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Original Article

Effects of holder pasteurization operating parameters on pasteurized raw milk quality

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ABSTRACT

A batch pasteurizer could be considered a closed system and it mostly makes use of heat, stirring, or agitation and speed variation as a process parameter for pasteurization. This study investigates if these parameters support the holder pasteurization method using White Fulani cow breed milk samples. The milk samples were pasteurized at 63°C for 30 minutes with slight but insignificant variations in pH values up to 7.55. The stirring speeds used in this study were 30 rpm, 36 rpm, and 42 rpm. The results showed a significant difference in the microbial loads and phytochemical values due to the study treatments. The microbial loads varied between 1.05* 10^4CFU/ml to 8.25*10^7 CFU/ml while the phytochemical values were between 0.12 mg/ml to 27.67 mg/ml. The milk samples were poor in phenol and flavonoid but their differences were significant at $p \leq 0.05$ after pasteurization. The speed of 30 rpm and 36 rpm did not show a significant difference at $p \leq 0.05$ in the fungi counts after the pasteurization. The blade shapes considered were anchor, helical, and vane. These blade shapes used contributed to the holder pasteurization process.

1. Introduction

Milk has to be processed by an appropriate heat treatment method to reduce microbial loads [1] and disrupt the activities of enzymes to a level acceptable for human consumption [2] to be stored. Milk pasteurization is a technique that combines heat treatment and time as variables to reduce or eliminate microorganisms' population to acceptable health and storage quality [3]. In this process, not all the microorganisms are eliminated and the heat treatments may induce several changes in milk quality while focusing on microbial load reduction [4][5]. During milk processing, stirring, agitation, shear, or high-pressure heat treatment may affect milk and milk products' physical properties like turbidity, viscosity, fat particle size changes, and creaming and may not have direct effects on milk chemical properties [6][7]. The process of stirring or agitation helps to improve the process reaction and homogenize milk components [8]. However, if the heating temperature is too high it may affect milk's antioxidants (e.g. phytochemicals), fat structure, protein profile, milk's texture, and colour [9][10][11]. Stirring is a vital aspect of handling milk products as it supports the production process. Stirring prevents acid formation during yogurt production, it supports the fermentation process of milk products and combines with it, to affect milk viscosity [12]. This study investigates if stirring supports batch pasteurization or has no effects on the overall nutritional quality of batch pasteurized milk samples.

In this study, batch or holder pasteurization involves processing milk samples at 63°C for 30 minutes. Pasteurization at a higher temperature can affect milk appearance, colour, flavour, and other sensory properties [13]. Pasteurization helps to maintain milk quality and in

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addition to the process, high sanitary levels and milking procedures are also considered to maintain good milk quality. Therefore, to maintain high nutritional value after pasteurization, all sanitary conditions must be observed [14][15].

2. Materials and Methods

2.1. Milk sample collection

White Fulani cow breed milk samples were manually collected early in the morning from the Fulani cattle farms in Oke-Odo, Ilorin, Kwara State, Nigeria. These milk samples were then sieved before being transported to the university of Ilorin food engineering laboratory in coolers laced side by side with ice blocks. Figures 1 and 2 show the batch pasteurizer setup.

2.2. Pasteurization procedure

A total of nineteen (19) samples were pasteurized. The pasteurization process involved heating the raw milk samples in the batch pasteurizer (Figure 1) at 63°C for 30 minutes, after which small pasteurized samples were taken through the pasteurizer tap for laboratory analysis. Eighteen of the samples were completely randomized, while one of the samples was pasteurized but unstirred (U) or agitated (one of the two controls). These treatments were carried out using a 3 x 3 x 2 factorial design with two replicates. The other control was the raw sample (R) with two replicates. The pasteurizer was designed with various detachable blades of various shapes to accommodate blade replacement. The detachable blades (Figure 3) were vane-shaped, helical (helix), and anchor shaped. These blade shapes helped to control the mixing pattern within the batch pasteurizer.

Nine small subsamples were taken from the 19 pasteurized samples for immediate microbial analysis. The remaining pasteurized 19 samples were stored in a refrigerator for 12 days after pasteurization before their nutritional and microbial analysis.

2.2.1. Microbial load determination

The media and culture preparation as well as the identification and characterization of fungi and bacteria isolates were done according to the technique described by Fawole and Oso [16].

