LWT - Food Science and Technology 154 (2022) 112895

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Duvan chvarci: Product characterization and comparison between traditional and industrial production

Mirjana Grujović [a,b,*], Tanja D. Žugić Petrović [b], Katarina G. Mladenović [a,b], Vladimir M. Tomović [c], Sunčica D. Kocić-Tanackov [c], Teresa Semedo-Lemsaddek [d]

[a] University of Kragujevac, Institute for Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia
[b] University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia
[c] University of Novi Sad, Faculty of Technology, Department of Food Preservation Engineering, Boulevard of Cara Lazara 1, 21000 Novi Sad, Serbia
[d] CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

ARTICLE INFO

Keywords:
Traditional meat product
Duvan chvarci
Sensory profile
Physicochemical and technological features
Microbiota

ABSTRACT

This research aimed to investigate and compare traditional products called "*duvan chvarci*" produced using pork meat and fat, originated from local households and industries. Physical and chemical analysis demonstrated differences among the examined products, mostly in total chloride content and TBARS values. Samples collected at local households showed finer color (higher lightness and yellowness) and sensory properties (rated as "extremely acceptable"), while industrial products were rated between "very acceptable" and "extremely acceptable". Microbiological analysis exhibited that enterobacteria, coagulase-positive staphylococci, *Salmonella* spp., *Shigella* spp., and fungi were absent from all meat products. Dominant microbiota was identified as *Lactobacillus* spp. and *Staphylococcus* spp. All tested isolates showed γ-haemolysis on blood agar plates. Tested *Staphylococcus* spp. were sensitive to novobiocin while *Lactobacillus* isolates demonstrated sensitivity to ampicillin, chloramphenicol, clindamycin, erythromycin, tetracycline, gentamycin, and streptomycin. None of the tested isolates showed full resistance to antibiotics. Overall, results indicated that *duvan chvarci* is a microbiologically safe product and provided the initial evidence regarding the physical, chemical, technological, and sensory properties of this widely consumed product in the Balkans.

1. Introduction

Traditional meat products are produced on a small scale, using ingredients and procedures from ancient times. Although these foods are associated with highly appreciated organoleptic features and nutritional value, producers must be able to satisfy consumer's expectations. Nowadays, the high demanding food market is quite challenging regarding sensory and nutritional aspects, as well as safety.

The production of traditional meat products has considerable potential in the Republic of Serbia and the Balkan peninsula, particularly in individual households (Karabasil et al., 2018). As a general rule, meat products are defined as foods that consist of or contain meat (Laranjo et al., 2017). Among artisanal meat products, the pork meat snack called "*chvarci*" (in Serbian: *чварци*) is greatly popular for consumption and trade. *Chvarci* are sometimes internationally recognized as pork rinds, or pork scratchings, but with a certain difference in texture and taste. Similar meat products are commercialized in Europe, USA and in some Asian countries. In the United Kingdom and USA, a similar product is sold as a snack, and it is known as "pork scratchings" or "cracklings". In Australia and New Zealand, crispy pork rinds are sold as "pork crackles". In Thailand, a similar product is called *"Kaeb Moo"* (Kitpot al., 2019; Sriwattana et al., 2012).

In Serbia, *chvarci* are made from pork meat and fat in a traditional way, by melting and frying the meat in the melted fat. There are mainly three types of *chvarci*, and overall variations depend on the size of each meat segment: large pieces, small pieces, or pieces like tiny threads, which are called in Serbian the "*duvan chvarci*". These products should demonstrate natural color, no detection of overburnt parts and rancidity, no contaminants (e.g. hairs or pebbles), must be crispy and have a distinctive odor and taste. The indications of *duvan chvarci* have been registered and protected in the Serbian intellectual property office (The Official Rule of RS,50/2019). In the Balkans, *duvan chvarci* are

* Corresponding author. University of Kragujevac, Institute for Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia.
E-mail address: mirjana.grujovic@pmf.kg.ac.rs (M. Grujović).

https://doi.org/10.1016/j.lwt.2021.112895
Received 6 September 2021; Received in revised form 23 November 2021; Accepted 29 November 2021
Available online 30 November 2021

traditionally manufactured in autumn and consumed throughout the winter. This foodstuff may be eaten on their own as a snack, served with a heated fruit brandy called "*rakija*", typical for the same region or as an ingredient in other food recipes (such as "*proja*" baked with *chvarci*) (The Official Rule of RS,50/2019).

The Serbian meat product *duvan chvarci* is often produced in local households, using ancient recipes. These meat products are widely accepted by consumers, leading to higher market demands, which could not be met by artisanal producers. Hence, at the present time, *duvan chvarci* is produced in local industries as well. The main differences between traditional and industrial production processes could be associated with stricter production conditions, applied in each industrial process stage (exact process time, temperature, sanitary condition, etc.), while artisanal production is more empiric and variable. Additionally, the demands regarding pig nutrition are higher in the industrial production. There is an assumption that the meat industry cannot respond to increases in demand, since increased production volume affects product's quality.

Since there is no scientific information about *duvan chvarci*, the aims of this research project were to investigate physicochemical, technological, and sensory characteristics, as well as microbiological properties, and to identify the dominant microbiota present in *duvan chvarci*. Moreover, this study further compared *duvan chvarci* produced in local households to those in meat industries, in order to assess whether massive production loads have an impact on the quality and acceptance of the product.

2. Material and methods

2.1. Preparation of traditional and industrial duvan chvarci

The *duvan chvarci*is traditionally made from Serbian pork meat, using specific procedures, which lead to particular organoleptic features. The artisanal manufacturing procedure consists of the following steps: the pork meat from the back is removed along with the fat, but without skin, and cut into large squares of about 6 × 6 cm in size. The shredded meat and fat are put into the container of an appropriate size, not overcrowded. Half a liter of water is added for each 8-liter of the container. A large mixing wood spoon is put in the middle of the container and the fire is lit. When the wooden spoon falls to the bottom of the container, the boiling mass needs to be mixed. At this point the added water has evaporated and the fat has melted, thus mixing has to be vigorous and constant, in order to avoid over burn. This process can take up to 2 h, on a moderate fire. This process will make the meat and fat characteristic texture, which can be finely chopped with a wooden spoon. At the end of the process the fat should be carefully drained (by gently pouring it with a spoon into a gauze), while the *duvan chvarci* remains in the pan. Subsequently, the same gauze is used to squeeze the *duvan chvarci* with a press. Once all the excess fat has been removed, the *duvan chvarci* returns to the pan and is submitted to heat and vigorous mixing for an extra 15 min, to obtain the beautiful characteristic golden color. After cooling, salt is added and the mixture in hand-homogenized.

