Physically and chemically modified starches as texturisers of low-fat milk gels

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ABSTRACT

In reduced fat-milk gels, lack of fat globules results in undesirable texture changes. The application of chemically modified (E-1412, E-1414, E-1422, E-1442), pregelatinised (PG), and both pregelatinised and chemically modified (PGE-1414) starches as fat replacers was evaluated. Skimmed milk was substituted with 2% of each starch. The pH and rheological evolution during the acidification process, viscosity of the stirred gels, texture of the set gels and synersis of all gels were measured. Skimmed and full-fat milk gels were included as controls. All starches had lower or higher pH values than skimmed or full fat milk gels during acidification. Gelation time was reduced for all starches compared with the skimmed milk gel. For both stirred and set milk gels with PG, PGE-1414, E-1422 and E-1442, viscosity/texture values were similar to or higher than those found for full fat milk gel. E-1412 had drastically increased synersis values when compared with both controls.

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1. Introduction

Yoghurt is a composite gel in which fat globules appear to act as fillers and copolymers forming casein-fat globule networks. The reinforcement functionality of fat globules has been shown to be largely dependent upon fat globule population density and, to a lesser extent, upon fat globule size (Xiong, Aguilera, & Kinsella, 1991). When preparing reduced or free fat-yoghurts, lack of fat globules results in textural changes (Houzé, Cases, Colas, & Cayot, 2005) that adversely affect mouthfeel and is not desired by consumers, as mouthfeel is an important parameter when it comes to yoghurt product quality. An interesting solution is to try to create alternative physical building blocks within the continuous phase, to mimic the functionality of the removed fat. Starch can be an option for fat replacement in low-fat-yoghurts.

The use of starch as a texturiser has already been tested in both stirred and set type yoghurts with levels ranging between 0.01% and 2%. Regarding stirred yoghurts, the effect of native (Keogh & O'Kennedy, 1998), chemically modified (Amaya-Llano, Martínez-Alegría, Zazueta-Morales, & Martínez-Bustos, 2008; Ares et al., 2007; Azim, Corredig, Koxholt, & Alexander, 2010; Lobato-Calleros, Ramírez-Santiago, Vernon-Carter, & Alvarez-Ramirez, 2014; Pang, Deeth, & Bansal, 2015; Pang, Deeth, Prakash, & Bansal, 2016; Williams et al., 2003) and physically modified (pre-gelatinised) (Morell, Hernando, Llorca, & Fiszman, 2015) starches has been reported. For set type yoghurts, the effect of native (Oh, Anema, Wong, Pinder, & Hemar, 2007; Schmidt, Herald, & Khatib, 2001; Zuo, Hemar, Hewitt, & Saunders, 2008) and chemically modified (Cui, Lu, Tan, Wang, & Li, 2014; Nguyen, Kravchuk, Bhandari, & Prakash, 2017; Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004; Schmidt et al., 2001) starches have also been tested, but the effect of physically modified starches has not been elucidated. In both stirred and set type yoghurts, when chemically modified starch, typically hydroxypropyl distarch phosphate (E-1442) was used, with the exception of the studies of Ares et al. (2007) that also used an oxidised starch (E-1404), Amaya-Llano et al. (2008) that used acid treated starches (E-1401), Azim et al. (2010) that used a cross-linked and stabilised waxy maize starch and Schmidt et al. (2001) that used acetylated cross-linked, hydroxypropylated, and hydroxypropylated cross-linked wheat starches, although they did not specify how the cross-linking and/or stabilisation was facilitated. In all these studies, a positive effect of starch on the viscoelastic properties of yoghurts was observed, but a full-fat yoghurt was not always used as a reference. In addition, the pH and rheological changes of the starch-enriched yoghurts occurring during the gelation process have only been reported by Oh et al. (2007), Pang et al. (2015, 2016) and Zuo et al. (2008).

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With the exception of the oxidised and acid treated starches, it can be said that all the chemically modified starches tested were stabilised cross-linked starches. However, there are several ways of stabilising or cross-linking starches, and more research can be done with other starches using different modification approaches. It is also interesting to note that little information is available about the use of pregelatinised starches as fat replacers in yoghurts, when this kind of starch has been tested both as a fat replacer in emulsions (Román, Martínez, & Gómez, 2015) and as a thickener in sauces (Román, Reguilón, & Gómez, 2018) with promising results. Furthermore, the combination of physical and chemical modifications may also provide added benefits, which to the best of our knowledge has never been tested.

