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MONITORING OF SENSORY QUALITY OF MILK BY SPECTROSCOPIC TECHNIQUES

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR MASTER PROGRAM IN BIOLOGICAL CHEMISTRY

BY

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ABSTRACT

The combined use of spectroscopic analysis and chemometric tools has wide application in evaluating and monitoring the product sensory qualities. The main intention of the work was to better understand the use of a spectroscopic technique in evaluating the changes in fluid milk when quality is reduced during storage or by special reasons. In addition, to study the possibility of correlating infrared (IR) spectroscopy results with the sensory quality changes in milk. This was done by storing milk samples for three consecutive weeks, agitating pasteurized milk with and without raw milk addition, exposing milk samples to different wavelengths of light (300-800 nm) using different color filters and storing milk samples at three different temperature for a week and one specific temperature for one day.

Stored milk samples at 4 ^{o}C were analyzed weekly with sensory and spectroscopic technique. Sensory analysis showed that dairy and age of milk had significant effect ($p<0.05$) on the quality of milk and on free fatty acids (FFA) formation. It was observed that low quality score was mainly due to off-flavor, harsh, rancid and sickening flavor. However, the sensory attributes didn't change very much even after the storage for three weeks. The calibration model ($R^2=0.382$) indicates that small changes in sensory quality is difficult to measure by FTIR spectroscopy.

Pasteurized milk when agitated at 15 ^{o}C for 0, 15 and 45 minutes with and without addition of 1% raw milk, the sensory analysis showed significant effect ($p<0.05$) between dairies and raw milk addition with quality score and rancid attribute. The FFA formation was greatly enhanced by raw milk addition rather than agitation time and the effect was clearly visible after 5 days. By IR spectroscopy, it showed that quality score had been affected by rancid off flavor. The calibration model with quality score and rancid was found to be $R^2=0.825$ and 0.801. It shows much information about sensory quality and rancidity which can be calibrated by FTIR.

Pasteurized milk when exposed to blue light (400-500 nm), it got absorbed by riboflavin and orange light (575-750 nm) absorbed by tetrapyrroles. Green light (450-600 nm) didn't promote photo oxidation of riboflavin and tetrapyrroles. Sensory analysis showed that filters used for light exposure and time interval had significant effect ($p<0.05$) on quality score and oxidized flavor in the milk. Reference sample showed high quality with less oxidation in compare to

unwrapped samples. It was observed that orange filter absorbing light longer than 575 nm induced more off flavor than blue filter.
However, by IR spectroscopy with calibration model R^2=0.391, it showed that low sensory quality, caused by oxidized off flavor was best measured when riboflavin was degraded by blue light. Tetrapyrrole degradation causing oxidized off-flavor, can probably not be measured by FTIR.

When pasteurized milk was stored at different temperatures, sensory analysis showed that dairy and temperature had significant effect on quality score. Milk samples stored at 4 ^{o}C for 7 days had better quality than 6 days at 4 ^{o}C plus 1 day at 17 ^{o}C. It was observed that quality score had been affected by bitter, sour, rancid and off-flavor defects. Furthermore, the calibration model from IR spectroscopy with quality score was found to be R^2=0.282.

Finally, the combined merged datasets of sensory and spectroscopy showed a good correlation model with quality score (correlation coefficients= 0.51).

In conclusion, FTIR technique can be useful to apply as screening tool for evaluating quality of milk samples. However, for the total sensory quality, this method can't be applicable as it can't detect all possible sensory defects in milk.

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Abbreviations

A.U.	Arbitrary Unit
CMS	Cell Match Solution
FTIR	Fourier Transform Infrared Spectroscopy
IDF	International Dairy Federation
IR	Infrared Spectroscopy
ISO	International Organization for Standardization
MFGM	Milk Fat Globule Membrane
MIR	Mid Infrared Spectroscopy
NIR	Near Infrared Spectroscopy
PCA	Principle Component Analysis
PCR	Principle Component Regression
PLS	Partial Least Squares Regression
UV	Ultra Violet

1. Introduction

The consumer preference for a food product is largely based upon its sensory characteristics. Accurate monitoring and control of sensory quality will facilitate the production of high-quality products which is vital for consumer satisfaction and thus for the revenue generation of a food company. Some of the factors that interfere the sensory quality of consumer milk are the quality of the raw milk, the physical, chemical and microbiological processes during the production and subsequent alteration of milk in the distribution chain. Sensory profiling allows various quality attributes to be identified and their intensity determined (Fagan et al., 2007). The descriptive sensory evaluation including different sensory attributes are traditionally assessed by using trained panelists. However, this method takes lot more time resulting expensive cost and lacks objectivity too (Fagan et al., 2007).

Therefore, the instrumental technique is preferred to be used for fast analysis, less operating cost and more objectivity while assessing product quality, including sensory quality (Fagan et al., 2007). Such an instrumental technique could assist producers to maximize yields, increase efficiency, reduce labor costs, optimize product quality, consistency, and increase customer satisfaction.

Combination of spectroscopic analysis and predictive mathematical models have wide use in controlling and monitoring the product quality throughout the value chain, which is developed using multivariate data analysis techniques such as partial least squares (PLS) regression. In particular, infrared spectroscopy has been considered to be the non-destructive technique which focuses on cost effective result and real-time analysis of both composition and quality (Fagan et al., 2007).

Infrared spectroscopic techniques i.e. near infrared (NIR) and mid infrared spectroscopy (MIR), are the preferred ones which need minimal volume of sample for analysis and low operating cost (Wu, Feng, & He, 2007). In addition, they can provide valuable information about qualitative attributes of food products without doing the classical analysis in laboratories that takes much time (Fagan et al., 2007). Comparing both techniques, the advantage of MIR bands over NIR is high molar absorptivity which makes the peaks more specific, sharp and sensitive while NIRS has overtone characteristic resulting low sensitivity (Wu et al., 2007). Determination of fat and protein contents in milk products is done in the mid-infrared region of 400 to 4,000 cm^{-1} (Wu et al., 2007)) and often applied for detecting compositional differences between samples. Chemometric model design is very essential for extracting the

relative quantitative information. However, it is time consuming, the analysis can be done fast once the model is established (Wu et al., 2007).

1.1. Milk Properties

Milk is considered as a variable biological fluid because of its changing characters with interspecies, breed type, health, nutritional status, stage of lactation, age and interval between milking (Fox & McSweeney, 1998). It possesses a neutral flavor profile due to its natural components such as proteins, fat, salts, milk sugar (lactose), and small amounts of other milk components that is pleasantly sweet, with no distinct aftertaste (Clark, Bodyfelt, Costello, & Drake, 2009). Depending upon the content of milk fat present, it can be divided into whole milk (3.5%), low fat milk (1-2%) and skim milk (< 0.5%).

1.1.1. Physical properties

There are two important physical properties that are responsible for the visual appearance of milk. Firstly, milk is a protein-stabilized emulsion of fat in a continuous aqueous phase. Secondly, it is a suspension of insoluble colloidal mineral particles. The light scattering caused by the insoluble colloidal minerals, protein, and fat particles enhances the property of milk regarding the opaqueness and white color of the milk (Clark et al., 2009).

1.1.2. Chemical and microbiological properties

Fresh milk consists of water, fat, protein, lactose, and minor mineral components in which the lactose available at an average concentration of 4.8% responses to a mild sweet taste. Likewise, fat present in milk enhances for the rich mouth feel of full fat milk in comparison to skim milk (Dunkley, 1982; Clark et al., 2009).

Milk when gets secreted within the secretory glands, it is typically sterile in nature. But it is supposed to get contaminated first with bacteria within the teat canal (Clark et al., 2009). Furthermore, the contamination and growth of microbial organisms is enhanced by the milk handling process on the farm including the equipment used for automated milking, milk handling lines, and refrigerated bulk milk storage tanks. Nutrients present in milk create an ideal growth medium for the wide spectrum of microbial flora too. Besides, psychotropic bacteria (i.e. *Pseudomonas* sp.) as a primary factor, other bacterial contaminants generated from soil, water, animal feed and animal faeces including gram negative rods (*Alcaligenes, Acenitobacter, Aeromonas,* and *Flavobacterium*), gram positive bacteria (*Bacillus, Clostridium, Lactobacillus, Streptococcus,* and *staphylococcus*), yeasts, and molds also play

significant role in spoilage of milk prior to pasteurization (Al-Qadiri, Lin, Al-Holy, Cavinato, & Rasco, 2008).

Pasteurization of milk is done to remove heat resistant pathogens; however, some pathogens can even survive this condition. The initial micro flora in pasteurized milk present is gram-positive thermoduric organisms available in raw milk. However, most strains of bacteria are not able to reproduce after pasteurization under refrigerated storage conditions like *Bacillus, Micrococcus, Enterococcus, Corynebacterium, Microbacterium, Arthrobacter, and Lactobacillus* (Champagne et al., 1994).

1.2. Milk composition

The major components present in milk are water, lactose, fat, and protein (casein dominates). Besides, those principal components in higher quantities, vitamins and minerals are important with respect to nutritive value and enzymes being catalysts of reactions are found in low amount (Walstra, Walstra, Wouters, & Geurts, 2014).

1.2.1. Lactose

Lactose or milk sugar is a reducing sugar or disaccharide composed of glucose and galactose. It is absorbed in the IR-spectrum within the wavenumber area 1038-1058 cm^{-1}.

1.2.2. Fat

Milk fat is largely composed of triglycerides, which partly gets solid at room temperature. Lipids are also used as alternative term, which constitutes esters of fatty acids. These are soluble in nonpolar organic solvents and insoluble in water. Other lipids present in milk include phospholipids, cholesterol, free fatty acids, monoglycerides, diglycerides. Fat A consisting of the C=O group lies within the wavenumber 1740-1756 cm^{-1} while Fat B consisting of the acyl chain (C-H) group lies within the wavenumber area 2838-2864 cm^{-1} and 2800-2824 cm^{-1} (Karoui, Downey, & Blecker, 2010).

1.2.3. Protein

Whey proteins, also called serum proteins and caseins are two different types of milk proteins where casein occupies 80% of the total volume. Caseins may be subdivided into five proteins: α_{s1}-, α_{s2}-, β-, gamma and k-casein (Varnam & Sutherland, 2001).

Moreover, milk contains numerous minor proteins including a wide range of enzymes. It is absorbed between wavenumber range 1485-1497 cm^{-1} and 1531-1551 cm^{-1}.

1.2.4. Minerals

Milk contains mainly potassium, sodium, calcium, magnesium, chloride, phosphate, and other elements in trace amount. The salts are partly ionized i.e. cationic while the organic acids occur largely as ions or as salts(i.e. citrate)(Walstra et al., 2014). However, the detection of minerals in spectroscopic analysis isn't known yet.

1.3. Sensory quality of milk

Sensory properties of milk, such as flavor, plays an important role in the dairy industry because they directly affect product quality and consumer acceptance. Milk flavor results mainly from proteins, lipids and carbohydrates, which are the precursors of aroma compounds. Milk has a bland, yet characteristic flavor, which is very susceptible to develop off-flavor. The way of handling, processing and storage determine the milk flavor. Oxidized and rancid are common milk off-flavors developing due to light oxidization, heat treatment, enzymatic and microbial activities, transfer of substances from the feed, and transfer of substances from the environment (Clark et al., 2009; Rodriguez Otero, Hermida, & Cepeda, 1994). Appearance, texture and flavor are the important basis for characterizing milk where whiteness, glossiness and transparency describe the appearance quality. Similarly, oxidized, salty, cooked, bitter and sweet characters define the flavor quality of milk (Phillips, Mcgiff, Barbano, & Lawless, 1995b). Milk after pasteurization might develop 'cooked' flavor which gets diminished in intensity or disappear during storage because of the divalent cations present in the milk (Clark et al., 2009). Similarly, the development of rancid flavor is possible even after pasteurization because of the heat resistant thermoduric psychrotrophic bacteria that releases lipases for lipolytic activity. The mixing of raw and homogenized milk with temperature fluctuation or agitation during processing also results in developing a different flavor called sickening. The homogenization of milk encourages less probability of developing metal induced, cardboard, or oxidized off-flavor while in reverse, enhances the development of light induced off-flavor ("Sunshine flavor") (Clark et al., 2009). The content of fat in milk plays a vital role in differentiating texture (Phillips, Mcgiff, Barbano, & Lawless, 1995a). Thus, whole milk with 3.5% fat possesses creamy and heavy mouth feel while low fat milk with less than 2.5% fat shows lighter and watery consistency (Saba, Moneta, Nardo, & Sinesio, 1998).

The dairy industry has used different systems for quality evaluation of milk. The International Dairy Federation has published standard methods IDF-99 some 30 years ago. Today's versions of these are published as ISO 22395 / IDF 99 (International Organization for Standardization & International Dairy Federation, 2009a; International Organization for Standardization & International Dairy Federation, 2009b; International Organization for Standardization & International Dairy Federation, 2009c). Scoreboard is often used by the trained judges in dairy companies for evaluating the defects in milk during sensory analysis. However, these methods have been criticized for its failure to predict consumer acceptance, its lack of objectivity in quality judgements, and the complexity in assignment of quantitative scores. In addition, the laboratory based method is time consuming and needs skilled manpower in execution (Blazquez, Downey, O'Donnell, O'Callaghan, & Howard, 2004; Fagan et al., 2007).

The sensory characteristics of any dairy product are mostly dependent on the quality attributes of the milk ingredients used for the production. This is especially important for fresh fluid milk. Some authors state that the ingredients are far better in quality than the finished milk products which they are made from (Clark et al., 2009). Some common defects due to conditions during production and storage are the main subject of this thesis.

1.4. Infrared Spectroscopy (IR Spectroscopy)

Infrared spectroscopy has a wide application in the structure and compound identification of samples available in any form (Stuart, 2004). In addition, this technique helps to quantify carbohydrates, proteins, fats and other constituents such as vitamins, minerals etc. present at low concentrations (Sun, 2008). Infrared spectroscopy is a technique that works on the principle of the vibrations of the atoms of a molecule (Stuart, 2004). Vibration of chemical bonds takes place at specific frequencies, which are determined by different factors. This includes constituent atomic mass, molecule shape, the bond stiffness, and the periods of the associated vibrational coupling.

There are some phenomena whose presence creates complication in the interpretation of MIR spectra. These include the overtone and combination bands and Fermi resonances. However, the identification of specific chemical groups can be possible because of the absorption frequencies which is the key role of FTIR spectroscopy (Karoui et al., 2010). When infrared radiation is passed through a sample, it results in obtaining an infrared spectrum determining what fraction of incident radiation being absorbed at a particular energy at the same time. Similarly, any peak that appears in an absorption spectrum at certain energy indicates about the frequency of vibration that belonged to a part of sample molecule (Stuart, 2004).

In practice, a sample can be measured by passing a beam of infrared light through the sample and the energy absorbed at each wavelength is recorded. This method can be done in two different ways. One is done by scanning through the spectrum with a monochromatic beam, which changes in wavelength over time. Second is done measuring all the wavelengths at the same time using a Fourier transform system. Thus, the effect of all the different functional groups result in to an absorbance (or transmittance) spectrum showing at which wavelengths the sample absorbs the infrared light. Finally, this helps in the interpretation of the chemical bonds (Sun, 2008).

Vibration of the biological molecule when irradiated by IR radiation occurs in the wavelength range of 2.5 to 25 μm (Stuart, 2004). As the molecule absorbs energy, the signal at this frequency decreases, leading to a peak in the spectrum.

1.4.1. Dispersive Infrared Spectrometers

This infrared spectrometer was first used in the field of obtaining IR absorbance spectrum with the use of prisms made of materials such as sodium chloride. However, this technology was no more in use after the introduction of grating instruments during 1960s that was cheaper and of better quality. The basic components in this spectrometer include the source, the monochromatic and the detector device. The monochromatic disperses source radiation using a dispersive element such as a prism or grating. This separates the components of polychromatic radiation based on their wavelength (Stuart, 2004).

When the energy that falls on the entrance slit is collimated on the dispersive element, dispersion is occurred. This results the dispersed radiation to reflect back to the exit slit, beyond which lies the detector. Moreover, the rotation of suitable component within monochromator helps to scan the dispersed spectrum across the exit slit. When there is absence of a sample, the detector receives radiation of approx. constant energy once the spectrum is scanned. This energy then moves to the detector through the sample. The entire sample spectrum can be obtained by the adjustment of a component within the monochromator and allowing the different wavelength to pass through the exit slit at a time (Stuart, 2004; Stuart & Ando, 1996). However, the instrument became less popular after the 1950s because of its defects like time consumption, sample overheating and damage due to repeated sample irradiation. Later, these limitations were overcome by the use of advanced instrument called Fourier- transform infrared spectrometer. Diffraction grating is replaced by an interferometer in the Fourier Transform spectrometer (Sun, 2008).

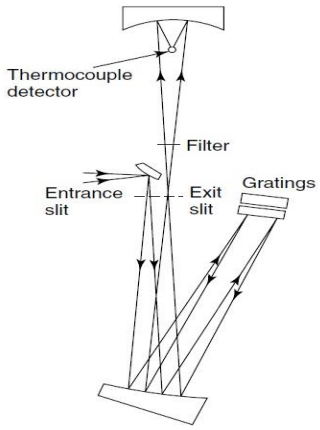

Figure 1: Schematic diagram showing the optical path of a double beam infrared spectrometer with a grating monochromator (Stuart, 2004; Stuart & Ando, 1996)

MIR represents the absorption spectrum of all the chemical bonds including infrared activity between 4000 and 400 cm^{-1} (Stuart & Ando, 1996). This region includes the electromagnetic spectrum segmented into four broad regions as the X-H stretching region (4000-2500 cm^{-1}), the triple bond region (2500-2000 cm^{-1}), the double bond region (2000-1500 cm^{-1}), and the finger print region (1000-400 cm^{-1}). The identification of fat is done by the acyl-chain (C-H) (3000- 2800 cm^{-1}), the C=O group (around 1750 cm^{-1} and C-O at around 1175 cm^{-1}) while protein is detected at the amide I (1653 cm^{-1}) and II (1567 cm^{-1}) (Karoui et al., 2010). The absorption bands present in the mid-infrared region, are identified and attributed to chemical groups (Karoui, Mazerolles, & Dufour, 2003).

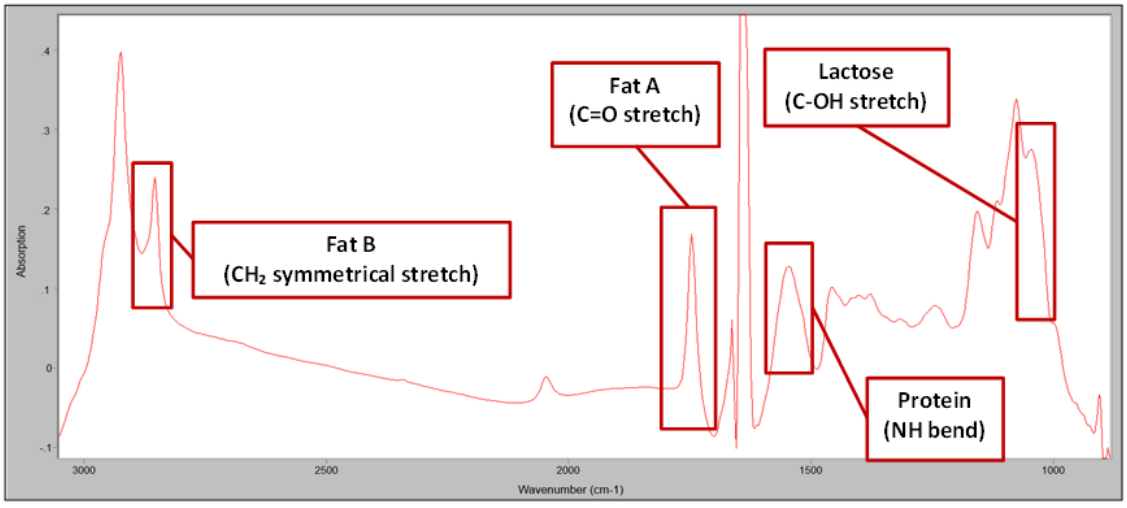

Figure 2: Typical milk spectra showing its major constituents with functional groups within the wavenumber range 4000-400 cm^{-1}

In order to study the difference in the spectral nature of different milk products, a few samples of milk products collected from local grocery were analyzed for demonstration purpose including ice-coffee (Iskaffe), chocolate milk, goat milk, ecological milk (Økologisk) and cream. There is a clear difference seen in the absorption spectras among these products within the wavenumber region 4000-400 cm^{-1}.

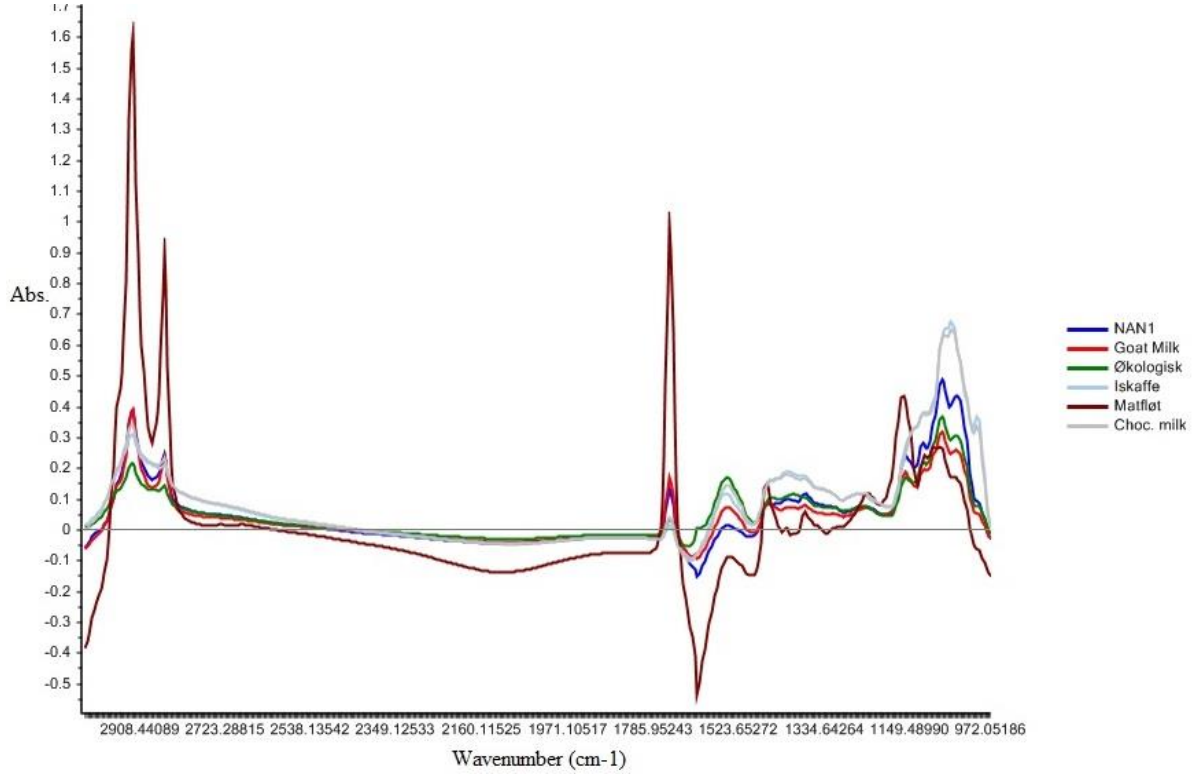

Figure 3: Spectra of different milk products showing absorption differences in functional groups within the wavenumber range 4000-400 cm^{-1}

1.4.2. Fourier Transform Infrared spectroscopy (FTIR)

The development of Fourier-transform spectrometers and the computer technology have helped to improve spectrum quality and data time turnover greatly. In addition, this technique has made the study of biological molecules rapidly in a easy way (Stuart, 2004).

It acquires the IR absorbance spectrum by the combined use of interferometer and the mathematical processes of Fourier transformation. It has many benefits as it requires very little volume of sample for analysis, less expenses to operate, doesn't heat sample and the resulting spectra can be received within short period of time (Stuart, 2004). The major advantages of FT-IR spectrometers over the conventional spectrometers include:

i) The higher signal to noise ratios for spectra recorded for the same measurement time, which is a consequence of both the concurrent

measurement of detector signal for all the resolution elements of the spectrum known as multiplex or Fellgett advantage and of the high optical throughput of the FTIR spectrometer known as throughput or Jacquinot advantage.

ii) The higher accuracy in frequency measurement for the spectra, which is a consequence of the use of a laser referencing the measurements made by the interferometer, known as laser interference or Connes advantage (Jaggi & Vij, 2006)

Principle

FT-IR spectroscopy with the use of the interferometer achieves the production of an IR radiation signal across all wavelengths. The basic components of this instrument include source, the interferometer, sample and detector. Here, the radiation coming from the source passes through an interferometer before reaching to the sample and then to the detector. Then the signals received are amplified where the filter eliminated high frequency contributions. Finally the data are converted to the digital form by an analog to digital converter and with the help of Fourier transform technique, the sample's spectrum is calculated from the interferogram using mathematical algorithms (Stuart, 2004; Stuart & Ando, 1996).

Figure 4: Basic components of an FTIR instrument (Stuart, 2004; Stuart & Ando, 1996)

The analysis process of FT-IR involves the following procedure:

The Source

The black body source usually Globar or Nerst is used for the mid infrared region. It emits the infrared energy, which passes through an aperture, and it controls the amount of energy that is presented to the sample.

Michelson Interferometer

The most commonly used interferometer in FTIR spectroscopy is a Michelson one, which consists of three components, a fixed mirror, a moving mirror and a beam splitter. Here the beam splitter is a semi reflecting transparent film which splits the beam into two equal parts with fifty percent of radiation each (Stuart, 2004). The composition of beam splitter depends upon the region that is to be examined. For the mid infrared region, infrared transparent substrate i.e. potassium bromide or caesium iodide are coated with germanium or iron oxide.

When the monochromatic radiation is passed to the beam splitter, 50 % of the incident radiation gets reflected from one of the mirrors while rest 50 % gets transmitted from the other. Finally, both beams return back to the beam splitter where they recombine and interfere. The beam emerging from the interferometer at 90 ^{o}C to the input beam is the transmitted beam which is detected in the FTIR spectrometry (Stuart, 2004). The transmitted beam passes though the sample where some of the energy is absorbed. The remaining portion reaches the detector and the interferogram is recorded. With the use of Fourier transformation, it is translated into sample absorbance spectrum.

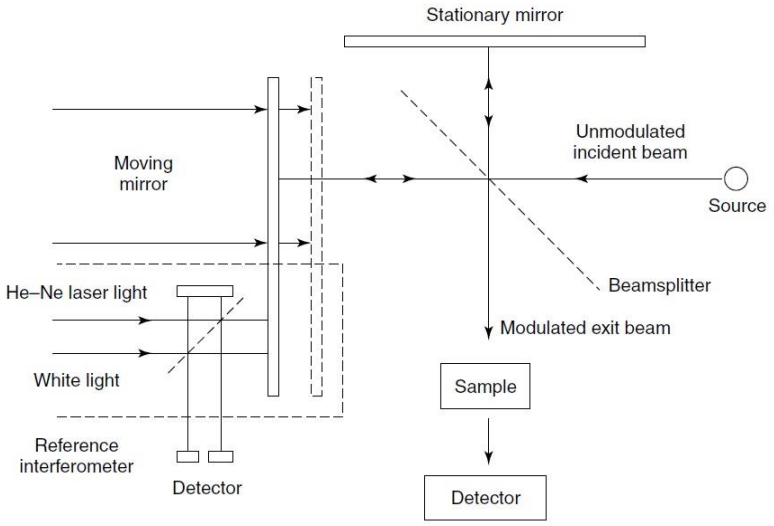

Figure 5: Schematic diagram of a Michelson interferometer (Stuart, 2004)

The Sample

The emitted beam enters the sample compartment and according to the need of analysis, this light is transmitted or reflected through the surface of the samples. Then the specific frequencies of energy that the sample possesses are absorbed here.

