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Jabuticaba (*Myrciaria jaboticaba* Vell. Berg) syrup as coloring and pH indicating agent for an intelligent sheep milk yogurt

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ABSTRACT

Consumers are demanding for healthier and safe products. In this context, we proposed the development of an intelligent sheep milk yogurt, with a natural coloring agent, that changes color as the acidity increases, an indicative of shelf life decay. For that propose, jabuticaba (*Myrciaria jaboticaba*) syrup (15%, 30%, and 45% soluble solids) was added to the milk before fermentation. Yogurts ability in reporting acidity by changing color was observed by correlating yogurt titratable acidity (Dornic) and pH with visual and colorimetric parameters (L, a, and b). Other physical-chemical parameters as fat, protein, moisture, and ash were also analyzed to evaluate the syrup addition effect on yogurts composition. Statistical analysis of the data was performed through analysis of variance and the means were compared by the Tukey test at 5% of probability. Results have indicated that intelligent yogurts naturally colored with jabuticaba syrup, was able to change color from light purple to dark purple pigmentation, indicating pH decrease. Jabuticaba syrup affected yogurts composition, increasing titratable acidity, fat and ash as jabuticaba syrup soluble solids concentration increased.

KEYWORDS: Acidification; color stability; physical-chemical composition.

INTRODUCTION

Nowadays, consumers have a positive perception of yogurt as a functional dairy product with health-promoting ingredients (CHOUCHOULI *et al.*, 2013). Yogurt is a product of the milk fermentation by the symbiotic action of the bacteria *Streptococcus salivarius subsp. Thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus*, with better digestibility and is more nutritious than milk (BOUDIER, J. F., and LUQUET, 1993; PANAHI *et al.*, 2017). When produced with sheep milk, present a denser protein network and highest gel firmness compared to goat and cow´s milk. It is also important to highlight that when compared with other species, the sheep milk has components of high nutritional value and health benefits (RIBEIRO *et al.*, 2007; NGUYEN, AFSAR, and DAY, 2018). Its shelf life is not exclusively related to deterioration but to a complex phenomenon and consumer perceptions of the yogurt, such as pH decrease, that increases consumer rejection up to 50% (CRUZ *et al.*, 2010).

Jabuticaba (*Myrciaria jaboticaba*) a dark-purple Brazilian native fruit (MANICA, 2000; NEVES *et al.*, 2018), has described potential health benefits (CLERICI and CARVALHO-SILVA, 2011; OLIVEIRA *et al.*, 2012), due to the high content of phenolic compounds such as anthocyanin acting as antioxidants, anti-inflammatories and antidiabetics (WU *et al.*, 2012; WU; LONG; KENNELLY, 2013; JASTER *et al*., 2018). Anthocyanins are also natural pigments with colors ranging from pink to blue (BORDIGNON JR. *et al.*, 2009). These compounds have a chimocromic behavior (VEIGA-SANTOS, DITCHFIELD, and TADINI, 2011; BORDIGNON JR *et al.*, 2009; MALACRIDA AND MOTTA, 2006), depending on the pH of the food matrix (MALACRIDA AND MOTTA, 2006).

Color is one of the most important quality parameters for consumers (JASTER *et al*., 2018). In this context, fruits with high anthocyanin content can be used as alternative natural food coloring, reducing the use of artificial colorants.

In order to produce an intelligent yogurt without artificial colorants, sheep milk yogurt was fermented with jabuticaba syrup and the yogurts ability to indicate pH decrease was evaluated. Yogurts ability in reporting acidity by changing color was observed by correlating yogurts titratable acidity (^{o}Dornic) or percentage (%) of lactic acid and pH with yogurts visual and colorimetric parameters (*L*, *a* and *b*). Fat, protein, moisture and ash were also analyzed to evaluate syrups addition effect on yogurts composition.

MATERIALS AND METHODS

To obtain the yogurt formulations were used as material raw frozen sheep milk, jabuticaba syrup (*Myrciaria jaboticaba Vell. Berg*), frozen yogurt as a starter culture. Starter culture was produced by inoculating commercial lyophilized ferment BioRich (Chr. Hansen Ind and Com. Ltda) containing *L. acidophillus* LA-5 ($1x10^6$ CFU/g), *Bifidobacterium* BB-12 ($1x10^6$ CFU/g) and *S Thermophillus* (amount not reported).

