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Fourier-transformed infrared (FTIR) spectroscopy for detecting milk powder addition in Cyprus goat milk: a preliminary study for future adulteration detection in halloumi cheese

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ORIGINAL ARTICLE

Abstract

This study was about the adulteration of milk with milk powder and serves as a precursor for future studies on halloumi cheese adulteration with milk powder. Three different commercial milk powders of different geographic origins were tested to check the impact of milk powder origin versus the amount of milk powder added in milk as an adulterant. Three different concentrations of skim milk powder (SMP) were added (0.5%, 1%, and 3%) to Cyprus goat milk, and the mixtures were then heated to 90˚C (a crucial step in halloumi cheese preparation). Fourier-transformed infrared (FTIR) spectra were collected after freeze-drying, and Partial Least Squares Discriminant Analysis, a supervised classification method, was applied in terms of chemometrics to test the classification of samples. The adulterated milk samples of this study are used in halloumi cheese making to check after cooking the discrimination of adulterated halloumi cheese at low percentage of adulteration with SMP by applying FTIR and chemometrics in future. Based on the present study, the origin of milk powder is not significant, and it is assumed that generally, for future studies the origin of milk powder will not affect sample classification.

Keywords: adulteration; chemometrics; FTIR spectroscopy; milk powder

Introduction

Food fraudulence has become a significant concern as many foods and beverages fail to comply with their labeling claims. This issue, driven by economic motives and the need to safeguard consumer health, has elevated food adulteration, counterfeiting, substitution, and mislabeling to critical topics in food safety and quality. Animal-derived products, such as milk, dairy products, meat and their products, eggs, and seafood, are among the most frequently adulterated food categories. To ensure fair competition and protect consumer rights, it is essential to detect all forms of adulteration in these products. The increasing globalization and complexity of food supply chains have exacerbated this problem, impacting food system at local, regional, and global levels (Smaoui *et al.*, 2023).

In the dairy industry, fraudulent practices include mislabeling and adulteration of milk and cheese with cheaper,

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lower-quality ingredients, compromising product integrity and consumer trust. Such practices involve the addition of milk powder to replace milk solids, use of starch or flour to improve texture or reduce costs, and substitution of high-value milk with more abundant and lower-priced alternatives, such as bovine milk in caprine–ovine products, which can mimic the characteristics of traditional cheeses (Abedini *et al.*, 2023; Capici *et al.*, 2015; Ionescu *et al.*, 2023; Smaoui *et al.*, 2023). This is particularly worrisome in cheese making, where product authenticity directly impacts quality and safety (Maritano *et al.*, 2024).

Goat milk, prized for its unique flavor and nutritional benefits, is increasingly popular for cheese production (Kováčová *et al.*, 2021). Its price varies significantly depending on regional availability, demand, and seasonality—spring and summer often see higher yields due to improved grazing conditions (Li *et al.*, 2022). In addition, organic goat milk usually commands a higher price due to the cost of organic farming practices (Arsenos *et al.*, 2021).

In terms of quality, goat milk typically contains higher levels of fat and protein than cow milk, contributing to cheeses with richer flavor and enhanced sensory properties. Its smaller fat globules promote a smoother and creamier texture, which is particularly valued in artisanal cheese production (Siddiqui *et al.*, 2024). Goat milk also imparts a characteristic tangy flavor that enhances cheese identity and is influenced by factors such as animal diet, breed, and farming practices. Additionally, goat milk is more easily digested by some consumers because of its distinct fatty acid composition, including medium-chain triglycerides associated with potential health benefits, making it a suitable option for individuals with lactose sensitivity or specific dietary preferences (Nayik *et al.*, 2022).

Moreover, cheese adulteration with milk powder involves the addition of milk powder to cheese products to increase volume or reduce production costs (Smaoui *et al.*, 2023). This practice can compromise the flavor, texture, and nutritional value of the cheese. In the case of halloumi cheese, the inclusion of milk powder in Protected Designation of Origin (PDO) halloumi violates the specific standards established by the PDO certification. According to the PDO requirements registered with the European Union (EU), halloumi must comply with strict guidelines regarding its ingredients and production methods, and no substitutes or additives, such as milk powder, are permitted (European Commission, 2021; Tarapoulouzi *et al.*, 2024a).

