Detection of adulterant in raw cow milk and its impact on Total Solids (TS) using Fourier Transform Infrared (FTIR) Spectroscopy

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Abstract

Fourier transform infrared (FTIR) spectroscopy is commonly used for milk analysis and the detection of certain milk adulterants. This study attempts to ascertain the accuracy of the FTIR machine in identifying adulterants as well as to detect the impact of adulterants in Total Solids (TS) value in raw cow milk samples. Milk samples were from samples received in Biochemistry Section, Veterinary Research Institute (VRI). Each adulterant's concentration set was based on the milk analyser's Limit of Detection (LOD). The coefficient of determination (R^2) between the actual (set of concentration) and predicted values showed that all adulterants achieved R^2 values greater than 0.99, with minimal error. Analysis showed that the TS value increased with increasing the concentration of the adulterant. Sodium bicarbonate, however, had the opposite effect. Coefficient corelation analysis between TS and adulterant having a high correlation value (more than 99.0%) for all adulterant tested. Overall, the FTIR machine proved a good accuracy in detecting a various of possible adulterants in milk. Additionally, this investigation demonstrates unequivocally that adding adulterants to milk significantly raises the milk's TS value. However, some adulterants are too dangerous to be ignored.

Keywords: FTIR, adulterants, raw cow milk, total solids.

Introduction

Fourier Transform Infrared Spectroscopy is a systematic procedure used to identify organic, polymeric as well as inorganic materials (https://rtilab.com/techniques/ftir-analysis/). FTIR spectrometers work by sending infrared light through a sample and detecting how the sample absorbs the wavelengths delivered. The light is first processed by an interferometer, producing a signal called an interferogram. As this signal interacts with the sample, some energy is absorbed, revealing information about the sample's molecular interactions (Undavalli et al., 2021). In this context, FTIR spectroscopy facilitates the swift and concurrent identification of adulteration and the forecasting of valuable parameters, irrespective type of milk (Sen et al., 2021). This FTIR technique is low operational cost and high analytical performance (Cassoli et al., 2011) with high accuracy (Cirak et al., 2018).

Food adulteration is primarily intended to modify the food products' quality in order to gain an economic advantage. Usually, such acts involve adding undeclared substances to increase weight or volume and replacing food that is of lower quality or worth. This also includes injecting fake chemicals and colorants to create a more appealing appearance (Fiorino et al., 2018; Pastor et al., 2019). Unlike contamination, this is deliberate.

Since ancient times, milk and other goods have been adulterated due to their widespread consumption, which could benefit dishonest manufacturers financially (Nicolaou et al., 2010). Adulterants in milk mostly consist of the incorporation of plant-based protein, milk from various species, whey addition, and dilution with water, collectively known as adulteration driven by economic motivations (Fischer et al., 2011; Singh and Gandhi, 2015). Such adulterations do not present any significant health danger. Among the major milk adulterants that present serious health dangers are ammonium sulphate, benzoic acid, boric acid, caustic soda, detergents, formalin, hydrogen peroxide, melamine, salicylic acid, sugars and urea (Singh and Gandhi 2015; Hussain et al., 2019).

Urea purposely added into a milk to stimulate a higher milk protein content (Santos et al., 2012), however, it can burden the kidneys since they must remove more urea from the body (Kandpal et al., 2012). In addition, milk's peroxides and detergents can result in gastrointestinal issues, including gastritis and intestinal inflammation (Singuluri and Sukumaran, 2014). According to Domingo et al. (2014), infants that consume melamine at levels higher than the safety limit may develop renal failure and death. In the meantime, potassium salts of nitrates and nitrites are added to products to enhance their flavor, color, and appearance as well as to prolong their shelf life (EFSA, 2017). Nitrates and nitrites in food have been connected by the International Agency for Research on Cancer to a higher risk of methemoglobinemia in infants and young children as well as gastrointestinal cancer. On the other hand, to keep the milk from spoiling and becoming sour, as well as to get rid of the dirt, sodium bicarbonate was added (Biswas 2019). However, Singuluri and Sukumaran, (2014) reported that bicarbonates and carbonate may interfere with hormone signals that control growth and reproduction. Other than that, water was added to enhance milk volume, which modifies its composition such as protein and total solids content. As a result, merchants unlawfully add thickening substances, like sugar to milk to increase its density and sweet taste, which are normally lost due to water adulteration (Jaiswal et al., 2016). Although there is no risk to human life, adulterating milk with sucrose can have a significant negative impact on the economy and is considered as dishonest dealings since it seriously betrays consumer trust (Balan et al., 2020).