2.2.2. Determination of Nutrients Quality

The protein, fat and phytochemical contents were determined as recommended by the AOAC method [17]. Also, the pH values were determined using the handheld HANA pH meter (HI19813-6, Romania, USA).

2.3. Statistical analysis

The statistical summary, analysis of variance (ANOVA), and Duncan's New Multiple Range Test (DNMRT) of the data generated was done using Statistical Package for Social Science (SPSS) 25.

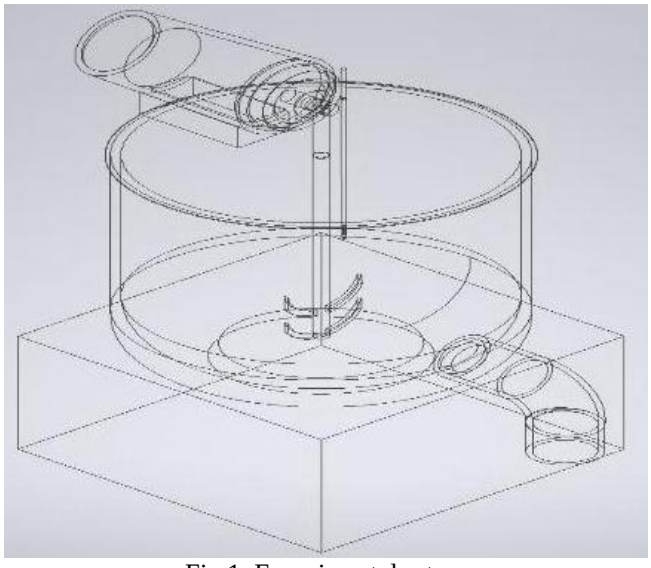

Fig 1. Experimental setup

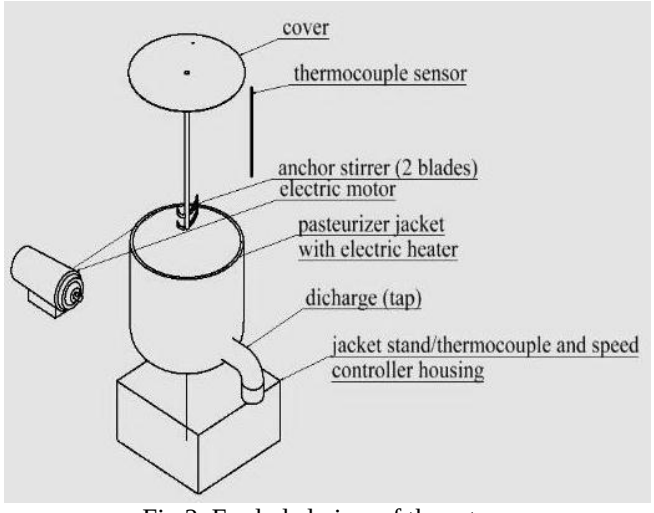

Fig 2. Exploded view of the setup

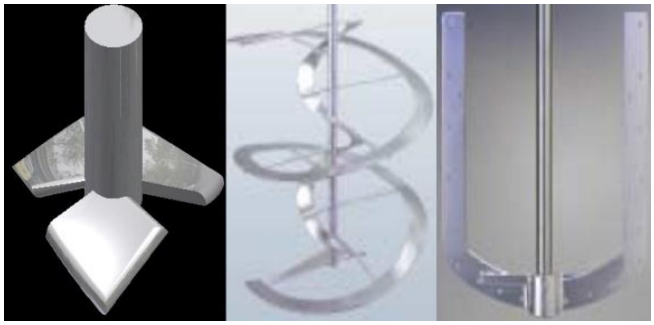

Fig 3. Vane, helical and anchor stirrer (left to right)

3. Results and Discussion

3.1. Effects of pasteurization parameters on microbial loads

The microbial loads were higher in the control samples (Table 1). The pasteurized samples analyzed immediately after pasteurization showed a significant reduction in microbial loads (Table 2) at $p \leq 0.05$. However, the milk samples showed an increase in microbial loads after storing them in the refrigerator for 12 days. This could as a result of post-pasteurization contamination [18].

