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The Combined Effect of Phytosterols and Lactulose Supplementation on Yoghurt Quality

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Abstract This study aimed to evaluate the physicochemical, microbiological, rheological and sensorial properties of yoghurt enriched with phytosterols (1.6%) and /or lactulose (6%), during refrigerated storage. Compared with the control yoghurt, postacidification and syneresis were reduced in all enriched samples. Besides, yoghurt starter counts were affected neither by phytosterols nor by lactulose addition, during storage. Otherwise, yoghurt samples enriched with both phytosterols and lactulose had a lower oxidationand better rheological properties. Also, their sensory characteristics were appreciated by consumers.

Keywords: *phytosterols, lactulose, yoghurt, quality*

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1. Introduction

Phytosterols are plant-originated fractions found in vegetable oils, seeds, nuts, cereals and beans [1]. The major phytosterols in nature are β-sitosterol, campesterol and stigmasterol [2]. They are saturated phytosterols that have no double bond in the ring structure. Phytosterols and stanols are structurally alike to cholesterol, so, they are distinguishable by the presence of additional methyl or ethyl-groups in their side chain [3]. Therefore, phytosterols are apt to substitute cholesterol during micelle formation in the intestine due to their higher hydrophobicity, thus, reducing cholesterol absorption [4]. In this regard, phytosterols play an important role in inhibiting the absorption of dietary cholesterol and lowering biliary one [5].

In recent years, functional foods have gained momentum, owing to their beneficial health effects. Functional foods may take different forms, i.e. either conventional, endowed with bioactive components, or enriched [6,7]. In this connection, the US Food and Drug Administration (FDA) and Committee on Foods of the European Unionapproved phytosterols as safe food ingredients[8,9].A daily intake of 1.7 g of phytosterols and stanols recommended achieving optimal cholesterol-lowering action[10].Therefore, the incorporation of phytosterols in foods such as margarine, dark chocolate and dairy products was largely studied [11,12,13]. However, the greater problems related to fortified foods with phytosterols are oxidative stability, quality of the product, as well as the functional role of these fractions into the products during their shelf life.

Further, phytosterols were associated to bioactives compounds such as probiotics [14,15] or prebiotics [16] in order to produce functional foods with more beneficial effects. In fact, in some studies, phytosterols were combined with few types of prebiotics such as inulin [16]. Prebiotics are substrates that are selectively utilized by host microorganisms conferring health benefits [17]. Besides, prebiotics are capable of decreasing triglycerides (TGs) by many mechanisms including *i)* decreased hepatic lipogenesis, *ii)* increased muscle lipoprotein lipase activity and *iii)* increased short chain fatty acids by bacterial fermentation of carbohydrates which is also capable of inhibiting TG synthesis [18,19]. Among the most famous prebiotics compounds, lactulose has potential benefits for consumers and may be useful to further the desirable health impact of food products, particularly yoghurt [20].

Yoghurt is one of the most popular fermented milk products as it provides nutritional and health advancing properties [21]. Yoghurt has the potential to prevent gastrointestinal disorders and reduce the risks of colon cancer [22]. Moreover, a high intake of saturated fatty acids increases the risk of cardiovascular diseases. For this reason, consumers have begun to demand foods that contain a low level of saturated fatty acids [23].

For the best of our knowledge, no studies have been conducted on the fortification of foods especially dairy products by both phytosterols and lactulose.

The purpose of this study was, therefore, to produce a new low-cholesterol yogurt by addition of phytosterols and/or lactulose. This work was initiated to evaluate the effect of phytosterols and lactulose supplementation alone, and their combination on yoghurt quality, during refrigerated storage. The physicochemical, rheological and sensorial properties, starter culture contents and oxidative stability of the fortified yoghurt samples were determined and compared with control yoghurt.

2. Materials

Phytosterols used in this study consisted of β-sitosterol, stigmasterol and campesterol at ratio of (~80 %), (~12 %) and (~8 %) respectively. It was a gift from Lipofoods (Gava Barcelona, Spain). Lactulose is provided by Chimica Mugello society (Italy). Skim milk (10% total solids) was obtained from a dairy plant 'Natilait'. Starter culture was obtained from DSM food specialties (the Netherlands). Skim milk powder was purchased from Ukrdelice Agricultural Company (Germany). All chemicals were of reagent grade and were used as such.

