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ORIGINAL ARTICLE

A simple and fast method for discrimination of phage and antibiotic contaminants in raw milk by using Raman spectroscopy

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Abstract Phage and antibiotic in raw milk poses significant risks for starter culture activity in fermented products. Therefore, rapid detection of phage and antibiotic contaminations in raw milk is a crucial process in dairy science. For this purpose, a preliminary novel method for detection of phage and antibiotic was developed by using Raman spectroscopy. *Streptococcus thermophilus* phages and ampicillin which are quite important elements in dairy industry were used as model. The phage and antibiotic samples were added to raw milk separately, and Raman measurements were carried out. The obtained spectra were processed with a chemometric method. In this study, it has been demonstrated that the presence of phage has a titer sufficient to stop the fermentation (10^7 pfu/ml), and antibiotic in a concentration which inhibits the growth of starter cultures (0.5 µg/ml) in raw milk could be discriminated through Raman spectroscopy with a short analysis time (30 min).

Keywords Raman spectroscopy · Bacteriophage · Antibiotic · Raw milk · Chemometric analysis

Introduction

Phages and antibiotics are among the main factors hindering the quality achieved in the manufacture of fermented dairy products. Phages, which affect *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains used for the production of cheese and yoghurt are the main ones causing problems in the dairy industry. In the presence of these phages in the environment, they slow down or stop the fermentation. In other words, the quality of the fermented product is directly related to the existence of bacteriophages in dairy environment (Kleppen et al. 2011). They may cause considerable financial loss in dairy factories since 0.1–10% of milk fermentations in dairy plants can be adversely affected by phages (Moineau and Lévesque 2005). The same situation can be seen in the case of antibiotics in milk. Antibiotics have been commonly used in dairy animals to treat or prevent diseases such as mastitis (McEwen and Fedorka-Cray 2002). Residues of those antibiotics in raw milk and milk products may lead to serious problems related to human health. Furthermore, their effect on starter cultures may result in low quality products or fermentation failures (Brady and Katz 1988). The ampicillin breakpoints were defined by the European Food Safety Authority (EFSA) as 1 µg/ml for *L. bulgaricus* and 2 µg/ml for *S. thermophilus* (Bories et al. 2008).

A wide variety of methods can be used for detection of antibiotics and phage. Currently, the most reliable method for phage detection is the double layer agar method. However, this method involves long incubation period required for phage plaques to form and appear, low phage titer, or inability to observe a clear plaque formation. Therefore, rapid identification technique for phages was needed. Among these, there are some studies based on

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impedimetric change (García-Aljaro et al. 2009), PCR (del Rio et al. 2007; Binetti et al. 2008), real-time PCR (Ly-Chatain et al. 2011), imaging ellipsometry (Qi et al. 2009) epifluorescence and atomic force microscopy (Zago et al. 2012), and Surface-Enhanced Raman Scattering (SERS) (Chen et al. 2015a). As in phage, many techniques have been used to analyze antibiotic residues in milk, such as microbiological and bioassay techniques (Althaus et al. 2009; Nagel et al. 2013), voltammetric electronic tongue system (Wei and Wang 2011), immunoassay and immunochromatographical techniques (Taranova et al. 2015; Song et al. 2015), digital-image-based colorimetry (Urapen and Masawat 2015), and mass spectrometry (Han et al. 2015). Several rapid test kits such as Delvotest SP Kit, Copan test (CH ATK), Charm Farm Test, Charm AIM-96 and BRTAIM have also been developed and used in dairy industry for detection of antimicrobial residues in milk, and many of them are based on the inhibition of microorganism growth (Molina et al. 2003; NaVrátiloVá 2008). Although sensitive and reliable results are obtained with these methods, they require extensive sample preparation procedures and trained personnel, as well as expensive consumables. Thus, there is a growing need for rapid and reliable analysis methods, and one of these methods is Raman spectroscopy. Raman spectroscopy can be regarded as a fingerprint that can provide extensive information about the electronic environment, structure and bonding properties of molecules (Das and Agrawal 2011). Besides being rapid, cost-effective, and non-destructive for the samples, Raman measurements do not require long sample preparation processes (Boyaci et al. 2015).