The Detector

Finally, the beam gets through the detector, which is designed for final measurement of the special interferogram signal. For the mid infrared region, two detectors are commonly used. First type is deuterated triglycine sulphate (DTGS) pyro electric detector that is heat resistant and placed in an alkali halide window. Second type is mercury cadmium telluride (MCT) photoconductive detectors that needs to be cooled at liquid nitrogen temperatures (Stuart, 2004).

The Computer

This special signal is first digitized and then passes to the computer where the Fourier transformation takes place. The final infrared spectrum is then used for interpretation and manipulation.

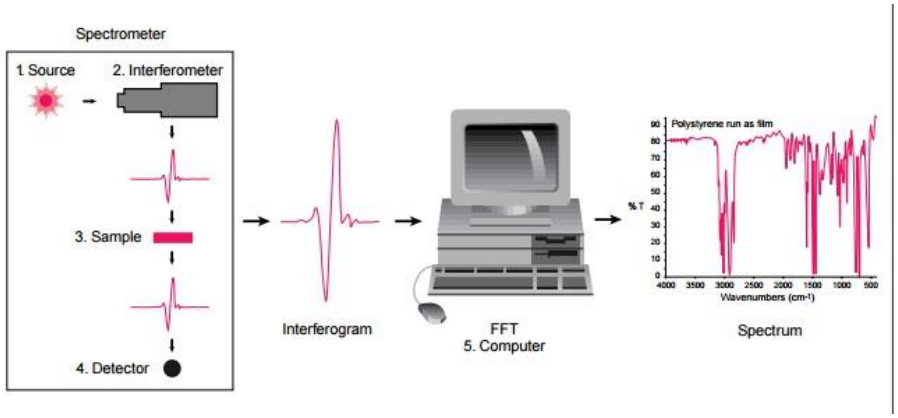

Figure 6: Different steps used in the analysis process of Fourier transform infrared spectrometry (http://mmrc.caltech.edu/FTIR/FTIRintro.pdf accessed on March, 2015)

Compositional analysis of milk and dairy products using MIR method is well established within the dairy industry. The multivariate data analysis or chemometrics is very beneficial in order to interpret the IR data because the spectrum usually contains numerous overlapping target wavelengths. Thus, linear regression can't be done using traditional univariate method.

Here the use of spectroscopic technique seems very appropriate for detecting the compositional variations between the samples with specific information about chemical groups (Wu et al., 2007). Moreover, one more advantage is, it doesn't need bulky time consuming chemical analysis in laboratories. This method assists in maximizing yields, increase efficiency with optimizing product quality and reducing labor cost.

1.5. Stability of Milk

1.5.1. Stability of milk under mechanical treatment

The mechanical handling of milk in dairy farms and during transport and production process contribute to increased lipolysis resulting in rancidity. Though it is detected only after free fatty acid crosses the flavor threshold (Escobar & Bradley Jr, 1990). The lipolyzed flavor detection threshold in milk was observed within the limit of 4.1 to 4.5 acid degree (Pillay, Myhr, & Gray, 1980). From the study, it was accepted that activation of the milk lipase system lead to lipolysis in raw milk during milking, processing i.e. foaming of milk, pumping, agitation in pipelines (Deeth & Fitz-Gerald, 1977). Lipolysis induced by shaking continued to exist even after the

milk had been cooled to low temperature. Thus, the more obvious was the change in odor, flavor and titratable acidity (Herrington & Krukovsky, 1939).

The mechanism of lipolysis due to lipase activation by agitation is rupture of the protective milk fat globule membrane (MFGM) (Deeth & Fitz-Gerald, 1978). Lipolysis result in the liberation of fatty acids that develop unpleasant off-flavors in milk and milk products (Deeth & Fitz-Gerald, 1977) especially rancid off flavor which is a matter of concern in dairy field (Deeth, 2006). Raw milk continued to rise in the titratable acidity when homogenized, followed by the development of a rancid flavor and this happens because of the lipase action normally present in all milk (Larsen, Trout, & Gould, 1941; Fitz-Gerald, 1995). Pasteurization of milk prevents the development of rancidity upon homogenization, so in order to study the effect of pasteurization Larsen, P. B. et al. (1941) conducted an experiment with mixtures of raw and pasteurized homogenized milk and the result obtained showed that rancidity was developed by the addition of raw homogenized milk. It is also stated that "mixtures of pasteurized and homogenized cream or milk with raw milk, raw skim milk or raw cream become rancid." (Dorner & Widmer, 1932). From the experiment carried out by Larsen, P. B. et al. 1941, small quantities of unhomogenised raw milk in homogenized pasteurized milk such as one to five percent were sufficient to produce an increase in acidity after three to five days of storage. However, the maximum acidity increment occurred when the ratio was one to one (Larsen et al., 1941). Several changes occur in milk because of lipase action. Apparently, all milk is capable of appreciable true lipolytic activity if subjected to suitable activating treatments. The acidity of raw and pasteurized milk was studied with increasing the temperature from 2 ^{o}C to 25 ^{o}C and 37 ^{o}C and shaking for 2 hours. From the experiment carried out, the acidity increased less with shaking at 2 ^{o}C than 25 ^{o}C and finally high increase in acidity at 37 ^{o}C. Lipolysis in milk depends upon the agitation mode, severity and duration of agitation when stored under standard conditions (Deeth & Fitz-Gerald, 1977). The degree of lipolysis activation also depends upon the temperature during agitation. From the study, it was observed that the lipolysis induced by agitation was high below 20 ^{o}C (Deeth & Fitz-Gerald, 1977).

1.5.2. Stability of milk under light exposure

Light plays a vital role in inducing chemical changes in food as well as dairy products and leading to formation of off-flavors and off-odors. It enhances degradation of lipids, vitamins and proteins (Herrington & Krukovsky, 1939; Intawiwat, Wold, Skaret, Rukke, & Pettersen, 2013).

Dairy industries use cartons for packaging milk that don't appear transparent and it's always an interesting topic in investigating the effects of light on such products. Studies were performed to investigate the effect of different colored light on quality deterioration in milk. Hansen et al., (1975) performed an experiment on homogenized milk packed in transparent polythene containers. He exposed them to different colored lights by covering the light source with different filters. He found that yellow and green filters gave the best protection against formation of off flavors while the pink filter for instance, gave less protection. In addition, he also observed the development of off flavors in whole milk after light exposure simulating commercial display cases for 2 to 4 hours (Hansen, Turner, & Aurand, 1975).

Dairy products contain several photosensitive pigments (riboflavin, porphyrin and chlorophyll compounds) that absorb in different wavelength regions of Ultra violet (UV) and visible light (Wold, Veberg, Lundby, Nikolai Nilsen, & Moan, 2006). Riboflavin absorbs wavelengths below 500 nm, which means it absorbs UV and blue light whereas tetrapyrroles (chlorophyll compounds and porphyrins) typically absorb light above 600 nm. Airado Rodriguez et al. (2011) found that milk exposed to wavelength longer than 575nm (orange light) developed significantly higher amount of sensory off flavors than wavelength shorter than 500 nm (blue light) (Airado Rodriguez, Intawiwat, Skaret, & Wold, 2011; Intawiwat et al., 2010).

Thus, excluding the UV and blue light below 500 nm and the orange light above about 600 nm seems to be a feasible approach to avoid the worst photo oxidation (Intawiwat et al., 2010). In studies, green light (450-600 nm) has been shown to give the least severe effect on photo oxidation in dairy products (Hansen et al., 1975; Intawiwat et al., 2010). Light exposure had a clear effect on the sensory properties of milk. Milk stored under all transparent filters (colored and non-colored) had significant higher oxidized/rancid flavor and odor than milk stored in darkness and in a carton.

Intawiwat, N. et al. (2010) conducted an experiment in which he found significant lower rancid (oxidized) flavor in milk samples exposed under light for 20 hours at 4 ^{o}C under red (570-800 nm) and green (500-800 nm) filters and also milk samples in dark than UV filters (300-800 nm). He also showed that the light transmission from green filter was less compared to UV1, UV2 and orange filters as it blocked light wavelengths shorter than 450 nm and wavelength longer than 600 nm, which prevented photo oxidation of riboflavin and tetrapyrroles. Hansen et al., (1975) showed that light activated flavor was found to be strong after eight hours of light exposure with fluorescent lamp, which persists after subsequent storage at 7.2 ^{o}C.

1.5.3. Stability of pasteurized milk stored at different temperature

Though pasteurized milk is safe to drink, it is a rich growth medium for microbial growth, which can lead to off flavor, coagulation and ropiness. However, the change in flavor needs some time to occur depending on the number and microorganism types, condition at which pasteurization was done and the temperature at which milk was stored (Petrus, Freire, Setogute, & Higajo, 2011). Thus, the microbial flora including gram positive and gram-negative bacteria in pasteurized milk plays a critical role in determining the shelf life. Pseudomonas spp. having short generation time (< 4 hours) (Samarija, Zamberlin, & Pogaiic, 2012a) particularly *Ps. fluorescens* plays a vital role in the spoilage of milk at refrigeration temperature (Roginski, Fuquay, & Fox, 2003; Roginski et al., 2003) while milk stored at temperature above 10 ^{o}C can get spoiled because of the presence of gram positive bacteria and enterobactericeae (Griffiths & Phillips, 1988). Some of the tests for predicting shelf life of milk include incubation followed by standard microbiological testing. These methods are applied for determining low levels of thermoduric gram-negative bacteria such as psychrotropic bacteria, coliforms, and pseudomonads. These organisms can however surpass pasteurization temperature and are most likely to grow under typical storage conditions (3-7 ^{o}C). They use large molecules of proteins and lipids for growth (Ledenbach & Marshall, 2010). The preferred method for assaying for specific spoilage microorganisms i.e. psychrotrophs in pasteurized milk and milk products include standard plate count (SPC) that has maximum limit of 10^6 cfu/ml (Petrus et al., 2011).

Because of difficulty in excluding heat resistant thermophilic microorganisms, it is a very difficult task for the production of sterile milk. In addition, studies suggest that these bacteria are difficult to exclude from raw milk, which also produce proteolytic enzymes. The presence of extra cellular heat resistant enzymes i.e. proteases, lipases (Roginski et al., 2003) even after the pasteurization leads to bitter flavor and coagulation (Adams, Barach, & Speck, 1975). Cromie, S.J. et al (1989) stated that the storage temperature after pasteurization had the greatest effect on bacterial growth and the growth was found to be more rapid at 7 ^{o}C than at 3 ^{o}C. He also observed rapid growth in the standard plate counts, psychrotrophs and anaerobe counts in milks pasteurized at 80 ^{o}C or higher during storage resulting shorter shelf life (Cromie, Schmidt, & Dommett, 1989).

1.6. Degradation Mechanism of Milk

1.6.1. Lipolysis

The breakdown of fats (lipids) catalyzed by lipase enzymes resulting the production of free fatty acids and partial glycerides is called lipolysis. These enzymes are still active after pasteurization and lead to break fats resulting free fatty acids (Champagne et al., 1994; Miller, Jarvis, & McBean, 2006). The important lipases present in milk includes lecithinase, a phospholipase which hydrolyses the protective membrane of lipid (Roginski et al., 2003). The effect on flavor because of these products is summarized as rancid, bitter, unclean etc. (Ray, Chatter Jee, Chakraborty, & Ghatak, 2013). Besides native lipase characterized as lipoprotein lipase, other enzymes present in bovine milk responsible for lipolysis includes somatic cell origin lipase and bacterial lipases (Santos, Ma, Caplan, & Barbano, 2003). Lipase action involved in lipolysis produces free fatty acids and also diglycerides and monoglycerides.

Triglycerides $\longrightarrow$ Free Fatty Acids + Diglycerides

Diglycerides $\longrightarrow$ Free Fatty Acids + Monoglycerides

Lipolysis can be divided in two categories:

1) Spontaneous Lipolysis 2) Induced lipolysis

Spontaneous Lipolysis

This lipolysis is initiated in milk of some individual cows when cooled below 15 ^{o}C after milking (Ray et al., 2013). The major factors that affect this lipolysis involves:

a) Stage of lactation

b) Feed and nutrition

c) Season

d) Milk production

e) Mastitis

Induced Lipolysis

This lipolysis is initiated when the milk lipase gets activated by physical, thermal and chemical means externally. The activation of lipases can happen by the following processes as:

(a) Agitation and foaming

The vigorous shaking/agitation producing foaming enhances lipolysis in raw milk. It depends on severity and duration of agitation, the amount of enzymes present. The temperature during agitation has also significant role in activating lipolysis. Study showed that the activation was high at 37-40 ^{o}C while least at <5 ^{o}C (Deeth & Fitz-Gerald, 1977; Ray et al., 2013). Similarly, different milking procedures, pumping with different rate and physical handling also influence the lipolytic activity in milk (Escobar & Bradley Jr, 1990; Ray et al., 2013). Similarly, homogenization, chemical activation and temperature activation has the important role in lipolysis activity.

(b) Homogenisation

Homogenization plays key role in the lipolysis of raw milk and cream. The factors that affect lipolysis in this process include pressure, time and temperature. In addition, fat content, storage temperature and storage time after homogenization also affect the lipolysis in homogenized raw milk (Ray et al., 2013). The mixing of homogenized pasteurized milk with unhomogenized raw milk can also lead to lipolytic activity considering the percentage of raw milk addition and storage time period (Larsen et al., 1941).

Similarly, temperature activation and chemical activation shows prominent role in the lipolytic activity of milk (Ray et al., 2013).

Lipolysis by bacterial lipases

Because of the pre and post manufacture contamination by microorganisms, microbial lipolysis resulting hydrolytic rancidity is produced (Ray et al., 2013).

Psychotropic bacteria (mainly gram negative) acts as dominant organisms in raw milk and cream. It goes on increase in number during storage at the farm and factory (Ray et al., 2013).

1.6.2. **Proteolysis**

Different proteinase enzymes isolated from pseudomonads are present in milk that degrade milk proteins. These proteases originate from either microbial contamination or transferred from blood to milk. These enzymes that are of bacterial origin produce undesirable changes in milk affecting k-Casein a lot while β-casein and as- casein in less amount. It is basically useful for cheese ripening with desirable changes in flavor and texture (Fox & Kelly, 2006; Ray et al., 2013). During ripening process, casein forms water soluble nitrogenous compounds i.e. peptides and amino acids which differs in solubility in water and other solvents (Sun, 2008). Proteolysis during storage leads to bitterness, which is caused by the accumulation of small peptides (Santos et al., 2003).

1.6.3. **Light Oxidation**

Milk when gets exposed to light leads to oxidation. This phenomena is catalytic effect of light which promotes the off flavor development. Photo oxidation can occur by the two ways as photolytic auto oxidation or photosensitized oxidation. Photolytic auto oxidation leads to free radicals formation initiated by the high energy light i.e. sun light (10,000- 100,000 lx) whereas, photosensitized oxidation occurs in visible light due to the presence of photosensitizers like riboflavin (Hui, Meunier Goddik, Josephsen, Nip, & Stanfield, 2004). These photosensitizers consists of two excited states namely singlet and triplet in which triplet possesses long lifetime and initiates oxidation (Airado Rodriguez et al., 2011).

Basically the effect of light oxidation depends upon the three conditions including the time length of light exposure, light intensity and the wavelength of light (DeMan, 1976). Sunlight, burnt and oxidized flavor are some of the flavor types mostly experienced during the light oxidation.

During oxidation process, riboflavin acts photosensitive pigments, which transfer into its excited state because of the light exposure. This excited compound reacts with fatty acids. This reaction results in fatty acids peroxides production. Furthermore, these peroxides break down to aldehydes and ketones that result to the unpleasant flavor.

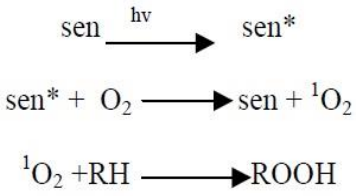

Figure 7: Riboflavin (sensitizer) activated by light (hv) reacts with oxygen (O_2).The excited riboflavin (Sen*) activates oxygen (O_2) forming singlet oxygen ($^1O_2$) and then reacts with fatty acids (RH), resulting fatty acid peroxides (Airado Rodriguez et al., 2011; DeMan, 1976)

1.6.4. Microbial growth in liquid milk products

The initial conditions by which microbial growth occur in collected raw milk includes transportation (uncleaned vehicles) and milk temperature during journey time. Besides, the storage temperature until the milk reaches to the processing plant is also a critical point. It's so because the changes in dairy industry practices have led the milk to be stored for long time before processing i.e. 5 working days per week, shortage of milk due to quota systems at certain times of year (Roginski et al., 2003). Microbiological spoilage in pasteurized milk occurs in two types. One includes the post process contaminates that occurred after heating and second one contains heat resistant bacteria which survived heating. Most gram-negative rod shaped psychrotropic bacteria dominates the post process contamination while enterobacteriaceae dominates the second type. The growth of psychrotropic bacteria is very high below about 8 ^oC while that of enterobacteriaceae grows well above 8 ^oC (Varnam & Sutherland, 2001). The key reason behind postpasteurization contamination is biofilms formed by *pseudomonas* spp. expel bacteria on gaskets in pasteurized milk pipelines. These bacteria grown in biofilms are even less affected by the sanitizer too (Roginski et al., 2003). Even if there is no contamination after postpasteurization, the native lipases and proteases activity affects the fluid milk shelf life (Santos et al., 2003). Among different psychrotropic bacteria, gram-negative Pseudomonas species possess short generation time of less than 4 hours for growth when kept at 4 ^oC for 8 days. But gram positive spore forming bacteria are lesser in number due to their longer generation time (about 8.5 hours) and long lag phase at temperature 2-7 ^oC (Samarija, Zamberlin, & Pogaiic, 2012b). Psychrotropic bacteria grows well including Bacillus at storage temperature below 5 ^oC. The presence of proteases and lipases in psychrotropic bacteria leads to proteolytic and lipolytic spoilage type that results various off-tastes, clot formation and also in some case, digestion of the protein (Varnam & Sutherland, 2001). These heat stable enzymes

are influenced by milk proteins that exert a stabilizing effect. However, the growth of psychrotropic bacteria or extracellular enzymes can be controlled by thermization (60-66 ºC), addition of CO_2, nitrogen additives, high pressure treatment etc. (Roginski et al., 2003).

2. Objective of this study

As shown, conservation of a good flavor profile along the dairy value chain is a great challenge. Many instances can lead to the development of off-flavors. The control of off-flavors traditionally is done by sensory evaluation with the above-mentioned drawbacks. The main goal hereby is to investigate whether it is possible to use common standard analytical process equipment to monitor the development of off-flavors in milk. In order to simulate and enhance spoilage processes, a series of experiments was designed.

Milk gets stress during processing and transportation. Therefore, to study this kind of spoilage, agitation of milk was done with Kitchen Aid stirrer. Similarly, the off-flavor development in milk because of the light exposure in groceries was studied by keeping the milk under different wavelengths of light. Further, the spoilage seen because of the storage problem was studied by keeping the milk in different temperatures for the provocation of bacterial growth.

Therefore, the main objectives were:

1) To investigate the use of a spectroscopic technique (mid infrared spectroscopy) to monitor the changes in fluid milk when quality is reduced during storage or the milk is damaged for other reasons.
2) To study the possibility of correlating IR spectroscopy results with the sensory quality changes of milk. The coupling of spectroscopic and sensory analysis would provide the advantage to know both chemical and sensory changes at once, which is not possible from one of the method only.

3. Materials and Methods

A series of 4 experiments was designed. In the first experiment, milk samples were collected from four different dairies in order to analyze the changes in milk properties on a weekly basis for three consecutive weeks. In the second experiment, milk samples collected from the dairies were altered with mechanical force using Kitchen Aid stirrer and allowed to store for nine days. Sensory as well as spectroscopic analysis was performed during the storage period. In the third experiment, milk samples collected were wrapped with different color filters i.e. blue, green and yellow and kept under the light in versatile environment test chamber for three different time intervals to observe the changes in chemical as well as sensory properties. At last,

temperature experiment was done with the milk samples collected from five different dairies. In this experiment, milk samples were kept in the incubator at 4, 7 and 10 ^{o}C for 7 days and at 17 ^{o}C for one day to provoke the growth of microorganisms. Bactocount as well as sensory and spectroscopic analysis was performed for the samples. The series of experiments are explained below.

Milk samples were used both for the sensory and spectroscopic analysis in all experiments.

3.1. Experiment 1: Storage of normal milk samples (unaltered)

Ordinary market milk samples from different dairy plants, varying production dates, were collected and stored in refrigerator for 3 weeks. Each sample was analyzed every week, 3 consecutive times.

Table 1: Milk samples collected from different locations for analysis without alteration is listed

Production plant	Packing type	Fat (%) present	Qty. Of samples collected/week	Total number of samples * number of assessments (3)
Q	Purepak	1.0	1	30
R	Purepak	1.2	1	30
S	Purepak	1.2	5	150
T	Tetrapak	1.2	5	150

The milk samples S and T were transported to the laboratory packed with ice bags in boxes to maintain the temperature between 2-4 ^{o}C, to avoid spoilage. While samples Q and R were collected from local grocery stores and then stored at 2-4 ^{o}C.

Table 2 : Design for the collection of milk samples, showing how many samples were analyzed per week

Months	Sept		Oct				Nov				Dec	
No. Of Weeks	1	2	3	4	5	6	7	8	9	10	11	12
Samples/week	12	12	12									
		12	12	12								
			12	12	12							
				12	12	12						
					12	12	12					
						12	12	12				
							12	12	12			
								12	12	12		
									12	12	12	
										12	12	12
Total/week	12	24	36	36	36	36	36	36	36	36	24	12

Three cartons of each sample was collected. One was used for both chemical and sensory analysis for that same week. Two were kept for analyzing in next two consecutive weeks. Around 200 ml volume of each milk sample was required for the chemical analysis performed under Lacto scope Fourier Transform Infra-Red (FTIR) for 15 replicates while approximately 250 ml volume for each sample was required for the sensory analysis.

3.2. Experiment 2: Mechanical alteration of pasteurized milk

Pasteurized milk samples used in the study was obtained from the grocery stores with three different dairies Q, R and S having two different expiry dates for each of them. Thus, six different individual samples were prepared for the same alterations. Commonly available kitchen stirrer was used for the agitation of milk samples for three holding times with and without addition of raw milk.

Table 3: The overall structure of the milk sampling design for mechanical alteration kept at 15 ^{o} C (except the reference samples kept at 4 ^{o}C)

Sr.No	Milk Sample	Expiry date	Raw milk addition	Agitation Time(Min)	Total samples
1	R	20-01-2015	0%	0	3
				15	
				45	
			1%	0	3
				15	
				45	
2	R	21-01-2015	0%	0	3
				15	
				45	
			1%	0	3
				15	
				45	
3	Q	20-01-2015	0%	0	3
				15	
				45	
			1%	0	3
				15	
				45	
4	Q	21-01-2015	0%	0	3
				15	
				45	
			1%	0	3
				15	
				45	
5	S	20-01-2015	0%	0	3
				15	
				45	
			1%	0	3
				15	

				45	
6	S	21-01-2015	0%	0	3
				15	
				45	
			1%	0	3
				15	
				45	
				Reference samples	6
				Total	42

The agitation of milk samples was done by the use of solid-state speed control Kitchen Aid (Model k5ss). The speed in this stirrer is adjusted by a device with no moving parts, which gives the mixer precise speed selection, smooth speed change over, and high reliability. It has a 10 step multispeed motor. It can hold up to maximum power of 300 watts and voltage 230.

Figure 8: Solid state speed control Kitchen Aid

Three different time intervals 0, 15 and 45 minutes was used for agitation after all the milk samples were tempered at 15^{o}C. Pasteurized milk was spiked with 0% and 1% raw milk to investigate the effect of enzymatic triggered.

Thousand millilitres of altered pasteurized milk (also including raw milk on it) were kept in 1000 ml clear, sterilized glass bottles (Schott Duran).

So agitation of 6 samples for three different time intervals made 18 samples themselves and addition of raw milk (0 and 1 %) to those samples collectively made 36 altered samples. Six more samples were kept as reference in the refrigerator at 4 ^{o}C making altogether 42 samples for analysis. The spectroscopic analysis of these samples was performed on first, second, fifth,

seventh and ninth day of milk alteration date while the sensory analysis was performed once on the fifth day.

3.3. Experiment 3: Pasteurized milk exposed to different wavelengths of light

Samples were exposed to five different wavelength combinations of light for 3 holding times. The test chamber with model MLR-351 from SANYO Company was used for the source of light in milk alteration by different wavelengths of light. It has an ideal quality in accurate temperature control and widely used in food industry for packaging, quality control and stability testing. The maximum limit of temperature in the cabinet is 56.2 ^{o}C while the lower limit is -10.6 ^{o}C. The versatile environment chamber with 15 fluorescent tube lights (i.e. FL40SS W37) was used for the light alteration. It was set up with a controlled temperature environment of 4 ^{o}C and 0% humidity having white light with color temperature of 4200 K.

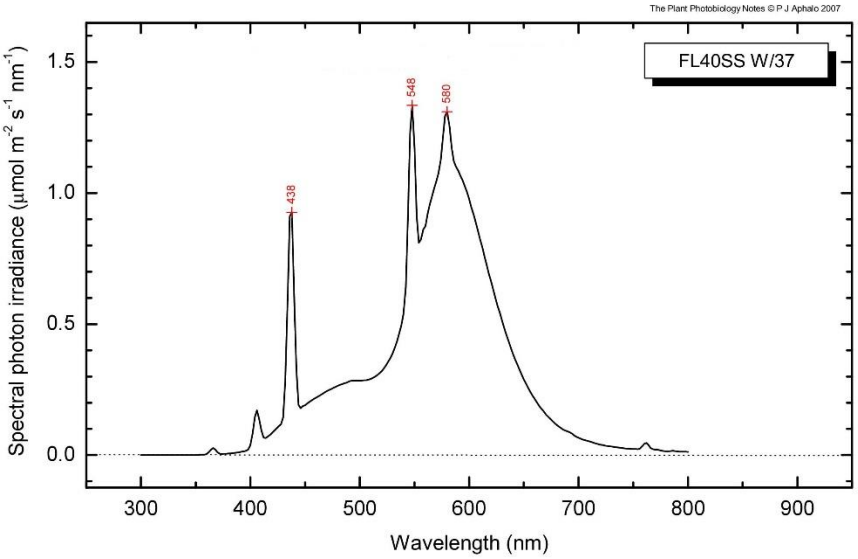

Figure 9: Light emitted from tube light (i.e. FL40SS W37) within the wavelength region 400-800 nm

Similarly, three different color filters from Bright Norway As were used for receiving different wavelengths of light during the experiment. Blue color filter with company code 118 Light blue, green filter with code 122 Fern Green and orange filter with code 105 Led were used for exposing the milk samples within the wavelength range of 300-800 nm.

