PAPER

EFFECTS OF LACTULOSE LEVELS ON YOGHURT PROPERTIES

O. BEN MOUSSA*, M. BOULARES, M. CHOUAIBI, M. MZOUGHI and M. HASSOUNA
Research unit Bio-preservation and valorization of agricultural products UR13-AGR 02, HigherInstitute of Food Industries of Tunisia, Carthage university, Tunisia
*Corresponding author: olfajamel@yahoo.fr

ABSTRACT

Therapeutic levels of lactulose were used with commercial starters (Yoflex 801, Yoflex 901 and Yomix 486) in yoghurt. In fact, Yoflex 801 was supplemented with 1.5% lactulose resulting in minor yoghurt quality alterations. This co-culture was retained to study the influence of lactulose levels (0, 4, 6, and 8 %) on yoghurt quality for 28 days at 4°C. Kinetic parameters, syneresis, proteolysis degree, and sensory characteristics were improved by increasing lactulose dose; thus, thixotropic and pseudoplastic gel was shown. Accordingly, functional yoghurt fermented with Yoflex 801, containing 4 to 6 % of lactulose, proved to be the most adequate choice.

Keywords: dose, lactulose, prebiotic, starter, yoghurt

1. INTRODUCTION

Yoghurt is one of the most popular fermented dairy products, widely consumed all over the world, owing to its nutritional and sensory characteristics solicited by consumers (Loveday*et al.,* 2013). It is produced by lactic fermentation of two specific strains: *Streptococcus thermophilus* and *Lactobacillus delbruekii subsp. bulgaricus* (CODEX STAN 243-2003). Yoghurt has nutritional and health benefits, such as improving digestibility and lactose utilization. It promotes gut health and has a hypocholesterolemic action (ADOLFSSON *et al.,* 2004; WEERATHILAKE *et al.,* 2014). Bioactive compounds such as probiotics and prebiotics are usually added in yoghurts to enhance its functionality, quality and therapeutic properties (ÖZER *et al.*, 2005; CRUZ *et al.*, 2013a).

PREBIOTICS ARE SUBSTRATES THAT ARE SELECTIVELY UTILIZED BY HOST MICROORGANISMS CONFERRING A HEALTH BENEFIT (GIBSON ET AL., 2017). Prebiotics cannot be digested by the enzymes of the human gastrointestinal tract, however, they are fermented in the large intestine by colonic microflora, producing lactic acid, short chain fatty acids (acetic, propionic and butyric) and gases (GARCIA *et al.*, 2008; NICHOLSON *et al.,* 2012). Therefore, intestinal pH is reduced and harmful and pathogenic microorganisms proliferation are inhibited (ROLIM, 2015; WANG, 2009). Also, prebiotics prevent diarrhea and other diseases like colon cancer (MANN *et al.*, 2007). Besides, they act in the absorption of calcium and establish favorable mechanisms to immunomodulation as well as beneficial effects on lipid metabolism and various cardiovascular risk factors (DELGADO *et al.*, 2011).

Prebiotics including lactulose, inulin and oligofructose are considered as bifidogenic factors (ROBERFROID, 2000; RAFTER *et al.*, 2007). Thus, they are used in the formulation of dairy products, such as fermented milk (ÖZER *et al.*, 2005), Italian cheese (FERRÃO *et al.*, 2016; BELSITO *et al.*, 2017; FERRÃO *et al.*, 2018), whey beverage (GUIMARAES *et al.*, 2018) and ice cream (BALTHAZAR *et al.*, 2017) in order to add a functional value to these products and improve their technological characteristics.

Lactulose is a prebiotic (FRIC, 2007) used as a drug to treat illnesses, particularly chronic constipation (AIDER and DE HALLEUX, 2007; LEE-ROBICHAUD *et al.,* 2010). Moreover, it stimulates the growth of bifidobacteria (PHARM and SHAH, 2008; OLANO and CORZO, 2009). In this regard, lactulose effects are dose dependent (BOTHE *et al.*, 2017), for instance, 2 g of administrated lactulose would increase the short-chain fatty acid levels of the intestinal content (MIZOTA *et al.*, 2002). Besides, bifidogenic effects of lactulose are acquired when 5 g of lactulose are consumed every day. Therefore, when bacterial counts of *Bifidobacterium*, *Lactobacillus* and *Anaerostipes* increase, subsequently, acetate, butyrate and lactate increase with a decrease of branched-chain fatty acids. Likewise, 7.5 g dose of lactulose, daily, allows decreasing ammonia levels (AGUIRRE *et al.*, 2014).

Accordingly, lactulose appears as an important food ingredient that might be further explored for the production of new functional foods, and thus its future large scale production for food and nutraceutical purposes is anticipated.

For the best of our knowledge, there are few researches about lactulose effects on technological properties of yoghurt starters as well as on yoghurt characteristics.

In this connection, with the present study we intend to formulate new functional yoghurt and explore the possible application of lactulose as a prebiotic agent in this product when varying his concentration.

Then, the first aim of this study is to chiefly evaluate the effect of lactulose on the acidification kinetics and post-acidification, syneresis, proteolysis degree and growth of three different commercial yoghurt starters during storage, in order to select yoghurt cultures, possessing a low affinity to lactulose, and thus yielding functional yoghurt similar to the conventional one, as requested by consumers. The second aim of this work is to evaluate the effect of the incorporation of lactulose at different doses on the quality of new developed yoghurt inoculated with the selected starter. The lactulose dose effect was determined on biochemical, microbiological, rheological, and sensory yoghurt properties when compared with control that lead to choose the most adequate concentration having the least effects during refrigerated storage.