Jabuticaba syrup concentration was adjusted to 60 °Brix using three treatments with different jabuticaba syrup soluble solids: 15% jabuticaba and 85% sucrose (T1), 30% jabuticaba and 70% sucrose (T2) and 45% jabuticaba and 55% sucrose (T3). Jabuticaba was thawed at the room temperature and washed with current water. Water and jabuticaba were weighed in ratio 1:1 and the mixture was boiled for 5 min and cooled to room temperature, then the liquid extract of jabuticaba (LEJ) was filtered by synthetic thin mesh previously sanitized. Soluble solids concentration (°Brix) and mass (g) in LEJ were determined using a digital refractometer (Reichert model r^2i 300) and a digital balance respectively. Then the mass of soluble solids (ss) was calculated, using the equation 1.

$$Mass_{ss}(g) = \left(\frac{LEJ_{Brix} \times LEJ_{mass}}{100}\right) \quad (1)$$

Where: Mass ss – soluble solids mass of liquid extract of jabuticaba; LEJ Brix – soluble solids concentration of liquid extract of jabuticaba and LEJ mass – the mass.

The amount of sugar required to obtain syrup with defined concentrations of soluble jabuticaba solids (15, 30 and 45 %) was calculated. Sugar was added in the LEJ and boiled, the concentrations of solids in the solution was monitored every 5 min until the 60 °Brix. Then the syrup was rapidly cooled and packaged into sterile glass bottles (500 ml) and stored at 4 °C until use.

For yogurt production, the milk was thawed for 24 h at 4 °C, followed by pasteurization at 82-90 °C for 30 min and then rapidly cooled in a water bath to 43 °C, as recommended by Behmer (1979). The jabuticaba syrup was added, using the mass balance described in equation 2:

$$B1 \times M1 + B2 \times M2 = B3 \times M3 \quad (2)$$

Where: B1: soluble solids concentration of syrup (60 °Brix); M1: the mass of the syrup (g); B2: soluble solids concentration of yogurt; M2: the mass of yogurt (g); B3: soluble solids concentration of yogurt sweetened (14 °Brix); M3: the mass of yogurt sweetened (g).

Jabuticaba syrup and milk were mixed and kept under constant stirring to obtain a homogenous mix. The amount of syrup added resulted in yogurt at 14 °Brix. Thereafter, the lactic ferment (3% w/w of frozen yogurt) was inoculated to the milk (43°C) under stirring. The inoculated milk was placed in plastic pots and incubated (Marconi incubator, model MA 032) at 43-45 ºC for 3-4 h. The fermentation was performed until 90 °D or 0.9% lactic acid was reached. After that the fermentation was interrupted and the yogurt was immediately cooled and stored at 4 ºC.

ABILITY IN REPORTING PH DECREASE

YOGURTS ABILITY IN REPORTING ACIDITY

Yogurts ability in reporting acidity by changing color was studied by correlating yogurts titratable acidity (°Dornic) or % of lactic acid and pH (direct measurement in a digital potentiometer) with yogurts visual and instrumental color parameters. Yogurts were artificially acidified with citric acid (99.5 %) diluted in water in ratio 1:1 resulting in approximately 50 % concentration of citric acid. Six (6) essay different pH environments (by adding 0, 5, 10, 15, 25 and 30 drops of citric acid 50 %) were performed. Samples were directly measured in Petri plates, using the CIE*Lab* system (*L** (lightness), *a** (green-red) and *b** (blue-yellow)), with a CR 400 colorimeter (DL65 Konica Minolta, Ltd Osaka, Japan).

PHYSICOCHEMICAL ANALYSIS

The physicochemical parameter: moisture content, ash values (gravimetric method), protein (Kjeldahl method) and fat content (Gerber method), were also analyzed (AOAC, 2005).

STATISTICAL ANALYSIS

Yogurt manufacturing was carried out in triplicate for each treatment (T1, T2, and T3), making nine experimental plots. All analyses were performed in triplicate. Physicochemical and colorimetric results were subjected to analysis of variance (ANOVA), means were compared using Tukey's test at a probability level of 95% ($p < 0.05$), test run with Assistat 7.7 beta statistical software (Campina Grande- PB, Brazil) (SILVA and AZEVEDO, 2016).

RESULTS AND DISCUSSION

PHYSICOCHEMICAL ANALYSIS OF YOGURT

The values obtained (mean ± standard deviation) in the physicochemical analysis of yogurt sweetened with jabuticaba syrup are given in Table 1.

Table 1. Mean values of physicochemical parameters of jabuticaba yogurt.