3. Methods

Yoghurt manufacture was made in laboratory scale. Raw cow milk with 5% (w/v) skim milk powder addition was pasteurized at 92°C for 3 min and cooled to 43°C. Then, milk was divided into 4 batches: untreated control, milk added with 1.6% phytosterols, milk added with 6% of lactulose and finally, milk supplemented with both phytosterols (1.6%) and lactulose (6%). Then, all samples were inoculated with 2% (v/v) of yoghurt starter culture including *S. thermophilus* and *L. bulgaricus* YS 131 (DSM, Netherlands), and mixed thoroughly. Samples were distributed into 200 mL sterilized bottle and incubated at 43°C until pH 4.6 is reached. After fermentation, yoghurt samples are cooled and stored in 4°C refrigerator.

3.1. Comparative Analysis of Yoghurt Samples

Yoghurt pH, Dornic acidity, syneresis, yoghurt starter's counts, peroxide value and TBArs, are measured at the 1st day of storage and each week during 4 weeks of refrigerated storage.

Sensory characterization, firmness and apparent viscosity were evaluated at 1st, 14th and 28th day of storage at 4°C. Before the analyses, except for the gel firmness and sensory characterization, the yoghurt samples were gently stirred to ensure homogeneity. All measurements were carried out in triplicate.

3.2. pH and Dornic Acidity

The pH values and the amount of Dornic acidity (as Dornic degree) of each yoghurt sample were measured according to AOAC [24].

3.3. Syneresis

The Susceptibility of yoghurt to syneresis was determined as described by [25]. A quantity of 10g of yoghurtwas centrifuged (Beckman USA) at 80000 rpm for 12 minutes and at 4°C. The clear supernatant was recovered and weighed, and then syneresis was calculated as mass of the separated serum from the gel after centrifugation, related to the total mass of gel that was centrifuged.

3.4. Yoghurt Starters' Enumeration

The counts of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* were taken using M17 and MRS medium respectively [26]. The results were expressed as colony-forming units per millilitre of yoghurt (cfu/mL).

3.5. Analysis of Lipid Oxidation Products

3.5.1. Lipid Extraction

Phytosterols were extracted according to a modified version of the Folch et al. [27] method. For lipids extraction, 20g ofyoghurt samples were homogenized with 400 mL chloroform/methanol solution (1:1, v/v) in a glass bottle with a screw cap. The bottle was kept in a thermostatic oven at 60°C for 20 min before adding 200 mL chloroform. After 3 min of homogenization, bottle content was filtered through Whatman Chr. 1, to eliminate solid residue. The filtrate was mixed thoroughly with 100 mL of KCl (1 M) solution and left overnight at 4°C. Then, lipid-containing phase was collected, treated with anhydrous sodium sulfate, and dried using a rotary evaporator.

3.5.2. Peroxide Value

Peroxide value (PV) was determined by the ferric thiocyanate standard method of Shantha and Decker [28]. During this assay, ferrous ions (Fe^{2+}) from ferrous chloride (1 mg/mL) were oxidized by the peroxides, from fat sample (25-35 mg), in ferric ions (Fe^{3+}), which forms a red complex in the presence of 50 µL ammonium thiocyanate (30 %, w/v). Then, the absorbance was measured at 530 nm (Jenway 6300, France). The blank was prepared in the same way as the sample. Peroxide value (expressed as meq O_2 kg/L of fat) was calculated as follow: Peroxide value = Absorbance x a / m x 55.84; with: a= standard curve slope and m = mass of lipid extract.

3.5.3. Thiobarbituric Acid Reactive Substances Value

Thiobarbituric acid reactive substances (TBARs) were determined according to a modified method proposed by Rossetti et al. [29]. The 2-thiobarbituric acid test is based on the formation of a pink colored complex resulting from the reaction between a malonaldehyde molecule and two 2-thiobarbituric acid molecules. The colored complex formed absorbed at 532-535 nm. The concentration of MDA in samples was expressed as mg / kg of yoghurt, using standard curve. 1,1,3,3-tetramethoxypropane (TMP), which is an MDA precursor, was prepared by the method of Sugiarto et al. [30] .

3.5.4. Rheological Measurements

The rheological properties were determined according to the method described by NGUYEN *et al.* [31] with a slight modification. A rotary viscometer Rheometric RM180 (Rheomat, Caluire, France), equipped with the coaxial cylinders' geometry was used to analyze flow curves of yoghurt samples. 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 done between 0.01 and 500 s^{-1}. The viscometer was controlled by RSI Orchestrator v6.5.8 software. Flow properties were assessed at the temperature of 4°C. A circulator bath (Julabo GmbH, Germany) was used to regulate temperature during

the rheological measurements. Area of thixotropic hysteresis loop as difference between the area under the up-flow curve and the down-flow curve was also computed by the use of RSI Orchestrator v6.5.8 software.

