The MUSKETEER project: Milk adUlteration detection using SpecKlE paTtern and machinE lEaRning

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ABSTRACT

The MUSKETEER (Milk adUlteration detection using SpecKlE paTtern and machinE lEaRning) project aims to address the global challenge of fighting milk adulteration, which poses significant health risks for consumers. Traditional methods for milk analysis (eg. ELISA, PCR, chemical tests) are complex, time- and money-consuming. The project goal is the development of a user-friendly platform that employs real-time artificial intelligence (AI)-based processing of speckle pattern (SP) images to identify adulteration in milk samples non-invasively. SP is the interference pattern produced when laser light illuminates a milk sample, which has a non-uniform refractive index distribution due to the presence of suspended particles. Images of SP acquired by an low-cost industrial camera are rich in information about the sample. In this work, we report an effective method to recognize different types of commercial cow milk and to identify milk dilution with water and 12.5% water-glucose solution. The average intensity and the dimension of the SP grains can be extracted from SP images. By considering both statistical parameters, our system can distinguish between different types of milk and detect diluted samples with both water and glucose, offering a reliable approach to address milk adulteration and ensure the integrity of dairy products on the market.

Keywords: Milk adulteration, scattering fluids, speckle pattern imaging, machine learning

1. INTRODUCTION

Milk, a mixture of lipids, proteins, and carbohydrates, is consumed worldwide and represent the primary ingredient in numerous dairy products. According to the EU Agri-Food Fraud Network (EU-FFN)1 and the Food Fraud and Quality Knowledge Center (KC-FFQ)2, milk is one of the products most frequently prone to food adulteration worldwide. The most common type of counterfeiting involves dilution with water, sugar syrups, or hydrogen peroxide, which are added to increase volume and, consequently, profit. Afterwards, milk could be contaminated with harmful substances (such as urea, melamine, detergents, formalin, chlorine, starch, oils) to simulate its original physical/chemical and nutritional characteristics. This type of chemical adulteration poses serious health risks for consumers, such as kidney and liver failure, gastrointestinal diseases, allergy, poisoning, and even cancer. On the other hand, the use of adulterated milk for production of dairy derivatives (cheese, cream, yogurt) leads to quality degradation of final products and constitutes an illegal food fraud.

Nowadays, reliable detection of milk adulteration is achieved by means of complex physical- chemical analyses, which can be performed only in specialized chemical laboratories. Common methods used to identify illegal additives in milk are cryoscopy3, mass spectrometry4, the Enzyme Linked Immunosorbent Assay (ELISA)5, Polymerase Chain Reaction (PCR) tests 6, and other chemical analyses 7, 8. Although highly sensitive and specific, chemical tests have significant drawbacks. Indeed, they need complex procedures for sample manipulation and the use of dangerous chemicals, to be handled by qualified personnel in dedicated laboratories. Moreover, they require the use of expensive and bulky instrumentation. Optical sensing techniques could represent a smart

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alternative to laboratory analyses since they are contactless, non-invasive, immune to electromagnetic interference and make use of non-ionizing radiation 9. However, while several conventional optical methods are efficient and suitable for analyzing transparent liquids, they cannot be applied to fluids such as milk, appearing as a turbid and opaque sample. This is due to light scattering that destroys light coherence and beam directionality, causing degradation of the signal-to-noise ratio. In this article, we introduce MUSKETEER as an innovative measurement system capable of detecting milk adulterations that exploits precisely the scattering of light by milk and is intended to be applied to opaque, diffusing fluids 10, 11.

