Journal of Food Composition and Analysis 32 (2013) 12–19

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original research article

The discrimination of raw and UHT milk samples contaminated with penicillin G and ampicillin using image processing neural network and biocrystallization methods

Sevcan Unluturk [a,*,1], Merve Pelvan [a], Mehmet S. Unluturk [b,1]

[a] Izmir Institute of Technology, Food Engineering Department, Urla, 35430 Izmir, Turkey
[b] Izmir University of Economics, Department of Software Engineering, Balcova, 35330 Izmir, Turkey

ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form 24 June 2013
Accepted 27 June 2013

Keywords:
Milk
Antibiotic residues
Penicillin G
Ampicillin
Food analysis
Food safety
Image processing
Neural networks
Biocrystallization
Veterinary residues
Food regulation issues
Food trade issues

ABSTRACT

This paper utilized a neural network for texture image analysis to differentiate between milk, either raw or ultra high temperature (UHT) with antibiotic residues (e.g., penicillin G and ampicillin) and milk without antibiotic residues. The biocrystallization method was applied to obtain biocrystallogram images for milk samples spiked with penicillin G and ampicillin at different concentration levels. The biocrystallogram images were used as an input for a designed neural network called the image processing neural network (ImgProcNN). The visual differences in these images that were based on textural properties, including the distribution of crystals on the circular grass underlay, the thin or thick structure of the crystal needles, and the angles between the branches and the side needles, were used to discriminate the antibiotic-free milk samples from samples with antibiotic residues. The visual description and definition of these images have major disadvantages. In this study, the ImgProcNN was developed to overcome the shortcomings of these visual descriptions and definitions. Overall, the neural network achieved an average recognition performance between 86% and 100%. This high level of recognition suggests that the neural network used in this paper has potential as a method for discriminating raw and UHT milk samples contaminated with different antibiotics.

1. Introduction

Milk is a major source of elements that are vital for proper nutrition and health. Cows that provide milk are often treated with antibiotics, especially cows suffering from mastitis. Low levels of antibiotics are used to increase the rate of weight gain or to improve the feed efficiency in cattle breeding (Gustafson and Bowen, 1997).

The excessive usage of antibiotics may lead to antibiotic residues in milk from 12 to 96 h following the injection, based on a variety of factors (Santos et al., 2007). These residues could cause allergic reactions in sensitive individuals, and the continued exposure to antibiotics could lead to the development of antibiotic resistance. In addition, antibiotic interference with the starter cultures that are used in dairy products negatively influences the coagulation process (Rinken and Riik, 2006).

To protect consumers, maximum residue limits (MRLs) for veterinary medicinal products in milk have been established by EU Council Regulation (EEC) No: 2377/90 (Zvirdauskiene and Salomskiene, 2007). According to the Turkish Food Codex Regulation No: 2002/30 Part 6, the residue levels of veterinary drugs in raw and drinking milk must not exceed the limits stated (KKGM, 2007).

Residue analysis must be periodically carried out to screen milk and milk products in the market. This includes both screening and confirmatory methods. Current methods for the detection of antibiotic residues are classified as spectrophotometric, chromatographic, and fluorimetric (Le Breton et al., 2007). Chromatographic analysis is a confirmatory method that identifies and quantifies the presence of antibiotic residues with a high degree of accuracy. However, this method has drawbacks. Chromatographic analysis is time-consuming, involves complex procedures, is damaging to the environment and requires a high level of expertise (Rinken and Riik, 2006). Therefore, simpler and more effective methods have been developed to reduce the time needed for testing. However, these methods also have limitations. Simpler methods are unable to quantify the concentration of antibiotics and have a relatively narrow antibiotic spectrum. Furthermore, these methods could produce false-positive or false-negative

* Corresponding author. Tel.: +90 232 7506317; fax: +90 232 7506196.
E-mail addresses: sevcanunluturk@iyte.edu.tr (S. Unluturk), mervepelvan@iyte.edu.tr (M. Pelvan), suleyman.unluturk@ieu.edu.tr (M.S. Unluturk).
[1] Both authors have contributed equally to this article.

0889-1575/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jfca.2013.06.007

results causing an unjustifiable waste of milk and economic losses (Kang et al., 2005).

In another study, we found that the beta-lactam group of antibiotics, penicillin G, ampicillin and amoxicillin, are commonly used for the treatment of dairy cattle (Yildiz, 2008). This study was based on the need to develop a new method to demonstrate the existence of the antibiotic residues of penicillin G and ampicillin in milk. Antibiotic residues may be found in milk at a level higher than the allowable limits (maximum residue limits (MRL)). However, this level is decreased by applying heat processes (e.g., UHT) and the antibiotic concentrations fall below the detection limits (Rinken and Riik, 2006). To overcome this detection problem, the innovative "biocrystallization" method was found to be suitable for detecting the presence of penicillin G and ampicillin residues in raw and UHT milk and discriminating the milk samples that are contaminated with these antibiotics. The biocrystallization method, also called "sensitive crystallization" or "cupric chloride crystallization", was originally introduced by E. Pfeiffer in the 1930s. This method is based on the crystallographic phenomenon that occurs after adding specific inorganic ionic or organic substances to an aqueous solution of dihydrate cupric chloride. This method creates crystallization images with reproducible textures that are formed during crystallization.

This method has been used to examine the effects of different farming systems, fertilization practices, and processing on the pictomorphological properties of agricultural and horticultural samples (Kokornaczyk, 2008). Crystallograms produced by using pure $CuCl_2$ exhibit a merely peripheral distribution of crystals on a circular glass surface. Biocrystallograms produced from biological substances, such as plant extracts, fruits, vegetables and milk, display crystal structures covering the whole of the glass underlay. These biocrystallograms exhibit a variety of macro and microscopic morphological features that reflect the specific admixed substances (Fig. 1a and b.). Morris and Morris (1941) modified the cupric chloride crystallization by adding purified egg albumin, the water soluble globulin of oats, white blood and the tobacco mosaic virus. The biocrystallogram images showed that the protein properties of these substances affect the biocrystallization patterns (Morris and Morris, 1941). Kahl (2007) used the biocrystallization method to authenticate agricultural products. The cupric chloride crystallization was used to differentiate milk and butter samples from different feeding regimes (Kahl et al., 2009). This method was successful in distinguishing the effects of different treatment methods on milk samples, such as raw and UHT milk, and could differentiate between milk samples that were homogenized at two different pressures (Huber et al., 2007).