3.5.5. Sensory Evaluation

During the storage period at 4°C (first, 14th and 28th days), the sensory characteristics of yoghurt samples were evaluated by 35 untrained panelists in a uniformly illuminated room at approximately 25°C. Each sample had a cod with a random six-digit number. All of them were served to panelists in a randomized order. The quality evaluated properties were: overall liking, odor, whey exudation, white color, sweet, acidic or bitter taste, nappy, creamy or fluid texture, thickness, and smoothness. Sensory scores of the yoghurts samples are done using a 9-point hedonic scale, ranging from 1(the least, the lowest) to 9 (the most, the highest) [32].

4. 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. Principal component analysis (PCA) biplots were done using XLSTAT version 2018.3 (Addinsoft USA). All the experiments were done in triplicate.

5. Results and Discussion

5.1. pH Variation and Post-acidification of Control and Enriched Yoghurts during Cold Storage

pH values and Dornic acidity of yoghurt samples were shown in Table 1. pH values of all yoghurts decreased during 28 days of storage period at 4°C. On the other hand, Dornic acidity increased for all prepared yoghurts, over the storage period. The Reduction of pH values and increasing in Dornic acidities were due to the growth of acid-forming bacteria which produced lactic acid during the storage period [33,34]. In phytosterols yoghurt, acidity and pH variations were the lowest over the storage period; followed by yoghurt with mixed lactulose and phytosterols. These data were in agreement with Izadi et al. [35] who reported that titratable acidity values were lower in yogurt enriched with phytosterols (1.4%); whereas, when milk fat content varied, acidity and pH values are not affected [36]. These findings may be due to the addition of oil in water emulsion in enriched yoghurt which induce differences within the food environment influencing the metabolism of microorganisms during storage [37]. Concerning pH and acidity values of lactulose yoghurt, there is no significant differences with control (P>0.05). These results were in accordance with the study of Cruz et al. [6] reporting that the addition of different doses (2 to 8%) of oligofructose as prebiotic has no effect on post acidification. Indeed, weak variations of pH and acidity during cold storage are desirable in the modern yoghurt industry. Overall, the combination of phytosterols and lactulose in yoghurt lead to pH and acidity values comprised between those obtained respectively for yogurts enriched with phytosterols alone and lactulose alone.

5.2. Syneresis Variation of Yoghurts during Storage

Syneresis levels increased in all samples overthe storage period (Table 1). In fact, the evolution of pH and Dornic acidity could change global charge of casein, it becomes negative, hence, capillary water kept between casein molecules. These findings are in a good agreement with Supavititpatana et al. [38] who reported that syneresis of yoghurt increases during the 21 days of storage. Moreover, it is noteworthy that syneresis values of phytosterols yoghurt with or without lactulose (respectively 58.92%±0.03, 56.41%±0.48 after 28 days) were significantly lower(P<0.05), than control yoghurt (60.11%±0.011 after 28 days). These results could be explained by the interactions between fat and protein network [35,39]; wherein, phytosterols made copolymer with casein to enhance the gel of yoghurt and reduce the syneresis levels. Regarding the effect of lactulose addition (6%), it reduced syneresis by 6.5%. This finding could be related to the effective role of prebiotics in increasing water holding capacity in the texture [40].Also, Estevez et al. [41] reported that when dry extracts increased syneresis decreased. Hence, lowest syneresis values were obtained in samples of yoghurt supplemented with lactulose and phytosterols together. Therefore, an accrued effect of these two bioactive compounds seems to be taken place, and yoghurt quality was improved.

Table 1. pH values, Dornic acidity (°D) and syneresis (%) of different yoghurt samples (Control yoghurt: (C), enriched with 1.6% of phytosterol (P), enriched with 6% of lactulose (L), enriched with both phytosterols and lactulose (P+L) during storage period (1st, 7th, 14th, 21st and 28th days)