1.1 Aim of MUSKETEER

In more detail, MUSKETEER (is to demonstrate an innovative, easy-to-use, economic, and portable smart platform capable of identifying milk adulteration. Additionally, the system will enable the remote and non-invasive detection of potentially hazardous chemical compounds in milk, requiring only a small sample of volume. This is achieved by) leverage the scattering of light by milk that is a liquid suspension of lipid micelles and caseins surrounded by a matrix composed of water, lactose, electrolytes, and proteins and strongly scatters coherent light. In fact, when it is illuminated by coherent radiation, light is scattered all around and if it is captured by a camera, the resulting images exhibit an interference pattern of bright spots on a dark background . This pattern is called SP and its distribution may appear random at first glance. Instead, SP images and video are dense in information that, by proper elaborations, could be correlated with the main properties of the milk sample under test. In a future stage, the project will also involve the use of machine learning and deep learning, to recognize different types of milk and adulterants based on the resulting SP. A key aspect of MUSKETEER will be the design of a portable and compact sensing device that can be easily used by non-expert personnel for on-site, real-time analyses during quality and safety checks on dairy products.

In this work, we present a proof of concept demonstrating the effectiveness of the system in detecting milk adulteration. Specifically, we show that milk dilutions with water and a 12.5% water-glucose syrup solution can be identified by extracting two parameters from SP images: average gray intensity and speckle grain size. These values are then analyzed to detect differences between samples using statistical hypothesis tests 12. This specific concentration of 12.5% glucose solution was chosen because its refractive index (RI) closely matches that of whole milk, making it difficult to detect through traditional refractometric testing.

2. MATERIAL AND METHODS

2.1 Experimental configuration

The instrumental configuration used for the excitation and the acquisition of SP images generated by milk samples is reported in Fig. 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 supplied through a current driver (LDC500, Thorlabs, NJ, USA) and connected to a temperature controller (PRO800, Thorlabs, NJ, USA) for thermal stabilization. An antireflection coating aspheric lens (C230260P-B,

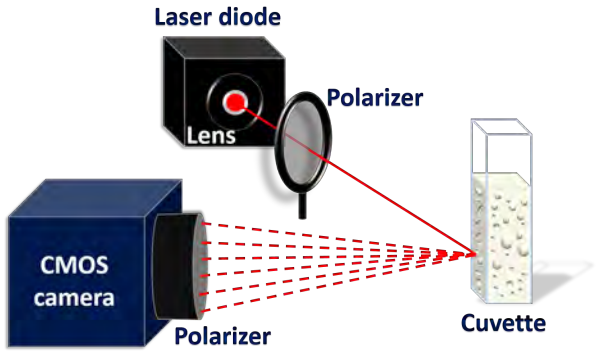

Figure 1. Scheme of the experimental setup for speckle pattern imaging.

Thorlabs, NJ, USA) is used to focus the radiation onto the surface of a transparent plastic cuvette (volume 4.5 mL and size $10 \times 10 \times 50$ mm) containing the milk sample, at an angle of about 30°. A linear polarizer (LPVISE100-A, Thorlabs, NJ, USA) is positioned after the lens to select only the main polarization component. Images and videos of the SP generated by the scattering samples are acquired using a monochrome CMOS camera (Grasshopper3 GS3-U3-41C6NIR, FLIR System Inc., OR, USA) located in front of the cuvette. The chosen camera orientation prevents Snell reflection from the cuvette wall from reaching and saturating the CMOS sensor. The camera sensor has a size of 11.26×11.26 mm, a total number of pixels of 2048×2048, and a pixel size of 5.5×5.5 μm. A second linear polarizer is placed before the CMOS sensor. The camera is connected via USB to a laptop, allowing data acquisition through proprietary software (Point Grey Flycap2). All measurements are conducted in a dark room.