Detecting cheese adulteration can involve various chemical analyses through gas chromatography (Saad *et al.*, 2023), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) (Ray *et al.*, 2023), advanced mass spectrometry such as liquid chromatography–tandem mass spectrometry (LC-MS/MS) (de Oliveira *et al.*, 2022), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Kritikou *et al.*, 2022), and spectroscopy such as Fourier-transform near-infrared (FT-NIR) (Visconti *et al.*, 2024) or Raman spectroscopy (Genis *et al.*, 2021). Furthermore, it is worth mentioning that spectroscopic techniques such as FT-NIR spectroscopy and Fourier-transformed infrared (FTIR) spectroscopy are increasingly used in dairy research (Karaziack *et al.*, 2024; Tarapoulouzi *et al.*, 2022; 2024b; 2024c).

Nowadays, chemometrics incorporates both supervised and unsupervised methods for analyzing and interpreting complex data, especially in the fields such as spectroscopy, analytical chemistry, and process monitoring. Unsupervised methods work on unlabeled data, focusing on identifying patterns, structures, or groupings within the dataset. Supervised statistical methods are used to classify data into groups. They build predictive models by maximizing separation between predefined classes (e.g., authentic and adulterated samples) while also explaining the variance in independent variables (FTIR spectra in this case) (Kharbach *et al.*, 2023). Partial Least Squares Discriminant Analysis (PLS-DA), a powerful supervised chemometric method, effectively addresses challenges such as multi-collinearity and high-dimensional data, making it particularly suited for analyzing complex mixtures, such as food and beverages. This technique not only distinguishes between classes with high accuracy but also provides quantitative insights, enabling the precise determination of adulteration levels within a sample. In addition, PLS-DA integrates seamlessly with spectroscopic data, enhancing its applicability in rapid and non-destructive testing methods. Its versatility and robustness have established it as a valuable tool for ensuring food authenticity and safety in modern quality assurance frameworks (Du, 2024). The combination of FTIR and PLS-DA provides high sensitivity and specificity for identifying adulterants in food products, ensuring reliable detection even at low concentrations. Additionally, this approach offers robust quantitative capabilities, enabling the accurate determination of adulterant levels within complex matrices. Together, FTIR and PLS-DA represent a fast, efficient, and cost-effective alternative compared to traditional methods, such as chromatography, which are often time-consuming and resource-intensive. These advantages make them particularly valuable for high-throughput analysis in quality control settings, contributing to improved food safety and authenticity verification across the supply chain (De Girolamo *et al.*, 2020; Tsagkaris *et al.*, 2023).

Milk powder is high in lactose, which provides more substrate for lactic acid bacteria (LAB) to ferment. Halloumi cheese is unique compared to other cheeses because it is typically made without starter cultures, meaning that acidification relies less on bacterial fermentation and more on direct heating process. Therefore, since production of halloumi cheese generally does not involve active bacterial fermentation, the addition of milk powder is unlikely to change significantly acidity during the cheese-making process itself. The main source of acid in halloumi comes from the addition of rennet and the heating process, not from lactose fermentation by bacteria (Papademas and Robinson, 1998). In addition, milk powder increases the protein and mineral content of milk (Alsaleem *et al.*, 2023), which enhance structure of curd and affect texture of cheese. Even though halloumi does not rely on bacterial fermentation, the addition of milk powder introduces more lactose into the mix. Lastly, the increased solids from milk powder can change the moisture content and texture of curds (Alsaleem *et al.*, 2023; Pinto *et al.*, 2007). The retention of water and residual whey, which may contain traces of lactic acid, could influence the perceived acidity. However, this impact is expected to be minimal, and thus negligible.

This preliminary study focuses on milk adulteration with milk powder as a potential issue in halloumi cheese production. Since halloumi manufacturers (if using milk powder) probably source different brands from various geographical origins, several factors—such as animal breeds, environmental conditions, and milk collection periods—may influence the FTIR spectral profiles of the final product. The primary objective of this study is to assess the impact of geographical origin on FTIR spectral variations by analyzing three different skim milk powders (SMPs). If geographical origin proves to be a more dominant factor than percentage of adulteration, classification based on origin, rather than adulteration level, may pose a significant challenge for future detection studies. The secondary objective is to determine whether very low levels of adulteration (0.5%, 1%, and 3% SMP) can be accurately detected using FTIR spectroscopy combined with chemometric analysis. Furthermore, the adulterated milk samples are used in halloumi cheese production to evaluate whether FTIR and chemometrics can still effectively discriminate adulterated cheese after the cooking process.