Parameters	Yoghurt samples	Day 1	Day 7	Day 14	Day 21	Day 28
pH	C	4.58 ± 0.01^{aA}	4.51 ± 0.01^{bA}	4.39 ± 0.00^{cA}	4.33 ± 0.03^{dA}	4.33 ± 0.01^{dA}
	P	4.69 ± 0.01^{aB}	$4. 57 \pm 0.00^{bB}$	4.51 ± 0.01^{cB}	4.45 ± 0.00^{dB}	4.42 ± 0.00^{dB}
	L	4.61 ± 0.01^{aC}	4.50 ± 0.01^{abC}	4.47 ± 0.02^{bC}	4.38 ± 0.01^{cC}	4.35 ± 0.00^{cC}
	P+L	4.66 ± 0.01^{aC}	4.6 ± 0.00^{bC}	4.47 ± 0.01^{cD}	4.44 ± 0.00^{cdC}	4.43 ± 0.01^{dC}
Dornic acidity (°D)	C	98.75 ± 0.25^{aB}	111.25 ± 2.2^{bA}	117.5 ± 2.70^{cA}	124.5 ± 0.5^{dA}	128.3 ± 0.20^{dA}
	P	92.5 ± 1.50^{aA}	104 ± 1.00^{bB}	112.5 ± 2.20^{cA}	120.65 ± 0.65^{dB}	125.65 ± 0.65^{eB}
	L	93 ± 1.00^{aA}	111 ± 0.40^{bA}	116.1 ± 0.80^{cA}	123.35 ± 0.50^{dAC}	127.1 ± 0.20^{eC}
	P+L	96.8 ± 1.70^{aB}	110.6 ± 1.00^{bA}	115.3 ± 0.80^{cA}	122.65 ± 0.75^{dC}	124.7 ± 0.4^{dA}
Syneresis (%)	C	57.57 ± 0.06^{aA}	59.12 ± 0.30^{bA}	60.46 ± 0.14^{cA}	64.32 ± 0.06^{dB}	60.11 ± 0.01^{cA}
	P	50.85 ± 0.18^{aB}	53.24 ± 0.30^{bB}	54.94 ± 0.01^{cB}	58.77 ± 0.16^{dB}	58.92 ± 0.03^{dB}
	L	51.14 ± 0.09^{aB}	52.88 ± 0.06^{bC}	53.40 ± 0.23^{cC}	57.74 ± 0.05^{dC}	57.74 ± 0.19^{eC}
	P+L	51 ± 0.31^{aB}	51.65 ± 0.11^{bD}	52.83 ± 0.04^{cD}	57.35 ± 0.05^{dD}	56.41 ± 0.18^{eD}

Different letters (a–b–c–d) and (A–B–C–D–E) represent significant differences (P<0.05) in the same rows and in the same column respectively.

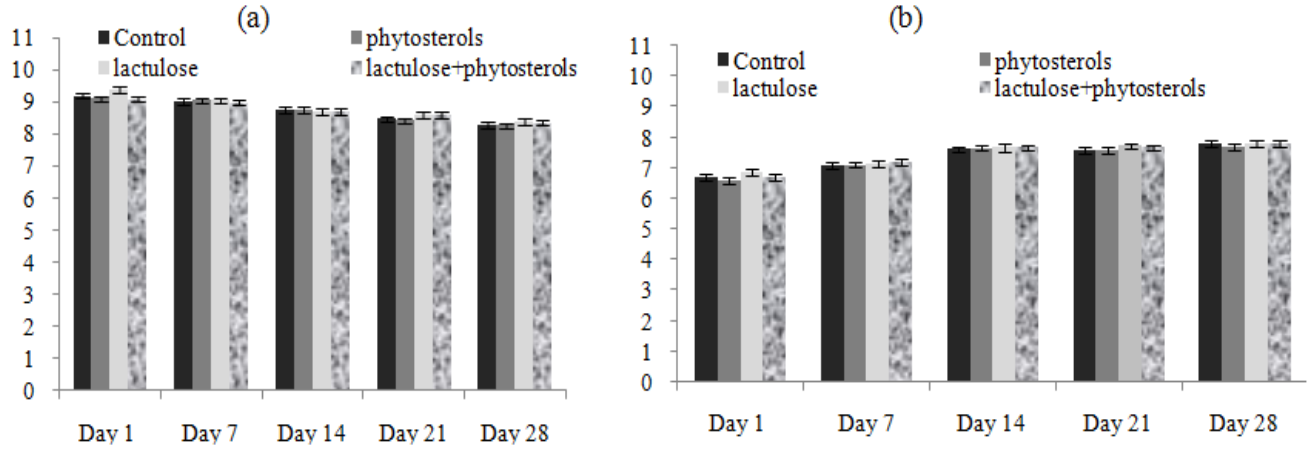


Figure 1. Viable counts of Streptococci (a) and Lactobacilli (b) (expressed as log cfu/mL) in yoghurt samples (enriched with 1.6% of phytosterols (P), and enriched with both phytosterols and lactulose (P+L) during storage period (first, seventh, 14th, 21st and 28th days)

