Fourier transform infrared spectroscopy investigation of batch pasteurized milk molecules

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Abstract

The study investigated the effects of stirring on the milk molecular arrangements in a batch pasteurized milk. Three variables were considered with 3x3x2 factorial levels. The stirrer shapes considered were anchor, vane and helical; the orientation was single and twice the stirrer shapes while the varied speeds were 30 rpm, 36 rpm and 42 rpm. Standard batch pasteurizing temperature of 63 degree Celsius for 30 minutes was considered. The experimental outcome at 95% significance showed that these treatments did not create new bonds, however, statistically significant changes were observed in few selected bond frequencies for NH, OH and CN. The experiment showed different organic molecules at different frequencies and wavelengths. The raw sample which was used as the first control, gave similar outcome as the second control which was raw milk pasteurized without stirring. The following frequencies were obtained for the raw sample with the other samples showing slight deviations in values after the decimal place; amine group ⟩N– H which stretch at frequency *(*3449.20 cm^{-1}*)*, methylene group, C–H *(*2920.45 cm^{-1}*)*, methyl, C–H stretch *(*2853.96 cm^{-1}*)*, terminal alkyne group, C□C, *(*2103.57 cm^{-1}*)*, secondary amine bend *(*1644.47 cm^{-1}*)* and aromatic ring, C=C–C *(*1460.83 cm^{-1}*)*. However, methyl, C–H bend of frequency 1365.85 cm^{-1}, is in the wave frequency as aromatic tertiary amine and primary amine, indicating their presence. Also, O–H group was observed at frequency 1248.70 cm^{-1} with secondary amine, CN stretch at frequency 1156.88 cm^{-1}.

Keywords: Bond, Molecular, Orientation, Shape, Speed

Introduction

Milk is an emulsion, containing fat globules that dispersed into liquid phase at temperature above 45°C and starts crystalizing at tempearature below this temperature. However, milk casein micelle is stable when exposed to heat for a longer period of time without coagulating (Hui et al., 2007). The stability of these nutritional and or molecular components to heat treatments, are of major concern. Too much heat treatment and improper handling as well as under treatment can either cause damage to these components or microbial or enzyme activities.

Food rheology deals with the deformation and flow of food materials due to the action of external forces. Fluid foods are heterogeneous materials, containing many molecules (Krokida et al., 2001). The modification of raw milk contents as seen in fermented milk according to Espírito-Santo et al., (2012) can influence it texture, rheology and sensory properties.When food is worked on, the molecular contents are rearranged to take new form in appearance or taste. This could be understood by an example of the shearing process, which according Teggartz and Morris (1990), influences the rheology and microstructure of ropy yoghurt, a product of raw milk. This subsequently influences the yoghurt casein content which

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influences the viscosity. However, Fellows (2000) stated that the action of a mixer does not have a direct effect on the nutritional makeup or the shelf span of a food but may indirectly affect the components by allowing thorough mixing as seen in food like bread, dough and pastries. In addition, for product like milk, rancid flavour may develop due to too much agitation of raw milk (Deeth and Fitz-Gerald, 2006).

In order understand the behavior of batch pasteurized milks' molecules to the treatment combinations of this study under consideration, which involved mixing and heat treatments, Fourier Transform Infrared (FTIR) spectroscopy, was employed. In other words, this experiment aimed at investigating milk bond behavior to mechanical (stirring) and heat treating (pasteurization). The FTIR is a nondestructive method for milk analysis that works by expressing vibrational modes of covalent bonds in molecules and it allows a quick analysis of several different components of using absorbs IR radiation (Etzion et al., 2004).