For industrial production only carcass meat from domestic pigs (over 150 kg) is used. The animals are submitted to a precise diet, which means that protein components should not contain fishmeal, and the vegetable ingredients have to be originated from a specific geographical region. Slaughtering and primary cooling (refrigeration until 7 °C) must be performed in registered facilities, taking into account good manufacturing practice (GMP), hygiene practice (GHP), sanitary standard operating procedures (SSOP) and the application of the concept of hazard analysis and critical control points (HACCP) (The Official Role of RS no. 91/2005, 30/2010, 93/2012 and 17/2019; The Official Role of RS, no. 50/2019).

Industrial procedure consists of the following steps: inspection, selection, and preparation of raw materials for melting; wetting of raw materials (50 L of water/100 kg of raw material); melting and frying in specific machines; straining and pressing; scattering and forming a thin layer; salting with1.8 ± 0.2% of salt; cooling to 4 °C; storing at ≤ 7 °C and appropriate packaging with a proper labeling. The final product must present a fibrous appearance and a light brown-yellow color.

For the present study, four types of samples were collected from southeastern Serbia: two artisanal, first from a local household near the city of Niš (Kamenica village (sample DCh1) and another from the Knez village (sample DCh2); while the two industrial samples were produced in meat industries from the city of Niš ("Mak International" (sample iDCh1) and "Dakom" (sample iDCh2)). The samples were collected in triplicate for every location. All samples, weighing 600 g, were aseptically collected and stored at −18 °C and analyzed under 72 h.

2.2. Physicochemical properties of duvan chvarci

2.2.1. Determination of water activity (a_w)

Water activity (a_w)was determined using a Testo 650 (Testo, Inc., 40 White Lake Rd, Sparta, NJ, USA) with a special probe. The procedure included filling the coarse chopped crackling sample into a measuring cup up to 2/3 of its height, placing it in the measuring part of the probe at constant room temperature (≈20 °C) until the equilibrium was reached, *ca*2 hours. The test was performed on three samples from each location in duplicate.

2.2.2. Determination of water content

Water content was determined by applying SRPS ISO 1442 (1997). The determination procedure consisted of the complete mixing of the homogenized test sample with quartz sand and drying to a constant mass at 103 ± 2 °C. A TGA701 thermogravimetric analyzer (LECO Corporation, St. Joseph, Michigan, USA) was used to determine the water content of individual crackling (expressed as a percentage). The test was performed on three samples from each location in duplicate.

2.2.3. Determination of total ash content

Total ash content was determined by applying SRPS ISO936 (1999). The determination procedure consists of measuring the cold residue, after drying, carbonizing, and annealing part of the test sample at 550 ± 25 °C, to a constant mass. A TGA701 thermogravimetric analyzer (LECO Corporation, St. Joseph, Michigan, USA) was used to determine the ash content of an individual crackling. Simultaneous analysis of moisture and ash content takes about 5 h, amid which the device automatically determines these parameters, while maintaining the appropriate temperature until a constant mass is reached. The total ash content of the sample is expressed in percent (%). The test was performed on three samples from each location in duplicate.

2.2.4. Determination of total nitrogen and total protein content

Non-protein nitrogen (NPN) content was attained by determining the total nitrogen content of filtrate, according to UNI ISO 937 (1991) by *Kjeldahl* method, after quantitative protein precipitation of 10% trichloroacetic acid (TCA) solution. Specifically, 10 g of the crackling sample was homogenized with 20 ml of 10% TCA using T18 Basic Ultra Turrax (IKA-Werke GmbH & Co. KG) for 60 s at 13 500 rpm. After the homogenization, samples were left for 2 h at 4 °C for precipitation. After filtration the nitrogen content of10 ml of the filtrate was determined by the *Kjeldahl* method. NPN content in the sample is expressed in mg/kg of dry matter (calculated by the previously described SRPS ISO 1442 (1997) method).

Total protein content was determined based on total nitrogen (TN) content, by applying UNI ISO 937 (1991) method by *Kjeldahl*, multiplied by a factor of 6.25. The determination principle consists of digesting the sample with concentrated sulfuric acid, using copper (II) sulfate as a catalyst to convert the total nitrogen into ammonium ions ($(NH_4)_2SO_4$). This is followed by alkalization with sodium hydroxide, distillation of the liberated ammonia into the excess boric acid solution, and titration with hydrochloric acid to determine the ammonia bound to boric acid.

Protein content of the sample is expressed in percentage (%). The test was performed on three samples from each location in duplicate.

2.2.5. Determination of free fat content

Free fat content was extracted from the sample using petroleum ether by Soxhlet apparatus (SRPS ISO 1444:98). The free fat content of the samples was expressed in percentage (%). The test was performed on three samples from each location in duplicate.

2.2.6. Determination of chloride content (NaCl)

Total NaCl content was determined using a standard reference method (SRPS ISO 1841–1:1999c). The results were expressed in percentage (%). The test was performed on three samples from each location in duplicate.

2.2.7. TBARS determination

TBARS (2-thiobarbituricacid reactive substances) test was performed according to the method of Botsoglou et al. (1994), with modifications described by Šojić et al. (2015). TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample (mg MDA/kg). The TBARS test was performed on three samples from each location in duplicate.

2.3. Technological properties of duvan chvarci

2.3.1. pH determination

Sample pH was measured using a Testo 205 portable pH meter (Testo AG, USA) equipped with a combined penetration tip, harboring a temperature probe. The pH value was measured in three samples from each location in duplicate.

2.3.2. Color determination

The color of each sample was measured immediately after slicing, according to the procedure described by Tomović et al. (2013). Color characteristics were expressed in the International Commission on Illumination (CIE $L^*a^*b^*$system). Based on the measured color parameters, and using mathematical formulas, the following color characteristics were determined:

- hue (h) = arctan (b*/a*)
- color saturation $(C^*) = ((a^*)^2 + (b^*)^2)^{0.5}$

Ten replicated measures of surface color were performed, and results were presented as mean values ± standard deviation.