5.3. Lactic Flora Growth in Yoghurts during Storage

As shown in Figure 1, yoghurt bacteria counts were conform to standards ($\geq 10^8$ cfu/mL for Streptococci and $\geq 10^5$ cfu/mL for Lactobacilli) [42]. However, *Lactobacillus bulgaricus* colony numbers (expressed in log cfu/mL) were weaker than *S. thermophilus*, as reported by other authors [42,43]. *S. thermophilus* counts decreased in all yoghurt samples, over 28 days of refrigeration storage. Indeed, lactic acid exerts an inhibiting effect on *S. thermophilus* and result in lowering their count [43,44,45]. Besides, *L. bulgaricus* numbers increased during shelf life in all samples to reach the highest count $7.7x10^7$ cfu/mL in lactulose enriched yoghurt. This result wasn't in line with those of Zare et al. [46] and Zhao et al. [45] who observed a decrease of *L. bulgaricus* number with time progress. In our study, high initial number of Streptococci ($>10^9$ cfu/mL) may be helpful for increasing number of Lactobacilli. Since, it's well known that *S. thermophilus* stimulates the growth of *L. bulgaricus* by the production of pyruvic acid, formic acid, folic acid, ornithine, long-chain fatty acids, and CO_2 [47]. More, starter behaviour is culture dependent. *L. bulgaricus* growth until shelf life is advantageous for two essential reasons. In one sense, it promote yoghurt flavor, in the second sense, yoghurt starter effect on human health is well known. Indeed, they improve digestibility, lactulose utilization and they promote gut health and have a hypocholesterolemic action [48,49]. Besides, differences in counts of both *S. thermophilus* and *L. bulgaricus* during yoghurt storage, with and without added phytosterols, at the same periods were not significant ($P>0.05$). Therefore, yoghurt bacteria growth were unaffected by phytosterols. It is owing to microbial metabolism; mainly, the capacity of some lactic acid bacteria to assimilate cholesterol [50,51]. As far as, no significant difference between lactic acid bacteria counts were get when lactulose was added. These data taken as a good deal with Ozer et al. [52] results who did not obtain any significant action of lactulose on the growth of commercial starter in yoghurt. Indeed, probiotic bacteria are more sensitive to prebiotic than technological lactic acid bacteria. Same occurred were observed in yoghurt samples added with both phytosterols (1.6%) and lactulose (6%).

5.4. Evolution of Lipid Oxidation in Yogurts during Refrigerated Storage

Lipid peroxidation in yoghurt samples containing phytosterols was monitored. Peroxide and TBARS values were used to investigate either primary or secondary oxidation of phytosterols.

Initial peroxide values (PV) of yoghurt samples enriched with phytosterols alone or with both phytosterols and lactulose were about 0.61 ± 0.01 meq O_2 / kg of fat and 0.46 ± 0.01 meq O_2 / kg of fat, respectively. These values increased significantly ($P<0.05$), during storage time at 4 °C, to reach respectively, 4.46 ± 0.01meq O_2 / kg of fat and 3.22 ± 0.02meq O_2 / kg of fat,as shown in Table 2. Indeed, primary oxidation of drinking yoghurt enriched with phytosterols and different flavors, trend during storage [53].

In regard to the secondary products of phytosterols secondary oxidation, malondialdehyde (MDA) production increased with storage time, they reach 0.87 ± 0.02 and 0.42±0.02mg MDA/kg respectively for yoghurts enriched with phytosterols or with both phytosterols and lactulose. Several previous studies had discussed secondary oxidative changes in yoghurts; Semeniuc et al. [53] and Citta et al. [54] reported that in all types of yoghurts tested, MDA formation increased during storage time shelf life. Serra et al. [55] indicated that lipid peroxidation depends on pH, temperature, material of packaging and milk quality. Moreover, in this study, we observed that PV and TBARS values of yoghurts supplemented with the combination of lactulose and phytosterols were significantly ($P<0.05$) lower than yoghurts enriched only with phytosterols. These findings involve that lactulose should be able to stimulate antioxidant activity of yoghurt starters. In fact, it is known that prebiotics stimulate growth and /or activities of lactic acid bacteria, depending on prebiotics as well as strain [56].

5.5. Rheological and Textural Properties

The results of rheological properties of formulated yoghurt were presented in Figure 2. It is clear that the apparent viscosities of all samples decreased when the shear rate increased from 0.01 to 500 s^{-1}. In our study, the highest viscosity values were observed in yoghurt when

