Madras Agric.J.,2024; https://doi.org/10.29321/MAJ.10.500002

Development of Rapid Grading Method For Quality Analysis of Ghee Using Fourier Transform Near Infra Red (FT-NIR) Spectroscopy

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Received: 17th August,2024

Revised: 30th August, 2024

Accepted: 21st September,2024

Abstract

Ghee is one of the important fat sources in our diet. The ghee loses its quality due to increased rancidity. Hence, a rapid grading method is required in the industry since the quality of the ghee varies according to its storage time in the market. The present study aimed to develop the rapid grading method using FT-NIR spectroscopy. The quality of the ghee was graded with two parameters mainly moisture and FFA. The FFA and moisture content of the ghee was analyzed with the chemical method, and it was used to develop the spectra for grading of ghee. The spectra of the samples were obtained and the model was calibrated and validated. The performance of the model was analyzed by R^2 and $RMSE_{cv.}$The coefficient of determination (R^2) was > 95 % for moisture and FFA showed higher accuracy. The Residual Predictive Deviation(RPD) of the model was 5.71 and 6.36 for moisture and FFA, respectively which is greater than 3 showed the excellence of prediction. The time taken for the FT-NIR to analyze the moisture and FFA of the unknown sample was 25 s. The FT-NIR was useful for rapid quality grading of ghee.

Key words: *Ghee, Moisture content, FFA, FT-NIR Spectroscopy*

Introduction

Ghee is the heat clarified product made by indigenous method mostly in Asia, Middle East and Africa which is obtained from the cow or buffalo milk. The clarified temperature ranged between 120 to 140 °C (Gemechu and Tola, 2017). According to FSSR-2011, ghee is defined as the heat clarified pure fat obtained exclusively from curd, milk, cooking butter, or cream, without the addition of any coloring agents or preservatives. It is a rich source of fat-soluble vitamins like A, D, E and K. It was ideal medium for deep frying.The quality of a commodity is a major factor that drives its export potential demand.

The chief component present in ghee is fat and moisture. The moisture content not more than 0.5 % and the total fat content ranged from 99-99.5 % (FSSAI, 2016). These two parameters are important for price fixation. Grading in ghee was mainly based on moisture content and Free Fatty Acid (FFA). Agmark grading is mainly based on the FFA. Agmark standard gives three standards of ghee including Special, General and Standard grades. According to Agmark the moisture content not more than 0.3 % and FFA not more than 1.4 % considered as special and moisture content not more than 0.3 % and FFA not more than 2.5 % considered as general and moisture content not more than 0.3 % and FFA not more than 3 % categorized as standard. The quality parameter of the ghee was mainly based on the free fatty acid and increased FFA content, it is an indicator of the deterioration of ghee and loss of quality. The ghee with unsaturated free radicals were highly susceptible to oxidative spoilage (Kumbhare *et al*.,2021). The estimation of the moisture content and FFA using chemical method is time consuming process and hence development of a non-destructive rapid method is necessary. FT-NIR (Fourier Transform Near Infra-Red Spectroscopy) is a simple instrument that facilitates the direct quantization of the samples fed into the imaging platform without any or minimum sample preparation (Yu *et al*., 2022; Wang *et al*., 2022, Vanitha *et al*., 2023 and Cevoli*et al*., 2024).

Pereira *et al.* (2019) estimated the adulteration of soybean oil with butter oil using FT-NIR and reported that FT-NIR was best fit for the analysis of the adulteration.Andrade *et al.* (2019) used the FTIR-ATR to determine the protein content to analyze the adulteration of whey protein concentrate in the samples.Antony *et al.* (2017) reported the FT-MIR spectra for ghee anhydrous fat to differentiate the ghee from other fat materials. It was observed that the rapid detection technique for the quality evaluation of the ghee samples were not explored much and

literatures were found to be very limited. Hence, the study was aimed to develop FT-NIR analysis for quick quality quantization of ghee in terms of moisture content and FFA.

Materials and method

Raw material:

Ghee samples of different brands were purchased in the local market of Chennai, India. The ghee was purchased with different manufacturing date (including expired ghee) of different brand to set a wide range array for the quality analysis of the sample. Samples were noted with the number to identify the brand name of the ghee.Totally, 30 samples were tested for moisture content and FFA. Table 1. represents the number of samples tested with the different ranges of moisture content and FFA to create a library of spectra on the ghee samples.