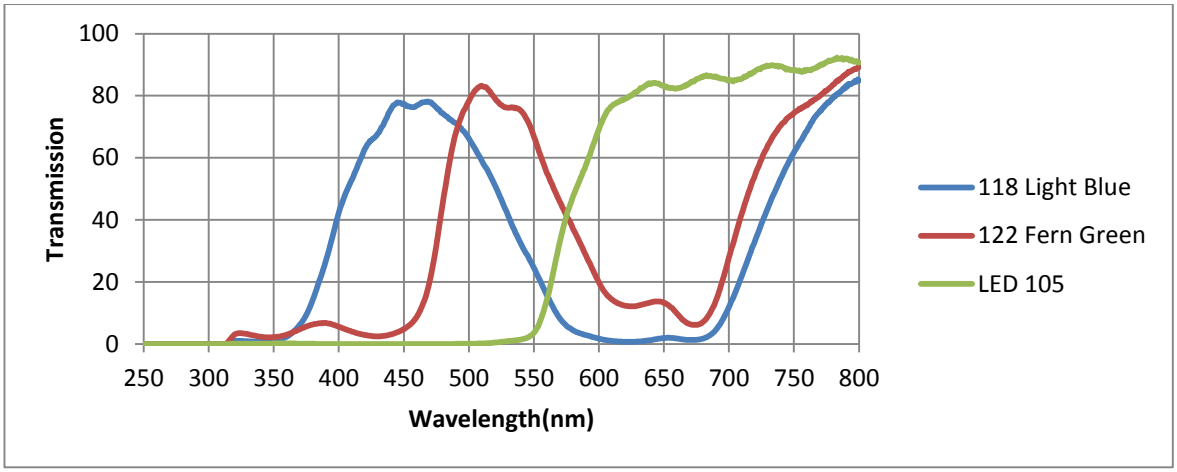

Figure 10: Different color filters showing the spectral range from 300 to 800 nm wavelength

Milk samples needed for the experiment was collected from the S dairy and the total quantity needed was 10 cartons of each specific production.

Table 4: The table shows the overall structure of the experimental design keeping them at 4 ^{o}C wrapping with different color filters

				Time period to store samples				
Sr. no	Sample name	Expiry Date	Color filters used	15 (min)	30 (min)	60 (min)	Total samples	Altogether
1	S	Date 1	Blue	1	1	1	3	
			Green	1	1	1	3	
			Orange	1	1	1	3	15
			Unwrapped	1	1	1	3	
			Aluminium foil	1	1	1	3	
2	S	Date 2	Blue	1	1	1	3	
			Green	1	1	1	3	
			Orange	1	1	1	3	15
			Unwrapped	1	1	1	3	
			Aluminium foil	1	1	1	3	
							Total	30

Two different milk productions, with successive best before dates from dairy S were collected. These samples were stored in refrigerator at 4 ^{o}C prior to the alteration. First, the milk samples were poured in 500 ml glass bottles (Schott Duran) sterilized in an autoclave at 135 ^{o}C for 3 minutes at 15 psi. Four individual samples were exposed to light of different wavelengths by wrapping them with blue color filter (300-550 nm), green color filter (450-600 nm), led orange filter (500-800 nm) and also left one set unwrapped for exposing to the full light spectrum.

As a reference, these two milk samples in their respective bottles were wrapped with aluminum foil for light exposure in the same light chamber.

These milk samples were exposed in light for three different time intervals i.e. 15, 30 and 60 minutes and stored them back to the refrigerator after the time duration.

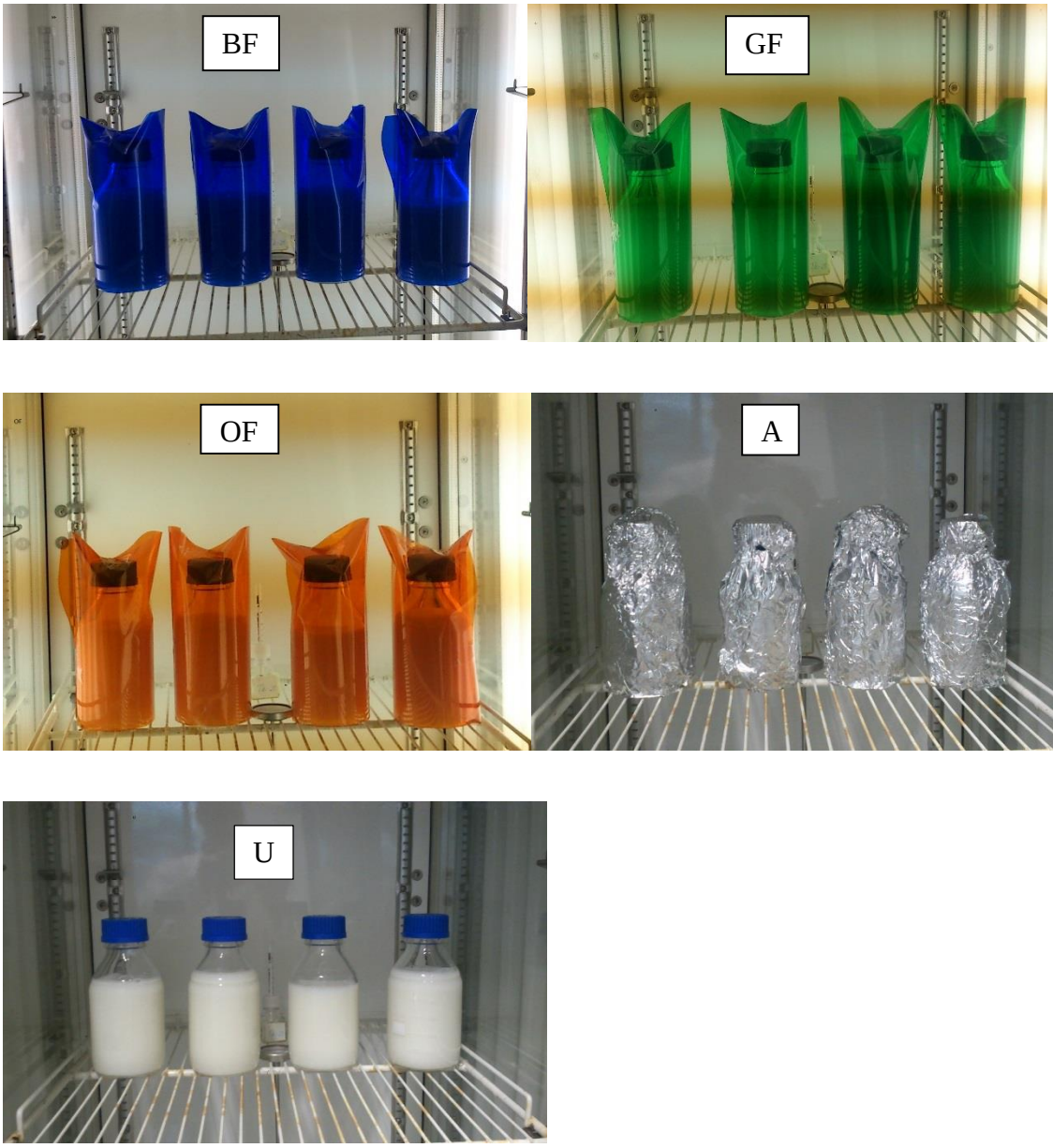

Figure 11: Milk samples used in the study. Milk was kept in sterilized glass bottles (Schott Duran) wrapped with three color filters. Sample code in each picture indicates as BF= Blue color filter, GF= Green color filter, OF =Orange color filter, A = Aluminium foil wrapped as reference and U= unwrapped milk samples for exposure to whole light spectrum

The total 30 samples were subsequently judged by the sensory panel and analyzed in Lacto scope after 24 hours. For giving training to the assessors, a calibration tasting session was held with the reference milk sample (bought from grocery), the bad and worst milk samples.

3.4. Experiment 4: Pasteurized milk alteration by different temperature

Milk from five different dairies were chosen for the alteration purpose. These are referred to with letters P, Q, R, S and T. Like the previous alteration experiment, two different dates of each dairy milk were chosen. Thus, the total samples became 10 from five different dairies.

Total cartons needed for the experiment = 5*2*4 = 40 cartons.

Table 5: The table shows the experimental setup of milk samples incubated at four different temperatures for 7 days

				Temperature chosen to store milk samples				
Sr.no	Sample name	Expiry date	Time period of incubation(Days)	4◦C	7◦C	10◦C	17◦C	Total samples
1	R	Date1	7	1	1	1	1	4
2	R	Date2	7	1	1	1	1	4
3	Q	Date1	7	1	1	1	1	4
4	Q	Date2	7	1	1	1	1	4
5	S	Date1	7	1	1	1	1	4
6	S	Date2	7	1	1	1	1	4
7	T	Date1	7	1	1	1	1	4
8	T	Date2	7	1	1	1	1	4
9	P	Date1	7	1	1	1	1	4
10	P	Date2	7	1	1	1	1	4
							Total samples	40
	Note: Milk samples for 17 ˚C are stored at 4 ˚C for 6 days and then kept at 17 ˚C for 1 day before analysis.							

These 10 different samples were kept in incubator at four different temperatures. They were incubated until 7 days for temperature at 4 ^{o}C, 7 ^{o}C and 10 ^{o}C. However, for 17 ^{o}C, samples were stored at 4 ^{o}C for 6 days and lastly at 17 ^{o}C for 1 day (24 hours).

In total 40 samples were subsequently judged by the sensory panel and analyzed for chemical change using lacto scope spectroscopy. All samples were analyzed after 7th day of incubation. In addition, bacto–count analysis was performed on eighth day.

3.5. Spectroscopic and Chemical Analysis

The Delta Lacto Scope FTIR Advanced, type FTA-3.X from Delta instrument was used for the spectroscopic analysis of milk samples. Its working mechanism is based upon hydraulic flow system with analyzing capacity of 120 samples per hour. It has an automatic function of zeroing, cleaning and sample heating. Calibrations are easily transferable due to the absolute wavelength reproducibility, thus cell match solution needs to be used for standardization.

Figure 12: Diagram of the FTIR instrument named Delta Lacto scope FTIR Advanced (Source:http://www.aicompanies.com/index.cfm/products/?productId=24 accessed on January, 2015)

CMS measurement is used before the start of analyzing milk samples as it helps to normalize the spectra. Mid-infrared spectra were collected over the range of 4000 to 400 cm^{-1}, with a resolution of 8 cm^{-1}. Before the start of mid infrared analysis, milk samples were taken in clear plastic containers with tight fitting lids and kept in water bath at 37 ^{o}C for about 10 minutes. Longer heating of milk samples might separate the butterfat leading to analysis problem, so they can't be heated longer than 30 minutes. Fifteen replicates were captured for each unaltered milk samples and these replicate spectras were averaged prior to data analysis.

3.5.1. Chemicals needed for cleaning Lacto scope FTIR

Decon 90 (4%) and Triton X-100 (0.1%) are the two cleansing agents used for cleaning the instruments in between analysis.

3.5.2. Cell Match solution (CMS Solution)

This solution is used before analyzing the milk samples in order to standardize the spectral curve. Because of the absolute wavelength reproducibility, calibrations can be easily transferable. It means the spectra of milk samples aren't fixed at the same position all the time. Thus, cell match solution needs to be used before every analysis for the standardization and needs to change silica often.

3.5.3. Water source

Water used for preparing cleansing solutions i.e. zero solution and cleaning solution was of Milli Q grade.

3.5.4. Calibrations of the instrument

CMS adjustment was performed at the very first to prevent shifting of the spectra. The measurement was accepted if it was within the limits and this method was continued every week before analysis of milk. Lacto scope FTIR Advanced can also judge on standard deviation of repeatability of Zero values. Practical setting for standard deviation is 0.01 for Fat, Protein and Lactose and solids and SNF to 0.15.

The instrument was set in resolution of 8 cm^{-1} with scans number 16, pump strokes 23, cell strokes 5 and CMS correction factor 0.998.

Chemical values of the chemical parameters like fat, protein, lactose, FFA etc. were obtained by reanalyzing the spectroscopic data obtained during the sample analysis, which had been calibrated from its manufacturer.

3.6. Bactocount analysis

The quantitative study of bacteria present in the milk was done by bactocount analysis. BactoCount IBC-M instrument was used for this purpose, which is an alternative method of total plate count. It works on the principle of flowcytometry technology used for instant count of milk bacteria. The quick count of individual bacteria was done by staining a suspension of cells and forced to pass through a capillary tube. It got illuminated in front of microscope objective and each passing cells started to be registered by photo electronics attached to the microscope. Samples could be prepared within 10 minutes and analyzing time in less than a minute (http://www.foss.dk/industry-solution/products/bactoscan-fc/ accessed on June 2015). Milk samples from five dairies P, Q, R, S and T with two different dates each were collected for the analysis.

3.7. Sensory analysis

3.7.1. Sample preparation

All the samples were kept in water bath at 16 ± 2^{o}C for about one hour before carrying out the sensory analysis based on the regulations of the ISO 22935-2:2009(E)/IDF 99-2:2009(E).

Plastic cups were used for serving the samples and they were labelled with three digit codes from a random number table. Clean dark plastics were used to cover them during tempering to avoid oxidation.

Samples were served in random order to each of the assessors, using a randomized serving scheme generated in the Eye Question software – also used for data collection from the assessors (Logic8, Wageningen, Netherlands).

3.7.2. Sensory evaluation

Four sensory experts were selected from TINE's sensory panel. Panellists were trained according to ISO 22935-2/IDF 99-2(International Organization for Standardization & International Dairy Federation, 2009a).

The round table discussion among the sensory experts was applied before every sensory session, in order to calibrate the scoring and use of nomenclature of defects.

The tests were conducted in partitioned booths with fluorescent lighting and eye question program was used for giving the numbers to the samples. Overall quality score, on a scale from 1 to 5 given to the dairy samples represents the deviation magnitude from the pre-established sensory specification ISO 22935-2:2009(E)/IDF 99-2:2009(E). Here scale 5 shows no deviation, scale 4 shows minimal deviation and at last, 1 shows very considerable deviation from the pre-established sensory specification (International Organization for Standardization & International Dairy Federation, 2009c).

Different sensory attributes were used for identifying and quantifying the defects while carrying out the sensory analysis of milk samples. The defects used are shown in the table below. Each defect attribute was rated on a scale from 1 (not present) – 9 (strongly present).

Table 6: Nomenclature or terms for defects in milk

S.no	Sensory attributes	S.no	Sensory attributes
1	Flocculants	16	Malty
2	Cream layer	17	Fruity
3	Cream adhesion	18	Cardboard
4	Coagulated	19	Feedy
5	Bicolored	20	Chemical
6	Foreign matter	21	Oxidized
7	Untypical color	22	Rancid
8	Sour	23	Salt
9	Sour off flavor	24	Uncharacteristic
10	Harsh	25	Watery
11	Bitter	26	Acid
12	Sickening	27	Goat flavor
13	Cooked	28	Cow shed
14	Burnt		
15	Off flavor		

Plastic cups filled with water to rinse their mouths between in-take of samples, empty cups to spit while rinsing their mouths and napkins were arranged for the panel members. In order to remove beany flavor while tasting, crackers were provided to them during the analysis period.

3.8. Mathematical Modeling

3.8.1. Multivariate data modelling

For the analysis of multivariate data, Principal component analysis (PCA) is commonly used which helps to get overview information in the data set. It uses the entire spectrum for the quantitative analysis without losing information and extract any information related to structural changes in milks (Esbensen, Guyot, Westad, & Houmoller, 2002). Information

included in variables are explained by the latent variables, also called components, scores or factor. PCA or data compression method helps to plot concentrated information from many variables in one, two or three dimensions. Most of the information is lying on the first dimension, while most share of the residual information is included in the second PC and so on. Loading plot helps to visualize the importance of variables and correlation with each other. It also helps to relate with samples too. The use of score plot helps to find the similarity and difference between the samples. Similarly, it is easy to see variables describing the related samples with the help of the corresponding loading plots. The below figure shows extracting scores principles in two PCs.

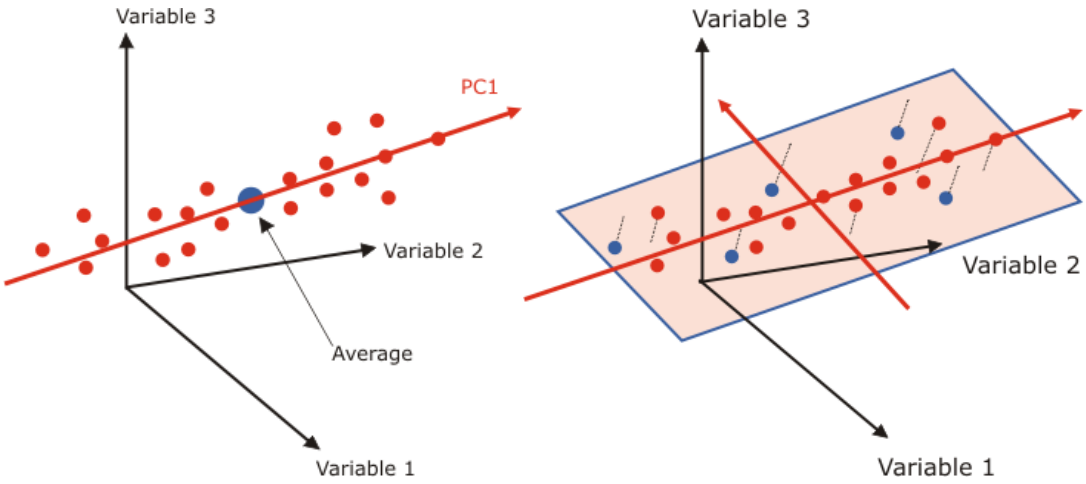

Figure 13: Scores from PCA. Left: The first principle component. Right: PCs PC1 and PC2. Illustration from Unscrambler 10.3 (CAMO Software AS, Oslo Norway)

Partial least square regression is widely used to establish a calibration model and to provide a correlation between reference data with spectral data (Al-Qadiri et al., 2008). The accuracy of the model can be evaluated using the coefficients of determination (R^2) between the predicted and measured values (Fagan et al., 2007). For the regression activities, factors from data compression are used as regressors when trying to model one or many regressands. The most often used methods are Partial Least Squares regression (PLS) and Principle Components Regression (PCR). PLS maximizes the covariance between X and Y. This method works in contrast to PCR, which first performs Principal Component Analysis (PCA) on X and then regresses the scores (T) against the Y data. A conceptual illustration for PLS is shown graphically below (Martens & Martens, 2001; Varnam & Sutherland, 2001).

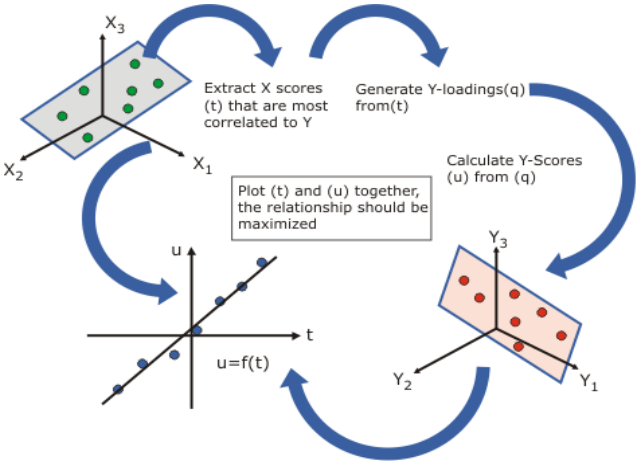

Figure 14: PLS procedure. Illustration from Unscrambler 10.3 (CAMO Software AS, Oslo, Norway)

The below figure shows the PCA score plot of different milk products analyzed as demonstration. From the geometrical viewpoint, two samples are considered as similar if the values of most of their variables are close to each other. On the other hand, they are considered as different if their values differ greatly for at least some of the variables. Chocolate milk, matfløte, goat milk, økologisk and NAN1 are spread in the plot, which signifies that they are different from each other. However, chocolate milk and ice-kaffe in the upper region and goat milk and økologisk milk in the below region seem to be similar as they fall close to each other while matfløte and NAN1 are typically different from others.

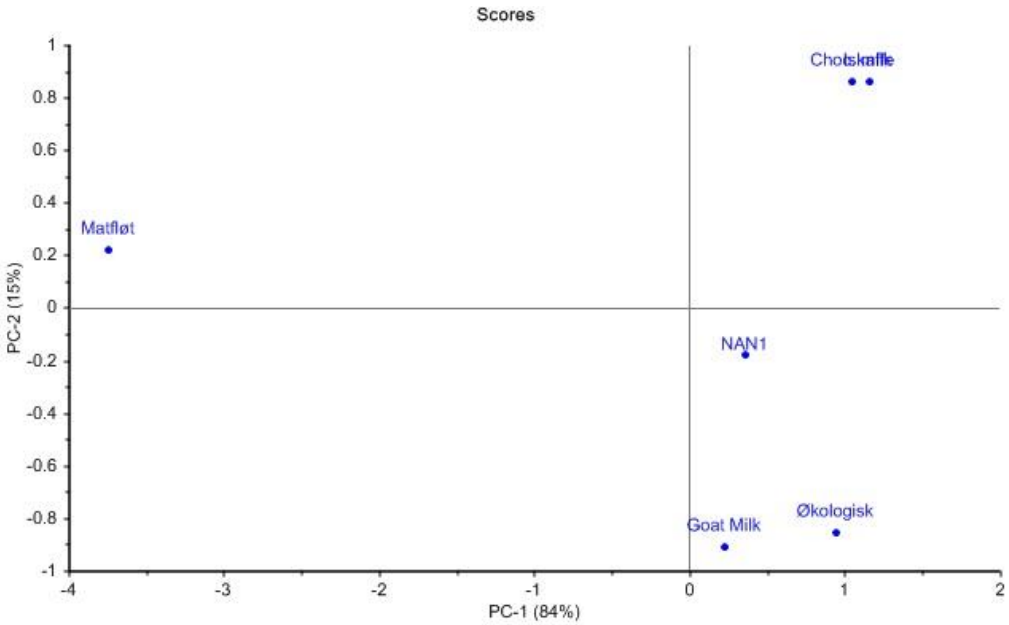

Figure 15: PCA score plot of the different milk products based on the spectroscopic data within the wavenumber range 4000-400 cm^{-1} as demonstration

Partial least squares as described earlier is used for establishing a correlation model between reference data and experimental data. The relation between chemical and spectroscopic data is shown in the below figure. This model helps to detect the different dairy constituents in the wavenumber range of 4000-400 cm^{-1}. For example, Fat A is detected at wavenumber 1724-1770 cm^{-1}, protein at 1458 cm^{-1} and lactose at around 2912 cm^{-1} . This model helps in the visualization of the structure in the data as the importance of individual variables is visualized more clearly in this plot.

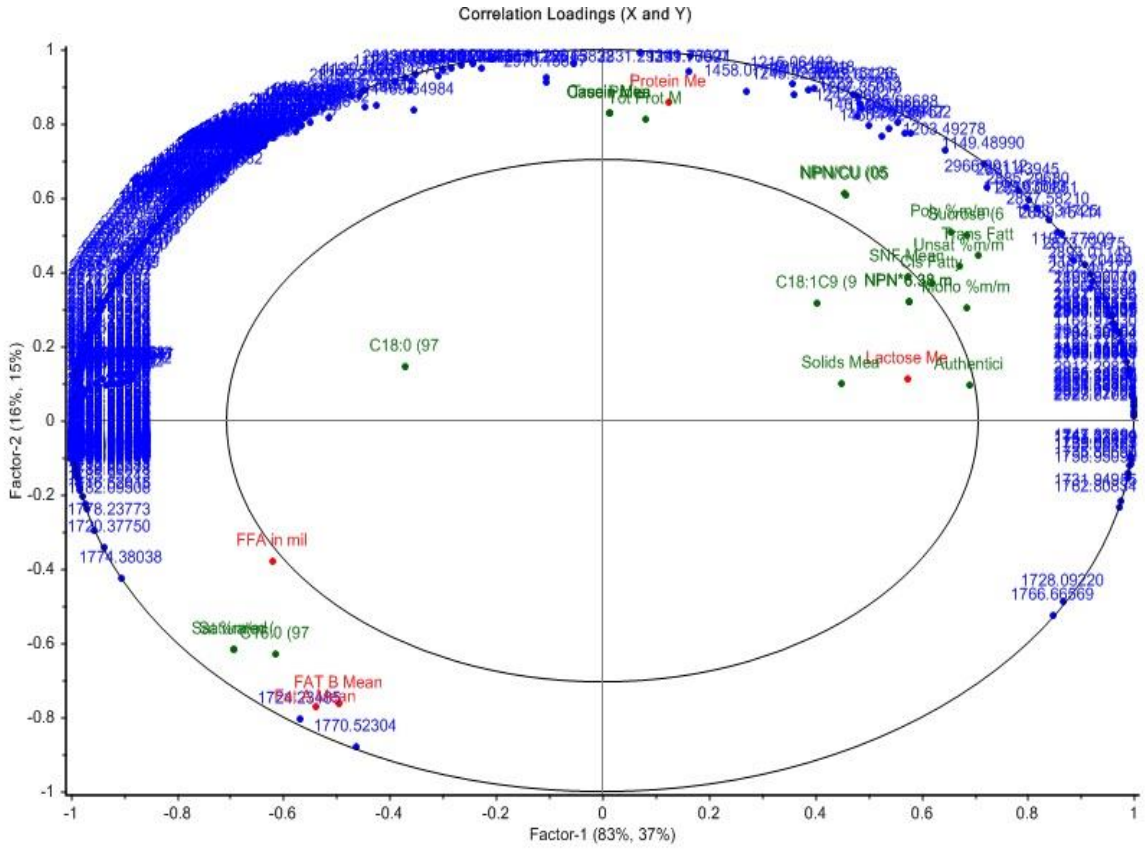

Figure 16: Partial Least Square Regression (PLSR) correlation loadings plot of chemical data of different milk products with spectroscopic data analyzed for demonstration purpose. Here, X-variable contains spectroscopic data (blue color) while Y-variable contains chemical data where red color represents participating data in the model and green color represents non-participating data.

The common feature of regression methods includes the predicted vs. reference plot that shows a straight-line relationship between predicted and measured values. The ideal condition for the relationship to show that the data is well modelled is slope equals to one and a correlation close to one. Likewise, the calibration variance gives the idea about fitting the calibration data to the

model. In the below plot, it shows the calibration model of different dairy products. However, the model wasn't good (R^2=0.002) as the sample quantity was very less (only six) and their chemical properties were different from each other. Therefore, it was very difficult to find a good model.