Parameters	Treatment		
	T1	T2	T3
pH	4.28a ± 0.06	4.12a ± 0.07	4.11a ± 0.04
Ash (%)	0.84b ± 0.01	0.95ab ± 0.05	1.08a ± 0.11
Moisture (%)	77.11a ± 0.97	77.37a ± 0.49	78.15a ± 0.44
Titratable acidity (°D)	90.00c ± 0.00	101.20b ± 7.41	114.75a ± 3.69
Lactic acid (%)	0.90c ± 0.00	1.01b ± 7.41	1.15a ± 3.69
Protein (%)	4.11a ± 0.40	4.12a ± 0.45	4.28a ± 0.10
Fat (%)	2.70b ± 1.07	4.63a ± 0.48	3.25ab ± 0.29

NOTE: Different letters in the rows indicate significantly different at 5% probability ($p<0.05$). T1; T2 and T3, correspond to yogurt with 15%, 30% and 45% soluble solids of jabuticaba respectively.

Jabuticaba syrup did not affect ($p<0.05$) on yogurts moisture and protein content (Table 1). Since milk was the same for all yogurts since jabuticaba has an insignificant amount of protein and soluble solids was fixed in 60 ^{o}Brix; it was expected no difference in protein and moisture.

Ash, titratable acidity, lactic acid and fat content were affected ($p<0.05$) (Table 1). Jabuticaba´s high mineral levels (2.8-3.8%) (ALEZANDRO *et al.*, 2013), especially calcium, magnesium, phosphorus, copper, and iron (LIMA *et al*., 2011), can explain the increase in ash content as increasing fruit concentration in the syrup.

Regarding fat content, the difference observed was not expected, since the same raw material (milk) was used for the preparation of yogurts. However, it is assumed that this difference is related to the difference in the sample homogenization process for the analysis.

Costa *et al*. (2013) report that milk solids content greatly influences acidity titratable of the yogurt. The carbohydrate content may justify the acidity differences of the different treatments, probably the lactose is responsible for reduction of the pH values of this study, since during the fermentation process, initial lactose metabolism result in the formation of glucose and galactose, culminating in the production of lactic acid, resulting in increased acidity and reduced pH (CRUZ *et al*., 2017).

The acidity (titratable acidity and lactic acid) of the yogurt showed a significant difference between the three treatments, revealing that the higher soluble solids of the fruit in the syrup added, more acid becomes the yogurts. Jabuticaba is a fruit rich in organic acids, which could have reflected in yogurts acidification (LIMA *et al*., 2011).

Although the acidity is inversely related to pH, no effect of syrup addition was observed ($p<0.05$) on yogurts initial pH. Two facts may explain this finding: the buffering capacity of the yogurts and the logarithmic scale that defines pH. According to KIM, OH, and IMM (2018) buffering capacity is related to the sum of individual ionizable acid-base groups, including salt, organic acids, and proteins (casein) as major contributors of this propriety in dairy products. Al-Dabbas, Al-Ismail, and Al-Abdullah (2011) reported that due to the ability of dairy products to bind or release ions, upon addition of acid or alkali, changes in pH values tend to be small. Schmidt *et al*. (1996) reported an increase in titratable acidity (% lactic acid) increasing of protein content in milk samples.

YOGURTS COLOR AND ABILITY IN REPORTING ACIDITY

The jabuticaba syrup, in all tested concentrations, was able to pigment the sheep milk yogurt without other coloring agent addition, although jabuticaba´s syrup solids soluble concentration resulted in different color parameters. The yogurt with 15% soluble solids of jabuticaba was the lightest purple, followed by yogurt with 30%, and the yogurt with 45% was the darkest purple. With the increase of acidity, the yogurt color (light purple) became more intense (dark purple) (Table 2).

Jabuticaba syrup addition resulted in an intelligent yogurt able of changing color related to pH decrease and a concentration dependence was observed. However, color change occurred in not specific pH, from 3.73 to 3.55. (Table 2). This fact could be due to anthocyanins acid-base balance chemical structure in different pH conditions, becomes more intense in low acidity medium.

The values obtained (mean ± standard deviation) of luminance (L^*), chromaticity (a^*) and (b^*) parameters in the colorimetric analysis of artificially acidified jabuticaba yogurt and their pH are shown in Table 2.