Material and methods

1. Experimental procedure

About 50 L of raw milk samples was bought from the local Fulani herders in Ilorin, and transported in ice block coolers to the Food Engineering, pilot plant of the University of the Ilorin, Nigeria, where the experiment took place. Two liters of milk was fed into the pasteurizer (Figure 1) and held for 30 minutes at 63 degree Celsius after the milk was screened using fine net to remove dirt. Total of 18 samples were pasteurized while varying the treatment combinations of speed, stirrer shapes and stirrer orientation, however, one sample was pasteurized without stirring which is used as a control in conjunction with the raw sample that had no treatment. A 3×3×2 factorial experiment in a Completely Randomized Design (CRD) was used in this research work. Few frequencies from the FTIR chat were subjected to statistical analysis using Statistical Package for Social Science (SPSS) 20.0 at 95% confidence.

Fourier transform infrared (FTIR) tests

The FTIR test was carried out in the Chemistry Laboratory of the University of Ilorin, Kwara State. The pasteurized milk samples were ran through the thermo scientific NICOLE iS5 FTIR spectrometer **(**Figure 2**)**. The spectrometer has a spectra range of 7800 to 350 cm^{-1}, sensitivity of 2200-2100 cm^{-1}, power source of 100 to 120 VAC, 50/60 Hz and a spectra resolution greater than 0.8 cm^{-1}.

Figure 1: Setup of the batch pasteurizer

Figure 2: Thermo Scientific Nicolet iS5 FTIR Spectrometer

Results and discussion

The milk sample pasteurized without stirring in Figure 3 which is the second control, the following were obtained; amine group $>N-H$ which stretch from aromatic amine to heterocyclic amine at frequency (3474.41 cm-1) when compared to the raw sample. The methylene group, C–H (2964.64 cm-1) and Methyl C–H stretch (2923.48 cm-1) remained in the same frequency range as the raw sample but with new frequencies. However, the terminal alkyne group, C□C, (2074.93 cm-1) showed characteristics aromatic combination band. Secondary amine, NH bend (1641.16 cm-1), aromatic ring stretch, C=C–C (1460.69 cm-1), methyl, C–H (1375.20 cm-1) and O–H (1245.38 cm-1) remained in their frequency range with secondary amine, CN stretch changing at frequency 1245.38 cm-1 changing from secondary aromatic amine to primary aromatic amine. Also, primary amine CN stretch was observed to remain in range at 1071.24 cm-1 while changing to secondary amine at 1163.06 cm-1 indicating that the amine group is increased or strongly influenced by the interactions between the variable parameters, with vinyl C–H out-of-plane bend being replaced by primary amine at frequency (1039.58 cm-1). These new frequencies indicate an increase or decrease in organic behaviour of the milk samples. In a study by Borgo et al., (2015) using pasteurized human milk; similar trends in change in frequencies were observed for various molecules in the pasteurized milk samples. However, from the study there were no variations in speed was reported rather only the effect of pasteurization on the donated human milk were reported to have caused these shifts in frequencies or bonds. Also, the amount of water in milk may affect it sensory properties, for milk product with high water content is more likely to coalesce than that with low water content (Rønholt et al., 2014) and while the milk fat is subjected to high temperature other than the room temperature, some fractions of the low melting fatty acids is likely to melt and escape from the molecular crystals (Rønholt et al., 2013). This may also explain while the milk molecules and the fatty acid groups responded to mechanical and heat treatment during batch pasteurization.

Vélez et al., (2010), in a similar study reported that pasteurization and agitation significantly affect the molecular properties of pasteurized milk. However, the extent of agitation as not reported. For the single anchor stirrer at 30 rpm in Figure 4, amine group $>N-H$ which stretch at frequency (3442.74 cm-1), methylene group, C–H (2917.15 cm-1), methyl C–H stretch (2853.83 cm-1) and remained in their frequency range with slightly different frequencies as observed in the two control samples. However, terminal alkyne group, C□C, (2090.77 cm-1) showed characteristics aromatic combination band. However, secondary amine bend (1637.99 cm-1), aromatic ring, C=C–C (1470.18 cm-1), methyl, C–H (1372.03 cm-1), O–H (1235.88 cm-1), secondary amine, CN stretch (1172.56 cm-1), primary amine CN stretch at 1096.57 cm-1 and 1017.41 cm-1 with vinyl C–H out-of-plane bend (966.76 cm-1) remained in frequency range with different frequency with different frequency band compared to two controls.