2. MATERIALS AND METHODS

2.1. Yoghurt manufacture and study design

For yoghurt production, 5% of skim milk powder was added to skimmed milk (not fat solid =10%). Thus, enriched milk was homogenized and heated to 95°C for 3 min. The pasteurized milk was then rapidly cooled down to 43 +/- 1°C and divided into six batches. Three control batches were inoculated with three combinations of frozen starters composed of two strains, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, at a concentration of 20 mL/L, which corresponds to an initial count ofabout 8 log cfu/mL. These technological starters named, respectively, Yoflex 901, Yoflex 801 and Yomix 486 were purchased from Chr. Hansen's Dairy Cultures (Hoersholm, Denmark). They are the most commonly used ones by dairy industries in Tunisia in terms of yoghurt manufacture. Thereafter, 1.5% of lactulose was added to each sample of the remaining batches (Chimica Mugello society, Italy), before inoculation with one of the three co-cultures. Subsequently, milk was distributed in sterilized sealed containers, incubated at 43°C until pH reached 4.6 and acidity reached 75 °D, and then cooled and stored at 4 °C. Finally, Dornic acidity, total solids, proteolysis degree, syneresis and lactic acid bacteria counts were determined after 24 h of production each week, during 4 weeks of refrigeration storage.
After highlighting the best co-culture for yoghurt with lactulose, in the first part of this study, we focused, in the second part, on the effect of lactulose dose. Accordingly, for yoghurt preparation, the same steps were applied as described above. In fact, four batches were prepared and supplemented with 0, 4, 6 and 8% of lactulose. Each sample was then inoculated with the selected starter. All previous analyses and viscosity measurements were performed on the obtained yoghurt samples. Sensory evaluation was also carried out at days 1, 14 and 28, during refrigeration storage.

2.2. Total solids, pH and Dornic acidity

Total solids, pH values and Dornic acidity (expressed as degree Dornic) were measured according to AOAC (1995) and AFNOR (1980), respectively. Kinetic parameters were also considered in this study: (*i*) the maximum acidification rate (Vmax), expressed in 10^{-3}pH units/ min, (*ii*) the time to reach the maximum acidification rate (Tvmax), and (*iii*) the time to complete the fermentation (TpH4.6), expressed in hours.

2.3. Syneresis

The gel was stirred at 4°C for 60 s and centrifuged for 20 min at 12,075 g in an ultracentrifuge (Beckman USA) (RINALDONI *et al.*, 2009). Syneresis (%) was calculated as mass of the separated serum from the gel after centrifugation, relating to the total mass of gel that was centrifuged.

2.4. Bacterial enumerations

The enumeration of lactic acid bacteria was performed by using De Man Rogosa and Sharpe agar (pH=6.2±0.2; Oxoid, France) after the incubation of plates at 45°C for 48h (GUIRAUD, 1998).

2.5. Evaluation of the proteolysis

For proteolysis determination, the fractional precipitation method, described by HASSOUNA *et al.* (1999), was used. Total nitrogen (NT) and soluble nitrogen at pH 4.6 (NS) were assayed after mineralization of organic nitrogen followed by distillation according to the kjeldahl reference method (AOAC, 1990). The protein content of yoghurt samples was calculated as follows: TN (g of nitrogen/100 mL of yoghurt) x 6.38 (Duncan *et al.*, 2008). Proteolysis degree = 100 x [NS (g of nitrogen/100 mL of yoghurt)/TN (g of nitrogen/100 mL of yoghurt)] as described by BOULARES *et al.* (2011).

2.6. Rheological measurements

The rheological properties were determined according to the method described by NGUYEN *et al.* (2015) with a slight modification. Briefly, flow curves of yoghurt samples were analyzed with a rotary viscometer Rheometric RM180 (Rheomat, Caluire, France), equipped with coaxial cylinders' geometry. The bob and the cup used had 15.18 (R_1) and 21 mm (R_2) radius, respectively, giving a ratio R_1/R_2=0.72. Viscosity measurements at increasing and decreasing strain rates were conducted between 0.01 and 500 s^{-1}. The viscometer was controlled by RSI Orchestrator v6.5.8 software. Flow properties were assessed at temperature 4°C. The regulation of temperature during the rheological measurements was obtained using a circulator bath (Julabo GmbH, Germany). The area of thixotropic hysteresis loop was determined using RSI Orchestrator v 6.5.8 software, which calculates the difference between the area under the up-flow curve and the down-flow curve.

2.7. Sensory evaluation

Throughout the storage period at 4°C (1^{st} , 14^{th} and 28^{th} day), the sensory properties of experimental yoghurts were evaluated by a jury of panelists consisting of 20 trained members (8 male and 12 female, aged between 24 and 45 years). The trained panelists were students from the Tunisian higher Institute of Food Industry and the training was conducted according to the method described by HOOTMAN (1992) and MEILGAARD *et al.* (2006). The test was performed inside a uniformly illuminated room, at approximately 25 °C. The obtained yoghurts were coded with a random six-digit number and served to panelists in a randomized order. The main descriptors, used to evaluate appearance, taste and texture, were sweet taste, bitter taste, mouth feel, granular texture, whey exudation, white color and overall acceptance, consisted in a 9-point scale (DANTAS *et al.,* 2016; SILVA *et al.,* 2018).

2.8. Statistical analysis

The obtained data were statistically evaluated using a one-way analysis of variance (ANOVA) with Ducan's test for mean comparison to highlight significant differences ($P < 0.05$) among yoghurt samples. All the experiments were carried out in triplicate.

