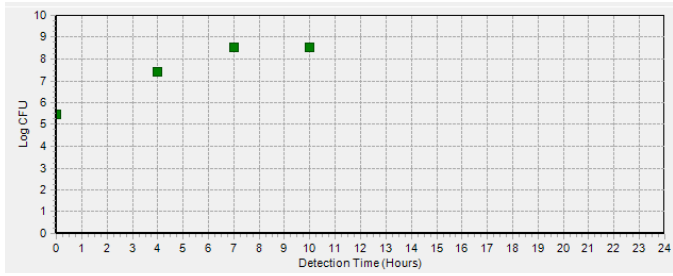
Figure 1: Calibration points: CFU in function of detection time in the case of *E. coli*

Calibration points for *Staphylococcus aureus* are shown in *Fig 2*. The correlation coefficient was 0.99 with detection time ranging from 0 to 10 hrs.
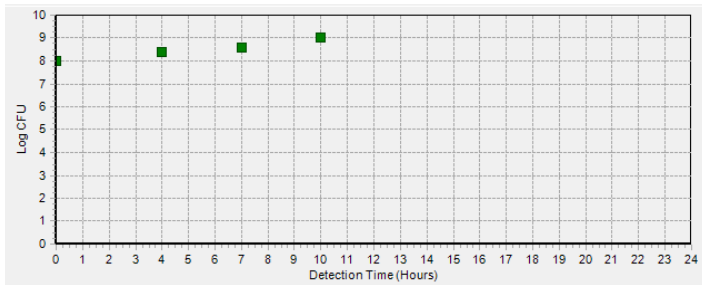

Figure 2: Calibration points in the case of S*taphylococcus aureus*

Calibration points for *P. aeruginosa* are shown in *Fig 3*. The correlation coefficient was 0.97 with detection time ranging from 0 to 10 hrs.
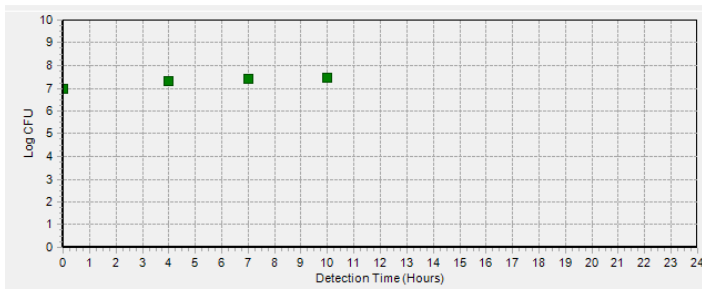

Figure 3: Calibration points in the case of *P. aeruginosa*

The obtained correlation coefficients were higher than the minimum allowed, and the calibration was accepted. The dairy samples have been analysed for the occurrence of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with the quantified Soleris® System. The results are shown in *Table 1*. The lack of good hygienic practices contributes to the high prevalence of foodborne pathogens.

Escherichia coli is the faecal indicator organism in processed milk products. *E. coli* have been detected in all ten samples with high count (*Table 1*).

Table 1: *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus* count in the dairy samples

Studied samples	E. coli CFU/g	Detection time (h)	P. aeruginosa CFU/g	Detection time (h)	Staphylococcus aureus CFU/g	Detection time (h)
fresh cheese 1	$1.4 \cdot 10^9$	10.1	$2.50 \cdot 10^7$	7.6	<10	ND
fresh cheese 2	$5.9 \cdot 10^8$	8.9	$2.30 \cdot 10^7$	6.7	<10	ND
feta-like cheese	$8.4 \cdot 10^{10}$	16	$3.40 \cdot 10^7$	10.4	<10	ND
fresh cheese 3	$1.3 \cdot 10^8$	6.7	<10	ND	<10	ND
salty type soft cheese	$2.6 \cdot 10^8$	7.7	$1.90 \cdot 10^7$	4.8	<10	ND
cottage cheese 1	$1.1 \cdot 10^7$	3.2	$1.80 \cdot 10^7$	4.5	<10	ND
cottage cheese 2	$1.9 \cdot 10^{11}$	17.2	$2.60 \cdot 10^7$	7.9	<10	ND
fresh cheese 4	$5.2 \cdot 10^8$	8.7	$4.20 \cdot 10^7$	12.3	$1.3 \cdot 10^8$	1.2
cottage cheese 3	$6.8 \cdot 10^8$	9.1	$2.20 \cdot 10^7$	6.3	<10	ND
fresh cheese 5	$6.4 \cdot 10^8$	9	$2.60 \cdot 10^7$	8	<10	ND

The most contaminated samples were one type of the cottage cheese and feta-like cheese. In the less contaminated sample cottage cheese 1, the *E. coli* count was also high. Detection time varied between 9 and 17 hrs (Fig 4). *Foti et al.* (2012) previously reported that the Soleris® test system could be applied for the assessment of *E. coli* occurrence in mozzarella cheese. The detection time of *E. coli* ranged from 11.4 to 19.1 hrs. The advantages resulting from the use of this alternative system are simplicity and rapidity.

Pseudomonas aeruginosa were found in nine samples. One of the tested dairy products (fresh cheese 3) was free of this bacteria. All positive samples have a high number of *Pseudomonas aeruginosa*. Detection times for *P. aeruginosa* ranged from 4.5 to 12.3 hrs in contrast with *Foti et al.* (2012), where these bacteria were not detected.