2.2 Milk sample preparation and data acquisition

Analyses were first conducted on three types of commercially available cow milk — whole milk (composition: 36 g/L of lipids, 34 g/L of proteins and 51 g/L of carbohydrates), partially skimmed milk (16 g/L of lipids, 34 g/L of proteins and 52 g/L of carbohydrates), and skimmed milk (<0.5 g/L of lipids, 33 g/L of proteins and 49 g/L of carbohydrates). All samples consisted of pasteurized long-life milk processed using ultrahigh-temperature (UHT) treatment. These samples differ mainly in their fat content. Indeed, lipid content tends to decrease by half from whole milk to partially skimmed milk, becoming nearly absent in fully skimmed milk. Milk dilutions were then prepared by starting from whole milk and gradually adding increasing volumes of deionized water. As a result, samples were created containing 90%, 80%, 70%, and 60% of whole milk, which were then analyzed. Using the same concentrations, other adulterated milk samples were prepared by incorporating varying amount of a 12.5% water glucose solution into whole milk. It is worth noting that the refractive index (RI) of this specific water-glucose solution is similar to that of cow milk. In more detail, fat micelles exhibit a RI n_L ranging from 1.458 to 1.460 RIU 13, while the surrounding liquid matrix has an RI n_M between 1.376 and 1.405 RIU, depending on its water, lactose, electrolyte, and protein content. Thus, lipids act as scattering particles inside the fluid, and the amount of scattered light depends both on the concentration of lipids and on the RI difference $\Delta n = |n_M - n_L|$. SPs were recorded for each prepared milk sample. In particular, a video consisting of 100 SP frames was captured in raw format with an 8-bit depth, resulting in images with 256 gray levels, ranging from 0 (representing black) to 255 (representing white). The laser driving current was set to 80 mA, and its temperature stabilized at 25°C. The camera exposure time and frame rate were set to 602 µs and 90 fps, respectively. An example of SP frame of whole milk is reported in Fig. 2 in false-color map.

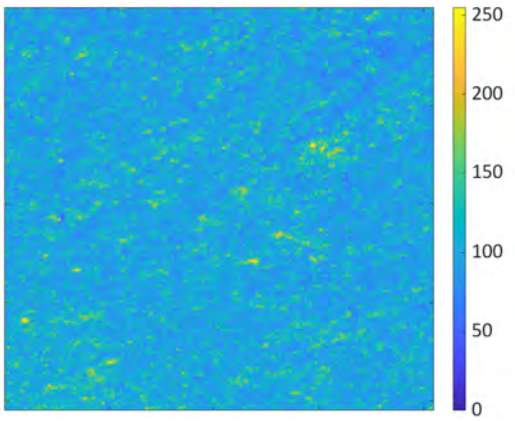

Figure 2. Example of a SP image of a 100% whole milk sample displayed in false colors. The intensity scale ranges from 0 (blue) to 255 (yellow), following the standard MATLAB parula colormap.

2.3 Data parameter extraction

The acquired SP images were then processed using a custom code in the MATLAB environment. For each SP image, the histogram representing the distribution of gray levels was computed, together with the average gray

level intensity $\bar{I}$, calculated using the following formula:

$$\bar{I} = \frac{1}{N}\sum_{k=1}^{N} I_k$$

where N is the total number of pixels in the image, and I_k represents the gray level value of the k-th pixel. Another parameter that we took into account is the 3D normalized autocovariance function $c_N(x, y)$ of the SP images. This is an informative parameter on the structure and texture of the images. The normalized autocovariance function is given by:

$$c_N(x, y) = \frac{c(x, y)}{max(c(x, y))}$$

where $c(x, y)$ is the non-normalized autocovariance function, that is calculated as:

$$c(x, y) = \mathcal{F}^{-1}\{|\mathcal{F}[I(x, y) - \langle I(x, y)\rangle]|^2\}$$

where $\mathcal{F}$ represents the Fourier transform, $\mathcal{F}^{-1}$ represents the inverse Fourier transform and $I(x, y)$ is the gray level matrix representing the SP frame 14. The dimensions of the 2D SP grains, which represent the brighter spots in the image, can be calculated as the Full Widths at Half Maximum (FWHM) on the 2D projections of the normalized autocovariance function $c_N(x, y)$. Specifically, it is possible to retrieve the dimension grain along the x-direction (Δx) as the FWHM of $c_N(x, y = 0)$, while the y-direction (Δy) as the FWHM of $c_N(x = 0, y)$. However, since Δx and Δy are highly correlated, only Δx has been reported and discussed in the following sections.