3. RESULTS AND DISCUSSION

3.1. Effect of lactic starter variation and lactulose addition on yoghurt quality

3.1.1 Effects on yoghurt fermentation

Changes in kinetic parameters of acidification during the fermentation of control and lactulose incorporated yoghurts, using three different commercial starters, are shown in Table 1.

Table 1. Kinetic parameters of acidification of yoghurt using different starter co-cultures with and without lactulose at 1.5%.

Yoghurt starters	Lactulose	Vmax (10^{-3}pHunits/min)	Tmax (hours)	TpH4.5 (hours)
Yoflex 801	Control	19.33±0.02	3.5±0.19	5±0.20
	with lactulose	19.33±0.01	2.5±0.17	4±0.22
Yoflex 901	Control	16.61±0.02	3.5±0.22	4.5±0.19
	with lactulose	19.67±0.03	3±0.25	4±0.27
Yomix 486	Control	20.00±0.01	3±0.24	4.5±0.21
	with lactulose	23.33±0.02	2±0.23	3.5±0.22

As expected, on the basis of chemical acidification reaction that underlies the fermentation process, pH dropped during 3.5–5 h (TpH4.6) to values of 4.6 in all experiments. Yomix 486 exhibited the fastest Acidifying kinetics (Vmax = 20±0.01 x 10^{-3} pH units /min; Tmax = 3 h); followed by Yoflex 801. In fact, according to Almeida *et al.* (2009), different acidification profiles of LABs depend on their peculiar capacity to use nutritive compounds of milk, which could account for the differences in the kinetic parameters observed amongst the various yoghurts. Thus, LABs capacity to produce lactic acid, which is the main product of the metabolic activity of starter cultures, depends on the strains and their associations (BÉAL *et al.*, 1999). Indeed, it is known that a synergic proto-cooperation between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* takes place during yoghurt fermentation (LOURENS-HATTINGH and VILJOEN, 2001). Furthermore, Vmax increased by the incorporation of lactulose (1.5%) (Except for Yoflex 801), reaching 19.67±0.03 and 23.33±0.02 x 10^{-3} pH units/min, in fermented yoghurt, respectively, with Yoflex 901 and Yomix 486. The time for reaching maximum acidification rate (T max) was reduced by 1h, 0.5 h and 1 h, respectively, for Yoflex 801, Yoflex 901 and Yomix 486. Besides, the time for reaching $T_{pH4.6}$ was reduced by 1h for Yoflex 801 and 0.5h for both Yoflex 901 and Yomix 486. These differences could be attributed to the high rate of *L. bulgaricus* in Yomix 486 starter and/or the differences between strains and species of LABs for lactulose metabolism.

3.1.2 Effects on yoghurt quality during storage

Quality parameters of yoghurts fermented by each of the three different lactic starters with or without lactulose at 1.5%, over 28 days of refrigeration storage, are illustrated in Table 2.

Total solids content of yoghurts were evaluated during refrigeration storage. Initial total solids levels were between 98±0.38 and 102±0.95 g/L for control yoghurts. However, after lactulose incorporation, these values reached 111±0.98 and 114±1.24 g/L. Hence, the presence of prebiotics increased the total solids content of milk bases. These results were in accordance with other studies reporting that the addition of prebiotics in mix increase total solids content (Aryana and MC GREW, 2007). However, no significant differences ($P > 0.05$) between total solids values during storage period were noted.

Postacidification of the yoghurts displayed an increase over the storage period. Dornic acidity values growths were 13.5, 18 and 19°D for control fermented yoghurt, respectively, with Yoflex 801, Yoflex 901 and Yomix 486. The values changed to be 14.5, 20 and 26.5°D, when lactulose was added. Indeed, acid-production trend during storage was similar to other research studies (ÇELIK, 2007). The lowest postacidification was obtained with Yoflex 801, against the highest one noted for Yomix 486 starter, especially in yoghurt, wherein lactulose (1.5%) was added. These findings suggest that *L. bulgaricus* strain of Yomix 486 was able to assimilate more lactulose than the other co-culture strains. Moreover, *L. bulgaricus* produces more lactic acid when lactulose is available (Hernandez-Hernandez *et al.*, 2012).

For syneresis (Table 2), a steady increase in all tested samples was recorded, with the progress of storage time until the 21st day. Syneresis levels increased from 62% to 73% and from 60% to 77%, respectively, for Yoflex 901 and Yomix 486. The use of Yoflex 801 culture was associated with weak whey separation, compared to the other starters, during all storage time. Syneresis values rose from 58% to 69%. These findings could be attributed to the capacity of each strain to produce exopolysaccharides. In fact, AMATAYAKUL *et al.* (2006) reported that syneresis could be reduced by starters producing exopolysaccharides. Besides, there were conflicting findings about fermentation parameters effects on molecular characteristics of exopolysaccharides (MENDE *et al.,* 2016). Furthermore, IBRAHIM (2015) noted that frail gel was obtained when the fermentation time of camel milk was long. The main reasons for syneresis might be ascribed to the structural rearrangements in casein micelles in the gel network and the rate of solubilization of colloidal calcium particles. In this study, a longer fermentation period was achieved by Yoflex 801 culture. However, higher syneresis was observed in yoghurt Yoflex 901 or Yomix 486 (Table 2). Therefore, the primary reason for higher syneresis was considered to be the type of strains in each co-culture.