In the literature, there are some studies which made use of Raman spectroscopy to monitor antibiotic and phage. Among them, studies having been carried out with phages were mostly related to structural changes (Rodríguez-Casado et al. 2001; Thomas Jr et al. 1982), genomic material (Incardona et al. 1987), or detection of phage amplification with a phage specific antibody presenting particle by using SERS (Voorhees 2014). Besides, the studies related with antibiotic were based on the detection of antibiotic residues in food samples by using SERS-active substrates (Chen et al. 2015b), differentiation of different/various penicillin, quantification of the level of penicillin in fermentation broths by using Raman spectroscopy and SERS (Clarke et al. 2005), and quantitative analysis of tetracycline with SERS- active micro-scale Au hollow spheres (Li et al. 2011). Although these studies provide an overall perspective, there is still a need for more detailed studies especially for the analysis of raw milk to be used in the production line and for distinction of phage and antibiotic in raw milk.

The aim of this study was to discriminate bacteriophage and antibiotic in raw milk. For this purpose, raw milk was contaminated with phages or antibiotic, and some pre-treatments were applied. After Raman spectra of the samples were collected and analyzed, phage and antibiotic samples were distinguished using a chemometric method, namely principal component analysis (PCA).

Materials and methods

Chemicals and growth media

Lactic acid (~90%), sodium dodecyl sulfate (≥99.0%), glycerol, 2-mercaptoethanol and ampicillin (anhydrous, 96–100%) were obtained from Sigma-Aldrich (St. Louis, MO); $CaCl_2$, $MgSO_4$, M17 Broth and Agar were purchased from Merck (Darmstadt, Germany). Modified M17 broth, modified M17 agar (1.5% w/v) and modified M17 soft agar (0.6% w/v) were used for titer detection of phages through using the double layer agar method (Acar-Soykut and Tunail 2015).

Bacterial cultures, phages and raw milk samples

The eight phages grew on their homologous commercial *S. thermophilus* host strain (B3, 231, 709) in modified M17 broth. All *S. thermophilus* host strains and Φ231-X9, Φ231-X21, Φ231-X23, ΦB3-X1, ΦB3-X11, 1ΦB3-X13, Φ709-X4, Φ1B3A were obtained from a previous study (Acar-Soykut and Tunail 2015; Kaleli et al. 2004). Φ709-B1 was taken from the culture collection unit of Hacettepe University, Food Research Center in Ankara, Turkey. The titers of phages were raised to approximately 10^8 pfu/ml by using the methods described in the literature (Acar-Soykut and Tunail 2015). The phages and cultures were kept at −20 °C in modified M17 broth containing 50% glycerol.

Eleven raw milk samples received from different dairy plants in Turkey were used in the experiments. One of them was chosen as the model raw milk sample. All phages and antibiotic were added into it in a concentration mentioned below. The other raw milk samples were used to verify the developed method.

Detection of antimicrobial activity of ampicillin on *S. thermophilus*

One of the commercial *S. thermophilus* strains (Chr. Hansen Lab., Denmark) was chosen to determine the antimicrobial activity of ampicillin; 100 µl bacteria was inoculated into 10 ml of modified M17 Broth, and then 100 µl 0.5 µg/ml ampicillin was added to the medium. As

a control group, 100 µl bacteria were inoculated into 10 ml medium without any antibiotic. Following this process, both the antibiotic-added and the antibiotic-free bacteria were incubated at 43 °C. For all the samples, 50 µl sample was taken every 30 min during the 5-h incubation, and they were diluted with 150 µl sterile modified M17 broth which were prepared as parallels. Absorption values of the samples at OD_{600} were recorded with an Agilent 8453 UV–visible spectrophotometer (Agilent Technologies, Inc. Santa Clara, CA) by using a micro cuvette cell.