3. PRELIMINARY RESULTS AND DISCUSSION

3.1 Experimental results for three commercial pure cow milk samples

First, whole milk, partially skimmed milk and skimmed milk were tested. SP images were acquired and, after outlier removal, the gray level histograms were retrieved from the valid frames. As an example, Fig. 3 reports histograms extracted from SP frames acquired for whole milk (green trace), partially skimmed milk (blue trace) and skimmed milk (red trace). From the histogram shapes, it is already possible to distinguish the three samples. In particular, the histograms tend to shift towards darker intensity values when the concentration of lipids decreases. This effect can be explained by considering that a lower fat content corresponds to a lower

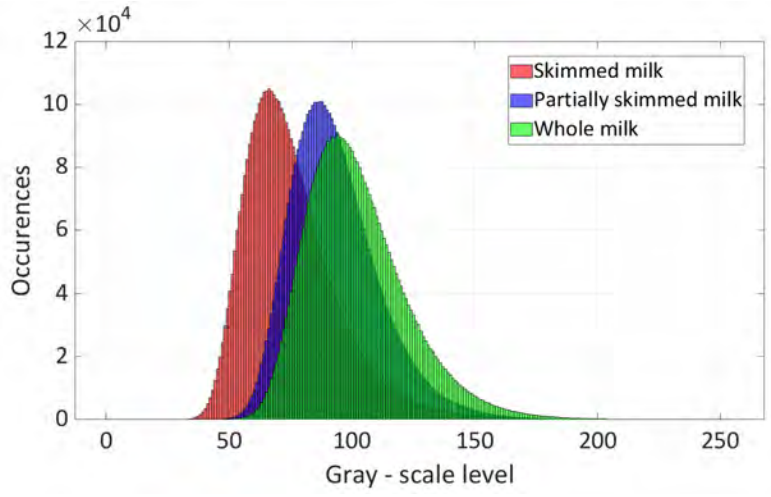

Figure 3. Histograms representing the gray level distributions for whole (green trace), partially skimmed (blue trace) and skimmed milk samples (red trace).

concentration of lipid micelles that act as scattering particles in the sample. Hence, for lower concentration of lipids the intensity of back-diffused light decreases. In particular, the average gray level intensity was found equal to 100.7 ± 1.3 , 91.1 ± 1.1, and 73.9 ± 1.1, for whole, partially skimmed, and skimmed milk, respectively.

An ANOVA (Analysis of Variance) test was conducted to assess whether the null hypothesis — stating no differences between groups — could be rejected. The ANOVA results ($F = 13.232$, $p \leq 0.001$) strongly rejected the null hypothesis, indicating statistically significant differences in the average intensity values among the three samples. To identify which samples differed from each other, a post-hoc Tukey HSD test was performed. This test, applied after ANOVA, confirmed that all three milk samples were statistically distinct ($p \leq 0.001$) based on the average intensity parameter.

Subsequently, the normalized autocovariance function was computed and the SP grain dimension Δx was determined as the FWHM of the 2-D function. The values of Δx were found to be 49.8 ± 7.1 μm for whole milk, 35.0 ± 2.6 μm for partially skimmed milk, and 27.5 ± 1.5 μm for skimmed milk. The ANOVA test (with a significance level of 0.05, an F-statistic of 802 and a p-value ≤ 0.001) together with the post-hoc Tukey test (p-value ≤ 0.001) confirmed that all three milk varieties were statistically distinguishable based on the speckle grain dimension Δx.

3.2 Experimental results for water and glucose adulteration in whole milk

Afterwards, dilutions of whole milk with water were investigated. The average gray-level intensities and the autocovariance functions were extracted from the SP frames after outlier removal. Experimental results are presented in Fig.4. Specifically, Fig.4(a) illustrates the average gray-level intensity, while Fig.4(b) depicts the speckle grain size, both shown as box plots for different milk concentrations. While the grain size steadily decreases with lower milk concentration, the average gray-level intensity initially increases for the 90% milk sample before decreasing further. This behavior arises from two opposing effects: dilution reduces lipid concentration, decreasing backscattered light intensity; at the same time, dilution increases the RI step $\Delta n = |n_M - n_L|$, enhancing the reflection coefficient at the lipid micelle–liquid matrix interface. For weak dilutions (as in the case of the 90% milk sample), the latter effect dominates. These results highlight the necessity of considering both speckle grain dimension, which seems to depend solely on lipid concentration, and gray-level intensity, to accurately detect

