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Stability of probiotic yogurt added with glucose oxidase in plastic materials with different permeability oxygen rates during the refrigerated storage

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ABSTRACT

The stability of probiotic yogurts added with glucose oxidase and packaged in different plastic packaging systems that present different oxygen permeability transfer rates (0.09, 0.2, 0.39 and 0.75 mL O_2/day) was evaluated during 28 days of refrigerated storage. Probiotic stirred yogurts were submitted to physicochemical (pH, proteolytic activity, dissolved oxygen) and microbiological analyses (yogurt bacteria, *Lactobacillus acidophilus* and *Bifidobacterium longum*) as well as the content of organic acids (lactic and acetic acid) and aroma compound (diacetyl and acetaldehyde) were assessed. Overall, yogurts packaged in plastic containers with lower oxygen permeability rates showed a higher extent of post-acidification, proteolysis and organic acid production. Additionally, these samples also presented a lower content of dissolved oxygen and a lower decrease of the probiotic bacteria count. No influence on the production of aroma compounds was observed. Our results suggest that the use of packaging systems with different oxygen permeability rates coupled with the addition of glucose oxidase presented an interesting technological option to minimize the oxidative stress in probiotic yogurts.

1. Introduction

The popularity of yogurts and fermented milks is suitable food matrices for supplementation of probiotic bacteria and an increasing number of studies have indicated the health benefits related to their regular consumption (Akalın, Unal, Dinkci, & Hayaloglu, 2012; Ebel, Martin, Le, Gervais, & Cachon, 2011; Ejtahed et al., 2012; Ibarra, Acha, Calleja, Chiralt-Boix, & Wittig, 2012; Sadaghdar, Mortazavian, & Ehsani, 2012; Wang et al., 2012). Moreover, optimization procedures have been tested in order to improve existing operational parameters for the development and processing of probiotic foods (Sanders & Marco, 2010).

In fact, the addition of probiotic bacteria in fermented milks and yogurts is a topic of relevance to both consumers and food companies (Giraffa, 2012). The processing and stability of probiotic yogurts, in turn, are multidimensional tasks due to various technological operations that probiotic bacteria are subjected to in the food matrix. Some of these conditions are related to acid stress, cold stress and oxidative stress (Granato, Branco, Cruz, Faria, & Shah, 2010). The oxidative stress, in turn, is induced by the formation of reactive oxygen species (ROS), such as superoxide ion or hydrogen peroxide, and it is crucial to maintain the viability of probiotic bacteria in yogurts once probiotic strains become a microbial group that presents a facultative or a strictly anaerobic behavior. They also do not produce endogenous antioxidant enzymes, such as catalase and superoxide dismutase (Plessas, Bosnea, Alexopoulos, & Bezirtzoglou, 2012).

The addition of glucose oxidase into yogurts during processing has shown to be a potential option to decrease the oxidative stress in probiotic yogurts. In general, high counts of viable strains – *Lactobacillus acidophilus* and *Bifidobacterium longum* – and slight changes in some physicochemical (Cruz et al., 2010; Cruz, Castro, Faria, Bogusz, 2012; Cruz, Castro, Faria, Lollo et al., 2012) and sensory (Cruz, Cadena, Faria, Bolini et al., 2012) parameters of yogurts have been observed along the refrigerated storage. However, any technology solution aiming at a positive impact on the functional aspects of probiotic yogurts should not bring additional financial impact, once it may represent a relative loss of market share. In this context, it would be interesting to investigate the effect of glucose oxidase on some quality parameters of probiotic yogurts packaged in different plastic containers (with different oxygen permeability transfer rates) throughout the refrigerated storage. The results of this study may be useful for food companies that produce functional milk-based products that aim to maintain the viability of the probiotic bacteria without changing the packaging system.

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2. Material and methods

2.1. Culture growth

Pure cultures of *Streptococcus thermophilus* TA 040, *Lactobacillus delbrueckii* ssp. *bulgaricus* LB 340, *L. acidophilus* La14 and *B. longum* Bl05 were obtained from Danisco (São Paulo, Brazil) and maintained at −80 °C. The *B. longum* strain was used in this study due to its higher sensitivity to oxygen as compared with other *Bifidobacterium* strains (Jayamane & Adams, 2009). Skimmed milk powder (Molico, São Paulo, Brazil), reconstituted to 11% w/v, was used to prepare the lactic and probiotic cultures. For the *B. longum* BL05, 0.05% w/v of cysteine (Sigma, São Paulo, Brazil) and 0.5% w/v of yeast extract (Oxoid, São Paulo) were added to the milk powder reconstituted to 11%, whereas for the other cultures, the reconstituted skimmed milk was supplemented with 2% w/v of glucose (Synth, São Paulo, Brazil). All the reconstituted milks were autoclaved at 115 °C/10 min, and the cysteine solution used was sterilized by membrane filtration.