Preparation of raw milk samples

In order to prepare raw milk samples, 5 ml raw milk was centrifuged at 10,000 rpm for 5 min at 4 °C for the separation of milk fat. After 300 µl 10% lactic acid solution was added to collapse casein micelles, milk was filtered using a coarse filter paper and then a 0.45 µm sterile syringe filter. For the present study, all of these pretreatments applied to the samples containing raw milk were called "milk pretreatments". All raw milk filtrates were prepared and collected in sterile tubes by using this procedure (Fig. 1S).

Control of raw milk samples for phage and antibiotic

Three strains of *S. thermophilus* (231, B3, 709) were selected as the test bacteria to control the phage presence in the raw milk samples. At this stage, 500 µl of raw milk filtrates were put into sterile Eppendorf tubes, and 200 µl of the host bacteria at log phase (OD_{600}) was added. After being left for 15 min for the adsorption, 1 ml of liquid medium was added, then it was left for 18 h at 43 °C for incubation. At the end of the incubation, 5 min centrifugation at 10,000 rpm was applied to remove cell residues and particles, and supernatants were collected. The phage enrichment process was repeated for three days. The spectrophotometric method was carried out with the same raw milk filtrate that is thought to contain phage, Φ1B3A as a positive control, and a negative control (host bacteria without phage). The sample and bacteria ($OD_{600} = 0.5$) were mixed at a volumetric ratio (5/1). Multiplicity of infection (MOI) of Φ1B3A was adjusted as 5. After the 15 min adsorption period, modified M17 Broth was added to promote the growth of phage and bacteria at 43 °C. Samples from this mixture were taken at 10 min intervals for the first hour and then 30 min intervals to monitor bacterial growth. Absorption values of the samples at OD_{600} were recorded with an Agilent 8453 UV–visible spectrophotometer (Agilent Technologies, Inc. Santa Clara, CA).

For the microbiological control of antibiotic, double layer agar method as described in Samac et al. (2003) and Charm CowSide® II (Charm Sciences Inc, Lawrence, MA) test kit were used. The unprocessed raw milk sample was directly put into vial provided with the test kit. As a control group, another vial containing 100 µL raw milk and 5 µL ampicillin was used. Both vials were incubated at 64 °C for 3 h, and the obtained results were evaluated.

Sample preparation for Raman analysis

All phage, blank (bacterial growth medium without phage) and antibiotic samples were prepared in the raw milk and, the same pretreatments mentioned above (Preparation of raw milk samples) were applied to the samples. For the preparation of phage samples (PS), 500 µl of each phage was added to 5 ml raw milk, and the milk pretreatments were performed. Sixteen phage samples including two parallel for each eight phages were prepared and collected in sterile tubes by applying this method. For the preparation of blank samples (BS), 75 µl host bacteria in log phase (OD_{600}) were inoculated in 1 ml fresh modified M17 Broth and then incubated at 43 °C for 18 h. After the incubation period, cultures were centrifuged at 10,000 rpm for 5 min in order to remove bacterial residues. The supernatants were collected in sterile tubes as blank samples. Then, 500 µl of each blank was added to raw milk, and the milk pretreatments were applied. Eighteen blank samples including 6 parallel for each host bacteria were prepared applying this procedure. For the preparation of antibiotic samples (A), 500 µl ampicillin was added to raw milk to a final concentration of 0.5 µg/ml, which is below the breakpoint values for *S. thermophilus*. The same procedure was applied on raw milk samples as mentioned above. Thirteen parallel raw milk samples contaminated with ampicillin were collected using this procedure. Other 10 raw milk samples—except the raw milk used as model—were also prepared by applying the described pretreatment procedure and collected in sterile tubes.

