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Effect of incubation temperature, heat treatment and milk source on the yoghurt kinetic acidification

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Abstract

Despite the crucial importance of the acidification step for the yoghurt manufacture, few research studies have deeply focused on the understanding and characterization of this biotechnological and complex phenomenon. In order to address this important topic, this paper aims to (a) evaluate the effect of the type of milk (caprine, bubaline and bovine), incubation temperature (39ºC, 42ºC and 45ºC) and the applied thermal process (T1: pasteurization at 72°C for 15s and T2: pasteurization at 72°C for 15s followed by additional thermal treatment at 90°C for 15min) on the acidification profile and kinetic parameters V_m (maximum acidification rate), T_m (time to reach V_m), and T_e(time to reach pH 4.6) and (b) to estimate the relationship between these selected kinetic parameters (V_m, T_m, T_e). Results showed that each type of milk presents a particular behavior during the acidification, which might be related to their chemical composition. It was also possible to observe that the type of milk has a significant effect on Vm and the thermal treatment can also influence the parameter T_m. Overall, our results suggest that the processing parameters already established for cow's milk cannot be directly extrapolated to other types of milk.

Introduction

Yoghurt is one of the major dairy products in the food market worldwide. Its production includes several steps, where the acidification phase is one of the most important ones and it substantially influences the quality of the final product (Horne, 1999). The milk fermentation is generally conducted with thermophilic lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. bulgaricus, but other lactic species can be present (Amirdivani and Baba, 2011).

Despite the fact that yoghurt production is mainly conducted with bovine milk, other milk sources such as caprine and bubaline milks are available in several parts of the world and present strategic and/ or technical advantages over the traditional cow's milk (Raynal-Ljutovac *et al*., 2008; Bezerra *et al*., 2012). The physicochemical and sensory parameters of fermented dairy beverages are affected by the type of milk used in the process, creating opportunities to produce final products with unique properties (Gomes *et al*., 2013). It has also been reported that the type of milk can influence the microbial metabolism, which means that the same microorganism applied to different milk sources can generate a final product with distinct chemical composition and different volatile compounds (Guler e Gursoy-Balci, 2011).

The acidification phenomenon is a biochemical process characterized by its complexity and includes several reactions where the starter culture produces lactic acid and aromatic and volatile compounds which bring the particular yoghurt identity. The acidity and low pH resultant from the lactic acid fermentation also induce significant structural changes that are responsible for the yoghurt texture and its unique rheological characteristics (Gastaldi *et al*., 1997; Lucey and Singh, 1998). Despite the recognized importance of the acidification step for the final product quality, most of the literature already published regarding the yoghurt manufacturing and characterization has mainly focused on technological aspects. Additional investigation about some important scientific aspects of the acidification phenomenon would bring deeper understanding about the parameters that would influence the quality of yoghurt.

There is some consensus that the milk composition, the applied thermal treatment and the incubation temperature would influence the acidification process and the characteristics of the final yoghurt (Jumah *et al*., 2001; Lucey, 2004a; Lee and Lucey, 2010; Loveday *et al*., 2013). However, the intensity of this influence and the way the association of all these factors would affect the coagulation process is particular to each type of milk and needs

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to be further investigated. It has been already reported that the thermal treatment would modify the protein structural organization, alter the saline balance, and inactivate some enzymes (Lucey and Singh, 1998) and it is also expected to cause a certain degree of protein denaturation, which would benefit the gel formation (Brabandere and Baerdemaeker, 1999). The incubation temperature also affects the microbial metabolism and the consequent lactic acid fermentation (Beal *et al*., 1999).

Therefore, the objective of this paper is to evaluate the effect of the type of milk (caprine, bubaline and bovine), the incubation temperature and the applied thermal process on the acidification process involved in yoghurt manufacture. Selected kinetic parameters V_m (maximum acidification rate), T_m (time to reach V_m), and T_e (time to reach pH 4.6) were evaluated and the relationship between these selected kinetic parameters was estimated. In addition, surface responses were used to better understand the importance of each parameter considered in this study for the yoghurt acidification. The results were presented and discussed in terms of both qualitative and quantitative aspects.