The phenomenon of the biocrystallogram is based on the ramification structure. The ramification structure extends from the center and develops in all directions until it reaches the periphery of the image (Fig. 2). In the initial stage, 1-zonal biocrystallogram, the transparent needles in star-like formations extend in all directions to the periphery by increasing the concentrations of biological substances with a fixed concentration of $CuCl_2$. In the second stage, 2-zonal structure, the needles are pointed predominantly on the vertical and horizontal axes that pass through the crystallization center, and the needles are transparent and of relatively equal length in the middle zone. The last stage of the biocrystallogram is divided into a 3-zonal structure: the central zone around the crystallization center, the median zone that contains the major ramification structure, and the marginal zone (Andersen, 2001). The location of the crystallization center does not correspond to the geometrical center. All biological or agricultural substances have unique biocrystallogram patterns with a distinct center coordination, branch distribution and needle variety. Andersen et al. (1999) indicated that out of several single organic compounds examined, proteins and *N*-containing compounds showed unique abilities to coordinate the crystal structures.

The quality of products can be assessed by evaluating the biocrystallogram images using both visual and computational analysis. The visual assessment method makes use of staff who are trained according to the ISO-norms in the visual evaluation and interpretation of images (Huber et al., 2010). Computational image analysis has become a possible method for the evaluation of biocrystallogram images because of developments in computer science. The computational image analysis procedure for the evaluation of biocrystallogram images should reflect all of the characteristics of a biocrystallogram as a three dimensional, colored crystal structure, coordinated with zones relative to a center (Meelursarn, 2007; Andersen et al., 1999). In contrast, previous studies have used a texture analysis that applied 32 grey levels and a single type of circular region-of-interest (ROI) approach (Meelursarn, 2007; Busscher et al., 2010). In a study using a different approach, a feed-forward neural network was applied to biocrystallization in conjunction with computer vision techniques to discriminate sweet red pepper products that were prepared by different methods, such as freezing and pureeing. In this case, a methodology known as the process neural network

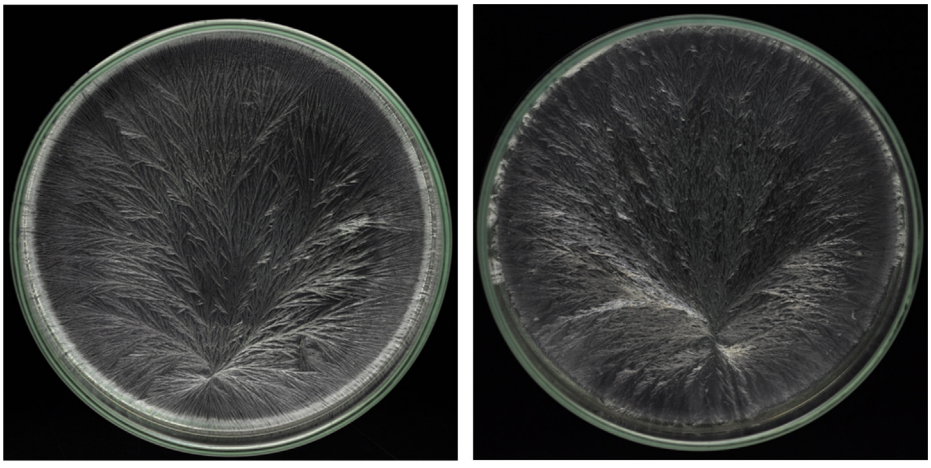

Fig. 1. (a) Raw milk biocrystallogram (b) UHT milk biocrystallogram (Pelvan, 2011).

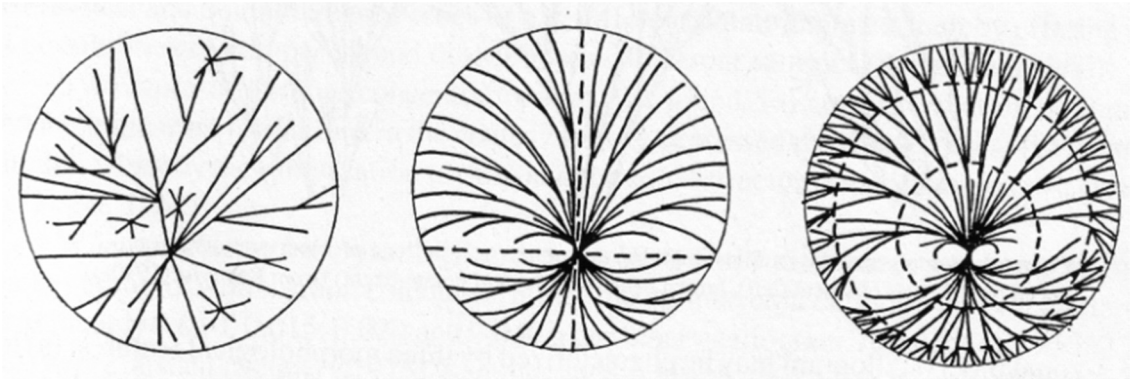

Fig. 2. The phenomena of the biocrystallogram image (divided into 3 stages of imaging on the basis of increasing the concentration of additives at certain concentrations of $CuCl_2$). From left to right: 1-zonal structure, 2-zonal structure, and 3-zonal structure (*Source*: Engqvist, 1970).

(ProcNN) has been able to achieve 100% recognition and has proved to be a useful method in the discrimination of red pepper products (Unluturk et al., 2011). This method not only provides information representing the visual characteristics but also discriminates between images that cannot be differentiated visually (Basset et al., 2000).

The intention of this paper is to develop a method for detecting antibiotic residues, namely penicillin G and ampicillin, in spiked milk samples by assessing biocrystallogram images using computational image processing. This method utilized a neural network, called the image processing neural network (ImgProcNN), which was supported with a computer vision technique to create an effective analysis tool.

The developed methodology was confirmed by comparing the biocrystallogram images obtained from naturally contaminated raw milk supplied from a cow treated with penicillin G.