In Malaysia, testing milk right at the farm is a simple but important step to make sure the milk collected is safe and genuine. By checking it early, any sign of adulteration can be caught before it goes for processing. Some local studies found that farmers, especially small-scale ones, still have issues with milk handling and quality control, showing that better awareness and monitoring at the farm level are needed (Nordin et al., 2019). Some studies from nearby countries also reported that farmers sometimes add substances such as detergent, urea, or formalin to keep the milk looking fresh or to stop it from spoiling too quickly. These practices not only risk consumer health but also reduce public confidence in local milk quality (Ibrahim et al., 2023). Encouraging basic on-site testing and giving farmers short, hands-on training could make a real difference in improving milk safety and strengthening trust in Malaysia's dairy industry (Garg and Mulla, 2024).

Several studies in Malaysia have applied the FTIR and ATR (Attenuated total reflectance)-FTIR methods, particularly for determining food authenticity. For example, Nurani et al. (2021) used FTIR to detect pork adulteration in roasted meatballs, while Salleh et al. (2019) analyzed goat milk from various breeds, and Azlin-Hashim et al. (2019) identified chemical composition and potential adulterants in coconut milk. For ATR-FTIR method, they used to detect and discriminate of palm cooking oil adulterated with indigestible plastic (Ismail et al., 2021), discrimination and quantification of UHT milk adulterated with urea (Tan et al., 2023) as well as to classified the raw bovine's milk samples based on their geographical origin in Peninsular Malaysia (Ketty et al., 2017). While, Julmohammad et al. (2024) used the ATR-FTIR approach to find residues of adulterants (melamin, formalin, detergent) in UHT milk. Although some of these studies are almost similar to the current study, but they used UHT milk as a sample while the current study using raw cow milk. Current study was conducted to proving the accuracy of the FTIR technique in detecting adulterants in raw cow milk as well as the impact in Total Solids (TS). Assessing the accuracy of this test enables effective monitoring of potential adulterant presence in field samples.This study also to prove that the results issued by the Biochemistry Section of VRI are accurate and practical.

Materials and methods

Chemical and concentration

Formaldehyde, potassium nitrate, sodium bicarbonate, sucrose, and urea were the five adulterants that were used. Each adulterant's concentration was chosen based on the Limit of Detection (LOD) (Table 1) as specified by the milk analyzer manufacturer.

Analysis of adulterant in milk

Raw cow milk samples were from samples received in Section of Biochemistry, Veterinary Research

Institute (VRI) from dairy cattle farmer around Perak. Milk samples then were stored in a 4°C refrigerator and should be used for testing within 24 hours. Before the test is performed, it is compulsory to place the milk samples in the water bath at 40°C for 5 minutes and mix gently to confirm a uniformly mixed sample. Next, milk samples were tested for the existence of adulterant, and samples that are free from adulterant were used for the test. Milk sample will be divided into five groups according to the type of adulterant. For each adulterant, five different concentrations will be prepared, and each concentration will be replicated five times. A total of 30 mL of fresh milk will be used for testing in each replicate. Milk samples were then poured into a falcon tube (50ml) and were mixed up with each concentration of adulterant up to 50ml. Each reading value of adulterant and milk composition of TS were recorded.

Table 1. Types of adulterants and concentration used.