In the perspective of public health, *Staphylococcus aureus* represents a serious health threat. In the tested samples, one type of cheese (fresh cheese 4) was detected as positive for this bacteria. There was detected a high count of $1.3 \cdot 10^8$.
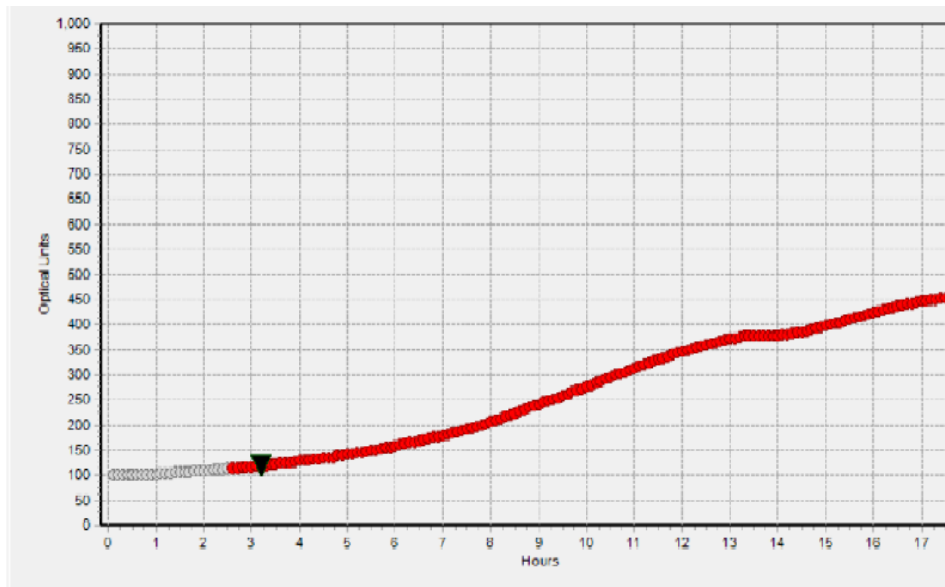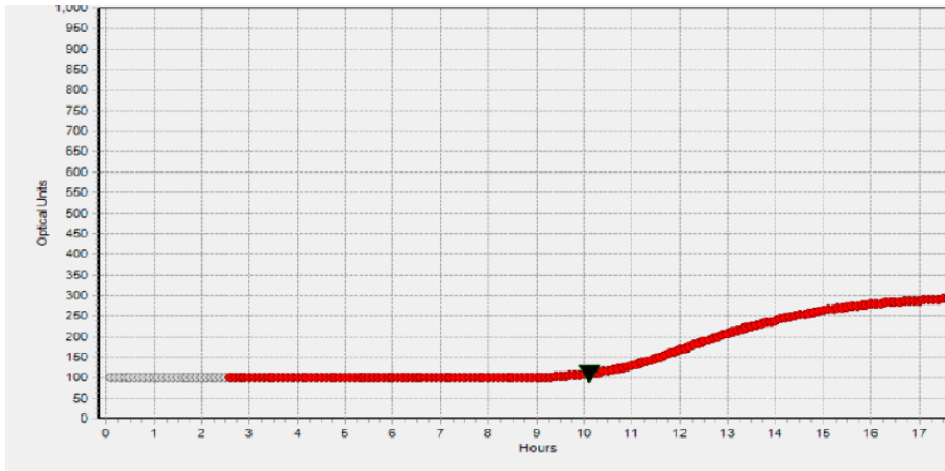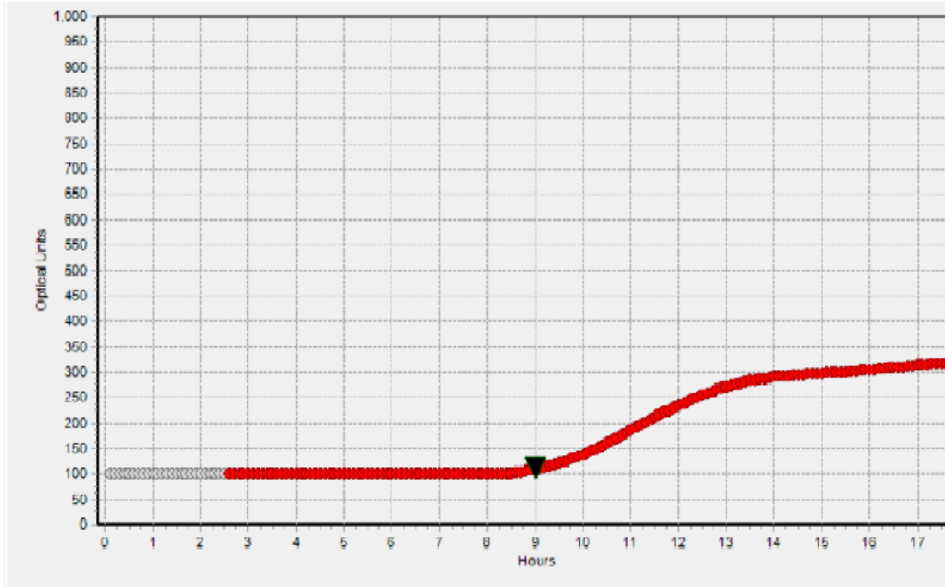
Figure 4: Detection of *E. coli* in fresh cheese 1, cottage cheese 1, fresh cheese 4, and cottage cheese 2

Globally, the occurrence of this bacteria was detected in various types of cheese. The high nutritional value (proteins, vitamins, and minerals) favours the growth and activity of *Staphylococcus aureus*. According to *Baran et al.* (2017), various causes are responsible for the staphylococcal intoxications transmitted by cheese. One of them is the raw milk contamination of *Staphylococcus aureus*, which can occur during cheese processing or after production.