As indicated in Table 2, at the 28th day, syneresis percentage exhibited little decrease. These results were in accordance with AKGUN *et al.* (2017) findings, pertaining to probiotic yoghurts. As determined by MENDE *et al.* (2016), medium acidity was linked to the interaction between polysaccharides molecules and protein network. Indeed, acidity affects protein network charges, and consequently their joining with polysaccharides would be modified as well.

Table 2. Variations in Total solids, postacidification, syneresis, proteolysis degree and lactic acid bacteria counts of yoghurt fermented using three different starters, with or without lactulose at 1.5%, for 28 days of storage at 4°C.

Parameters	Starters	Lactulose (1.5%)	Storage period (days)				
			1	7	14	21	28
Total solids	Yoflex 801	Control	98±0.38	98±0.88	99±0.84	99.4±0.85	99.18±1.22
		With lactulose	111±0.98**	111.5±0.97**	112±0.94**	113±1.24**	112±0.85**
	Yoflex 901	Control	99±0.99	99.9±0.56	101±0.85	100±0.76	101.1±0.97
		With lactulose	113±0.74**	113.5±0.54**	112±0.65**	112±0.85**	114±1.14**
	Yomix 486	Control	99±1.12	102±0.54	101±0.68	100±0.75	102±0.95
		With lactulose	111±0.98**	112±0.65**	113±0.94**	114±1.24**	113.2±0.85**
Dornic acidity	Yoflex 801	Control	78±0.43	82±0.72*	85±0.45*	89±0.66*	92.5±0.34*
		With lactulose	81.5±0.28**	84±0.63*	90±0.52*,**	95±0.38*,**	96±0.71**
	Yoflex 901	Control	76±0.50	84±0.52*	86±0.34*	87±0.35*	94±0.38*
		With lactulose	76.5±0.91	92±0.45*,**	93±0.34**	94±0.92**	96.5±0.26**
	Yomix 486	Control	79±0.5	90±0.52*	94±0.81*	93±0.73	98±0.90*
		With lactulose	81±0.38**	91±0.27*	96±0.63*	101±0.63*,**	107.5±0.38*,**
Syneresis (%)	Yoflex 801	Control	58±0.05	60±0.02*	67±0.1*	67±0.01	66±0.4*
		With lactulose	63±0.01**	63±0.01**	67±0.12*	69±0.02*,**	68±0.3*,**
	Yoflex 901	Control	62±0.03	67±0.01*	69±0.1*	73±0.01*	70±0.2*
		With lactulose	63±0.02**	68±0.02*,**	70±0.08*,**	74±0.02*,**	72±0.1*,**
	Yomix 486	Control	60±0.01	65±0.1*	70±0.01*	77±0.3*	74±0.5*
		With lactulose	61±0.01**	66±0.01*,**	75±0.5*,**	79±0.1*,**	75±0.7*
Proteolysis degree (%)	Yoflex 801	Control	33±0.71	36±0.57*	39.5±0.42*	43±0.57*	44.5±0.71*
		With lactulose	40±0.57 **	42.6±0.42*,**	43.75±0.35*,**	46±0.71*,**	49.5±0.71*,**
	Yoflex 901	Control	39±0.28	41±0.99	43.9±0.28	46.8±0.58*	49.2±0.14*
		With lactulose	44±0.71**	45±0.2	49.2±0.14*,**	50.49±0.58**	52.99±0.56*,**
	Yomix 486	Control	28±0.42	32±0.56*	38±0.35*	43.9±0.57*	49±0.71*
		With lactulose	30±0.42**	38±0.35*,**	46±0.5*,**	48±0.71*,**	56±0.56*,**
LAB counts (log cfu/mL)	Yoflex 801	Control	8.70±0.3	8.75±0.1	9.08±0.1*	9.39±0.3*	9.60±0.1
		With lactulose	8.85±0.1	9.22±0.24*,**	9.32±0.2**	9.55±0.2*	9.85±0.1*,**
	Yoflex 901	Control	9.05±0.1	9.15±0.1*	9.45±0.17*	9.65±0.34	9.80±0.22
		With lactulose	9.15±0.2	9.45±0.3	9.75±0.12**	9.90±0.19	10.10±0.13*,**
	Yomix 486	Control	9.01±0.2	9.32±0.2*	9.84±0.1*	9.94±0.2	10.4±0.1*
		With lactulose	9.22±0.2	9.58±0.14*,**	10.1±0.12*,**	10.12±0.24	10.9±0.16*,**

Data are presented as the mean±SD of three separate experiments. *, significant differences between storage period ($P< 0.05$); **, significant differences between control and supplemented yoghurt at the same storage time ($P< 0.05$).

Otherwise, yoghurts incorporating 1.5% lactulose had higher syneresis values at each storage period. However, these differences were not significant ($P > 0.05$) compared to control, except for Yomix 486 samples, which recorded the highest syneresis percentage. This might be assigned to lower pH obtained when lactulose was added, which caused an unstable gel network with a continuous changing arrangement, thus, resulting in disturbed protein micells as described by DONKOR *et al.* (2007) and MÖLLER and VRESE (2004).

Yoghurt protein content was 4.6%.This value is in compliance with the standard (CODEX STAN 243-2003), which requires content in minimal equal protein of 2.7 %. Table 2 presents proteolysis degree obtained in yoghurt supplemented with 1.5% and fermented with Yoflex 801, Yoflex 901 and Yomix 486. As expected, proteolysis degrees increased for all yoghurt samples, for 28 days of refrigeration storage. These results induced extracellular proteases activity of lactic acid bacteria through the storage period (YUKSEL and ERDEM, 2010). Besides, NIELSEN *et al.* (2009) proved that proteases are active during refrigeration storage. Further, proteolysis degrees were higher, at each storage period, when 1.5% of lactulose was added. Similar results were obtained by YUKSEL and ERDEM (2010) and DONKOR *et al.* (2007). In fact, they also demonstrated that proteolysis levels depend on the nutrients available to proteolytic microorganisms.