Details of adulterants used	Limit of detection (%)	Set of concentration (%) (based on limit of detection)
Urea, BioXtra, pH 7.5-9.5 (20°C, 5M in H_2O)-Sigma	0.04	0.02, 0.04, 0.08, 0.16, 0.32, 0.64
ACS Reagent Sucrose-Sigma Aldrich	0.25	0.13, 0.25, 0.5, 1.0, 2.0, 4.0
Sodium bicarbonate, ≥99.5%, Reagent Plus-Sigma	0.06	0.03, 0.06, 0.12, 0.24, 0.48, 0.96
Potassium nitrate	0.02	0.01, 0.02, 0.04, 0.08, 0.16, 0.32
Analytical grade 37% formaldehyde solution (formalin)-MERCK Jermany	0.07	0.04, 0.07, 0.14, 0.28, 0.56,

Milkoscan ™ Mars Machine

Milkoscan™ Mars Machine was developed in response to test raw milk for milk composition (total solids, protein, solid-non-fat, fat, lactose, freezing points) and adulteration. This machine has capable to detect six (6) parameters of adulterant namely urea, sodium bicarbonate, potassium nitrate, formalin, sucrose and freezing point (water addition). This machine equipped with infrared technology called Fourier Transform Infrared (FTIR). The milk analyser has the capability to perform self-validation prior to use. In addition, the instrument is calibrated using standard samples provided by the manufacturer.

Statistical analysis

The data were examined by coefficient determination in regression analysis using excel Microsoft Office LTSC Professional Plus 2021 to evaluate the relationship between the percentage of adulterant concentration tested and the value of adulterant reading detected in milk using the Milkoscan machine.

Accuracy test was done by measured the standard error of estimation (Se). While the impact of the addition of adulterant on the TS value was confirmed through Dunnett's test ($p<0.05$) using the Statistical Analysis SPSS version 22.

Results and discussion

The linearity was verified by examining the concentration percentage for every adulterant. The coefficient determination (R^2) between the actual (set of concentration) and predicted values and the calibration curve's linear regression equation demonstrated that all adulterants performed best with the R^2 value were more than 0.99 and the accuracy was nearest to zero. (Table 2). With an R^2 of 1.00, potassium nitrate shows a prefect of a model between the actual and predicted values of the concentration. It was followed by sodium bicarbonate and sucrose, formaldehyde and lastly was urea. While for the highest accuracy was also found in potassium nitrate (0.002), followed by formaldehyde and sodium bicarbonate (0.006), both of which had the same accuracy value and the last two were sucrose (0.035) and urea (0.080).

Table 2. Coefficient determination ($R^{2)}$ and the accuracy value for each adulterant.

Adulterants	Linear regression equation	Coefficient determination (r^2)/%	Standard error of estimation (SE)-accuracy
Urea (% w/v)*	Y=0.8783x + 0.0053	0.9993/ 99.93%	0.008
Sucrose (% w/v)*	Y=0.9463x + 0.113	0.9997/ 99.97%	0.035
Sodium bicarbonate (% w/v)*	Y=0.0024x - 0.0089	0.9997/ 99.97%	0.006
Potassium nitrate (% w/v)*	Y=1.0115x + 0.005	1.00/ 100.00%	0.002
Formaldehyde (% v/v)*	Y=0.9815x – 0.004	0.9995/ 99.95%	0.006

Previous researchers in their study proved that the infrared technique is very robust, sensitive (Mabood et al., 2019; Coitinho et al., 2017; Hansen and Holroyd, 2019) and accurate (Cassoli et al., 2011) in identify the existence of adulterants in milk. In this study, R^2 value showed an equal or slighter differences between the actual and predicted value of concentration. This indicates that the performance of milkoscan machine using FTIR technique is good in detecting adulterant in milk. Coitinho et al. (2017) found that the use of FTIR techniques in detecting formaldehyde and sodium bicarbonate in raw milk showed excellent sensitivity with sensitivity values >84% and 100% respectively. In accordance with the study led by Cassoli et al. (2011) on raw milk using the FTIR technique to detect sodium bicarbonate at concentrations of 0.05%, 0.10% and 0.25%, they found the value of R^2 was 0.9883 and the accuracy value (Se) was 0.005, where in the recent study, the accuracy value is almost the same which is 0.006. Meanwhile Saha and Thangavel, (2018) reported that R^2 value (0.9952) have a strong correlation between the predicted and expected values on addition of formaldehyde spike at 0-20% v/v concentration using FT-NIR spectroscopy on raw milk. Tests on urea in milk samples using the FTIR technique at concentrations of 0, 275ppm, 420ppm, 550ppm, 825ppm and 1100ppm found 100% sensitivity, 91% specificity with an R^2 value of 0.98 (Hansen and Holroyd, 2019). While for sucrose in raw milk, using the FT-MIR technique, R^2 is equal to 0.997 (Bassbasi et al., 2014). However, for nitrate, the test conducted by Gan et al. (2020) to detect nitrate in aqueous solutions using FTIR-ATR spectroscopy at high concentrations of 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/L -1 and low concentration 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 mg/L-1 found R^2 for high concentration group is 0.9578 while for low concentration group is 0.9865. All of these studies were accordance with current study in terms of R^2 infrared technique in detecting the presence of adulterant.