2. Materials and methods

2.1. Materials

The raw milk samples were collected from a single cow from a farm located in Gulbahce, Izmir, Turkey, between March 2010 and October 2011. Prior to measurements, the raw milk samples were preserved with a final concentration of 0.02% Bronopol (PESTANAL® Code: 32053, Sigma-Aldrich) and stored at +4 °C. A number of UHT whole cow milk samples of the same brand were purchased from a local store in Izmir, Turkey during the interval period from 2010 to 2011. All milk samples were screened using the New SNAP Beta-Lactam Test Kits (Idexx Laboratories, Inc., USA) for antibiotic residues following the instructions of the manufacturer.

2.2. Biocrystallization

The biocrystallization method comprises several steps. These include preparing the samples, mixing an aqueous solution of the sample extract and $CuCl_2{\cdot}2H_2O$ in a glass Petri dish at a defined ratio, drying the glass Petri dishes in an air climate chamber at a constant temperature and humidity to obtain clear biocrystallograms, acquiring images under dark illumination and finally evaluating the images of the biocrystallograms by means of computational image processing. Biocrystallization is a highly sensitive method that can be affected by both the physical conditions of the drying (crystallization) chamber and the concentration matrix. Regarding the solution concentration matrix, the milk concentration, the $CuCl_2{\cdot}2H_2O$ concentration, the optimal mixing ratio between milk and copper chloride (volume of milk/volume of $CuCl_2{\cdot}2H_2O$) and the volume of the mixture are important factors that influence the crystallization process. In the crystallization step, a known amount of milk sample (0.02–0.5%) and aqueous $CuCl_2{\cdot}2H_2O$ solution (5–15%) were mixed in a clean glass Petri dish at a defined mixing ratio (milk/$CuCl_2{\cdot}2H_2O$) and dried in an air climate chamber (Memmert-HCP108, Germany) adjusted to a certain temperature and relative humidity. The temperature and humidity inside the cabin were monitored using a thermocouple and a hygrometer. The variations in temperature and humidity were ±0.1 °C and ±1%, respectively. Factors including the amount of mixture (4–8 mL), the mixing ratio (1/1–3/1), the drying temperature (25–40 °C) and the relative humidity (40–60%) affected the ramification patterns of the biocrystallograms. The biocrystallization process was optimized, and the important factors affecting the ramification patterns of the biocrystallograms and their levels were determined (Table 1) (Pelvan, 2011). These levels were used to generate biocrystallograms having the optimum observable dendritic pattern during crystallization, i.e. the drying period.

For the biocrystallization method, penicillin G potassium salt (Code: 46609, Lot4016X) and ampicillin trihydrate (Code: 46061, Lot 2316X) (Vetranal analytical standard Sigma-Aldrich GmbH Quality Assurance) were chosen as target antibiotics. Raw milk and UHT milk samples were spiked with penicillin G and ampicillin prepared in the concentrations of 2, 4, and 8 ng mL^{-1}. The antibiotic free raw and UHT milk samples (as controls) and samples spiked with penicillin G (2, 4, and 8 ng mL^{-1}) and ampicillin (2, 4, and 8 ng mL^{-1}) were diluted with Milli-Q water to provide a milk concentration of 0.5%.

Copper(II) chloride dihydrate ($CuCl_2{\cdot}2H_2O$) (102733, Merck) was used for the biocrystallization process. An aqueous solution of $CuCl_2{\cdot}2H_2O$ was prepared with Milli-Q water at a concentration of 5%.

Circular glass Petri dishes with a diameter of 100 mm and a thickness of 2 mm were washed in regular dish detergent and rinsed with de-ionized water at 98 °C before being cleaned with 99.5% Ethanol (Merck) and dried in an oven (Nuve EN-050, TURKEY) at 50 °C for 1 h.

Table 1
Optimum values of important factors affecting biocrystallization process.

Factor	Optimum values
Milk concentration (%)	0.5
$CuCl_2{\cdot}2H_2O$ concentration (%)	5
Mixing ratio (milk/$CuCl_2{\cdot}2H_2O$)	3/1
Amount of mixture (mL)	6
Drying temperature (°C)	40
Drying humidity (RH %)	60
Drying time (h)	22

In the crystallization step, 0.5% milk sample and aqueous 5% $CuCl_2{\cdot}2H_2O$ solution were mixed in a cleaned glass Petri dish at 3/1 mixing ratio (milk/$CuCl_2{\cdot}2H_2O$) to provide a total amount of 6 mL. The sample was then dried in an air climate chamber (model HCP 108, Memmert GmbH+Co.KG, Germany) at 40 °C and 60% relative humidity for 22 h. In this study, we used a standard air climate chamber with two rectangular trays, which differed from the air climate chamber used by Kahl (2007). To avoid the serious problem of vibration during the drying period, several air chambers were tested, and the chamber with the lowest amount of vibration was selected for use in the studies. In this selected air climate chamber, the vibrations resulted in approximately two or three failed integrated biocrystallograms. The number of failed biocrystallograms in this air climate chamber was much lower than the number of failed biocrystallograms obtained by using the other chambers. Additionally, the setting accuracy of the temperature and the humidity inside of the air cabin was in the range that was declared by the manufacturer. Kahl (2007) used a specially designed climatic chamber for biocrystallization, which was optimized to control physical conditions such as vibration. This climatic chamber had a daily capacity of 43 plates. In contrast, our chamber capacity was only 15–16 plates. Plates were arranged in two rows in each tray. Dendritic patterns were formed during crystallization from an aqueous solution containing milk and $CuCl_2$. Sixty biocrystallograms were prepared for each of the milk samples spiked with the target antibiotics (2 different milk samples ×2 different antibiotics × 4different concentrations) (Fig. 3) and the raw milk samples naturally treated with penicillin G (Fig. 4). Thus, a total of 1020 biocrystallogram images were obtained. Multi-centered and malformed biocrystallogram images were discarded. The center of the glass dish was marked prior to the drying step. The crystallization center of the images to the periphery of the image and the center of the glass dish along the vertical axis were measured and the average was calculated. The single centered images with crystallization centers closest to the average were selected.