Materials and methods

Milk supply

Fresh bubaline and caprine milks were obtained from two different farms, both of them in Northeastern Brazil. The total solids of milk were evaluated by method 990.19, AOAC (1998). Bubaline milk (total solids 17.53±1.26%) was obtained from Murrah buffalos and the caprine milk (total solids 12.17±1.39%) from the mixture of Saanen and Murciana goats. All animals exhibited good health and were managed under intensive feeding conditions. Bovine milk (total solids 11.50±1.70%) was obtained from the local market from the mixture of several breeds.

Yoghurt production

Two heat treatments (T1 – pasteurization:72oC, 15s; T2 – pasteurization 72°C, 15s followed by additional heat treatment 90°C/15min) and three incubation temperatures (39, 42 and 45°C) were applied to each type of milk, according to the complete factorial design outlined in Table 1, consisting of 18 experimental groups. The lyophilized Y4.50B starter culture (Sacco, Brazil) containing Streptococcus salivarius subsp. thermophilus and Lactobacillus delbruekii subsp. bulgaricus (0.02 U/L), referred in the text as St-Lb, was used to milk inoculation. The mixtures were transferred to sterile glass containers and incubated in temperature-controlled chambers (Tecnal, Brazil) until a final pH 4.6 was reached. The entire experimental process was repeated four times.

Table 1. Experimental conditions applied for yoghurt made with each type of milk (bovine, caprine and bubaline)

Incubation temperature (IT)	Heat treatment (HT)
39° C	T1
42° C	T1
45° C	T1
39° C	T2
42° C	T2
45° C	T2

T1: pasteurization at 72°C for 15 s and T2: pasteurization at 72°C for 15 s followed by additional thermal treatment at 90°C for 15min

pH measurement

The pH changes during fermentation were monitored every 30 minutes by means of a glass electrode pH meter (HI9020 pH meter, Hanna Instruments, RI, USA).

Acidification kinetics evaluation

The following parameters were considered responses that characterized the process kinetics: (a) maximum acidification rates (V_m) which were calculated from pH–time curves according to the equation $Vm = (\frac{dpH}{dt})_{max}$ and expressed in absolute values (m unit.pH /min); (b) T_m, the time at which the maximum acidification rate was observed (hours) and (c) the time at which pH 4.6 was reached (T_e, hours).

Statistical evaluation of the effects

Four batches were prepared for each experimental condition and all analyses were carried out in triplicate (N = 12). Results were expressed as mean ± SD. In order to evaluate the effect of different experimental conditions, the t-Student distribution was applied considering $p<0.05$. The analysis was performed using coded units. Three independent variables (factors) were considered for the surface response analysis: the type of milk (1-bovine, 2-caprine and 3-bubaline), thermal treatment (1-T1 and 2-T2) and incubation temperature (1-39°C, 2-42°C and 3-45°C). The considered responses were V_m (maximum acidification rate), T_m (time to reach V_m), and T_e(time to reach pH 4.6). The deletion of terms was applied to remove the statistically non-significant terms. However, when the exclusion of such terms decreased R^2 (adjusted) and increased the estimator of the variance S, the term was maintained.

Results and discussion

Acidification profiles during yoghurt fermentation

Different acidification profiles were obtained for each type of milk and for the distinct experimental conditions. Figure 1 illustrates the acidification profiles of bovine, caprine and bubaline milks submitted to T1 and T2 treatments and incubated at 39°C, 42°C and 45°C. It was observed that the pH decrease for bubaline milk is slower when compared to the other two types of milk. Ahmad *et al*. (2008) reported a similar tendency when comparing the buffalo's milk and cow's milk acidification. According to the authors, the higher buffer capacity of buffalo's milk can be explained by its composition in acido-basic compounds, higher casein content and increased concentration of inorganic phosphate.