The microbial loads decreased significantly in Figure 4 when the data generated from the experiment were compared to the raw sample (R) and the sample pasteurized without stirring (U) at $p \leq 0.05$ after 12 days of storage. Table 3 and Table 4 show these variations. At speeds of 30 rpm and 36 rpm, the decrease in fungi counts remained insignificant at $p \leq 0.05$ (Table 5). Also, the pH varied from 6.30 to 7.55 after the 12 days of storage, but these variations were not significant at $p \leq 0.05$ for speeds of 30 rpm and 36 rpm (Table 4). The pH also remained insignificant for the anchor and vane-type blade at $p \leq 0.05$. The pH values increased 12 days after pasteurization. This could result from lactic acid production and post-contamination of the milk samples [19].

At the batch temperature of 63°C and holding time of 30 minutes, stirring components showed reducing effects on the microbial loads. These loads varied between $5.75*10^5$ CFU/ml to $1.0*10^4$ CFU/ml after 12 days of storage. These reducing effects might have been indirect effects due to the interactions between speed, the blade type, and the number of blades on a stirrer. This is because the samples pasteurized without stirring showed more microbial loads when compared to other samples pasteurized involving stirring.

This study agrees with Bhanduriya *et al.* [20] and Sunmonu *et al.* [21], as they reported a significant reduction in microbial values due to holder pasteurization. Lee *et al.* [22] are of the opinion that if raw milk initially has a low level of microbial load, pasteurization might have no significant effect on the milk samples. However, the interactions between speed and number of stirrer blades were not significant on yeast changes and the corresponding pH (Table 4 and Table 5). The controlled samples pasteurized without stirring were more viscous at the bottom of the pasteurizer with some parts getting burnt at the base. This was a possibility of lack of stirring effects during holder pasteurization.

Table 1: Data generated for the controlled samples

Sample	TVC	TCC	LBC	FC	YC	pH
R	$8.25*10^7$	$4.55*10^7$	$2.85*10^6$	$5.45*10^7$	$3.15*10^6$	6.50
PU	$5.75*10^5$	$3.90*10^5$	0.00	$1.15*10^6$	$1.00*10^5$	6.30

Where (R) is the raw sample and the (PU) is the sample pasteurized without stirring

*Total viable counts (TVC), Total coliform counts (TCC), Lactobacillus counts (LBC), Fungi Counts (FC), Yeast counts (YC), Number of blades (BN), Speed in revolution per minute (S)

Table 2: Summary statistics of data generated without storage for single blade

Blade	BN	S	TVC	TCC	LBC	FC	YC	pH
Anchor	1	30	1.80±0.07	1.35±0.01	0.00±0.00	1.90±0.00	0.98±0.00	6.50±0.00
		36	2.95±0.01	1.68±0.04	0.00±0.00	0.00±0.00	1.58±0.00	6.70±0.00
		42	2.90±0.07	1.75±0.07	0.00±0.00	1.28±0.04	1.15±0.07	6.50±0.00
Helix	1	30	3.10±0.04	1.93±0.04	1.28±0.04	1.53±0.04	0.55±0.07	6.50±0.00
		36	2.66±0.01	1.94±0.01	0.00±0.00	1.52±0.02	0.65±0.00	6.50±0.00
		42	4.31±0.08	2.53±0.04	0.00±0.00	1.13±0.04	1.75±0.07	6.50±0.00
Vane	1	30	2.75±0.00	1.15±0.07	1.25±0.07	2.03±0.04	1.65±0.07	6.50±0.07
		36	2.16±0.01	1.35±0.07	1.15±0.07	1.43±0.04	1.00±0.07	6.30±0.07
		42	2.93±0.04	1.33±0.04	1.05±0.07	1.21±0.01	1.00±0.00	6.40±0.00

Total viable counts (TVC*****10^5CFU/ml), Total coliform counts (TCC*****10^5CFU/ml), Lactobacillus counts (LBC*****10^5CFU/ml), Fungi Counts (FC*****10^5CFU/ml), Yeast counts (YC*****10^4CFU/ml), Number of blades (BN), Speed in revolution per minute (S)

Table 3: Summary statistics of the microbial data generated after 12 days of storage