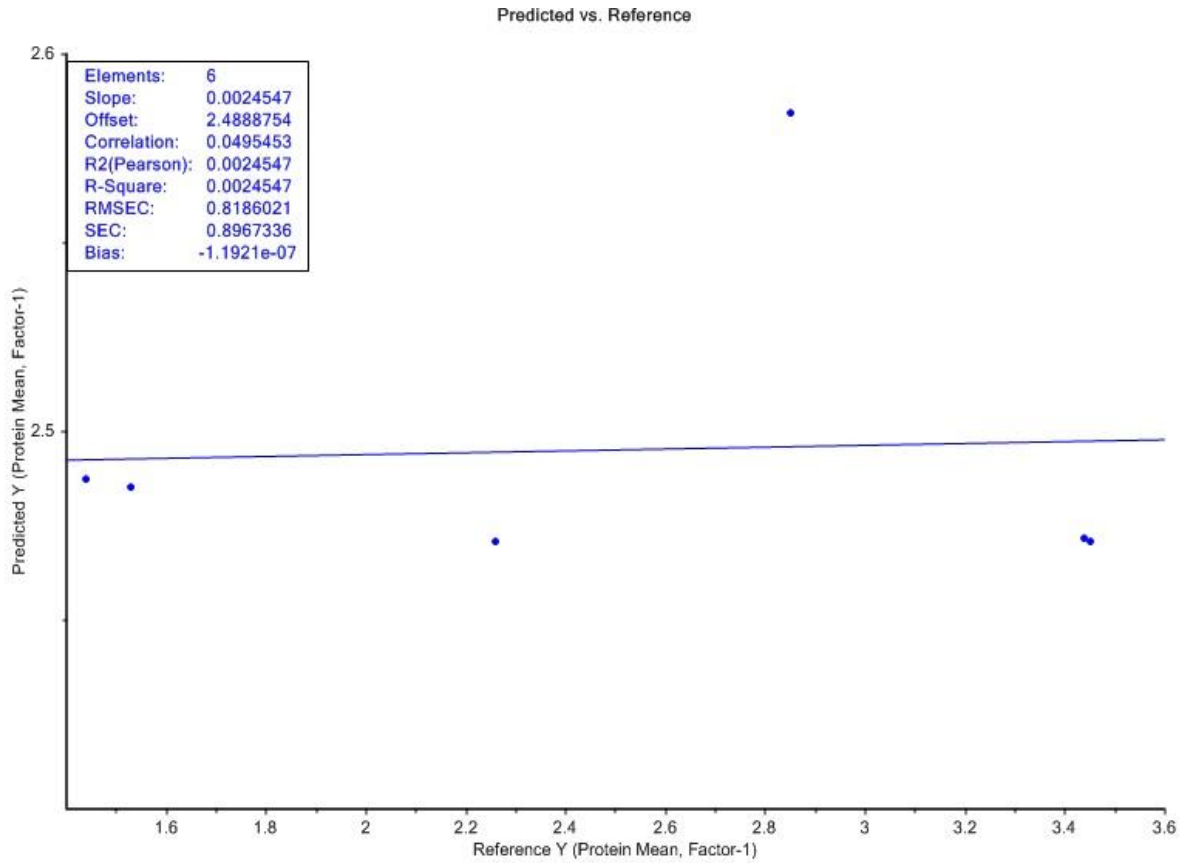

Figure 17: Predicted vs. reference plot for PLS calibration samples of different dairy products based on chemical data on Y-variable and spectroscopic data on X-variable

Therefore, Partial least squares (PLS) regression was used to find correlations between spectral data and sensory assessed attributes. The PLS regression was carried out by using the Unscrambler version 10.3 (64 bit) software (Camo Software AS, Oslo, Norway).

Significance testing of the sensory analysis was carried out within each treatment using ANOVA test i.e. general linear model (GLM) Analysis of Variance (Anova) using Minitab (version 16.0; PNAgent) to establish significant differences, followed by the Tukey honestly significantly different test.

4. Results and discussion

The project was conducted in two phases. In one phase, milk samples were collected for 10 weeks starting from September to December. The weekly collected milk samples were stored for three weeks to analyze milk spectra during storage along with sensory evaluation data. While in second phase, three different alteration experiments were conducted before analyzing milk spectra and sensory quality.

4.1. Experiment 1: Storage of normal milk samples (unaltered)

Milk samples from four different dairies Q, R, S and T were collected in a weekly basis for three consecutive weeks. These collected samples were then performed sensory analysis and spectroscopic analysis on the same day. The rest samples were then analyzed in the following weeks.

The below figure represents the average milk spectra of stored normal milk samples showing absorbance (Abs.) in Y- axis and Wavenumber (cm^{-1}) in X-axis. The spectra was collected from 960 cm^{-1} to 1612 cm^{-1} and 1701 cm^{-1} to 3028 cm^{-1}. The regions between 400-960 cm^{-1}, 1612-1701 cm^{-1} and 3028 - 4000 cm^{-1} were eliminated because of the interference by water molecule in the spectra.

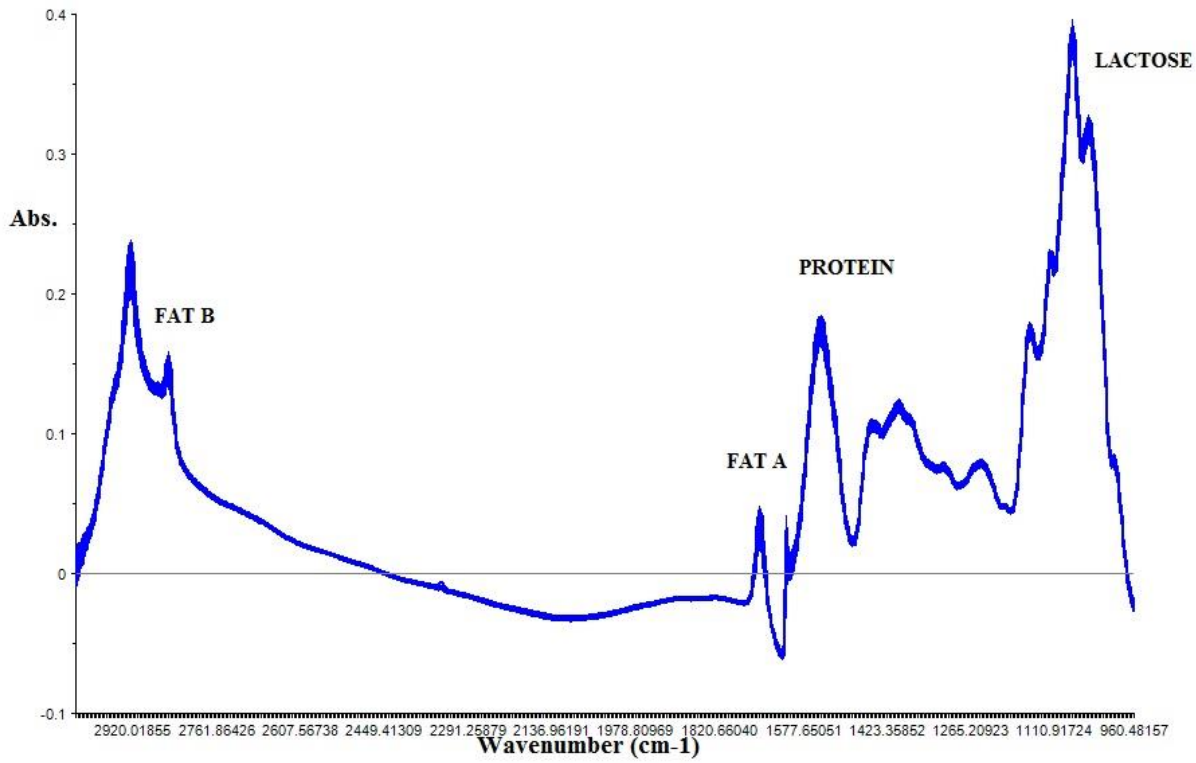

Figure 18: Average milk spectra from the stored normal milk samples held for three weeks

Table 7: P-values based on sensory scores for sensory attributes and FFA (calibration) with significant effect in one or more experimental factors for unaltered milk samples

Factor	Quality score	Flocculants	Cream layer	Sour off flavor	Bitter	Sickening	Rancid	FFA
Dairy	<0.001	0.439	0.022	0.016	0.066	0.018	0.135	0.000
Analyzing week	0.058	0.015	0.358	0.01	0.572	0.376	0.05	0.677
Age of Milk	<0.001	0.151	0.009	0.65	0.025	0.137	0.003	0.001
Dairy*Analyzing week	0.304	0.524	0.003	0.002	0.099	0.282	0.004	0.139
Adjusted R^2:	0.124	0.006	0.062	0.074	0.049	0.024	0.06	0.7542

Dairy and age of milk had a significant effect on the sensory quality score of the milk and on FFA formation measured by FTIR spectroscopy. Rancid taste showed significant effect with age of milk as well as analyzing week, and there was an interaction effect between dairy and analyzing week (table 7).

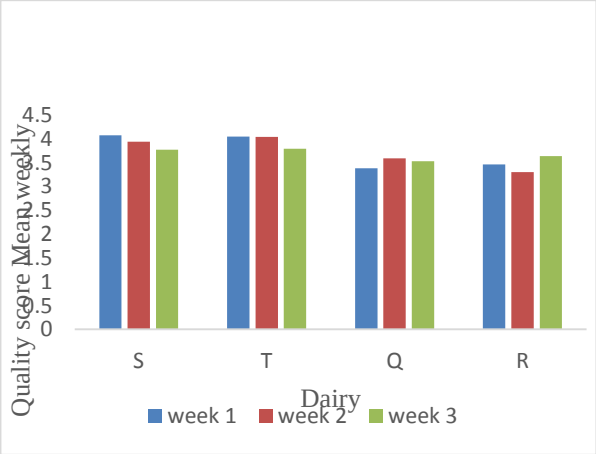

Figure 19: Quality score mean of milk weekly w.r.t different dairies when stored for three weeks

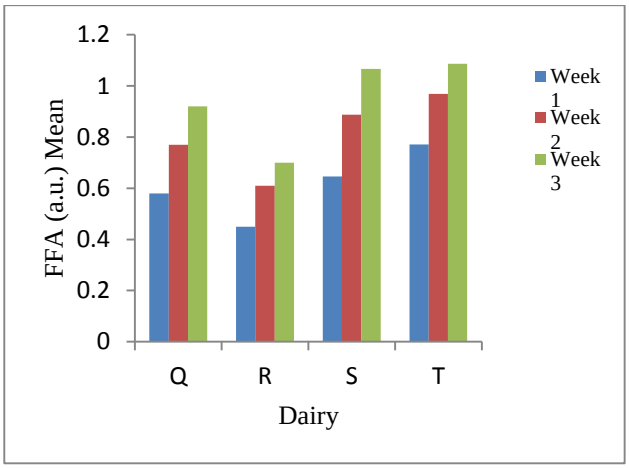

Figure 20: FFA formation in milk from four different dairies w.r.t weekly storage

Likewise, the milk quality and FFA development did not have significant effect with analyzing week (table 7) as the quality remained quite high even after three weeks of storage (figure 19). However, the quality of milk including the rancid development showed significant effect with the age of milk during storage (table 7). Among four different dairies, S and T were found to have slightly better quality in first and second week than the other two dairies Q and R according to the sensory data obtained (figure 19). The reason might be S and T milk samples

were collected directly from dairy, which had better cooling system and kept in cold condition all the time. While, Q and R milk samples were collected from the local groceries which mightn't get better cooling system than dairy while carrying or transport. Thus, the storage condition might have played the key role in the quality of milk samples. At the same time, the splitting of FFA (a.u.) from fat was observed during the storage period of three weeks (figure 20). Nevertheless, the increase in FFA was on average around 0.1 per week. Thus, there was no significant effect with analyzing weeks (table 7). For comparative study, the splitting of fat into free fatty acids with respect to time interval (weekly analysis) was higher in milk from dairy S and T in compared to the dairies Q and R (figure 20).

One of the causes for the increment in FFA formation from week one to three for all dairy milk samples might be due to the presence of endogenous milk enzymes. Phospholipases present in milk are capable to rupture the phospholipids of the MFGM, enhancing the milk fat to lipolytic attack (Ray et al., 2013). Milk samples used for the experiment contained 1.2% fat for R, S and T dairy while 1% fat for dairy Q. However, milk from S & T dairies showed a tendency of increasing FFA (figure 20) from the very first week compared to the other dairies indicating fast lipase activation.

The regression plot between free fatty acid (FFA) versus quality score, bitter and rancid were plotted to show the relationship between these sensory attributes.

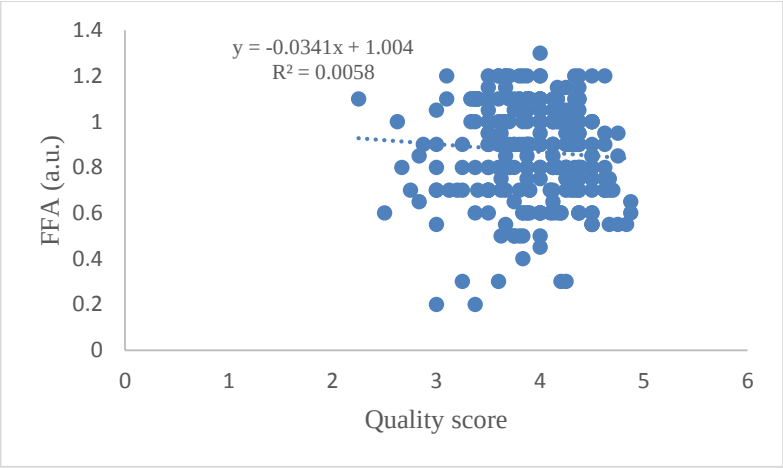

Figure 21: Regression plot showing relation between quality score and FFA

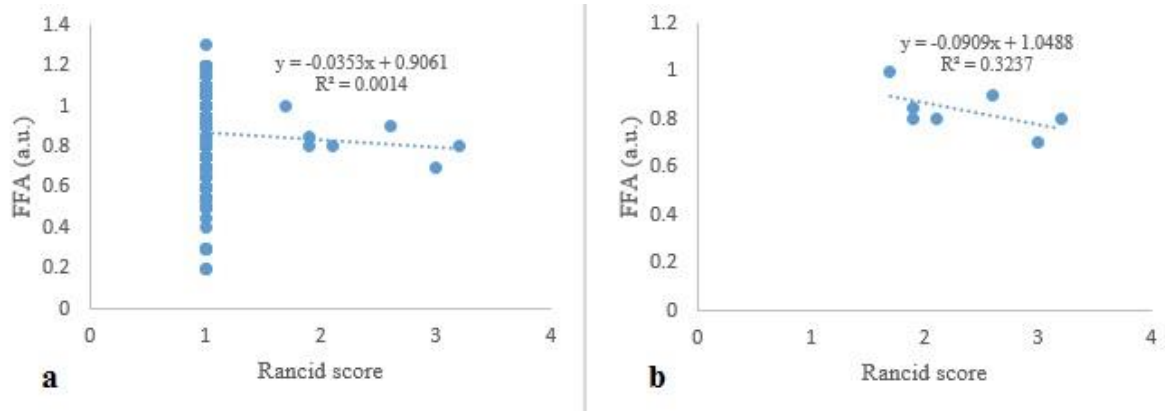

Figure 22 (a & b): Regression plot showing relation between all rancid scores and FFA; rancid score >1 and FFA (rancid scale goes from 1-9; 1= no rancid taste)

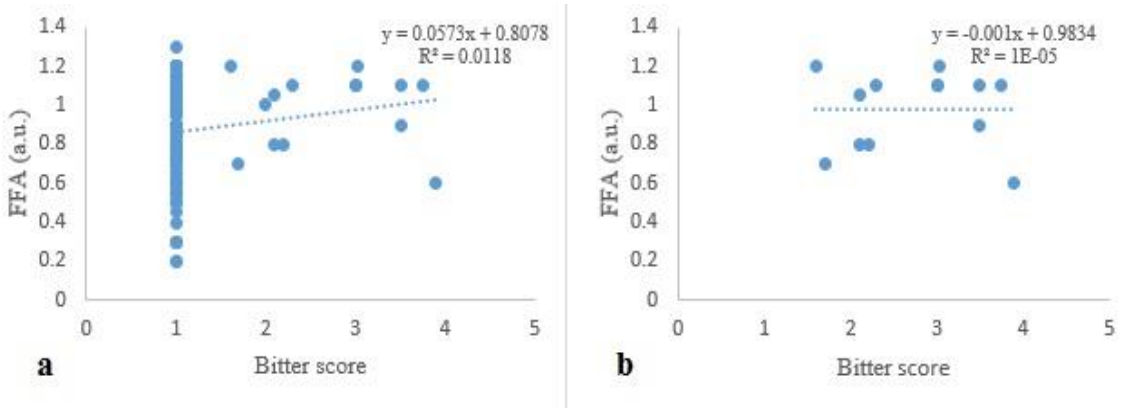

Figure 23 (a & b): Regression plot showing relation between bitter score and FFA; bitter score >1 and FFA (bitter scale goes from 1-9; 1= no bitter taste)

Similarly, the regression plot between analyzing week versus FFA and quality score, age of milk versus FFA and quality score were plotted to show the relation between these factors.

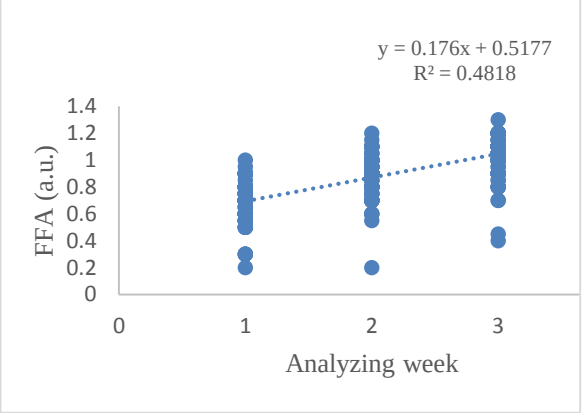

Figure 24: Regression plot showing relation between analyzing week and FFA

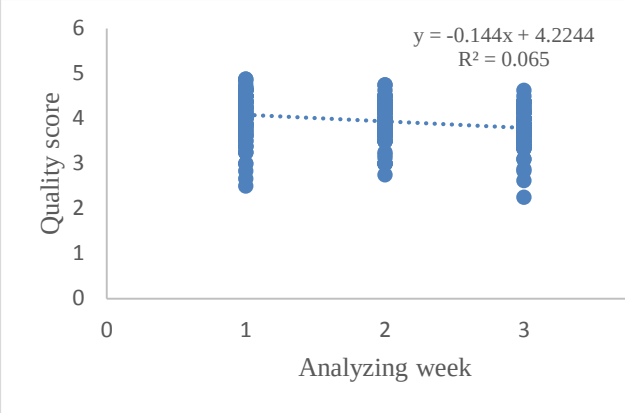

Figure 25: Regression plot showing relation between analyzing week and quality score

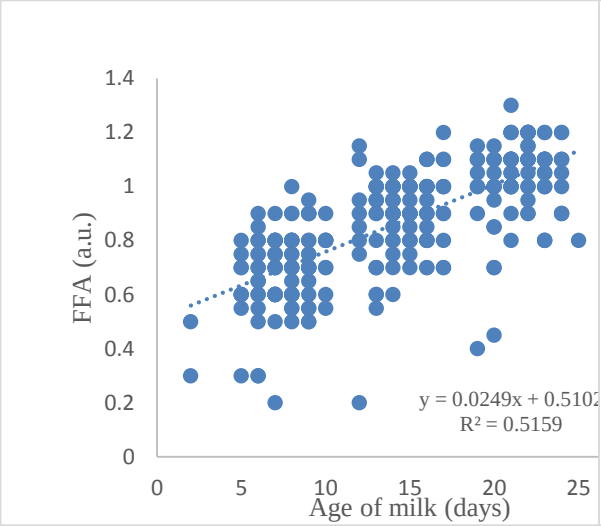

Figure 26: Regression plot showing relation between age of milk (days) and FFA

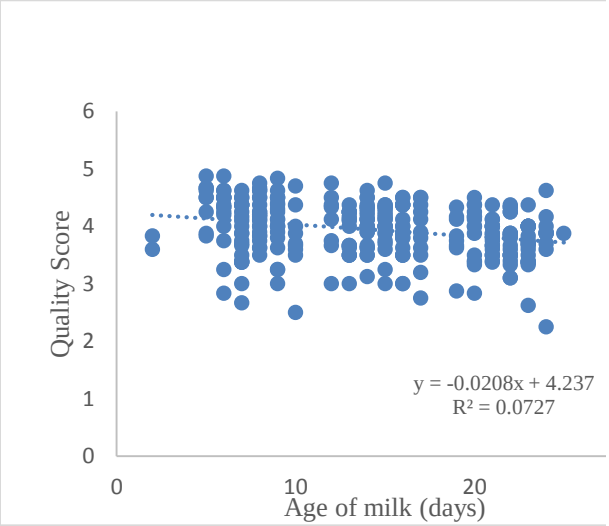

Figure 27: Regression plot showing relation between age of milk (days) and sensory quality score

The figures 21-27 drawn above signify that these sensory attributes weren't well correlated to the possible explanation variables, like FFA and age of milk. The threshold values for FFA in milk for lipolyzed flavor detection was within the range of 4.1 to 4.5 acid degree (Pillay et al., 1980). However, FFA measurement in the instrument wasn't calibrated and thus comparison between experiments could only be done. The average FFA value observed was very less compared to experiment 2 (mechanical alteration). Therefore, there was no correlation with FFA. The sensory data obtained from the sensory analysis apparently was more complex and affected by several factors. This might include the quality of raw milk during collection,

processing condition, storage condition after pasteurization in and outside dairy. We could expect that the quality of milk should have changed more by the storage for long time. However, the sensory result shows that quality of milk was kept good for the analyzing period and there wasn't so much change in its quality with storage as expected. There was also not much increase in sensory defects. That's why; sensory panel couldn't find any significant difference in most of the sensory attributes in the milk samples stored between week one to three.

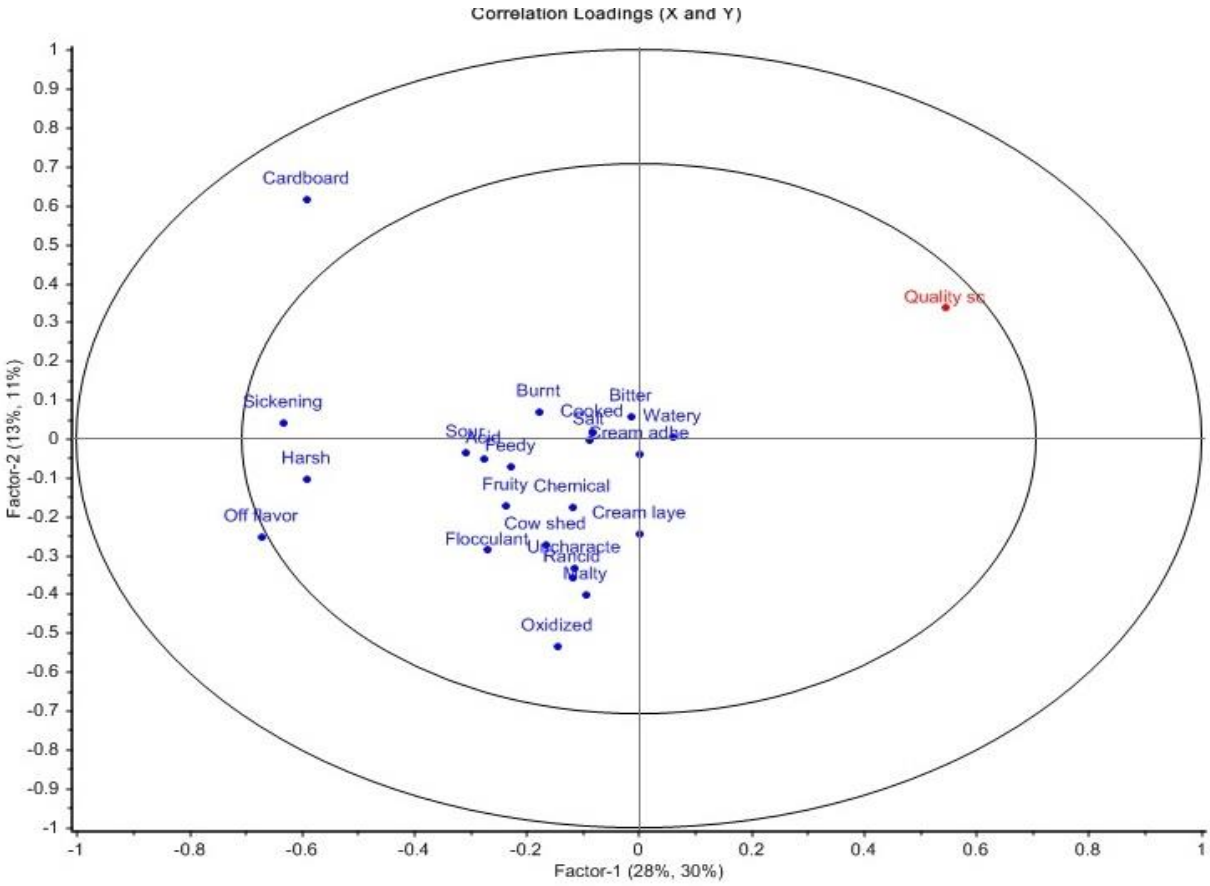

Figure 28: Partial Least Square Regression (PLSR) correlation loadings plot of unaltered milk sensory profile data with Quality score. X-variables: Sensory profile data while Y-variable: Quality score

Quality score in the correlation loadings plot showed the strong negatively correlation with the defects. The most important defects were off-flavor, harsh, sickening and cardboard. While, burnt, cooked, bitter, cream and watery attributes had no correlation with the quality score of milk samples (figure 28). When there was high value for quality score or say, quality of

samples was good, then the value for defects like off-flavor, harsh, sickening and cardboard flavor became less.

Spectroscopic data of stored milk were analyzed simultaneously with sensory analysis every week for three successive weeks.

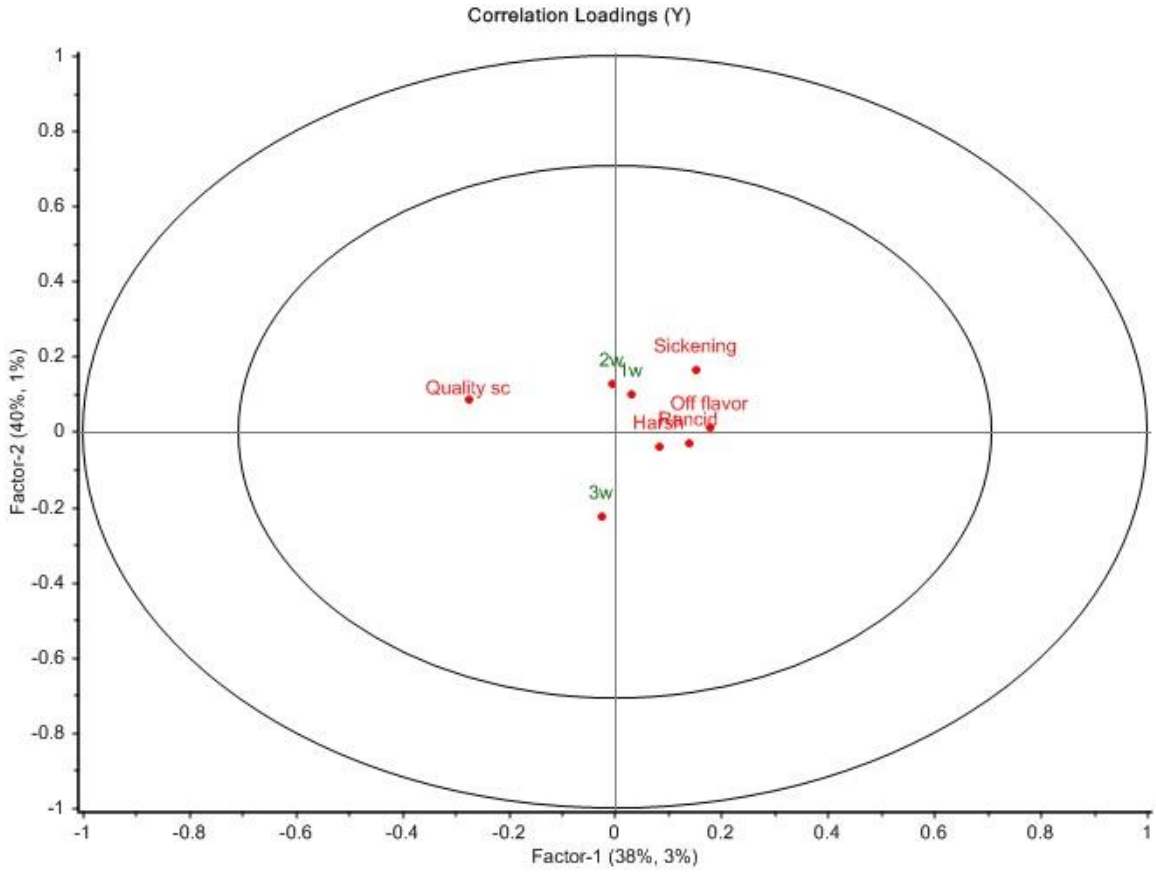

Figure 29: Partial Least Square Regression (PLSR) correlation loadings plot of unaltered milk spectroscopic data with analyzing weeks and sensory attributes. X-variables: Spectroscopic data while Y-variable: Sensory attributes with dummy variables

Quality score in the correlation loadings plot appeared to be strongly negatively correlated with off-flavor, harsh, rancid and sickening as shown above. Sickening was seen in first analyzing week while harsh and rancid was seen in third week of analysis.