Although important differences were observed for the investigated experimental conditions, all acidification profiles exhibited three distinct and noticeable phases. Initially, a slight decrease in pH was shown, followed by a second period where the pH values decreased faster and a steep curve inclination was detected. Finally, the third acidification phase was characterized by a tendency to stabilization, with little variation in the pH levels. This behavior was previously reported by Brabandere and Baerdemaeker (1999) and Jeanson *et al.* (2009) and they are justified by several chemical and biochemical reactions that take place during the lactic acidification process (Lucey, 2004b).

Acidification kinetics

The acidification kinetics was evaluated in regard to the maximum acidification rate (V_m), time to reach V_m (T_m) and time to reach pH 4.6 (T_e), which represents the isoelectric point of milk proteins. These results and the total solids of each type of milk are shown in Table 2. These results will be discussed in combination with the statistical evaluation of the effects.

Table 3 summarizes the estimated regression coefficients of the polynomial models for the response variables, along with the corresponding R^2 and Figures 2A, 2B and 2C show the surface response obtained in this study. All generated models adequately explain the variation of the responses with R^2 higher than 0.90 and non-significant lack of fit. It is worth noting that no total solids adjustment was made in this study. This experimental design was chosen in order to provide a better understanding of what would be obtained with milk collected in routine conditions.

Overall, lower V_m values were observed for the

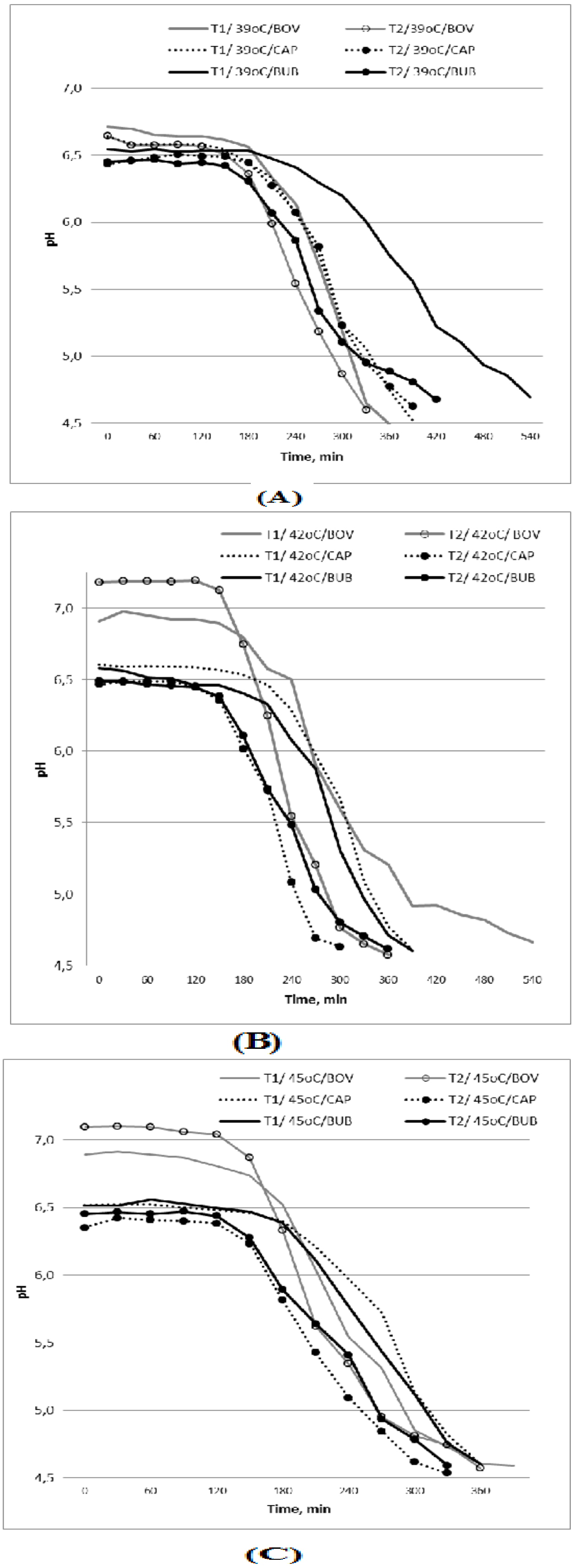


Figure 1. Acidification profiles of bovine (BOV), caprine (CAP) and bubaline (BUB) milks submitted to T1 (pasteurization at 72°C for 15 s) and T2 (pasteurization at 72°C for 15 s followed by additional thermal treatment at 90°C for 15min) treatments and incubated at 39°C (A), 42°C (B) and 45°C (C)