Blade	BN	S	TVC	TCC	LBC	FC	YC	pH
Anchor	1	30	4.60±0.00	2.95±0.07	1.45±0.07	5.80±0.00	1.20±0.00	7.30±0.00
		36	5.20±0.00	3.55±0.07	2.45±0.07	2.00±0.00	3.95±0.07	7.20±0.00
		42	5.75±0.07	3.90±0.00	2.85±0.07	5.45±0.07	3.15±0.07	6.80±0.00
	2	30	5.80±0.00	2.65±0.07	2.95±0.07	3.35±0.07	1.65±0.07	7.50±0.00
		36	3.45±0.07	1.75±0.07	1.30±0.00	2.90±0.00	2.40±0.00	7.25±0.07
		42	7.15±0.07	4.00±0.00	2.05±0.07	4.00±0.00	1.15±0.07	7.55±0.07
Helix	1	30	3.05±0.07	1.95±0.07	1.25±0.07	2.75±0.07	1.00±0.00	7.45±0.07
		36	8.65±0.07	4.90±0.00	0.00±0.00	4.50±0.00	3.20±0.00	7.40±0.00
		42	8.25±0.07	4.55±0.07	0.00±0.00	1.15±0.07	1.00±0.00	7.40±0.00
	2	30	6.45±0.07	3.75±0.07	0.00±0.00	1.20±0.00	0.00±0.00	7.35±0.07
		36	4.65±0.07	2.40±0.00	2.05±0.07	1.80±0.00	1.15±0.07	7.50±0.00
		42	3.90±0.00	1.75±0.07	0.00±0.00	0.00±0.00	0.00±0.00	7.40±0.00
Vane	1	30	4.75±0.07	3.00±0.00	2.95±0.07	4.05±0.07	4.55±0.07	7.05±0.07
		36	2.15±0.07	1.30±0.00	1.10±0.00	4.45±0.07	1.80±0.00	7.25±0.07
		42	2.05±0.07	1.40±0.00	1.00±0.00	5.20±0.00	1.05±0.07	7.40±0.00
	2	30	3.75±0.07	1.95±0.07	0.00±0.00	2.55±0.07	1.95±0.07	7.30±0.00
		36	7.75±0.07	4.30±0.00	3.55±0.07	3.85±0.07	4.30±0.00	7.35±0.07
		42	5.85±0.07	3.45±0.07	1.65±0.07	1.45±0.07	1.80±0.00	6.95±0.07

*Total viable counts (TVC*****10^5CFU/ml), Total coliform counts (TCC*****10^5CFU/ml), Lactobacillus counts (LBC*****10^5CFU/ml), Fungi Counts (FC*****10^5CFU/ml), Yeast counts (YC*****10^4CFU/ml), Number of blades (BN), Speed in revolution per minute (S)

Table 4: Multivariate analysis of the microbial data generated

Parameter	DV	TVC	TCC	LBC	FC	YC	pH
S	F	483.071	247.300	258.700	246.778	160.473	10.125
	Sig.	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
B	F	1653.500	540.400	3024.100	5627.444	245.798	58.500
	Sig.	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
N	F	528.286	90.000	10.000	9025.000	158.519	40.500
	Sig.	0.000*	0.000*	0.005*	0.000*	0.000*	0.000*
S*B	F	968.750	388.000	332.050	2688.444	52.868	12.938
	Sig.	0.000*	0.000*	0.001*	0.000*	0.000*	0.000*
S*N	F	323.214	93.900	1107.100	576.333	5.357	0.375
	Sig.	0.000*	0.000*	0.000*	0.000*	0.015	0.693
B*N	F	3840.929	1890.000	46.900	313.000	69.426	55.500
	Sig.	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
S*B*N	F	4198.036	2133.300	1679.950	959.333	126.403	63.187
	Sig.	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

**Significant at p ≤0.05, S-Speed B-Blade shape, Number of blades*

Table 5: New Duncan multiple range test for the microbial data

		TVC	TCC	LBC	FC	YC	pH
Speed	30 rpm	4.7333a	2.7083a	1.4333a	3.2833a	1.7333a	7.3250a
	36 rpm	5.7333b	3.033b	1.7417b	3.2500a	2.7000b	7.3250a
	42 rpm	5.3083c	3.175c	1.2583c	2.8750b	1.3583c	7.250b
Blade	Anchor	5.3250a	3.1333a	2.1750a	3.9167a	2.1500a	7.2667a
	Helix	5.8250b	3.2167b	0.5500b	1.9000b	0.9583b	7.4167b
	Vane	4.3833c	2.5667c	1.7083c	3.5917c	12.5750c	7.2167a
Blade Numbers	1	3.0556a	3.0556a	1.4500a	3.9278a	2.3278a	7.2500a
	2	2.8889b	2.8889b	1.5056b	2.3444b	1.5333b	7.3500b