Table 2. Mean values of colorimetric analysis of yogurts sweetened with jabuticaba syrup.

pH	T1		
	L*	a*	b*
4.63	72.03a ±0.64	9.46b ± 0.23	0.748a± 0.75
4.09	72.52a ± 0.72	11.27b ± 0.30	0.25a ± 0.77
3.67	72.56a ± 0.88	12.74c ± 0.22	0.16a ± 0.86
3.45	72.57a ± 0.10	14.38b ± 0.50	0.19a ± 0.79
3.22	71.39a ± 0.96	15.78b ± 0.49	0.17a ± 0.88
2.96	70.98a ± 1.90	17.96a ± 1.59	0.25a ± 0.81
	T2		
	L*	a*	b*
4.63	61.50b±0.44	10.67a±0.10	-1.95c±0.01
4.09	62.43b±0.45	12.96a±0.59	-2.43c±0.05
3.67	62.18b±0.75	15.54a±0.53	-2.67c±0.13
3.45	61.39b±0.93	16.91a±0.88	-2.76c±0.09
3.22	61.12b±0.69	18.86a±0.12	-2.55c±0.42
2.96	60.59b±1.83	20.33a±1.43	-2.38c±0.43
	T3		
	L*	a*	b*
4.63	58.20c±0.64	10.97a±0.24	-1.05b±0.14
4.09	58.63c±0.44	12.39a±0.18	-1.15b±0.08
3.67	58.57c±0.28	13.93b±0.26	-1.11b±0.08
3.45	58.47c±0.44	14.78b±0.21	-1.12b±0.05
3.22	58.32c±0.61	16.22b±0.09	-1.08b±0.03
2.96	57.53b±1.09	18.19a±1.56	-0.98b±0.23

NOTE: T1; T2; T3 are yogurt with 15%, 30% and 45% soluble solids of jabuticaba respectively. The different letters in the rows indicate significantly different at 5% probability ($p < 0.05$).

In general, for all treatments, *a** values were closer to the red, *b** values were closer to the blue, with low lightness, indicating dominant purple coloration, increasing with higher soluble solids concentrations in the syrup, which would lead to higher pigments concentration (SILVA *et al.*, 2017).

A significant difference was observed for *L** values (scale ranges from 0 to 100, from black to white). Yogurts with 45% soluble solids of jabuticaba presented a darkest ($p < 0.05$) appearance, with lower *L** values, probably due to jabuticaba higher total solids content, resulting in less light reflection (JASTER *et al.,* 2018; CALDEIRA *et al.*, 2010).

A correlation was observed between yogurts increasing acidity and the positive *a** values (towards red). On the other hand, for the *b** values, while for 15% soluble solids the *b** values were positive (towards the yellow), as increasing soluble solids, a negative *b** values (toward the blue) ($p<0.05$) was noticed.

Since Rodas et al. (2001) reported the pH from 3.6 to 4.3 the limit in which lactic acid bacteria develop without any impairment, the pH in which the jabuticaba syrup was able to report pH decrease is totally pertinent.

CONCLUSIONS

Jabuticaba syrup is able to pigment the sheep milk yogurt and can be used as a natural coloring. Regardless of the concentration of soluble jabuticaba solids present in the syrup added in yogurt, the color change was noticeable for all treatments by acidifying the yogurt.

The jabuticaba yogurts can be considered an intelligent product due to the capacity to visually report a decrease in pH, in additional can be considered as an indicator of shelf life quality.

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Xarope de jabuticaba (*Myrciaria jaboticaba Vell. Berg*) como corante e indicador de pH para um iogurte inteligente de leite de ovelha

RESUMO

Os consumidores estão exigindo produtos mais saudáveis e seguros. Nesse contexto, propusemos o desenvolvimento de um iogurte inteligente de leite de ovelha, com um corante natural, que muda de cor à medida que a acidez aumenta, um indicativo de deterioração da vida útil. Para essa proposta, o xarope de jabuticaba (*Myrciaria jaboticaba*) (15%, 30% e 45% de sólidos solúveis) foi adicionado ao leite antes da fermentação. A capacidade dos iogurtes em relatar acidez alterando a cor foi observada correlacionando a acidez titulável de iogurte (°Dornic) e o pH com os parâmetros visuais e colorimétricos (L, a e b). Outros parâmetros físico-químicos como gordura, proteína, umidade e cinzas também foram analisados para avaliar o efeito da adição de xarope na composição dos iogurtes. A análise estatística dos dados foi realizada através da análise de variância e as médias foram comparadas pelo teste de Tukey a 5% de probabilidade ($p < 0,05$). Os resultados indicaram que os iogurtes inteligentes, coloridos naturalmente com xarope de jabuticaba, foram capazes de mudar a cor de roxo claro para pigmentação roxo escuro, indicando diminuição do pH. O xarope de jabuticaba afetou a composição dos iogurtes, aumentando a acidez titulável, a gordura e as cinzas à medida que a concentração de sólidos solúveis em xarope de jabuticaba aumentou.

PALAVRAS-CHAVE: Acidificação; estabilidade da cor; composição físico-química.

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