2.4. Sensory analysis of duvan chvarci

Sensory analysis of four different samples of *duvan chvarci* was performed with the commission of 20 trained individuals', representing distinct age groups. The laboratory for sensory analysis at Faculty of Technology in Novi Sad was designed according to Radovanović and Popov-Raljić (2001). A point system of analytical descriptive tests with a scale from 0 to 5 was used, where each evaluation represents a certain level of quality. Thus, the lowest grade 0 represents products with visible mechanical and/or microbiological damage, grade 1 represents altered and atypical properties of an unacceptable product, grade 2 represents pronounced to very pronounced defects and shortcomings of product quality, grade 3 represents certain defects and defects of quality, grade 4 represents noticeable deviations or slight defects in quality, while grade 5 represents exceptional, typical sensory properties and optimal level of quality. Individuals were asked to evaluate the following features: external appearance and coating, appearance and composition of the cross-section, color and sustainability of the color, odor and taste, texture, and juiciness. Each of these features was rated from 1 to 5, as previously described, and the score was shown as mean value from 20 repetitions.

2.5. Microbiological analysis of duvan chvarci

The microbiological testing was conducted according to the following methods: SRPS EN ISO 4833-2 (2017), for the total number of aerobic mesophilic bacteria;ISO 21528-2 (2017), for the total number of enterobacteria; SRPS EN ISO 6888-2 (2009), for the detection of coagulase-positive staphylococci; ISO 13721 (1995), for detection of lactic acid bacteria (LAB); and SRPS ISO 21527-1 (2011) for the detection of yeasts and molds. The detection of species from the genera *Salmonella* and *Shigella* was conducted by using SS agar (Torlak, Belgrade, Serbia) (Mokhtari et al., 2012).

Preliminary identification of the *Staphylococcus* isolates to the genus level was performed as follows: Gram-straining, morphological characteristics, catalase and coagulase assays, the ability of growth in nutrient broth at different temperatures (15 °C and 45 °C), nitrate reduction, hydrolysis of arginine and esculin, haemolysis on blood agar plates, fermentation of selected sugars, synthesis of exopolysaccharides (EPS), lipolytic activity, proteolytic activity (Kaban & Kaya, 2008). Isolated *Staphylococcus* were further identified, to the species level, using API Staph (BioMerieux, Montalien-Vercien, France) tests.

Preliminary identification of lactic acid bacteria (LAB) to the genus level occurred as follows: growth at 15 and 45 °C in MRS broth, growth at 4 and 6.5% NaCl in MRS broth, production of carbon dioxide from glucose by subculturing the isolates in MRS broth with Durham's tubes, growth, and production of slime on MRS agar with sucrose (20.0 g/L), L-arginine and esculin hydrolysis (Isenberg, 1992; Mundt, 1986; Prescot et al., 1996, pp. 685–688). Isolated *Lactobacillus* strains were further identified, to the species level, using API 50CH (BioMerieux, Montalien-Vercien, France) tests.

Final microbial identification was achieved using MALDI-TOF mass spectrophotometry, as described in Muruzović et al. (2018). The results were expressed by MALDI-bio Typer matching scores (ranging from 0.000 to 3.000), which indicated the similarity of the unknown bacterial profile to available profiles on the MALDI-bio Typer software database. Matching score values ≥ 2.00 were taken as correct identification to the species level.

2.5.1. Safety aspect of tested isolates

In order to evaluate the safety aspect of tested isolates, their ability of synthesizing extracellular proteins, named haemolysins, on blood agar plateswere investigated (Yasmin et al., 2020). Resistance of isolated CNS strains to novobiocin (30 μg) has been examined using the disc diffusion method (Bauer et al., 1966). *Lactobacillus* isolates were tested on their sensitivity to ampicillin, chloramphenicol, clindamycin, erythromycin, tetracycline, gentamycin, kanamycin, and streptomycin (Sigma-Aldrich, St. Louis, MO, United States) by using the microdilution method with resazurin, where the minimum inhibitory concentration (MIC) was determined (Sarker et al., 2007). The antibiotics were in concentration range from 0.06 to 128 μg/mL.

2.6. Statistical analysis

All data were presented as means ± standard deviations, using Microsoft Excel (Redmond, Washington, DC, USA). Paired sample T-test (IBM SPSS Statistics 20) was used for comparison among *duvan chvarci* from distinct productions. A principal component analysis (PCA) was used to reveal the associations of the different physical and chemical compositions and TBAR values among *duvan chvarci* samples.

3. Results

3.1. Physicochemical and technological properties of duvan chvarci

The differences in physical and chemical characteristics among different types of *duvan chvarci* are presented in Table 1. The total water, ash, chloride, and nitrogen contents were higher in samples iDCh1 and

Table 1
Physicochemical properties of *duvan chvarci*.

Parameters	*Duvan chvarci* samples			
	iDCh1	iDCh2	DCh1	DCh2
Water activity (a_w)	0.84 ± 0.00^a	0.86 ± 0.00^a	0.87 ± 0.00^a	0.80 ± 0.01^a
Total water content (%)	32.6 ± 0.13^a	32.0 ± 0.10^a	25.7 ± 0.05^b	17.6 ± 0.09^c
Total ash content (%)	5.9 ± 0.01^a	5.3 ± 0.01^a	2.9 ± 0.04^b	3.0 ± 0.00^b
Total nitrogen content (mg/kg)	3.63 ± 0.13^a	2.89 ± 0.10^b	0.70 ± 0.00^c	2.36 ± 0.07^d
Total protein content (%)	34.6 ± 0.24^a	35.1 ± 0.23^a	46.6 ± 0.45^b	44.4 ± 0.10^c
Free fat content (%)	22.9 ± 0.02^a	24.0 ± 0.30^b	24.7 ± 0.01^b	32.5 ± 0.19^c
Total chloride content (%)	3.87 ± 0.05^a	3.61 ± 0.02^a	1.88 ± 0.01^b	1.60 ± 0.03^b
TBARS (mg MDA/kg)	0.759 ± 0.005^a	0.664 ± 0.013^a	2.000 ± 0.014^b	2.788 ± 0.010^b
pH values	6.16 ± 0.04^a	6.25 ± 0.05^a	5.92 ± 0.07^b	5.9 ± 0.10^b

Values are presented as mean ± SD; means ± SD with different superscript letters in the same row differ significantly ($P < 0.05$).

iDCh2 while total protein content was higher in DCh1 and DCh2 ($P < 0.05$).

