Journal of Fluorescence (2019) 29:485–493
https://doi.org/10.1007/s10895-019-02359-3

ORIGINAL ARTICLE

Thermal Effects on Biochemical Signatures of UHT, Pasteurized and Domestically Boiled Buffalo Milk Detected by Synchronous Fluorescence Spectroscopy

Hina Ali[1] · Muhammad Saleem[1] · Rahat Ullah[1] · Saranjam Khan[2] · Babar Manzoor Atta[1] · Muhammad Bilal[1]

Received: 13 December 2018 / Accepted: 20 February 2019 / Published online: 3 March 2019

Abstract
Thermal treatment of milk is performed to limit bacterial growth and make it safe for human consumption. To increase the shelf life of milk, either ultrahigh temperature (UHT) or pasteurization techniques are employed that destroy the microorganisms. In this study, the synchronous front face fluorescence spectroscopy was employed for comparative study of raw, UHT treated, pasteurized and conventionally boiled milk at 93 °C (domestic boiling). Principal Component analysis clearly showed distinct clustering of UHT milk due to formation of Maillard reaction products. Fluorescence emission peak at 410 nm showed irreversible change in peak intensity attributed to conformational changes in protein due to UHT treatment while pasteurization and domestic boiling showed reversible changes when milk was cooled down to 10 °C. Furthermore, fluorescence emission peaks at 410 nm previously assigned to vitamin A has also been discussed.

Keywords Thermal treatment · Conventional boiling · Fluorescence spectroscopy · Vitamin A · Lipids · Proteins

Introduction

The milk from buffalo or bovine to buyer's home has been a tradition in the Sub-continent and most of the population prefers to buy fresh raw buffalo milk from milkman. Raw milk is vulnerable to protein hydrolysis due to proteolytic enzymes (plasmin) and bacterial proteases [1]. Oxidation of lipids also occurs in raw milk during storage and influenced by many factors like exposure to light, oxygen, metal ions and content of low molecular weight antioxidants etc. [2]. On the other hand, the demand for packaged and pasteurized milk is getting high throughout the world with the changing life style. The market for Ultra high temperature (UHT) treated milk was worth $60.8 billion as calculated in 2012 and was expected to reach approximately $137.7 billion in 2019 [3]. The reason for high demand of UHT milk is due to increased shelf life of milk as it can easily be stored outside refrigerator for up to 3 months, if remain unopened. Moreover, poor infrastructure for cold storage and transportation are driving the market for UHT milk.

UHT is the sterilization process with optimal combination of processing time and temperature to maximize destruction of microorganism, milk is heated at high temperature i.e. 280° F (138° C) for short duration (2–4 s) to kill any bacteria (harmful pathogens). On the other hand, another thermal treatment to reduce number of pathogens is Pasteurization in which either milk is heated at low temperature for long time (145° F or 63° C for 30 min) or commonly at high temperature for short time (160° F or 71° C for 15 s) followed by rapid cooling [4].

Milk is a good source of proteins, B vitamins and minerals; study reported that commercial processing of milk causes no drastic effect on milk fat, fat soluble vitamins and minerals while protein and water-soluble vitamins are affected [5]. Other studies reported that due to high temperature treatment of milk, non-enzymatic reaction occurs between free amino group of protein (mainly lysine residues) and carbonyl group of reducing sugar (lactose) resulting in different Maillard reaction products (MRPs). Such reaction between sugar and proteins also occurs in the body (bloodstream) through a glycation process producing harmful compounds also known as advanced glycation end products (AGEs) in vivo. In the early stage of Maillard reaction,

✉ Hina Ali
hinali991@hotmail.com

[1] National Institute of Lasers and Optronics (NILOP), Lehtrar Road, Islamabad, Pakistan

[2] Department of Physics, Islamia College, Peshawar, Pakistan

Amadori products (lactulosyllysine) and later heterocyclic nitrogenous brown colored compound melanoidins are produced [6]. Acid hydrolysis of lactulosyllysine results in the formation of furosine (Nε-2- furoylmethyl-l-lysine) which is stable MRP and carboxymethyl-lysine (CML) is produced at the later stages as advanced MRP. These chemical markers are proposed to assess the effects of thermal treatment [7, 8]. This complex process not only occurs in milk but also in different food processed at high temperature or during storage to generate different taste and aroma [9].

Amadori products change the bioavailability of lysine milk protein and one of the breakdown products from them is HMF (Hydroxymethylfurfural) which acts as chelating agents thus blocks the mineral availability also. Lysine (an essential amino acid) damage due to Maillard reaction has been well reported and its loss increases during thermal treatment [10–12]. Moreover, dietary MRPs add to AGEs thus accumulation of such compounds in living organisms may lead to onset of metabolic complications [9].

Due to health risks posed by MRPs, its detection in thermally treated milk is done with biochemical reactions [13, 14]. Fluorescence spectroscopy has also been employed as a reliable and potential technique for molecular characterization and chemical analysis of dairy products as it contains intrinsic fluorophores with specific excitation and emission spectra. Intrinsic fluorophores in dairy products are amino acids such as tryptophan, tyrosine and phenylalanine in proteins, vitamins A and B2 (riboflavin), NADH and various compounds in low concentration [15]. The assessment of MRPs with quick analysis has been established through fluorescence spectroscopy [16–18]. Quality control of milk is also being assessed with the production of MRPs; hence furosine, HMF and lactulose concentrations are best indicators of MRPs and increase during heat treatment [19–22].

Thermal treatment also affects heat labile vitamins, proteins and riboflavin. Fluorophores like alkaline phosphatase, NADH and flavin adenine dinucleotide (FADH) that act as coenzymes along with tryptophan fluorescence emission have been reported to determine whey protein (β-lactoglobulin) in heat treated milk samples [23]. Many other studies have also focused on fat, one of the main constituents of milk; studies reported the fluorescence emission of fat soluble vitamin A at 410 nm when excited at 325 nm [15, 24–28]. Thus, fluorescence spectroscopy has a potential to determine chemical alterations during storage and thermal treatment in dairy products.