Raman spectra were collected using 200 µl of the prepared samples. Several chemical and physical treatments were applied to the samples in order to detect phage presence in raw milk occurring due to spectral differences. Sample spectra were also collected in the presence of different chemicals such as β-mercaptoethanol (5%), SDS (10%). All solutions were added to the samples in a volumetric ratio of 1:2. The samples were also measured after being left in boiling water for 5 min and cooling to room temperature. After all these processes, SDS was chosen, and all measurements were performed with the addition of 100 µl 10% SDS.

Raman measurement and chemometric analysis

Raman measurement was carried out using a DeltaNu Examiner Raman Microscopy system (Deltanu Inc.,

Laramie, WY) with a 785 nm laser source and a cooled charge-coupled device (CCD, at 0 °C) detector. The amount of the sample measured in the Raman vessel was 300 µl. SDS was added to all samples in a volumetric ratio of 1:2. The spectra were obtained in the range of 200–2000 cm^{-1} at a resolution of 2 cm^{-1} with constant measurement parameters (20 s integration time, five measurements for every sample, coaddition of five measurements, and 100 mW laser power).

PCA is a linear transformation method of multidimensional data into different coordinates based on minimum correlation and maximum variance where the data is most spread. For the present study, a PCA model was developed in order to analyze large numbers of Raman spectra of the samples. Stand-alone Chemometrics Software (Version Solo 6.5 for Windows 7, Eigenvector Research Inc., Wenatchee, WA) was used for the PCA analysis.

Results and discussion

Antimicrobial activity of ampicillin on *S. thermophilus*

In the present study, an ampicillin concentration below the breakpoint values given for *S. thermophilus* was chosen, and the growth of the cultures in the antibiotic-added and antibiotic-free media were monitored in OD_{600} for 5 h (Fig. 1). Up to 150 min, no significant difference between samples with and without antibiotic was observed due to the lag phase (the period during which the bacteria adapts to the environment). However, the difference was evident when the bacteria are in log phase and begin to multiply in a logarithmic trend. As apparent in Fig. 1, the ampicillin at the concentration below breakpoint values has completely stopped the growth of culture, while the bacteria have showed a favorable development in an antibiotic-free medium. In one of the previous studies, it has also been demonstrated that the concentrations below maximum residue levels (MRL) could inhibit the growth of *S. thermophilus* (Tosi et al., 2007).

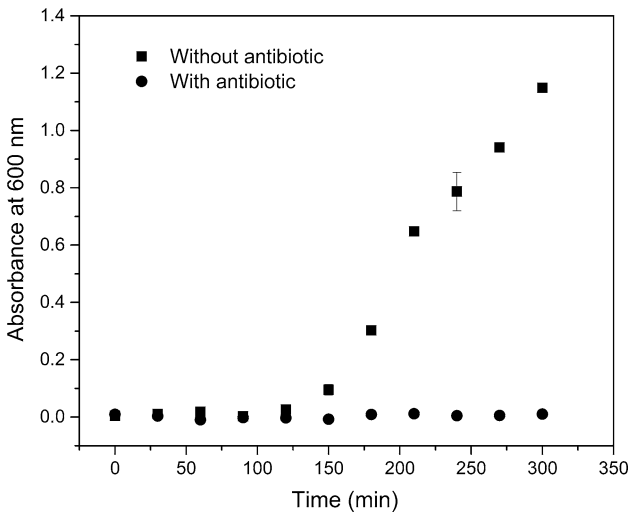

Fig. 1 UV–Vis absorption values for the culture media with and without antibiotic

Control of raw milk samples for phage and antibiotic

The presence of phage or phages in milk is a serious problem. For detection of their presence, three strains of *S. thermophilus* (231, B3, 709) were treated with raw milk samples. These three bacteria were mixed and incubated at 43 °C for 18 h with eleven different raw milk samples. The phage existence was controlled by monitoring the plaque formation in the area of the dropped sample for both raw milk samples and control groups. For the raw milk samples, some lysis plaques indicating the presence of phage were observed only in the plates belonging to B3 strains. However, the titer of these detected phages were considerably low (approximately 4×10^1 pfu/ml), which couldn't affect the process of fermented dairy products and does not cause fermentation delays or failures (Binetti et al. 2008).