Results revealed that the Soleris® Test System as a rapid, alternative method can be used for microbial quality determination. It is remarkable that the sensitivity of this method ranges to 10^8 CFU/ml (*Jasson et al.*, 2010). In three cases, the bacterial count was higher than the aforementioned value, while these samples were also examined with the traditional plate count method in order to confirm the results – the results turned out to be identical.
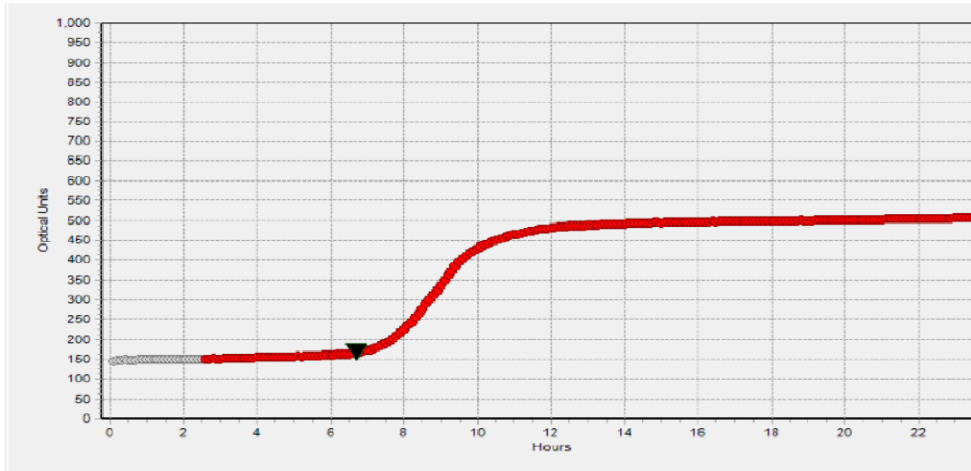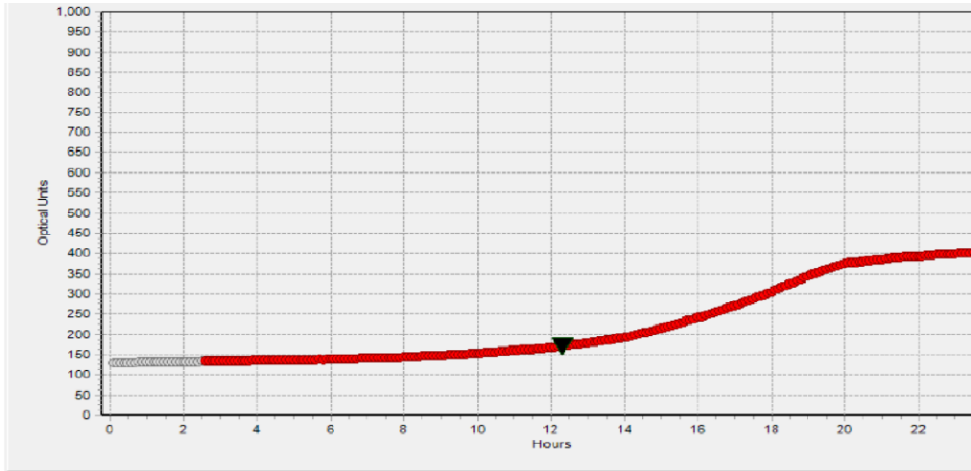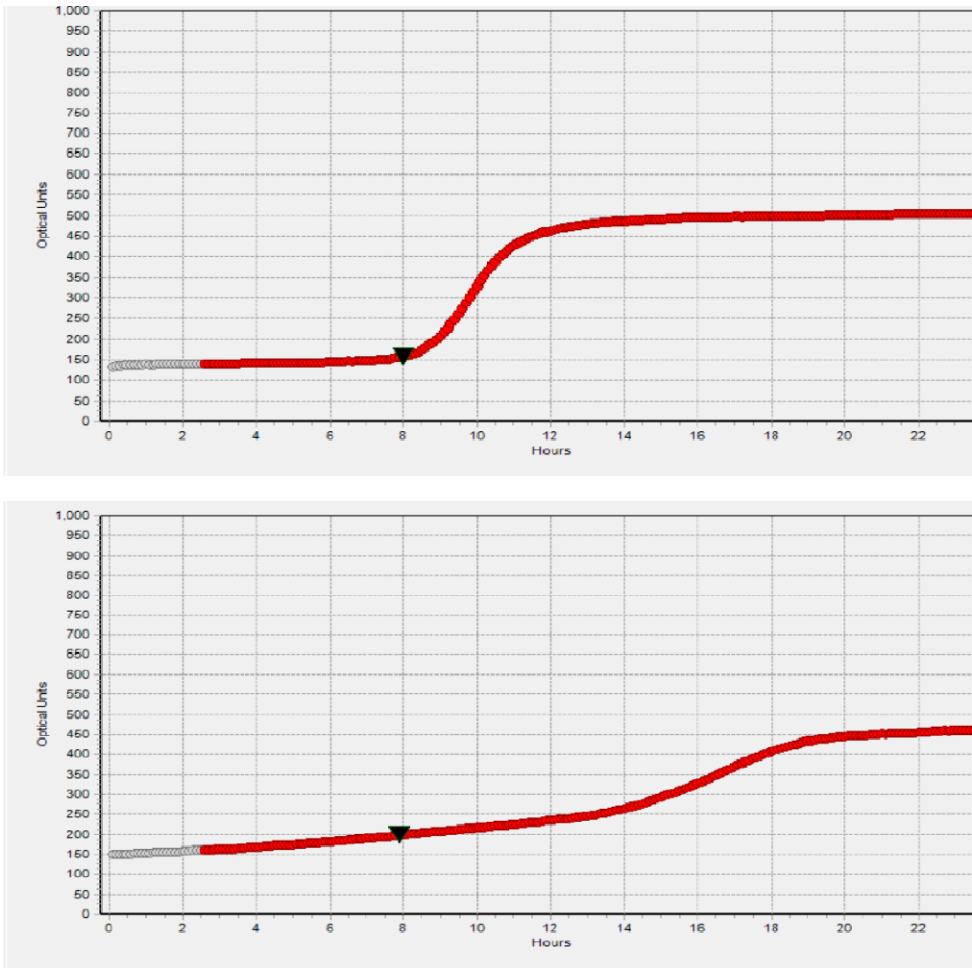
Figure 5: Detection of *Pseudomonas aeruginosa* in fresh cheese 1, fresh cheese 3, fresh cheese 5, and cottage cheese 3
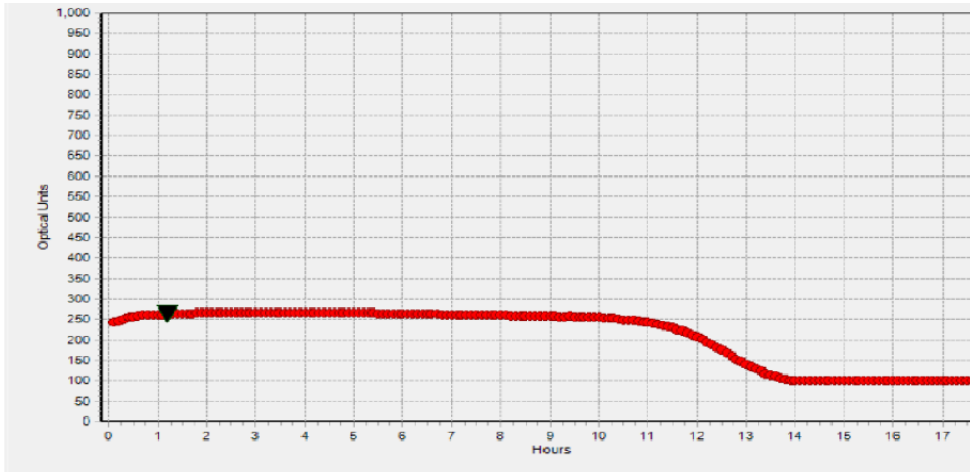
Figure 6: Detection of *Staphylococcus aureus* in fresh cheese 4

4   Conclusions

The Soleris® Automated System was successfully calibrated. Our results indicate that the Soleris® Automated System can be utilized as an alternative method to the standard plate count methods for the quantitative detection of *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in different dairy products. Our findings also revealed that dairy products sold by weight obtained from local stores and open-air public markets were highly contaminated. Besides poor hygienic practices during production, storage and distribution can also act as sources of contamination.

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Studies on the heat and disinfectant resistance of a spore-forming spoilage bacterium

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Abstract. Heat resistant thermophilic spore-forming bacteria, such as *Aeribacillus* (*A.*) *pallidus*, may contaminate the surfaces in food facilities resulting food spoilage of the products. The aim of this work was to determine the heat and disinfectant resistance of an *A. pallidus* strain that was isolated from a canning factory environment. Compared to other heat-resistant spore-forming bacteria, it did not prove to be very resistant to heat with a D_{10}-values of *A. pallidus* from 12.2 min to 2.4 min (at 102 °C and at 110 °C), with a calculated z-value of 11.6 °C. Not only spores but vegetative cells showed resistance against all investigated disinfectants.

1 Introduction

Thermophilic, endospore-forming bacteria can cause serious problems in different fields of food industry by their ability to form resistant spores and

biofilms (*Flint et al.*, 2001; *Kilic et al.*, 2017). Endospores of bacteria are able to survive extreme environmental conditions. They are highly resistant to preservation techniques, e.g. to heat treatment, drying, irradiation, pressure treatment, or chemicals. Thermophilic bacteria are able to grow at 70 °C. The first research about their characterization was performed by *Miquel* in 1888. Since then, many strains of thermophilic bacteria belonging to the *Bacillus* and *Clostridium* genera have been characterized (*Maugeri et al.*, 2001; *Belduz et al.*, 2003). Recently, several bacteria of Bacillus genus have been reclassified, such as *Alicyclobacillus*, *Paenibacillus*, *Brevibacillus*, *Aneuribacillus*, *Virgibacillus*, *Salibacillus*, *Gracilibacillus*, *Ureibacillus*, and finally *Geobacillus*. Thermophilic bacteria in the 1. and 5. genetic groups were classified into the *Geobacillus genus* (*Iida et al.*, 2005).

Thermophilic bacteria have an optimal growth temperature range between 45 and 70 °C, wherefore they are isolated from hot environments such as hot springs, geothermally heated soils, shallow marine hot springs, petroleum reservoirs, deep-sea hydrothermal vents, the leachate of a waste pile from a canning factory, hot water pipelines, heat exchangers, waste treatment plants, etc. (*Rahman et al.*, 2004; *Bae et al.*, 2005). Microbial spoilage contributes to the vast amount of food that is wasted and the associated financial losses. Spoilage of heat-treated food products are usually provoked by spore-forming bacteria as a result of the resistance of spores to high temperatures commonly used to preserve foods. Canned food products generally undergo spoilage by thermophilic bacilli. *Denny* (1981) had demonstrated that thermophilic bacteria were the prime cause of spoilage of canned corn. Spoilage of heat-treated dairy products is often caused by thermophilic spore-formers. It was demonstrated by *Scheldeman* and co-workers (2006) that spoilage spore-formers, e.g. *Bacillus licheniformis* and *Aeribacillus pallidus*, were most frequently isolated from farms and may cause spoilage of treated milk.