For the single anchor stirrer at 36 rpm in Figure 5, amine group $>N-H$ which stretch at frequency (3471.21 cm-1), methylene group, C–H (2923.48 cm-1), methyl C–H stretch (2856.99 cm-1), terminal alkyne group, C□C, (2087.60 cm-1), secondary amine bend (164.16 cm-1), aromatic ring, C=C–C (1467.02 cm-1), methyl, C–H (1375.20 cm-1), O–H (1245.38 cm-1), secondary amine, CN stretch (1159.89 cm-1), primary amine CN stretch at 1071.24 cm-1 and 1033.25 cm-1 with vinyl C–H out-of-plane bend (988.92 cm-1) remained in frequency range with different frequency band compared to the two controls. This increase or decrease in the frequency of stretch indicate an increase or decrease in the molecular contents such as protein value, phenol value or amino groups etc. Kaylegian et al. (2009) and Khan et al. (2018) reported similar molecular behaviour for pasteurized milk on fatty acid chain length and trans fatty acid.

Li-Chan (2007) reported the spectrum behaviour in pasteurized milk using Raman Spectroscopy which makes use of high photon energy light similar to Fourier transform spectroscopy, observed changes in the frequency bands of heated milk for 80 degree Celsius for 60 minutes, for phenol, C-H groups and polypeptide chain whose bands moved from 935 cm-1 to 944 cm-1 at pH 7.0 which is similar to this study. This is also similar to Larsson (1996) who reported similar changes in milk fat. Major changes were also observed in bovine serum albumin and oil, for C-H bending of as well as C=C, and COOR stretching modes of the oil (Meng et al., 2006). The oil and water interface in milk reduces when heat is applied, thereby increasing cohesive interfacial film around oil droplets. For this reason, milk proteins are useful as emulsifiers in foods (Holt and Rogniski, 2001). In addition, stretching or compression of these bonds makes it easy to adjust milk molecular profile.

However, similar trends can be seen in all the other samples in this study, as they all remain in their frequency range with differences in their frequency bands. Wen et al. (2012) stated that any treatment or technique that disturbs the balance among milk components will impact directly, the rheological properties of milk product such as yoghurt, like the increase in protein content leads to an increase in thixotropy of the yoghurt and the increase or decrease of milk fat will increase or decrease the viscoelastic properties of yoghurt. Furthermore, this study shows that, a new substance or molecular bond has not been created, rather adjustments in the frequencies values either to stretch or compress the carbon to hydrogen bond (C–H), nitrogen to hydrogen bond $>N-H$ and oxygen to hydrogen bond (O–H) etc., due to the study treatment. This shows that each experimental treatment has an individual effect on the milk samples as could be observed in Table 1 and 2, with few exceptions. This can be thought of as the rearrangement of bonds rather the creation of new bonds.