lactulose and phytosterols were combined, followed by those enriched with only lactulose. Therefore, the flow curves of all yoghurt samples were apparently improved by incorporation of lactulose and phytosterols. All yoghurts showed non Newtonian pseudoplastic or shear thinning flow behavior. These results are in a great agreement with those obtained by Izadi et al. [57] who indicated that the apparent viscosity of yoghurt incorporated with phytosterols increased with phytosterols concentration and storage time. Moreover, Ilicic et al. [58] reported also that lactulose addition increased the consistency of yoghurt samples. As suggested by Sahan et al. [59] that apparent viscosity can raise over storage time due to the rearrangement of protein and protein-protein contacts. The power law was used to explain the flow curves of the investigated yoghurt samples. The rheological parameters were illustrated in Table 3. From these results, the index flow values (n) were reduced with storage time. Then, all formulated samples confirmed non-Newtonian flow behavior ($n<1$). Concerning consistency coefficient and firmness, they are supposed as the essential parameter for yoghurt texture. In general, both consistency and firmness of all samples were significantly improved during storage time at 4°C ($P<0.05$). We noted that the highest values were obtained for yoghurts enriched with both lactulose and phytosterols. During 28 days of refrigeration storage, these values varied respectively from 2.40 ± 0.1 to 2.74 ± 0.1 Pa.s^n, and from 94.86±2.9 to 98.16±3.46 N, respectively for consistency and firmness. These results were in concordance with Izadi et al. [35] who reported that phytosterols increased firmness of yoghurt until day 21 of storage. Similarly, lactulose incorporation resulted in higher firmness of yoghurt, reflecting weak gel attributable to compatibility between caseins, polysaccharides and phytosterols. This enhancement is probably most likely due to the interactions between exopolysaccharides, phytosterols and the milk protein network. Similar improvement in the firmness of yoghurt with exopolysaccharides producing lactic acid bacteria was shown by Shihata and Shah [60] and Prasanna et al. [61]. However, there was no significant variation between control and phytosterols sample in terms of firmness value during 14 days storage.

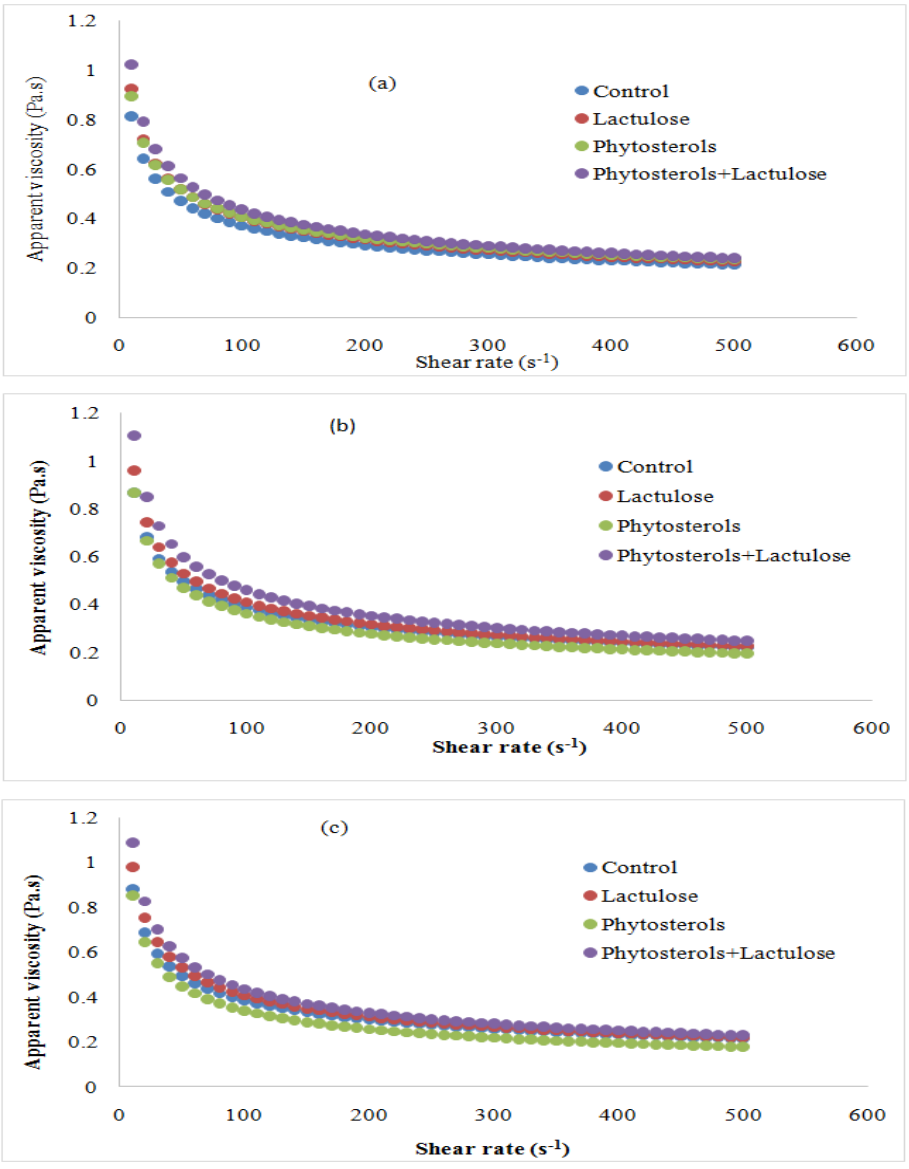


Figure 2. Apparent viscosity vs. shear rate curves of yoghurt samples (Control yoghurt: (C), enriched with 1.6% of phytosterol (P), enriched with 6% of lactulose (L), enriched with both phytosterols and lactulose (P+L)) during storage period ((a)1day, (b)14days, and (c) 28days)