Moisture content

The moisture content of the ghee samples was assessed using hot air oven method. The porcelain dish was used to analyze the moisture content of the ghee. A sample weight of 5 g was taken in each porcelain dish. Then the dish was placed in hot air oven for drying. The constant temperature of 105± 2 $^{\circ}$C was used in the hot air oven for all samples. After 60 minutes, the samples were placed in the desiccator and weight of the sample was measured. The weight of the samples measured until constant weightswere obtained. The initial and final weight of the sample was noted, and the amount of moisture present in the ghee sample wasfound (Arulkumar *et al.*,2024).

$$\text{Moisture Content, (\%)} = \frac{(initial\ weight - final\ weight)}{(initial\ weight)} \times 100$$

Free Fatty Acid

As per the FSSAI (2016) guidelines,the titration method was used to determine the FFA of ghee. For titration, 5 gram of ghee sample measured and poured in the 250 mL Erlen-mayer flask with 50 mL of Alcohol Ether mixture and 0.1 mL of phenolphthalein indicator. This mixture was further titrated against 0.1N KOH, the appearance of permanent pale pink colour denoted the endpoint and persisted for more than 10 seconds.

$$\text{FFA value of ghee}=\frac{V\times T\times 56.1}{W}$$

Where,

V signifies the volume of standard potassium hydroxide in mL, T signifies the normality of the potassium hydroxide solution and W signifies the weight of the sample in g.

Fourier Transform Near Infrared Spectroscopy:

This experiment was carried on Fourier Transform Near Infrared Spectroscopy (Bruker optics, MATRIX-I, Germany) Figure.1. Software used for this experiment was Opus (version 7.2, Bruker optics, Germany). The multivariate analysis was conducted using QUANT software (version 7.2, Bruker Optics, Germany), which employs PLSR (Partial Least Squares Regression) to create the model. For FT-NIR spectroscopy, diffuse reflectance spectra were collected using an integrated sphere. The measurements were taken at a resolution of 8 cm^{-1} within a wavelength range of 12500-3600 cm^{-1}, with each spectrum comprising 64 scans. A tungsten halogen lamp served as the light source, while a Michelson interferometer with a beam splitter and a lead sulfide (PbS) detector was used to measure light interference through the ghee sample. The interferogram produced was then transformed into a spectrum using Fourier transformation.

Fig.1. FT-NIR Spectroscopy

Spectra Recording

The samples were placed in a sample holder, and the spectra obtained were recorded. The spectra of each sample were replicated thrice, and each replication was done by shuffling the sample thrice. Totally, 90 spectra of ghee samples were recorded.

Calibration and validation

Quant 7.2 software was utilized for multivariate analysis employing Partial Least Squares Regression (PLSR). PLS algorithm was used for the calibration model. The validation of PLS model was conducted through the leave-one-out cross-validation approach. After validation, the prediction results were consolidated with the validation outcomes. The performance of the developed model was evaluated based on the minimal standard error of cross-validation ($RMSE_{CV}$) and higher coefficient of determination (R^2) (Hashem *et al.*, 2022).

For the study, 30 ghee samples were taken. For all 30 samples moisture and Free Fatty Acid content was estimated by conventional wet chemistry method. Before the analysis spectra from ghee, the values obtained from conventional analysis for moisture content and FFA% were used for calibrating the instrument and model development.The performance of the final model was assessed using coefficient of determination (R^2) and root mean square error of cross-validation (RMSECV). Partial Least Squares Regression (PLSR) was performed with Quant software (Version 7.2) (Medeiros *et al.*, 2023). The Sum of Squared Errors (SSE) was calculated as the quadratic sum of the residual values, where each residual represents the difference between the actual value and the predicted value.

$$\text{SSE} = \Sigma(Residual)^2$$

$$\text{RMSEE} = \sqrt{\frac{1}{(n-r-1)}} * SSE$$

Where,

RMSEE denotes the root mean square error of estimation, n denotes the sample number and r denotes the rank

$$R^{2=} \left(1-\frac{SSE}{\Sigma(yi-ym)^2}\right) * 100$$

Where, ym is the mean of true value of all sample and yi is the mean of sample i

$$\text{RMSE}_{CV} = \sqrt{\Sigma(y\bar{\imath} - yi)^2/n}$$

Where,

$y\bar{\imath}$ represents the reference measurement result for sample i, yi represents the estimated result for the sample when the model is developed and n represents the number of samples.