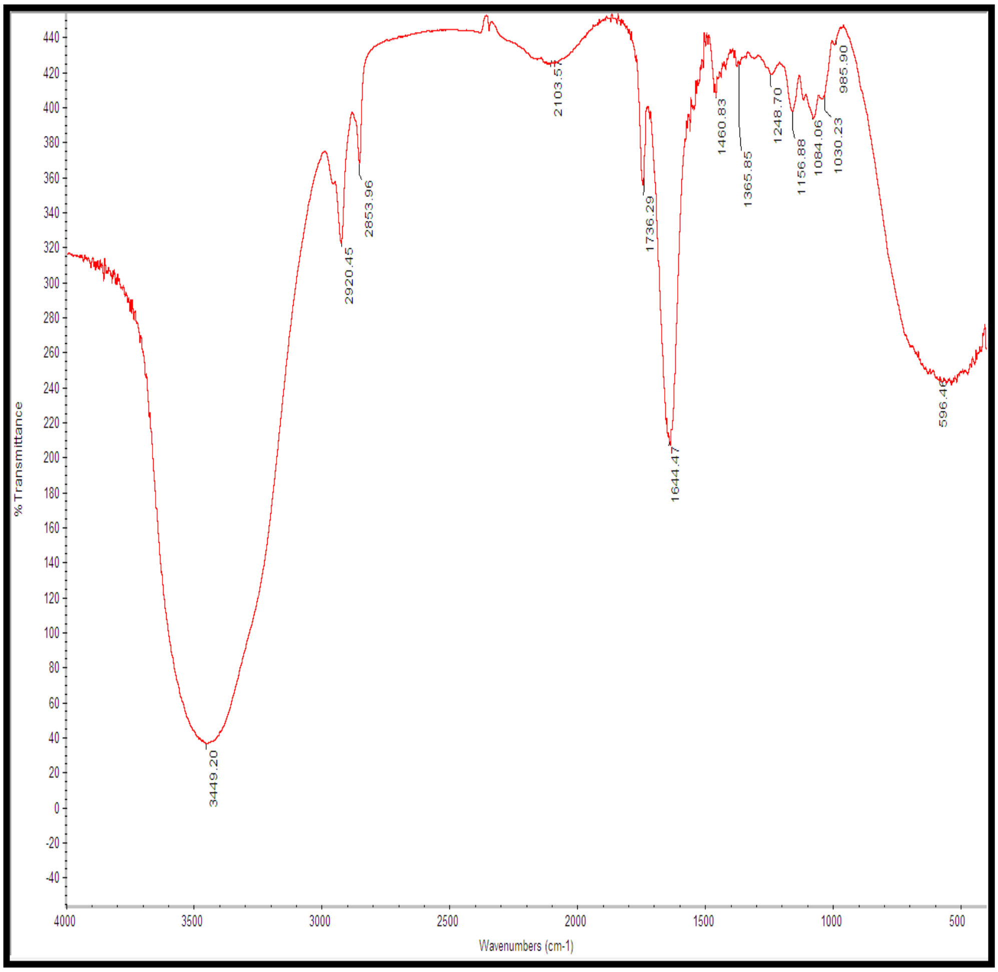

Figure 3: Fourier transform infrared spectroscopy chart of the raw milk sample

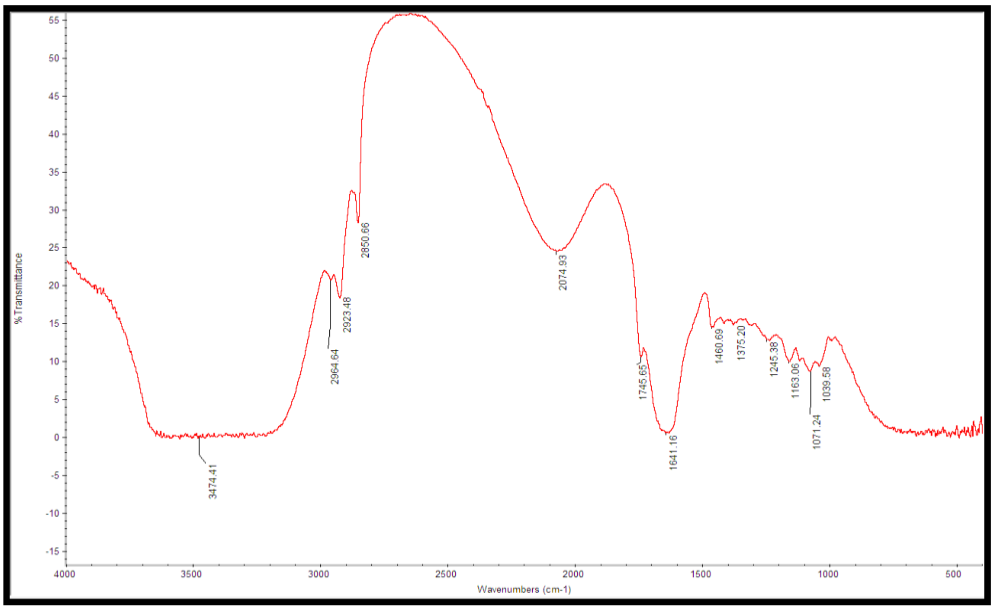

Figure 4: Fourier transform infrared spectroscopy chart of the milk pasteurized without stirring