Table 2. Peroxide values (expressed as meq O_2 kg/L of fat) and TBARs (expressed as mg MDA / kg of yoghurt) of yoghurt samples (enriched with 1.6% of phytosterol (P), and enriched with both phytosterols and lactulose (P+L) during storage period (first, seventh, 14th, 21st and 28th days)

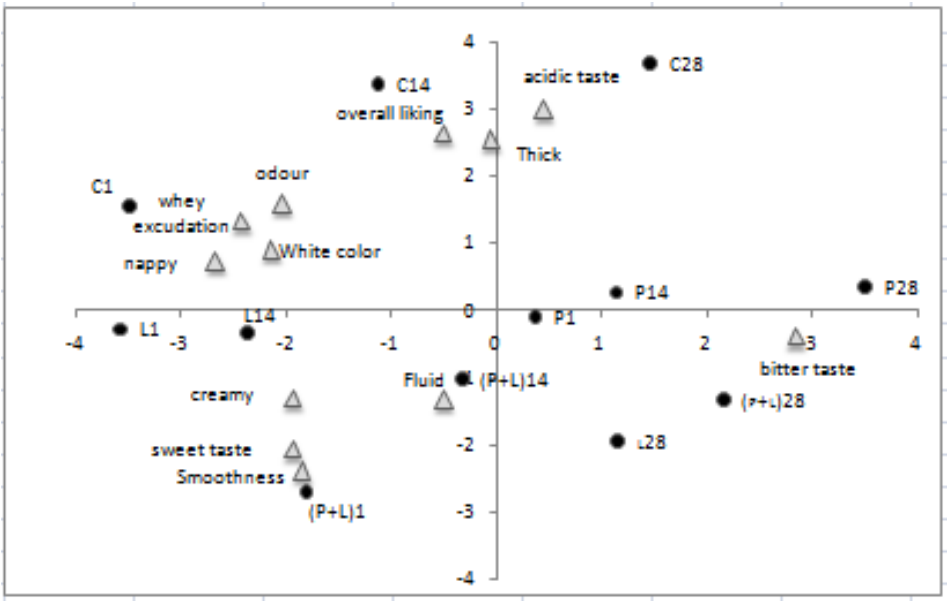
oxidation parameters	Yoghurt samples	Day 1	Day 7	Day 14	Day 21	Day 28
PV	P	0.61 ± 0.01^{aA}	1.34 ± 0.01^{bA}	1.65 ± 0.01^{cA}	2.45 ± 0.01^{dA}	4.47 ± 0.01^{eA}
	P+L	0.46 ± 0.01^{aB}	1.12 ± 0.01^{bB}	1.26 ± 0.01^{cB}	1.39 ± 0.01^{dB}	3.22 ± 0.02^{eB}
TBARs	P	0.18 ± 0.01^{aA}	0.22 ± 0.01^{bA}	0.63 ± 0.02^{cA}	0.84 ± 0.01^{dA}	0.87 ± 0.02^{dA}
	P+L	0.16 ± 0.02^{aB}	0.20 ± 0.01^{aB}	0.26 ± 0.02^{bB}	0.35 ± 0.01^{cB}	0.43 ± 0.02^{dB}

Different letters (a–b–c–d) and (A–B) represent significant differences (P<0.05) in the same rows and in the same column respectively.

Table 3. Rheological parameters and firmness values of different yoghurt samples (Control yoghurt: (C), enriched with 1.6% of phytosterol (P), enriched with 6% of lactulose (L), enriched with both phytosterols and lactulose (P+L) during storage period (1st, 14th and 28th days)