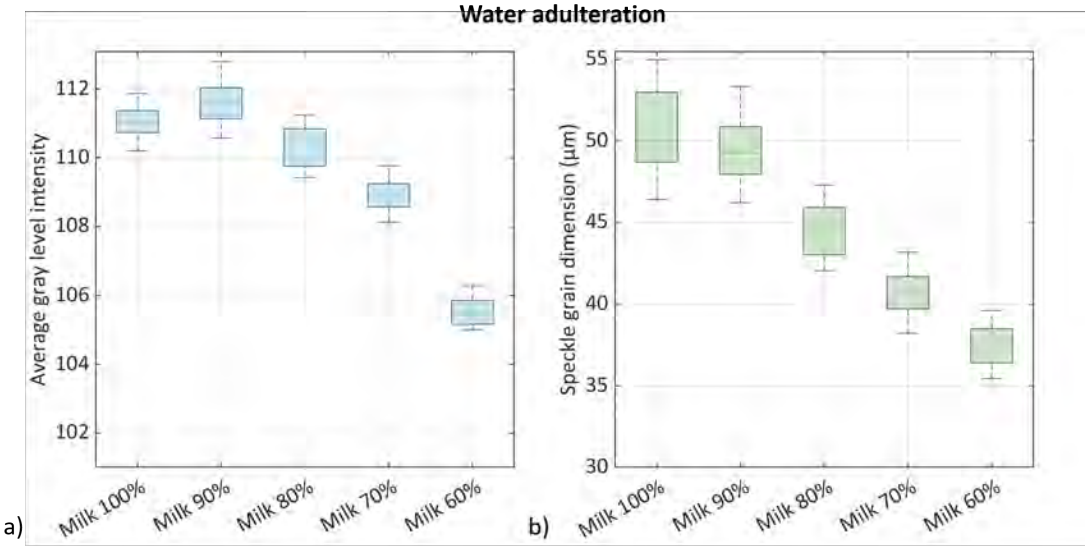

Figure 4. Box plots illustrating the variations in (a) average gray-level intensity and (b) SP grain size for different water concentration in milk samples.The grain dimension decreases consistently with lower milk content, whereas the average gray-level intensity shows a slight increase at a low water concentration (10% water added to 100% milk sample) before decreasing with the milk content.

milk dilutions. Additionally, intensity measurements can be influenced by external factors such as laser power variations, detector sensitivity, and cuvette imperfections. An ANOVA test on the average intensity values (significance level 0.05) yielded an F-statistic of 942 and a p-value ≤ 0.001, confirming significant differences among the five samples. A subsequent Tukey test (p-value ≤ 0.05) consistently rejected the null hypothesis. The same statistical tests were then applied to the speckle grain dimension Δx, again rejecting the null hypothesis with a p-value ≤ 0.001, confirming that all dilutions could be distinguished using this parameter. However, despite this distinction, it is important to note that the behavior of the SP grain dimension Δx remains monotonically decreasing with increasing water concentration, whereas no such trend is observed for the intensity parameter.

The samples of milk adulterated with water-glucose were then tested as previously done. A box plots representing the average gray-level intensity and SP grain dimension for glucose-adulterated milk samples is shown in Fig. 5. As in the previous adulteration case, Fig.5(a) reports the average gray-level intensity, while Fig.5(b) represents the SP grain size, both shown as box plots for different milk concentrations. The image clearly illustrates that both parameters follow a decreasing trend as the concentration of water-glucose solution increases. An ANOVA test was conducted on the average intensity values, considering a significance threshold of 0.05. Results show an F-statistic of 230 and a p-value ≤ 0.001, indicating a significant difference among the five samples. A Tukey test was applied to the same data, using a significance threshold of 0.05. The null hypothesis was consistently rejected in all cases except for milk 100%–milk 90%. Therefore, this pair of concentrations, based on just the average gray-level intensity of SP images, could be confused. Thus, we conducted the same statistical tests (ANOVA and Tukey post-hoc tests) using speckle grain size Δx as the experimental parameter to determine whether all milk samples mixed with the glucose solution could be discriminated. The results of the ANOVA test satisfactorily rejected the null hypothesis (p-value≤ 0.001), as did the Tukey test for pairwise group comparison, which rejected the null hypothesis for all pairs, again using a significance threshold of 0.05. As in the case of pure water-diluted samples, the use of the parameter Δx, which represents the SP grain size, allows the recognition of whole milk and all glucose-adulterated samples.

