Authenticity assessment of goat milk: detecting dilution with cow milk by ML-enhanced speckle pattern imaging

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ABSTRACT
Dilution of precious goat milk with cheaper cow milk represents one of the most common types of food fraud in the dairy industry. Existing methods for detecting the presence of cow milk mainly rely on chemical tests or spectroscopic analyses, which require dedicated laboratory facilities, expensive instrumentation, complex sample preparation, and long waiting times for results. We propose an innovative method based on the combination of speckle pattern imaging and artificial intelligence models to detect the adulteration of goat milk with cow milk. The instrumental setup we developed is based on a low-cost semiconductor laser and an industrial CMOS camera. We analyzed five milk samples and applied well-established machine learning models, achieving an accuracy of 96.9 % on the test set. These results are very promising and pave the way for the development of new opto-electronic systems to fight milk adulteration.

1. INTRODUCTION

Goat milk is highly valued for its nutritional content, digestibility, and hypoallergenic properties, making it the preferred choice for consumers who are allergic or intolerant to cow milk [1]. Due to its limited availability and higher production cost, goat milk is typically more expensive than cow milk. Unfortunately, this creates the condition for fraudulent practices: one of the most common is the adulteration of goat milk through the addition of cheaper cow milk to dairy derivatives (especially cheese) that are falsely labeled and sold as "pure goat" products [2], [3]. This practice is referred to as "sophistication", which is the deliberate adulteration of a precious substance with another one of inferior quality. Sophistication represents a food fraud against consumers, since, in many European countries, legislation requires producers to clearly indicate the type of milk used for the production of dairy products. Moreover, substitution of goat milk with cow milk may also pose dangerous health consequences for individuals allergic to bovine milk proteins.

For all these reasons, the identification of cow milk in goat milk has received great attention in recent years. This type of economically motivated adulteration is often difficult to detect visually

or organoleptically, due to the similar appearance and texture of different types of milk. Consequently, there is a growing interest in the development of innovative methods for the authenticity assessment of dairy products and the detection of milk adulteration. Many methods have been proposed in the scientific literature to detect the presence of adulterants in milk [4] and, in particular, to reveal cow milk addition in precious goat milk. The majority of the studies in this field rely on the use of polymerase chain reaction (PCR) bioassays [5], immunoassays [6], surface plasmon resonance (SPR) [7], and, above all, chemical tests [8]–[11]. These methods are highly sensitive and specific, but they require complex pre-treatment of the sample to be done in specialized laboratories by qualified technicians, thus requiring high costs and long waiting times.

As an alternative, many optics-based solutions have been proposed. Indeed, optical radiation allows non-invasive contactless analyses, and manipulation of the sample is usually not required. These solutions mainly rely on near-infrared (NIR) and mid-infrared (MIR) spectroscopy. These techniques are based on measuring the absorbance or reflectance of light by a sample in the wavelength range from 800 nm to 2500 nm and from 2500 nm to

25 000 nm, respectively. These techniques have been successfully adopted to detect and identify the presence of microplastics in liquids such as milk or seawater [12], [13], or even to measure milk properties [14]. Specifically in the field of milk sophistication, in [15] NIR spectroscopy was combined with Principal Component Analysis (PCA) and Partial Least Squares (PLS) analysis with a limit of detection of 1.0154 g of cow milk added to 100 g of goat milk. Du et al. combined MIR spectroscopy with PLS analysis, demonstrating that the effect of diluting goat milk with cow milk is particularly evident in the spectral bands from 2000 nm and 2700 nm and 3300 nm to 5880 nm [16]. Other works exploit Raman spectroscopy, a vibrational spectroscopic technique adopting laser light to detect molecular vibrations, providing a chemical fingerprint of components in the sample [17], [18]. These optical techniques require bulky and very expensive instrumentation, and they are not suitable for frequent on-site tests of milk at production sites.