In recent years, there has been evidence of contamination of canned food (especially canned corn) products by new spore-forming, thermophilic bacteria from the genus *Aeribacillus* (previously *Geobacillus*). Members of the genus *Aeribacillus* are aerobic, thermophilic, alkalitolerant, motile, and Gram-positive rods (0.8–0.962–5 nm) that occur singly, in pairs, or in chains. The reason for the spoilage of ready canned corn by *A. pallidus* can be the improper performance of thermal processes due to the incorrect relation between heating time and temperature. For that reason, investigation of the heat resistance of *A. pallidus* spores and determination of decimal reduction time (D-value) are high concerns for the canning industry. In comparison with other thermophilic bacteria from the genus *Geobacillus*, there are no reported D-value data for

A. pallidus in canned corn products.

Therefore, the aim of this study was to determine the influence of heat treatment and sanitizers on the survival of spores of *Aeribacillus pallidus*.

2   Materials and methods

2.1   Bacterial strain

In this study, *Aeribacillus pallidus* T6 3 (NCAIM B.01143) culture was used. The strain was isolated from spoiled canned corn products. Isolation and storage of *A. pallidus* culture was done using Casein-peptone Soymeal-peptone Agar (CASO agar, Merck).

2.2   Preparation of spore suspensions

Agar slants of *A. pallidus* cells were flooded with sterile distilled water, and then 2 ml of suspension was transferred on the surface of CASO agar in 200-mm-diameter Petri dishes.

Inoculated Petri plates were incubated at $55\,^{\circ}\mathrm{C}$ for 48 hours and then were transferred to a refrigerator and stored at $15\,^{\circ}\mathrm{C}$ for 72 hours. Spores then were collected by scrapping the surface of the agar with sterile metal spatula, suspended in sterile distilled water, and washed three times by centrifugation $(4{,}000 \times \mathrm{g}$ for 10 minutes). Spore suspensions were stored at $4\,^{\circ}\mathrm{C}$ until they were used. The number of spores in a suspension was determined by the pour-plate method, and it was 8×10^9 CFU/ml. Suspensions were diluted to obtain approximately 8×10^7 CFU/ml. Activation of spores was done by heating spore suspension in water bath at $80\,^{\circ}\mathrm{C}$ for 10 minutes.

2.3   Heat treatment analysis

Canned corn brine (pH 6.11 ± 0.18) was used as a heating medium. Heating experiments were carried out in small glass vials. After filling them with 2.5 ml of spore suspension, vials were sealed with gas burner flame. Thermal inactivation was performed in temperature-controlled oil bath (Memmert, Model ONE 7, Germany). The samples were heated in the oil bath at temperatures of $102\,^{\circ}\mathrm{C}$, $104\,^{\circ}\mathrm{C}$, and $110\,^{\circ}\mathrm{C}$. The sample temperature was monitored continuously using Testo 110-1 channel NTC Thermometer with needle-type sensor. Triplicate samples were removed from the bath every 2 minutes, at 0, 2, 4, 6, and 8 minutes. After removal, the samples were immediately immersed into

cold water. The viable spores were counted by triplicate plating on CASO agar and incubated at 50 °C for 2 days.

2.4　Calculation of D10-values

D_{10}-values were calculated using the average slope ($D_{10} = -1/$slope) for each temperature treatment.

2.5　Disinfection efficacy test

2.5.1　Disinfection test against vegetative cells

A. pallidus was inoculated on CASO agar and incubated at 50 °C for 24 hours. The turbidity of the culture was set to OD 1 (the initial cell count was 3.2×107 CFU/ml). 400 μl of corn brine was applied to clean, pre-sterilized stainless steel coupon surfaces, and be distributed evenly throughout the coupons (8×6 cm). The brine was dried on the surface in a laminar box, and 400 μl of cell suspension (10^7 cell/ml) was uniformly dispersed on the surfaces and dried in a laminar box. 400 μl of disinfectant suspension was applied to the surfaces, and cells were removed from the surface with a swab soaked in an inactivating solution after the time of exposure (*Table 1*).

Table 1: Recommended parameters for the industrial application of disinfectants

Name	Concentration	Contact time (min)	Temperature (°C)
Apesin DSR	0.5%	30	25
Chlor-sept	1:10	1	25
Descosal	1%	30	25–30
Idro 86	3–5%	rinse as desired	
Innofluid-MF-M	2% (generally)	5–30	35–50
	8% (egg-contaminated surfaces)		
Megabrite	1–2% (generally)	10–30	30–80
	5–20% (heavy contamination)	15–60	30–80
Rimadet-SR-310	3–5%	20	25
Wunder	1:10	10	25–30

The surviving cells recovered by the swab were placed in an inactivating solution (1.56 g sodium thiosulfate ($Na_2S_2O_2 \cdot 5H_2O$), 0.07 g of lecithin, 0.1 mL Tween 80/100 mL PBS) and vortexed. After serial dilution, the number of surviving cells was determined by plate counting on CASO agar. Incubation

was carried out at 55 °C for 24–48 hours. As a control, clean brine-free surfaces were applied with the same procedure.

2.5.2   Disinfection test against spores

A. pallidus spores were heat-activated before disinfection test (10 minutes at 80 °C). Inoculation and treatment of spore suspension was carried out similarly as described in 2.5.1.

3   Results and discussion

3.1   The heat resistance of *Aeribacillus pallidus* spores

Thermal inactivation tests of *A. pallidus* spores at three different temperatures (102 °C, 104 °C, and 110 °C) in corn brine have been performed. Decimal reduction times (D_{10}-values) were calculated by linear regression analysis ($D_{10} = -1$/slope of a plot of log surviving cells versus time). Survival curves of *A. pallidus* spores at different temperatures are shown in *Figure 1*.
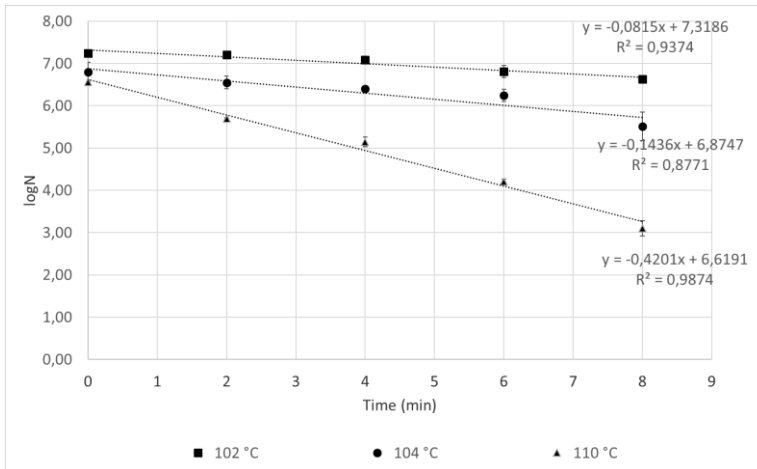

Figure 1: Survival curves of *Aeribacillus pallidus* spores in corn brine at different temperatures

Table 2 summarizes the D-values obtained at different heating temperatures in corn brine.

