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Organic and conventional milk – insight on potential differences

1. Introduction

The consumption of organic food, including fruits, vegetables, cereals and animal origin products showed an increasing trend in many markets, especially in the developed countries. This is associated with the fact that consumers believe organic food products are healthier, safer and better for the environment and animal welfare than conventional food. This perception justifies the premium price for organic food products (Aryal *et al.*, 2009; Willer and Schaack, 2015). Organic primary production is defined as a system of farm management and food production that combines best environmental practices, a high level of biodiversity, the sustainable cultivation systems, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes (EU, 2007; Serbia, 2010).

Organic foods of animal origin are obtained derived from animals which are fed using organic feed the whole year long. The organic dairy farming is perceived to be kinder to animals and environment, without the application of antibiotics, synthetic growth promoters and other synthetic chemicals during organic milk production (Galgano *et al.*, 2016; Schwendel *et al.*, 2015). It is important to keep good health of animals, applying different preventive and natural means of defense, such as choosing suitable breeds and rearing practices. The usage of immunological veterinary medicinal products such as phyto-therapy products or homeopathic products vaccines is allowed (Winter and Davis, 2006). It is of note that EU rules allow usage of synthetic chemical allopathic medicinal products, including antibiotics, under strict conditions, for the immediate treatment of an illness and to prevent the animal from suffering. If this is applied more than three treatments per year, milk derived from these animals are not to be sold as organic (EC, 2008; Schwendel *et al.*, 2015). The cattle feeding system must be based on maximum use of pasture and cattle must have access to pasture whenever possible. Minimally 60% of dry matter of feeding ration has to be roughage, fresh or dried fodder, or silage (EC, 2008). In organic dairy farming, the usage of genetically modified organisms and their products are prohibited. Products that should be used for cleaning and disinfection should have minimal effect on the environment and dairy production. To sell organic milk and to use the organic logo, farmers must be certified (EU, 2007; Serbia, 2010).

Composition of bovine milk is influenced by many different factors, and it is dependent on both individual animal and environment, such as feed, breed, management, season, etc. Many different studies have been investigating if there is a difference in milk composition and quality between organic and conventional farming (Čuboň *et al.*, 2008; Ellis *et al.*, 2006; Mullen *et al.*, 2013; Müller and Sauerwein, 2010; Zagorska and Ciprovica, 2008; Zwald *et al.*, 2004). Nevertheless there is no clear conclusions that can be made (Schwendel *et al.*, 2015).

The aim of this study was to investigate if there is a difference in hygiene parameters of raw milk produced in organic and conventional farm of similar size. Additionally, we aimed to determine if there are differences in pasteurized organic and conventional milk samples delivered to the market.

2. Material and methods

2.1. Raw milk samples

Raw milk samples were analysed at the reception of dairy plant. The study involved samples of raw milk from one certified farm producing organic milk (ORM – organic raw milk) and

two farms producing only conventional milk (CRM – conventional raw milk). The organic herd size was 945 Holstein and Brown Swiss cows, while herd size was 550 and 690 Holstein cows for the two conventional herds. Mean milk yield per cow was 6,265 kg for the organic herds, and mean milk yield per cow was 6,869 kg (in the range 6,738–7,001) for two conventional herds. A total number of 1,076 samples were collected on a daily basis during the year 2014, with 237 samples of raw milk from organic farm and 839 samples of raw milk from two conventional farms. These samples were analysed for aerobic colony count (ACC), somatic cell count (SCC), acidity, temperature, fat and protein content.

Samples of raw milk were analyzed within the plant laboratory for the aerobic colony count (ACC) using the standard ISO 4833:2003. The results obtained for ACC in raw milk were expressed as log CFU/ml. Somatic cell count was determined using Fossomatic Minor (Foss, Denmark). The SCC was calculated using the formula $\log_2(\text{SCC}/100.000)+3$ to obtain the logarithmic values in linear score (Mullen *et al.*, 2013; Radostits *et al.*, 2006). The acidity of milk was analysed by titratable method and expressed in Soxlet-Henkel degrees (°SH). The milk fat content of liquid milks was determined according to the Gerber method (IDF, 2008). The proteins were analyzed by the Kjeldahl method (ISO, 2008). The significance of difference between means was analysed using t-test (Microsoft Excel 2007).

2.2. Pasteurized milk samples

Samples of organic and conventional pasteurized milk with 2.8% declared milk fat (CPM and OPM, respectively) were bought from the market in November 2015. The dairy factory that was involved in first part of this study was the producer of both types of pasteurized milk samples. In total three samples of CPM and three samples of OPM were randomly taken from the market and analyses were performed in duplicate. These samples were analysed for Raman spectroscopy, colour change and sensorial analysis.