There was not a significant difference between samples in water activity ($P > 0.05$), while free fat content depends on the type of the sample, presumably due to the fact that fat is added into the mixture throughout the artisanal preparation process.

Lipid oxidation was evaluated by determining the levels of TBARS (mg malondialdehyde/kg) assay (Table 1). In this study, the samples of DCh1 and DCh2 demonstrated a significant ($P < 0.05$) increased TBARS values compared to the samples iDCh1 and iDCh2. TBARS values were in the range of 0.664 up to 2.788 mg MDA/kg.

The pH values of investigated samples were between 5.9 and 6.25, with a significant difference between samples from local industry and those produced in local households regarded to pH values ($P < 0.05$).

Color is one of the most important parameters by which consumers evaluate meat and meat product's quality (Cachaldora et al., 2013). The color parameters, lightness (CIE L^* value), redness (CIE a^* value), yellowness (CIE b^* value), color saturation (CIE C* value), and metric hue (h^0 value) are shown in Table 2. As explained above, *duvan chvarci* must present a yellow or golden color, therefore, sample DCh1, produced in a local household, possessed best color properties, regarding lightness and yellowness.

The results of PCA for chemical characteristic and TBARS values of the examined samples of *duvan chvarci* are presented in Fig. 1. The first two Principal Components (PCs) were assumed with a total variation of 98.42%. Quantitative composition of water activity (WA), the total water content (TWC), total ash content (TAC), total protein content

Table 2
Color parameters of *duvan chvarci*.

Color parameters	*Duvan chvarci* samples			
	iDCh1	iDCh2	DCh1	DCh2
CIE L^* value	34.74 ± 6.00^a	40.08 ± 5.03^b	43.84 ± 3.13^c	38.86 ± 4.28^d
CIE a^* value	8.85 ± 1.95^a	10.02 ± 1.26^b	9.81 ± 0.71^b	10.58 ± 1.38^b
CIE b^* value	19.48 ± 3.95^a	23.13 ± 3.72^b	27.70 ± 2.20^c	25.42 ± 4.62^d
CIE C* value	21.43 ± 4.26^a	25.24 ± 3.69^b	29.40 ± 2.25^c	27.55 ± 4.75^d
h^0 value	65.53 ± 2.96^a	66.32 ± 2.99^a	70.46 ± 1.03^b	67.18 ± 1.76^{a,c}

Means ± SD of the surface of *duvan chvarci* with different superscript letters in the same row differ significantly ($P < 0.05$).

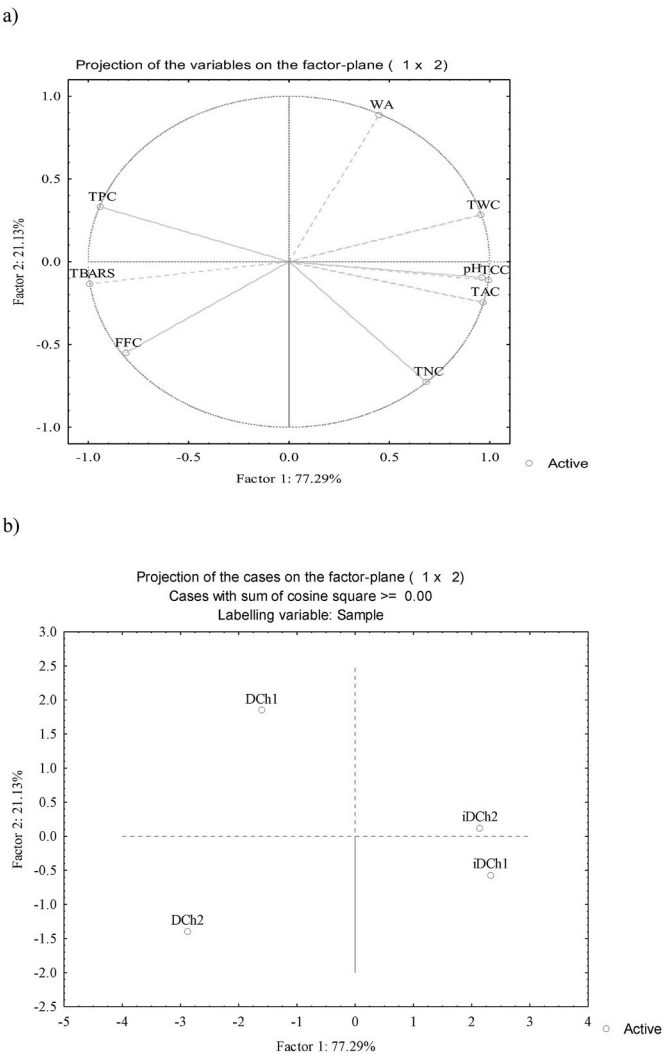

Fig. 1. a) Biplot for PCA model for the dependence of physicochemical characteristic and TBARS values in different *duvan chvarci* samples; b) Principal components based on physicochemical characteristic and TBARS values in different *duvan chvarci* samples.

(TPC), free fat content (FFC), total chloride content (TCC), total nitrogen content (TNC), pH and TBAR values correlated significantly in the samples of *duvan chvarci*. Factor loadings for TBARS (0.990), TPC (0.966) and FFC (0.812) had a positive value, while TCC (−0.993), TAC (−0.966), pH (−0.960), TWC (−0.957), TNC (−0.686) and WA (−0.449) had a negative value.

Therefore, PCs differentiate *duvan chvarci* based on chemical characteristics and TBARS values. Along with the Principal Component (1 and 2), axes exhibited that the *duvan chvarci* from local households (DCh1 and DCh2) differed from *duvan chvarci* from local industries (iDCh1 and iDCh2). The iDCh1 and iDCh2 samples from local industries were distinct by the highest values of pH, TCC, and TAC, while DCh1 and DCh2 samples from local households were differentiated by the highest values of TBARS. The pH, TCC, and TBARS were one of the examined compounds that contributed to the highest variability of *duvan chvarci* (Fig. 1a). As it can be seen in Fig. 1b, samples iDCHh1 and iDCh2 from local industries were much similar, compared to samples DCh1 and DCh2 from local households. Presumably this was a reflection of the specific production procedure, which is required to be followed in the industrial production.