Meanwhile, for the analysis of the impact of adulterant on total solids (TS), this study showed that each concentration tested for sucrose and formaldehyde have a significant different with control group at p value <0.05. Whereas for urea and potassium nitrate, there were no significant different with the control group at the lowest concentration; 0.02% (p=0.076) and 0.01% (p=0.337) respectively as well as sodium bicarbonate at 0.03% (p=0.157) and 0.06% (p=0.056) concentration. Generally, analysis showed that the TS value increased with increasing the concentration of the adulterant. Yet, sodium bicarbonate had the opposite effect (Table 3).

Table 3. Significant level of adulterants for each concentration and TS value.

Variable	**Control group (Average value)**	**Adulterants / Concentrations**					
Total Dissolved Solid (TDS)		Urea %					
	12.728*	0.02	0.04	0.08	0.16	0.32	0.64
		12.774	12.792*	12.848*	12.902*	13.028*	13.292*
		Sucrose%					
	13.058*	0.13	0.25	0.5	1.0	2.0	4.0
		13.25*	13.414*	13.662*	14.12*	16.094*	16.932*
		Sodium bicarbonate%					
	12.508*	0.03	0.06	0.12	0.24	0.48	0.96
		12.482	12.476	12.410*	12.252*	11.946*	11.318*

	Potassium nitrate%					
	0.01	0.02	0.04	0.08	0.16	0.32
13.288*	13.318	13.364*	13.448*	13.640*	13.888*	14.358*
	Formaldehyde%					
	0.04	0.07	0.14	0.28	0.56	-
12.864*	12.988*	13.054*	13.284*	13.724*	14.610*	-

*There is a difference between control and adulterant (Dunnett, $p<0.05$)

Adulteration is the process of adding varied inexpensive adulterants to milk in order to enhance its quantity and increase profits (Chugh and Kaur, 2022). Several researchers conducted a study on adulteration in milk and they discovered that the milk adulterated with urea, sucrose, sodium bicarbonate, potassium nitrate and formalin would change or alter the milk composition (Gahukar, 2013; Balan et al., 2020; Biswas, 2019; Brkić et al., 2022; Reddy et al., 2017). Recent research revealed that the inclusion of sucrose to the milk sample increase the total solids value and this aligns with the study done by Balan et al. (2020) who claimed that sucrose is frequently added to raise the milk's total solids content in order to counteract the dilution caused by adding water. For the urea test, the value of total solids shows a slight increase with each increase in concentration. In line with Ahirwar et al. (2015)'s study, they discovered that milk tainted with 0.5, 1.0, 3.0, 5.0, 7.0 percent of urea increased the value of total solids. Study by Supapong and Cherdthong (2020) although the method is slightly different from the current study which is adding 20g/kg urea in the diet of lactating cows, the result shown was dramatically increased the milk fat and total solids.

Milk kept at ambient temperature and in a refrigerator showed no variation in the total solids value of milk with formalin at a rate of 0.1/ 25 ml (Bector and Narayanan,1973). A study by Ranvir et al., (2015) on milk mixed with 0.4% formalin found that the percentage of fat was significantly decrease ($p<0.05$) while the total solids, lactose and protein values remain unchanged. These results were contradicted with our findings where total solids values were significantly increased ($p<0.05$) along with the increase in the concentration of formaldehyde tested. However, El-Shabrawy and El-Fadaly (2006) reported that roughage treated with formaldehyde (concentrate feed mixture-CFM) were significantly increase the percentage of total solids by the methods of AOAC (1980). In this study, potassium nitrate rises the total solids value for each increase in concentration tested. However, Stewart and Merilan (1958) reported that giving potassium nitrate to the diet of lactating cows (25g KNO_3/cwt) had no adverse effect on milk composition; milk fat and total solids even reduce milk production. Although the experiments conducted are different, it can be observed that the effect of potassium nitrate on total solids is in contrast with the current study.