2.3. Image capture

After the crystallization step, digital images of the biocrystallograms were captured using a Nikon D90 camera fitted with a 60 mm f/2.8D AF Micro-Nikkor lens with the following mode specifications: ISO 250, record mode HQ (JPEG file format 1/8 compression), aperture priority F 5.6, exposure −0.3 eV and focusing mode single AF. The camera was positioned vertically over the sample at a 40 cm distance. The angle between the camera lens, the lighting source, and the illumination were fixed, and the same settings were used for all of the sample images. For this

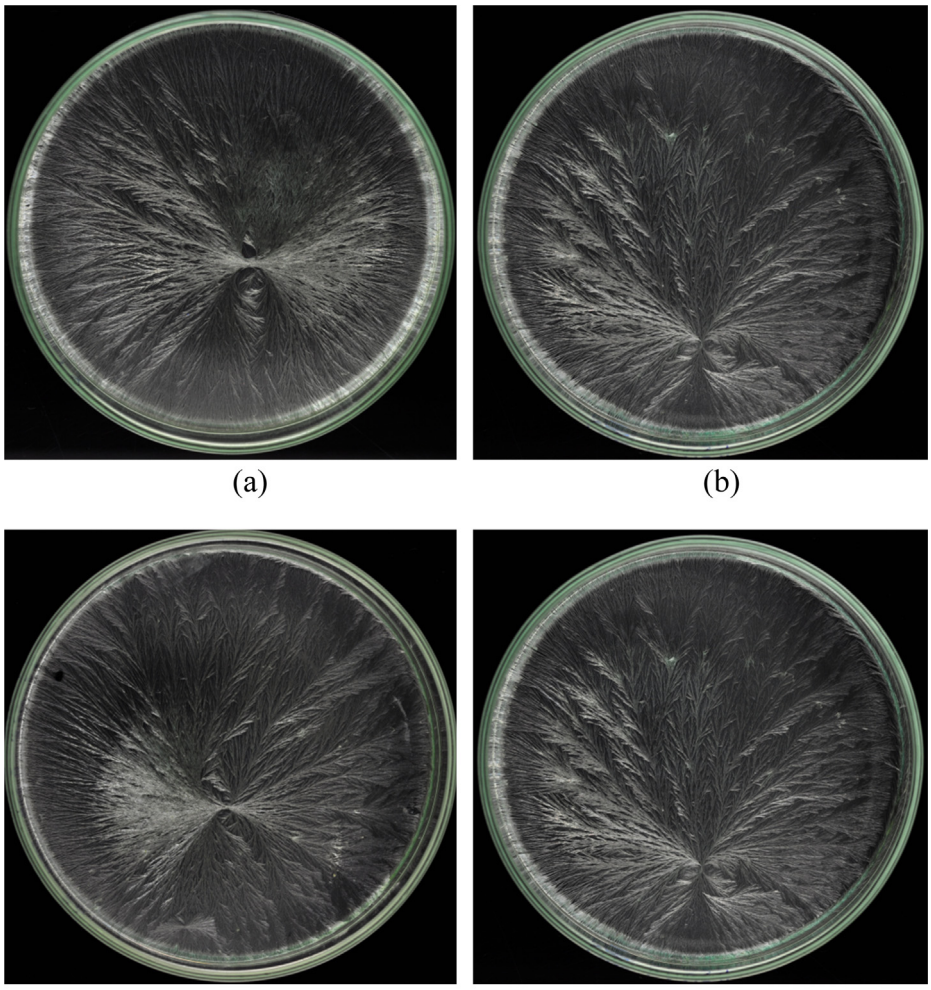

Fig. 3. Biocrystallogram images of (a) raw milk, (b) raw—2 ng mL^{-1} penicillin G, (c) raw—4 ng mL^{-1} penicillin G, and (d) raw—8 ng mL^{-1} Penicillin G.

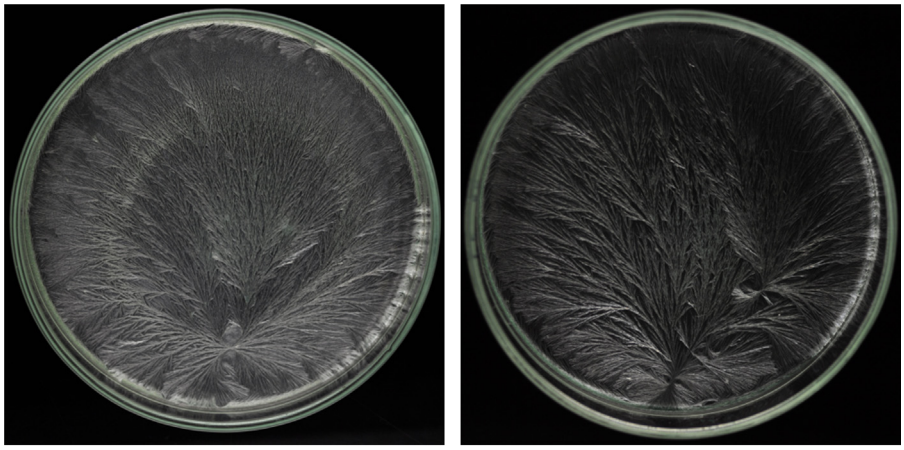

Fig. 4. Biocrystallogram images of (a) raw milk, (b) raw milk obtained from a cow treated with Penicillin G.

purpose, a wooden light box (dimensions 390 × 390 × 160 mm, $l \times w \times h$) providing dark illumination was constructed as described by Huber et al. (2010). The images were transferred and stored on a PC as a JPEG format of "high resolution" and "superfine quality".

2.4. Neural network (ImgProcNN) and image processing

Four ImgProcNNs were used to classify the raw/UHT milk samples and samples containing either penicillin G or ampicillin at three different concentrations: 2, 4, and 8 ng mL^{-1}. The fourth ImgProcNN was used to classify different sample types in a single neural network. For these ImgProcNNs, a set of 123, 90, 126, and 243 images were utilized to train the corresponding neural network for discrimination of penicillin G containing milk samples. A new set of 15 images for each class was then prepared for testing. The same type of neural network was used for ampicillin detection in raw/UHT milk samples. For these ImgProcNNs, a set of 119, 124, 153, 230 biocrytallogram images were utilized to train the corresponding neural network for discrimination of ampicillin containing milk samples. A new set of seven images for each class was then prepared for testing. In contrast, only one ImgProcNNs was created to classify raw milk and raw milk naturally contaminated with penicillin G. For this ImgProcNN, a set of 64 images was used in the training phase. A new set of 15 images for each class was then prepared for the testing phase.