Among optical methods, speckle pattern (SP) imaging represents an interesting alternative. SP analyses can be performed in a contactless and non-invasive manner, even with low-cost instrumentation, and they do not require sample preparation. SP is the granular interference generated when coherent light illuminates a target object with roughness of the same order of magnitude as the wavelength [19]. The statistical properties of interference SP depend on the composition and structure of the target source. Traditionally, static SP has been applied to study the roughness of surfaces [20], the granularity of powders [21], and the structure of biological tissues [22]. In the last few years, SP imaging has also revealed potential for studying fluids. Indeed, opaque liquids, suspensions, or emulsions (such as milk) generate SP, too, since they contain particles floating in a liquid matrix. Scattering elements in such liquids are subject to Brownian motion; the SP they generate is dynamic and, thus, more challenging to be investigated. A few works reported in the literature focus on SP imaging, also combined with artificial intelligence (AI) to investigate suspensions or microplastics [23], [24]. In our previous works, we have exploited SP imaging to reveal cow milk dilution [25], adulteration with water and glucose [26], and even to distinguish between samples of raw cow milk [27].

This work demonstrates the use of SP imaging combined with an AI-based analysis to investigate the authenticity of goat milk and detect adulteration with cheaper cow milk. Besides our latest advancements in the field of SP imaging, this work is a further step towards the full comprehension of the capabilities of the proposed measurement pipeline, because mixtures of milks are more difficult to detect than dilutions. Our opto-electronic configuration for SP generation and acquisition is low-cost and compact, potentially portable, and easy-to-use also by non-technically expert users. After conducting multiple experimental campaigns on five samples of goat and cow milk, we have extracted 12 statistical features of interest from every collected SP image and used them to train and test 10 well-established machine learning (ML) algorithms to prove the potential of the method.

2. OPTO-ELECTRONIC SETUP

The opto-electronic instrumental configuration used for the excitation and the acquisition of SP images produced by milk samples is shown in Figure 1. A semiconductor laser diode (L658P040, Thorlabs, NJ, USA) is used as a coherent light source to irradiate the samples. It emits a maximum optical power of 40 mW at the wavelength of 658 nm. It is powered by a current driver (LDC500, Thorlabs, NJ, USA) and thermally stabilized to the temperature of 25 °C by means of a temperature controller (PRO800, Thorlabs,
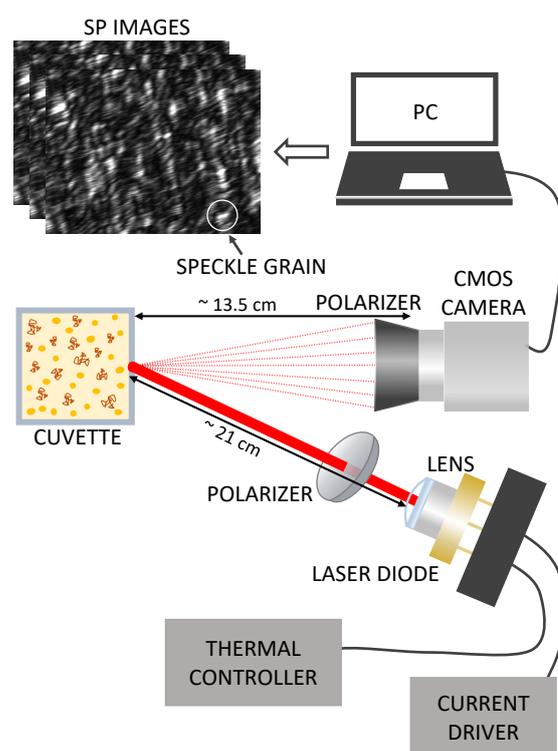

Figure 1. Opto-electronic setup used to excite and collect the SP images. Light emitted by the laser diode is polarized and focused onto the surface of the cuvette containing the milk sample under test. Generated SP images are collected by means of an industrial CMOS camera.