The D-values of *A. pallidus* ranged from 12.2 to 2.4 min (at 102 °C and at 110 °C), and the calculated z-value of the test strain was 11.6 °C.

Table 2: D_{10}-values of *Aeribacillus pallidus* spores at different temperatures in corn brine

Heating temperature (°C)	D10-values (min)
102	12.2
104	7.1
110	2.4

In comparison with literature data, the *A. pallidus* strain proved to be not extreme heat resistant in corn brine. *Geobacillus stearothermophilus*, a thermophilic spore-forming bacterium, has a D-value of 62.04 min at 112.8 °C, 18.0 min at 115.6 °C, and 3.33 min at 121.1 °C in aqueous suspension, with a z-value of 8.3 °C (*Feeherry et al.*, 1987).

Warth (1978), when examining the heat tolerance and optimal growth temperature of *Bacillus* species, found that there is a relationship between optimal growth temperature and heat resistance. The composition of the culture medium has no effect on maximum growth temperature. However, the composition of the heat treatment medium (pH, water activity, etc.) greatly influences bacterial heat tolerance. *Lopez et al.* (1996) showed that the heat tolerance of *B. stearothermophilus* spores was reduced by 7 to 23 times when the pH of the heat treatment medium was lowered from 7 to 4.

3.2 Disinfection efficacy tests

3.2.1 Efficacy of different disinfectants against *A. pallidus* vegetative cells

The metal coupons used in surface tests were designed to model the surface of industrial production lines, both in the form of clean and organic soil-contaminated surfaces.

Table 3 shows the extent of disinfectant-induced decrease in the number of cells between the initial cell count and the number of cells recovered after plating.

In general, disinfection treatment resulted in a mean 2.9 log reduction.

Against vegetative *Aeribacillus pallidus* cells, the most effective agents were Chlor-sept, Innofluid-MF-M, and Megabrite.

Chlor-sept disinfectant was examined at the specified concentration and at two end contact times (1 min, 30 min) as per the manufacturer's instruction.

Table 3: Cell count reduction of *A. pallidus* vegetative cells during disinfectant treatments

	Disinfectant		Reduction (Log N)	
Name	Used concentration (%)	Contact time (min)	Clean surface	Surface with brine
Apesin	0.5	30	2.37	2.37
Chlor-sept	10	1	2.32	2.01
		30	3.73	3.36
Descosal	1	30	2.43	2.20
Idro-86	5	15	2.47	2.74
Innofluid-MF-M	2	10	2.47	2.32
	8	5	3.51	3.3
Megabrite	2	10	3.46	3.39
Rimadet	3	20	2.7	0.54
Wunder	10	10	3.01	2.84

For Innofluid, using the two concentrations (2%, 8%) given by the manufacturer, 8% treatment with only 5 minutes exposure time was more effective than 10 minutes contact time with 2% concentration. Megabrite has been shown to be effective at 2% concentrations for 10 minutes. The acidic detergent, Wunder, caused a reduction of 3 orders of magnitude in 10% concentration. The Rimadet chemical (3%) on clean surface resulted a considerable cell count reduction in contrast with the organic-loaded surface, which showed the lowest efficiency. In the case of Apesin, the agent showed very low activity against the test strain, with no difference between the clean and soiled surfaces. Descosal (1%) also had low efficacy, similarly to the previous agent, after 30 minutes of exposure. In the case of Idro-86, however, unexpected results were observed. After disinfection treatment, the number of surviving cells was higher on the clean surface than on the brine-containing surface.

Overall, the number of cells decreased several orders of magnitude; however, the lethal effect of the chemicals on the vegetative form of bacteria was not remarkable. The high survival rate is partly due to the presence of spores in the cell mass produced during vegetative cell culturing (the initial cell suspension showed the presence of spores after spore stain; results are not shown).

3.2.2 Efficacy of different disinfectants against *A. pallidus* spores

The results of the disinfectant test against spores are summarized in *Table 4*.

In the number of spores, 2–3 log reduction was observed generally after treatment. The greatest reduction was caused by the chlorine-containing Chlor-sept

as well as by Innofluid and Megabrite. Among them, the most effective one was the application of 10% Chlor-sept solution for 30 minutes – both on clean and organic soiled surfaces. Surprisingly, despite the efficacy of Innofluid, increasing the exposure time did not affect further the decrease in spore count. Megabrite was as effective against spores as against vegetative cells after only 2% treatment for 20 minutes.

In the case of Idro-86, a small but measurable difference could be observed between the results of the two surfaces. At 10% concentration, Wunder resulted a moderate reduction in cell number on a clean surface, while the surface exposed to organic matter showed less efficiency. Rimadet was the least efficient at 3% concentration on both clean and dirty surfaces. In the applied concentration, Descosal and Apesin were moderately effective against spores.

Table 4: Spore count reduction of *A. pallidus* during disinfectant treatments

Disinfectant			Reduction (Log N)	
Name	Used concentration (%)	Contact time (min)	Clean surface	Surface with brine
Apesin	0.5	30	3.06	2.68
Chlor-sept	10	1	2.44	1.51
		10	2.62	2.25
		30	3.52	3.3
Descosal	1	30	2.40	2.28
Idro-86	5	15	2.65	2.73
Innofluid-MF-M	8	5	2.89	2.87
		10	2.98	2.93
Megabrite	2	20	3.20	3.10
Rimadet	5	20	1.78	1.59
Wunder	10	10	3.15	2.62

Compared with other literature data, a similarly low spore count reduction was observed by *Guan* and co-workers (2013) for the heat resistant spore-forming bacterium *Geobacillus stearothermophilus*. Disinfectants reduced the spore counts on heavy-organic-load-containing surfaces by only less than 2 $\log_{10}$ within 2 hours of exposure.

In most cases, resistance decreased with increasing concentration, but increasing exposure time did not cause significant changes in either vegetative cells or spores. As expected, endospores proved to be more resistant to the vegetative form although the degree of resistance was not considerable.

4   Conclusions

Because of the adhesive characteristics of spore-forming bacteria, they are ubiquitous in the food industry, in raw materials, ingredients, packaging materials, environment, and processing lines. Therefore, the contamination of end-products can easily occur. Due to their heat resistance, commonly used pasteurization processes fail to kill spores. Moreover, heat resistance may vary within the strains of a species and may differ according to the physiological state of cells and the composition of the food product (e.g. composition, a_w, pH). Therefore, further research is needed to determine the resistance properties of spoilage spore-formers.

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Energy drink consumption pattern and the effect of consumption on university students' blood pressure and heart rate

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Abstract. Energy drink (ED) consumption, even mixed with alcohol, is popular among adolescents and young adults. The side effects of ED are attributed to their active ingredients and their cumulated effect. A cross-sectional study to identify university students' ED consumption habit was realized. A small sample size (n = 10) experiment examining the effect of ED consumption on arterial blood pressure and heart rate was carried out.