RPD was residual predictive deviation calculated to the ratio of Standard error to standard error of cross validation (SD_{cv}), when RPD is ≥ 3 considered as excellent prediction (Hashem *et al.*, 2022).

Result and Discussion

Spectra Recording

The light beams from interferometer passed through the sample were detected by lead sulphide detector. The detected signals are fourier transformed to obtain the spectrum. The spectra ranged from 12000 to 4000 cm^{-1}. Figure 2 illustrates the spectra recorded for all 30 samples.

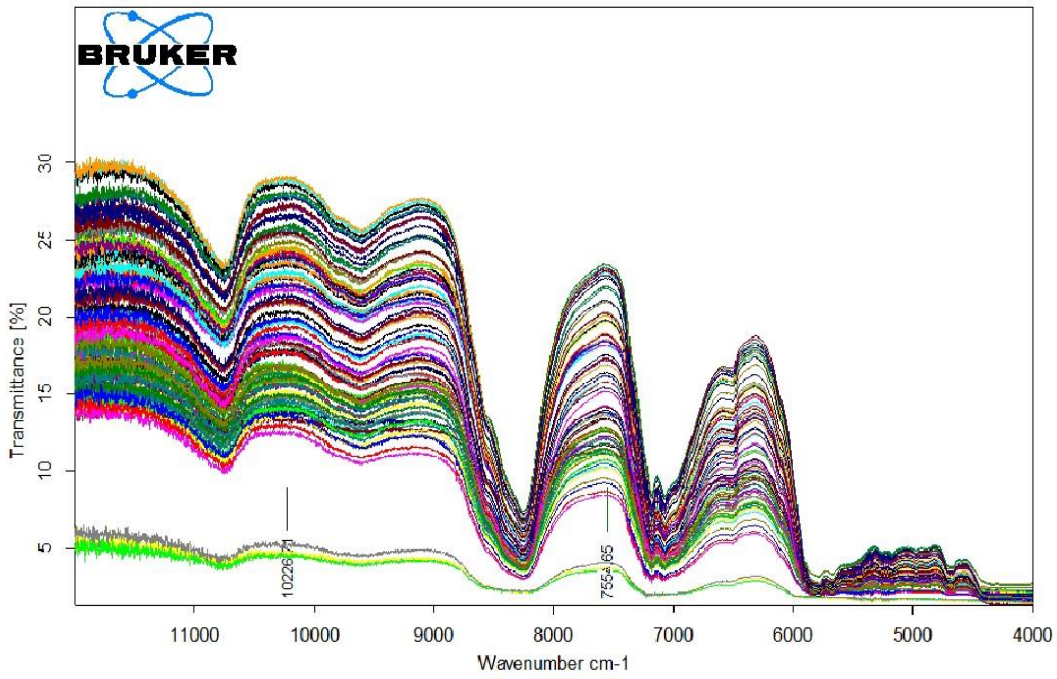

Fig.2 Spectra for Ghee samples

Quality evaluation of ghee using FT-NIR

Fourier Transform Near Infrared Spectroscopy was used to develop a non-destructive quality analysis of ghee. The result obtained from the conventional analysis of moisture content and Free Fatty Acids was used for this model development. The values obtained from the conventional analysis were fed into the system to the respective spectra, which were recorded. The recorded spectrum is shown in Figure 3.