3.2. Sensory analysis of duvan chvarci

Crispy and fried products are highly appreciated by consumers, due to crunchiness and typical aroma. The 5-point hedonic scale test was acquired for the evaluation of sensory characteristics towards the *duvan chvarci* samples. Overall liking, size, appearance, odor, texture, color, crispness, and taste of the samples were evaluated. The sensory panel results are shown in Fig. 2. The samples of *duvan chvarci* iDCh1 were quite uneven in the size, presented some moisture, were not crispy, too salty, and very dark. The *duvan chvarci* iDCh2 were quite uneven in size, there were many large pieces, they looked like *duvan chvarci* but with little moisture. The sensory panel described them as "very good". The *duvan chvarci*DCh1 and DCh2 were quite uneven in size, looked like *duvan chvarci*, but presented large pieces. The panel described them as "very good". Generally, products from local households rated with 5score, while products from the local industries were rated between 4 and 5. We assumed that *duvan chvarci* from the local industries, especially iDCh1 samples, demonstrated lover sensory scores since they were over fried, and their taste was not exceptional.

3.3. Microbiological analysis of duvan chvarci

Microbiological results are presented in Table 3. The total aerobic mesophilic bacteria ranged between 4.16×10^4 and 4.82×10^4 CFU/g. The total number of LABs ranged between 2.78×10^4 and 3.39×10^4 CFU/g. Since the coagulase-positive staphylococci were negative in the analyzed samples, the prevalence of coagulase-negative staphylococci (CNS) was reported. The number of CNS ranged between 3.23×10^4 and 3.72×10^4 CFU/g. Generally speaking, the number of every tested group of bacteria was higher in samples from local households (DCh1 and DCh2). No *Enterobacteriaceae*, yeasts or molds were detected in the samples under analysis.

Preliminary characterization and identification of staphylococci was performed by conventional microbiological tests. Among them, 83 isolates, which were Gram-positive, catalase-positive, and coagulase-negative, were further analyzed. Biochemical tests using API-Staph identified them as *Staphylococcus xylosus* and *Staphylococcus equorum* (results shown in Table 4). The identification was confirmed by MALDI-TOF mass spectrophotometry, with score values ranging between 2.089 and 2.391.

Table 3
Microbiological parameters in *duvan chvarci*.

Duvan chvarci samples	iDCh1	iDCh2	DCh1	DCh2
Total aerobic mesophilic bacteria	$4.19 \pm 0.34 \times 10^4$ [a]	$4.16 \pm 0.28 \times 10^4$	$4.63 \pm 0.48 \times 10^4$	$4.82 \pm 0.12 \times 10^4$
Total lactic acid bacteria	$3.10 \pm 0.62 \times 10^4$	$2.78 \pm 0.44 \times 10^4$	$3.39 \pm 0.52 \times 10^4$	$3.33 \pm 0.68 \times 10^4$
Coagulase-negative staphylococci	$3.23 \pm 0.12 \times 10^4$	$3.34 \pm 0.23 \times 10^4$	$3.63 \pm 0.26 \times 10^4$	$3.72 \pm 0.34 \times 10^4$
Total enterobacteria	n.d.	n.d.	n.d.	n.d.
Salmonella spp. and *Shigella* spp.	n.d.	n.d.	n.d.	n.d.
Yeasts and molds	n.d.	n.d.	n.d.	n.d.

[a] CFU/g of *duvan chvarci*, average values of three independent experiments; n.d. – microorganisms not detected.

Among all LAB isolates that were Gram-positive and catalase-negative, 80 of them were identified to the genus level, by using the tests which results are presented in Table 5. Preliminary identification to the species level was achieved using the API 50 C H. The isolates were identified as *Lactobacillus sakei* and *Lactobacillus curvatus* (results presented in Table 6), followed by confirmation using the MALDI-TOF mass spectrophotometry, with score values ranging between 2.15 and 2.384.

The results indicated that all tested isolates showed γ-haemolysis on blood agar plates. The results indicated the sensitivity of CNS isolates to novobiocin (inhibition zones from 22 to 28 mm). The sensitivity of *Lactobacillus* isolates to clinically relevant antibiotics was checked according to LAB resistance criteria proposed for antibiotics of human and veterinary importance by the European Food Safety Authority (EFSA). Tested *Lactobacillus* isolates were highly susceptible to chloramphenicol, clindamycin, tetracycline, gentamycin, erythromycin, and streptomycin (MIC ranged from <0.06 to 2 μg/ml). The resistance of these isolates was noticed against kanamycin (MIC ranged from 16 to > 128 μg/ml).

As referred above and shown in Table 7, among all *duvan chvarci* samples a total of 163 isolates were recovered, 80 belonging to the LAB group and 83 to CNS group. Generally speaking, the number of CNS was higher, compared to the number of LABs. When comparing samples, the number of bacteria recovered (LAB and CNS) was higher in samples from local households (DCh1 and DCh2), in comparison with industrial samples, probably due to the specific qualities of the production