From the total number of 240 interviewed students, 87.1% consumed ED at least once, and one third of them did so on a monthly basis. Students consume energy drinks mainly for its taste, very rarely for studying. Differences in consumption place preferences were observed between sexes, females preferring bars, while males the dormitory. We have demonstrated the increase of systolic blood pressure (SBP) for one type of energy drink in young and healthy volunteer students. Other changes in blood pressure and heart rate were not observed.

1 Introduction

Energy drinks are a relatively new product category on the food market. The first energy drink was launched in Japan in 1960 followed by the appearance of Red Bull on the European market in 1987 (*Zucconi et al.*, 2013). A unanimous definition is still missing. Such drink category includes beverages that contain various ingredients, including: caffeine, herbal extracts, B vitamins, amino acids (taurine), amino acid derivatives (carnitine), sugar derivatives (glucuronolactone), and sugars or sweeteners (*Malinauskas et al.*, 2007; *Zucconi et al.*, 2013). Energy drinks may contain from 70 mg to 400 mg/L caffeine (*Zucconi et al.*, 2013). Energy drinks are most frequently used as energizers, stimulants, and performance enhancers. They should not be confused with isotonic beverages and sports drinks, which are considered functional foods. Energy drinks are not recommended to be consumed during physical activities, such as exercising or making physical effort, because the fluid loss and sweating can lead to an intense dehydrated condition and sodium imbalance (*Alsunni*, 2015).

There is a growing demand of energy drinks on a global scale due to the increased consumption in the past two decades mainly among adolescents and young adults. The global sale was 14.68 billion litres in 2018. The major market of energy drinks is North America followed by Asia-Pacific and Europe. On the global market, there are hundreds of different brands with major players such as Red Bull, Monster Beverages, and Rockstar Inc. (Mordor Intelligence, 2019).

Major companies have a very effective advertising, involving movie stars and famous people influencing mainly the vulnerable age-groups such as adolescents and young adults. They are the main targets of the marketing slogans, easily impressionable by the allocated effects to these drinks, such as boosting performance, feeling more energetic, reducing fatigue, helping to stay awake, etc. The susceptibility of adolescents and young adults are confirmed by the literature data. According to a European survey covering 16 Member States including Romania and Hungary, 30% of adults (18–68 years) and 68% of adolescents (10–18 years) interviewed were energy drink consumers (*Zucconi et al.*, 2013).

The side effects of energy drinks are attributed to their active ingredients such as caffeine, taurine, and glucuronolactone and their cumulated effect with other substances (*Itany et al.*, 2014). Therefore, it is required on the European market that beverages containing at least 150 mg/L caffeine to be labelled as "High caffeine content" (Directive 2002/67/EC). Caffeine has numerous

effects on the organism such as vasoconstriction due to antagonized adenosine receptors, increased basal metabolic rate caused by the upregulated circulation of cathecolamines, and water and sodium secretion (*Persad*, 2011; *Oprea et al.*, 2019). Taurine is a normal constituent of human cells; it was associated with several physiological functions such as neuro- and cellular membrane modulation or the modulation of Ca^{2+} levels (*Oprea et al.*, 2019). Energy drink consumption in excess may cause cardiovascular, neurological, psychological, gastrointestinal, metabolic, and renal diseases (*Lee et al.*, 2005; *Bichler et al.*, 2006; *Alsunni*, 2015; *Shah et al.*, 2016; *Utter et al.*, 2017).

Young adults tend to mix energy drinks with alcohol. In the USA, the prevalence of alcohol mixing with energy drink (AMED) varied between 8.1 and 64.7% and in Australia between 21.1 and 77% (*Verster et al.*, 2018). In an expanded European study, co-consumption with alcohol showed similar values: 56% among adults versus 53% among adolescents (*Zucconi et al.*, 2013). The potential risks of AMED are the following: increased total alcohol intake, masked intoxication effects and increased risk-taking behaviour (*McKetin et al.*, 2015; *Verster et al.*, 2018; *Benson et al.*, 2019).

The aim of the study was to identify energy drink consumption habits and their effect on arterial blood pressure and heart rate in the context of possible risk of cardiovascular diseases among university students of Sapientia Hungarian University of Transylvania.

2 Materials and methods

Questionnaire survey

A cross-sectional study was conducted among the students of Sapientia Hungarian University of Transylvania (Cluj-Napoca), Faculty of Economics, Socio-Human Sciences and Engineering, Miercurea Ciuc. The data were collected online via Google anonymous questionnaire during the spring of 2019. The questionnaire consisted of 19 questions on students' socio-demographic characteristics, habits, energy-drink-related habits, and health-impairment-related knowledge. The questionnaire was filled by 240 (27.97%) students from the 858 total active students of the faculty in the academic year 2018–2019.

Subjects

The energy drink consumption study was set up with a group of twelve volunteer students recruited from Sapientia Hungarian University of Transyl-

vania, Faculty of Economics, Socio-Human Sciences and Engineering, Miercurea Ciuc. Exclusion criteria were: cardiovascular diseases and certain kind of chronic diseases. Before starting the experiment, volunteers received verbal and written instructions, and they signed a statement of consent on study participation and on using their data anonymously for the purposes of our study. Before the experiment, participants were asked not to consume any caffeine-containing drinks for 12 hrs before the study. After the experiment, we managed to collect full data on 10 students.

Study protocol

For the experiment, three popular energy drinks and a control drink was purchased in a shop. The ingredients of these drinks are shown in *Table 1*.

Each volunteer consumed 250 mL drink (three types of energy drink and one control drink) on experimental days. Arterial blood pressure (systolic SBP and diastolic DBP) and heart rate (HR) were measured before consuming and 20 min after consuming the drinks. Arterial blood pressure and heart rate were measured with a Beurer Blood Pressure Monitor. All measurements were performed on the arm of the non-dominant hand and were repeated three times.

Table 1: Ingredients in 100 g drink used during the experiment

Ingredient	Control drink	Drink 1	Drink 2	Drink 3
Caffeine	–	29 mg	32 mg	33 mg
Taurine	–	400 mg	400 mg	250 mg
Sugar	9.09 g	10.22 g	11 g	10.93 g
Ginseng extract	–	–	–	40 mg
Niacin	–	9.82 mg	8 mg	10.16 mg
Vitamin B-6	–	2.16 mg	0.8 mg	1.01 mg
Vitamin B-12	–	1.97 mg	0.2 µg	2.57 mg
Pantothenic acid	–	–	2.0 mg	–

Statistical analysis

All the data were processed in Microsoft Excel by calculating the mean and standard deviation. To interpret the results of two samples, paired t-test and chi-square test were used, performed with Past3 and SPSS (Version 22) software. P-values lower than 0.05 were considered as significant.

3   Results and discussions

Socio-demographic characteristics, participants' habits and energy drink consumption pattern

The questionnaire was filled by a total number of 240 (27.97% of the totality of faculty's students) students of Sapientia Hungarian University of Transylvania. Students' socio-demographic characteristics are presented in *Table 2*. Of the participants, 159 (66.2%) were females and 81 (33.8%) were males. Participants included in data analysis ranged from 18 to 30 years of age. The age distribution was the following: 45 students (18.8%) were under 20 years, 157 students (65.4%) were between 20 and 24 years, and 38 students (15.8%) were above 24 years. Regarding accommodation, the participants shared residency with parents, lived in dormitories or in lodgings approximately in the same proportion (30%, 35.4%, 29.2%), whereas 5.4% commuted from home to university. Concerning the parents' educational level, 58.8% of the mothers and 66.3% of the fathers were high-school graduated.