Mean with the same alphabets are not significantly different vertically

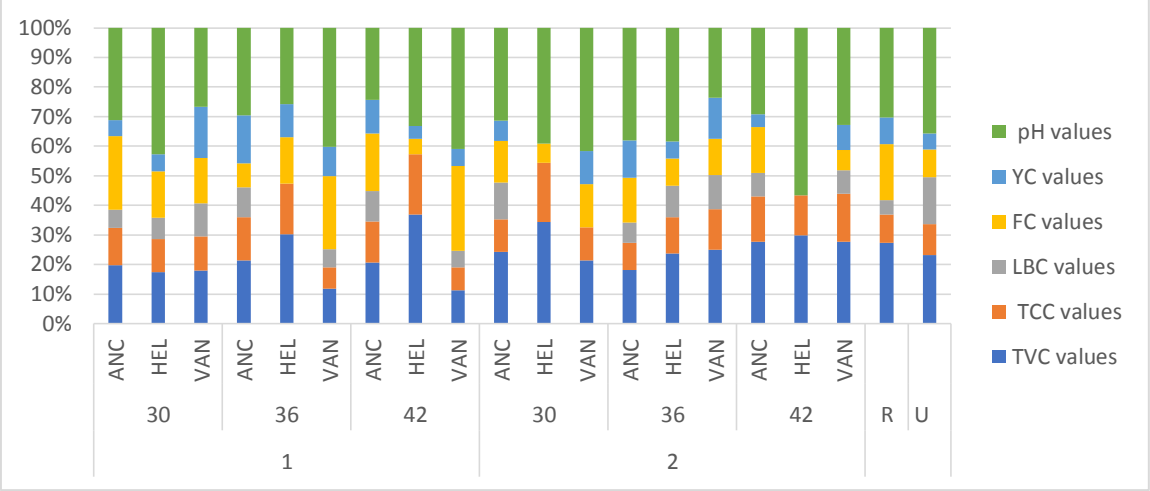

Fig 4. Microbial comparison between the raw, pasteurized unstirred, and other samples after 12 day

3.2. Effects of pasteurization parameters on protein and fats

The average initial protein content from the raw samples was 3.47 mg/ml while the average protein content for the samples pasteurized without stirring was 2.64 mg/ml. After pasteurization, the protein values varied between 2.04 mg/ml to 3.74 mg/ml (Table 6). These variations were significant at $p \leq 0.05$ for the interaction between speed, the number of blades, and blade types (Table 7). During pasteurization, milk protein is thermally unstable [23]. Milk protein changes when subjected to heat treatment and it leads to a thermal breakdown of the protein profile [24]. This could be a possible cause of changes in protein values during this study.

There was a significant difference (at $p \leq 0.05$) in the fat contents after pasteurization with the milk fat content ranging from 1.68 mg/ml up to 5.50 mg/ml (Table 6). This is in agreement with Pestana et al. [25]. Similarly, Tadjine et al. [26] in their studies reported significant changes in fat and protein contents for pasteurized cow and goat milk. This study agrees with their results for a batch pasteurizer.

3.3. Effects of pasteurization parameters on milk phytochemicals

Phytochemicals get into cow's milk through their feeds and according to place and dairy management [27]. Lorençoni et al. [28] reported that pasteurization reduces phenolic contents. However, in this study, the phenolic contents were very low and varied between 0.12 mg/ml to 1.96 mg/ml. The effects of pasteurization due to the pasteurizer's operating parameters were significant at $p \leq 0.05$ (Tables 6 and 7). The helical and vane blade stirrer showed slightly higher phenolic contents at the optimal speed of 42 rpm. According to Chávez-Servín et al. [29], pasteurization reduces phenolic contents in milk. This could be a possible reason for the low phenolic contents in the pasteurized samples.