Table 2. Kinetics acidification parameters for bovine (BOV), caprine (CAP) and bubaline (BUB) milks submitted to different thermal treatment (T1 and T2) and incubation temperatures (39°C, 42°C, 45°C)

Treatment/Incubation temperature/type of milk	Total solids (g/100g)	V_m ($\times 10^{-3}$upH/min)	T_m (h)	T_e (h)
T1/39° C/BOV	11.5 ± 1.3	16.67 ± 0.01^{b}	5.0 ± 0.0^{b}	9.0 ± 0.1^{a}
T1/42° C/BOV	11.5± 1.3	$19.89\pm0.30^{a,b}$	4.5 ± 0.1^{c}	6.0 ± 0.1^{c}
T1/45° C/BOV	11.5± 1.3	16.44 ± 0.77^{b}	4.0 ± 0.0^{c}	6.5 ± 0.1^{c}
T2/39° C/BOV	11.5± 1.3	$14.89\pm0.19^{b,c}$	4.0 ± 0.2^{c}	5.5 ± 0.1^{c}
T2/42° C/BOV	11.5± 1.3	23.44 ± 0.38^{a}	4.0 ± 0.0^{c}	6.0 ± 0.1^{c}
T2/45° C/BOV	11.5± 1.3	23.67 ± 0.33^{a}	3.0 ± 0.0^{c}	6.0 ± 0.1^{c}
T1/39° C CAP	12.2± 1.4	17.22 ± 0.52^{b}	5.0 ± 0.0^{b}	$7.5\pm0.0^{b,c}$
T1/42° C CAP	12.2± 1.4	$19.44\pm0.30^{a,b}$	5.5 ± 0.0^{b}	$6.5\pm0.0^{b,c}$
T1/45° C CAP	12.2± 1.4	$19.56\pm0.51^{a,b}$	5.0 ± 0.2^{b}	6.0 ± 0.1^{c}
T2/39° C CAP	12.2± 1.4	$19.44\pm0.96^{a,b}$	5.0 ± 0.0^{b}	$6.5\pm0.1^{b,c}$
T2/42° C CAP	12.2± 1.4	21.56 ± 0.38^{a}	4.0 ± 0.1^{c}	6.0 ± 0.0^{d}
T2/45° C CAP	12.2± 1.4	13.89 ± 0.18^{c}	3.0 ± 0.1^{d}	6.0 ± 0.1^{d}
T1/39° C BUB	17.5± 1.7	11.11 ± 0.31^{d}	7.0 ± 0.1^{a}	9.0 ± 0.0^{a}
T1/42° C BUB	17.5± 1.7	6.78 ± 0.30^{e}	5.0 ± 0.0^{c}	$6.5\pm0.0^{b,c}$
T1/45° C BUB	17.5± 1.7	12.22 ± 0.01^{d}	5.0 ± 0.2^{b}	6.0 ± 0.1^{c}
T2/39° C BUB	17.5±1.7	17.44 ± 0.17^{b}	4.5 ± 0.2^{c}	7.0 ± 0.0^{b}
T2/42° C BUB	17.5± 1.7	15.11 ± 0.39^{c}	4.5 ± 0.0^{c}	6.0 ± 0.1^{c}
T2/45° C BUB	17.5± 1.7	15.78 ± 0.73^{c}	4.5 ± 0.1^{c}	5.5 ± 0.0^{d}