Materials and Methods

Samples preparation

Pasteurized and homogenized goat milk was purchased from a local supermarket. The use of pasteurized milk in the production of halloumi cheese is permitted under EU Regulation 2021/591, which outlines the specific requirements and standards for its designation as a PDO product. Three different brands of SMPs, all coming from cow milk, were obtained from SMP1 – Romania, SMP2 – Spain, and SMP3 – Belarus, and these were selected to have a similar production date (within 1 month) in order to maximize the probability that the milk used in their production was obtained under similar climatic conditions during production and collection. The nutritional composition of the three SMPs was similar, as presented in Table 1.

Owing to the fact that adulteration of halloumi cheese with milk powder occurs at very low levels and that available analytical methods are unable to detect reliably the concentrations below 3%, three concentrations of SMP were added to goat milk in this study: 0.5%, 1%, and 3% (w/v). Each concentration was prepared in triplicate. Therefore, 30 samples (3 SMPs × 3 concentrations × 3 replicates, and 3 replicates × 0% SMP) were prepared. The mixtures were stirred and heated up to 90°C, which is the heating temperature of halloumi recipe. Moreover, because heating influences the FTIR spectral profiles of adulterated samples, all samples in this study were heated after the addition of milk powder. Freeze drying of the samples was performed as described in Tarapoulouzi *et al.* (2020) by using a LyoDry Compact Benchtop Freeze Dryer (Mechateck Systems, United Kingdom).

FTIR measurements

Three replicates were taken for each sample by using an FTIR 8900 (Shimadzu), as described by Tarapoulouzi *et al.* (2020). Like in Du (2024), three subregions were

Table 1. Nutritional composition of three SMPs.

	g fat/100g	g carbohydrates/100g	g proteins/100g	g salt/100g
SMP1	1	52	35	1.1
SMP2	1	54.5	32.5	1.3
SMP3	1.5	52	31.8	1.5

investigated considering their importance in classifying the samples, such as the whole spectrum (3,800-550 cm^{-1}), and the subregions 3,800-2,800 cm^{-1} and 1,800-550 cm^{-1}. From the whole spectrum, the spectral region between 2,800 cm^{-1} and 1,800 cm^{-1} was excluded because it contained the absorption band of atmospheric carbon dioxide, particularly at 2,360 cm^{-1}. This region was also excluded because of the absence of significant peaks. Its inclusion would simply add "noise" and take up computing effort with no benefit (Tarapoulouzi *et al.*, 2020). The software KaleidaGraph (Synergy) was used to display the spectra.

Chemometric analysis

All spectra were first imported into Excel for preprocessing before conducting chemometric analysis. Multivariate statistical analysis took place by applying initially the chemometric method principal component analysis (PCA) for visualizing the relationships between observations in terms of the most important patterns in the data. Afterwards, PLS-DA was carried out using the SIMCA software (version 18.0.1; Umetrics, Sweden) for classification. Data scaling to unit variance (UV) and mean-centering were applied prior to chemometric analyses.

In scatter plots, the ellipse represents Hotelling's T^2 confidence region, a multivariate generalization of Student's *t*-test that provides 95% Confidence Interval (CI) for observations. Components are extracted features or directions in the data that represent its most relevant information, often used in building and validating models. Parameters t[1] and t[2] refer to scores on the first and second principal components, respectively. In more detail, t[1] (on the *x*-axis) is the score of each data point on the first principal component (PC1). PC1 is the direction in the data that accounts for the largest variance. In addition, t[2] (on the *y*-axis) is the score of each data point on the second principal component (PC2). PC2 accounts for the second-largest variance and is orthogonal to the first component. Furthermore, model quality was assessed using the parameters R^2X and R^2Y, which represented cumulative modeled variation in X and Y, respectively, and Q^2, which estimates the model's predictive ability through cross validation analysis of variance (CV-ANOVA) (Oliveri *et al.*, 2020).