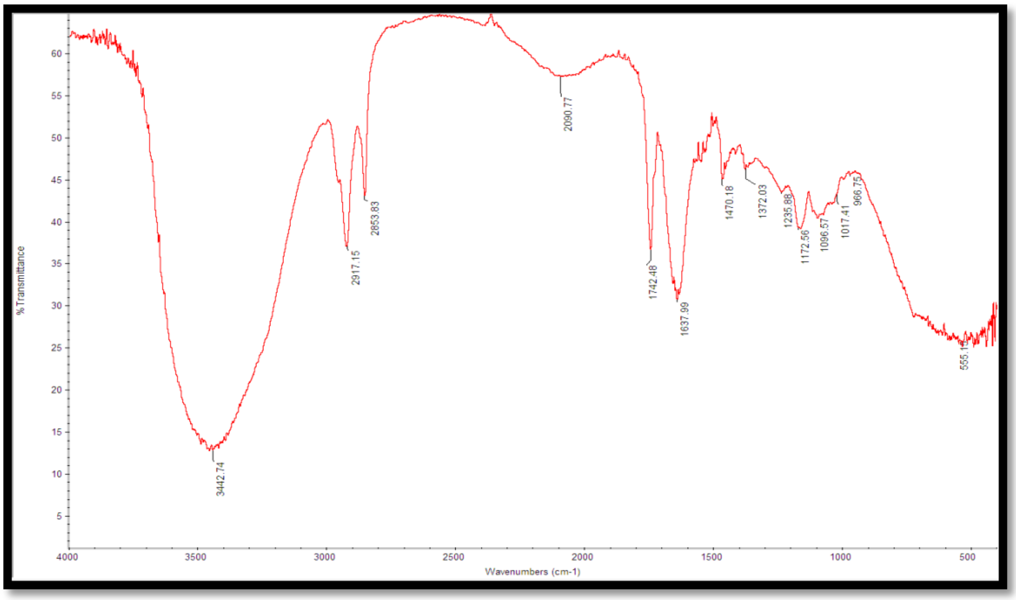

Figure 5: Fourier transform infrared spectroscopy chart of the interactions between single anchor stirrer at 30 rpm