In the image processing neural network (ImgProcNN) technique, images obtained from milk samples spiked with different amount of penicillin G and ampicillin were captured and saved in RGB (red, green, blue) space. For each component in RGB space, we performed two calculations: the mean and the standard deviation. The mean characterizes the average color properties of the milk samples, while the standard deviation provides a measure of color variation. Because three digital values were assigned to every pixel of color image of the sample, a total of six color features were calculated for the whole image. In other words, the mean and the standard deviation of each color component (R, G, B) were calculated for each image (Zheng et al., 2005).

All of the algorithms for the pre-processing of full images were written in MATLAB R2009b (The Math Works, Inc., MA, USA). Each 2731 × 2745 pixel RGB image that had a circular shape was cropped to a 1771 × 1511 pixel RGB image with a rectangular shape (Fig. 5). These final rectangular images were chosen to include the center region of the original biocrystallograms. Then, those images were used as the input for the neural networks. The number of hidden neurons in these neural networks was chosen as 20. During the training phase, we would increment the number of hidden neurons by two every 400 epoch if the learning process did not improve (Islam et al., 2009). For the training algorithm, we used the back propagation algorithm (Cichocki and Unbehauen, 1993; Freeman and Skapura, 1991). Fig. 6 shows a three layer neural network that is designed to classify the milk samples. The output of the hidden neuron of this neural network, y_j, is computed as:

$$y_i = \phi\left(\sum_{i=1}^{N} X_i w_{ji}^{h} + \theta^{j}\right) \tag{1}$$

where X_i is the mean or the variance of the biocrystallograms (μ_k, σ_k where k is R, G, B), N is 6, w_{ji}^{h} is the weight from jth hidden neuron to the ith input neuron, θ^{j} is the bias unit weight for the jth hidden neuron and the activation function for the hidden layer, $\phi()$, is a tangent hyperbolic function defined as:

$$\phi(z) = \frac{e^{z} - e^{-z}}{e^{z} + e^{-z}} \tag{2}$$

The output of the neural network, o, is calculated as:

$$o = \phi\left(\sum_{j=1}^{L} y_j w_{1j}^{o} + \beta\right) \tag{3}$$

where β is the bias unit weight for the output neuron, L is the number of hidden neurons (which is 20 for this neural network), and the term w_{1j}^{o} is the weight from the output neuron to the jth hidden neuron.

These ImgProcNNs were used to replace the human visual decision-making process.

3. Results

In all of the images (e.g., Figs. 3 and 4), each pixel has three components corresponding to red, green, and blue. We reduced the image size to 1771 × 1511, as in Fig. 5, and we calculated the mean and the standard deviation of the red, green and blue components of these images. As a result, we extracted six features for each image. These features were the input to the feed-forward neural network. The fully inter-connected feed-forward neural network that was used for raw milk and raw milk spiked with 2 ng mL^{-1} penicillin G classification is depicted in Fig. 6. In Fig. 6, μ_R, σ_R, μ_G, σ_G, μ_B, σ_B are the mean and the standard deviation values for red,

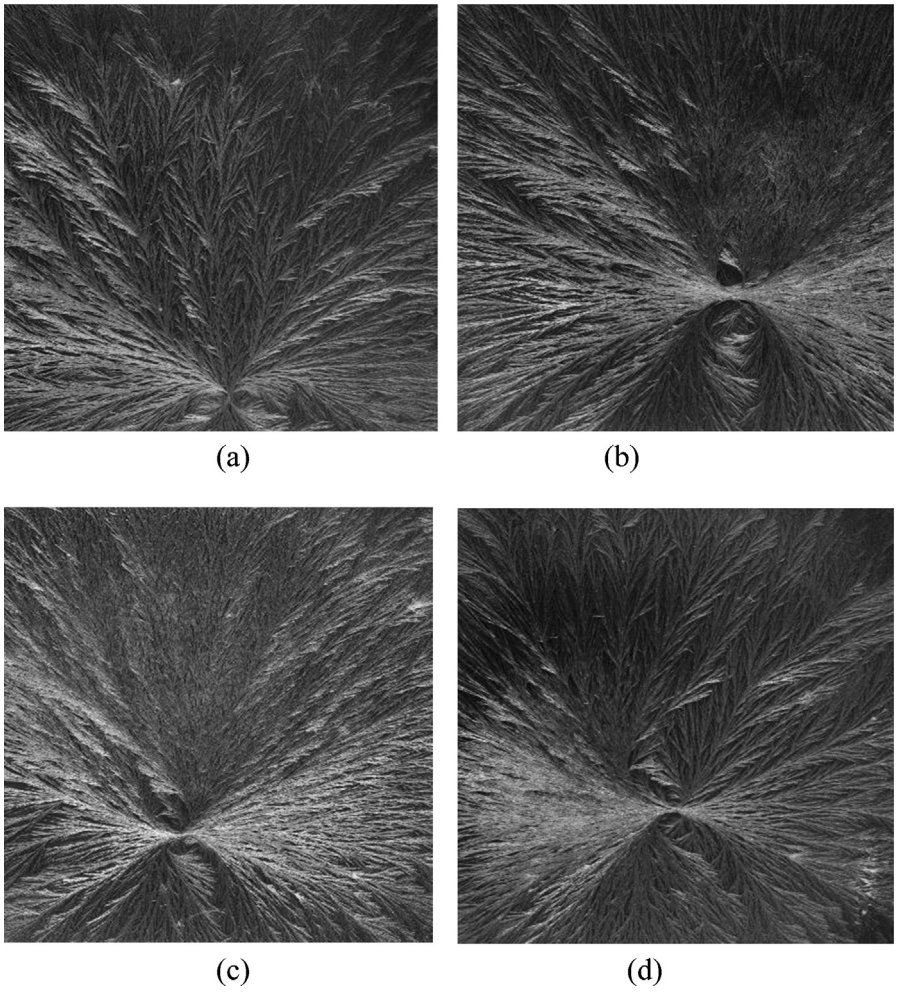

Fig. 5. Reduced image size (1771 × 1511) for (a) raw milk, (b) raw—2 ng mL^{-1} penicillin G, (c) raw—4 ng mL^{-1} penicillin G, and (d) raw—8 ng mL^{-1} penicillin G.

green and blue components of the RGB images. There was one output node, and the value of the node was 0.9 if the features belong to one type of class such as raw milk, or else −0.9 if the features belong to another type of class such as raw milk containing 2 ng mL^{-1} penicillin G.