2.3. Raman spectroscopy analysis

Raman spectra of OPM and CPM samples were collected by Raman microscope (HORIBA Jobin Yvon Xplora; HORIBA Jobin Yvon S.A.S., Villeneuve-d'Ascq, France). Excitation light of the laser (785 nm) was focused through the 10x objective and the scattered light was detected with thermoelectrically cooled CCD device (Syncerity, HORIBA Scientific, Edison, New Jersey, USA), operating at -60°C. Fifty spectra in the 600 – 3200 cm^{-1} Raman shift range were acquired for each sample, in order to capture variation due to potential micro domain inhomogeneity. Acquisition time for each spectrum was 60s. All measurements were conducted at room temperature. The Raman system was operated using LabSpec 6 software (Horiba Scientific, Villeneuve-d'Ascq, France).

Collected Raman spectra were pre-processed prior to comparison. First the cosmic spikes were removed with the dedicated feature in the LabSpec 6 software. To cancel out variations between the individual spectra the background due to fluorescence of the sample or fluctuation of the temperature on the CCD was removed employing a method based on the SNIP clipping algorithm (Morháč *et al.*, 1997) and then the spectra were normalised. Following that, the spectral regions 600 to 1850 cm^{-1} and 2600 to 3150 cm^{-1} were cut. Finally, in order to investigate the differences between the milk samples, mean spectra were compared by calculating difference spectrum. The statistical treatment of the data, except for cosmic spike removal, was conducted in R statistical software (R Development Core Team, 2014), employing “hyperSpec” package (Beleites and Sergo, 2009).

2.4. Determination of color by Computer vision system (CVS)

Visual color of samples was measured based on Hunter color parameters (L^* , a^* and b^*) using CVS. A digital camera featuring a 10.2 Megapixel CCD sensor was used for image acquisition. The camera was located vertically at a distance of 30 cm from the sample. The camera setting was the following: shutter speed 1/6s, manual operation mode, aperture Av F/11.0, ISO velocity 100, flash off, focal distance 30mm, lens DT-S18-70mm f 3.5-5.6. Lighting was achieved with four fluorescent lamps. The camera was calibrated with a 24 color chart X-Rite Colorchecker; the CCD sensor was adjusted using the standard colour rendition chart Colorchecker Passport (Michigan, USA).

The Colorchecker was photographed using the implemented CVS to obtain the input device RGB signals in the theoretical range of 0–255 (the RGB values are expressed as sRGB D65 and CIE Lab D50 2° observer). The monitor with a sRGB gamut (Standard RGB) was calibrated with X-Rite i1 Display Pro device by selecting white chromaticity at 6500 K (illuminant D65), gamma at 2.2 and white luminance at 140 cd/m² and the i1Profiler 1.5.6 software was used to create the ICC monitor profile. We employed the Adobe Photoshop CC (64 bit) software for image analysis. The L^* , a^* , and b^* values were measured on the digital image of the sample visualized on the monitor by pointing the cursor at the centre of the area (11x11 pixels) to be evaluated by clicking on it. The L^* , a^* and b^* values from RGB images were measured from RAW photographs.

2.5. Sensorial analysis

A total of 85 assessors (30 male and 55 female) participated in the triangle test for organic and conventional pasteurized milk samples. For one participant, the odd product was the same for the two replications, but the six triads (AAB, ABA, BAA, BBA, ABB and BAB) were counterbalanced over participants at each replication (ISO, 2004; Sauvageot *et al.*, 2012). Tests were carried in the Laboratory for Sensory analysis (Faculty of Agriculture, University of Belgrade), in darkened conditions. Samples were presented in white plastic cups and 50 mL of samples were presented in 100 mL cups. Cooling temperature of milk was 4°C. Samples were served immediately after opening.

Instructions to the participants included (i) a presentation of the task; (ii) the obligation to evaluate the samples in the imposed order; (iii) the obligation to give a response; (iv) the possibility to give their opinion of the degree of difference between the sample they chose and the others by circling one of the following descriptors which most closely describes the intensity of difference (“0” = none; “1” – very slight; “2” – slight; “3” – moderate; “4” – large; “5” – extreme).

For the similarity test, the percent correct above chance (symbolized by p_d) was fixed at 10%. When data are analyzed by replication, the critical number of correct responses to reject null hypothesis at $\alpha=0.05$ was obtained from the binomial distribution (Sauvageot *et al.*, 2012).