In this study we have focused on the biochemical changes in the composition of milk that have occurred during domestic and commercial thermal processing of milk by applying Synchronous fluorescence spectroscopy (SFS). Simultaneous scanning of excitation and emission wavelength with continuous wavelength interval denoted by $\Delta\lambda$ occurs in SFS. SFS portrays a chemical profile of complex mixtures of molecules that overlaps in classical emission fluorescence [14]. We have analyzed raw and thermally treated buffalo milk and observed reversible and irreversible biochemical changes in proteins that helped us in re-assigning the previously reported fluorescent peaks.

Materials and Methods

Sample Preparation

Top brands of UHT treated and pasteurized buffalo milk of different batches were purchased from market. Fresh raw milk samples from farmers were also collected and brought to laboratory in a cool environment then boiled to 93° C ± 2 °C for 10 min, which is a common domestic practice before milk consumption. The milk samples have been taken from different retailers and different batches (UHT and pasteurized) whereas raw milk samples from farmers and milk shops repeatedly to confirm the reproducibility of results.

Acquisition of Fluorescence Spectra

Fluorescence excitation and emission from all milk samples was acquired on FluoroMax®-4 (HORIBA scientific, Jobin Yvon, USA) with a software fluoroEssence™. The system is equipped with front face cuvette holder adjusted at an angle of 56° to incident excitation radiation. A continuous light excitation source is a 150-W ozone-free xenon arc-lamp. The signal detector is a photomultiplier tube. Synchronous fluorescence spectroscopy is used for detailed chemical characterization of complex samples having mixture of fluorescent compounds. This technique involves the simultaneous scanning of excitation and emission monochromators with a constant wavelength interval ($\Delta\lambda$) between them. All milk samples were recorded at excitation wavelength ranging from 200 to 650 nm with 1 nm increment in a 3 ml quartz cuvette. The spectra for milk samples were recorded for excitation and emission wavelengths with a constant $\Delta\lambda$ of 10 nm and tested range was set from 10 to 100 nm. The excitation and emission slits width were set at 3 nm and 2 nm respectively. Emission fluorescence of 114 milk samples was recorded in duplicate for authenticating the data.

Pre-Processing of Spectral Data

All the emission spectra were preprocessed by using self-written codes in MatLab (release MATLAB 2014a) for removing the noise and normalizing of all the spectra. Principal component analysis (PCA) was also applied to analyse the pre-processed data for classification based on their spectral similarities and dissimilarities using MatLab (2014a).

Results and Discussion

Spectroscopic Analysis

Synchronous spectra recorded for milk samples were initially tested for excitation and emission wavelength with constant intervals of 10 nm using offset range of 10 to 100 nm. The value of wavelength interval (Δ λ) was set at 90 nm, as it generated optimum fluorescence intensity and discrimination of all fluorophores present in the milk samples without any noise. In this study we are discussing effect of thermal treatment on different intrinsic fluorophores previously reported for milk.

The fluorescence of folded protein in raw milk is mostly due to aromatic amino acid i.e. tryptophan (Trp) residue of β-lactoglobulin (β-Lg) [29]. The emission peak for Trp was observed at 380 nm with λ_{ex} 290 nm in SFS (Fig. 1), contrary to the previous studies that reported emission at 330–340 nm for protein due to Trp [14, 30, 31]. In conventional fluorescence spectroscopy, we have also observed the excitation spectrum for Trp at 290 and 322 nm for buffalo milk when emission was set at 340 nm (data not shown) for the same set of samples. Shifting of emission peak from 340 nm to 380 nm in SFS might be due to cumulative effect of intrinsic fluorophores like interaction of other whey proteins with β-Lg in a specific environment. A shoulder has also been seen at 370 nm possibly assigned to vitamin B6 (Pyridoxine) previously reported to generate fluorescence emission at 370 nm when λ_{ex} was 250 or 325 nm [32, 33].

Slight increase in Trp and vitamin B6 fluorescence intensity in pasteurized milk and increase in vitamin B6 intensity only after conventional boiling was observed as compared to UHT milk where sharp decrease was seen (Fig. 2). Previous study on goat and bovine milk reported that fluorescence intensity of Trp increased with increasing temperature [34]. The slight increase in Trp intensity in pasteurized and conventionally boiled milk corresponds to the unfolding of whey protein and exposure of Trp residues, whereas very high temperature could lead to protein denaturation that resulted in lesser intensity of Trp fluorescence in UHT treated milk [35]. Very slight shift in Trp fluorescence emission peak towards lower wavelength in all thermally treated milk samples was seen that might be due to tryptophan exposure to more hydrophobic environment as reported [36]. Thermal treatment causes exposure of buried hydrophobic groups of protein that fluoresce differently from hydrophilic ones [14, 37]. Moreover, thermal treatment above 60 °C induces denaturation of whey proteins (β-lactoglobulin and α-lactalbumin), amino acid side chain modification, enzyme inactivation and casein micelles alteration. These changes result in the altered functionality of milk ingredients but are also dependent on temperature, time, pH and original composition of milk [37].

Fluorescence emission peak at 410 nm has been ascribed to fat soluble vitamin A with excitation maximum at 325 nm in many previous studies [15, 24, 26, 28]. There are multiple forms of vitamin A but it mostly occurs in the form of retinol [38]. Positive correlation has also been reported between this vitamin peak and fat intensity [39]. However, a discrepancy also lies with pure vitamin A excitation and emission peaks at 325/470 nm in ethanol [40] and 346/480 nm in iso-octance [41]. However it shifts to lower wavelength at 463 nm in biological system due to binding with retinol binding protein