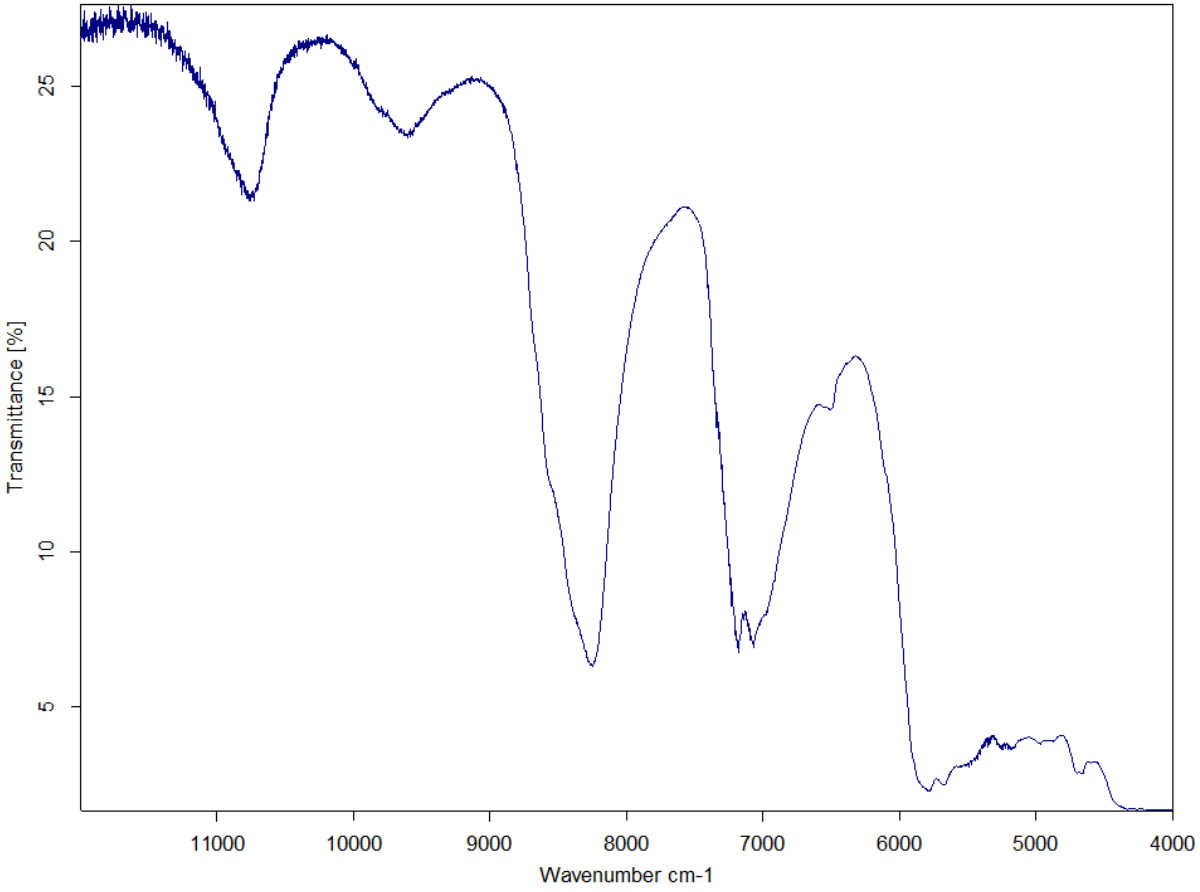

Fig.3 Spectrum of Ghee sample (Preprocessed)

Estimation of moisture content in ghee by FT-NIR spectroscopy:

Moisture contents of 30 samples were estimated by using oven drying method and the values were incorporated and the spectra obtained were recorded. The data obtained from conventional method ranged from 0.004 to 1.810 %. Then, these values were cross-validated using FT-NIR spectroscopy. The R^2 value and RMSECV value obtained for moisture is given in the (Table.2) and the graph was plotted between the predicted value and measured value (Fig

4).The R^2 value was 96.93 % and $RMSE_{cv}$ was 0.0222. The RPD value was 5.71 which≥ 3 categorized as excellent.