Glucono-delta-lactone (GDL) can be used to simulate yoghurt acidification and reduce variation caused by differences in culture performance between trials (Grygorczyk & Corredig, 2013; Lucey, Tamehana, Singh, & Munro, 1998). In fact, some studies were performed using GDL instead of culture fermentation when studying the behaviour of starches (Azim et al., 2010; Oh et al., 2007; Pang et al., 2016, 2015; Zuo et al., 2008).

The novelty and aim of the study was to assess the behaviour of three chemically modified starches that have not been studied before (distarch phosphate esterified with sodium trimetaphosphate, acetylated distarch phosphate or acetylated distarch adipate), a pregelatinised starch, a chemically modified (acetylated distarch phosphate), and pregelatinised starch. The starch E-1442 (hydroxypropyl distarch phosphate) was also included in the study to serve as a comparator with existing studies. Skimmed milk was substituted with 2% of each starch. The pH and rheological change during the acidification process, viscosity of the stirred acid milk gels, texture of the set acid milk gels and syneresis of all samples were measured. Two controls were included (skimmed milk and full-fat milk).

2. Materials and methods

2.1. Materials

Whole (26 g of fat, 40.9 g of carbohydrate and 24.1 g of protein per 100 g of powder) and skimmed (1 g of fat, 54.5 g of carbohydrate and 32.5 g of protein per 100 g powder) milk powders, were purchased from Central Lechera Asturiana (Granda, Asturias, Spain; composition data provided by the producer). Pregelatinised starch PREGEFLO MM (PG), pregelatinised and chemically modified starch PJ-30 (pregelatinised acetylated distarch phosphate, PGE-1414) and chemically modified starches CJ-5025 (acetylated distarch phosphate, E-1414), CH-2020 (acetylated distarch adipate, E-1422), PI-10000 (distarch phosphate esterified with sodium trimetaphosphate, E-1412), CR-2010 (hydroxypropyl distarch phosphate, E-1442) and glucono-delta-lactone (GDL) were provided by Roquette Laisa (Lestrem, France). From this point forward, chemically modified starches will be referred to by their E number, and physically modified starch will be designated as PG.

2.2. Methods

2.2.1. Sample preparation

Eight different samples were prepared with a total solid content of 15%. Ingredient ratios of each sample are shown in Table 1.

Milk and starch powders were reconstituted in distilled water at ambient temperature for 60 min under constant magnetic stirring and held overnight at 4 °C. Then, they were heated to and held at 85 °C with overhead stirring for 30 min before being cooled in ice to the incubation temperature (30 °C). GDL (2%, w/w) was then added and the slurries were kept under stirring for 2 min and 15 s.

Table 1
Amounts of different ingredients used for preparing gels samples.[a]

Sample	Total solids (%)	Whole milk powder (%)	Skimmed milk powder (%)	Modified starch (%)	Distilled water (%)	GDL (%)
FFM	15	15	0	0	83	2
SM	15	0	15	0	83	2
2% PG	15	0	13	2	83	2
2% PG+E-1414	15	0	13	2	83	2
2% E-1414	15	0	13	2	83	2
2% E-1422	15	0	13	2	83	2
2% E-1412	15	0	13	2	83	2
2% E-1442	15	0	13	2	83	2

[a] Abbreviations are: FFM, full-fat milk; SM, skimmed milk; PG, pregelatinised starch; PGE-1414, pregelatinised acetylated distarch phosphate; E-1414, acetylated distarch phosphate; E-1422, acetylated distarch adipate; E-1412, distarch phosphate esterified with sodium trimetaphosphate; E-1442, hydroxypropyl distarch phosphate.

Samples were incubated at 30 °C for 6 h starting from min 3 after GDL addition. Then samples were refrigerated at 4 °C for 2 or 9 days before instrumental measurements of the set acidified milk gels. For stirred acidified milk gels, samples were stirred with an electric wire whisk (Infinyforce blender, Groupe Seb Moulinex, Ecully, France) for 1 min at speed 2 after 1 day of refrigeration. The stirred gels were again refrigerated at 4 °C for 1 or 8 days before instrumental measurements. Two batches were prepared.