Table 2: Participants' socio-demographic characteristics

Characteristics	No. (n = 240)	Percentage
Gender		
Male	81	33.8%
Female	159	66.3%
Age-groups		
< 20	45	18.8%
20–24	157	65.4%
> 24	38	15.8%
Accommodation		
At home with the family	72	30%
Live in dormitory	85	35.4%
Live in lodgings	70	29.2%
Commute	13	5.4%
Mother's educational level		
Primary-school graduate	14	5.8%
High-school graduate	141	58.8%
College/university graduate (including postgraduate degrees)	85	35.4%
Father's educational level		
Primary-school graduate	11	4.6%
High-school graduate	159	66.3%
College/university graduate (including postgraduate degrees)	70	29.1%

Table 3 presents students' cigarette, alcohol, and energy drink consumption patterns. More than half of the students (58.3%) are non-smokers, and 41.7% are smokers. 88.3% of the students reported that they consumed alcohol with variable frequency, while 11.7% did not consume any alcohol. The majority of the students (87.1%) consumed at least once, whereas 12.9% never tried it out. Of those who never tried it, 87.1% considered energy drink unhealthy. A similarly high proportion of students that consumed at least once energy drink was observed in Hungary (95.3%; *Dojcsákné Kiss-Tóth & Kiss-Tóth*, 2018), Italy (75.8%; *Vitiello et al.*, 2016), *Lebanon* (63.6%; *Itany et al.*, 2014), and the USA (70.1%; *Pettit & DeBarr*, 2011). A significant difference between our results and those obtained at a Turkish university was observed, where 53.5% of the interviewed students never tried consuming any energy drinks (*Bulut et al.*, 2014).

Table 3: Participants' habits regarding smoking, alcohol, and energy drink consumption

Characteristics	No. (n = 240)	Percentage
Cigarette smoking		
Non-smokers	140	58.3%
Smokers	100	41.7%
Alcohol use		
Not using	28	11.7%
Using	212	88.3%
Energy drink consumption		
Consumed at least once	209	87.1%
Never tried	31	12.9%
Main reason for NOT trying ED		
No particular reason	4	12.9%
Consider unhealthy	27	87.1%

The pattern of energy drink consumption by the faculty students is presented in *Table 4*. The highest proportion of students consume one energy drink per month (67%), 19.1% of the surveyed students consume energy drinks on a weekly basis, while 5.8% of them does it on a daily basis. Other studies report different energy drink consumption frequency from our data: 51% of USA students consume more than one energy drink per month (*Malinauskas et al.*, 2007), while those from Lebanon consume less than one energy drink per month (*Itany et al.*, 2014). The weekly consumption of energy drinks was

observed in a similar proportion in Danish (*Friis et al.*, 2014), Italian (*Vitiello et al.*, 2016), and Hungarian (*Dojcsákné Kiss-Tóth & Kiss-Tóth*, 2018) students (15.8%, 15.8%, 14.4%), which correlates with our data.

Table 4: Participants' habits of energy drink consumption

Characteristics	No. (n = 209)	Percentage
Frequency of ED consumption (n = 209)		
>1 bottle/month	17	8.1%
1 bottle/month	140	67%
>1 bottles/week	22	10.5%
1 bottle/week	18	8.6%
1 bottle/day	6	2.9%
>1 bottle/day	6	2.9%
Main reason for consuming EDs		
Good taste	118	56.5%
Energy need	22	10.5%
Studying	8	3.8%
To stay awake for hours	26	12.4%
Reduce fatigue	27	12.9%
Other reason	8	3.8%
Time of first experience with EDs		
In primary school	69	33%
In high school	119	56.9%
In college/university	21	10%
Place of first time for trying ED		
At home	39	18.7%
Bar	38	18.2%
Coffee shop	11	5.3%
At a party	20	9.6%
Other places	14	6.7%
Not remember	87	41.6%
Companion when first tried an ED		
None	29	13.9%
Friend(s)	163	78%
Family members	15	7.2%
Not remember	2	1%
Type of preferred ED		
Hell	144	71.6%
Red Bull	37	18.4%
Monster	12	6%
Crazy Wolf	4	2%
Burn	1	0.5%
Other	3	1.5%
Place of consumption		
Bar	81	39.1%
At home	46	22.2%
In dormitory	42	20.3%
Coffee shop	13	6.3%
Other	13	6.3%
At university	7	3.4%
At work	5	2.4%

For more than half of the interviewed students (56.5%), the main reason for consuming energy drinks was their taste. In a Turkish study, 48.3% (*Sema & Çakir*, 2011) while in an Italian study 31% of the students consumed energy drinks for their taste (*Scuri et al.*, 2018). One eighth of our students consume energy drinks to reduce fatigue (12.9%) and to stay awake (12.4%). Similar values (10.3%) for reducing fatigue were observed in Turkish students (*Bulut et al.*, 2014), whereas the ratio of students consuming energy drinks for staying awake was lower (5%) in a Turkish study (*Sema & Çakir*, 2011) and higher (33%) in an Italian study (*Scuri et al.*, 2018). Our data show that one tenth (10.5%) of students consume energy drinks to feel more energetic. This reason was mentioned in a higher proportion (15.9%) among Turkish students (*Sema & Çakir*, 2011) and Italian students (32.5–44%) (*Vitiello et al.*, 2016; *Scuri et al.*, 2018). The smallest proportion of the students (3.8%) consume energy drinks to boost performance during studying. Similar data (4%) were obtained by *Sema & Çakir* (2011) among Turkish university students. In other studies, this reason was determining for a higher proportion of students (14%–28.6%) (*Bulut et al.*, 2014; *Vitiello et al.*, 2016; *Scuri et al.*, 2018). The most frequent places of consumption are bars (39.1%), homes (22.2%), and dormitories (20.3%). Our data correlates with those obtained at the Turkish university (*Sema & Çakir*, 2011).

The first experience with energy drinks was in high school for more than half of the students (56.9%) in the companion of friends (78%). At a Turkish university, energy drink consumption took place later in life, as university students (58.9%), in the companion of friends (59.2%) (*Sema & Çakir*, 2011).

The most popular energy drink brands among the faculty students were Hell (71.6%) and Red Bull (18.4%). The popularity of Red Bull was greater in studies from Italy, Turkey, and Lebanon (51.7–73.6%) (*Sema & Çakir*, 2011; *Itany et al.*, 2014; *Scuri et al.*, 2018). The popularity of the Hell energy drink in our region can be explained by the Eastern European (Hungary) origin of the company.

Energy drink consumption frequency differs significantly between males and females, being higher in females for bottle per month and lower for bottle per day consumption (*Table 5*).

Malinauskas et al. (2007) reported in a study based on American students that significantly more females (53%) than males (42%) consumed energy drinks. Other studies highlight the difference between sexes in favour of the male gender, which is more exposed to consumption (*Pettit & DeBarr*, 2011; *Bulut et al.*, 2014; *Friis et al.*, 2014; *Itany et al.*, 2014; *Vitiello et al.*, 2016).