2.2. Yogurt processing

The yogurt processing is published elsewhere (Cruz et al., 2010; Cruz, Cadena, et al., 2012; Cruz, Castro, Faria, Bogusz, et al., 2012; Cruz, Castro, Faria, Lollo, et al., 2012). A total of 1% v/v of *S. thermophilus* TA040, 1% v/v of *L. delbrueckii* spp. *bulgaricus* LB340, 3% v/v of *L. acidophilus* La14 and 3% v/v of *B. longum* Bl05 was used, which corresponds to an initial dosage of about 7.7, 8 and 8 log CFU.mL^{-1}, respectively. The fermentation was performed at 45 °C and the pH was monitored until it reached 4.6. After the gel stirring reached 10 °C, a solution of 250 ppm of glucose oxidase (Glucomax CO, Prozyn, São Paulo, Brazil, powered, food grade system, added with catalase in the formulation) was added to the product and the content was stirred manually; indeed, a recent study reported adequate results using this amount of enzyme covering all the technological parameters of the yogurt quality (Cruz, Castro, Faria, Bogusz, et al., 2012). The stirring process is the last step that incorporates oxygen during yogurt processing, and it is in this stage that the glucose oxidase was added, so more consistent results could be obtained. Finally, the probiotic stirred yogurts were packaged in four different plastic systems (PI, PII, PIII and PIV, respectively) and kept under refrigeration (3–5 °C) for 28 days. The yogurt processing was repeated three times, and the physicochemical and microbiological analyses were performed at 1, 7, 14, 21 and 28 days of refrigerated storage. Monolayer polypropylene (PP) or PP coextruded with ethylene vinyl alcohol (EVOH) cups (100 mL) was used to pack the samples. Such materials, commercially used for yogurt and other dairy foods, were obtained by donation from Dixie Toga, São Paulo, Brazil, coded as PI, PII, PIII and PIV, and presented an oxygen permeability rate (TPO_2) of 0.09, 0.2, 0.39 and 0.75 mL O_2/day, respectively.

2.3. Microbiological analysis

A 1 mL sample of yogurt was transferred to a screw-capped tube containing 9 mL sterile 0.1% w/v peptone water. Further dilutions were made from this original dilution and the microbial counts were carried out in duplicate by using the deep plating technique. The yogurt and probiotic bacteria were enumerated using a methodology published elsewhere (Sohrabvandi, Mortazavian, Dolatkhahnejad, & Monfared, 2012). All the culture media were previously tested aiming to guarantee their selectivity towards the microorganism desired. The bacteria count after the fermentation ranged from about 9.0 log CFU.mL^{-1} for yogurt bacteria and 8.0 log CFU.mL^{-1} for probiotic bacteria.

2.4. Physical–chemical analysis

The probiotic stirred yogurts were submitted to the following analysis at 1, 7, 14, 21 and 28 days of refrigerated storage: pH (Marshall, 1993), proteolytic activity (Church, Swaisgood, Porter, & Catignani, 1983), dissolved oxygen (Cruz et al., 2010), lactic acid and acetic contents (Donkor, Henriksson, Vasiljevic, & Shah, 2005) and diacetyl and acetaldehyde level (Condurso, Verzera, Romeo, Ziino, & Conte, 2008).

2.5. Statistical analyses

The experiment was repeated three times. The results were initially submitted to a one-way analysis of variance, followed by Tukey's test. All the analyses were carried out using the software XLSTAT for Windows 2012 with 95% of significance (Adinsoft, Paris, France).