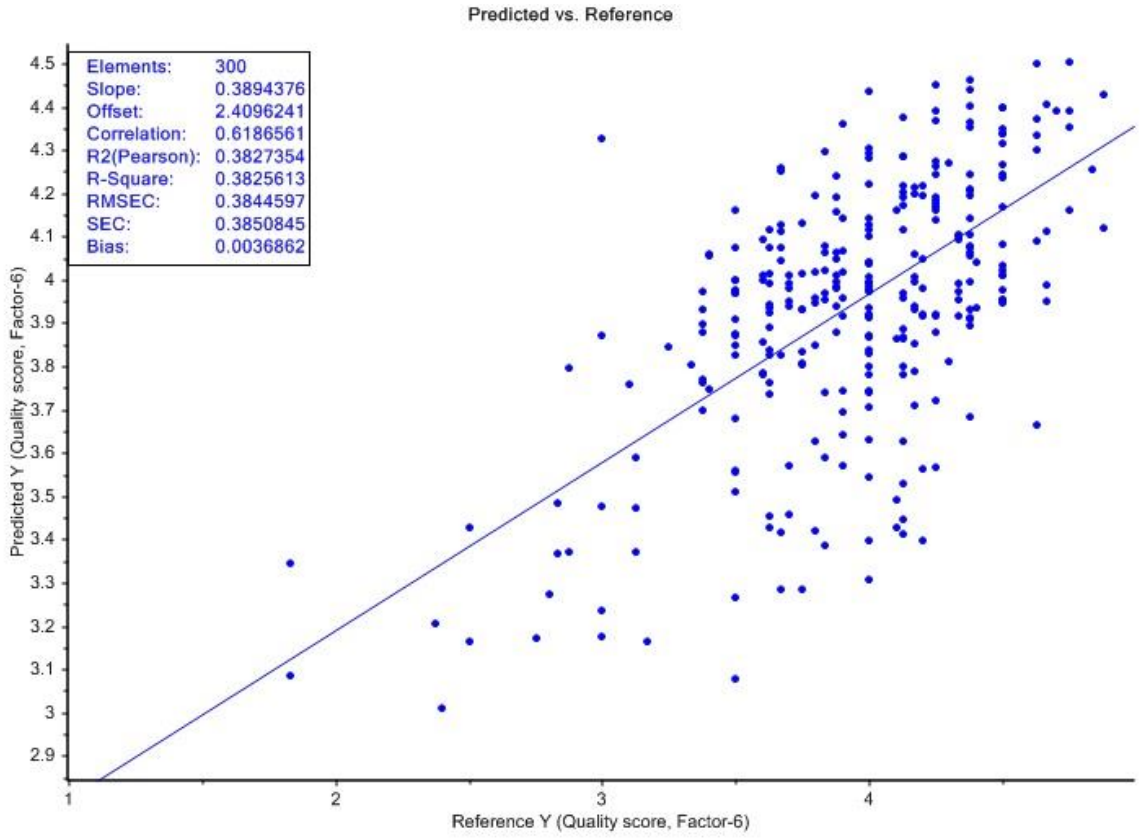

Figure 30: Predicted vs. Reference plot for PLS calibration samples based on spectroscopic data with quality score of unaltered milk samples. X-variable: spectroscopic data while Y-variable: Quality score. Correlation coefficients: 0.618

For the different sensory defects including rancid, bitter etc., the spectroscopic analysis didn't provide any information. The instrumental analysis result indicates that the sensory attributes didn't change so much when stored for a long period of time as there was no positive correlation between them. The calibration model with quality score (R^2=0.382) showed that the small changes in sensory quality is difficult to measure by FTIR spectroscopy. It can be observed from the above plot that many samples weren't so well predicted. Thus, overall correlation was unsatisfactory (figure 30).

4.2. Experiment 2: Mechanical alteration of pasteurized milk

For the mechanical alteration experiment, three dairies Q, R and S were selected for the milk samples as the quality of milk for S and T dairy seemed similar when analyzed for three weeks and weekly pattern of FFA development was almost same (figure 19, 20). In addition, it was difficult for the sensory panel to judge many samples in one session. So in order to have a good sensory analysis design, milk from three dairies were collected.

Table 8: P-value based on mechanical sensory scores for sensory attributes with significant effect in one or more experimental factors

	P-value					
Factor	Quality Score	Harsh	Sickening	Off flavor	Cardboard	Rancid
Assessor (random)	**<0.001**	**<0.001**	0.113	**0.015**	**<0.001**	**0.003**
Dairy	**<0.001**	0.306	0.467	0.355	**0.014**	**<0.001**
Raw Milk Addition (%)	**<0.001**	**0.041**	**0.020**	0.971	0.302	**<0.001**
Time stirring (min)	0.272	0.107	0.950	0.225	0.121	0.450
Dairy*Raw Milk	0.196	0.522	0.247	0.096	0.735	**0.015**
Dairy*Time stirring	0.249	0.967	0.642	0.341	0.663	0.442
Raw Milk Addition (%) *Time stirring(min)	0.872	0.221	0.692	0.623	0.398	0.653
Adjusted R^2:	0.364	0.191	0.012	0.073	0.062	0.229

There is significant effect between dairies and raw milk addition with quality score and rancid attribute. However, the quality of milk including rancid attribute didn't show significant effect with increase in agitation time (table 8). The reason might be the stirring time used for samples during experiment was too short or the force applied during agitation was less to show the effect. However, the change in quality of milk, rancid and sickening attributes could be easily observed after the addition of (1%) raw milk in pasteurized milk (figure 31, 32 & 33) where milk from dairy S possessed high rancid taste and Q being the least rancid (figure 34). Dairy and raw milk addition showed a significant effect with FFA formation ($p<0.05$) while time stirring showed no effect on it.

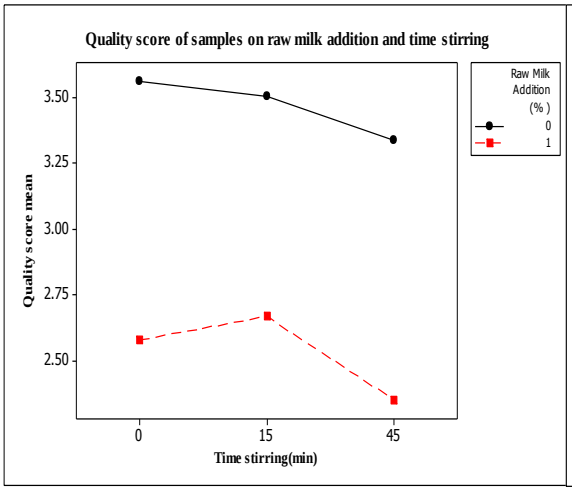

Figure 31: Quality score vs. time stirring

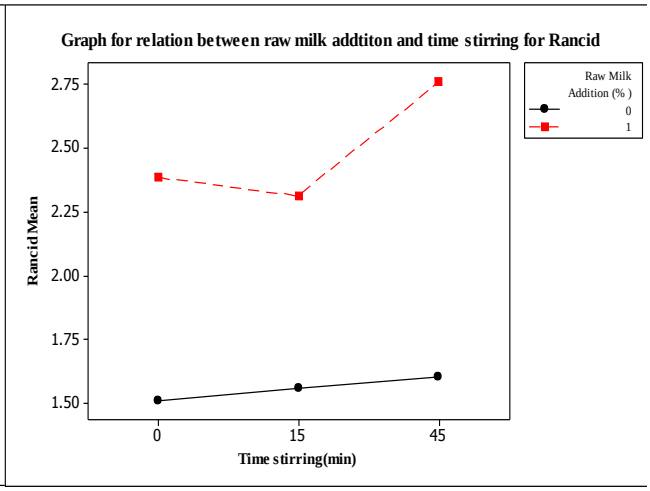

Figure 32: Raw milk addition vs. time stirring for rancid

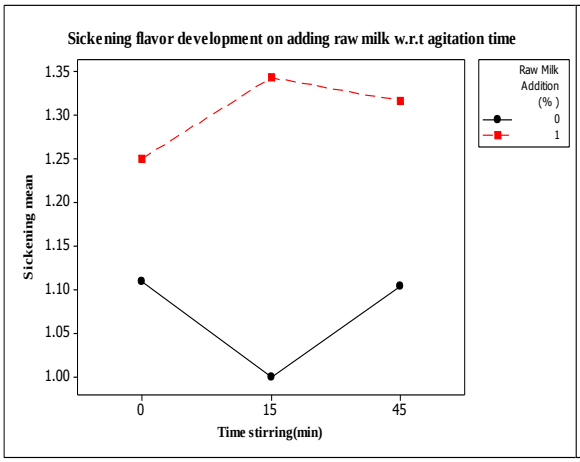

Figure 33: Raw milk addition vs. time stirring for sickening

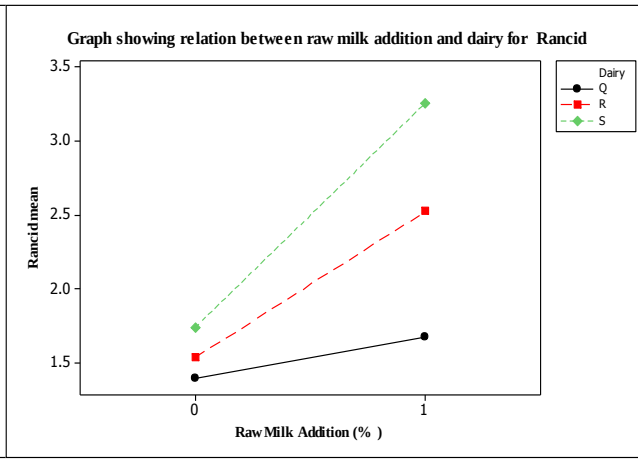

Figure 34: Raw milk addition vs. dairy for rancid

From the grouping information obtained using one way Anova Tukey method, it showed that milk from S dairy was significantly different from Q while R milk showed similarity with both S and Q.

Table 9: Grouping information of dairy milk using Tukey Method

Dairy/Brand	Mean	Grouping
S	2.41	A
R	1.95	A B
Q	1.48	B

The regression plot between sensory attributes Quality score and rancid taste drawn below shows the negative correlation between each other (figure 35). It means the quality score of the samples was greatly affected by the rancid defect.

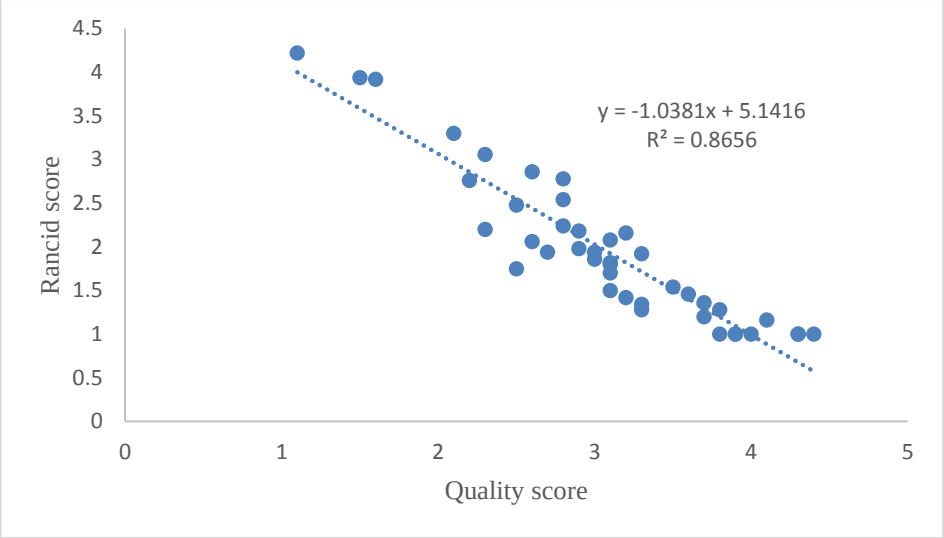

Figure 35: Regression line showing the relation between quality score and rancid score of milk samples

The pattern of FFA formation shows that the splitting of fat to FFA was greatly enhanced by the addition of raw milk rather than the time interval for agitation (figure 36). In addition, the storage time after agitation showed significant result in increasing FFA value. The pattern of FFA increment w.r.t storage period showed least FFA formation at day one while highest value at day nine (figure 36). Likewise, milk from S dairy developed high FFA on 9th day of storage compared to other dairies at the same storage time (figure 37).

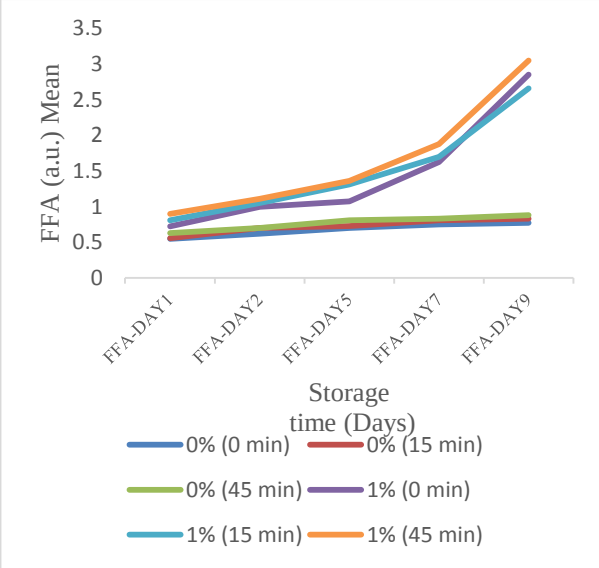

Figure 36: FFA mean of (0% & 1%) raw milk added milk samples with different agitation time for storage period (day1-9)

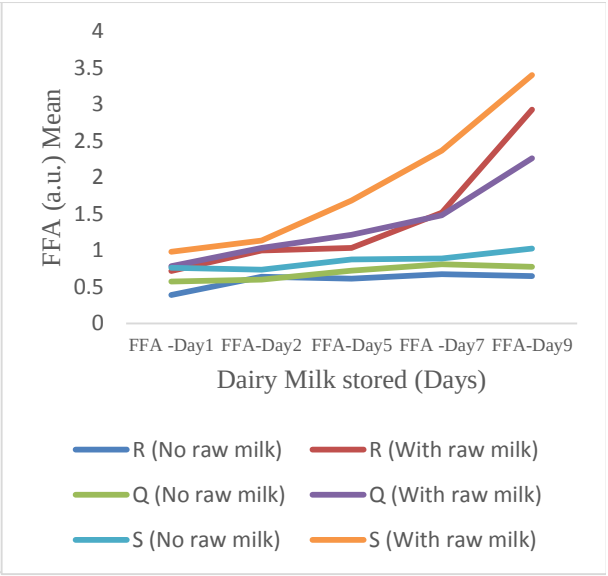

Figure 37: FFA mean of different dairy milk with and without raw milk for storage period (day 1-9)

Among the different milk samples studied, milk from dairy S (1.2%) shows high rancid taste (figure 34) upon the addition of raw milk. This shows that the rupture of MFGM in S milk took place fast compared to other dairy milk due to lipase activation (Larsen et al., 1941; Deeth & Fitz-Gerald, 1978). Agitation process should have significant role in lipolysis process (Deeth & Fitz-Gerald, 1977; Larsen et al., 1941) and development of sickening flavor (Clark et al., 2009). However, the result indicates that the liberation of fatty acids without raw milk addition was low in the milk samples (figure 34). There wasn't so much change seen in the pattern of fatty acid formation between one to ninth day of analysis. The reason might be the pasteurized milk didn't contain lipase enzymes enough to get activated during agitation or agitation time wasn't enough to breakdown MFGM (Deeth & Fitz-Gerald, 1978). Nevertheless, when 1% raw milk was added, there was significant increase in FFA formation after five days of storage. This result is much more similar with the one done by (Larsen et al., 1941) where it took 3-5 days for increasing acidity by adding 1% raw milk in pasteurized milk. Increase in FFA formation lowered the quality of milk (figure 30) because of the change in taste and odor. In addition, the lipolysis process seemed to be continued during storage at refrigerated temperature (figure 37). The acidity decreased by 0.88 when 2% tributyrin added to raw whole milk was stored at 2 ^{o}C for 72 hours after the agitation at 25 ^{o}C for half an hour while the

acidity decreased only by 0.27 for the same condition without 2% tributyrin ester (Krukovsky & Sharp, 1938).

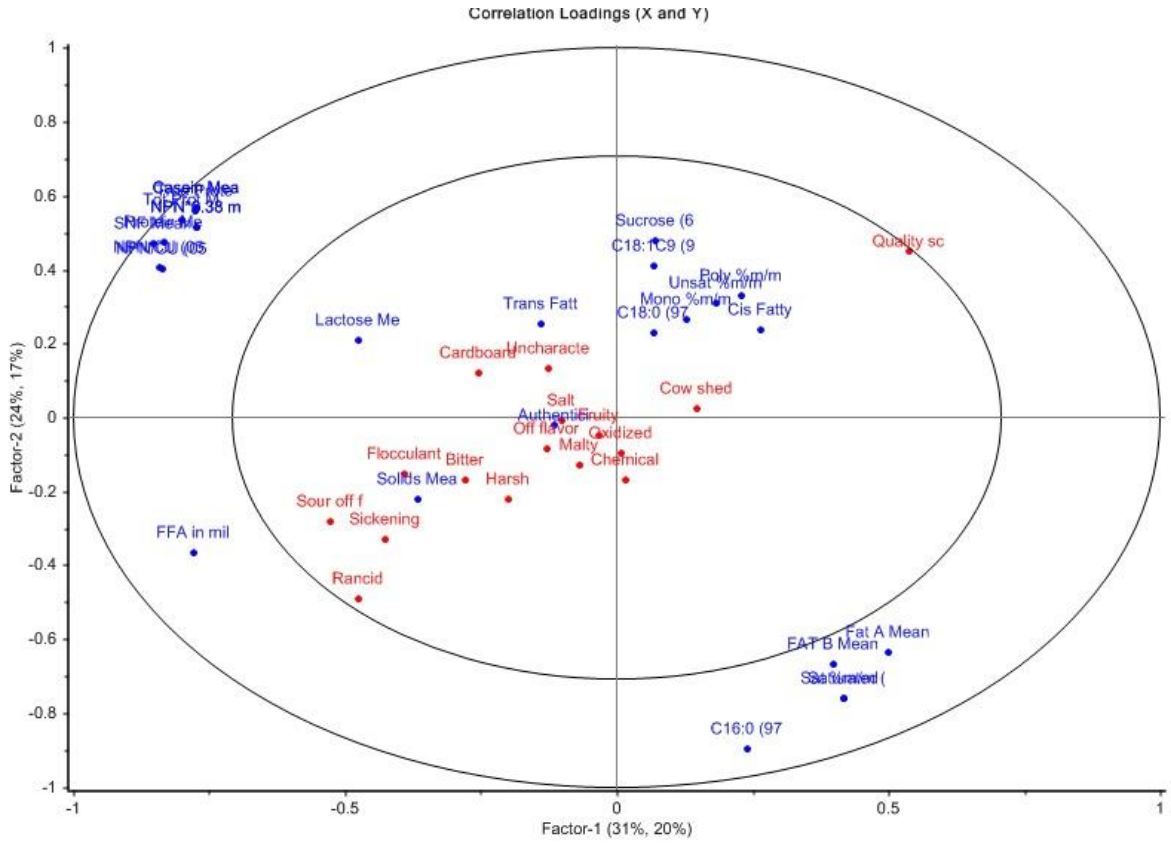

Figure 38: Correlation loadings from PLS of mechanical altered milk samples with Chemical data vs. Sensory profile data. X-variable: Chemical data (blue color) while Y-variable: Sensory profile data (red color)

Here in the graph, it shows that rancid and FFA formation in milk were the sensory defects that affected in lowering the quality score and sickening flavor had a negative correlation on the quality score of milk samples. While, rancid taste was positively correlated with free fatty acid in milk (FFA) indicating that formation of FFA in milk enhanced rancid taste. This happened possible because of the addition of raw milk in homogenized pasteurized milk susceptible for lipolysis due to lipase action (Larsen et al., 1941).

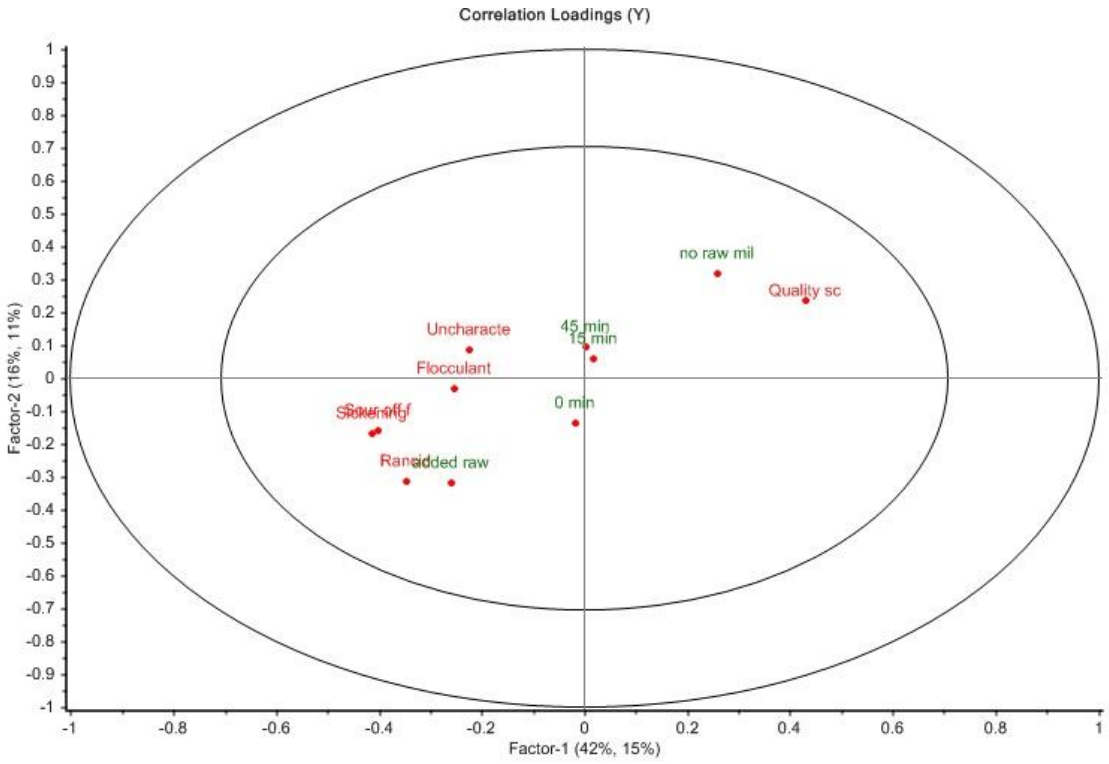

Figure 39: Partial Least Square Regression (PLSR) correlation loading plot of mechanical altered milk samples with spectroscopic data vs. quality score data. X-variable: Spectroscopic data and Y-variable: sensory attributes (red color) with dummy variables (down weighted, green color)

It shows that addition of raw milk lead to sensory defects like poor quality, rancid and sickening taste, as these attributes were closer among themselves while milk samples without raw milk addition lie closer to quality score indicating no change in the quality of milk. However, the agitation time parameter located in the central area indicating no positive correlation with rancid and sickening.