Values in the same column with different letters are significantly different ($p<0.05$).
T1: pasteurization at 72°C for 15s and T2: pasteurization at 72°C for 15s followed by additional thermal treatment at 90°C for 15min. V_m: maximum acidification rate; T_m: time at which the maximum acidification rate is observed; T_e: time at which pH 4.6 was reached

bubaline milk, followed by goat milk and cow's milk (Table 2). The bovine and caprine milks presented closer results (Figures 2A and 2B), but the bubaline milk showed an inverse tendency with lower Vm values and a minimum response when T1 treatment was applied (Figure 2C). The regression coefficients of the models (Table 3) also showed a significant effect of the milk source on Vm results ($p<0.05$) which is in agreement with the lower curve inclination observed for buffalo's milk (Figures 1A, 1B and 1C). Similarly to what Ahmad *et al*. (2008) and Menard *et al.* (2010) observed, the total solids content of buffalo's milk used in this study is higher than goat and cow's milk. According to Gastaldi *et al.* (1997) and Varghese and Mishra (2008), the buffering capacity is proportional to the total solids content of milk. Thus, the higher total solid content of buffalo milk might explain the lower acidification rates after incubation at 39°C.

The experimental Vm values for cow's milk ranged from 14.89×10^{-3} and 23.67×10^{-3} upH/min and they are close to what Almeida *et al*. (2009) and Espírito Santo *et al*. (2012) reported for fermented bovine milk by different lactic bacterial strains. Almeida *et al*. (2008) showed lower V_m values (15.05×10^{-3} upH/min to 16.80×10^{-3} upH/min) for milk whey acidified by a combination of *Streptococcus* and *Lactobacillus* strains at 42°C.

Higher V_m values were observed for T2 samples (Table 2). Comparing the effect of thermal treatment on Vm values within each milk specie, the higher significant differences were observed for buffalo's milk ($p<0.05$). Heating of milk is an important processing variable for the preparation of yogurt and it influences the physical properties and microstructure of yogurt (Lee and Lucey, 2010). When the pH of preheated milk decreases during fermentation, denatured whey proteins aggregate and extensive croos-linking between whey proteins and caseins occurs (Lee and Lucey, 2004). We hypothesize that the partial protein denaturation promoted by the thermal treatment T2 is enough to easy the coagulation process and promote higher maximum acidification rates (Brabandere and Baerdemaeker, 1999).

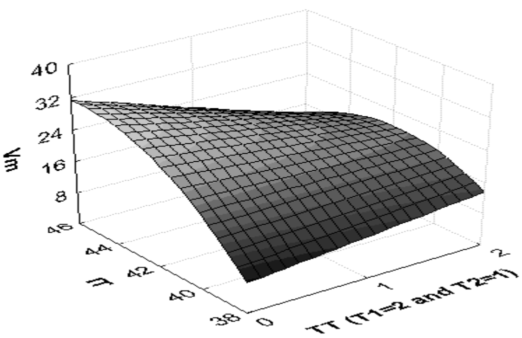
Also, the lowest V_m value ($p<0.05$) for caprine milk were observed by applying the treatment T2 at the higher incubation temperature (45°C). Although

Table 3. Regression coefficients of the surface response models

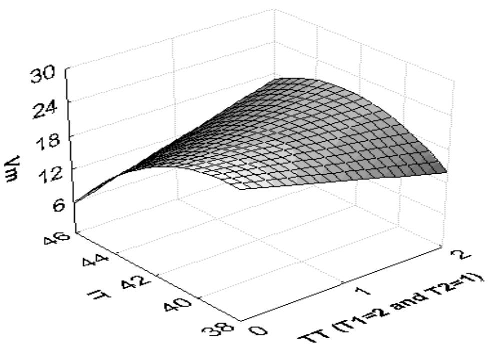
Factors	Responses		
	V_m	T_m	T_e
Constant	819.24**	206.17**	730.83**
TT	-504.22	-65.28**	-195.39**
MS	-1276.90**	-218.00	-372.00
$(MS)^2$	491.32	73.00	91.00
IT	-42.65	-9.50	-33.50**
$(IT)^2$	0.54	0.11	0.39
TT x MS	-18.38	-0.83	2.83
TT x $(MS)^2$	4.98	0.17	-0.67
TT x IT	24.97	3.11	8.86**
TT x $(IT)^2$	-0.30	-0.04	-0.10
MS x IT	65.44	10.50	17.23
MS x $(IT)^2$	-0.81	-0.12	-0.20
$(MS)^2$ x IT	-24.55	-3.50	-4.19
$(MS)^2$ x $(IT)^2$	0.30	0.04	0.05
R^2	0.875	0.830	0.930