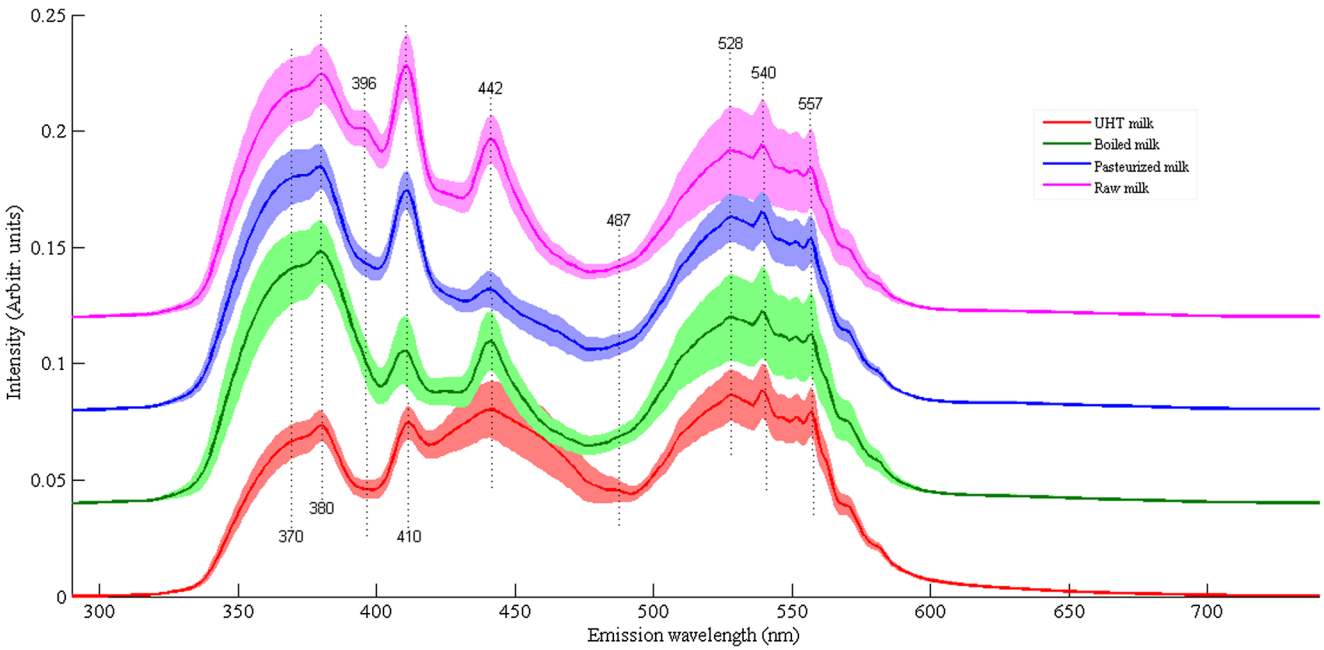

Fig. 1 Synchronous fluorescence mean spectra of different thermally processed and raw milk samples

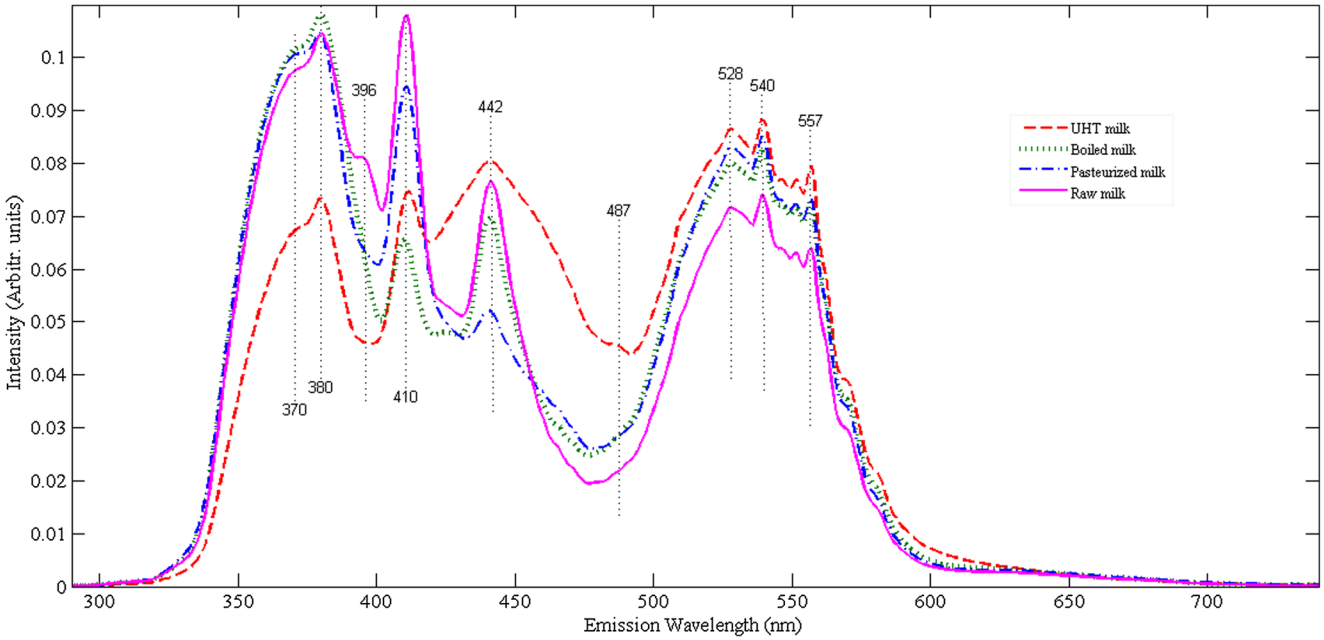

Fig. 2 Comparative fluorescence spectra of different thermally processed and raw milk samples

[42]. Therefore, we performed an experiment to reconfirm this peak for vitamin A.