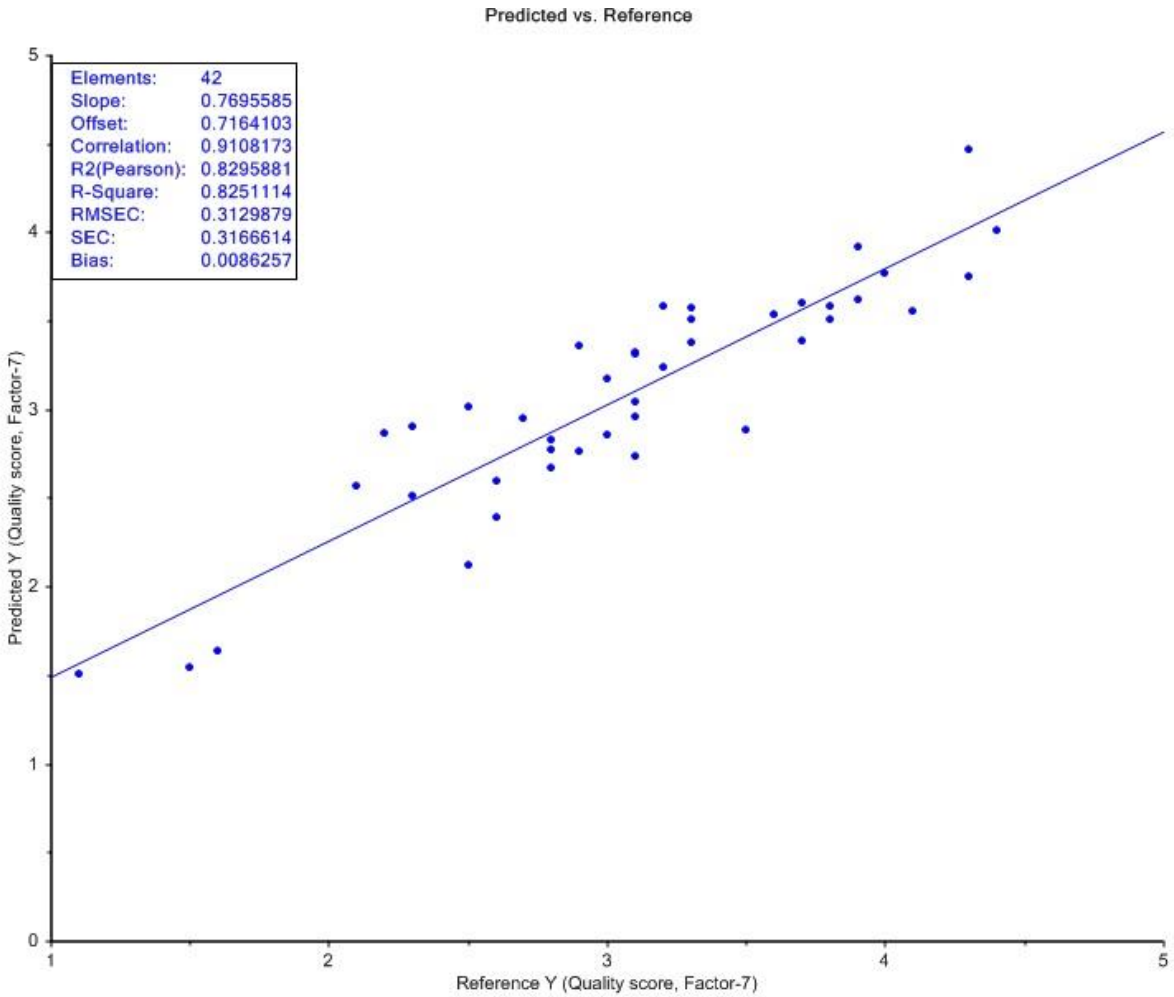

Figure 40: Predicted vs. reference plot for PLS calibration samples based on spectroscopic vs. quality score of mechanical altered milk samples. X-variable: Spectroscopic data while Y-variable: Quality score. Correlation coefficients: 0.91

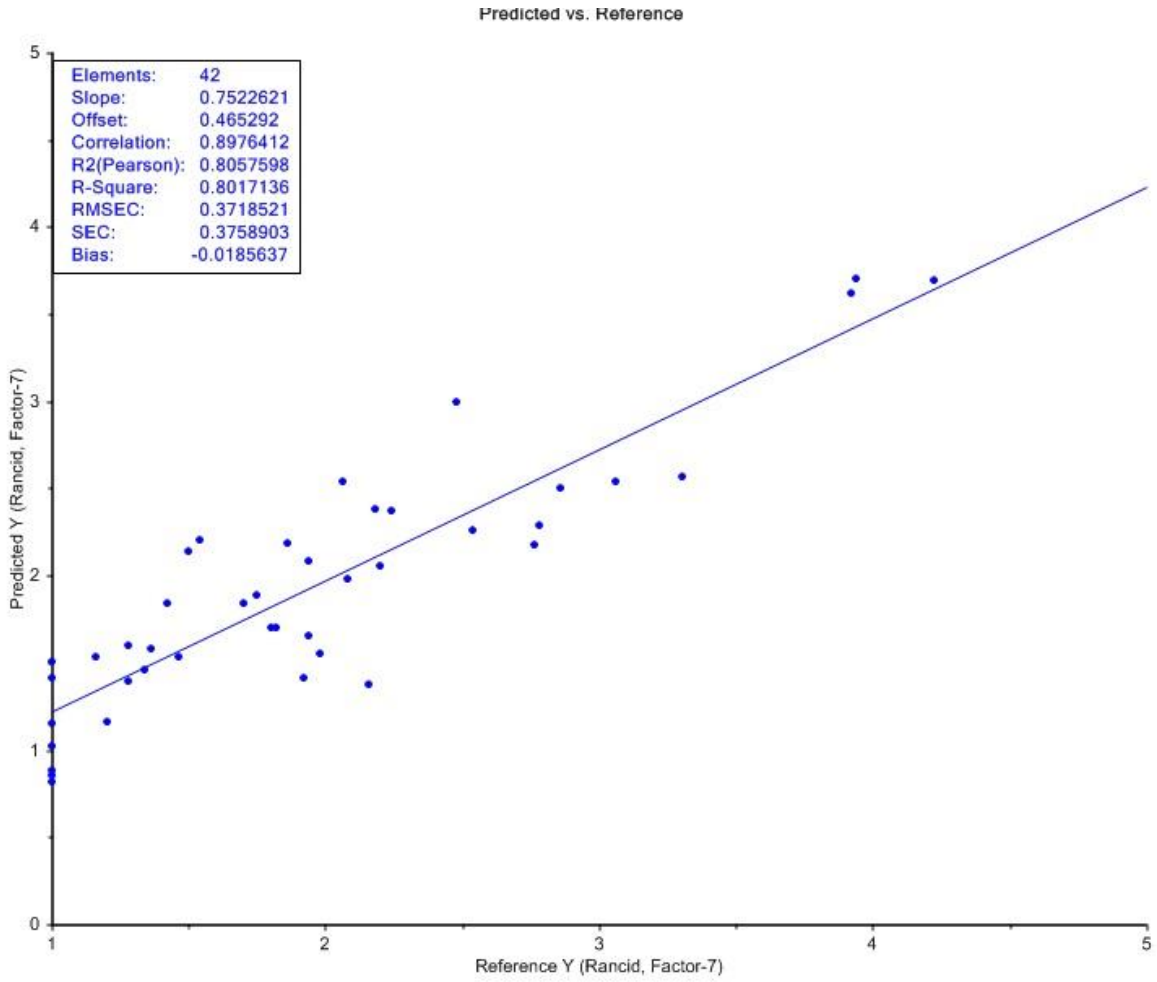

Figure 41: Predicted vs. reference plot for PLS calibration samples based on spectroscopic vs. rancid score of mechanical altered milk samples. X-variable: Spectroscopic data while Y-variable: Rancid score. Correlation coefficients: 0.89

The spectroscopic analysis data showed a good calibration model with quality score (R^2=0.825) and rancid (R^2=0.801) indicating much information about sensory quality and rancidity which can be calibrated by FTIR. The reason behind a good pattern might be due to rancid being the main factor which was affecting the quality score of milk and it was due to FFA formed by lipolysis in the presence of lipase enzymes (Deeth, 2006).

4.3. Experiment 3: Pasteurized milk exposure to different wavelengths of light

Milk from only one dairy S was selected for the light alteration experiment. Previously, milk samples from three dairies were selected to expose in light for three holding times and the quantity of samples was so large that it was difficult for the sensory panel to analyze in one

session. In addition, the quality of milk was bad enough due to long time exposure in light. Thus, the experiment was carried out again reducing sample size and time for light exposure.

Table 10: P-value based on sensory scores for sensory attributes with significant effect in one or more experimental factors for light altered milk samples

	P-value				
Factor	Quality score	Oxidized	Off flavor	Cardboard	Harsh
Filter	**<0.001**	**<0.001**	0.514	0.845	0.637
Time	**<0.001**	**<0.001**	0.192	0.134	0.886
Production date	**<0.001**	**<0.001**	0.198	0.701	0.830
Assessor	0.062	0.586	0.517	0.426	**0.019**
Filter*Time	0.908	0.891	0.585	0.969	0.234
Filter*production date	0.469	0.036	0.514	0.703	0.355
Adjusted R^2:	0.4912	0.5575	0.000	0.000	0.0511

Filters used during light exposure, time interval and production date of milk had a significant effect on the quality score of the milk and on oxidized flavor. It shows that the reference sample had higher quality score compared to other milk samples, and unwrapped sample got the lowest quality score (figure 42). Comparing among the color filters, the milk samples kept in light with green and blue color filters had higher quality score than the one with orange. The period for light exposure also gives valuable information indicating that the quality of milk went down with increasing the time for light exposure (figure 43). The milk samples at 15 minutes showed better quality score than 30 and 60 minutes.

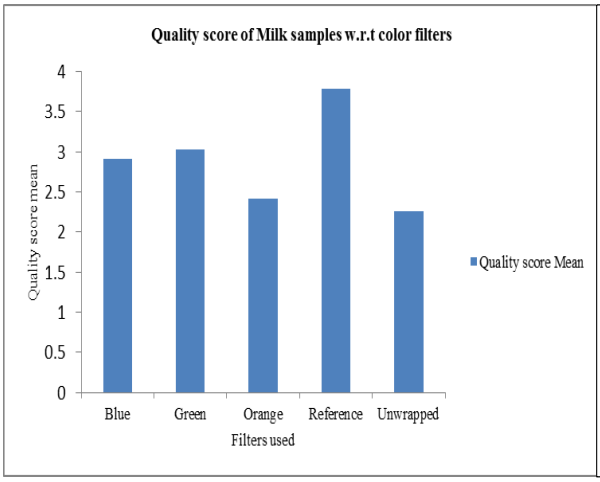

Figure 42: Quality score of milk sample vs. color filters

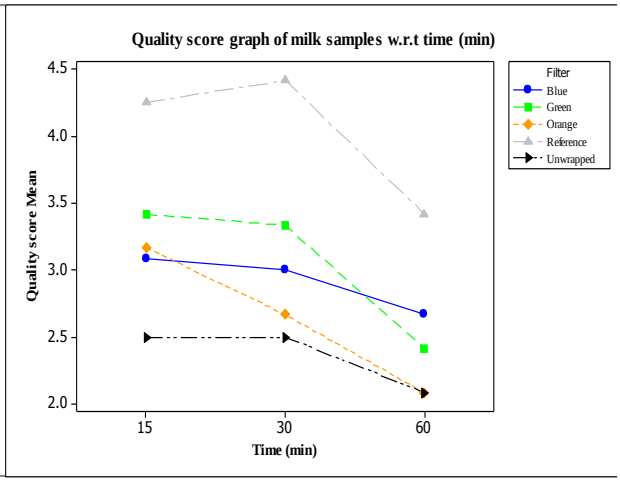

Figure 43: Quality score vs. time exposure under light

Similarly, the below figure 44 represents the intensities for the oxidized flavor where unwrapped milk samples were found to be more oxidized and the reference sample being the least oxidized. The tendency for oxidation process increases when the milk samples were kept under light for long time (figure 45).

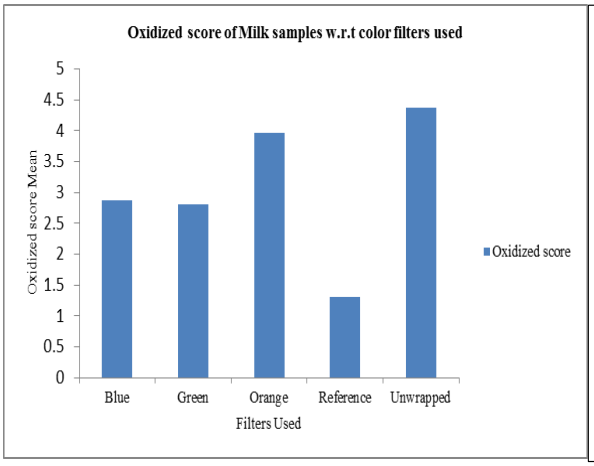

Figure 44: Oxidized intensity vs. color filters

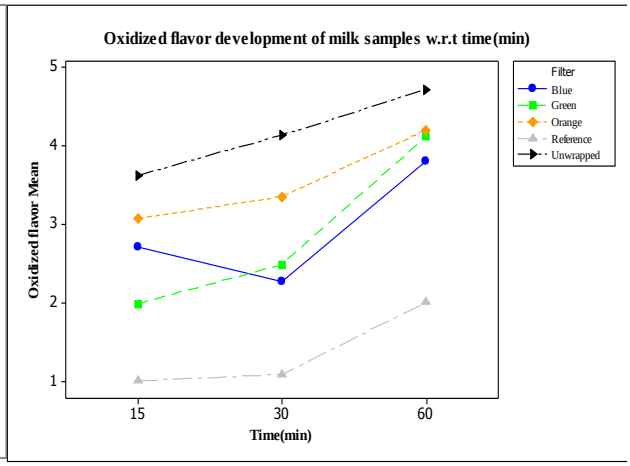

Figure 45: Oxidized intensity vs. time exposure under light

Figure 44 shows that the unwrapped milk samples developed higher oxidized flavor than other milk samples from the beginning of light exposure. The reference samples were found to have less oxidized flavor, which is expected. Likewise, the milk samples with green filter showed least oxidized effect while orange filter showed high photo-oxidation. The study done by J.P. Wold in 2010 also resulted the lowest light intensity in green filters among different filters used in his experiment inducing less photo-oxidation (Intawiwat et al., 2010). This signifies

that the green filter gives the best protection against forming off flavor (Hansen et al., 1975). Similarly, the cause for the high photo-oxidation in orange filter samples is due to the degradation of tetrapyrroles which typically absorb light above 600 nm responsible for sensory off flavor (Airado Rodriguez et al., 2011).

The regression plot between sensory attributes quality score and oxidized flavor drawn below shows that the quality of milk samples was affected only by oxidation (oxidized flavor). It shows the negative correlation between each other (figure 46).

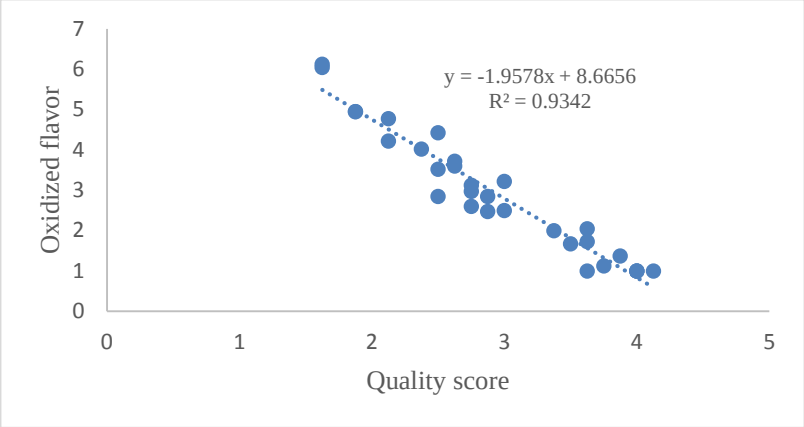

Figure 46: Regression line showing the relation between oxidized flavor intensity and quality score of milk samples

From the grouping information obtained using one way Anova Tukey method, it showed that reference milk sample is significantly different from unwrapped milk sample while milk samples with different color filters were at the same quality level differing from the reference and unwrapped one. In addition, it shows that orange filter were not different from the unwrapped one for quality score.

Table 11: Grouping information of quality score of milk samples under different color filters using Tukey Method

Filter	Mean	Grouping
Reference	3.81	A
Green	3.06	B
Blue	2.96	B
Orange	2.52	B C
Unwrapped	2.29	C

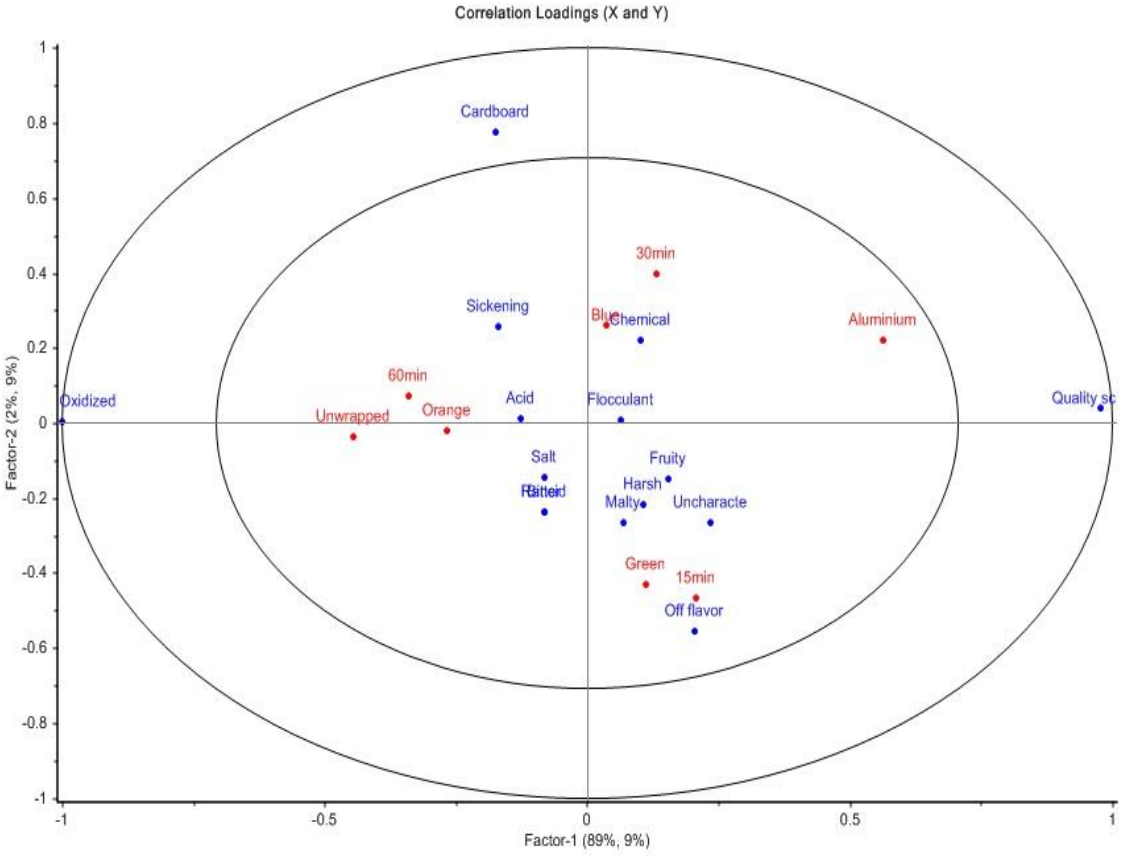

Figure 47: Partial Least Square Regression (PLSR) correlation loadings plot of light altered sensory profile data with experimental factors as dummy variables. X-variables: sensory data (blue) while Y-variable: Color filters used and time interval as dummy variables (red)

Quality score in the correlation loadings plot appeared to be strongly negatively correlated with oxidized flavor. While, sickening, harsh, flocculent, acid and fruity attributes were projected at the center, which indicates that these attributes had no correlation with quality score and couldn't be interpreted well. Aluminum wrapped milk samples were prone to have high quality score while unwrapped milk samples were more susceptible to have oxidized flavor (figure 47 and 48). Similarly, milk samples with orange filter were more prone to have oxidized flavor with light exposure of 60 minutes (figure 48).

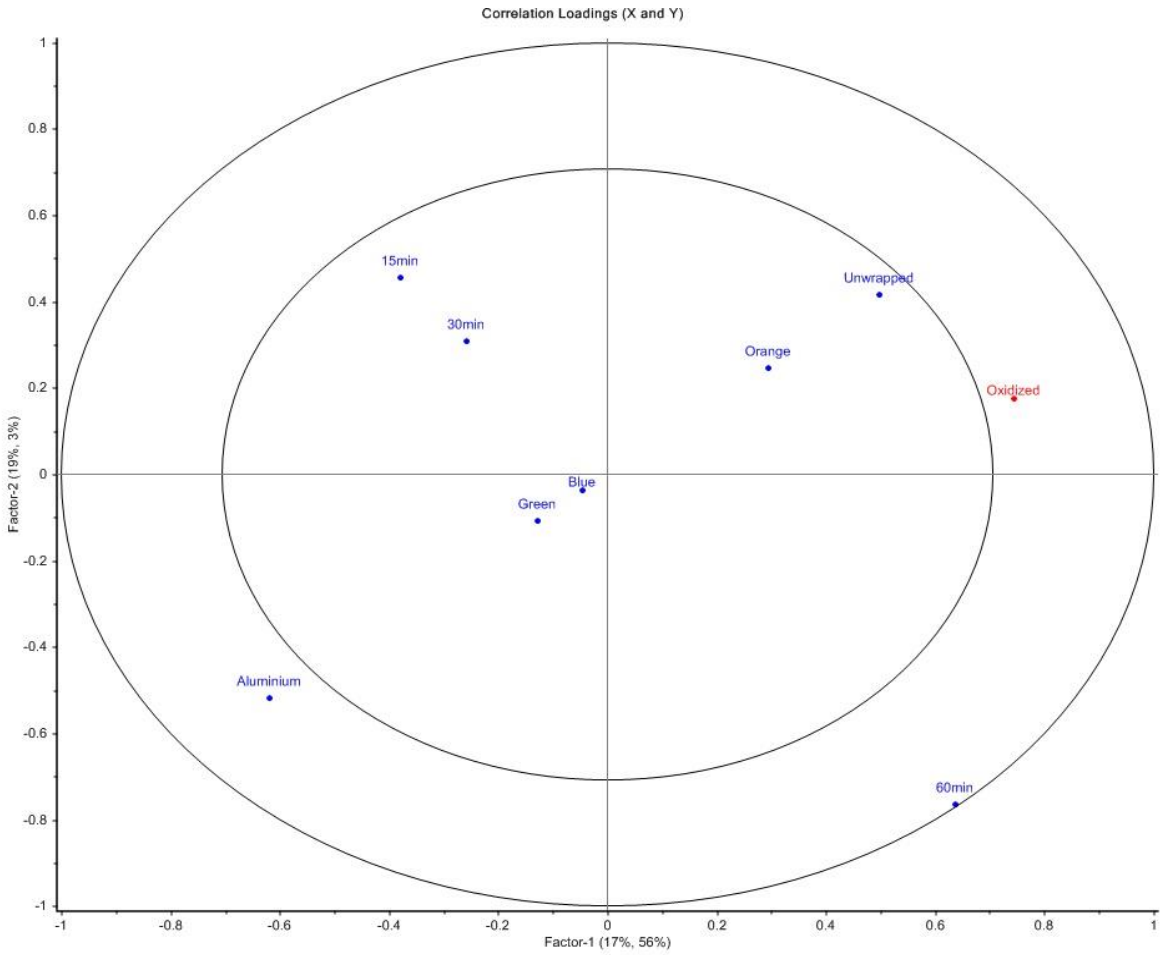

Figure 48: Partial Least Square Regression (PLSR) correlation loadings plot of light altered sensory attribute (Oxidized) with dummy variables. X-variables: Oxidized score (red) while Y-variable: color filters and time interval as dummy variables (blue)

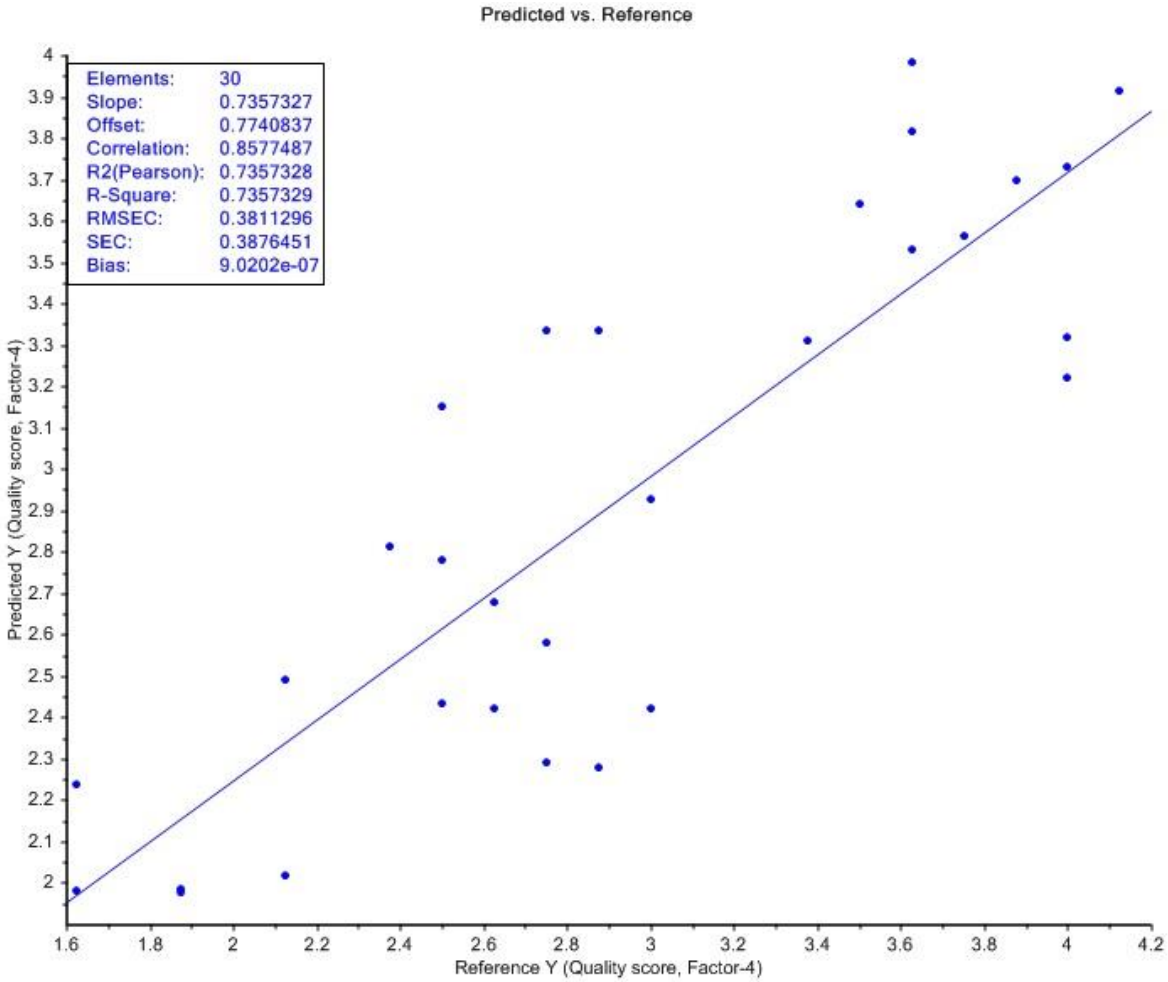

Figure 49: Predicted vs. reference plot for PLS calibration samples based on quality score of light altered milk samples. X-variable: Spectroscopic data. Y variable: Quality score. Correlation coefficients: 0.86

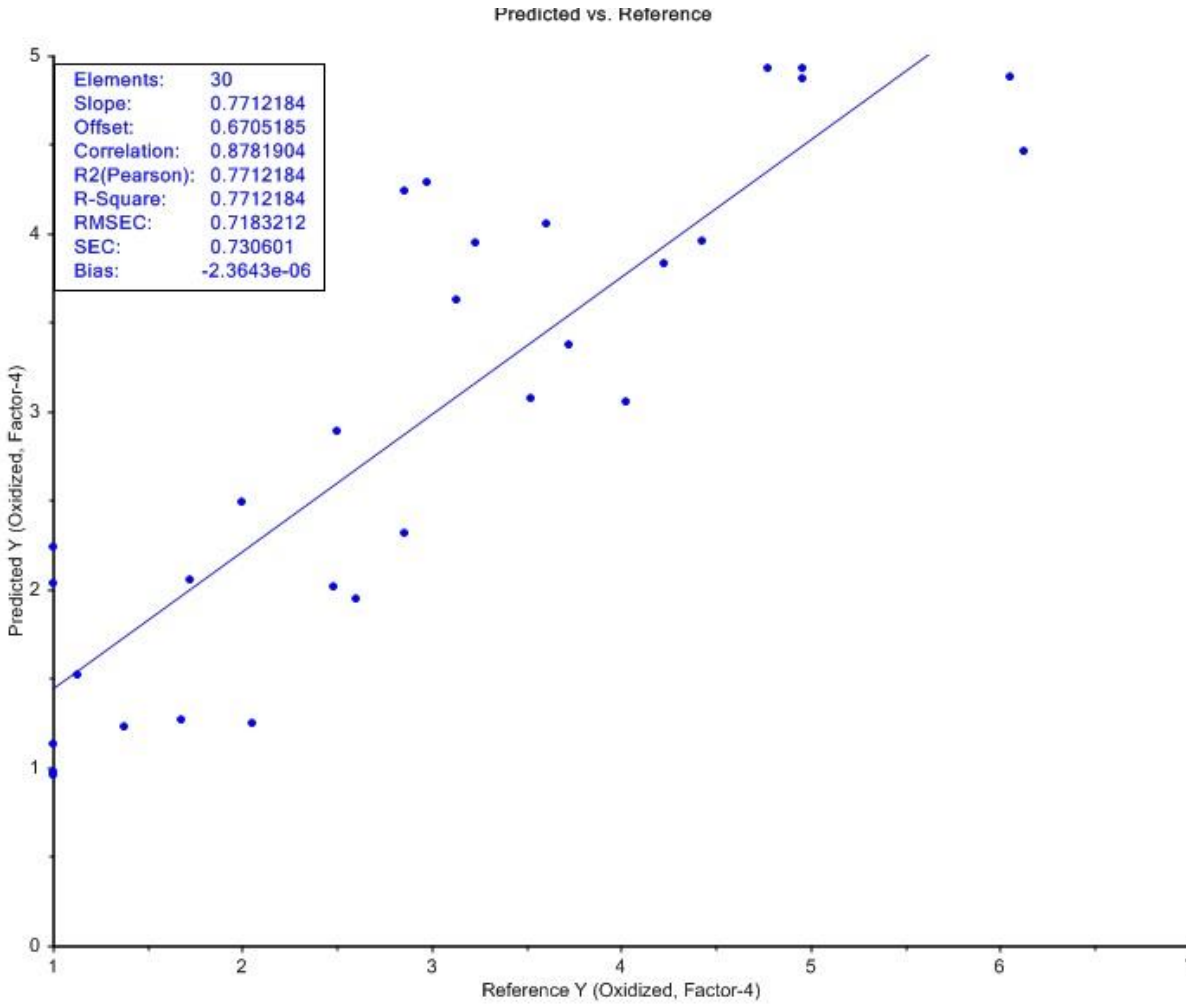

Figure 50: Predicted vs. reference plot for PLS calibration samples based on oxidized score of light altered milk samples. X-variable: Spectroscopic data. Y variable: Oxidized score. Correlation coefficients: 0.88

Figure 49 and 50 above shows that the spectroscopic data provided a good calibration model with quality score (R^2=0.73) and oxidized attribute (R^2=0.77) indicating that oxidized defect was the factor affecting quality score of milk samples. Likewise, calibration model with blue filter R^2= 0.39 showed that low sensory quality caused by oxidized off flavor was best measured when riboflavin was degraded by blue light, which absorbs the light below 500 nm (Intawiwat et al., 2010); explained by IR spectra. However, it didn't show any information about orange filtered milk samples that absorbed light between 575-750 nm resulting tetrapyrrole degradation (Airado Rodriguez et al., 2011). While, sensory analysis results shown in figure 46 and 47 indicates that milk samples with orange filter were more oxidized indicating tetrapyrrole degradation by absorbing the light above 600 nm. Sensory analysis from Airado Rodriguez et al., (2011) also showed that wavelengths longer than 575 nm induced significantly

more off-flavors than wavelengths shorter than 500 nm indicating tetrapyrrole degradation. Here by spectroscopic analysis, it showed that riboflavin degradation by blue light could be best measured by this method. While, sensory analysis showed that tetrapyrroles were found to degrade more by orange light than blue light. Thus, this contradiction in result indicates that IR spectroscopy isn't a good option for measuring the photo-oxidation in milk.