Storage period (days)	Yoghurt samples	Flow index n	Consistency coefficient k (Pa.s^n)	Firmness (g)
1	C	0.66 ± 0.02^{aA}	1.86 ± 0.04^{aA}	80.05 ± 3.10^{aA}
	P	0.66 ± 0.03^{aA}	1.92 ± 0.10^{aA}	86.70 ± 2.60^{aA}
	L	0.64 ± 0.02^{abA}	2.12 ± 0.06^{bA}	90.10 ± 4.14^{bA}
	L+P	0.62 ± 0.02^{bcA}	2.40 ± 0.10^{cA}	94.86 ± 2.90^{bA}
14	C	0.65 ± 0.03^{aA}	1.94 ± 0.08^{aAB}	81.30 ± 4.33^{aAB}
	P	0.58 ± 0.01^{cB}	2.08 ± 0.08^{abB}	87.30 ± 2.45^{aA}
	L	0.63 ± 0.02^{abAB}	2.25 ± 0.12^{bB}	92.40 ± 2.60^{bA}
	L+P	0.60 ± 0.02^{dA}	2.65 ± 0.10^{cB}	95.70 ± 4.12^{cA}
28	C	0.66 ± 0.03^{aA}	2.02 ± 0.06^{aBC}	83.46 ± 1.86^{aBC}
	P	0.60 ± 0.03^{bB}	2.15 ± 0.06^{bcBC}	90.23 ± 3.12^{cB}
	L	0.60 ± 0.01^{bB}	2.35 ± 0.09^{bC}	94.08 ± 2.02^{bAB}
	L+P	0.58 ± 0.02^{bcA}	2.74 ± 0.10^{dBC}	98.16 ± 3.46^{dAB}

Different letters (a–b–c–d) represent significant differences (P<0.05) in the same column at a same storage stage for different samples.Different letters (A–B–C–D) represent significant differences (P<0.05) in the same column for the same sample at different storage period.



C1, C14, C28 (control at 1, 14 and 28 days); L1, L14, L28 (enriched with lactulose at 1, 14 and 28 days); P1, P14, P28 (enriched with phytosterols at 1, 14 and 28 days) and (P+L)1, (P+L)14, (P+L)28 (enriched with both lactulose and phytosterols at 1, 14 and 28 days).

Figure 3. Principal components analysis of sensory attributes and yogurts samples (△: sensory attributes; ●:yoghurt samples)

5.6. Sensory Characteristics

Sensory characteristics of yoghurt samples at 1st, 14th and 28th day of storage at 4°C, were subjected of principal components analysis (PCA). PCA biplot is shown in Figure 3 reporting 63% of total variation, with factor1 (x-axis) explaining 35.05% of the data and factor 2 (y-axis) explaining 27.95%. Factor 1 was mainly represented by bitter taste versus odor, whey exudation, white color, sweet taste, creamy behavior and smoothness, based on high absolute values of attributes (Table 4). Besides, overall liking, acidic taste, thick smoothness and sweet taste characterized factor 2. During storage of all samples, odor, whey exudation, white color, nappy and creamy behavior, sweet taste and smoothness were the most affected. Bitter taste was highly affected only in phytosterols yoghurts. Parsa et al. [62] assert that storage time is a limiting factor for liking yoghurts. More, Göler-Akin and Akin [63] explained similar results by acidity increase and acetaldehyde content decrease over storage period. Moreover, we observed, at the first day of storage, that control yoghurt exhibited good odor, color,

nappy behavior and whey exudation. On the other hand, lactulose yoghurts (until 14th day) had good smoothness, creamy texture and sweet taste. Indeed, lactulose had a considerable sweetness power [64]. When phytosterols was added, previous cited characteristics were affected since the beginning of the storage, and bitter taste was affected too. These data were in a good agreement with our previous findings showing an oxidation in yoghurts added with phytosterols alone. Nevertheless, Izadi et al. [35] noted that, if only 0.5% of phytosterols were added in yoghurts, no sensory changes overview. Moreover, as can be looked in Figure 3, yoghurt samples prepared with both phytosterols and lactulose are located between lactulose and phytosterols samples.

Table 4. Factor loadings of sensory attributes for principal components

Sensory attributes	PC1	PC2
overall liking	-0,163	**0,750**
Odour	**-0,650**	0,451
Whey excudation	**-0,772**	0,378
White color	**-0,682**	0,256
sweet taste	**-0,618**	**-0,588**
acidic taste	0,141	**0,847**
bitter taste	**0,903**	-0,112
Nappy	-0,850	0,207
Thick	-0,019	**0,721**
Fluid	-0,162	-0,379
Creamy	**-0,618**	-0,370
Smoothness	**-0,590**	**-0,678**

6. Conclusions

The supplementation of yoghurt with both phytosterols and lactulose enhanced its quality by reducing postacidification, syneresis and improving firmness and consistency coefficient. Furthermore, the addition of phytosterols and/or lactulose in yoghurts did not affect significantly cell counts. This research demonstrated that lower peroxidation of phytosterols was obtained by adding lactulose concurrently. Also, sensory properties of yoghurts enriched with both phytosterols and lactulose were sustainable. It's advisable in the future to add antioxidants and vitamin sources to protect phytosterols from oxidative damage and increasing bioactive potential of the product. Other aspects may be also considered such as shelf-life of phytosterols-lactulose enriched yoghurt.

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