NJ, USA). The laser beam is focused by means of an antireflection coating aspheric lens (C230260P-B, Thorlabs, NJ, USA) onto the surface of a transparent plastic cuvette (with dimension of $10 \times 10 \times 50$ mm³ and a total volume of approximately 4.5 mL), containing the milk sample under test, at an angle $\delta = 30°$. A linear polarizer (LPVISE100-A, Thorlabs, NJ, USA) is located after the lens to select only the main polarization component. The laser beam irradiating the cuvette has a diameter of about 1 mm. Because of the angle of incidence δ, the laser spot on the cuvette wall has an elliptical shape with minor axis of length equal to $d = 1$ mm (as the laser beam diameter) and major axis of length $D = d / \cos(\delta) \approx 1.15$ mm. Moreover, due to the strong attenuation caused by scattering, the penetration depth is around 2 mm. Hence, the sample volume irradiated by the laser and actually needed for the measurement is around 6 µL. Images and videos of the SP produced by the milk samples are collected with a monochrome CMOS camera (U3-38J0XCP-M-NO, by iDS Imaging Development Systems GmbH, Germany) positioned in front of the cuvette. The camera was oriented in this way to prevent specular reflections from the cuvette wall from reaching and saturating the CMOS sensor. The camera sensor has a surface of 5.603 mm × 3.155 mm, a total number of pixels of 3864 × 2176, and a pixel size of 1.45 µm × 1.45 µm. A second linear polarizer (equal to the first one) is placed in front of the CMOS sensor. The camera is connected via USB to a laptop, allowing data acquisition through proprietary software.

3. MILK SAMPLES

Cartons of whole cow milk (sample M1) and goat milk (sample M2) were purchased at a local supermarket. Two brands were selected, being some of the most popular among Italian consumers. Both cow and goat milk were sterilized, pasteurized, and treated
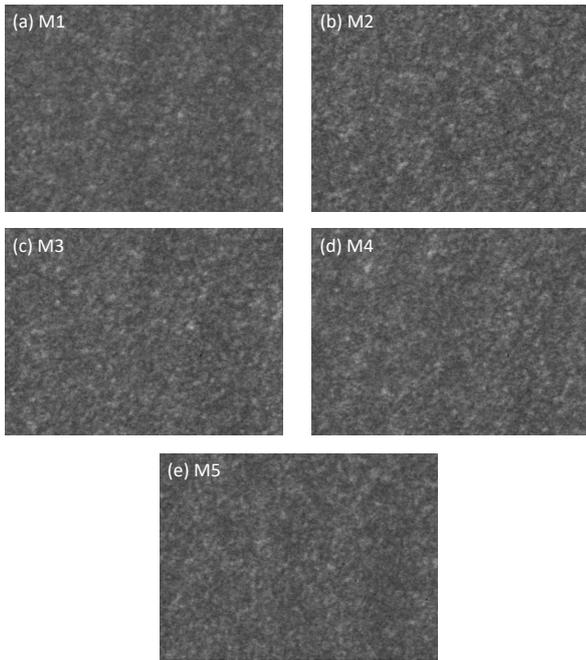
Figure 2. Example of typical SP images acquired during experimental testing. (a) SP image generated by M1 (cow milk); (b) SP image generated by M2 (goat milk); (c) SP image generated by M3 (85 % of goat milk mixed with 15 % of cow milk); (d) SP image generated by M4 (70 % of goat milk mixed with 30 % of cow milk); (e) SP image generated by M5 (55 % of goat milk mixed with 45 % of cow milk). The five images look almost identical to visual inspection.

with an ultra-high temperature (UHT) process. The composition of whole cow milk reported on the carton label is as follows: 35 g/L of lipids, 34 g/L of proteins, and 51 g/L of carbohydrates (mainly lactose). The composition of whole goat milk reported on the carton label is as follows: 35 g/L of lipids, 33 g/L of proteins, and 45 g/L of carbohydrates (mainly lactose). It can be noted that the composition of cow and goat milk is very similar in terms of lipid and protein concentrations, while goat milk has a slightly lower content of carbohydrates. However, it is well-known from the literature that cow and goat milk have substantial differences in terms of their microscopic molecular structure. For example, goat milk fat globules are significantly smaller on average and have a smaller range of sizes compared to cow milk fat globules [28]. Also, the structural composition of the proteins is different [29]. To test the sophistication of goat milk with cow milk, 3 mixtures of the two types of milk were then prepared by mixing 85 % of goat milk with 15 % of cow milk (sample M3), 70 % of goat milk with 30 % of cow milk (sample M4), and 55 % of goat milk with 45 % of cow milk (sample M5), respectively. As described in detail in [25], milk is a highly scattering fluid because it is an emulsion and can be considered as a suspension of particles (fat globules and casein micelles) floating in a liquid matrix of water, carbohydrates, and soluble proteins. Hence, the features of the generated SP images are strongly influenced by the milk samples' composition, as well as by the concentration, structure, and dimension of lipid and protein particles [30], [31].