Flavonoid contents in this study were low. This could be from the cow feed, breeds, and breeding schemes. The differences in the flavonoid contents in the milk samples after pasteurization were significant at $p \leq 0.05$ compared to the control samples. This could be an effect of pasteurization [28].

Table 6: Summary statistics of the nutritional data generated

Blade	BN	S	Protein (mg/ml)	Fat (mg/ml)	Flavonoid (mg/ml)	Phenol (mg/ml)
Anchor	1	30	3.15±0.02	4.26±0.00	24.30±0.05	0.18±0.00
		36	3.47±0.00	4.04±0.00	20.96±0.06	0.12±0.01
		42	3.74±0.02	4.88±0.08	25.06±0.09	0.39±0.01
	2	30	3.39±0.08	2.36±0.00	20.32±0.24	0.55±0.03
		36	3.29±0.10	1.68±0.00	25.53±0.11	1.89±0.00
		42	2.44±0.59	2.02±0.00	26.47±0.06	0.90±0.02
Helix	1	30	2.77±0.04	2.46±0.00	26.97±0.38	0.63±0.00
		36	2.61±0.01	3.70±0.00	26.62±0.02	0.24±0.02
		42	2.11±0.03	2.91±0.00	26.90±0.00	1.12±.02
	2	30	2.04±0.00	2.80±0.00	27.23±0.08	2.08±0.06
		36	2.70±0.08	2.91±0.00	27.67±0.00	0.52±0.02
		42	3.33±0.13	2.23±0.00	26.70±0.00	1.53±0.02
Vane	1	30	2.64±0.00	2.46±0.00	21.69±0.00	0.30±0.00
		36	2.34±0.48	2.22±0.00	22.80±0.00	0.29±0.00
		42	2.05±0.00	2.24±0.00	22.54±0.03	1.96±0.02
	2	30	2.58±0.00	4.71±0.00	24.20±0.02	0.23±0.02
		36	2.68±0.01	3.72±0.06	25.12±0.10	0.60±0.00
		42	2.95±0.06	3.38±0.01	26.59±0.00	0.97±0.02

*Number of blades (BN), Speed in revolution per minute (S)

Table 7: Multivariate analysis of the nutritional data generated

Parameter	DV	Flavonoid	Phenol	Protein	Fat
S	F	250.790	2567.688	0.873	360.920
	Sig.	0.000*	0.000*	0.433	0.000*
B	F	1353.137	1020.198	59.080	1005.480
	Sig.	0.000*	0.000*	0.350	0.000*
N	F	522.717	4517.768	0.917	2779.704
	Sig.	0.000*	0.000*	0.000*	0.000*
S*B	F	129.628	2815.133	2.953	1717.179
	Sig.	0.000*	0.000*	0.045*	0.000*
S*N	F	242.060	1329.004	4.956	1927.363
	Sig.	0.000*	0.000*	0.018*	0.000*
B*N	F	196.811	2745.693	17.065	26650.303
	Sig.	0.000*	0.000*	0.000*	0.000*
S*B*N	F	205.976	1145.403	26.433	87.066
	Sig.	0.000*	0.000*	0.000*	0.000*

Significant at $p \leq 0.05$

Table 8: New Duncan multiple range test for the nutritional data

		Protein	Fat	Flavonoid	Phenol
Speed	30 rpm	2.7623a	3.1755a	24.1184a	0.6622a
	36 rpm	2.8495a	3.0464b	24.7846b	0.6104b
	42 rpm	2.7719a	2.9431c	25.7110c	1.1442c
Blade	Anchor	3.2478a	3.2066a	23.7733a	0.6738a
	Helix	2.5944b	2.8359b	27.0164b	1.0178b
	Vane	2.5414b	3.1225c	23.8243a	0.7253c
Blade Numbers	1	2.7662a	3.2416a	24.2047a	0.5802a
	2	2.8228b	2.8684b	25.5380b	1.0310b

Mean with the same alphabets are not significantly different vertically

4. Conclusions

Stirring may not induce any direct chemical reaction in a batch pasteurizer, however, it contributes to an effective pasteurization process. It may also help to avoid burning milk products inside the batch pasteurizer and maintain homogenous heat transfer within the pasteurizer. Blade and speed selection are important parameters to be considered in a large batch pasteurization tank for quality milk products.

5. Declaration of conflicts of interest

The authors of this manuscript declare that there is no conflict regarding this study.

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