3. Results and discussion

3.1. Raw milk quality

The results obtained for raw milk produced in one organic and two conventional farms were presented in Table 1. Raw milk samples obtained from conventional farms were significantly lower in fat content than samples from organic farms ($P < 0.05$). The possible reason can be found in the diet composition in two farming system. Diet at the conventional farms consists of the greater percentage of concentrates, which is associated with the decline in milk fat concentration (Rosati and Aumaitre, 2004).w Additionally, the greater fat content in organic

farms could be related to the fact that organic herd consisted of Holstein and non-Holstein breeds (Brown Swiss), whereas conventional herds had mainly Holstein breeds (Nauta *et al.*, 2009). Our results are in line with those presented by Zagorska and Ciprovica (2008), for organic and conventional milk samples obtained in Latvia. Nevertheless, Müller and Sauerwein (2010) and Kuczyńska *et al.* (2012) reported that there is no difference in fat content related to organic or conventional production system in Germany and Poland, respectively.

Although our results indicated that raw milk samples produced in organic farm had greater percentage of protein compared to milk produced in conventional farms, this difference was not statistically significant ($P < 0.05$). Several studies reported that there is no difference in protein composition between organic and conventional raw milk samples (Müller and Sauerwein, 2010; Toledo *et al.*, 2002), although other studies showed variable results (Galgano *et al.*, 2016; Schwendel *et al.*, 2015). Composition of milk, including fat and protein content, is very dependent on the cow diet composition, breed, individual animal genetics, management, stage of lactation, and season (Coppa *et al.*, 2013; Larsen *et al.*, 2010), and therefore it is difficult to conclude that obtained differences in milk composition are attributed to the farming system alone.

The somatic cell count (SCC) in milk presents an important indicator for the udder health. The therapy to treat mastitis usually includes a large proportion of antibiotic drugs in conventional farms, while organic production should reduce the usage of antibiotics. It is of note that the specific rules and regulations on organic farming differ among countries (Schwendel *et al.*, 2015). Due to restrictions that the organic standards impose on treatment options for diseased animals, organic farms need to put more emphasis on prevention rather than treatment of diseases. To account for limited usage of antibiotics, organic production should include animal optimal care, adequate feed and housing to keep animals healthy. Our results indicated that SCC was significantly lower in the raw milk samples produced in organic farm compared to conventional farms (Table 1, $P < 0.05$). In organic herds, the milk yield was lower compared to conventional herds, which might explain obtained differences in SCC (Busato *et al.*, 2000). Other possible reason could be related to feeding practices, different attitude and disease management (Sundrum, 2001). Similar results to those obtained in this study were also reported in Sweden (Hamilton *et al.*, 2006; Toledo *et al.*, 2002), Norway (Garmo *et al.*, 2010), and Slovakia (Čuboň *et al.*, 2008). Within the current European and Serbian legislation, organic farmers can still manage mastitis due to the fact that cows can receive two conventional treatments per year and the treatment of a disease episode of mastitis is seen as one treatment procedure (EU, 2007).

The application of good hygiene practices is required at both farming systems. Nevertheless, our results indicated that parameters such as acidity of milk or “freshness” indicator, temperature and ACC for raw organic milk were significantly different from those obtained for conventional milk samples ($P < 0.05$). Although the temperature of both conventional and organic raw milk samples are in line with the current legislation (EU, 2004; Serbia, 2011), significantly lower temperature at the milk delivery was seen for organic milk samples ($<5^{\circ}\text{C}$) compared to conventional milk samples ($>7^{\circ}\text{C}$). Additionally, ACC for ORM samples were lower for approx. 1 log CFU/ml compared to CRM samples. This is related to the lower temperature that was observed for ORM samples, as the bacterial count in bulk milk is affected by the temperature (O’Connell *et al.*, 2016). Additionally, the difference might indicate better hygienic conditions during milking in organic farm, as ACC is a parameter usually associated with direct contamination in the dairy farm environment such as air, soil, workers hygiene, faeces, grass and excretion from the udder of an infected animal (Angulo *et al.*, 2009; Elmoslemany *et al.*, 2010; Murphy and Boor, 2000). Other points where

contamination of raw milk may occur are storage equipment in the farm, during transportation and at a dairy plant level (Millogo *et al.*, 2010).

The possible explanation for these differences might be found in the fact that selected organic farm was more frequently audited by certification body and dairy factory itself. It is of note that this farm is the only certified organic farm in the Republic of Serbia, and consequently more attention is paid from both farm owner and other stakeholders, to keep all hygiene parameters at the highest level possible.