4. EXPERIMENTAL DATA COLLECTION

A total of 20 experimental measurement campaigns were conducted, involving 6 milk cartons to include greater sample variability in the analysis. Specifically, 3 cartons of cow milk (cartons 1, 2, and 3) and 3 cartons of goat milk (cartons 4, 5, and 6) were opened and tested. Campaigns 1–11 were carried out using carton 1, carton 4, and mixtures of the two. Campaigns 12–15 involved carton 2, carton 5, and their mixtures. Campaigns 16–20 were conducted using carton 3, carton 6, and mixtures of the two. During each experimental campaign, each sample was inserted into 3 different cuvettes (to include the variability of the container). For every cuvette, 50 SP images were collected. Hence, for every campaign, 750 SP frames were acquired (50 SP images × 3 cuvettes × 5 samples). Since 20 campaigns were carried out, we obtained a total of 15000 SP images. During the experimental procedure, the laser current was set to 67.7 mA, the camera frame rate to 25 fps, and the exposure time to 1700 μs. All measurements were carried out at room temperature, over a time interval of 5 days.

5. DATASET CREATION AND ML MODELS

Collected SP images were elaborated to extract 12 relevant statistical features used to populate the database for training and testing the ML models. 8 features (mean intensity, i.e. mean gray level value of the image, standard deviation of the image intensity, image median, image kurtosis, image skewness, constrast of the image, and size of the SP grains along the x- and y-direction) are commonly used in SP-based analyses [32], [33]. 4 further features were extracted from the so-called Gray-Level Co-Occurrence Matrix (GLCM) (a matrix retrieved from the original SP image, which represents the distribution of co-occurring pixel values in a given direction) since they proved to be very representative in our previous works [25]–[27]. These features are GLCM contrast, GLCM correlation, GLCM energy, and GLCM homogeneity. The obtained dataset has a dimension of 15000 rows (corresponding to the number of SP images collected during the 20 campaigns) by 12 columns (corresponding to the number of features). We then divided it into the training set and the test set, following the commonly used 75 %–25 % split ratio. We included campaigns 1–15 in the training set, while campaigns 16–20 were assigned to the test set, ensuring that the data used during testing had never been seen by the models before.

Data standardization was conducted using z-score. The data contained in the training set were standardized (feature by feature) using the mean and standard deviation obtained from the training set itself. On the other hand, the data of the test set were standardized using the same mean and standard deviation from the test set. SP image processing and data standardization were carried out in MATLAB (R2023b version) environment. We evaluated 10 well-established classification models using MATLAB Classification Learner app, which provides a user-friendly environment for training, validating, and comparing different ML models. In detail, we have tested and trained the following models: (1) linear discriminant analysis, (2) quadratic discriminant analysis, (3) efficient logistic regression, (4) efficient linear support vector machine (SVM), (5) medium gaussian SVM, (6) fine gaussian SVM, (7) coarse gaussian SVM, (8) ensemble of boosted decision trees, (9) ensemble of bagged decision trees, (10) ensemble of subspace discriminants.