2.2.2. pH measurement

The pH changes during the acidification process (from minute 3 to minute 360) caused by GDL were continuously monitored at 30 °C, with a pH meter equipped with a glass electrode. One measurement was done per batch.

2.2.3. Rheology

Rheological measurements were performed with a controlled stress rheometer (Haake RheoStress 1, Thermo Fischer Scientific, Scheverte, Germany) equipped with a Phoenix II P1-C25P unit to control temperature, and a concentric cylinder system (Z34 DIN Ti) with 1 mm gap.

To assess viscoelastic changes of acid milk gels, starch-milk slurries were first mixed with the GDL for 2 min and 15 s at ambient temperature. Then, the slurries were placed in the rheometer and covered with Panreac vaseline oil (Panreac Química S.A., Castellar del Vallés, Spain) to avoid drying. Tests were started 3 min after GDL was added to the samples. Changes in the viscoelastic properties were evaluated at a constant shear strain of 0.5% and a frequency of 1 Hz for 360 min, at a temperature of 30 °C (Zuo et al., 2008). Viscoelastic properties of the samples were quantified by measuring the storage or elastic modulus (G′) and loss or viscous modulus (G″) as a function of time. One measurement was done per batch.

Viscosity of the stirred gels was assessed after 2 and 9 days of storage at 10 °C, as this is the usual temperature at which these kinds of products are consumed (Morell et al., 2015). Gels were gently stirred using a stainless-steel spatula to eliminate phase separation before taking measurements. The rheometer was programmed to equilibrate the sample for 60 s, followed by an increase in shear rate from 0.1 to 500 s^{-1} over 4.5 min then a decrease from 500 to 0.1 s^{-1} for another 4.5 min. Samples were covered with Panreac vaseline oil to avoid drying. Two measurements were done per batch.

Viscosity data were then fitted in the power law equation (Keogh & O'Kennedy, 1998):

$$\eta = \frac{k}{\dot{\gamma}^n} \tag{1}$$

Where η is the apparent viscosity (Pa s), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the flow consistency index (Pa s^n) and n is the flow behaviour index.

2.2.4. Texture and syneresis

Two hundred grams of the different mixtures with GDL were poured into two cylindrical plastic containers (65 mm diameter and 60 mm height) (for texture) and 15 g (W_0) in to 50 mL FALCON® centrifuge tubes (Corning, New York, USA) (for syneresis). Containers were stored at 30 °C for 6 h. Prior to analysis, samples were stored for 2 days at 4 °C. Syneresis was also measured after 9 days of storage at 4 °C.

Back extrusion assays were performed using a TA.XT2i Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK). Tests were carried out using a 50 mm-diameter cylindrical probe at a constant crosshead velocity of 1 mm s^{-1} to a sample depth of 30 mm, followed by a return to the original position. Four parameters were extracted from the force—time curve obtained: firmness (maximum positive force registered in the compression), consistency (positive area under the curve), cohesiveness (maximum negative force which indicates the resistance to withdrawal from the sample of the extrusion disc being lifted) and viscosity index (negative area under the curve) (Cevoli, Balestra, Ragni, & Fabbri, 2013; Ciron, Gee, Kelly, & Auty, 2010).

For syneresis, FALCON® tubes were centrifuged for 10 min at 2500×g. The separated liquid was then removed and weighed. Syneresis was calculated as:

$$Syneresis = \frac{W_1}{W_0} * 100 \tag{2}$$

where W_1 is the weight of the separated liquid after centrifugation and W_0 the initial weight of the sample.

All measurements were conducted at room temperature immediately after removal from refrigeration. All tests were made in duplicate per batch.

2.3. Statistical analysis

Data were studied using one-way analysis of variance (simple ANOVA). When significant ($p < 0.05$) differences were found, Fisher's least significant differences (LSD) test was performed to determine the differences among means. Statistical analyses were completed using Statgraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, Virginia, USA).