It is different with the sodium bicarbonate test on the value of total solids in milk. According to the current investigation, adding sodium bicarbonate lowers the total solids value. Despite the research method conducted by Hadjipanayiotou (1988) was different, where they fed livestock (goats) a supplement containing sodium bicarbonate, they found that the total solids value was increased. Similar to the research that Sarwar et al., (2007) conducted also by feeding livestock (buffalo) with sodium bicarbonate supplements of 1.0% and 1.50% were able to increase the total solids value by 85% and 92% respectively. Although these two studies differ in the type of test, they prove that sodium bicarbonate was able to have an effect on the value of total solids even though the effect was in contrast with the current study.

Table 4 showed the results of the correlation coefficient analysis between adulterants and the TS value. Formaldehyde has the highest percentage value, 100.00%, followed by sucrose, sodium bicarbonate, urea and potassium nitrate. A strong correlation ($r > 0.99$) was observed between total solids (TS) and all types of adulterants with p value <0.05.Therefore, introducing adulterants to milk can raise the TS value. However, Malaysia Food Act (1983) and Food Regulations (1985) (MoH, Malaysia) has restricted that all milk products, raw milk or fresh milk from containing any added water, permitted food additives, other additives or antibiotic effects. This clearly show that, in Malaysia, all dairy products must be free from any foreign material in order to ensure quality and customer safety.

Table 4. Correlation coefficient (R) between adulterants and TDS value.

Adulterant	R	Percentage (%)	P value
Urea-% w/v	0.9970	99.70%	0.000
Sucrose- % w/v	0.9999	99.99%	0.000
Sodium bicarbonate- % w/v	0.9980	99.80%	0.000
Potassium nitrate- % w/v	0.9960	99.60%	0.000
Formaldehyde- % v/v	1.000	100.00%	0.000

This study supports earlier findings demonstrating the reliability of *Fourier Transform Infrared Spectroscopy* (FTIR) in detecting adulterants in raw milk. Cassoli et al., (2011) confirmed that FTIR accurately identifies compounds such as sodium bicarbonate and sodium citrate, validating its analytical sensitivity to compositional changes. The current results further indicate that spectral variations reflect real chemical alterations within adulterated milk, highlighting FTIR's potential as a tool for both detection and quality assessment. Consistent with Balan et al., (2020), who used ATR-FTIR with multivariate analysis to detect sucrose adulteration, this study observed that changes in the fingerprint region of spectra correspond closely with solute concentration. Rather than focusing solely on prediction accuracy, the present work links variations in total solids (TS) content to corresponding spectroscopic responses, offering a deeper understanding of adulterant–matrix interactions. In line with Ceniti et al., (2023), these findings underscore FTIR's growing importance in milk quality monitoring and its potential role in supporting authenticity assurance in the dairy industry.

Although FTIR spectroscopy is well known for being a fast and reliable tool to screen adulteration in milk, its practical application still faces a few challenges. As discussed by Windarsih et al., (2021), many calibration models are built using milk samples that have been intentionally spiked in laboratory settings. Such samples, while useful for controlled studies, do not always reflect the complexity of fresh or commercial milk.

In reality, factors such as the cow's breed, type of feed, storage temperature, and seasonal variations can all alter the milk's chemical makeup and, in turn, affect its spectral behaviour. These variations can reduce the accuracy of models when they are applied to real-world samples (Poonia et al., 2017; Ceniti et al., 2023). Moreover, Xue et al., (2021) noted that FTIR can only identify compounds that already exist in its spectral database. This means it might miss new or unknown adulterants that do not match any stored patterns. Therefore, confirmatory tests using chromatographic or mass spectrometric methods such as GC–MS or LC–MS remain necessary to verify FTIR results and ensure analytical reliability.

Conclusion

Overall, the FTIR method proved a good accuracy in detecting a various of possible adulterants in milk. Additionally, this investigation demonstrates unequivocally that adding adulterants to milk significantly raises the milk's TS value. However, some adulterants that were added to increase the quality of milk are too dangerous to be ignored. To guarantee the safety, the authorities must rigorously inspect the milk's quality prior to its public marketing.

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Conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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