6. RESULTS

An example of the typical SP images that we collected for the 5 tested samples is reported in Figure 2. A simple visual inspection of the images does not allow for any clear distinction between them. Therefore, using ML algorithms becomes fundamental to properly distinguish the samples. As a preliminary step, we applied PCA for visualization purposes, to project the original 12 features into a new set of uncorrelated components that retain most of the variance in the data and that capture the majority of
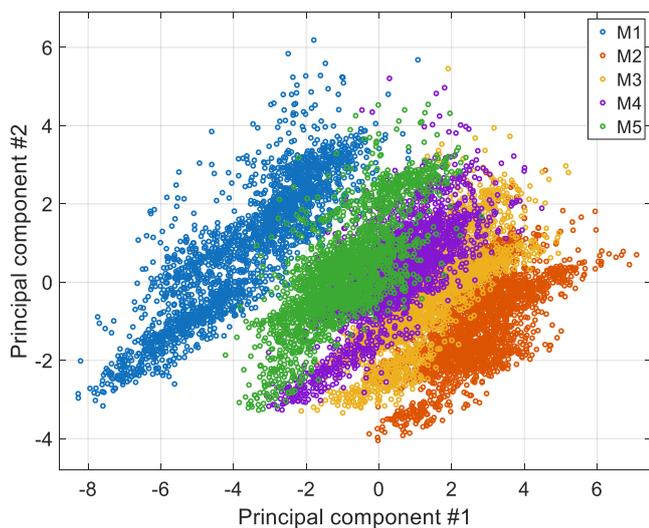
Figure 3. Scatter plot showing the distribution of data represented by means of the first and second principal components (principal components #2 vs principal components #1). Blue circles represent whole cow milk (M1), the orange circles represent whole goat milk (M2), the yellow circles represent the sample containing 85 % of goat milk and 15 % of cow milk (M3), the purple circles represent the sample containing 70 % of goat milk and 30 % of cow milk (M4), and the green circles represent the sample containing 55 % of goat milk and 45 % of cow milk (M5), respectively.

Figure 4. Confusion matrix obtained by applying the trained quadratic discriminant analysis model to the test set. Average accuracy is 96.9 %. Positive predictive Vvalues (PPV), false detection rate (FDR), true positive rate (TPR), and false negative rate (FNR) are reported for a better understanding of results.

the information from the original features. In particular, we considered the 2D lower-dimensional space to explore the structure and potential clustering patterns of the data. Figure 3 shows the 2D scatter plot of the first and second principal components for all the instances contained in the complete dataset. The principal components were computed in MATLAB environment. Here, they are ordered according to the percentage of data variance they explained; the first two explain more than 90 % of data variance. In the figure, the blue dots represent whole cow milk (M1), the orange dots represent whole goat milk (M2), the yellow dots represent the sample containing 85 % of goat milk and 15 % of cow milk (M3), the purple dots represent the sample containing 70 % of goat milk and 30 % of cow milk (M4), and the green dots represent the sample containing 55 % of goat milk and 45 % of cow milk (M5), respectively. It is interesting to observe that every milk sample corresponds to a different cluster, even though several outliers belonging to different classes appear overlapped, in particular for M3, M4, and M5. The blue data cloud and the orange one are the farthest, since they correspond to cow and goat milk, respectively. The clusters of the three other samples (M3, M4, M5) are reasonably located in between; in particular, when the concentration of cow milk in the sample increases, the cluster tends to move far away from that of M2 and closer to that of M1. Hence, the cluster of M3 (containing 85 % of goat milk) is closer to M2, while the cluster of M5 (containing only 55 % of goat milk) is closer to M1.

Afterwards, we proceeded with the ML analysis. All models were trained and tested using the original 12 features of the dataset, rather than the principal components. The best-performing model, in terms of accuracy, proved to be the quadratic discriminant analysis classifier. The hyperparameters of the algorithm are the default ones set by MATLAB (preset = "quadratic discriminant", covariance structure = "full", PCA = "disabled"). This model reached an average accuracy of 96.9 %, an extremely good result considering that SP images obtained from different milk samples (shown in Figure 2) cannot be distinguished with traditional approaches, making AI a necessity for the correct classification of the