In some previous studies, it was reported that B3 strains were quite sensitive to the phages found in dairy environments, thus they could be detected as a result of the enrichments. In a similar study, this strain was shown to be highly sensitive to the phages (Kaleli et al. 2004).

The phage existence was controlled by the spectrophotometric method. As can be seen in the Fig. 2a, negative control containing only host bacteria demonstrated an expected growth curve during the 240 min incubation period, while the suspicious sample gave lower absorbance values pointing to the declining growth rate of the bacteria and the presence of phage in the sample. However, the titer of the phages existing in the raw milk was not high enough to stop bacterial growth and thus the fermentation process. The positive control showed the impact of the ΦB3-X11 phage (have a titer approximately 10^8 pfu/ml) on the growth of bacteria as can be seen in detail in Fig. 2b. Bacterial growth was inhibited completely in the presence of sufficient phage in the environment.

The presence of antibiotic in milk was controlled with commercial antibiotic kits and by applying a microbial method. In this study, microbial inhibition kits were used to demonstrate that the raw milk samples used for the analysis were antibiotic-free. The blue/purple color of the control group shows that in the existence of antibiotic, no bacterial growth was observed in the vial. In addition, yellow/green color of the vial indicates a negative result, which means

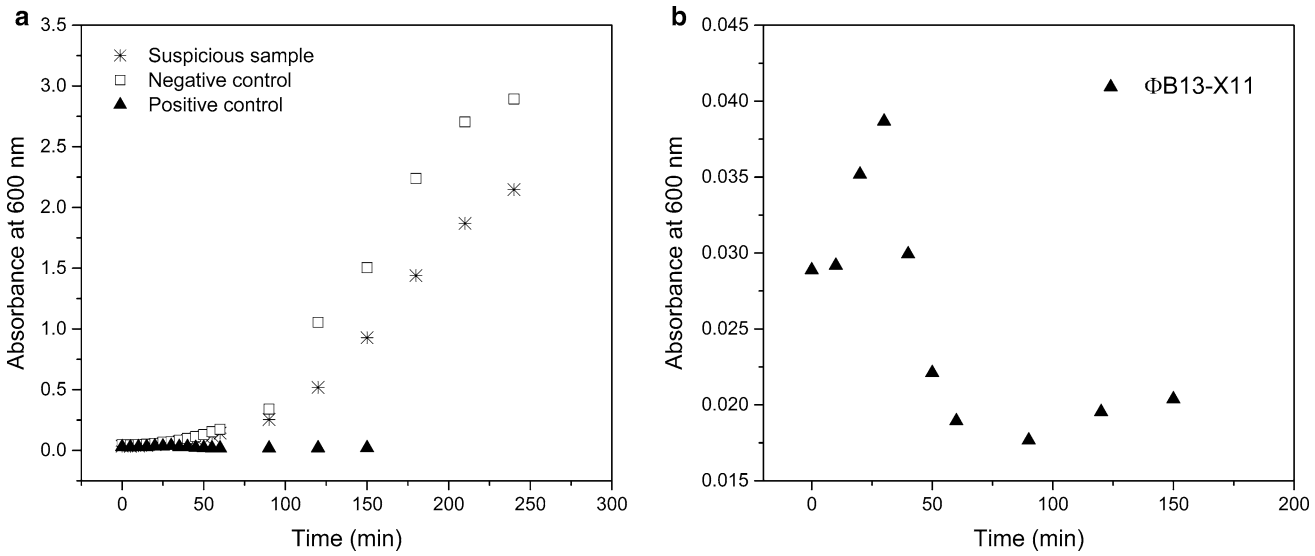

Fig. 2 UV–Vis absorption values for the suspicious sample, negative and positive control (**a**) and detailed graph of the sample containing ΦB3-X11 phage used as positive control (**b**)

the raw milk used in the analysis does not contain antibiotic above the MRLs. For the microbial control of the antibiotic presence in raw milk samples, plates were examined after the incubation, and no zones indicating the antimicrobial activity of antibiotics were observed.