Lactic acid bacteria counts were converted to log scale and reported in Table 2. Even with the addition of lactulose, LAB count was maintained over10^8 cfu/mL. This result was in good agreement with the Codex Alimentarius Commission (CODEX STAN 243-2003), which established that the counting of lactic acid bacteria must be over 10^7 cfu/mL. It is concluded that LAB counts become higher in yoghurt samples incorporating lactulose. Indeed, over the storage period, their counts increased by 0.9, 0.75 and 1.39 log cfu/mL, in control fermented yoghurts, respectively, with Yoflex 801, Yoflex 901 and Yomix 486. These increases became 1, 0.95 and 1.68 in yoghurts, wherein lactulose (1.5%) was added. These results were consistent with TABATABAIE and MORTAZAVI (2008) who reported that in yoghurt containing lactulose (1 and 3%) during 5 weeks of cold storage, the survival of *L. rhamnosus* LBA and *B. bifidum* CECT considerably improved. RASTALL and MAITIN (2002) found that the highest count of bifidobacteria was noted when adding xyloligosaccharide and lactulose, however, the largest increase in lactobacilli was obtained when adding FOSs. Thus, generally, lactulose was a more effective growth promoter for lactic strains compared to inulin. On the other hand, differences were not significant at each storage period. Indeed, probiotic bacteria metabolise prebiotics more than yoghurt starters.

Furthermore, when lactulose was incorporated, the lowest LAB counts changes were obtained in yoghurts with Yoflex 801. However, greater proliferations were noted for Yoflex 901 and Yomix 486 starters. Therefore, it can be concluded that lactulose had high prebiotic effect on Yoflex 901 and Yomix 486, followed by Yoflex 801. Indeed, it seems that the stimulatory impacts of prebiotics on lactic acid bacteria viability depends on several factors such as strain type and final pH.

Hence, low stimulation of starter bacteria, low postacidification, low proteolysis and low syneresis were sought to obtain functional food, having similar characteristics to the conventional food. Hereinafter, Yoflex 801 is chosen to be used in the rest of the study.

3.2. Characteristics of yoghurt added with different dose of lactulose

3.2.1 Effects of lactulose dose on yoghurt fermentation and post-acidification

Concerning acidification kinetics, shown in Table 3, it was observed that lactulose yoghurts exhibited higher Vmax than the control; values obtained ranged from 19.33±0.02 to 25±0.02 10^{-3}pH units/min, respectively, for control fermentation milk and samples added with 8% of lactulose. However, the time (Tmax) to reach Vmax was 3.5 h for control, 3 h for both 4% and 6% of lactulose and 2.5 h for 8%. Moreover, the time to reach pH= 4.6 was 5 h for control, 4 h for both 4% and 6% of lactulose and 3.5 h for 8%. These findings were in accordance with those of ÖZER *et al.* (2005), revealing that inulin and lactulose addition at different concentrations reduced the incubation period of yoghurt.

In this regard, lactic acidity values increased significantly ($P < 0.05$) during refrigeration storage in all yoghurt samples (Table 4). Indeed, metabolism of yoghurt bacteria continued during the 28 days of storage at 4°C, as shown previously in the first part of the study. Moreover, when lactulose was supplemented, overall postacidification increased weakly (1 to 3°D). This data was in agreement with CRUZ *et al.* (2013b) results, reporting that the supplementation of different doses (2, 4, 6 and 8%) of oligofructose as prebiotic has no significant effect on post acidification. These findings are desirable in modern yoghurt industry, and endorse the choice of Yoflex 801 as starter in this study. However, OLIVEIRA *et al.* (2011) proved that the addition of lactulose in skim fermented milk by probiotic LAB in coculture with *S. thermophilus* decreased pH at the final period of storage, indicating a bifidogenic effect for *Bifidobacterium lactis*.

Table 3. Kinetic parameters of acidification of yoghurt fermented with Yoflex 801 and added with lactulose at different doses (0, 4, 6 and 8%).

Lactulose dose (%)	Vmax (10^{-3}pHunits/min)	Tmax (hours)	TpH4.5 (hours)
Control	19.33±0.02	3.5±0.19	5±0.2
4	20±0.01	3±0.17	4±0.22
6	21.6±0.03	3±0.21	4±0.21
8	25±0.02	2±0.23	3.5±0.19

3.2.2 Effects of lactulose dose on yoghurt quality during storage

The parameters of control yoghurts and those obtained at different lactulose concentrations (4, 6 and 8%) fermented with selected Yoflex 801 starter, for 28 days at 4°C, are presented in Table 4. Total solids content of the four obtained yoghurts displayed an increase (P <0.05) when lactulose concentrations rose. Values varied from 97.5±0.9 g/L (control sample) to 178±0.9 g/L (8% lactulose). Indeed, DE CASTRO *et al.* (2008) reported that the addition of prebiotic was associated with a total dry extract increase. Moreover, these findings outlined that lactulose was still in yoghurts and would be available for consumers as prebiotic, in order to improve health.

Table 4. Variations in post-acidification, total solids, syneresis, proteolysis degree and lactic acid bacteria counts of yoghurt containing various doses of lactulose (0, 4, 6 and 8%) and fermented with Yoflex 801, for 28 days of storage at 4 °C.