In addition, the proportion of misclassified samples was calculated. Misclassification table is a tool used in classification problems to summarize the performance of a model by comparing predicted class labels to actual (true) class labels. It provides a detailed breakdown of how well the model classifies each category (Wang *et al.*, 2021).

Results

FTIR measurements

The average FTIR spectra from the four sample categories (0%, 0.5%, 1%, and 3% (w/v) SMP) are presented in Figure 1. Lactose-specific absorption bands were identified at 850, 900, and 1,130-1,040 cm^{-1}, corresponding to C–O, C–C, and C–H groups. Vibrations from the lactose carbohydrate ring appeared in the region of 800–1,000 cm^{-1}, while the 1,030–1,150 cm^{-1} region was associated with C–O groups in lactose.

Milk proteins showed characteristic absorption bands at 700, 1,260, 1,320, 1,550, and 1,635 cm^{-1}. More particularly, the band near 700 cm^{-1} was linked to the N–H group of amides, while peaks at 1,260, 1,550, and 1,635 cm^{-1} were attributed to C–H and N–H vibrations of tertiary amides, N–H groups of secondary amides, and the C=O group in primary amides. Protein-related vibrations were observed in the ranges 1,680–1,630 cm^{-1} (amide I), 1,570–1,510 cm^{-1} (amide II), and 1,470–1,240 cm^{-1} (amide III), corresponding to C=O stretching and N–H and C–N bending.

Absorption bands related to milk fat appeared at 790, 1,165, 1,375, 1,460, 1,745, 2,850, 2,915, and 3,265 cm^{-1}. Peaks at 1,165, 1,375, and 1,460 cm^{-1} were associated with C–O groups, CH_3 and CH_2 bending, and the C=O band, respectively. The CH_2 groups contributed to bands at 2,850 cm^{-1} and 2,915 cm^{-1}, which also marked

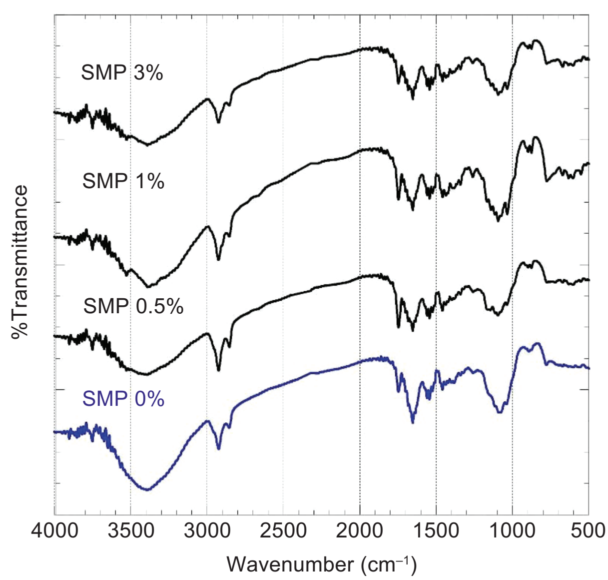

Figure 1. Average FTIR spectra from the four sample categories (such as 0%, 0.5%, 1%, and 3% (w/v) SMP) of the present study.

significant variations in spectral fingerprints. The observations of this study in terms of absorption of lactose, proteins, and fat agree with the findings of Du (2024).

Instead of whole SMP or semi-SMP, skim powders were used to check if adulterants with minimum compositional characteristics could be investigated. Other reasons include the fact that SMP is more commonly used in halloumi cheese adulteration because of its cost-effectiveness and neutral flavor profile. Its low-fat content allows manufacturers to increase protein levels and improve curd yield without disrupting the traditional fat-to-dry matter ratio, thereby mimicking the desired texture and firmness of authentic halloumi. In contrast, semi-SMP probably is less used because its higher fat content can alter the cheese's texture and flavor and is more expensive. However, adulteration with milk powders can compromise the authentic characteristics of halloumi, traditionally made from sheep and goat milk, and may violate PDO standards where applicable.

Chemometric analysis of data

In this study, chemometrics was primarily used for an initial visualization of the samples in terms of differentiation at three concentrations of adulteration with SMP, and not to produce a robust model, as the number of observations was too small (i.e., 30 samples). A loading plot (not shown) revealed that among the three different FTIR wavenumber regions treated in this study (i.e., 3,800-550 cm^{-1}, 3,800-2,800 cm^{-1}, and 1,800-550 cm^{-1}), only the subregion 1,800-550 cm^{-1} was significant and gave the best outcome in terms of identification of adulterated samples. This finding aligns with the recent study conducted by Du (2024).