Raman spectra of the samples and PCA analysis

The raw Raman spectra of the samples were collected between 200 and 2000 cm^{-1} as shown in Fig. 3. The six Raman bands at 248, 350, 492, 1064, 1340 and 1367 cm^{-1} which were remarkable with their high intensities show spectral differences between phage, antibiotic, blank and raw milk. In addition, there are also a lot of differences in the bands with relatively low intensities. The bands located at 813, 875, 1064, 1126 and 1439 cm^{-1} are similar to that of raw milk spectra which were given in a previous study (Khan et al. 2014). According to previous reports about Raman spectra of lactose, it is thought that four peaks observed at 875, 946, 1010 and 1125 cm^{-1} could indicate the presence of lactose found in milk (Li et al. 2015).

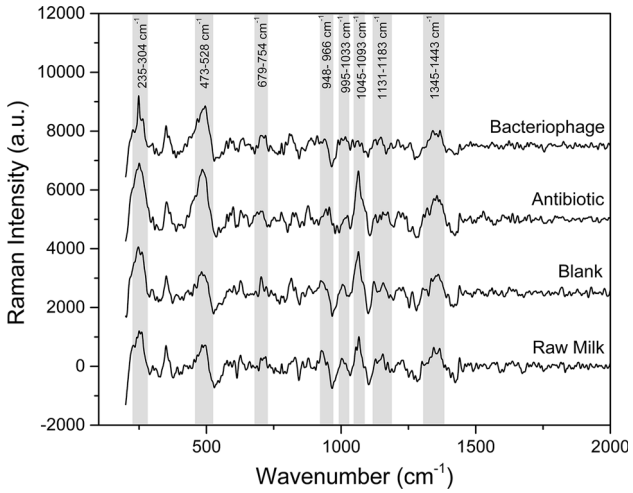

Fig. 3 Raman spectra of bacteriophage, antibiotic, blank and raw milk samples. All bacteriophage, antibiotic and blank samples were prepared in raw milk at certain concentrations and the spectra of the raw milk samples containing these components were analyzed

As a result of the milk pretreatments, it was observed that phage titers showed 1-log reduction, which means the phage samples used in the Raman measurement had a titer of approximately 10^7 pfu/ml. Detecting phage presence in a sample by using Raman spectroscopy and characterizing the obtained spectrum of phages is a challenge due to the scarcity of the studies on this subject. No specific spectrum belonging to the phages used in this study has been found, thus a variety of pre-processes was performed in order to show spectral differences between the samples with and without phages. At the beginning, Raman measurements were carried out using only raw milk, BS, PS and A without adding another solution, and a spectral separation between the groups could not be observed. Therefore, it was decided to add β-mercaptoethanol solution to the samples before performing Raman measurements; however, the result was the same and no significant difference could be obtained. As other pre-processes such as heat treatment did not work well, SDS was applied to all samples and the best spectral differences were obtained by using SDS without heat treatment.

The band positioned at 1065 cm^{-1} is mostly assigned to C–C *trans* bond of SDS (Picquart and Laborde 1986), and this high-intensity band cannot be seen in the spectrum of phage due to the interaction of SDS with phage proteins.

SDS denatured them without any need for heat treatment as mentioned in the previous studies (Roy et al. 2012), and the difference between samples could easily be observed. The band at 1012 cm^{-1} is unique for the phage spectrum and can be related to the spectrum of HK97 phage which was in the same order and the family that of *S. thermophilus* phages (Benevides et al. 2004; Němeček et al. 2009). For antibiotic samples, an ampicillin concentration below all breakpoint values (0.5 μg/ml) was chosen to detect low levels of antibiotic and to discriminate antibiotic and bacteriophage in raw milk. It was previously reported that the strong band which has the highest intensity at 483 cm^{-1} belongs to δHCC + δCCN(lactam) + πCNCO + δ_{ip} lactam in ampicillin (Baraldi et al. 2014).