Storage period (days)	Lactulose dose (%)	Dornic acidity	Total solids (g/L)	Syneresis (%)	Proteolysis degree (%)	LAB counts (log cfu/mL)
1	Control	79±0.13	97.5±0.9	61±0.12	32.38±0.71	8.35±0.14
	4	80.33±0.2*	125.33±1.2*	58±0.5*	37.1±0.56*	8.56±0.22*
	6	81±0.2*	155.5±0.8*	56±0.1*	42±0.42*	8.77±0.5
	8	82±0.2*	178±0.9*	53±0.18*	45.3±0.14*	8.86±0.12
7	Control	83.66±0.12**	98±0.85	62±0.16**	35.5±0.28**	8.48±0.2
	4	86.33±0.49*,**	127±1.24*	60±0.2*,**	38.3±0.78*	8.79±0.18*
	6	88.66±0.23*,**	155.66±1.22*	59±0.5*,**	44±0.59*,**	9.07±0.1*
	8	90.33±0.45*,**	168±0.85*	55±0.8*,**	47.5±0.71*,**	9.13±0.1**
14	Control	86±0.39**	98±0.97	65±0.12	37.8±0.35**	8.86±0.14
	4	88.33±0.23*,**	123±1.24 *	62±0.3*,**	40.4±0.58*	9.08±0.22*,**
	6	89±0.25*,**	155±1.9*	62±0.4**	45±0.28*	9.3±0.2**
	8	90±0.13*,**	170±1.74*	57±0.18*,**	49.5±0.5*	9.38±0.1**
21	Control	90.66±0.1**	99±0.95	68±0.1	41±0.35**	9±0.1**
	4	93.33±0.2*,**	124±0.99*	63±0.24*,**	42.6±0.42*,**	9.11±0.12
	6	94.6±0.12*,**	153±1.14*	64±0.1*,**	47±0.14*,**	9.31±0.13*,**
	8	94.33±0.17*,**	171±1.41*	59±0.18*,**	50.6±0.58*	9.56±0.1**
28	Control	94.66±0.39	98.8±0.85	70±0.09	43.8±0.28	9.66±0.14**
	4	97±0.25*,**	125±1.9*	65±0.02*,**	45±0.42*	9.87±0.2**
	6	97.33±0.1*,**	152±1.37*	65±0.01**	48.5±0.71*	10.01±0.2**
	8	100.66±0.49*,**	169±1.25*	60±0.03*,**	52.49±0.35*,**	10.16±0.24**

Data are presented as the mean±SD of three separate experiments.

*, significant differences between lactulose dose at the same storage time ($P< 0.05$); **, significant differences between the same dose of lactulose at different storage periods ($P< 0.05$).

Furthermore, initial syneresis values varied from 53±0.18 % to 61±0.12 %. Besides, whey separation increased significantly (P <0.05) during storage in all samples, and decreased with lactulose dose increase. Syneresis reached 60±0.03 % to 70±0.09 at the28th day of cold storage (Table 4). These results could be elucidated by the effective role of prebiotics in increasing water-holding capacity in the texture (REID et al., 2003). Moreover, some studies revealed that using prebiotic compounds, such as inulin and lactulose at optimum concentrations, might reduce the percentage of syneresis. In addition, these findings could be related to the total solids. In fact, when dry extracts increased, syneresis decreased (ESTEVEZ et al., 2009). Thus, lactulose levels would improve yoghurt quality by reducing syneresis, which is not sought by dairy industry.

On the other hand, during storage, proteolysis degrees increased significantly (P < 0.05). These findings are in line with our previous results and suggest that although lactulose dose did not affect LAB growth, it was involved into their proteolytic activity, as reported by ÖZER et al. (2005). However, it is noteworthy that proteolysis would generate free amino-acids, which improve the sensory properties of dairy fermented products.

Further, LAB counts over the storage period (Table 4), increased in all samples. Correspondingly, lactulose dose weakly affected LAB growth. These results were in good agreement with ÖZER et al. (2005) findings, who did not note any significant effect of lactulose (2.5%) on the growth of yoghurt starter bacteria. Likewise, those data asserted previous results when different starters were used with 1.5% of lactulose.

3.3. Rheological properties variation

In this study, as shown in Table 5, the results revealed that the increase of lactulose concentration and storage period give rise to the increase of the yield stress values (0.11±0.02-0.44±0.01 Pa), consistency coefficients (1.96±0.04 - 3.62±0.03 Pa.s^n) and hysteresis area, and the slow decrease of flow index values. This can be explained by the breakdown of the yoghurt structure during storage after shear. Indeed, the increase of consistency values of the formulated samples could be assigned to the increase of the total solid content in lactulose yoghurts, especially when lactulose dose ranged from 4% to 8 % (P < 0.05). Therefore, an increase in lactulose concentration was accompanied with an increase in pseudoplasticity. Moroever, lactulose contributed in forming the best structural arrangement in the enriched yoghurts. Thus, its addition increased the rates of aggregation and curd firming reactions in the casein gels, which was in line with the result reported in previous work (ARANGO *et al.*, 2013).

The two-way ANOVA test was made to ascertain the effects of the storage period, lactulose concentration as well as the interaction between the storage period and lactulose concentration on rheological parameters (yield stress, flow index, consistency coefficient and hysteresis area). The influence of both factors on each variable tested was clear with P values < 0.05, except for flow index, which had no significant P values (P > 0.05) in terms of storage period and interaction between the storage period and lactulose concentration.

On the other hand, flow curves of control and 8% lactulose enriched yoghurts, shown in Fig. 1, yield hysteresis loops. All samples exhibited thixotropic behavior as illustrated in other studies, covering set yoghurts (CIRON *et al.,* 2012; ESPÍRITO-SANTO *et al.,* 2013 and ILICIC *et al.,* 2014).