3. Results and discussion

3.1. The pH development and viscoelastic changes during acidification

Changes in pH of the milk gels during acidification is shown in Fig. 1. For all samples, a pronounced decrease was observed for 60—80 min. From this point the decrease was still noticeable but less progressive. This general pH evolution is because of the rapid hydrolysis of GDL to gluconic acid (Lucey et al., 1998). Along the curves, lower values were found for full-fat milk (FFM) gel compared with skimmed milk (SM) gel, this is in agreement with the results of Xu, Emmanouelidou, Raphaelides, and Antoniou (2008). Regardless of the type of starch used, a decrease in pH throughout the acidification process was observed when using SM as a reference. This agrees with studies conducted using potato
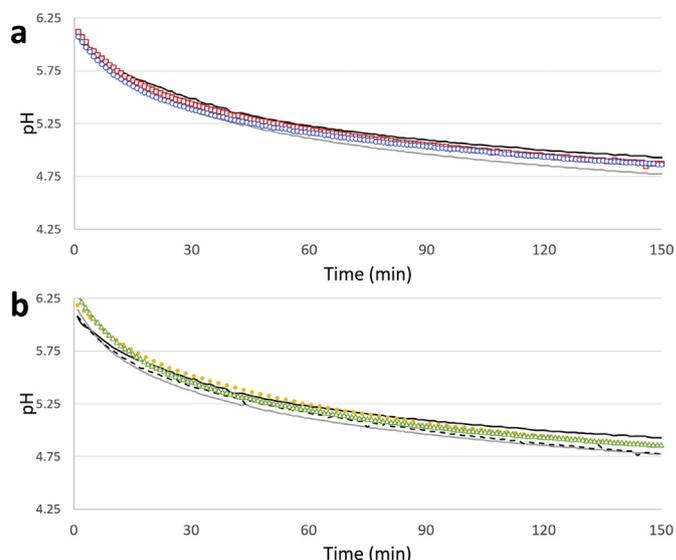
Fig. 1. The pH evolution during acidification of milk gels until min 150 of (a) ——, full-fat milk;—— skimmed milk; ----, skimmed milk + 2% pregelatinised starch; □, skimmed milk + 2% pregelatinised acetylated distarch phosphate (PGE-1414); ○, skimmed milk + 2% acetylated distarch phosphate (E-1414) and (b)——, full-fat milk; ——, skimmed milk; ----, skimmed milk + 2% distarch phosphate esterified with sodium trimetaphosphate (E-1412); ●, skimmed milk + 2% acetylated distarch adipate (E-1422); △, skimmed milk + 2% hydroxypropyl distarch phosphate (E-1442).

starch by Oh et al. (2007). Other studies (Azim et al., 2010; Zuo et al., 2008) did not find differences in pH development when starch was present, although starch percentage was not higher than 1% inclusion and in our case it was 2%. The pH values at min 180 and min 360 of the acidification process (Table 2) showed that for both times all starches had a value between the pH values of FFM and SM, with the exception of the pH of E-1412 for min 360, which did not present significant differences from the FFM gel.

Rheological change in the samples during the acidification process is shown in Fig. 2. Gelation time (gT) (Table 2), defined as the time at which a sustained and pronounced increase in the shear modulus G' is observed above the baseline noise (1—2 Pa) (Koh, Matia-Merino, & Dickinson, 2002), was longer for SM gel than for FFM gel. The gT was clearly influenced by the presence of starches. Regardless of the type, starch shortened gT compared with the SM milk gel. With the exception of E-1414, which presented a significantly longer gT than FFM, incorporation of the different starches to skimmed milk yielded approximately equal gT values compared with that of FFM. This is most likely due to the fact that starches were gelatinised in the heating step, and uptake of water by the starch granules during swelling resulted in an increase in the protein concentration in the continuous phase, leading to a stronger gel network (Oh et al., 2007).

As for the pH at which this gelation occurs (pHgt), also presented in Table 2, for all the samples it was between 5 and 5.5, in agreement with other authors that studied heated milk samples (Oh et al., 2007). The pHgT was significantly higher for FFM than for SM gel. As stated before, all starches reduced the gT with respect to the SM gel, but this was not the case for pHgt, as some of them even increased it (PG, PGE-1414 and E-1422), which is also in agreement with Oh et al. (2007).

No significant differences were found between the pHgt of the FFM gel and any of the starch-containing milk gels.