After identification of the peaks in the obtained spectra with Raman, PCA was used for the evaluation of them. PCA was applied to the whole spectra between 200 and 2000 cm^{-1} to discriminate between phage and antibiotic samples. There were 58 samples including 16 phages (2 parallel for each *S. thermophilus* phage), 18 blank (6 parallel for each *S. thermophilus* blank), and 13 antibiotics and 11 raw milk. Various preprocessing techniques such as normalization, auto-scaling, mean center, baseline correction, and derivative were applied to the obtained data of the sample spectrums in order to improve the efficiency of the model and to observe a better distinction. Best discrimination between the groups was obtained only through a combination of the two preprocesses; auto-scaling and first derivative, respectively. Then, the model was established, and the principal component scores were used to locate different sample groups on the plot (Fig. 4.). The plot was drawn with the first principal component which explained 20.01% of the total variance, and the second principal component which explained 15.54% of the total variance. RMSEC value of the model was 0.075, and RMSECV value of the model was 0.088. The score plot drawn with the previously specified features have clearly showed the distinction between the sample groups. Phages were located on the left upper side, while antibiotic samples clustered on the lower side of the graph. The blanks appeared on the right lower side, and the raw milk samples were positioned in the middle part of the plot. In this way, the samples containing phage and antibiotic in raw milk were effectively distinguished through the combination of Raman spectroscopy with chemometric analysis.

The antibiotic presence in the 10 raw milk samples was checked both with antibiotic test kits and by using microbiological methods, and it was seen that raw milk samples did not contain any antibiotic. When the spectrum data of these samples processed with PCA, 10 raw milk samples were found to be located in the same place with the model raw milk sample in the PCA score plot, which means that the PCA model confirmed the results obtained previously

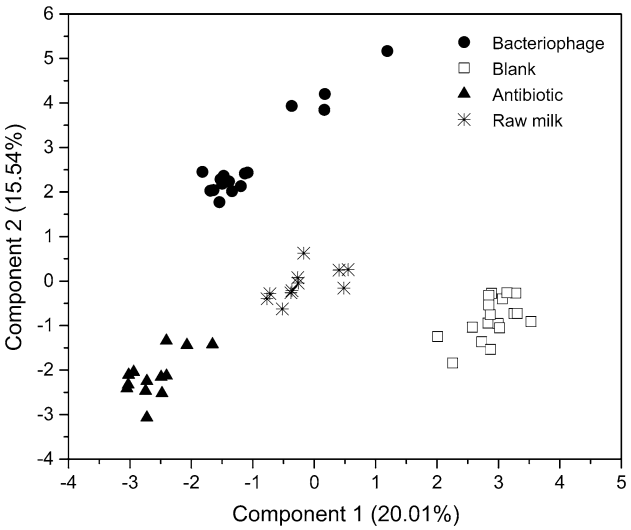

Fig. 4 PCA score plot for Raman spectra of raw milk samples containing bacteriophage, antibiotic and blank. All raw milk samples containing bacteriophage, antibiotic and blank were discriminated by using a PCA model

for antibiotic. The phage presence of the 10 raw milk samples was also monitored using microbiological methods. One of the raw milk samples was found to contain phage in a very low titer (approximately 4×10^1 pfu/ml), and the others were found to be phage-free. In the PCA score plot (Fig. 4), it has been found that phage-free raw milk samples were easily separated from others. However, phage-containing sample (4×10^1 pfu/ml) were located in the same place with phage containing model samples. This situation is quite promising. Because the sample can easily be separated from phage-free environments even the phage titer is very low in the sample. However, the inability to know exactly how much of the phage is in the environment will cause confusion in terms of precautions that can be taken in the factory environment. Therefore, the development of this model with different chemometric methods and/or pre-treatments is required for the separation of phages in different titers.