Table 5. Variations in Rheological parameters of yoghurt containing various doses of lactulose, for 28 days of storage at 4 °C.

Storage time (days)	Lactulose dose (%)	Yield stress σ_0 (Pa)	Flow index n	Consistency coefficient k (Pa.s^n)	Hysteresis area A (Pa/s)	R^2
	Control	0.11±0.02^a	0.65±0.02^a	2.05±0.04^a	1170.05±30.10^a	0.993
1	4	0.15±0.02^{ab}	0.63±0.02^a	3.13±0.03^b	1256.45±24.20^b	0.987
	6	0.18±0.01^b	0.61±0.01^a	3.27±0.07^{bc}	1290±20.02^b	0.996
	8	0.21±0.01^b	0.60±0.01^a	3.41±0.05^c	1319.30±26.22^b	0.975
	Control	0.12±0.01^a	0.66±0.02^a	2.13±0.05^a	1193.06±40.46^a	0.966
7	4	0.19±0.02^b	0.61±0.02^{ab}	3.22±0.03^b	1268.21±35.87^a	0.973
	6	0.26±0.01^c	0.58±0.02^{ab}	3.45±0.05^c	1277.09±34.91^a	0.992
	8	0.32±0.01^d	0.57±0.02^b	3.52±0.04^c	1394.20±30.33^b	0.991
	Control	0.14±0.02^a	0.64±0.03^a	2.21±0.05^a	1292.50±13.70^a	0.989
14	4	0.25±0.02^b	0.61±0.01^{ab}	3.38±0.06^b	1289.68±19.88^a	0.995
	6	0.31±0.03^{bc}	0.56±0.01^{ab}	3.48±0.05^{bc}	1314.59±16.64^a	0.970
	8	0.40±0.03^c	0.54±0.02^b	3.64±0.06^c	1349.70±15.50^b	0.989
	Control	0.15±0.0^a	0.64±0.02^a	2.10±0.04^a	1291.18±15.72^a	0.964
21	4	0.28±0.02^b	0.60±0.01^{ab}	3.40±0.03^b	1296.26±15.93^{ab}	0.989
	6	0.34±0.02^b	0.57±0.01^{bc}	3.48±0.02^b	1302.53±113.08^{ab}	0.976
	8	0.44±0.01^c	0.53±0.01^c	3.62±0.03^c	1303.46±12.66^b	0.985
	Control	0.13±0.01^a	0.68±0.03^a	1.96±0.04^a	1287.73±14.28^a	0.989
28	4	0.30±0.01^b	0.61±0.01^{ab}	3.38±0.04^b	1288.34±15.01^a	0.974
	6	0.36±0.01^b	0.55±0.03^b	3.50±0.05^{bc}	1299.87±13.53^{ab}	0.997
	8	0.43±0.03^c	0.52±0.02^b	3.64±0.04^c	1312.22±14.13^b	0.966

Data are presented as the mean±SD of three separate experiments.
Different superscript letters indicate statistically significant ($p<.05$) differences in a column at the same storage time.

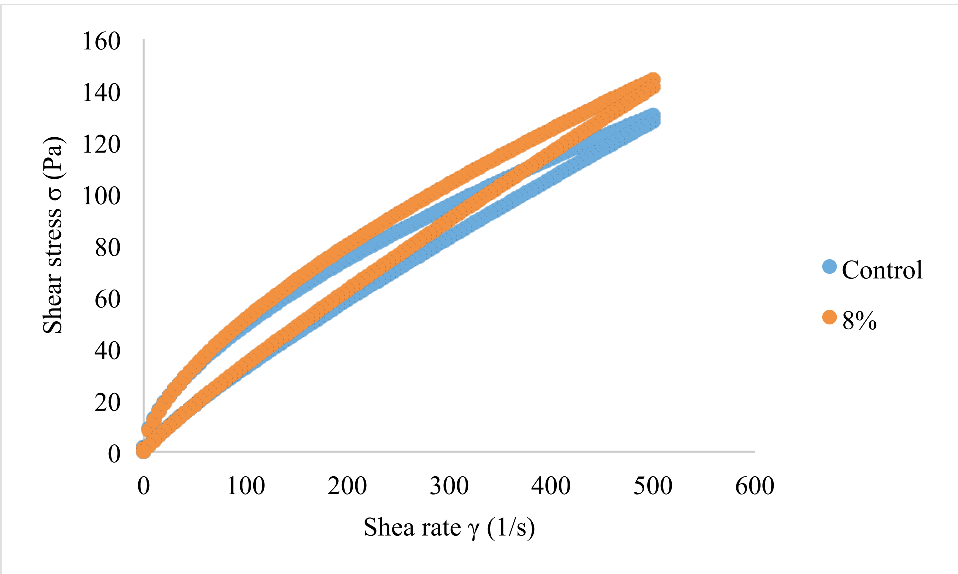

Figure 1. Hysteresis loops of set yoghurts (control and 8 % of lactulose) after 1 day of storage at 4°C.

STEFFE (1996) reported that thixotropic property is observed particularly in fragile structures and the three-dimensional network formed is completely destroyed as in the case of set yoghurts. Accordingly, it is clear that the sample enriched with lactulose has shear stress values, higher than those found in the control. In fact, yoghurts viscosities increased with the increase of lactulose concentration.