4.4. Experiment 4: Pasteurized milk alteration by different temperature

Milk samples from five different dairies were kept in 4, 7 and 10 ^{o}C for 7 days and in 17 ^{o}C for 1 day before analysis. Then, the effect of temperature treatment was investigated afterwards with sensory, bacto-count and spectroscopic analysis.

Table 12: P-value based on sensory scores for sensory attributes with significant effect in one or more experimental factors for temperature altered milk samples

	P-value			
Factor	Quality score	Oxidized	Rancid	FFA
Assessor	<0.001	<0.001	0.208	-NA
Dairy	**0.036**	0.844	0.233	**<0.001**
Temp	**0.001**	**0.002**	0.248	**<0.001**
Replicate	0.931	0.869	0.607	**<0.001**
Dairy*Temp	0.116	0.302	0.864	**<0.001**
Dairy*Replicate	0.804	0.720	0.964	**<0.001**
Temp*Replicate	0.368	0.320	0.061	**<0.001**
Adjusted R2	0.222	0.197	0.000	0.925

Dairy and temperature used to store milk samples had a significant effect on the quality score of the milk and on FFA formation. It is significant that the milk samples for all dairies at 4 ^{o}C had higher quality score that the one at 17 ^{o}C for 1 day (figure 52 and 53). In terms of dairy, Q showed the highest milk quality while R being the lowest one (figure 51).

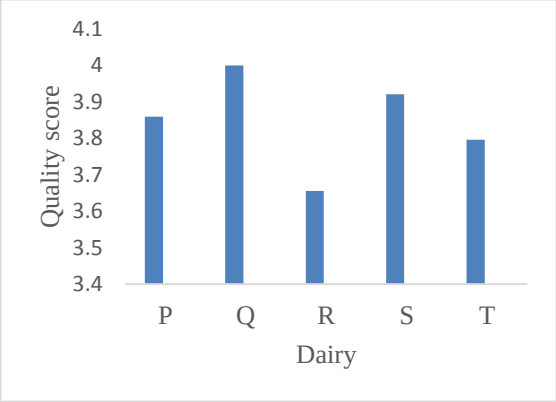

Figure 51: Quality score of milk samples from different dairies

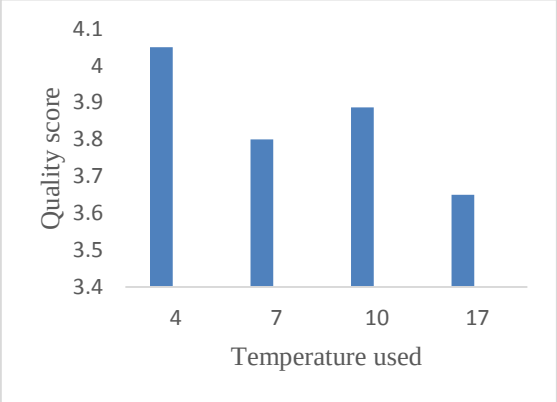

Figure 52: Quality score of milk samples when stored at temperatures 4, 7 and 10 °C for 7 days / 17°C for 1 day.

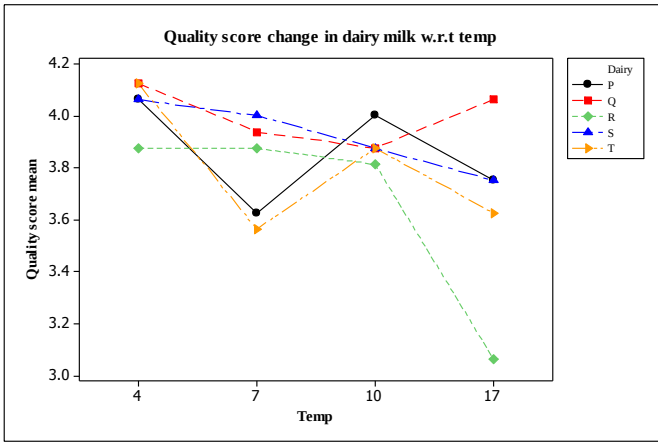

Figure 53: Quality score change seen in dairy milk samples when stored in different temperature

From the grouping information obtained using one-way Anova Tukey method, it shows that milk samples stored at 4 ^{o}C for 1 week were significantly different from 17 ^{o}C for 1 day (table 13).

Table 13: Grouping information of milk samples quality score w.r.t different temperatures using Tukey Method

Temperature	Mean	Grouping
4	4.05	A
10	3.89	A B
7	3.80	A B
17	3.65	B

Similarly, the milk samples exposed to 17 ^{o}C for 1 day developed higher rancid flavor than the one kept at 4 and 7 ^{o}C for a week (figure 54). It might be due to the bacterial induced lipase action. The same character of rancid flavor was obtained when pasteurized milk stored at 7 and 25 ^{o}C was analyzed on 5th and 6th day of storage (Zahar, Tatini, Hmama, & Fousshi, 2011). But, it can't be confirmed as there was not much variation in rancid mean value and also the free fatty acid of dairy milk wasn't correlated with rancid score (figure 55 and table 12).

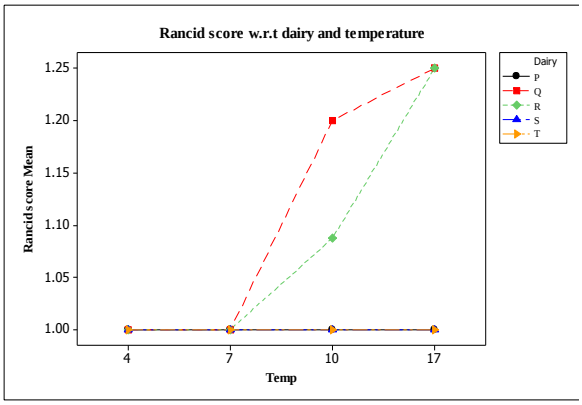

Figure 54: Rancid score w.r.t dairy and temperature

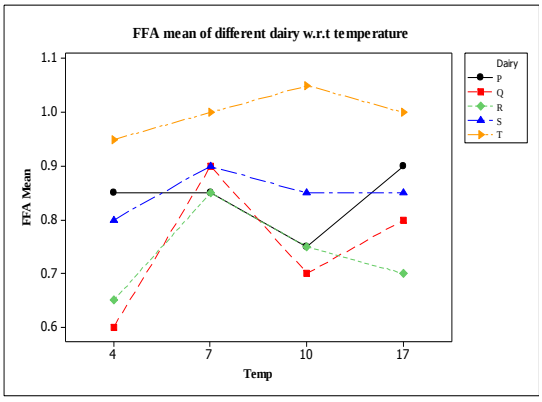

Figure 55: FFA mean value w.r.t dairy and temperature

Temperature alteration in milk samples didn't give good results in terms of microbial growth from the Bactocount analysis as it didn't show a uniform pattern of growth. However, it might be due to nature of bacteria as they have their own growing temperature (figure 56).

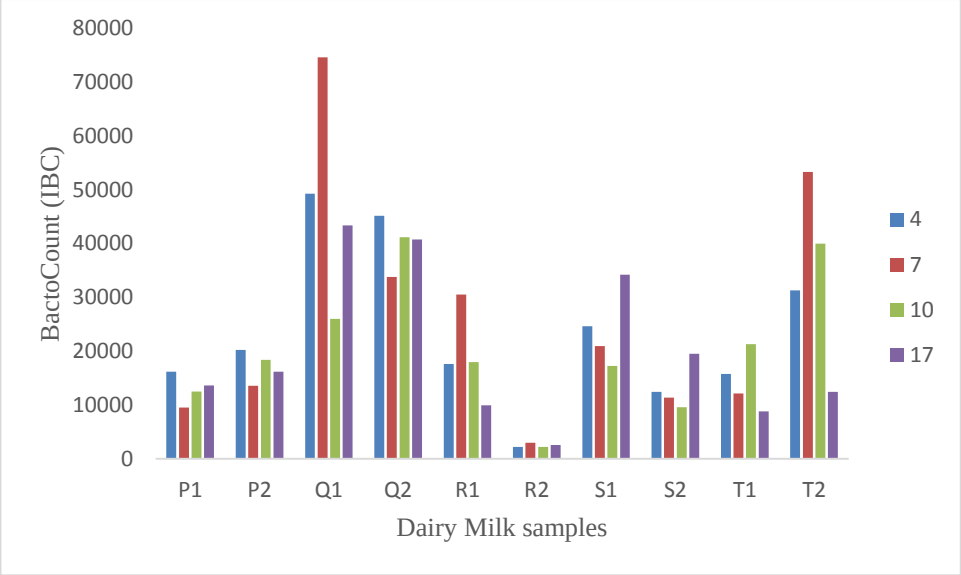

Figure 56: Bacto-count result of milk samples from different dairies when stored for various temperature. Here, 1 and 2 numbered for different dairies represent two different dated milk samples

The pattern of microbial growth in different dairy milk samples was inconsistent when stored for various temperatures. Bactocount IBC is normally used for the fast counting of individual bacteria in raw milk. However, the milk samples used for the bactocount were pasteurized and homogenized. This might be one of the reasons for the scattered result of bacterial growth. In addition, there might be different bacterial species activated during storage at different temperature. However, the identification wasn't possible with this method. The growth of bacteria was found high at 4 ^{o}C for P and Q2 samples, while R, Q1 and T2 milk samples had highest microbial growth at 7 ^{o}C. Likewise, S dairy samples found to have high growth at 17 ^{o}C and T1 at 10 ^{o}C. This shows that there was no significant pattern of bacterial growth when stored in different temperature. This can be due to many reasons. It might be that the analysis method was not exact for these samples. Or it can just be random effects in bacterial growth, as there was only 2 replicates per dairy / temperature. And the bacteria species present in each of the milk samples from the beginning can vary, which may give different effects. Those bacteria which were found high at 4 ^{o}C might be Pseudomonas species as these gram negative species possess short generation time of less than 4 hours for growth when kept at 4 ^{o}C for 8 days (Samarija et al., 2012b). Psychrotropic bacteria are more susceptible to grow at 7 ^{o}C or below (Champagne et al., 1994) while enterobactericeae grows well above 8 ^{o}C (Varnam & Sutherland, 2001). Therefore, the bacterial growth observed below 8 ^{o}C might be psychrotropic species while above 8 ^{o}C might be enterobacteriaceae. Cromie, S.J. et al (1989) stated that the storage temperature after pasteurization had the greatest effect on bacterial growth and the

growth was found to be more rapid at 7 ºC than at 3 ºC. This information seems relevant for milk samples from R, Q1 and T2 dairy.

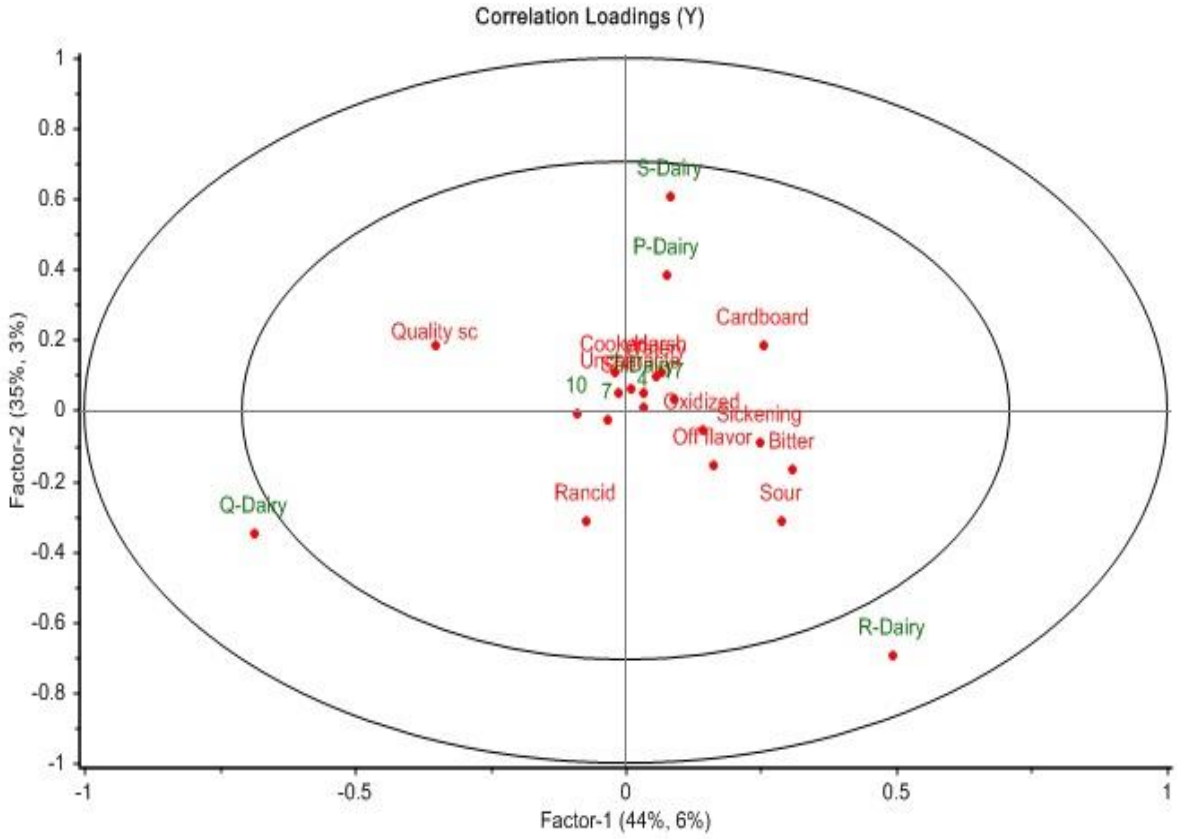

Figure 57: Partial Least Square Regression (PLSR) correlation loadings plot of temperature altered sensory attributes with dummy variables. X-variables: Spectroscopic data while Y-variables: sensory profile data (red) with dummy variables (down weighted, green color)

Quality score had negative correlation with different sensory defects like sour, rancid, off flavor, bitter, sickening. The reasons for this might be due to different nature for each sample. It might be due to endogenous enzymes or native enzymes present earlier in the milk samples responsible for lipolysis or microbial growth even after pasteurization (psychrotrophs, thermophilic) producing enzymes (proteases) responsible for bitter flavor due to proteolysis or growth of different microorganisms during storage in different temperatures. Among different dairies, P, S and T milk quality seemed to be more similar in nature as opposed to Q and R dairy milk (figure 57).

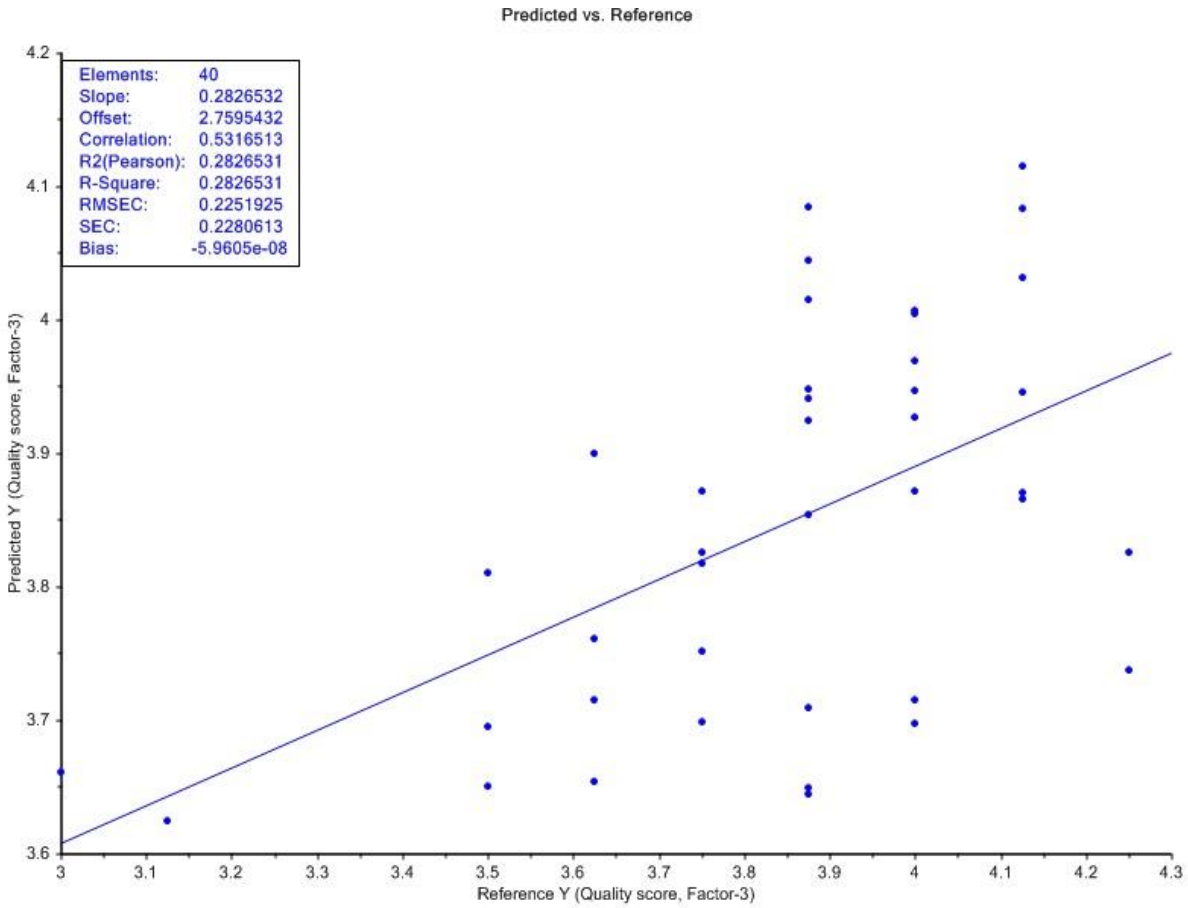

Figure 58: Predicted vs. Reference plot for PLS calibration samples based on sensory scores of temperature altered milk samples. X-variable: spectroscopic data. Y variable: sensory score. Correlation coefficient: 0.53

The calibration model with quality score (R^2=0.28) shows that small changes in quality was difficult to measure by FTIR spectroscopy method. In addition, most of the samples weren't so well predicted including other sensory defects. Thus, overall correlation wasn't satisfactory.

4.5. General calibration model of all experiments

The main objective of our study was to correlate sensory data obtained during the sensory analysis with spectroscopic data obtained from the LactoScope FTIR analysis. Hence, the spectroscopic data of all four experiments in conjunction with partial least square regression was used to predict several sensory attributes of milk samples. The sensory and spectroscopic data of all of the individual experiments were merged and analyzed to see the correlation between them, in order to make general calibration model for milk.

The below figure 59 represents the average milk spectra for each of the experiments showing absorbance (Abs.) in Y –axis and Wavenumber (cm^{-1}) in X-axis. As we can see, there is no visible difference between spectra.

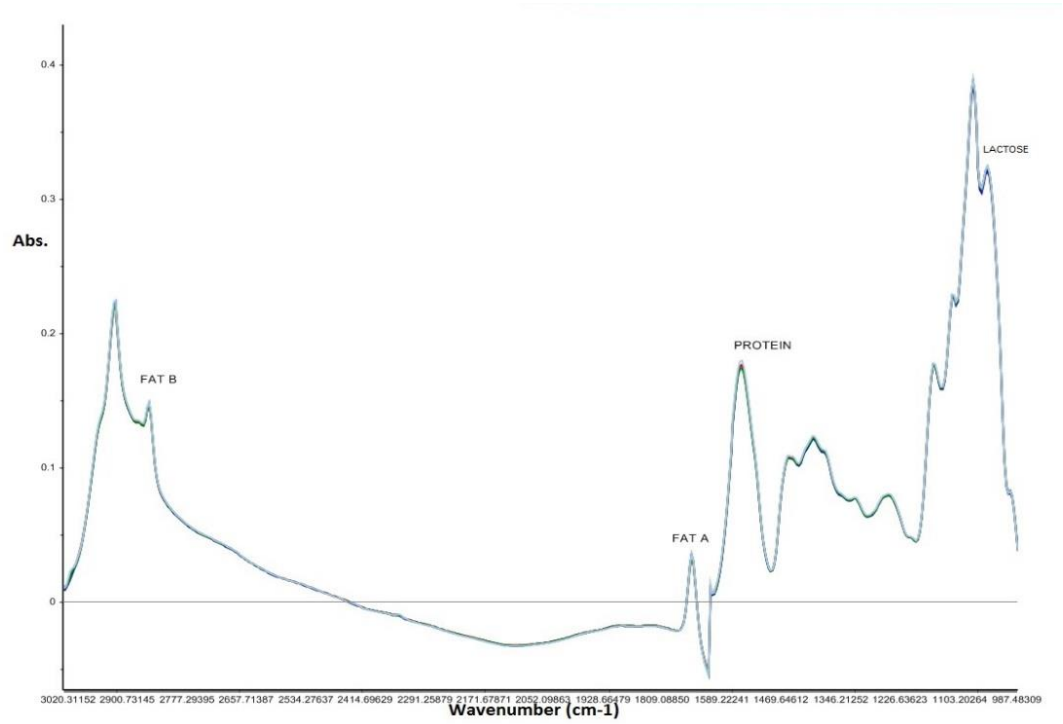

Figure 59: Average spectra from each of the experiments: 1) Average spectrum from unaltered milk experiment, 2) Average spectrum from mechanical experiment, 3) Average spectrum from light experiment, 4) Average spectrum from temperature experiment

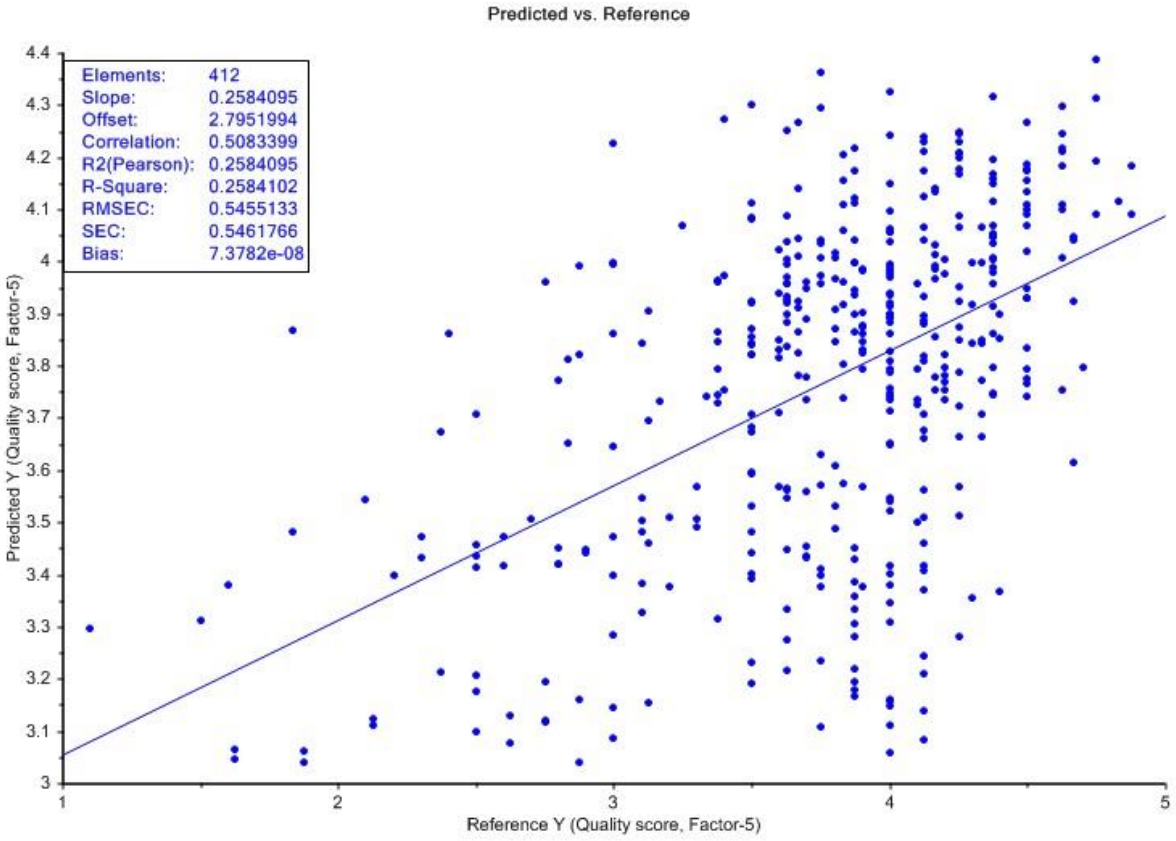

Figure 60: Predicted vs. Reference plot for PLS calibration samples based on sensory scores vs. spectroscopic data of all four experiments. X-variable: spectroscopic data. Y variable: sensory score. Correlation coefficient: 0.51

The accuracy evaluation of each model can be done using the coefficients of determination (R^2) between the predicted and measured values (Fagan et al., 2007).