Vitamin A is a fluorescent molecule and its excitation and emission depend upon triglycerides in fat globules [43]. In order to validate the emission peak at 410 nm, we added commercially available drops of vitamin A in raw milk with three different concentrations i.e. C1 (800 IU), C2 (1600 IU) and C3 (2400 IU). Synchronous fluorescence spectra were recorded after thorough mixing of sample in comparison with raw milk as depicted in Fig. 3. The effect of adding vitamin A in raw milk was emergence of fluorescence emission peak at 460 nm that positively correlated with increasing concentration of vitamin A. However, gradual decrease was observed in peak intensity at 410 nm, thus suggesting that the peak at 410 nm doesn't originate due to vitamin A fluorescence. The proposed mechanism behind observed stepwise decrease in the emission peak at 410 nm might be the fluorescence quenching in the presence of increased concentration of added vitamin A as both emission peaks (410 and 460 nm) share the same excitation wavelength (325 nm). Whereas decrease in

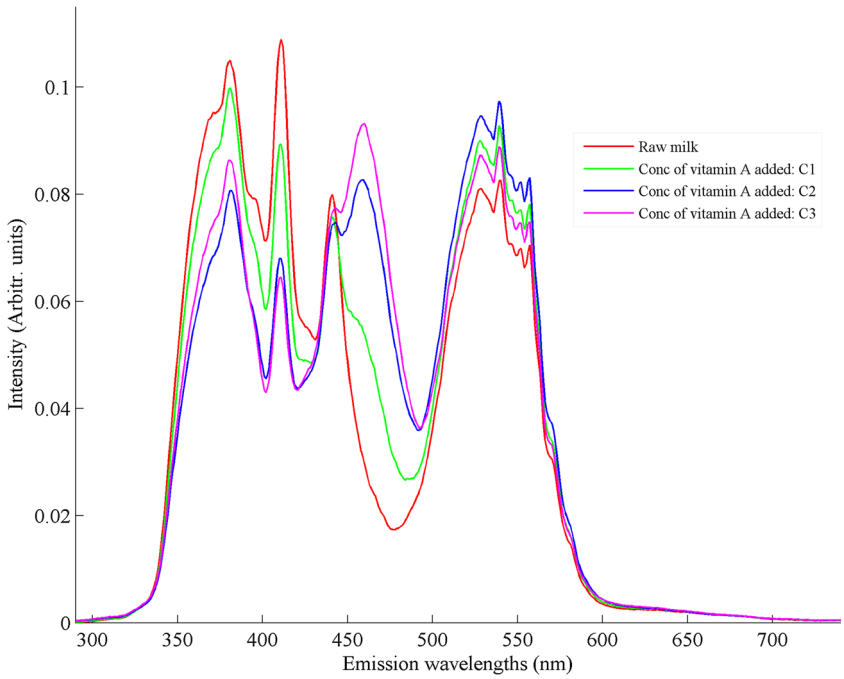

Fig. 3 Fluorescence emission spectra of raw milk with three different concentrations of vitamin A added. Emission peak at 460 nm originated after adding vitamin A showing successive increase in intensity with increasing concentration of vitamin A

Trp fluorescence emission in the milk sample is attributed to the conformational changes in protein structure due to change in the surrounding medium with the addition of exogenous vitamin A.

Interestingly a reversible change was observed for the fluorescence emission at 410 nm in boiled milk when cooled down to 10 °C by placing it in fridge. To reconfirm this change, raw milk was boiled at 93 °C for 10 min then gradually cooled down; fluorescence spectra of these milk samples were recorded at different temperatures i.e. 30 °C, 20 °C and 10 °C. The comparative spectra for the peak at 410 nm at different temperatures clearly showed the reversible biochemical changes in this region (Fig. 4) after thermal treatment below 100 °C and same happened in pasteurized milk. Hence, we, suggest that this peak belongs to protein as refolding of milk protein to its native structure at low temperature has also been observed in the previous study [35]. The fluorescence peak with $\lambda_{ex} = 325$ nm and $\lambda_{em} = 410$ nm might be due to dityrosine as Tyrosine (amino acid) is vulnerable to Reactive oxygen species (ROS) leading to the formation of dityrosine [44], formed during exposure of milk to light. Dityrosine is considered as a marker for protein oxidation and its fluorescence emission has been recorded at 410 nm [45]. Riboflavin based photo-oxidation in the presence of oxygen resulted in the formation of dityrosine that originates from intercrossing of tyrosine in milk [45–47]. Dityrosine has also been reported in humans as a marker for oxidative stress [44].

Our results are in accordance with the previous study [48] published in 1974 in which crude lipid extract from milk did not show the fluorescence of retinol (vitamin A). It was masked by the fluorescence generated by unidentified substance at 415 nm that was removed after saponification to obtain the emission of retinol at 480 nm. Later, invitro studies [49, 50] in 1990 and 1994 were conducted on the binding affinity of milk protein (β-Lg) for retinol and fatty acids, retinol was proposed to be embedded in hydrophobic pockets of β-Lg. In these studies, fluorescence of β-Lg–retinol complex showed emission at 475/480 nm and the conformational changes (protein foldings) in native environment completely dropped the fluorescence emission. We may assume that the above mentioned unidentified substance (λ_{em} at 415 nm) that quenched retinol fluorescence was protein. This region shares the same excitation wavelength (325 nm) with vitamin A, therefore fluorescence emission peak at 410 nm might be the probable reason for considering it from vitamin A in all the published studies on milk or cheese due to embedded retinol in β-Lg.

Boiling of milk sample at 93 °C caused a sharp decrease in fluorescence intensity of the peak at 410 nm and same effect was observed in UHT milk, whereas slight decrease was shown in pasteurized milk samples. In raw milk, the enzyme Lactoperoxidase (Lp) in the presence of reactive oxygen species (generated due to lipid oxidation) can cause production of dityrosine. A study on milk has also reported the generation of dityrosine as endogenous Lp enzyme was activated in unpasteurized milk after addition of H_2O_2 [47]. UHT milk lacks Lp as it is degraded during thermal treatment that's why low concentration of dityrosine was detected in UHT milk. This phenomenon needs further study and clarifications.