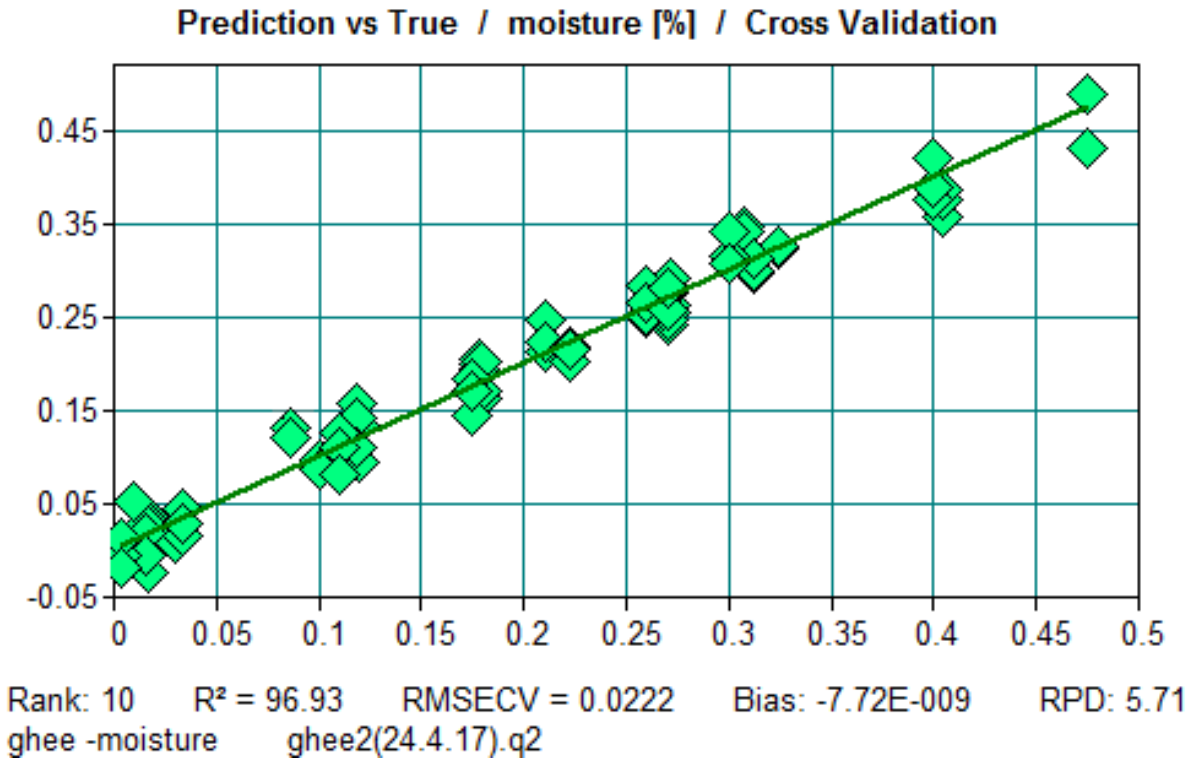

Fig.4. Linear plot between predicted and measured values of moisture content

Estimation of Free Fatty Acid % in ghee using FT-NIR spectroscopy:

The FFA content of 30 samples was estimated using the titration method and incorporated into the recorded spectra. The data obtained from the conventional method ranged from 0.561 to 3.553 %. Then these values were cross-validatedwith FT-NIR spectroscopy. The R^2 value and RMSECV value obtained for FFA is given in the (Table 2).The R^2 value was 97.53 % and $RMSE_{cv}$ was 0.103.The RPD value of the FFA was 6.36 which was ≥ 3, categorized as excellent. The graph plotted between the measured and predicted value for FFAis given in (Fig 5).