Initially, it was observed that the three SMPs exhibited similar FTIR spectra, a finding supported by chemometric analysis. For example, samples containing 0.5% SMP1, 0.5% SMP2, and 0.5% SMP3 were grouped into the same class. This led to the first conclusion that the geographic origin of milk powder and variations among different SMPs are not significant at the concentrations and with the spectroscopic method used in this study. Consequently, the second conclusion was that the geographic origin of SMPs is unlikely to influence results in future studies. Lastly, all samples were classified into four distinct classes based on SMP concentration of 0%, 0.5%, 1%, and 3% (w/v).

Second, chemometric treatment of the FTIR data showed that one sample out of nine from the 0.5% concentration group was an outlier and excluded. As can be seen in Figure 2, the most homogeneous group is the 0.5% SMP, in contrast to the groups of 1% SMP and 3% SMP. This may suggest that as the concentration of SMP increases, the complexity of the food also increases. A more heterogeneous food product, with greater complexity or variation in the interactions among its components, results in increased variability in its properties. This suggests that higher concentrations of SMP may influence the food's structure, behavior, or composition in ways that introduce greater diversity among samples. Such effects are important for understanding the sensory qualities, stability, and processing behavior of foods with varying SMP concentrations. The produced model had R^2X = 0.992, R^2Y = 0.843, and Q^2 = 0.831. R^2X value indicates that the model explains 99.2% of the variance in predictor variables (X-block). The model captures a very high proportion of variability in the independent data. R^2Y = 0.843 shows that the model explains 84.3% of the variance in response variables (Y-block). It shows a good fit between the model and the response data, but there is still 15.7% of variance that is not explained by the model. A Q^2 value of 0.831 indicates good predictive performance, as values >0.5 typically suggest that the model can generalize well.

It has to be noted that improvement in R^2 and Q^2 values highlights the enhanced validity of the model following the outlier exclusion described in the above paragraph. Specifically, R^2X increased from 0.980 to 0.992, indicating a more comprehensive representation of the input data. R^2Y showed a substantial rise from 0.761 to 0.843, demonstrating improved class discrimination. Most importantly, the predictive ability of the model, as reflected by Q^2, increased from 0.734 to 0.831. These enhancements underscore the positive impact of the outlier exclusion, leading to a more reliable and robust PLS-DA model. Furthermore, the misclassification table (presented as Table 2) shows a total of 96.55% correct classification of the samples in their groups, and the model can become bigger in the future with more samples in each category.

A PLS-DA model with 30 samples, four classes, and an overall correct classification of 96.55% is a good outcome, but it generates several critical considerations to ensure its robustness.

Discussion

Key authenticity challenges include verifying geographical origin, production methods, farming practices, species identification, processing techniques, and detecting adulterants. Developing precise and automated analytical methods, combined with advanced chemometric tools, offers effective solutions for identifying food fraudulence