Maillard-reaction products in the milk samples were recorded at λ_{ex} 360 nm and emission wavelengths ranging from 380 to 480 nm. Maximum emission for MRPs is denoted by 440 nm in various studies [51, 52]. In another study [17] on

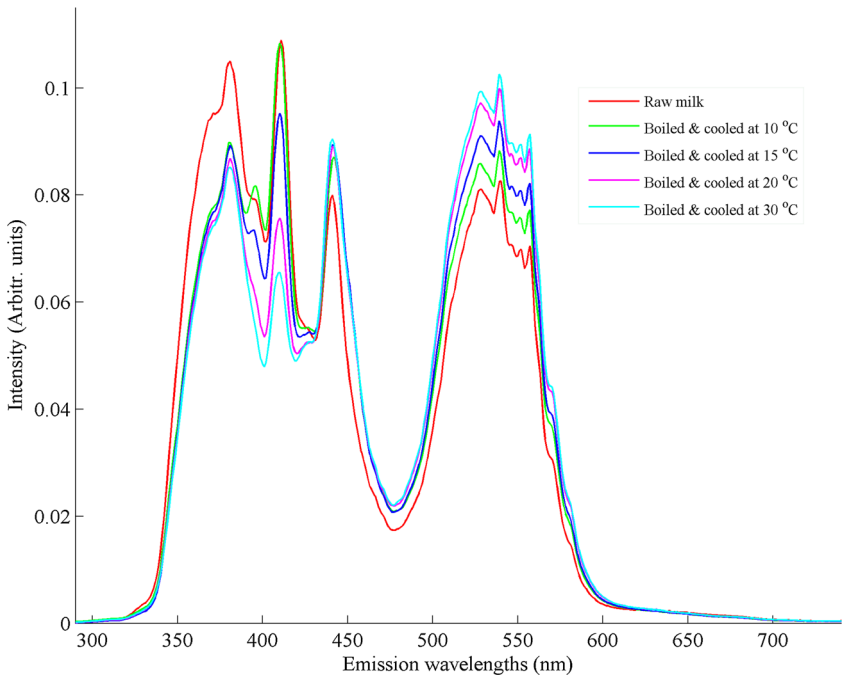

Fig. 4 Fluorescence emission spectra of raw milk and boiled milk that cooled down to 10 °C after boiling showing reversible changes in peak at 410 nm

raw buffalo milk correlated the sharp peak at 440 nm with NADH, [53] reported this peak as of fat soluble vitamin A as observed in buffalo milk while a small hump in cow milk is suggestive of less quantity of vitamin A in it. Cow milk has yellowish tinge as it contains beta carotene (precursor of vitamin A) which lacks in buffalo milk. Exposure of milk to light and air (oxygen) results in the formation of singlets reactive oxygen that hydrolysed fatty acids in milk. We suggest this peak as oxidized product of fatty acid as has also been reported for edible oils [54]. The presence of peak at 440 nm in raw milk might be due to oxidative damage to fatty acids to form hydroperoxides in the presence of light and air. Agitation and high temperature induce lipolysis and rancidity of free fatty acids [55]. Slight decrease in fluorescent intensity of this peak occurred after conventional boiling at 93 °C but such degradation is more obvious in pasteurized milk samples despite of low thermal treatment i.e.72 °C. It might be due to the mixing of cow milk in commercially available pasteurized milk samples having low fluorescence intensity of this peak [53]. The increased fluorescence signals and bandwidth in UHT processed milk sample in this region corresponds to cross linked compounds for MRPs [1] as discussed earlier.

Another small peaks with emission at 487 nm can be seen only in UHT milk samples (Fig. 2), which is suggestive of vitamin A. Irreversible Protein denaturation occurred in UHT milk samples exhibited by low intensity at 410 nm emission peak due to high temperature treatment. This protein denaturation might cause partial dissociation of retinol-β-Lg complex liberating the retinol that generated faint fluorescence at 487 nm.

Lastly a broad noisy band ranging from 520 to 560 nm with three emission peaks at 528, 540 and 557 nm have been observed. The suggestive emission peak for Riboflavin (vitamin B_2) is located at 520 nm in milk [14, 25, 56]. Increased fluorescence intensity was observed for this region in boiled, pasteurized and UHT milk samples that showed oxidation in milk after thermal treatment as compared to raw milk samples. The spectrum of riboflavin is a good indicator for oxidation in processed cheese and milk as it acts as a singlet oxygen sanitizer in the presence of light (non-enzymatic) to cause protein oxidation [1].

Statistical Analysis

Figures 1 and 2 show that the fluorescence spectra of UTH treated milk are clearly different from raw, boiled and pasteurized milk samples. However, fluorescence spectra of raw, boiled and pasteurized milk differed at few fluorescence emission bands. Therefore, apparently it looks difficult to conclude about the molecular changes occurred in boiled milk in comparison with non-heated raw milk. In order to classify all four milk types, principal component analysis (PCA) was applied on all the data which nicely separated UTH treated milk from the rest as depicted in Fig. 5. Principal component analysis is a non-supervised statistical procedure which uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of uncorrelated variables called principal components. These principal components are calculated based on the variance in the data. When these principal

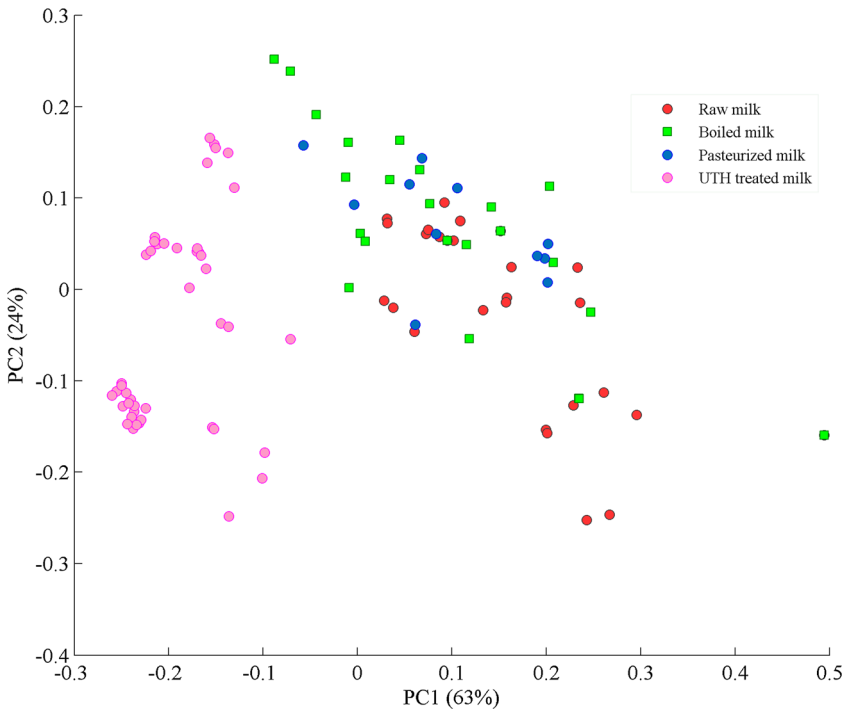

Fig. 5 PCA (Principal component analysis) score plot of fluorescent milk data based on Principal Component 1 (PC1) and Principal Component 2 (PC2) showing clear separation of UHT milk samples from rest of milk samples

components are plotted as a scatter plot, they nicely separate different set of data.