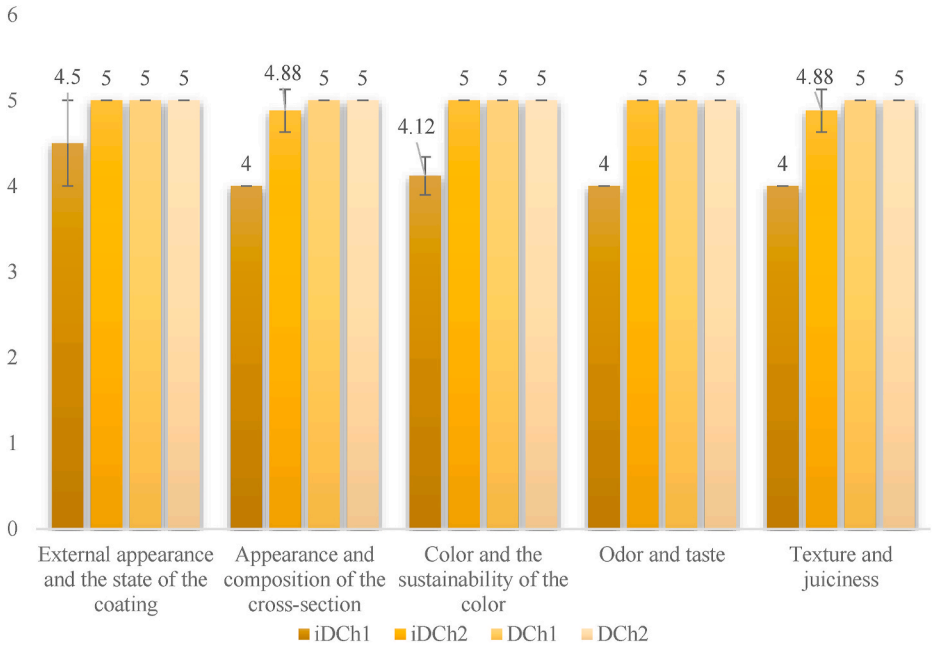

Fig. 2. Sensory quality properties of *duvan chvarci* (evaluated on 5-point hedonistic scale).

Table 4
Preliminary identification of CNS isolated from *duvan chvarci* by physiological characterization and API Staph test.

Tests	*Staphylococcus xylosus*	*Staphylococcus equorum*
Morphology	Cocci	cocci
Growth at 15 °C	+	+
Growth at 45 °C	+	+
Proteolytic activity	–	–
Lipolytic activity	+	+
Production of EPS	–	–
Alkaline phosphatase	+	–
Urease	+–	+–
Nitrate reduction	+	+
Glucose	+	+
Fructose	+	+
Lactose	+–	–
Maltose	+	+
Rafinose	–	–
Xylose	+	+
Mannose	+	+–
Mannitol	+	+
Trehalose	+	+
Sacharose	+	+
β-glucosidase	+	+
Hydrolysis of arginine	+	+
Hydrolysis of esculin	+	+
Coagulase	–	–

"+" - positive reaction; "– "– negative reaction; "+– "– weakly positive; "S" – sensitive.

Table 5
Physiological characteristics of LAB isolated from *duvan chvarci*.

Tests	*Lactobacillus sakei*	*Lactobacillus curvatus*
Morphology	Rods	rods
Growth at 15 °C	+	+
Growth at 45 °C	+	+
Growth in 4% NaCl	+	+
Growth in 6.5% NaCl	+	+
Hydrolysis of arginine	–	–
Hydrolysis of esculin	–	+
Production of CO_2	–	–
Black zone on bile esculin agar	–	–
Slime on MRS agar	–	–
Proteolytic activity	–	–
Lipolytic activity	–	–

"+" - positive reaction; "– "– negative reaction.

processes, as explained above.

4. Discussion

Duvan chvarci, a Serbian traditional product from pork meat and fat has not been investigated prior to this paper, therefore, these results are presented for the first time to the scientific community. Moreover, this study compares *duvan chvarci* produced in distinct settings, traditional households, and industries, as well.

Duvan chvarci is a specific product originated from Serbia and the Balkan Peninsula. There are some similar products in the world, but none possess the same features. For example, crispy pork rind, called *Kaeb Moo* from Thailand, consists of only skin layer or the subcutaneous fat (Sriwattana et al., 2012). The *duvan chvarci* consists of pork meat and fat, so this is the initial difference. The *Kaeb Moo* water and fat contents are between 0.3-2.3 and 20.0–36.5%, respectively, while the degrees of lipid oxidation were in the range of 0.10–2.57 mg MDA/kg; while the water content of *duvan chvarci* ranged between 17.6 and 32.6%, which is higher compared to *Kaeb Moo*. Free fat content of *duvan chvarci* ranged between 22.9 and 32.5%, similar to *Kaeb Moo.* Lipid oxidation of *duvan chvarci* ranged between 0.664 and 2.788 mg MDA/kg, which is also similar to *Kaeb Moo.* However, we do not have information about some other chemical components of *Kaeb Moo,* therefore, it may be concluded that the fat profile of the products is similar, while the water content differs.

Comparison between the chemical characteristics of *duvan chvarci* from local households and industry, led to the conclusion that samples iDCh1 and iDCh2, from local industries are much similar, compared to samples DCh1 and DCh2, from local households. The reason might be due to the fact that in the industries, the processing conditions must be defined and precisely followed, while in local households, conditions

Table 6
Preliminary identification of *Lactobacillus* spp. after API 50 C H test.

Characteristics	*Lactobacillus sakei*	*Lactobacillus curvatus*
Glycerol	–	–
Erythritol	–	–
D-arabinose	–	–
L-arabinose	+	+–
D-ribose	+	+
D-xylose	+	–
L-xylose	–	–
D-adonitol	–	–
Methyl-α-D-glucopyranoside	–	–
Methyl-β-D-xylopyranoside	–	–
D-galactose	+	+
D-glucose	+	+
D-fructose	+	+
D-mannose	+	+
L-sorbose	–	–
L-rhamnose	+	–
Dulcitol	–	–
Inositol	–	–
D-mannitol	+	–
D-sorbitol	+	–
N-acetylglucosamine	+	+
Amygdalin	+	–
Arbutin	+	+–
Salicin	+	+
D-cellobiose	+	+
D-maltose	+	+–
D-lactose (bovine origin)	+	+–
D-melibiose	+	–
D-saccharose	+	+–
D-trehalose	+	+–
Inulin	–	–
D-melezitose	+	–
D-raffinose	–	–
Amidon (starch)	–	–
Glycogen	–	–
Xylitol	–	–
Gentiobiose	+	–
D-turanose	+	+–
D-lyxose	–	–
D-tagatose	+	–
D-fucose	–	–
L-fucose	–	–
D-arabitol	–	–
Potassium gluconate	+	–
Potassium 2-ketogluconate	–	–
Potassium 5-ketogluconate	–	–

"+" - positive reaction; "– "– negative reaction; "+– "– weakly positive.

Table 7
Distribution of isolates among *duvan chvarci* samples.