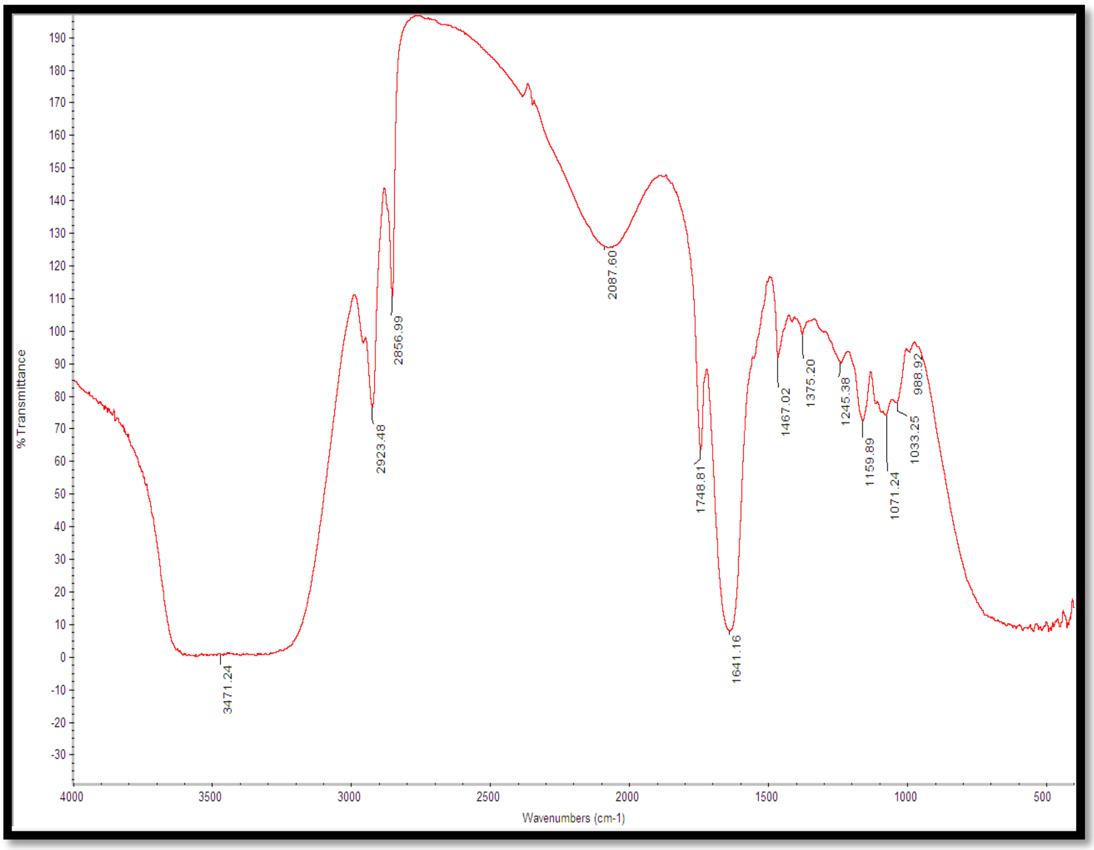

Figure 6: Fourier transform infrared spectroscopy chart of the interactions between single anchor stirrer at 36 rpm

Table 1: Summary of the analysis of variance of the N-H, O-H and CN stretch bond frequencies

Source	Dependent Variable	df	F	Sig.
M-A	N-H	2	1.548E4	0.000*
	O-H	2	7.400	0.005*
	CN Stretch	2	3.426	0.055
N-B	N-H	1	8.384E4	0.000*
	O-H	1	18.148	0.000*
	CN Stretch	1	20.773	0.000*
S-C	N-H	2	1.331E5	0.000*
	O-H	2	12.680	0.000*
	CN Stretch	2	9.071	0.002*
A* B	N-H	2	3.425E4	0.000*
	O-H	2	3.187	0.065
	CN Stretch	2	17.307	0.000*
A * C	N-H	4	5.637E4	0.000*
	O-H	4	22.014	0.000*
	CN Stretch	4	17.329	0.000*
B * C	N-H	2	1.004E5	0.000*
	O-H	2	21.886	0.000*
	CN Stretch	2	13.089	0.000*
A * B * C	N-H	4	3.643E4	0.000*
	O-H	4	14.769	0.000*
	CN Stretch	4	15.939	0.000*
Error	N-H	18		
	O-H	18		
	CN Stretch	18		
Total	N-H	36		
	O-H	36		
	CN Stretch	36		

Keys: Stirrer shapes (A), Stirrer orientation (B), Speed (C)

Table 2: New Duncan multiple range test for some molecular bond frequencies

	Factors	N-H	O-H	CN stretch
	Anchor stirrer	3.4564a	1.2403a	1.1620ab
	Helical stirrer	3.4554b	1.2389a	1.1630a
Stirrer shapes (A)	Vane impeller	3.4485c	1.2425b	1.1607b
	Single stirrer	3.4476a	1.2390a	1.1636a
Stirrer orientation(B)	Double stirrers	3.4592b	1.2422b	1.1603b
	30 rpm	3.4420a	1.2382a	1.1610a
	36 rpm	3.4511b	1.2406b	1.1607a
Speed (C)	42 rpm	3.4670c	1.2429c	1.1641b

Conclusion

In conclusion, it could be inferred from study that heat treatment in combination with medium to slight agitation and whatever the shape or orientation of the stirrer in a batch pasteurizer may be, will likely not damage the molecular arrangement or cause an untold reaction in batch pasteurized milk sample.

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