The higher pHgt when using pregelatinised starches can be the result of a more disrupted starch structure related with the fact that the starch was already gelatinised before the heating step, and this was reported by McPherson, Bailey, and Jane (2000) when

Table 2
The pH data of the milk gels, gelation time and final G′ values.[a]

Sample	pH at min 180 of acidification process	pH at min 360 of acidification process	Gelation time (gT) (min)	pH at gelation time (pHgt)	G′ values at min 360 (Pa)
FFM	4.70 ± 0.03^a	4.43 ± 0.00^a	36.50 ± 9.20^a	5.31 ± 0.15^{bcd}	516.00 ± 77.78^{bc}
SM	4.91 ± 0.01^e	4.68 ± 0.00^c	79.00 ± 12.73^c	5.14 ± 0.04^a	240.75 ± 72.3112^a
2% PG	4.85 ± 0.00^d	4.57 ± 0.01^b	42.50 ± 3.54^{ab}	5.34 ± 0.06^{bcd}	188.50 ± 27.58^a
2% PGE-1414	4.80 ± 0.00^{bc}	4.56 ± 0.01^b	38.00 ± 2.83^{ab}	5.36 ± 0.04^{cd}	742.50 ± 21.92^d
2% E-1414	4.8 ± 0.02^{cd}	4.59 ± 0.04^b	56.00 ± 9.90^b	5.19 ± 0.06^{ab}	404.50 ± 92.631^b
2% E-1422	4.78 ± 0.04^{bc}	4.54 ± 0.03^b	40.50 ± 3.54^{ab}	5.41 ± 0.04^d	658.50 ± 20.51^{cd}
2% E-1412	4.76 ± 0.01^b	4.48 ± 0.04^a	43.00 ± 9.99^{ab}	5.29 ± 0.04^{abcd}	534.00 ± 140.01^{bc}
2% E-1442	4.80 ± 0.01^{bc}	4.57 ± 0.01^b	54.50 ± 3.54^{ab}	5.23 ± 0.03^{abc}	437.50 ± 48.79^b

[a] Abbreviations are: FFM, full-fat milk; SM, skimmed milk; PG, pregelatinised starch; PGE-1414, pregelatinised acetylated distarch phosphate; E-1414, acetylated distarch phosphate; E-1422, acetylated distarch adipate; E-1412, distarch phosphate esterified with sodium trimetaphosphate; E-1442, hydroxypropyl distarch phosphate. Data are expressed as means ± SD of duplicate assays. Samples with the same superscript letter(s) did not present significant differences ($p > 0.05$).
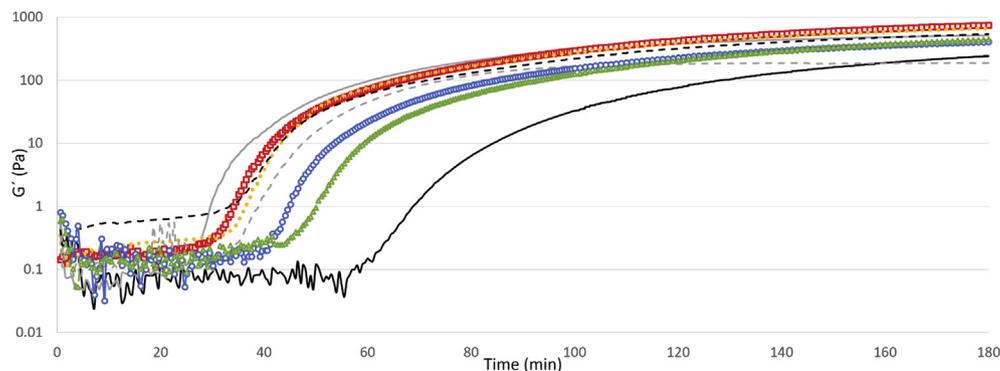
Fig. 2. Elastic modulus evolution during acidification process of milk gels: ——, full-fat milk; ——, skimmed milk; ----, skimmed milk +2% pregelatinised starch; ----, skimmed milk +2% distarch phosphate esterified with sodium trimetaphosphate (E-1412); □, skimmed milk + 2% pregelatinised acetylated distarch phosphate (PGE-1414); ○, skimmed milk + 2% acetylated distarch phosphate (E-1414); ●, skimmed milk + 2% acetylated distarch adipate (E-1422); △, skimmed milk + 2% hydroxypropyl distarch phosphate (E-1442).

examining the pasting properties of pregelatinised starches. For starch E-1422, introduction of acetyl groups into starch molecules, by acetylation, leads to structural reorganisation due to steric hindrance; this results in repulsion between starch molecules, thus facilitating an increase in water percolation within the amorphous regions of granules and a subsequent increase in swelling capacity (Lawal, 2004).