3.2. Pasteurized milk quality

Many conventional farms of different sizes deliver raw milk to dairy factory that was involved in this study. All conventional raw milk deliveries are pooled together and processed into different dairy products, including pasteurized milk. As already mentioned, in Republic of Serbia currently only one farm is producing milk according to organic production rules, and therefore all milk collected from this farm is used for further processing into organic dairy products.

Along with the differences that might be seen in the raw milk quality among organic and conventional milk samples, more important is to determine if there are some differences in the final product that is available on the market. As pasteurized milk is one of the most important and most often consumed dairy products, it was selected as a product to determine possible differences among conventional and organic farming practices. In a selected moment, three pasteurized milk samples from conventional and three pasteurized milk samples organic farms were collected from retail and analyzed. All pasteurized milk samples had total bacterial count of <2.5 log CFU/ml (Smigic *et al.*, 2012). The study involved analysis Raman spectroscopy, color and sensorial analysis.

3.2.1. Raman spectroscopy

Mean spectra of OPM and CPM pasteurized milk samples as well as the difference spectrum (OPM - CPM) are shown in **Error! Reference source not found.** The Raman spectra of both organic and conventional milk comprise of bands positioned at 1748 cm^{-1} , 1658 cm^{-1} , 1551 cm^{-1} , 1445 cm^{-1} , 1299 cm^{-1} , 1265 cm^{-1} , 1130 cm^{-1} , 1008 cm^{-1} in low wavenumber window and at 2935 cm^{-1} , 2889 cm^{-1} , 2854 cm^{-1} in high wavenumber window. The peaks observed in the Raman spectra of milk samples belong predominantly to the lipid molecules. Bands at 1748 cm^{-1} , 1658 cm^{-1} , 1445 cm^{-1} , shoulder at 1299 cm^{-1} , the prominent peak at 1265 cm^{-1} and the one at 1130 cm^{-1} arise from C=O stretching vibration of carboxyl group, C=C cis double-bond stretching vibration of RHC=CHR, C-H scissoring of $-\text{CH}_2$, C-H twisting of the $-\text{CH}_2$ group, bending at the cis double bond in R-HC=CH-R and C-C stretching respectively (El-Abassy *et al.*, 2011; Gallier *et al.*, 2011). In the high wavenumber region, the peaks at 2854 cm^{-1} , 2889 cm^{-1} and 2935 cm^{-1} are attributed to CH_2 and CH_3 symmetric and anti-symmetric stretching (Gallier *et al.*, 2011). Additionally, carotenoid peak can be observed at 1551 cm^{-1} . Minor band at 1006 cm^{-1} comes from phenylalanine ring breathing mode – band attributed to proteins (Socrates, 2001).

Simple visual comparison of the mean Raman spectra gives sufficient evidence that the two spectra are almost identical. Moreover, the difference spectrum verifies such assertion, since the subtraction of two spectra would certainly highlight variations in the relative intensities between them. Even though no prominent difference features have been found, lower wavenumber region looks “flatter” than the higher, but this comes from more pronounced spectral noise from 2600 to 3150 cm^{-1} which in turn gives noisier higher wavenumber region.

3.2.2. Color change

Instrumental color differences were observed between OPM and CPM samples. All pasteurized milk samples originating from organic farm had a significantly higher L* value (95.00) than those samples from conventional farms (94.00), indicating that OPM is more white compared to CPM samples. This was unexpected, due to the fact that color of milk from cows fed silage and grain (conventional diet) seems to be whiter compared to cows fed with green pasture (organic diet) (Solah *et al.*, 2007). On the other side, Campbell *et al.* (2003) reported that milk fortified with Conjugated Linoleic Acid was less white than typical milk of the same fat concentration. They have explained this by the fact that fortified milk is high in unsaturated bonds, while typical milk is naturally rich in saturated fat, which is responsible for the whiter appearance. Therefore, the whiter color of OPM in this study might be related to higher percentage of saturated fatty acids (data not shown) compared to CPM. Our results indicated no difference among OPM and CPM samples regarding b* and a* values.

3.2.3. Sensorial analysis

A similarity test is used when the interest is to demonstrate that the test samples are indistinguishable. Its objective is to determine that no perceivable difference exists between two samples (ISO, 2004). All together the 170 responses led to 55 correct answers (31/85 in first replication and 24/85 in the second replication). According to the results, with 95% confidence no more than 10% of the population is able to detect a difference. Average rank for correct answers was (1.91 ± 1.04) with no statistically significant difference between correct / incorrect answers ($P < 0.05$). To account for obvious color difference among two milk types, as also confirmed by color analysis, which could results in incorrect results, the sensorial analysis was performed in darkened conditions.