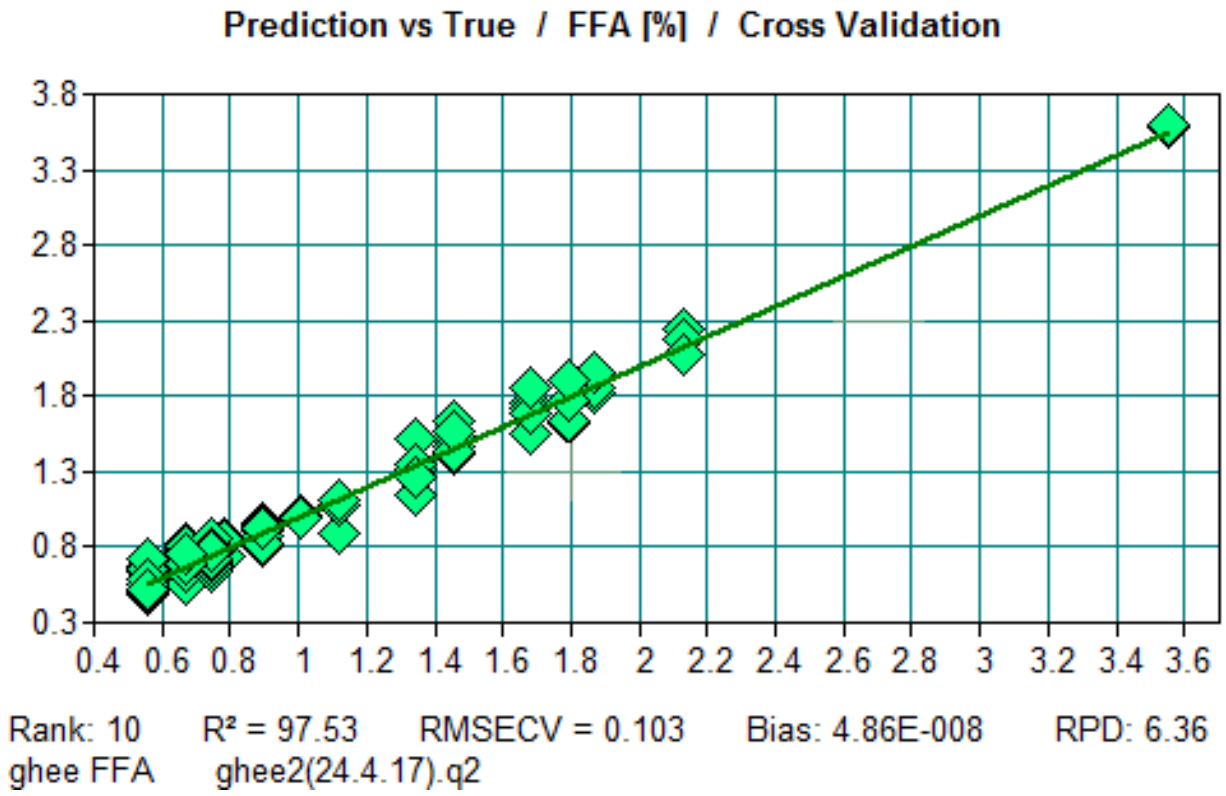

Fig.5 Linear plot between predicted and measured values of FFA percentage

4.5 Grading of ghee using FT-NIR:

Using this developed model, ghee can be graded based on their quality parameter. Fourier Transform Near Infrared Spectroscopy can analyze the unknown sample in 25 s and estimate the value of moisture content and Free Fatty Acid (%) in the test ghee sample. Based on these values, ghee sample can be graded into Standard, General and Special grades. This rapid analysis technique helps to check the specification for the large scale producers and dairy industries.The test results of the unknown sample through a dialogue box of FT-NIR are given in Fig.6.