Greater uptake of water by these starches during swelling would result in an increase in the protein concentration in the continuous phase, leading to a stronger gel network. Nevertheless, how crosslinking is achieved and whether the chemically modified starch(s) is pregelatinised also seems to play a role. In this way, starch E-1414, also an acetylated starch, but cross-linked with phosphate instead of adipate, did not increased the pHgt, although it did in combination with the pregelatinisation treatment.

After this pronounced increase in G′ values, a progressive increase was observed for all the samples, with a more pronounced increase for FFM than for SM gel. At the end of the curve (see values in Table 2), G′ values increased for starches with respect to SM gel, with the exception of 2% PG milk gel, which showed a G′ value similar to that of the SM gel. Gels made with starch PGE-1414 showed final G′ values higher than FFM, while E-1422, E-1412, E-1442 and 1414 showed no significant differences with FFM gels, but higher values than SM gels. When comparing the milk gels with E-1422 or E-1414, acetylated starches that only differ on the way that crosslinking is made, the gel with E-1422 presented a higher G′ value. Therefore, it can be stated that if crosslinking is done with adipate, G′ of gels is higher than if the crosslinking is done with phosphate. Nevertheless, the highest G′ value was the 2% PGE-1414 milk gel, made with a starch in which crosslinking was done with phosphate, indicating the potential of combining both chemical

and physical modifications in starches used as substitutes for fat in milk gels.

3.2. Viscosity of acidified milk gels

Flow behaviour index (n) and viscosity at a shear rate of 50 s^{-1} (η_{50}) are shown in Table 3. Viscosity, at a shear rate of 50 s^{-1}, has been suggested to have a good correlation with perceived thickness, stickiness and sliminess for a wide range of food products (Shama & Sherman, 1973; Wood, 1968), and was previously used as a viscosity reference in yoghurts by Nguyen et al. (2017). Viscosity of the stirred acidified milk gels was measured after 2 and 9 days of storage, all the samples exhibited thixotropic (data not shown) and shear thinning behaviour, typical for this type of product, and can be explained by the alignment of biopolymer molecules with the field of shear and disruption of weak physical interactions responsible for biopolymer—biopolymer interactions (Lobato-Calleros et al., 2014). For both flow behaviour index and viscosity at a shear rate of 50 s^{-1} no significant differences were found between FFM and SM gels, but the FFM gel had an increase in n at day 9, while the SM gel remained constant.

The addition of starches resulted in an increase of n at day 2, although PG and E-1414 did not show significant differences with respect to SM gel. Among starches, gels with E-1422 (the only starch used in this research with cross-linking made with adipate) gave the highest n value, followed by gels with E-1442 and PGE-1414. In general, no significant differences were found between days for each starch, with the exception of starch E-1412, which presented a significantly lower value compared with the previous day, and starches E-1414, and E-1442 that exhibited slightly increased value.

Table 3
Viscosity parameters of stirred milk gels.[a]

Sample	n		η_{50} (Pa s)	
	Day 2	Day 9	Day 2	Day 9
FFM	0.68 ± 0.01[aA]	0.71 ± 0.02[bcB]	0.56 ± 0.02[abA]	0.63 ± 0.02[bB]
SM	0.69 ± 0.01[abA]	0.69 ± 0.01[cdA]	0.48 ± 0.03[aA]	0.53 ± 0.02[abB]
2% PG	0.70 ± 0.01[bA]	0.67 ± 0.01[bA]	0.64 ± 0.03[bcA]	0.52 ± 0.18[abA]
2% PGE-1414	0.76 ± 0.01[dA]	0.78 ± 0.01[eA]	1.27 ± 0.07[eA]	1.48 ± 0.07[dB]
2% E-1414	0.70 ± 0.01[bA]	0.73 ± 0.01[dB]	0.84 ± 0.03[dA]	0.96 ± 0.02[cB]
2% E-1422	0.84 ± 0.01[fA]	0.85 ± 0.01[fA]	1.46 ± 0.20[fA]	1.41 ± 0.02[dA]
2% E-1412	0.73 ± 0.01[cB]	0.63 ± 0.03[aA]	0.70 ± 0.02[cB]	0.44 ± 0.05[aA]
2% E-1442	0.81 ± 0.01[eA]	0.84 ± 0.01[fB]	1.23 ± 0.02[eA]	1.38 ± 0.06[dB]