The back propagation learning algorithm was used to estimate the hidden layer, the output layer weights and the biases for the optimal design of the neural network. If the input image belongs to raw milk (Type-1), then the output neuron is expected to be bigger than zero. A value of zero is chosen for the threshold to help decide whether the input image belongs to the raw milk sample (Type-1) or the 2 ng mL^{-1} sample (Type-2). This threshold value is found using the probability density function (pdf) of the training milk samples for raw milk and 2 ng mL^{-1}. The probability density function (pdf) is estimated using the Parzen method (Masters T., 1993). Hence, an estimate of the density function from the samples can be obtained from:

$$f_j(y) = \frac{1}{n\sigma_j} \times \sum_i \Phi\left(\frac{y - y_i}{\sigma_j}\right), \quad j = \text{Raw milk or 2 ng ml}^{-1} \qquad (4)$$

where $y_i\{i = 1, 2, \ldots, n\}$ is the neural network output for the raw milk samples or the 2 ng mL^{-1} samples, Φ is the Gaussian density function (i.e. $\Phi(y) = \exp(-y^2)$) and the constant σ_j is chosen to be 0.25 for the experimental data ($j = 1$ for raw milk, and $j = 2$ for 2 ng mL^{-1}). If the same penalty or the cost assumed with a miss-detection and the false alarm rate, the threshold value is at the point of intersection of the two probability density functions, which is zero as shown in Fig. 7.

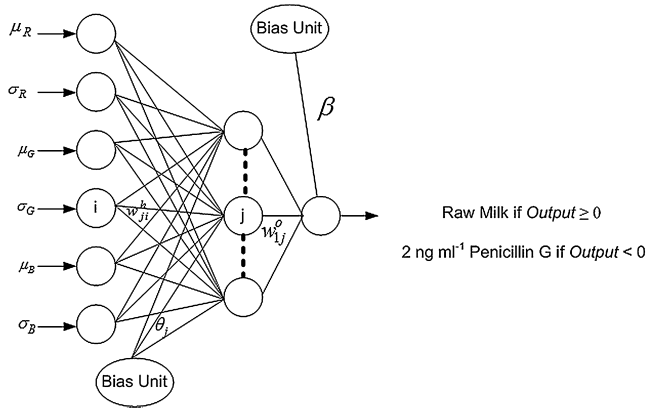

Fig. 6. Single output image processing neural network (ImgProcNN) for raw and UHT milk, 2 ng mL^{-1} penicillin G or 2 ng mL^{-1} ampicillin containing milk samples.

Table 2 shows the testing statistics of the neural network (Fig. 6) applied to discriminate raw and UHT milk samples (antibiotic free) from those containing different concentrations of penicillin G and ampicillin residues. Table 2 also shows the training and testing statistics for the neural network to distinguish between raw milk and milk samples obtained from a cow treated with penicillin G. In the present study, 5000 epoch were used to train the above neural networks to reach a sum-squared error of 0.01. An epoch is defined as the presentation of the entire training set to the ImgProcNN, and the sum-squared error is defined as a

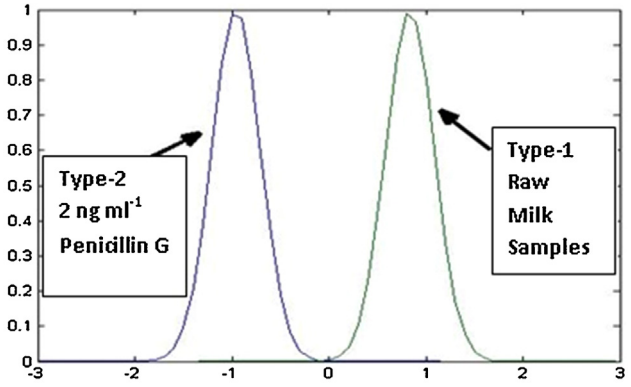

Fig. 7. The decision threshold is found to be zero, if the testing output is less than zero, then the input image belongs to 2 ng mL^{-1} or else it belongs to the raw milk samples.

measure of how well the ImgProcNN is doing at a particular point during its training (Masters, 1993; Cichocki and Unbehauen, 1993; Freeman and Skapura, 1991).

In the testing phase, 15 samples were used for each class (raw milk, 2, 4, and 8 ng mL^{-1} penicillin G containing raw milk, and 2, 4, and 8 ng mL^{-1} ampicillin containing raw milk). These test samples were not used during the training phase. For testing, 93%, 97% and 97% recognition performances were reached for raw milk samples contaminated with different amount of penicillin G by the corresponding neural networks (Table 2). In the UHT milk samples that contain different concentrations of penicillin G, 97% recognition performance was achieved.

On the other hand, we obtained 97%, 97% and 97% recognition performance in raw milk samples contaminated with ampicillin by the same ImgProcNN (Fig. 6). In the case of UHT milk treated with ampicillin at different concentrations, seven images were used in the testing phase for each class (UHT milk, and UHT samples containing 2, 4, and 8 ng mL^{-1} ampicillin). These test images were not used during the training phase. For testing, we reached 93%, 100% and 93% recognition performances by the corresponding neural networks.

In the case of the cow treated with penicillin G, 15 samples that were not used during the training phase were utilized for each class (raw milk, and milk that had been naturally contaminated with penicillin G). In this case, we reached 97% recognition performance using the neural network shown in Fig. 6.

We trained one more neural network where we used the same number of inputs (i.e. six), but the number of outputs was increased

Table 2
The testing statistics for ImgProcNN (penicillin G, ampicillin and naturally contaminated with penicillin G).

Neural network type	Testing recognition (%)*
ImgProcNN (raw milk and raw—2 ng mL^{-1} penicillin G)	93
ImgProcNN (raw milk and raw—4 ng mL^{-1} penicillin G)	97
ImgProcNN (raw milk and raw—8 ng mL^{-1} penicillin G)	97
ImgProcNN (UHT milk and UHT—2 ng mL^{-1} penicillin G)	97
ImgProcNN (UHT milk and UHT—4 ng mL^{-1} penicillin G)	97
ImgProcNN (UHT milk and UHT—8 ng mL^{-1} penicillin G)	97
ImgProcNN (raw milk and raw—2 ng mL^{-1} ampicillin)	97
ImgProcNN (raw milk and raw—4 ng mL^{-1} ampicillin)	97
ImgProcNN (raw milk and raw—8 ng mL^{-1} Ampicillin)	97
ImgProcNN (UHT milk and UHT—2 ng mL^{-1} ampicillin)	93
ImgProcNN (UHT milk and UHT—4 ng mL^{-1} ampicillin)	100
ImgProcNN (UHT milk and UHT—8 ng mL^{-1} ampicillin)	93
ImgProcNN (Raw milk and milk naturally contaminated with penicillin G)	97

* 5000 epoch were used to train the neural networks to reach a sum-squared error of 0.01.