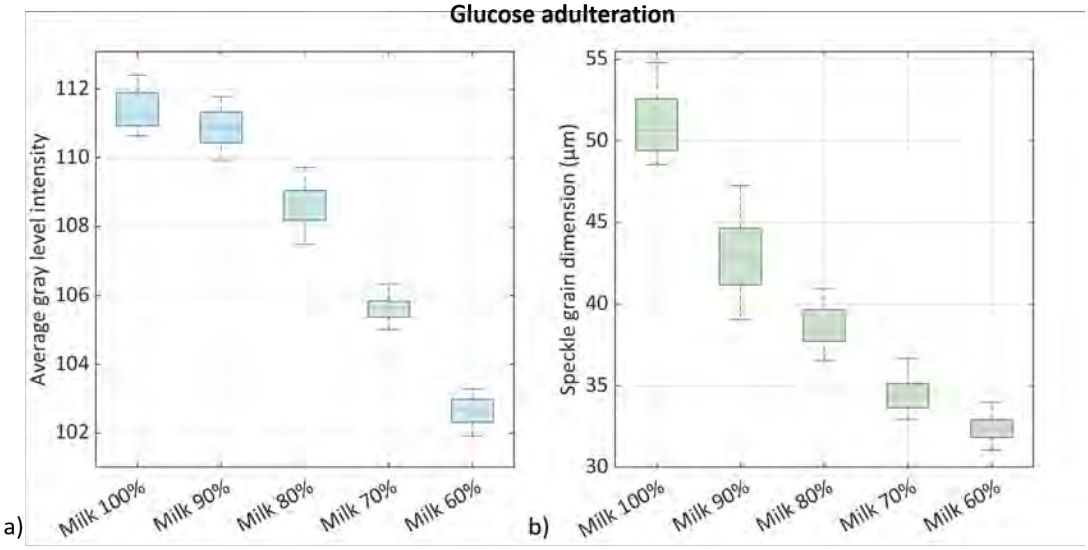

Figure 5. Box plots illustrating the variations in (a) average gray-level intensity and (b) speckle grain size for different 12.5% glucose solution concentration in milk samples. Both parameters decrease consistently with increasing water–glucose solution content (thus decreasing in milk content).

3.3 Comparison with RI measurements

It could be argued that adulteration should affect the RI of the final product and that milk adulteration could be recognized by means of refractometric measurements. We thus measured the RIs of the samples of adulterated milk. Fig. 6 reports box plots representing the RI values measured for various adulterated milk samples, in both

adulteration cases. In particular, Fig. 6 (a) is relative to the adulteration of whole milk with water. The RI trend shows a monotonic decrease for increasing amount of water (or decrease of whole milk fraction). Similarly, it was shown in the previous section that the average intensity of SP images decreases as the water concentration increases, as the size of the SP grain does. By contrast, in Fig. 6 (b) shows that RI values are similar for all samples of glucose-adulterated milk. This happens because the measured RI of whole milk ($n_{milk} = 1.3527$ RIU) and that of 12.5% glucose solution ($n_{glu12.5\%} = 1.3513$ RIU) are very similar: this kind of adulteration cannot be recognized by means of an RI measurement. Instead, the intensity of SP and the grain dimension decreases when the glucose solution content increases. Due to the limited RI measurements, normality assumptions were not met, requiring a nonparametric approach. A Kruskal–Wallis test (significance level 0.05) confirmed significant differences among water-adulterated samples (p-value ≤ 0.05), suggesting that RI can be used to detect milk dilution with water. However, for glucose-adulterated samples, the test yielded a p-value of 0.91, indicating no significant distinction, thus proving RI unsuitable for detecting this type of adulteration.