[a] Abbreviations are: n, flow behaviour index; η50, viscosity at a shear rate of 50 s-1;FFM, full-fat milk; SM, skimmed milk; PG, pregelatinised starch; PGE-1414, pregelatinised acetylated distarch phosphate; E-1414, acetylated distarch phosphate; E-1422, acetylated distarch adipate; E-1412, distarch phosphate esterified with sodium trimetaphosphate; E-1442, hydroxypropyl distarch phosphate. Data are expressed as means ± SD of duplicate assays. Samples with the same superscript lowercase letters in a column and different uppercase letters in a row (analysing separately n and η50) did not present significant differences ($p > 0.05$).

Table 4
Texture parameters of set milk gels.[a]

Sample	Firmness (N)	Cohesiveness (N)	Viscosity index (N s)	Consistency (N s)
FFM	4.11 ± 0.42[cd]	2.02 ± 0.11[b]	4.45 ± 0.17[b]	108.46 ± 7.84[d]
SM	3.15 ± 0.07[a]	1.46 ± 0.03[a]	3.34 ± 0.15[a]	84.04 ± 1.06[a]
2% PG	3.88 ± 0.04[bc]	3.20 ± 0.11[d]	6.82 ± 0.07[d]	103.50 ± 2.33[cd]
2% PGE-1414	4.49 ± 0.153[e]	3.67 ± 0.12[f]	7.47 ± 0.47[e]	118.76 ± 3.91[e]
2% E-1414	3.87 ± 0.15[b]	2.61 ± 0.22[c]	6.45 ± 0.79[cd]	101.77 ± 4.09[bc]
2% E-1422	4.33 ± 0.06[de]	3.53 ± 0.30[ef]	7.00 ± 0.39[de]	116.56 ± 2.99[e]
2% E-1412	3.73 ± 0.12[b]	2.56 ± 0.14[c]	6.06 ± 0.29[c]	96.13 ± 3.67[b]
2% E-1442	4.33 ± 0.08[de]	3.36 ± 0.14[de]	6.99 ± 0.15[de]	114.93 ± 2.53[e]

[a] Abbreviations are: FFM, full-fat milk; SM, skimmed milk; PG, pregelatinised starch; PGE-1414, pregelatinised acetylated distarch phosphate; E-1414, acetylated distarch phosphate; E-1422, acetylated distarch adipate; E-1412, distarch phosphate esterified with sodium trimetaphosphate; E-1442, hydroxypropyl distarch phosphate. Data are expressed as means ± SD of duplicate assays. Samples with the same superscript letters in a column did not present significant differences ($p > 0.05$).

Surprisingly, SM gels had viscosity η_{50} values that were not significantly lower than FFM gels values for both days 2 and 9. This can be related with the higher protein content of skimmed milk (see Materials and Methods section), as a gel with a cross-linked microstructure is formed by de-natured whey proteins associated with the casein micelles that governs gel strength and texture (Lee & Lucey, 2003; Lucey, Munro, & Singh, 1998). For day 2, all the starches, with the exception of PG starch, increased the viscosity compared with SM and FFM gels, which could be related with starch retrogradation increasing the viscosity of the aqueous phase of the system with time.

The lack of viscosity increase when using PG starch is likely related with the pregelatinisation treatment, that increases the solubility and a decreases the cold thickening capacity of this starch (Doublier, Colonna, & Mercier, 1986). As with n values, this effect was more evident when starch E-1422 was used, followed by gels with E-1442 and PGE-1414. After 9 days of storage, SM and FFM milk gels increased η_{50} compared with day 2, while E-1412 milk gel showed a particularly lower η_{50} when compared both with values on day 2 and with the values on day 9 for the other starches, which is in agreement with the n results. The decrease in η_{50} seems to be related with increased propensity of the gel network to expel water (syneresis) as storage time increased (Table 5). A relationship between increased syneresis and viscosity decrease was previously reported by Lobato-Calleros et al. (2014). This indicates that this starch is not suitable for stirred acidified milk gels, since it does not improve characteristics when compared with SM milk gel. E-1414, PGE-1414 and E-1442 increased viscosity at day 9, with the last two even equalling viscosity values for the gels with E-1422, which did not change in viscosity over time.