Type of sample/ bacteria	iDCH1	iDCH2	DCH1	DCH2	The number of isolates
Staphylococcus xylosus	7	5	6	7	25
Staphylococcus equorum	12	14	17	15	58
Lactobacillus sakei	7	6	8	9	30
Lactobacillus curvatus	11	15	12	12	50
Total number in each sample	37	40	43	43	Total: 163

depend on the host who manufactures the *duvan chvarci*.

Based on the results obtained from the analysis of *duvan chvarci*'s color, it may be concluded that this parameter depends on the production process ($P < 0.05$). The lightness (CIE L^* value) and yellowness (CIE b^* value) depend on the time of cooking. The redness (CIE a^* value) is quite similar between samples, while color saturation (CIE C* value) and metric hue (h^0 value) also depends on the production process. Sample DCh1, produced in a local household, exhibited the best color properties, as explained in the result section.

There were many important sensory attributes linked with *duvan chvarci,* i.e. hardness, crispness, salty, crispy pork rind flavor, oil odor, oil flavor, burnt odor, and rancidity. The consumer acceptance test using a 5-point hedonic scale was carried out with respect to the external appearance and state of the coating, appearance and composition of the cross-section, color and viability of the color, odor, and taste, texture, and juiciness. Products from local households (DCh1 and DCh2 samples) were scored with 5, while products from local industry (iDCh1 and iDCh2 samples) were rated between 4 and 5. The consumers were not informed about the products or origin of what they were consuming (blind samples); however, they certainly recognized something that "looked like *duvan chvarci*", due to its specific and recognizable odor and taste.

Microbiological safety analysis indicated that *Enterobacteriaceae*, coagulase-positive staphylococci, yeast, and molds, as well as *Salmonella* spp., and *Shigella* spp. were absent from all samples. The total aerobic mesophilic bacteria ranged between 4.16×10^4 and 4.82×10^4 CFU/g. The total number of LAB ranged between 2.78×10^4 and 3.39×10^4 CFU/g. The number of CNS ranged between 3.23×10^4 and 3.72×10^4 CFU/g. The dominant microbiota among lactic acid bacteria was identified as *L. sakei* and *L. curvatus*, while among CNS, *S. xylosus* and *S. equorum* were the predominant species. Overall, the dominant microbiota in *duvan chvarci* is similar to other meat products (Alfaia et al., 2018; Geeraerts et al., 2020; Mrkonjic Fuka et al., 2020). Tested isolates showed γ-haemolysis on blood agar plates; CNS isolates were sensitive to novobiocin while *Lactobacillus* isolates demonstrated sensitivity to the most tested antibiotics (the exception was kanamycin), while full resistance was not noticed. Based on the results obtained, it may be concluded that products from local households and meat industries are both microbiologically safe for consumption, with a minor difference in quantitative, but not in qualitative microbiological community.

5. Conclusions

This research project evaluated for the first time the physicochemical, technological, and sensory properties, as well as the microbiological safety of traditionally and industrially made pork meat and a fat product called *duvan chvarci*. The results indicated significant differences between samples iDCh1 and iDCh2, from local meat industries, compared to samples DCh1 and DCh2, from local households, in chemical and technological parameters, as well as in sensory profile. In fact, samples iDCh1 and iDCh2 from local meat industries are more analogous to each other, probably due to similar production conditions. Traditional products are much different regarding chemical parameters, which can be explained by production process variations in the household. Besides manufacture specifications, industrial *duvan chvarci* are packed, while artisanal products are consumed within few days of production. Both products under analysis were considered to be microbiologically safe for consumption, dominant microbiota belonging to LAB and CNS. It is also relevant to highlight that the sensory panel attributed higher scores to the artisanal products.

Overall, the presented study leads to significant results. The first scientific report on differences between *duvan chvarci* produced in local households and in the meat industry, may be used to help establish specific parameters for product quality and development. Hence, further research should focus on the improvement of the production process in the industry (in order to meet consumer's demands) and shelf-life extension of traditionally made *duvan chvarci* through the investigation of packaging conditions. Moreover, the technological properties of LAB and CNS isolates need to be investigated, in order to evaluate putative usage in the meat industry as starter cultures.

CRediT authorship contribution statement

Mirjana Ž. Grujović: collected the samples of duvan chvarci; done microbiological analysis and statistics, Formal analysis. **Tanja D. Žugić Petrović:** and collected the samples of duvan chvarci; done microbiological analysis and statistics, Formal analysis. **Katarina G. Mladenović:** done microbiological analysis and statistics, Formal analysis. **Vladimir M. Tomović:** and done the chemical and sensory analysis, Formal analysis. **Sunčica D. Kocić-Tanackov:** done the chemical and sensory analysis, Formal analysis. **Teresa Semedo-Lemsaddek:** Supervision, All authors take charge of the design and preparation of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements –

This investigation was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-9/2021–14/200122). Teresa Semedo-Lemsaddek is financially supported by Portuguese national funds through FCT - Fundação para a Ciência e a Tecnologia under the Transitional Standard - DL57/2016/CP1438/CT0004.

References

Alfaia, C. M., Gouveia, I. M., Fernandes, M. H., Fernandes, M. J., Semedo-Lemsaddek, T., Barreto, A. S., & Fraqueza, M. J. (2018). Assessment of coagulase-negative staphylococci and lactic acid bacteria isolated from Portuguese dry fermented sausages as potential starters based on their biogenic amine profile. *Journal of Food Science, 83*, 2544–2549. https://doi.org/10.1111/1750-3841.14298

Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*, 493–496.

Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measurement of lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry, 42*, 1931–1937. https://doi.org/10.1021/jf00045a019

Cachaldora, A., Garcia, G., Lorenzo, J., & Garcia-Fontan, M. C. (2013). Effect of modified atmosphere and vacuum packaging on some quality characteristics and the shelf-life of "morcilla". *A Typical Cooked Blood Sausage. Meat Science, 93*, 220–225. https://doi.org/10.1016/j.meatsci.2012.08.028

Geeraerts, W., De Vuyst, L., & Leroy, F. (2020). Ready-to-eat meat alternatives, a study of their associated bacterial communities. *Food Bioscience, 37*. https://doi.org/10.1016/j.fbio.2020.100681

Isenberg, H. D. (1992). Clinical microbiology procedures handbook. *American Society for Microbiology, 1*(2), 1–18. Washington DC, USA.

ISO 13721. (1995). *Meat and meat products – enumeration of lactic acid bacteria – colony-count technique at 30 degrees C*.