Table 14: PLS prediction result for sensory attributes using mid- infrared spectroscopy

Sensory attributes	RMSEC	Slope	Correlation	R^2
Quality score	0.54	0.26	**0.51**	**0.26**
Oxidized	0.58	0.29	**0.53**	**0.29**
Rancid	0.38	0.10	**0.31**	**0.10**
Off-flavor	0.54	0.06	0.24	0.06
Cardboard	0.72	0.08	0.29	0.08
Sickening	0.59	0.06	0.24	0.06
Bitter	0.12	0	0.04	0
Harsh	0.54	0.05	0.22	0.05

The summary table of PLS prediction result shows a good pattern of correlation of samples with a value of 0.51 and 0.53 for quality score and oxidized. This indicates that the models

allowed for discrimination between high and low values (Quality score and oxidized). Compared to other attributes, rancid had also considerable correlation (r =0.31). Thus, quality score, oxidized and rancid were most successfully predicted attributes by mid infrared spectroscopy (appendix: table A6).

From the model based on all experiments, it showed a good calibration model between spectroscopic and quality score with a correlation coefficient value (r)=0.51 and it was observed from the correlation loadings that quality score was mostly affected by rancid and oxidized flavor, which was natural from the experiments contained in the dataset. From the FTIR spectroscopy, the sensory attributes that were most successfully predicted were quality score, oxidized, and rancid. While, sensory defects like cream adhesion, cream layer, sour off-flavor, bitter, cooked, burnt, malty, feedy, chemical, salt, uncharacteristic, watery, acid, coagulated, bicolored, foreign matter, untypical color, cow-shed and goat flavor couldn't be detected because the defects weren't present in many of the samples. Therefore, it was not possible to model with spectroscopic analysis in our experimental series.

5. Conclusion

Since the sensory analysis is time consuming, needs extra labor cost and can't determine the compositional variations in milk samples, the use of spectroscopic technique is very essential to study the changes in milk when quality is reduced by storage or by some alteration methods. Therefore, this research work has attempted to evaluate the possibility of correlating IR spectroscopy results with the sensory quality changes of milk.

Among the four different laboratory scale experiments conducted in two phases, milk samples were stored for three consecutive weeks without alteration and analyzed every week in first phase. From the sensory analysis, it was found that dairy and age of milk had a significant effect ($p< 0.05$) on the quality of the milk and on FFA formation. Furthermore, milk from S and T dairies didn't seem to change in quality even after storage for two weeks compared to Q and R dairy during storage. It might be due to the reason that S and T dairy samples got better cooling system from dairy to laboratory throughout the whole period. However, Q and R samples didn't get the cooling system while bringing from grocery to the laboratory. Furthermore, quality score showed strongly negative correlation with off-flavor, harsh, rancid and sickening flavor. The calibration model with quality score (R^2=0.382) indicates that small changes in sensory quality is difficult to measure by FTIR technique.

The regression plots between quality score and sensory defects like rancid, bitter, FFA etc. didn't show any correlation between these attributes which indicates that the sensory attributes didn't change so much when stored for a long period.

In second phase, among three laboratory experiments performed, one of them was mechanical alteration of pasteurized milk with kitchen aid stirrer at 15 ^{o}C for three holding times. It showed significant effect ($p<0.05$) between dairies. Raw milk addition enhanced the reduction in sensory quality score and increased rancid defect. Nevertheless, increase in agitation time showed no significant effect with the quality of milk including rancid taste. Upon addition of 1% raw milk in pasteurized milk, dairy S showed high rancid taste and dairy Q less. Furthermore, the splitting of fat to FFA was more enhanced by the raw milk addition than the agitation time and the effect was more visibly seen after 5 days storage. Quality score decreased due to defects like rancid and sickening. Though, a good calibration model of spectroscopic data with quality score (R^2=0.825) and rancid (R^2=0.801) was obtained indicating that these attributes can be calibrated by FTIR.

Third experiment was performed exposing pasteurized milk to five different wavelength areas of light for three exposure times. Color filters used during light exposure and time interval showed significant effect ($p<0.05$) on quality score and oxidized flavor. Reference sample had high quality score and was less oxidized while unwrapped sample had the lowest quality score and were highly oxidized. Green filter samples had higher sensory quality than orange filter. Likewise, the milk samples at 15 minutes of light exposure showed higher quality than 60 minutes exposure. The results obtained from sensory and spectroscopic analysis was found to be contradictory. From the sensory result, it showed that samples with orange filter were more oxidized indicating tetrapyrrole degradation while from the spectroscopic analysis with calibration model (R^2= 0.391), it showed that low sensory quality due to oxidized off flavor was best measured when riboflavin was degraded by blue light. The main cause of the sensory effects probably was degradation of tetrapyrrole, in the milk samples wrapped with orange filter absorbing light above 600 nm. A good calibration model was obtained between spectroscopic data and quality score (R^2=0.735) and with oxidized (R^2=0.77).

Finally, a fourth experiment was performed altering pasteurized milk by different temperature. It showed that dairy and temperature factors had significant effect on the quality score of milk and on FFA formation. Milk samples at 4 ^{o}C for 7 days were of better quality compared to the samples treated at 4 ^{o}C for 6 days and 1 day at 17 ^{o}C and dairy Q showed better milk quality compared to other dairy samples. Likewise, milk samples kept at 17 ^{o}C developed higher rancid

flavor than the one kept at lower temperature. There might have several factors affecting the result. It might be due to the endogenous enzymes for lipolysis or the presence of bacteria after pasteurization releasing enzymes for proteolysis or the growth of microorganisms at different temperature. Furthermore, dairy P, S and T seemed to be similar and different from Q and R dairy milk. A calibration model obtained between spectroscopic data and quality score (R^2=0.282) wasn't a good model indicating that changes in sensory quality was difficult to measure by FTIR and other sensory attributes weren't so well predicted.

From the model based on all experiments, it showed a good correlation model between spectroscopic data and quality score with a correlation coefficient value of 0.51 and it was observed from the correlation loadings that quality score was mostly affected by rancid and oxidized flavor, which was natural from the experiments contained in the dataset. From the FTIR spectroscopy, the sensory attributes that were most successfully predicted were quality score, oxidized, and rancid.

Regarding the aim of the study, it was interesting to see the correlation between the sensory and spectroscopic data. For mechanical and light altered experiments, high correlations between quality score and spectroscopic data was observed. However, for temperature altered experiment, the correlation between spectroscopic and sensory data wasn't good. It might be due to many different factors affecting the results. That's why the correlation between them was n't high.

In conclusion, FTIR technique can be useful to apply as screening tool for evaluating quality of the milk samples. However, for the total sensory quality, this method can't be applicable as it can't explain all possible sensory defects.

Recommendation

The quality of milk samples used for the experiments didn't change very much except experiment 2 and the intensity of defects wasn't found so high. Thus, the calibration model obtained wasn't good.

To overcome this issue, quantity of samples need to be increased by creating more experimental designs and increasing the intensity of defects so that better calibration model can be made.

It can be done by increasing storage time and temperature (up to 3-5 weeks for experiment 1 and 4 to get worst milk), increasing the agitation force to examine the role of agitation in FFA formation (experiment 2).There could also be made more experiments with oxidization with more sensory replicates or assessors to get more accurate sensory scores.

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7. Appendix

Table A1: FFA data from the mechanical alteration of milk with and without addition of raw milk with respect to the storage time

Sr.no	Sample	FFA Day1	FFA-Day2	FFADay5	FFA - Day7	FFA-Day9
1	R1-8-n-0	0.3	0.6	0.6	0.6	0.6
2	R1-8-r-0	0.6	0.9	1.0	1.3	2.6
3	R1-8-n-15	0.4	0.7	0.5	0.6	0.7
4	R1-8-r-15	0.8	0.9	1.1	1.4	2.4
5	R1-8-n-45	0.5	0.7	0.6	0.7	0.7
6	R1-8-r-45	0.8	1.0	1.0	1.3	2.3
7	R1-8-n-0	0.4	0.6	0.5	0.6	0.7
8	R2-7-n-0	0.3	0.7	0.5	0.7	0.5
9	R2-7-r-0	0.6	1.0	0.8	1.6	3.3
10	R2-7-n-15	0.3	0.5	0.8	0.7	0.6
11	R2-7-r-15	0.7	1.0	1.1	1.5	3.0
12	R2-7-n-45	0.5	0.7	0.7	0.8	0.6
13	R2-7-r-45	0.8	1.2	1.2	2.0	4.0
14	R2-7-n-0	0.4	0.6	0.7	0.7	0.8
15	Q1-8-n-0	0.5	0.6	0.7	0.6	0.6
16	Q1-8-r-0	0.7	1.0	1.1	1.5	2.2
17	Q1-8-n-15	0.6	0.7	0.7	0.9	0.6
18	Q1-8-r-15	0.7	1.2	1.3	1.3	1.8
19	Q1-8-n-45	0.6	0.6	0.8	0.8	0.7
20	Q1-8-r-45	0.8	1.1	1.2	1.5	2.1
21	Q1-8-n-0	0.6	0.4	0.7	0.7	0.6
22	Q2-7-n-0	0.6	0.5	0.7	0.9	0.7
23	Q2-7-r-0	0.8	0.9	1.2	1.4	1.5
24	Q2-7-n-15	0.6	0.7	0.7	0.8	1.0
25	Q2-7-r-15	0.8	1.0	1.2	1.4	2.8
26	Q2-7-n-45	0.6	0.7	0.8	1.0	1.0
27	Q2-7-r-45	0.9	1.0	1.3	1.8	3.2
28	Q2-7-n-0	0.5	0.6	0.7	0.8	1.0
29	T1-8-n-0	0.7	0.7	0.8	0.8	1.0
30	T1-8-r-0	1.0	1.1	1.4	2.1	3.3
31	T1-8-n-15	0.7	0.7	0.9	1.0	1.1
32	T1-8-r-15	1.0	1.1	1.3	1.8	2.4
33	T1-8-n-45	0.8	0.7	0.9	0.8	1.1
34	T1-8-r-45	1.1	1.2	1.4	1.8	2.7
35	T1-8-n-0	0.8	0.7	0.8	0.9	0.9
36	T2-7-n-0	0.7	0.7	0.8	0.8	0.9
37	T2-7-r-0	0.9	1.1	2.0	2.8	4.4
38	T2-7-n-15	0.8	0.9	0.8	0.9	1.0
39	T2-7-r-15	0.9	1.1	1.9	2.8	3.6
40	T2-7-n-45	0.8	0.8	1.1	0.9	1.2
41	T2-7-r-45	1.0	1.2	2.1	2.9	4.0
42	T2-7-n-0	0.8	0.7	0.9	1.0	1.0

Table A2 (1): Sensory analysis result of mechanical altered milk samples analyzed on fifth day

Sample Name	Average of Quality score	Average of Harsh	Average of Bitter	Average of Sickening	Average of Cardboard	Average of Oxidized	Average of Rancid
Q1-1.1	3.9	1	1	1	1.4	1	1
Q1-1.2	3.1	1.28	1.48	1.8	1	1	1.7
Q1-1.3	3	1	1	1	1.28	1	1.94
Q1-1.4	3.1	1.58	1	1.42	1.5	1.22	1.5
Q1-1.5	3.7	1	1	1.4	1.42	1.42	1.2
Q1-1.6	2.6	1	1	1.2	1	1	2.06
Q1-1.7	4	1.4	1	1	1	1	1
Q2-1.1	4.3	1	1	1	1	1	1
Q2-1.2	2.5	1.525	1	1.275	1	1.4	1.75
Q2-1.3	3.2	1.32	1	1	1	1	2.16
Q2-1.4	3.3	1.58	1.38	1	1	1	1.28
Q2-1.5	3.3	1.24	1	1	1	1.18	1.34
Q2-1.6	2.7	1.4	1	1	1	1.32	1.94
Q2-1.7	4.4	1	1	1	1	1	1
R1-1.1	3.6	1.52	1.22	1	1	1	1.46
R1-1.2	3.5	1.14	1	1	1	1.06	1.54
R1-1.3	3.8	1.4	1	1	1.22	1	1.28
R1-1.4	2.3	1.94	1	1.62	1	1	3.06
R1-1.5	3.1	1	1	1	1.3	1.12	2.08
R1-1.6	2.5	1.52	1	1	1	1	2.48
R1-1.7	2.9	1.84	1	1	1.66	1	1.98
R2-1.1	2.3	1	1	2.2	1	1	2.2
R2-1.2	2.8	1	1	1	1	1	2.54
R2-1.3	4.1	1.3	1	1	1	1	1.16
R2-1.4	2.8	1	1	1	1	1.82	2.24
R2-1.5	3.9	1.2	1	1	1.24	1	1
R2-1.6	2.1	1.34	1.24	1.24	1.9	1	3.3
R2-1.7	4.3	1	1	1	1	1	1

Table A2 (2): Sensory analysis result of mechanical altered milk samples analyzed on fifth day

Sample Name	Average of Quality score	Average of Harsh	Average of Bitter	Average of Sickening	Average of Cardboard	Average of Oxidized	Average of Rancid
T1-1.1	2.8	1	1	1	1.22	1	2.78
T1-1.2	2.2	1.52	1	1.4	1	1	2.76
T1-1.3	3.1	1.5	1	1	1.32	1	1.8
T1-1.4	3	2.18	1	1	1.62	1	1.86
T1-1.5	3.1	1	1	1.22	1.64	1	1.82
T1-1.6	2.6	1.22	1	1.42	1.6	1	2.86
T1-1.7	3.3	1.68	1	1	1.46	1	1.92
T2-1.1	3.7	1.6	1	1	1.68	1	1.36
T2-1.2	1.1	1	1.34	1	1	1	4.22
T2-1.3	3.8	1.36	1	1	1.54	1	1
T2-1.4	1.5	1.62	1.28	2.02	1.12	1	3.94
T2-1.5	2.9	1	1	1	1.42	1	2.18
T2-1.6	1.6	2.02	1	2.04	1.52	1	3.92
T2-1.7	3.2	1	1	1.12	1.54	1	1.42
Grand Total	3.07416	1.31388	1.04641	1.17512	1.22967	1.05885	1.95407

Table A3 (1): Sensory analysis result of milk samples kept at different temperatures analyzed after seventh day

Sample Name	Average of Quality score	Average of Sickening	Average of Cardboard	Average of Oxidized	Average of Salt	Average of Uncharacteristic
P1-1.1	4	1	1	1	1.425	1.45
P1-1.2	3.5	1	1.5	1.725	1.4	1.4
P1-1.3	4	1	1	1	1.425	1.425
P1-1.4	3.625	1.5	1.725	1	1	1.475
P2-1.1	4.25	1	1	1	1	1
P2-1.2	3.625	1	2.05	1	1.175	1.425
P2-1.3	3.75	1	1	1	1.225	1.225
P2-1.4	3.625	1.275	2.125	1	1	1
Q1-1.1	4.125	1	1	1	1	1
Q1-1.2	3.875	1	1.925	1.225	1	1
Q1-1.3	3.875	1	1.175	1	1	1
Q1-1.4	4.125	1	1.125	1	1	1
Q2-1.1	4.125	1	1.5	1.525	1	1
Q2-1.2	4	1	2.35	1	1	1
Q2-1.3	3.875	1.5	1	1	1	1
Q2-1.4	4	1	1.5	1.5	1	1
R1-1.1	3.5	1	1.4	1	1	1
R1-1.2	3.875	1.175	1	1	1	1
R1-1.3	3.875	1	1	1	1	1
R1-1.4	3.125	1.25	1.65	1.775	1	1
R2-1.1	4.25	1	1.5	1	1	1
R2-1.2	3.875	1	1	1	1	1
R2-1.3	3.75	1	2.525	1.325	1	1
R2-1.4	3	1.3	1.975	2	1	1

Table A3 (2): Sensory analysis result of milk samples kept at different temperatures analyzed after seventh day

Sample Name	Average of Quality score	Average of Sickening	Average of Cardboard	Average of Oxidized	Average of Salt	Average of Uncharacteristic
S1-1.1	4	1	1.975	1	1	1
S1-1.2	4	1	1.425	1	1	1
S1-1.3	3.875	1	2.55	1	1	1
S1-1.4	3.875	1	1	1.775	1	1
S2-1.1	4.125	1	1.925	1	1	1
S2-1.2	4	1	2.275	1	1	1
S2-1.3	3.875	1	1	1	1	1
S2-1.4	3.625	1	1.475	1.875	1	1
T1-1.1	4.125	1	1.5	1	1	1
T1-1.2	3.75	1	2	1.525	1	1
T1-1.3	4.125	1	1.275	1	1	1
T1-1.4	3.75	1.225	1	1.525	1	1
T2-1.1	4	1	1.15	1	1	1
T2-1.2	3.5	1.525	2	1	1	1
T2-1.3	3.875	1	2.075	1	1	1
T2-1.4	3.75	1	2	1.65	1	1
Grand Total	3.84688	1.06875	1.54125	1.18563	1.04125	1.06

Table A4 (1): Sensory analysis result of milk samples exposed to light of different wavelengths analyzed after 24 hours

Sample Name	Average of Quality score	Average of Harsh	Average of Sickening	Average of Oxidized	Average of Cardboard	Average of Uncharacteristic
T1B-1.1	3.625	1	1	2.05	1	1
T1B-1.2	3.375	1	1	2	1.5	1
T1B-1.3	3.625	1	1	1.725	1	1.3
T1G-1.1	3.625	1	1	1	1	1.525
T1G-1.2	3.875	1	1	1.375	1	1.3
T1G-1.3	2.625	1.55	1	3.6	1	1
T1O-1.1	3.5	1.725	1	1.675	1	1
T1O-1.2	2.875	1	1	2.475	1	1
T1O-1.3	2.5	1	1	2.85	1	1
T1R-1.1	4.125	1	1	1	1	1
T1R-1.2	4	1	1.65	1	1	1.525
T1R-1.3	4	1	1	1	1.75	1
T1X-1.1	2.75	1	1.175	3.125	1	1
T1X-1.2	3	1	1	3.225	1	1
T1X-1.3	2.375	1	1.5	4.025	1.5	1

Table A4 (2): Sensory analysis result of milk samples exposed to light of different wavelengths analyzed after 24 hours

Sample Name	Average of Main points	Average of Harsh	Average of Sickening	Average of Oxidized	Average of Cardboard	Average of Uncharacteristic
T2B-1.1	2.5	1	1	3.525	1	1
T2B-1.2	2.75	1	1	2.6	1.15	1
T2B-1.3	1.875	1	1	4.95	1.375	1
T2G-1.1	2.75	1.475	1	2.975	1	1
T2G-1.2	2.875	1	1	2.85	1.15	1
T2G-1.3	2.625	1	1	3.725	1	1
T2O-1.1	2.5	1	1.475	4.425	1	1
T2O-1.2	2.125	1	1	4.775	1.625	1
T2O-1.3	1.625	1	1.3	6.125	1.225	1
T2R-1.1	4	1	1	1	1	1
T2R-1.2	3.75	1	1.5	1.125	1.3	1
T2R-1.3	3	1.275	1	2.5	1	1
T2X-1.1	2.125	1	1	4.225	1	1
T2X-1.2	1.875	1.775	1	4.95	1.475	1
T2X-1.3	1.625	1	1	6.05	1	1.725
Grand Total	2.92917	1.09333	1.08667	2.93083	1.135	1.07917

Table A5: Scaling chart for quality control and descriptive analysis of milk

Quality score scale 1-5 (5 = milk according to the specifications):

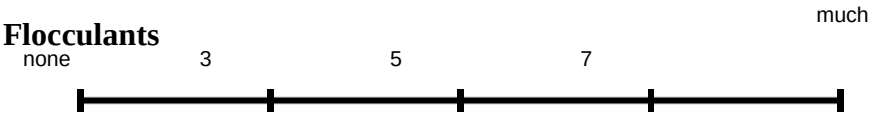
	5 points	**4,5 points**	**4 points**	**3,5 points**	**3 points**	**2,5 points**	**2 points**	**1,5 points**	**1 point**
Quality score	○	○	○	○	○	○	○	○	○

Comments:

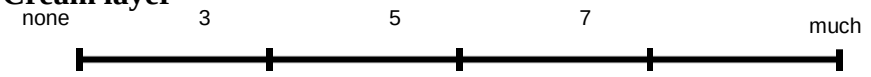
_____

Reply only if you have a remark:

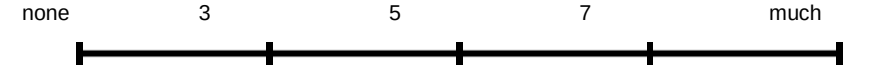
Flocculants



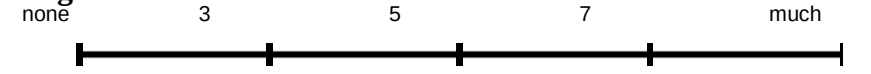
Cream layer



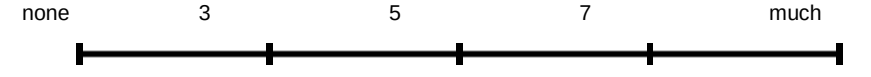
Cream adhesion



Coagulated



Bicolored



Foreign matter

none 3 5 7 much

Untypical color

none 3 5 7 much

Comments:

_____

Taste attributes

Sour

none 3 5 7 much

Sour off flavor

none 3 5 7 much

Harsh

none 3 5 7 much

Bitter

none 3 5 7 much

Sickening

none 3 5 7 much

Cooked

none 3 5 7 much

Burnt

much

none 3 5 7

Off flavor

none 3 5 7 much

Malty

none 3 5 7 much

Fruity

none 3 5 7 much

Cardboard

none 3 5 7 much

Feedy

none 3 5 7 much

Chemical

none 3 5 7 much

Oxidized

none 3 5 7 much

Rancid

none 3 5 7 much

Salt

none 3 5 7 much

Uncharacteristic

much

none 3 5 7

Watery

none 3 5 7 much

Acid

none 3 5 7 much

Goat flavor

none 3 5 7 much

Cow shed

none 3 5 7 much

Comments:

_____

Figure A6: Occurrence of sensory defects in milk samples during experiment1 (stored milk), experiment 2 (mechanical alteration), experiment 3 (light alteration) and experiment 4 (temperature alteration) observed during sensory analysis

Attributes	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Quality score	1			
Flocculants	1	1	1	
Cream layer	1			
Cream adhesion				
Coagulated				
Bicolored				
Foreign matter				
Untypical color				
Sour	1			
Sour off flavor	1	1		
Harsh	1	1	1	1
Bitter	1	1	1	1
Sickening	1	1	1	1
Cooked	1			1
Burnt				1
Off flavor	1	1	1	1
Malty	1	1	1	
Fruity	1		1	
Cardboard	1	1	1	1
Feedy				
Chemical	1		1	
Oxidized	1	1	1	1
Rancid	1	1	1	
Salt	1	1	1	1
Uncharacteristic	1	1	1	1
Watery				1
Acid			1	
Goat flavor				
Cow shed	1			

Appendix A7: BactoCount result of milk samples from five dairies stored at four different temperature

Sample code	Sample name	Temp. used(°C)	IBC	LOG (IBC)	(K)IBC	LOG(IBC)
P1-1.1	P1-4	4	15784	4.198217	15	1.176091
P1-1.2	P1-7	7	12115	4.083323	12	1.079181
P1-1.3	P1-10	10	21324	4.328869	21	1.322219
P1-1.4	P1-17	17	8791	3.944038	8	0.90309
P2-1.1	P2-4	4	31269	4.495114	31	1.491362
P2-1.2	P2-7	7	53309	4.726801	53	1.724276
P2-1.3	P2-10	10	40014	4.602212	40	1.60206
P2-1.4	P2-17	17	12434	4.094611	12	1.079181
Q1-1.1	Q1-4	4	49298	4.692829	49	1.690196
Q1-1.2	Q1-7	7	74621	4.872861	74	1.869232
Q1-1.3	Q1-10	10	25984	4.414706	25	1.39794
Q1-1.4	Q1-17	17	43381	4.6373	43	1.633468
Q2-1.1	Q2-4	4	45187	4.655014	45	1.653213
Q2-1.2	Q2-7	7	33773	4.52857	33	1.518514
Q2-1.3	Q2-10	10	41157	4.614444	41	1.612784
Q2-1.4	Q2-17	17	40774	4.610383	40	1.60206
R1-1.1	R1-4	4	17586	4.245167	17	1.230449
R1-1.2	R1-7	7	30515	4.484513	30	1.477121
R1-1.3	R1-10	10	17974	4.254645	17	1.230449
R1-1.4	R1-17	17	9902	3.995723	9	0.954243
R2-1.1	R2-4	4	2188	3.340047	2	0.30103
R2-1.2	R2-7	7	2940	3.468347	2	0.30103
R2-1.3	R2-10	10	2202	3.342817	2	0.30103
R2-1.4	R2-17	17	2572	3.410271	2	0.30103
S1-1.1	S1-4	4	24656	4.391923	24	1.380211
S1-1.2	S1-7	7	20931	4.32079	20	1.30103
S1-1.3	S1-10	10	17257	4.236965	17	1.230449
S1-1.4	S1-17	17	34197	4.533988	34	1.531479
S2-1.1	S2-4	4	12446	4.09503	12	1.079181
S2-1.2	S2-7	7	11385	4.056333	11	1.041393
S2-1.3	S2-10	10	9547	3.979867	9	0.954243
S2-1.4	S2-17	17	19489	4.28979	19	1.278754
T1-1.1	T1-4	4	16156	4.208334	16	1.20412
T1-1.2	T1-7	7	9537	3.979412	9	0.954243
T1-1.3	T1-10	10	12495	4.096736	12	1.079181
T1-1.4	T1-17	17	13594	4.133347	13	1.113943
T2-1.1	T2-4	4	20217	4.305717	20	1.30103
T2-1.2	T2-7	7	13578	4.132836	13	1.113943
T2-1.3	T2-10	10	18376	4.264251	18	1.255273
T2-1.4	T2-17	17	16158	4.208388	16	1.20412

Appendix 8: Information on tube light used in versatile environment test chamber

Specification	
Light color	White
Color temperature	4200 k
Length (mm)	1198
Glass tube diameter (mm)	28
Weight (g)	230
Base	G13
Rated lamp power (watt)	37
Lamp current (A)	0.410
Luminous flux (lm)	3100
Nominal life (hour)	12000
Fit lighting tube	FG-4P