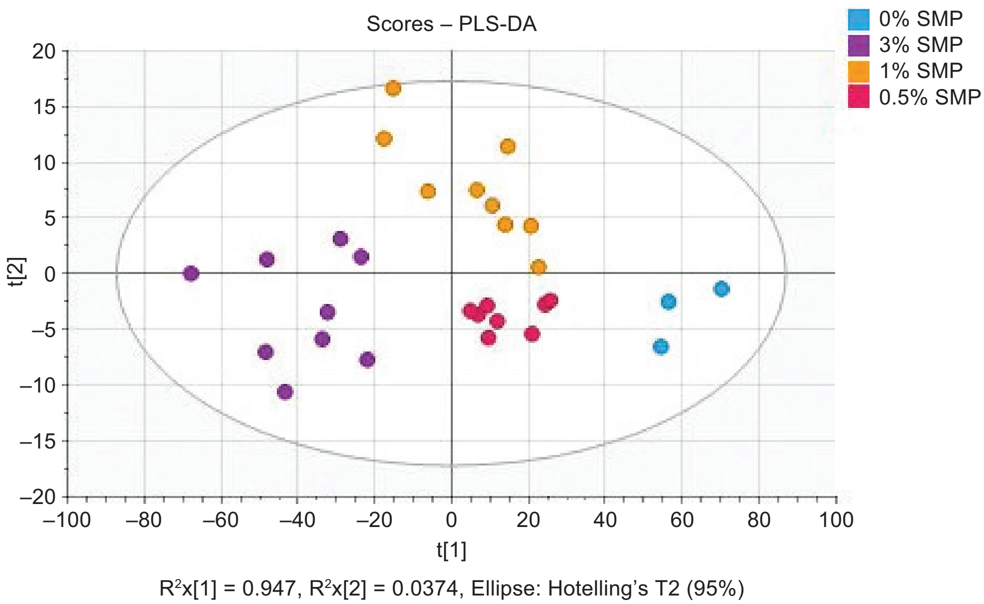

Figure 2. Score scatter plot (t[2]/t[1]) for the model with four groups, as produced by using the most important FTIR subregion 1,800-550 cm^{-1}.

Table 2. Misclassification table of partial least squares discriminant analysis (PLS-DA) produced by using the subregion 1,800-550 cm^{-1}.

PLS-DA	Members	Correct	0% SMP	3% SMP	1% SMP	0.5% SMP
0% SMP	3	100%	3	0	0	0
3% SMP	9	100%	0	9	0	0
1% SMP	9	88.9%	0	0	8	1
0.5% SMP	8	100%	0	0	0	8
Total	29	**96.55%**				
Fisher's prob.	1.9e-13					

(Smaoui *et al.*, 2023). These challenges are particularly significant in safeguarding the integrity of traditional products, such as halloumi cheese, where authenticity is closely tied to its cultural and economic values. One notable issue is the fraudulent addition of milk powder or other noncompliant ingredients that undermines quality standards and consumer trust (Smaoui *et al.*, 2023; Tarapoulouzi *et al.*, 2024b).

To address these concerns, developing precise, automated, and reliable analytical methods has become imperative. Chemometric analysis, in combination with spectroscopic techniques enables, nowadays, the processing of complex datasets, enhancing the ability to distinguish between genuine and adulterated products with high accuracy (Cruz *et al.*, 2024; Tarapoulouzi *et al.*, 2023). Moreover, spectroscopy and chemometrics are cutting-edge technologies as they offer robust solutions for detecting inconsistencies and identifying food fraudulence (Tarapoulouzi *et al.*, 2024c). Subsequently, their incorporation into food authenticity verification not only helps in combating fraudulence but also ensures compliance with PDO, protected geographical indication (PGI), and other certification standards. As consumer demand for authenticity and quality grows, leveraging these sophisticated tools will be essential for preserving the heritage and reputation of traditional products such as halloumi while protecting public health and ensuring fair trade practices (Tarapoulouzi *et al.*, 2024a; Tsagkaris *et al.*, 2023).

Conclusions

In the present study, three commercial milk powders of different geographical origins were evaluated to determine whether milk powder origin is a more significant factor than the concentration of milk powder in milk adulteration. If origin were the dominant parameter, samples would be classified according to geographical origin. However, the results demonstrated that milk powder origin was not a significant factor, as samples were correctly classified based on adulteration level. Specifically, relative adulteration levels of 0%, 1%, and 3% (w/v) SMP were successfully discriminated using FTIR spectroscopy coupled with chemometric analysis. These findings suggest that, in general, the origin of milk powder is less influential than the level of adulteration with SMP for detection by FTIR in combination with PLS-DA. This highlights the effectiveness of FTIR spectroscopy combined with chemometrics as a powerful tool for detecting milk fraudulence involving addition of milk powder. Compared to traditional techniques, such as chromatography, the proposed methodology is faster, more cost-effective, and better suited for high-throughput adulteration screening. Furthermore, chemometric analysis revealed that the FTIR spectral subregion between 1,800 and 550 cm^{-1} was sufficient for discriminating adulterated samples. Using this subregion with PLS-DA resulted in a classification accuracy of 96.55%. Although preliminary, these results clearly demonstrate that halloumi cheese adulterated with low levels of SMP can be reliably detected using FTIR spectroscopy combined with chemometric analysis.

Author Contributions

All authors contributed equally to this article.

Conflict of Interest

The authors had no relevant financial interests to disclose.

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