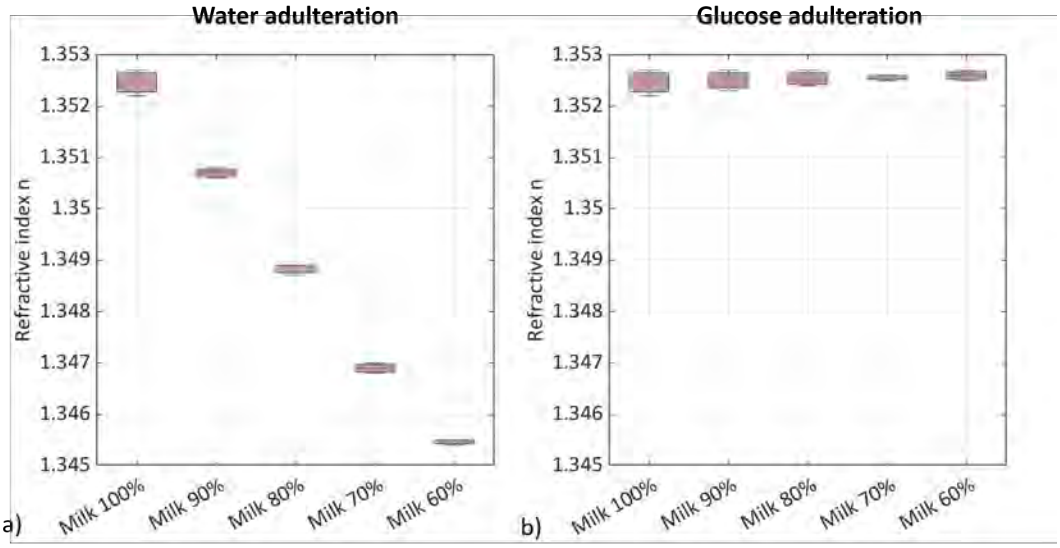

Figure 6. RI measurements for (a) whole milk adulterated with water and (b) milk adulterated with 12.5% glucose syrup. In the first case, the RI values decrease (as with the SP parameters), whereas in the case of glucose adulteration, the RI measurements remain unchanged. This highlights the ineffectiveness of RI measurements in detecting this type of milk counterfeiting.

4. CONCLUSION

In this work, we have proposed a simple and cost-effective optoelectronic configuration to generate SP by irradiating samples of milk with a laser diode and acquire SP images with a CMOS camera. In particular, we have exploited the system to investigate commercial cow milks (whole, partially skimmed, and skimmed milk) with the aim of distinguishing different types of dairy fluids and revealing if samples of whole cow milk have been adulterated by mixing with water and 12.5% glucose solution. From the recorded SP experimental data, we extracted the average gray level intensity and the dimension of the speckle grain. It has been shown that only by considering both parameters together can these adulterations be detected. Additionally, we compared our technique with refractive index (RI) measurements performed using a commercial refractometer.We demonstrated that glucose addition cannot be detected solely through RI tests. These findings indicate that refractometry is not a reliable approach for detecting more sophisticated adulterations. In contrast, the analysis of average SP image intensity and SP grain size revealed statistically significant differences. In summary, our innovative SP-based system to reveal milk adulteration has several strengths. It is simple to use and low cost, making it accessible and easily adaptable for practical applications. Indeed, our system was designed for fast onsite test: it could be exploited inside dairy factories or milk distribution plants. In future studies integration of

artificial intelligence techniques could facilitate automatic and real-time sample recognition, improving efficiency and user-friendliness. The proposed system has great potential in becoming a reliable approach to address milk adulteration and ensure the integrity of dairy products on the market.

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