A non-linear relationship was detected between shear stress (σ) and shear rate ($\dot{\gamma}$). These findings were in accordance with those of SAH*et al.* (2016), CUI*et al.* (2014) and CIRON *et al.* (2012). Based on the values of R^2coefficient, the HERSCHEL-BULKLEY model was found to be a better-fit model for flow curves (R^2> 0.96) and only rheological parameters of this model are presented in this study (Table 5). The obtained data were fitted to HERSCHEL–BULKLEY model according to:

$$\sigma = \sigma_0 + K\dot{\gamma}^n$$

Where σ σrepresents the shear stress (Pa), k is the consistency coefficient ($Pa.s^n$), $\dot{\gamma}$ is the shear rate (s^{-1}), σ_0 is the yield stress and n is the flow behavior index (dimensionless). Moreover, the plot of the shear stress against shear rate of the yoghurt samples under investigation yielded a flow index n of less than 1 (thinning fluid) (0.53±0.01 - 0.68±0.03), indicating that their flow behavior had a non-Newtonian profile.

3.4. Sensory evaluation

Table 6 represents comparative sensory analysis among yoghurts supplemented with different doses of lactulose (0, 4, 6 and 8%) using scoring methodology, after storage for days 1, 14 and 28.

Table 6. Variations in sensory evaluation of yoghurt containing various doses of lactulose (0, 4, 6 and 8 %) for 28 days of storage at 4°C.
*: significant differences between lactulose doses at the same storage time (P< 0.05).

Storage period (days)	Lactulose dose (%)	Sweet taste	Bitter taste	Mouth feel	Granular texture	Whey exsudation	White Color	Overall acceptance
1	Control	1.9±0.4	2.28±0.7	5.11±0.5	5.18±0.75	3.9±0.8	2.95±0.8	3±0.35
	4	2.78±0.3*	2.63±0.5	3.15±0.6*	4±0.65	4.56±0.4	3.72±0.6	3,65±0.25*
	6	4.95±0.5*	2.85±0.35	4.88±0.7	2.65±0.75	4±0.3*	5.44±0.5	4,43±0.6
	8	6.84±0.6*	2.48±0.2	6.58±0.25	1.79±0.8	2.9±0.38*	5.31±0.3	6,2±0.39
14	Control	2.45±0.6	3.22±0.3	3.58±0.25	4.2±0.24	4,78±0.32	3.3±0.2	3,11±0.32
	4	5±0.35	3.25±0.25	3±0.7	3.9±0.9	3,75±0.35*	3.25±0.7	3.65±0.62
	6	6±0.5	2,5±0.24*	3.65±0.4	4.45±0.75	4.56±0.56	3.6±0.56	3.4±0.23*
	8	6±0.7	2.99±0.3*	5.15±0.8*	1.72±0.65	3.14±0.38	2.91±0.34	5.65±0.39*
28	Control	2.1±0.2	2,5±0.3	4.29±0.65	4.09±0.9	4.72±0.42	3.34±0.85	3.11±0.8
	4	3±0.35*	2.78±0.24	4.5±0.45	3.5±0.3	3.5±0.6	4.28±0.77	3.5±0.77
	6	3.78±0.4	2.3±0.35	5±0.35*	3.4±0.6	4.52±0.9	4.27±0.36	4±0.65
	8	5.3±0.3*	2.85±0.7	7.1±0.5*	2.9±0.8	4.4±0.42	4.1±0.39	4.34±0.55

The panelists could identify differences (*P* < 0.05) in the sweet taste during storage. Moreover, when lactulose dose increased, sweet taste score increased, being from 1.9±0.4 to 6.84±0.6 at the first storage day, respectively, for control yoghurt and when 8 % of lactulose was added. Indeed, lactulose had a considerable sweetness power (WESTHOFF *et al.* 2000). The bitter taste and color scores of the yoghurt samples were not affected by lactulose addition. Otherwise, lower score of granular texture and whey exudation were obtained in yoghurt with higher lactulose dose. Besides, mouth feel was better, when lactose dose or storage period increased, especially for 6 and 8% of lactulose. The overall appreciation increased when lactulose dose increased. Scores reached, at the first day, 6.2±0.39 for yoghurt with 8% of lactulose against 3±0.35 for control yoghurt. This is probably ascribed to the sweetness power of lactulose. Literatures about the effects of prebiotics on sensory attributes of fermented milk products are rather conflicting. SEYDIN *et al.* (2005) found that yoghurts containing inulin had good flavor and smooth texture. Further, HAYDARI *et al.* (2011) reported that increasing the concentration of prebiotics led to a weaker sensorial gel firmness and scoopability probably ascribed to depletion flocculation of milk proteins during fermentation. Except for inulin, increasing the concentration of prebiotics resulted in less smooth oral texture, and, likewise, higher concentration of prebiotics possessed less flavor acceptability and total acceptability.

4. CONCLUSIONS

Starter type had significant effects on kinetic parameters, postacidification, syneresis and proteolysis degree of yoghurt containing 1.5% of lactulose. Thus, Yoflex 801 was the adequate co-culture to use in prebiotic yoghurt supplemented with lactulose. With respect to lactulose therapeutic dose, increased levels (4, 6 and 8%) reduced syneresis and improved sensory characteristics. However, concerning rheological characteristics, yoghurts supplemented with lactulose had a weak gel, with a thixotropic and pseudoplastic behavior, peculiarly 8% of lactulose. Hence, minor quality alterations were obtained with 4% or 6% of lactulose. Based on the result found in this study, it is concluded that yoghurt fermented with Yoflex 801, and supplemented with 4% or 6 % of lactulose could have interesting outcomes with respect to functional food production and preservation.

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