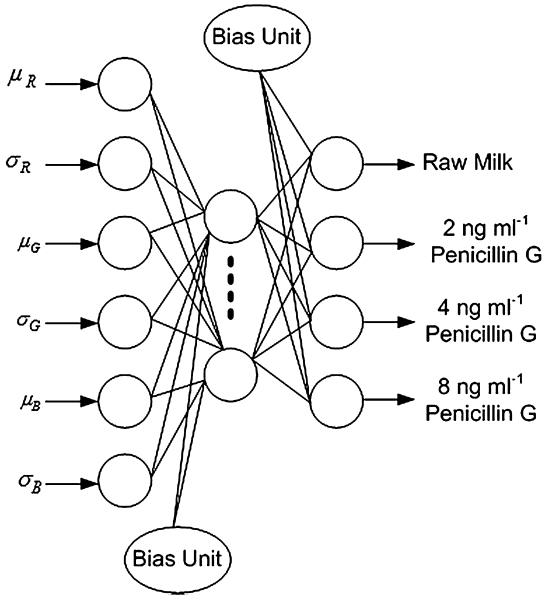

Fig. 8. Multi-output image processing neural network (ImgProcNN) for raw milk/UHT milk, 2, 4, and 8 ng mL^{-1} penicillin G/ampicillin containing milk samples.

to 4 (Fig. 8). Each output corresponds to each class, raw milk or UHT milk; raw or UHT milk containing 2, 4, and 8 ng mL^{-1} of either penicillin G or ampicillin. We needed 65 hidden neurons, and we spent 100,000 epoch to reach the sum squared error of 0.01. When the input set was belong to raw milk, the first output was 0.9 and all the other outputs were −0.9. If the input set belonged to a milk type having 2 ng mL^{-1} penicillin G or ampicillin, then the second output was 0.9 and all the other outputs were −0.9, etc. During testing, we again used 15 samples for each class, and we reached an 88% recognition overall. We created the same type of neural network as shown in Fig. 8 for the images obtained from the milk samples spiked with ampicillin. Again, we obtained an 88% recognition level.

We applied the same neural network as depicted in Fig. 8 for UHT milk samples contaminated with different concentrations of ampicillin. During testing, we used seven images for each class, and we achieved 89% recognition overall. For the UHT milk containing different concentrations of penicillin G, we reached 86% of recognition for the corresponding neural network.

When comparing the different neural networks (Figs. 6 and 8), it can be seen that the number of epochs and the number of hidden neurons were increased in the neural network described in Fig. 8. However, the recognition performance was decreased because the necessary number of classifications for a single neural network increased with the number of outputs.

We reached a recognition performance between 86% and 100% for each neural network type during testing. This high level of recognition suggests that ImgProcNN and the RGB color values is a promising method for analyzing biocrystallogram images to determine the presence and concentration of penicillin G or ampicillin in raw/UHT milk.

Performance of the ImgProcNN was further evaluated using the data in the matrix given in Table 3, which shows the confusion matrix for the Raw Milk-Raw Milk Naturally Contaminated with

Table 3
Confusion matrix for testing results of two class classifier, ImgProcNN.

		Predicted	
		Negative	Positive
Actual	Negative	15	0
	Positive	1	14

penicillin G ImgProcNN classifier. According to Table 3, the ImgProcNN does not confuse the two classes and can distinguish between raw milk and raw milk that has been naturally contaminated with penicillin G.

4. Discussion and conclusion

A biocrystallization method was used to create 1020 biocrystallogram images of different milk samples to detect antibiotic residues in raw or UHT milk. However, the required information was not readily quantifiable from these images by visual means, and there were no uniquely recognizable features. Therefore, a neural network was appealing for classifying these images because of the trainable features of a neural network. We developed two neural networks to detect the presence of antibiotic residues in raw/UHT milk samples. We used biocrystallogram images of different milk samples spiked with specific amounts of antibiotics for these neural networks. The optimal values for the neural network weights were estimated using the back propagation algorithm. Biocrystallization images were utilized to train and test the image processing neural networks. These networks showed classification performances between 86% and 100%. Parallel classification performance was also achieved when we trained the neural network. These results are significant and suggest that neural networks are potentially useful for detecting varying amounts of penicillin G and ampicillin in milk. However, it is difficult to make comparisons due to the lack of similar studies in the literature.

The image processing neural network offers practical advantages such as real-time processing, adaptability, and training capability. It is important to note that similar neural networks can be used to classify images of food grains. For instance, neural networks are used to detect contaminated food products, evaluate the surface quality of raw food materials and determine the quality features of foods, such as object recognition, geometrical parameters, and surface color. Additionally, neural networks are useful in other areas, such as medical ultrasonic imaging for tissue characterization and diagnosis and industrial defect discrimination.

It is important to emphasize that the biocrystallization method combined with the image processing neural network (ImgProcNN) is not being proposed to replace the existing rapid screening and confirmatory chromatographic methods used to detect antibiotic residues in milk. However, the given methodology could eliminate some disadvantages of the existing methods, such as the limited storage time and the tendency of minor compounds in milk (e.g., somatic cells) to give false-positive results. Chromatographic methods are expensive tests that require expertise, sample preparation and the use of considerable amounts of environmentally hazardous chemicals. However, biocrystallization supported with ImgProcNN uses only one chemical compound ($CuCl_2$) at a low concentration, making this method considerable less expensive than the other tests. Additionally, qualitative results can be obtained for contaminants of milk even when they are under detection limits. Additionally, such tests may eliminate unnecessary testing of the milk samples by chromatographic methods, consequently reducing the cost of these confirmatory tests. The proposed method shows considerable potential for detecting penicillin G and ampicillin contaminations in milk samples. Currently, the main disadvantage of this method is the additional analysis time. Further research on reducing the crystallization time and on adapting the test for cases of other possible contaminants and milk from different origins are needed.