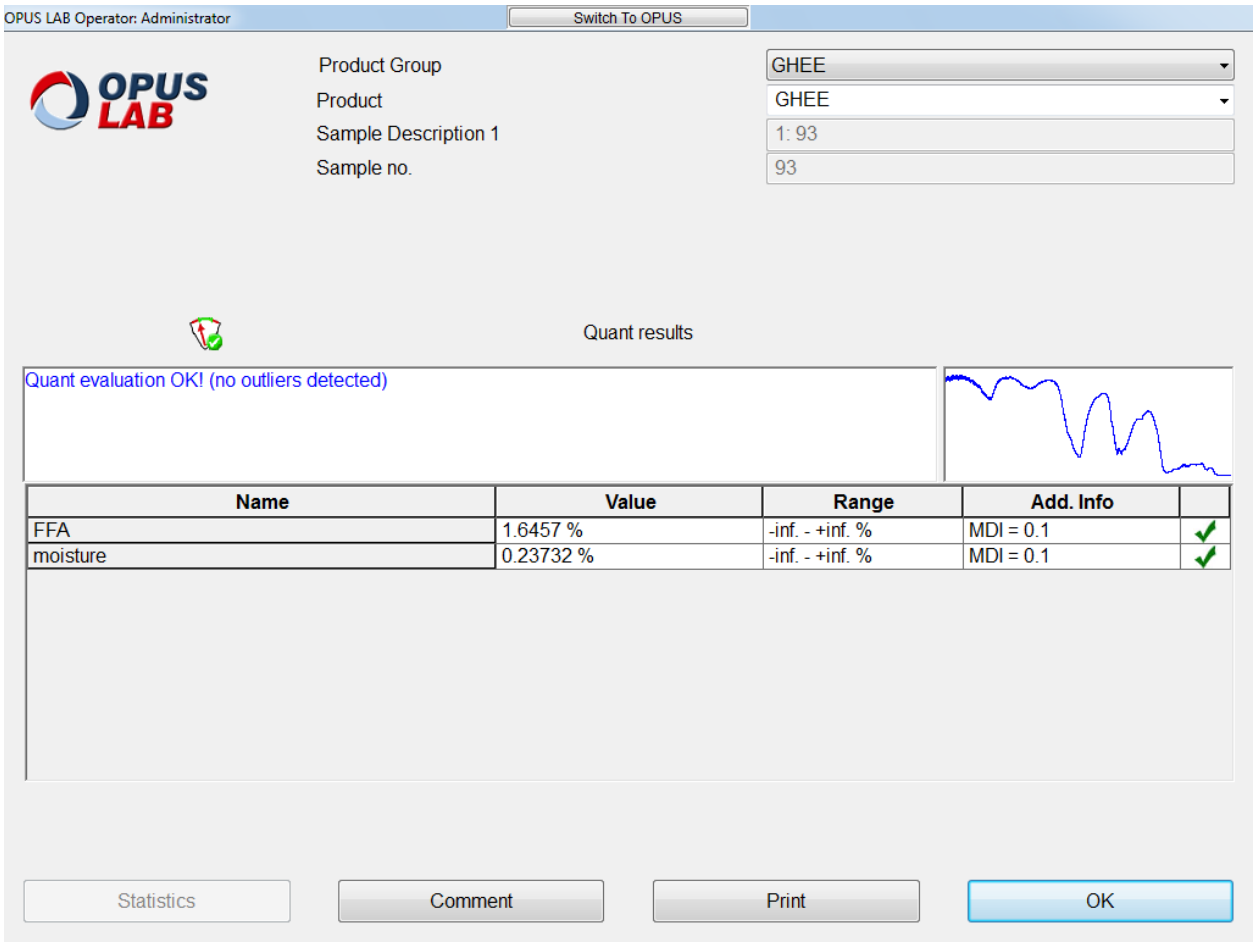

Fig.6.Values of FFA and Moisture percentage in unknown ghee sample using FT-NIR spectroscopy

Conclusion

The existing measurement methods for grading of commercial ghee based on the quality parameters are slow, destructive, and requires substantial manual effort. As a result, there is an increasing need for faster and less labor-intensive analysis techniques. The present study aimed to develop a non-destructive, rapid and computerized method for ghee grading based upon FFA and moisture content.The ghee samples were analyzed for both moisture content and FFA % by using conventional method (wet chemistry). The R^2 values were 96.93 % and 97.53 % and the values for $RMSE_{cv}$ were 0.0222 and 0.103 and RPD values were 5.73 and 6.36 for moisture and FFA, respectively. Fourier Transform Near Infrared Spectroscopy could be used as a non-destructive and reliable method to determine the ghee quality

parameters.The developed grading method estimated the values of moisture content and FFA in 25 s for unknown samples.

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Table.1 Moisture content and FFA range of the samples

Quality Indicators	**Range**	**Number of samples**	**Total**
Moisture content, (%)	Below 0.3	**25**	**30**
	Above 0.3	**5**	
Free fatty acid, (%)	Below 1.4	**15**	**30**
	1.5 to 2.5	**8**	
	2.6 to 3	**4**	
	Above 3	**3**	

Table.2 Statistical Measures for the Models on Moisture and FFA

S. No	**Statistical Measures**	**Moisture content,(%)**	**Free fatty acid,(%)**
1	R^2	96.93	97.53
2	$RMSE_{CV}$	0.0222	0.103
3	Bias	$-7.726 \times 10^{-0.009}$	$4.86 \times 10^{-0.008}$
4	RPD	5.71	6.36