The clear separation of UTH treated milk from raw, boiled and pasteurized milk is explained by the score plot between first two principal components PC1 and PC2 that showed variance as 63% and 24% respectively (Fig. 5). These principal components have been calculated based on dissimilarities in spectral data that comes up from protein denaturation and production of Maillard reaction products at high temperature in UTH treated milk. In addition, there is a nice distribution of data points of raw, boiled and pasteurized milk in such a way that raw milk data points are slightly clustered on one side of boiled and pasteurized milk samples which are almost mixed due to few spectral variations. However, UTH treated milk data points are completely separated from the rest of three types of milk samples.

It is further confirmed with the PCA factors loadings that were obtained from normalized fluorescent spectra of raw and thermally treated milk samples as presented in Fig. 6. UHT milk is separated from other milk samples with main loadings with positive bands at 366, 380, 410 nm and negative bands at 432 and 462 nm that correspond to Trp, dityrosine and Maillard reaction products respectively. MRPs in UHT milk are generated due to interaction with lactose and protein as mentioned earlier alongwith decrease in Trp and dityrosine intensity accounted to high thermal treatment. PC2 showing negatively contributing components with a slight shift in protein (dityrosine) band having emission at 411 nm in UHT treated milk and the Maillard reaction products band at 442 nm. Another positive loading with a broad region of riboflavin fluorescence also contributed to discriminate milk samples with relative intensity variation subjected to thermal treatment.

Conclusion

This study demonstrated that the fluorescence emission peak at 410 nm in milk samples indirectly corresponds to vitamin A (previously reported in many studies). The previous studies showed that originally vitamin A generates fluorescence from 460 to 480 nm and is embedded in fatty acids and both have binding affinity with β-Lg. In milk, this fluorescence is masked by the fluorescence generated at 410 nm. We suggest this peak at 410 nm belongs to protein as depicted reversible change in fluorescent intensity that was temperature dependent. It may be β-Lg–retinol complex or retinol binding protein in which tyrosine residues may be cross-linked to synthesize fluorescent molecule (dityrosine) that generates fluorescence at 410 nm. Dityrosine is a biomarker for protein oxidation and has been reported in foodstuff like milk and milk derived products. Still this area needs further research and confirmation.

Thermally processed sterilized milk either UHT or pasteurized is consumed throughout the world due to safe for transportation to far distances and to retailers shop for consumers without spoilage as compared to raw milk. Though the milk subjected to these heat treatments has increased shelf life but on the other hand is subjected to chemical alteration and non-enzymatic browning. Sugar and protein rich milk generates Maillard reaction products due to high temperature treatment. Raw milk on the other hand is conventionally boiled at home before consumption has less effect on nutritional quality as

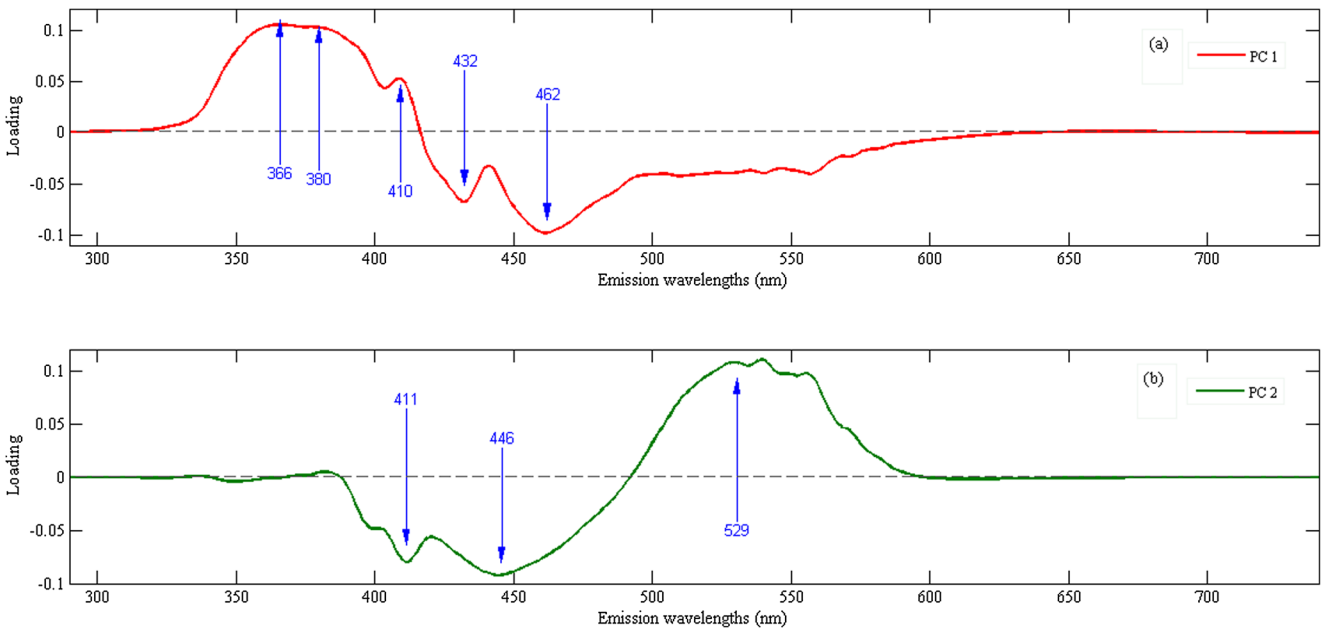

Fig. 6 PCA loadings for the significant components used to discriminate florescence spectrum that classified different milk samples

compared to UHT milk. Nutritional quality of even raw milk ingredients like proteins and lipids are affected by heat, light and oxygen as observed using fluorescence emission spectra and have also been reported in literature. Therefore, great care should be taken in transportation of milk like in chillers to far off places and pasteurized milks should be preferred as compared to UHT milk as it showed less nutritional loss. Still there is a room for further study in this area regarding proteolysis and oxidation of proteins and lipids respectively in raw milk.