Table 5: Frequency of energy drink consumption among energy drink consuming university students

Frequency	% males	% females	χ^2	p (sex)
> 1 bottle/month	7.7	8.4	0.03	0.85
1 bottle/month	57.7	72.5	4.85	< 0.05
>1 bottle/week	15.4	7.6	3.11	0.07
1 bottle/week	9	8.4	0.02	0.88
> 1 bottle/day	3.8	2.3	0.42	0.51
1 bottle/day	6.4	0.8	5.59	< 0.05

$n = 78$ males, 131 females, $\chi^2(1, N = 209)$

Table 6 presents the data on genders regarding the main reason of energy drink consumption. Differences between the sexes were not statistically significant for this issue. The same results were obtained for American students by *Malinauskas et al.* (2007).

Table 6: Situation of energy drink consumption among energy drink consuming university students

Situation	% males	% females	χ^2	p (sex)
To stay awake for hours	16.7	10.7	1.55	0.21
Good taste	59	55	0.32	0.57
Energy need	9	11.5	0.31	0.57
Reduce fatigue	7.7	15.3	2.57	0.10
Studying	3.8	3.8	0.00	0.99

$n = 78$ males, 131 females, $\chi^2(1, N = 209)$

Differences between the sexes regarding the preferences for the place of consumption were observed (*Table 7*), females opting more often to consume energy drinks in bars while males in dormitories.

Table 7: Place of consumption of energy drinks among energy drink consuming university students

Place of consumption	% males	% females	χ^2	p (sex)
Bar	26.3	46.6	8.27	< 0.01
At home	19.7	13	0.42	0.51
In dormitory	32.9	13	11.79	< 0.001
Coffee shop	7.9	5.3	0.53	0.46
At work	2.6	2.4	0.02	0.87
At university	2.6	3.8	0.20	0.64

$n = 104$ males, 145 females, $\chi^2(1, N = 207)$

Effects of consumption on blood pressure and heart rate

The baseline characteristics of volunteers are listed in *Table 8*. Originally, five female and seven male volunteers were involved in the study, but complete datasets were obtained only for five participants from each gender. The mean age of the volunteer students was 21.4 ± 2.31 years. Nine of them had normal body mass index (BMI), and one was overweight.

Table 8: Study subjects

Parameters	Volunteer Group
Sex	
Female	5
Male	5
Age, years	21.4 ± 2.31
Height (m)	1.74 ± 0.075
Weight (kg)	70.3 ± 10.73
BMI	
> 24.9	1
18.5–24.9	9
< 18.5	0

Table 9 describes the blood pressure (SBP, DBP) and heart rate (HR) parameters observed during the experiment. All parameters were recorded in triplicate before and after energy or control drink consumption. Statistically significant changes were observed in heart rate in the case of the control drink, whereupon 20 minutes after consumption the heart rate declined (*Fig. 1*).

The second energy drink (Drink 2) used in the experiment caused statistically significant increase in volunteers' systolic blood pressure (*Table 9, Fig. 1*).
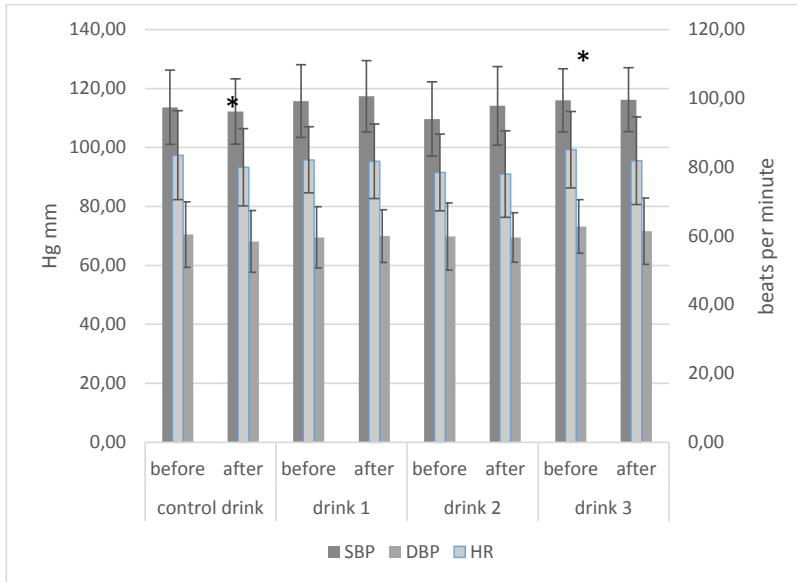
Figure 1: Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) among participants (n = 30, 10 volunteers, 3 repeats)

* Significance (at $p < 0.05$) was only stated in the case of HB (decreased) in control drink and in the case of SBP in Drink 2 (increased)

Similarly to our findings, other studies report no significant difference in heart rate after energy drink consumption (*Shah et al.*, 2016; *Nowak et al.*, 2018). In contrast with our data, a significant heart rate decline was observed by *Hajsadeghi et al.* (2016) in the case of 44 volunteer students from Iran after consuming energy drinks.

Significant increase in systolic blood pressure (SBP) was observed in the case of 38 German students after 1 hour of 750–1000 ml energy drink administration (*Basrai et al.*, 2019) and 34 Canadian volunteers after 30 minutes of 500 ml energy drink consumption (*Shah et al.*, 2019), which is in accordance with our results for Drink 2. No significant changes in systolic blood pressure was observed by *Hajsadeghi et al.* (2016) and *Nowak et al.* (2018), whereas the latter study reported a significant increase in diastolic blood pressure after the consumption of three doses of energy drinks.

Table 9: Blood pressure and heart rate data recorded during the experiment

Parameters	Control drink (n = 30, 10 volunteers, 3 repeats)			Drink 1			Drink 2			Drink 3		
	Before ED	After ED	p	Before ED	After ED	p	Before ED	After ED	p	Before ED	After ED	p
SBP (mm Hg)	113.63 ± 12.58	112.20 ± 11.08	0.349	115.77 ± 12.31	117.40 ± 12.12	0.293	109.67 ± 12.57	114.13 ± 13.33	< 0.05	116.03 ± 10.73	116.20 ± 10.85	0.240
DBP (mm Hg)	70.47 ± 11.12	68.13 ± 10.45	0.302	69.50 ± 10.39	69.93 ± 8.96	0.681	69.80 ± 11.37	69.47 ± 8.4	0.820	73.20 ± 9.1	71.60 ± 11.28	0.261
HR (beats per minute)	83.47 ± 12.95	79.97 ± 11.21	< 0.05	82.13 ± 9.63	81.70 ± 10.85	0.743	78.47 ± 11.19	77.97 ± 12.60	0.611	85.10 ± 11.13	81.90 ± 12.74	0.093

Data on both increase and no difference in blood pressure and heart rate were published in the literature, being highly influenced by the amount of energy drink consumed and the timing of measurements after consumption.

4   Conclusions

Energy drinks are popular among the examined faculty students; as shown in our study, 87.1% of the students consumed ED at least once. Two-thirds of the consumers use one bottle per month – significantly more females, while significantly more males consume ED with daily frequency. Students consume energy drinks mainly for its taste and very rarely to enhance learning efficiency. Differences between the sexes in consumption place preferences were observed: females preferring bars while males dormitories.

We demonstrated the increase in systolic blood pressure (SBP) in young and healthy volunteer students for one type of energy drink. Other changes in blood pressure and heart rate were not observed. Due to the fact that the experiment depends on the type and quantity of energy drinks and exposure time, future investigations are necessary on a larger population.

Acknowledgements

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