Overall, the results of the present study encourage us to suggest that the combined methodology has the potential to be generally applied for discrimination and classification purposes in the area of food research.

Acknowledgment

The authors wish to thank Izmir Institute of Technology, Biotechnology and Bioengineering Research and Application Center for the use of their facilities.

References

Andersen, J-O., 2001. Development and Application of the Biocrystallization Method. Report no. 1. Biodynamic Research Association, Denmark.
Andersen, J-O., Henriksen, C.B., Laursen, J., Nielsen, A.A., 1999. Computerized image analysis of biocrystallograms originating from agricultural products. Computers and Electronics in Agriculture 22, 51–69.
Basset, O., Buquet, B., Abouelkaram, S., Delacharte, P., Culioli, J., 2000. Application of texture image analysis for the classification of bovine meat. Food Chemistry 69, 437–445.
Busscher, N., Kahl, J., Andersen, J-O., Huber, M., Mergardt, G., Doesburg, P., Paulsen, M., Ploeger, A., 2010. Standardization of the biocrystallization method for carrot samples. Biological Agriculture and Horticulture 27, 1–23.
Cichocki, A., Unbehauen, R., 1993. Neural Networks for Optimization and Signal Processing. John Wiley and Sons Inc, New York, NY, USA.
Engqvist, M., 1970. Gestaltkrafte des Lebendigen. Klostermann, Frankfurt am Main. .
Freeman, J.A., Skapura, D.M., 1991. Neural Networks Algorithms, Applications, and Programming Techniques. Addison-Wesley Publishing Company, Boston, MA.
Gustafson, R.H., Bowen, R.E., 1997. Antibiotics use in animal agriculture. Journal of Applied Microbiology 83, 531–541.
Huber, M.A.S., Doesburg, P., van de Vijver, P.L., 2007. Biocrystallisations—Milk Treatments: Milk Treatment Experimental Results. Rapport M26. Louis Bolk Instituut, Driebergen, The Netherlands.
Huber, M.A.S., Anderson, J-O., Kahl, J., Busscher, N., Doesburg, P., Mergardt, G., Kretschmer, S., Zalecka, A., Meelursarn, A., Ploeger, A., Nierop, D., van de Vijver, L.P.L., Baars, E.W., 2010. Standardization and validation of the visual evaluation of biocrystallization. Biological Agriculture and Horticulture 27 (1) 25–40.
Islam, M.M., Sattar, A., Amin, F., Yao, X., Murase, K., 2009. A new adaptive merging and growing algorithm for designing artificial neural networks. IEEE Transactions on Systems, Man, and Cybernetics Part B: Cybernetics 39 (3) 705–722.
Kahl, J., 2007. Entwicklung, in-house Validierung und Anwendung des ganzheitlichen Verfahrens Biokristallisation für die Unterscheidung von Weizen-, Möhren- und Apfelproben aus unterschiedlichem Anbau und Verarbeitungsschritten. Habilitationsschrift. Ph.D. ThesisUniversität Kassel, Germany.
Kahl, J., Busscher, N., Doesburg, J., Mergardt, G., Huber, M., Ploeger, A., 2009. First tests of standardized biocrystallization on milk and milk products. European Food Research and Technology 229, 175–178.
Kang, J.H., Jin, J.H., Kondo, F., 2005. False-positive outcome and drug residue in milk samples over withdrawal times. Journal of Dairy Science 88 (3) 908–913.
KKGM, 2007. Koruma and Kontrol Genel Mudurlugu. In: T. C. Tarim ve Koyisleri Bakanligi. Hayvansal kokenli gidalarda veteriner ilacları maksimum kalinti limitleri tebligi. Turkish Food Codex, Retrieved December 7, 2011 fromhttp://www.resmigazete.gov.tr/eskiler/2002/04/20020428.htm#5.
Kokornaczyk, M.O., 2008. Quality comparison of organic and conventional wheat by the use of common and holistic methods of analysis. Ph.D. ThesisThe University of Pisa, Italy.
Le Breton, M.H., Savoy-Perroud, M.C., Diserens, J.M., 2007. Validation and comparison of the copan milk test and delvotest SP-NT for the detection of antimicrobials in milk. Analytica Chimica Acta 586, 280–283.
Masters, T., 1993. Practical Neural Network Recipes in C++. Academic Press, New York, NY, USA.
Meelursarn, A., 2007. Statistical evaluation of texture analysis data from the biocrystallization: effect of image parameters to differentiate samples from different farming systems. Ph.D. ThesisUniversität Kassel, Germany.
Morris, D.L., Morris, C.T., 1941. The modification of cupric chloride crystallization patterns by traces of proteins. The Journal of Biological Chemistry 141, 515–521.
Pelvan, M., 2011. Determination of antibiotics in raw and UHT milk samples by the image forming method of biocrystallization. M.Sc. ThesisIzmir Institute of Technology, Turkey. http://iyte.mitosweb.com/eng/browse/79979/
Rinken, T., Riik, H., 2006. Determination of antibiotic residue and their interaction in milk with lactate biosensor. Journal of Biochemical and Biophysical Methods 66, 13–21.
Santos, M.S., Henriques, M., Duarte, C.A., Esteves, I.V., 2007. Development and application of a capillary electrophoresis based method for the simultaneous screening of six antibiotics in spiked milk samples. Journal of Chromatography A 71 (2) 731–737.
Unluturk, S., Unluturk, M.S., Pazir, F., Kuscu, A., 2011. Process neural network method: case study I: discrimination of sweet red peppers prepared by different methods. EURASIP Journal on Advances in Signal Processing, http://dx.doi.org/10.1155/2011/290950, Article Number: 290950.
Yildiz, O., 2008. Investigation of physical and chemical properties of milk containing antibiotics. M.Sc. ThesisIzmir Institute of Technology, Turkey.
Zheng, C.X., Sun, D.W., Zheng, L.Y., 2005. Correlating color to moisture content of large cooked beef joints by computer vision. Journal of Food Engineering 77 (4) 858–863.
Zvirdauskiene, R., Salomskiene, J., 2007. An evaluation of different microbial and rapid tests for determining inhibitors in milk. Food Control 18, 541–547.