Acknowledgements The authors are thankful to our Scientific Assistants namely Mrs. Fatima Batool (SA-II) and Mr. Muhammad Irfan (SA-II) for their help in collection of milk samples and experimentation.

References

1. Wiking L, Nielsen JH (2004) The influence of oxidation on proteolysis in raw Milk. J of Dairy Res 71(2):196–200
2. Nielsen JH , Ostdal H, Andersen HJ (2002) The influence of ascorbic acid and uric acid on the oxidative stability of raw and pasteurized milk. In: Morello Mj, Shahidi F, Ho CT, editors. Free radicals in food: Chemistry, nutrition and health effects. Washington, DC: ACS Symposium Series, American Chemical Society 126–137
3. Global UHT Milk Market is Expected to Reach USD 137.7 Billion by 2019: Transparency Market Research (2014), http://www.transparencymarketresearch.com/. Accessed July 2018
4. Ritota M, Di Costanzo MG, Mattera M, Manzi P (2017) New trends for the evaluation of heat treatments of Milk. J of Anal Methods in Chem 2017:1–12
5. Sharma SK, Mittal GS, Hill AR (1994) Effect of milk concentration, pH and temperature on k-casein hydrolysis at aggregation, coagulation and curd cutting times of ultrafiltered milk. Milchwissenschaft 49:450–453
6. Tamanna N, Mahmood N (2015) Food processing and Maillard reaction products: effect on human health and nutrition. Int J of Food Sci & Tech https://doi.org/10.1155/2015/526762
7. Rufiàn-Henares JA, Guerra-Hernández E, García-Villanova B (2002) Maillard reaction in enteral formula processing: furosine, loss of o-phthaldialdehyde reactivity, and fluorescence. Food Res Int 35:527–533
8. Bastos DM, Monaro É, Siguemoto É, Séfora M (2012) Maillard Reaction Products in Processed Food : Pros and Cons. Food Industrial Processes - Methods and Equipment Croatia: InTech. pp 281–300
9. Barbosa JHP, Oliveira SL, Seara LT (2008) The role of advanced glycation end-products (AGEs) in the development of vascular diabetic complications. Braz Arch Endocrin Metab 52:940–950
10. O'Brien J, Morrissey PA (1989a) Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit Rev Food Sci Nutr 28:211–248
11. O'Brien J, Morrissey PA (1989b) The Maillard reaction in milk products. Intl Dairy Fed Doc 238:53–61
12. Van Boekel MAJS (1998) Effect of heating on Maillard reaction in Milk. Food Chem 62:403–414
13. Shimamura T, Uke H (2012) Maillard reaction in Milk - effect of heat treatment. Milk protein W. L. Hurley, ed. InTechOpen Science, London, UK pp147–158
14. Boubellouta T, Dufour É (2008) Effects of mild heating and acidification on the molecular structure of Milk components as investigated by synchronous front-face fluorescence spectroscopy coupled with parallel factor analysis. Appl Spectrosc 62:490–496
15. Karoui R, Dufour E, De-Baerdemaeker J (2007) Monitoring the molecular changes by front face fluorescence spectroscopy throughout ripening of a semi-hard cheese. Food Chem 104:409–420
16. Bosch L, Alegría A, Farré R, Clemente G (2007) Fluorescence and color as markers for the Maillard reaction in Milk-cereal based infant foods during storage. Food Chem 105:1135–1143
17. Kulmyrzaev AA, Levieux D, Dufour É (2005) Front-face fluorescence spectroscopy allows the characterization of mild heat treatments applied to Milk. Relations with the denaturation of Milk proteins. J of Agricultural and Food Chem 53:502–507
18. Herbert S, Riou NM, Devaux MF, Riaublanc A, Bouchet B, Gallant DJ, Dufour E (2000) Monitoring the identity and the structure of soft cheeses by Fluorcence spectroscopy. Lait 80:621–634
19. Corzo N, Lopez-Fandino R, Delgado T, Ramos M, Olano A (1994) Changes in furosine and proteins of UHT-treated milks stored at high ambience temperatures. Z Lebensm Unters Forsch 198:302–306
20. Corzo N, Delgado T, Troyano E, Olano A (1994) Ratio of lactulose to furosine as indicator of quality of commercial milks. J Food Protect 57:737–739
21. Andrews GR (1984) Distinguishing pasteurized, UHT and sterilized milks by their lactulose content. J Soc Dairy Technol 37:92–95
22. DeRafael D, Villamiel M, Olano A (1997) Formation of lactulose and furosine during heat treatment of milk at temperatures of 100–120 °C. Milchwissenschaft 52:76–78
23. Shaikh S, O'Donnell C (2017) Applications of fluorescence spectroscopy in dairy processing: a review. Current Opinion in Food Sci 17:16–24
24. Karoui E, Riaublanc A (1997) Potentiality of spectroscopic methods for the characterization of dairy products. Lait 77:657–670
25. Karoui R, Dufour E, Pillonel L, Schaller E, Picque D, Cattenoz T, Bosset J-O (2005) The potential of combined infrared and fluorescence spectroscopies as a method of determination of the gerographic origin of emmental cheeses. Int Dairy J 15:287–298
26. Karoui R, Laquet A, Dufour E (2003) Fluorescence spectroscopy: a tool for the investigation of cheese meltings correlation with rheological characteristics. Lait 83:251–264
27. Kulmyrzaev A, Dufour E, Noël Y, Hanafi M, Karoui R, Qannari EM, Mazerolles G (2005) Investigation at the molecular level of soft cheese quality and ripening by infrared and fluorescence spectroscopies and chemometricssrelationships with rheology properties. Int Dairy J 15:669–678
28. Hougaard AB, Lawaetz AJ, Ipsen RH (2013) Front face fluorescence spectroscopy and multi-way data analysis for characterization of Milk pasteurized using instant infusion. LWT - Food Sci and Tech 53:331–337
29. Albani JR, Vogelaer J, Bretesche L, Kmiecik D (2014) Tryptophan 19 residue is the origin of bovine β-lactoglobulin fluorescence. J of Pharm and Biomed Anal 91:144–150
30. Dufour E, Mazerolles G, Devaux MF, Duboz G, Duployer MH, Riou MN (2000) Phase transition of triglycerides during semi-hard cheese ripening. Intl Dairy J 10:81–93
31. Liu X, Metzger LE (2007) Application of fluorescence spectroscopy for monitoring changes in nonfat dry Milk during storage. J of Dairy Sci 90:24–37
32. Hui Y, Xue X, Lan H, Hou Z (2016) Study on fluorescence spectra of B vitamins 160–165.International conference on mechanics,