3. Results and discussion

3.1. Postacidification, oxygen levels, and proteolysis

Table 1 shows the mean values of pH, dissolved oxygen and proteolysis of the probiotic yogurt packaged in different plastic containers throughout the refrigerated storage. Overall, yogurts packaged in plastic containers with lower oxygen permeability rate showed a higher extent of post-acidification and proteolysis as well as a lower content of dissolved oxygen during the refrigerated storage ($p<0.05$). Indeed, P1 (permeability of 0.09 mL O_2/cup.day) presented a considerable variation in pH (4.61–3.96), dissolved oxygen content (6.05–4.15 ppm O_2) and proteolysis (0.542–0.777), respectively, while yogurt P4 (permeability of 0.75 mL O_2/cup.day) presented a higher variation in pH (4.61 to 4.34), dissolved oxygen content (6.46 to 9 ppm of O_2) and proteolysis (0.577 to 0.70$_2$).

These data suggest that there was a synergism between the packaging system and the glucose oxidase added to the products. The complete removal of oxygen by glucose oxidase is difficult to be attained, and this fact may be explained by two reasons: i) the permeability through the packaging system is multidirectional; ii) there is little substrate (glucose) available in the products and, consequently, there is a considerable loss of enzymatic activity along the storage period (Cruz, Castro, Faria, Lollo, et al., 2012). Herein, the use of a plastic system that presents a low oxygen permeability transfer rate may be interesting once it may decrease the amount of oxygen that permeates the packaging system

Table 1
pH, oxygen content and proteolysis in probiotic yogurts added in different plastic packaging materials along the refrigerated storage.

	Days	pH	Oxygen	Proteolysis
PI	1	4.61^{Aa}(0.46)	6.05^{Aba}(0.32)	0.607^{Aba}(0.031)
PII	1	4.63^{Aa}(0.37)	5.95^{Aa}(0.35)	0.543^{Aa}(0.027)
PIII	1	4.62^{Aa}(0.38)	6.75^{aAa}(0.12)	0.547^{Bb}(0.045)
PIV	1	4.61^{Aa}(0.40)	6.46^{Ab}(0.31)	0.577^{Ac}(0.034)
PI	7	4.15^{Ad}(0.51)	5.10^{Bac}(0.24)	0.673^{Ac}(0.021)
PII	7	4.26^{Aa}(0.27)	4.60^{Bac}(0.27)	0.549^{Ba}(0.033)
PIII	7	4.57^{Aa}(0.37)	5.55^{ABb}(0.29)	0.593^{Ab}(0.022)
PIV	7	4.56^{Bb}(0.42)	6.85^{Babc}(0.20)	0.595^{Bc}(0.022)
PI	14	4.04^{Bc}(0.41)	4.80^{aB}(0.34)	0.716^{Ad}(0.012)
PII	14	4.10^{Bd}(0.37)	4.65^{Aa}(0.21)	0.616^{Ad}(0.036)
PIII	14	4.55^{Aa}(0.28)	5.55^{Bb}(0.25)	0.660^{Ba}(0.034)
PIV	14	4.26^{Bb}(0.36)	7.00^{Bb}(0.38)	0.652^{Aa}(0.027)
PI	21	3.94^{Cbc}(0.29)	4.40^{Bb}(0.35)	0.764^{Ab}(0.024)
PII	21	4.15^{Bbc}(0.27)	6.05^{aB}(0.34)	0.698^{Bc}(0.033)
PIII	21	4.33^{Cd}(0.34)	5.50^{aC}(0.33)	0.703^{Ad}(0.026)
PIV	21	4.42^{Aa}(0.38)	8.30^{Aa}(0.21)	0.694^{Aa}(0.031)
PI	28	3.96^{Bb}(0.41)	4.15^{Bb}(0.34)	0.777^{Bab}(0.030)
PII	28	3.99^{Cbc}(0.46)	5.85^{Bbc}(0.28)	0.714^{Cbc}(0.028)
PIII	28	4.25^{Cc}(0.48)	7.00^{Cbc}(0.19)	0.706^{Bc}(0.037)
PIV	28	4.34^{Cc}(0.33)	9.00^{Dc}(0.25)	0.702^{Bc}(0.033)

Values are means with standard deviations in brackets. Different lowercase letters in the same column indicate presence of statistical difference ($p<0.05$) among treatments (yogurts). Different capital letters in the same column indicate presence of statistical difference ($p<0.05$) along the storage days. pH is admensional. Dissolved oxygen is expressed in ppm (mg.L^{-1}). Proteolysis is expressed in absorbance$_{340}$. Analysis is performed in duplicate. PI, PII, PIII, and PIV = see text for codification.