samples. In more detail, Figure 4 shows the confusion matrix of the classification model obtained on the test set. Four prediction metrics typically used in ML are also reported: true positive rate (TPR), false negative rate (FNR), positive predicted value (PPV), and false detection rate (FDR). TPR (also called recall or sensitivity) is the percentage of actual positive cases correctly identified by the model classifier, while FNR represents the proportion of actual positive cases that the model incorrectly predicted as negative. PPV (also known as precision) is the proportion of predicted positives that are actually true positives. FDR (FDR = 1 - PPV) is the percentage of predicted positives that are actually false positives. From Figure 4, we can observe that the PPV and TPR are equal to 100 % for M1, meaning that the model always correctly recognizes cow milk and never predicts goat milk or mixtures as cow milk. Most importantly, the PPV for M2 is also extremely high (98.1 %). This indicates that among all the instances that the model classified as pure goat milk, only 1.9 % of predictions were wrong. In other words, in only 1.9 % of the occurrences the ML model mistakenly predicted another sample as pure goat milk. These misclassifications occurred only for M3, that is the mixture containing the lowest percentage of adulterant (the cow milk), and thus more difficult to distinguish. Looking at the mixture samples (M3, M4, M5), we observe that they are correctly classified (in 92.3 %, 96.9 %, and 96.0 % of cases, respectively), and, very importantly, only M3 is rarely predicted as M2 (in 0.3 % of cases). On the other hand, M4 and M5, which contain a higher percentage of cow milk with respect to M3, are never predicted as M2. Moreover, the model only rarely makes confusion between M3, M4, and M5, meaning that it is also able to correctly classify the percentage of cow milk added to goat milk. To gain a deeper understanding of the classification process, we also computed the Shapley values for the model. These are attribution scores, grounded in game theory, that quantify the impact of each feature on the model's predictions for each class. A high Shapley value (in modulus) indicates that a feature had a strong influence on the model decision process. Specifically, for the quadratic discriminant analysis classifier, the feature with the highest modulus of the Shapley value is the mean intensity (approximately 0.18), closely followed by GLCM contrast, standard deviation of im-

age intensity, and GLCM energy. All other features have Shapley values below 0.05 in modulus. Relying predominantly on the mean intensity feature is not the optimal choice, as the intensity of SP images can be affected by external or spurious factors unrelated to the intrinsic characteristics of the samples. Therefore, incorporating additional statistical features enhances both the robustness and generalization ability of the model. In addition, in our previous studies [27], we observed that removing even a single feature can lead to a drop in the overall classification accuracy. Thus, despite some features having lower influence, we chose to retain all of them.

In conclusion, the ML analysis applied to the statistical features extracted from the SP proved to be an interesting and promising tool to distinguish different types of milk and detect the sophistication of precious goat milk by cheaper cow milk.

7. CONCLUSION

Addition of cow milk to more valuable goat milk is a very common type of food fraud. In this work, we proposed the use of SP imaging in combination with a ML classification based on statistical features extracted from the SP images to detect counterfeiting of goat milk with cheaper cow milk. We have proposed a low-cost simple opto-electronic setup based on the use of a semiconductor laser and an industrial CMOS camera. In our analysis, we considered whole goat milk, whole cow milk, and 3 mixtures of the two. We conducted an extensive experimental study, carrying out 20 experimental measurement campaigns and building a dataset of 15000 instances. From every SP image, we extracted 12 statistical features that we used for training and testing of 10 ML algorithms. We observed that the quadratic discriminant analysis is the best-performing model for our purpose, with an average accuracy of 96.6 %. With the designed measurement system and data analysis pipeline, we could correctly identify mixtures of cow and goat milk, and even identify the correct concentration of the two types of milk in the majority of cases. Our technique is based on the integration of SP imaging and ML-based analysis, representing an innovative low-cost alternative to chemical tests and spectroscopic measurements, since it does not require expensive and bulky instrumentation or milk samples manipulation. In the future, we will carry out further analyses to quantify the limit of detection of our methods and enhance detection resolution. We will also test additional brands of milk to further investigate the generalization ability and the practical applicability of our method. Moreover, we plan to exploit our ML-enhanced SP imaging platform to analyze milk samples contaminated with more toxic and dangerous chemical substances, such as melamine, starch, whey, hydrogen peroxide, chlorine, starch, and oils, which are common adulterants used to counterfeit milk.

AUTHORS' CONTRIBUTION

Irene Bassi: data curation, formal analysis, investigation.

Cristina Nuzzi: conceptualization, methodology, validation, writing – review & editing.

Simone Pasinetti: conceptualization, funding acquisition, project administration.

Sabina Merlo: conceptualization, funding acquisition, project administration.

Valentina Bello: conceptualization, methodology, supervision, validation, visualization, writing – original draft.

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