4. Conclusion

The results observed in this study indicated that there are some differences in raw milk samples that have originated from organic or conventional farm, mainly related to fat content, SCC, acidity, temperature and ACC. The possible explanation for determined differences in acidity, temperature and ACC is related to hygienic conditions during milking in organic farm, as this farm is more frequently controlled and audited by both organic certification bodies and dairy companies itself. Additionally, there have been some differences in the pasteurized milk samples, mainly in the color of milk.

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Table 1. Raw milk composition of milk produced in organic and conventional farms

Parameter	Raw milk type (mean ± standard deviation)	
	Organic raw milk ORM	Conventional raw milk CRM
Fat (%)	3.80±0.13 ^a	3.72±0.19 ^b
Protein (%)	3.26±0.10	3.19±0.11
Non fat dry matter (%)	8.61±0.14 ^a	8.41±0.12 ^b
Titrateable acidity (SH)	6.01±0.07 ^a	5.80±0.21 ^b
Temperature (°C)	4.68±0.46 ^a	7.31±1.02 ^b
TPC (log CFU/ml)	4.13±0.33 ^a	5.00±0.21 ^b
SCC (log cells/ml)	4.51±0.26 ^a	5.56±0.42 ^b

^{a, b}Means within the same row with different letters are statistically different ($p < 0.05$).

Table 2. Color values (L*, a*, and b*) of OM and CM milk samples.

Colour parameter	Pasteurized milk type (mean ± standard deviation)	
	Organic pasteurised milk OPM samples	Conventional pasteurised milk CPM samples
L*	95.00±0.00 ^a	94.00±0.00 ^b
a*	0.00±0.00	0.00±0.00
b*	1.00±0.00	1.00±0.00

^{a, b}Means within the same row with different letters are statistically different ($p < 0.05$).

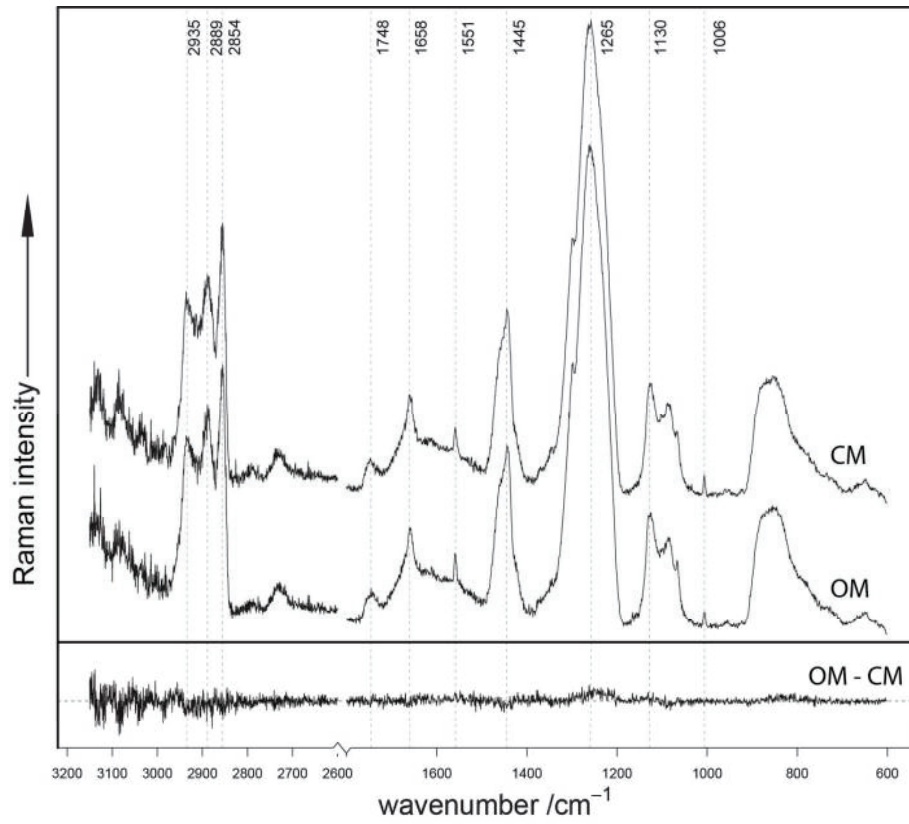


Figure 1. Mean spectrum of organic pasteurized milk (OPM) compared to mean spectra of conventional pasteurized milk (CPM) in order to investigate spectral differences by calculating the difference spectrum (OPM-CPM).