Materials and structural engineering (ICMMSE). Atlantis Press: 160–165
33. Yang H, Xiao X, Zhao X, Hu L, Lv C, Yin Z (2016) Study on fluorescence spectra of thiamine, riboflavin and pyridoxine. Seventh international symposium on precision mechanical measurements, edited by Liandong Yu, 99030H · SPIE vol.9903. https://doi.org/10.1117/12.2211248
34. Pásztor-Huszár K (2008) Protein changes of various types of Milk as affected by high hydrostatic pressure processing. Corvinus University of Budapest, Budapest, Dissertation
35. Akkerman M (2012) The effects of heating processes on Milk whey protein denaturation and rennet coagulation processes. Aarhus University, Denmark, Dissertation
36. Andersen CM, Mortensen G (2008) Fluorescence spectroscopy: a rapid tool for analyzing dairy products. J of Agri and Food Chem 56:720–729
37. Augustin MA, Udabage P (2007) Influence of processing on functionality of Milk and dairy proteins. Advances in Food and Nutr Res 53:1–38
38. Dufour E, Riaublanc A (1997) Potentiality of spectroscopic methods for the characterisation of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks. Lait 77:657–670
39. Andersen CM, Frøst MB, Viereck N (2010) Spectroscopic characterization of low- and non-fat cream cheeses. Intl Dairy J 20:32–39
40. Duggan DE, Bowman RL, Bradie BB, Udenfriend SA (1957) Spectrometric study of compounds of biological interest. Arch. Biochem. Biophys 68:1–14
41. Christensen J, Nørgaard L, Bro R, Engelsen SB (2006) Multivariate auto-fluorescence of intact food systems. Chem Reviews 106: 1979–1994
42. Goodman S, Leslie RB, Frythe T (1971) Fluorescence studies of human plasma retinol-binding protein and of the retinol-binding protein-prealbumin complex. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 260:670–678
43. Herbert S, Riou NM, Devaux MF, Riaublanc A, Bouchet B, Gallant DJ, Dufour E (2000) Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. Lait 80:621–634
44. Heineckes JW, Li W, Daehnke HL, Goldstein JA (1993) Dityrosine, a specific marker of oxidation. J Biol Chem 268:4069–4077
45. Bekard IB, Dunstan DE (2009) Tyrosine autofluorescence as a measure of bovine insulin fibrillation. Biophys J 97:2521–2531
46. Dalsgaard TK, Nielsen JH, Brown BE, Stadler N, Davies MJ (2011) Dityrosine, 3,4 Dihydroxyphenylalanine (DOPA), and radical formation from tyrosine residues on Milk proteins with globular and flexible structures as a result of riboflavin-mediated photo oxidation. J of Agri and Food Chem 59:7939–7947
47. Østdal H, Bjerrum MJ, Pedersen JA, Andersen HJ (2000) Lactoperoxidase-induced protein oxidation in Milk. J of Agri and Food Chem 48:3939–3944
48. Thompson JN, Erdody P, Maxwell WB, Murray TK (1972) Fluormetric determination of vitamin a in dairy products. J Dairy Sci 55:1077–1080
49. Dufour E, Haertlle T (1990) Alcohol-induced changes of b-lactoglobulin/retinol binding stoichiometry. Protein Eng 4:185–190
50. Dufour E, Genot C, Haertle T (1994) B-Lactoglobulin binding properties during its folding changes studied by fluorescence spectroscopy. Biochem Biophysics Acta 1205:105–112
51. B-Aragon I, Nicolas M, Metais A, Marchond N, Grenier G, Calvo DA (1998) Rapid fluorimetric method to estimate the heat treatment of liquid milk. Int. Dairy J 8:771–777
52. Feinberg M, Dupont D, Efstathiou T, Louâpre V, Guyonnet J-P (2006) Evaluation of tracers for the authentication of thermal treatments of milks. Food Chem 98:188–194
53. Ullah R, Khan S, Ali H, Bilal M, Saleem M (2017) Identification of cow and Buffalo Milk based on Beta carotene and vitamin-a concentration using fluorescence spectroscopy. PLoS One 18;12: e0178055. https://doi.org/10.1371/journal.pone.0178055
54. Kyriakidis NB, Skarkalis P (2000) Fluorescence spectra measurement of olive oil and other vegetable oils. J of AOAC Intl 83:1435–1439
55. Ahmad I, Shaheen M, Mijid N, Ali S, Rashid AA (2011) Lipolysis , hydrolytic and oxidative rancidity in raw milk 59–62. In: Conference proceedings
56. Skibsted LH (2000) Light induced changes in dairy products. In packaging of milk products. Int. dairy fed. Brussels, Belgium 346: 3–9