Earlier antibiotic residues determination from food matrix using Raman spectroscopy has been tried (Clarke et al. 2005; Chen et al. 2015b). However, while evaluating fermented dairy products, the factors that prevent the growth of starter cultures needs to be understood. The microbial inhibition kits are commonly used but necessitate long analysis time. In addition to this, microbial inhibition kits cannot specify whether the inhibition of the starter culture was due to antibiotic or phage (Cháfer-Pericás et al. 2010; NaVrátiloVá 2008). Also, there is no specific method for controlling the phage presence in the raw milk received in the factory. Microbiological methods and acidification test for the determination of the phage are also time consuming procedures which may last at least one day. For this

reason, investigations have been performed for rapid detection of phage (del Rio et al. 2007; Binetti et al. 2008; García-Aljaro et al. 2009; Qi et al. 2009; Ly-Chatain et al. 2011). Among these studies, PCR and real-time PCR methods based on DNA are the most preferred methods where phage detection time could be reduced to 30 min and detection limit could be 10^2 pfu/ml (del Rio et al. 2007; Binetti et al. 2008). However, methods based on DNA have some disadvantages: (1) First, phage DNA should be isolated in high quality and purity from food environment which is a mixed matrix. (2) There is a need for trained staff to work with these devices and interpret the results. (3) The cost of consumables is high. (4) Since it is the (only) determining factor in selection of phage specific primer, phage design is very important. It means that detection of all the present phages might not be possible. In another study, where impedimetric change was taken as basis, the effect of phages with different titers on biofilm formed by *E. coli* was investigated. However, phage presence could only be observed at the end of 6 h, and a calibration based on phage was not obtained. The method is less favorable in terms of time than the acidification test performed in some factories (García-Aljaro et al. 2009). The detection limit was 10^9 pfu/ml in the phage detection system using imaging ellipsometry method. In addition, in order to detect phage with this method, the corresponding phage antigen must be obtained. Therefore, this method is phage-specific and will cause some problems such as inability to detect other phages in the environment (Qi et al. 2009). Chen et al. (2015a) prepared a SERS substrate using the surface they developed in their study and made use of this surface to differentiate three phages effective against *E. coli*. In this very impressive study, three phages were washed and concentrated to remove the matrix effect by two-hour centrifugation twice a day. Although Raman spectroscopy was used in our study, neither a surface to increase the signal nor a concentration/washing to get rid of the matrix effect was needed.

When these methods were compared with the method developed in this study, it was the phage-specific materials such as primer and antibodies were also not required to be synthesized. Moreover, the dairy plants may not have their own phage collection or experienced personnel for microbiological analysis. In the companies that are conscious about phages, starter cultures are put into rotation at certain time intervals, and hygienic measures are taken. However, such measures are not always beneficial. Therefore, with this study, it has been shown that by Raman spectroscopy it is possible to detect not only the presence of phage with a titer of 10^7 pfu/ml, which is sufficient to fail the fermentation, but also antibiotic which is in a concentration below the MRLs and inhibits the growth of starter cultures (0.5 μg/ml) in raw milk in 30 min.

Conclusion

The combination of Raman spectroscopy with chemometric analysis was used to analyze raw milk samples mixed with bacteriophages, their blanks and antibiotic. Raman spectra of all samples were evaluated with PCA in order to discriminate each sample group. A clear differentiation between phage and antibiotic was achieved by using the developed PCA model. Raman spectroscopy combined with chemometric methods were found to be more useful for rapid and sensitive analysis of phage and antibiotic in raw milk compared to traditional analysis methods based on microbial inhibition. Although the antibiotic-related studies using Raman spectroscopy can be found easily, phage studies using Raman spectroscopy are quite limited in the literature. The present study suggests a new approach to detect phage and antibiotic presence in raw milk, but further studies are needed to detect and discriminate the low titers of phage contamination in raw milk more precisely.

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