** $P \leq 0.05$

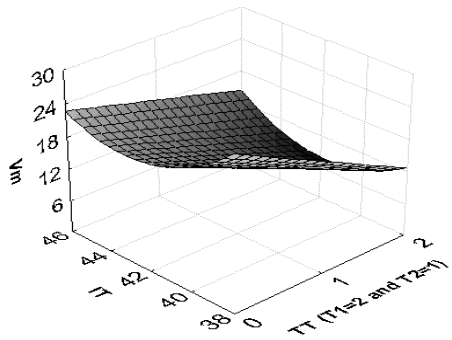
TT (thermal treatment): T1: pasteurization at 72°C for 15 s and T2: pasteurization at 72°C for 15 s followed by additional thermal treatment at 90°C for 15 min; MS (milk source): caprine, bubaline and bovine; IT (incubation temperature): 39ºC, 42ºC and 45ºC; V_m: maximum acidification rate; T_m: time at which the maximum acidification rate is observed; T_e: time at which pH 4.6 was reached.



(A)



(B)



(C)

Figure 2. Response surface plots for the maximum acidification rate (V_m) as a function of the thermal treatment (TT) and the incubation temperature (IT) for (A) bovine milk, (B) caprine milk, and (C) bubaline milks. T1: pasteurization at 72°C for 15 s and T2: pasteurization at 72°C for 15 s followed by additional thermal treatment at 90°C for 15 min.

there are few studies on the effect of pH on the heat stability of goat's milk, Montilla and Calvo (1997) have reported lower goat's milk stability during heat treatments when compared to cow's milk. Therefore, the combination of T2 treatment and higher incubation temperature may have exerted a significant influence on the coagulation process and decreased the acidification rates.

In regard to the quality of the final product, intermediate acidification rates are preferable, because they lead to appropriate and regular acid production, and result in more homogeneous structure and greater viscosity of the yoghurt (Beal *et al*., 1999; Kristo *et al*., 2003; Jacob *et al*., 2011).

The time at which maximum acidification was reached (T_m) was similar to what Almeida et al. (2009) found for bovine milk fermented with traditional yoghurt culture (St-Lb), but higher than Espírito Santo (2012) observed for probiotic yoghurt. To the best of our knowledge, Tm has not been reported for buffalo's and cow's milk before.

The time to reach pH 4.6 (T_e) is often referred as the time to reach the end of yoghurt fermentation (Kristo *et al.*, 2003). The thermal treatment, the incubation temperature and the combination of both factors influence T_e (Table 3). The longest time to reach T_e (9 h) was observed for cow's and buffalo's milks when the lowest incubation temperature (39°C) was used in the experiments ($p<0.05$). The reduction of incubation temperature causes a clear increase in the time required to reach pH 4.6, which is justified by a decrease in lactic acid metabolic activity. Haque *et al.* (2001) also concluded that the reduction of incubation temperature leads to a marked effect on the ability of the lactic bacteria to convert lactose to lactic acid.

Conclusion

In this study, the kinetic parameters V_m, T_m and T_e were presented for bovine, caprine and bubaline milks. To the best of our knowledge, T_m and T_e were shown for the first time for goat's and buffalo's milks. Our results also indicate that the type of milk, heat treatment and incubation temperature has a significant effect on the acidification process of yoghurt. The presented data indicate that each type of milk presents a specific behavior during the acidification process, which depends mainly on inherent characteristics of each milk source. Therefore, the parameters of the acidification process, which are well established for cow milk, cannot be directly extrapolated to other milk sources such as buffalo or goat milk.

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