ISO 21528-2. (2017). *Microbiology of the food chain – horizontal method for the detection and enumeration of Enterobacteriaceae – Part 2: Colony-count technique*.

Kaban, G., & Kaya, M. (2008). Identification of lactic acid bacteria and gram-positive catalase-positive cocci isolated from naturally fermented sausage (Sucuk). *Journal of Food Science, 73*(8), 385–388. https://doi.org/10.1111/j.1750-3841.2008.00906.x

Karabasil, N., Bošković, T., Tomašević, I., Vasilev, D., Dimitrijević, M., Katanić, N., & Antić, D. (2018). Production of traditional meat products in small and micro establishments in Serbia: Current status and future perspectives. *Acta Veterinaria Brno, 68*(4), 373–390. https://doi.org/10.2478/acve-2018-0031

Kitpot, T., Sriwattana, S., Angeli, S., & Thakeow, P. (2019). Evaluation of quality parameters and shelf life of Thai pork scratching "Kaeb Moo". *Journal of Food Quality*, 1–9. https://doi.org/10.1155/2019/2421708, 2019.

Laranjo, M., Talon, R., Lauková, A., Fraqueza, M. J., & Elias, M. (2017). Traditional meat products: Improvement of quality and safety. *Journal of Food Quality*. https://doi.org/10.1155/2017/2873793

Mokhtari, W., Nsaibia, S., Majouri, D., Ben Hassen, A., Gharbi, A., & Aouni, M. (2012). Detection and characterization of *Shigella* species isolated from food and human stool samples in Nabeul, Tunisia, by molecular methods and culture techniques. *Journal of Applied Microbiology, 113*(1), 209–222. https://doi.org/10.1111/j.1365-2672.2012.05324.x

Mrkonjic Fuka, M., Tanuwidjaja, I., Zgomba Maksimovic, A., Zunabovic-Pichler, M., Kublik, S., Hulak, N., Domig, K. J., & Schloter, M. (2020). Bacterial diversity of naturally fermented game meat sausages: Sources of new starter cultures. *LWT-Food Science and Technology, 118*. https://doi.org/10.1016/j.lwt.2019.108782

Mundt, J. O. (1986). In I. P. H. A. Sneath, N. S. Mair, M. E. Sharpe, & J. G. Holt (Eds.), *Bergey's manual of systematic bacteriology: Vol. 2. Lactic acid streptococci* (pp. 1064–1071). Baltimore: Williams and Wilkins.

Muruzović, M., Mladenović, K., Djilas, M., Stefanović, O., & Čomić, L. (2018). *In vitro* evaluation of antimicrobial potential and ability of biofilm formation of autochthonous *Lactobacillus* spp. and *Lactococcus* spp. isolated from traditionally made cheese from Southeastern Serbia. *Journal of Food Processing and Preservation, 42*(11), 1–10. https://doi.org/10.1111/jfpp.13776

Prescot, L. M., Harley, J. P., & Klein, D. A. (1996). *Microbiology* (3rd ed.). London: Wm. C. Brown Publishers.

Radovanović, R., & Popov-Raljić, J. (2001). *Sensory analysis of food products*. Novi Sad: Faculty of Agriculture, Belgrade, Faculty of Technology (in Serbian).

Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtitre plate based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods, 42*, 321–324.

Šojić, B., Tomović, V., Kocić-Tanackov, S., Škaljac, S., Ikonić, P., Džinić, N., Živković, N., Jokanović, M., Tasić, T., & Kravić, S. (2015). Effect of nutmeg (*Myristica fragrans*) essential oil on the oxidative and microbial stability of cooked sausage during refrigerated storage. *Food Control, 54*, 282–286. https://doi.org/10.1016/j.foodcont.2015.02.007

SPRS ISO 1444. (1998). *Meat and meat products - determination of free fat content*.

Sriwattana, S., Utama-ang, N., Thakeow, P., Senapa, J., Phimolsiripol, Y., Surawang, S., Pongsirikul, I., & Angeli, S. (2012). Physical, chemical, and sensory characterization of the Thai-crispy pork rind 'Kaeb Moo. *Chiang Mai University Journal of Natural Sciences Special Issue on Agricultural & Natural Resources, 11*(1), 181–191.

SRPS EN ISO 4833-2. (2017). *Microbiology of the food chain - horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 degrees C by the surface plating technique*.

SRPS EN ISO 6888-2. (2009). *Microbiology of the food chain - horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -Part 2: Technique using rabbit plasma fibrinogen agar medium*. ISO 6888-2: 1999.

SRPS ISO 1442. (1998). *Meat and meat products - determination of moisture content (Reference method)*.

SRPS ISO 1841−1. (1999c). *Meat and meat products – determination of chloride content – Part 1: Volhard method*.

SRPS ISO 21527-1. (2011). *Microbiology of food and animal feeding stuffs - horizontal method for the enumeration of yeasts and molds - colony count technique in products with water activity greater than 0.95*.

SRPS ISO 936. (1999). *Meat and meat products - determination of total ash*.

The Official Role of RS no 91/2005, 30/2010, 93/2012 and 17/2019 - Veterinary law.

The Official Rule of RS,50/2019 - Rules on the quality of ground meat, semi-finished meat, and meat products (in Serbian).

Tomović, V. M., Jokanović, M. R., Petrović, L. S., Tomović, M. S., Tasić, T. A., Ikonić, P. M., Šumić, Z., Šojić, B., Škaljac, S., & Šošo, M. (2013). Sensory, physical, and chemical characteristics of cooked ham manufactured from rapidly chilled and earlier deboned *M. semimembranosus*. *Meat Science, 93*, 46–52. https://doi.org/10.1016/j.meatsci.2012.07.015

UNI ISO 937. (1991). *Meat and meat products - determination of nitrogen content (reference method)*.

Yasmin, I., Saeed, M., Khan, W. A., Khaliq, A., Chughtai, M., Iqbal, R., Tehseen, S., Naz, S., Liaqat, A., Mehmood, T., Ahsan, S., & Tanweer, S. (2020). *In vitro* probiotic potential and safety evaluation (hemolytic, cytotoxic activity) of *Bifidobacterium* strains isolated from raw camel milk. *Microorganisms, 8*(3), 354.