3.3. Texture of acidified milk gels

Table 4 shows texture results for the acidified milk gels. All the measured parameters were significantly higher for the FFM gel compared with the SM gel. Regarding firmness, it can be observed that all starches improved this parameter compared with SM gel, although E-1414 and E-1412 milk gels had significantly lower firmness than the FFM milk gel, while PGE-1414 milk gel showed significantly higher values. The other three starches (PG, E-1422 and E-1442) did not exhibit differences from the FFM milk gel, which agrees with the results of the stirred gels, as these three starches presented the highest viscosities compared with the other milk gels.

Alternatively, all starches boosted cohesiveness compared with both SM and FFM gels, with PGE-1414 and E-1422 boosting this parameter the most and E-1442 s the most. Other authors already reported an increase in firmness and cohesiveness when adding E-1442 starch (Pang et al., 2015) and a modified tapioca starch (Sandoval-Castilla et al., 2004). They attributed this to the increased viscosity of the samples by starch molecules binding to and orienting water, which consequently dampened the effect of applied stress, with the starch molecules functioned as "fillers".

Milk gels containing E-1414, E-1422 or E-1442 starches increased the milk gel consistency above the value of FFM milk gel, while PG gave a value not significantly different from it. The other two starches (E-1414 and E-1412), cross-linked with phosphate, showed a lower consistency than FFM, although all values where significantly higher than the one for SM gel. For viscosity index, samples containing starches showed higher values than the FFM gel. Starches presented a similar trend as that observed for firmness or cohesiveness, with E-1412 and E-1414 gels presenting the lowest values, and PGE-1414, E-1422 and E-1442 the highest, although no significant differences were found among E-1422, E-1442, E-1414 and PG milk gels.

In general, it can be observed that, regarding texture results, the presence of all starches helped to increase the values of the measured parameters, although milk gels with E-1412 and E-1414 (both of them cross-linked with phosphate) were the ones with the poorest behaviour.

Table 5
Syneresis of set and stirred milk gels.[a]

Sample	Set day 2	Set day 9	Stirred day 2	Stirred day 9
FFM	1.9 ± 0.27[a]	1.65 ± 0.23[a]	3.82 ± 0.34[a]	4.37 ± 0.35[ab]
SM	2.73 ± 0.29[abc]	2.76 ± 0.16[bcd]	13.68 ± 4.63[d]	8.57 ± 3.56[d]
2% PG	2.88 ± 1.48[abc]	3.63 ± 1.28[d]	8.23 ± 2.57[ab]	7.78 ± 4.88[cd]
2% PGE-1414	3.80 ± 0.94[c]	3.51 ± 0.52[d]	8.23 ± 0.38[c]	7.71 ± 1.00[cd]
2% E-1414	3.48 ± 0.54[c]	3.16 ± 0.34[cd]	5.24 ± 0.61[ab]	5.18 ± 1.02[bc]
2% E-1422	2.38 ± 0.34[ab]	2.02 ± 0.39[ab]	3.64 ± 1.65[a]	2.80 ± 0.73[ab]
2% E-1412	21.605 ± 1.01[d]	29.48 ± 1.10[e]	6.89 ± 0.10[bc]	17.62 ± 0.84[e]
2% E-1442	3.20 ± 0.25[bc]	2.40 ± 0.25[abc]	2.73 ± 0.58[a]	2.21 ± 0.6[a]

[a] Abbreviations are: FFM, full-fat milk; SM, skimmed milk; PG, pregelatinised starch; PGE-1414, pregelatinised acetylated distarch phosphate; E-1414, acetylated distarch phosphate; E-1422, acetylated distarch adipate; E-1412, distarch phosphate esterified with sodium trimetaphosphate; E-1442, hydroxypropyl distarch phosphate. Data (expressed as %) are means ± SD of duplicate assays. Samples with the same superscript letters in a column did not present significant differences ($p > 0.05$).

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3.4. Milk gels syneresis

Syneresis of the different milk gels can be found in Table 5. Spontaneous whey separation is related to an unstable network, which can be due to an increase in the rearrangement of the gel matrix, and furthermore negatively affects consumer perception of yoghurt, as they will think the product is deteriorated (Lobato-Calleros et al., 2014). For both stirred and set milk gels, FFM had significantly lower values than SM gels, with the exception of stirred milk gels on day 2, for which no significant differences were found. This is because in the presence of more fat globules, protein adsorbs on the surface of the fat globules, making milk fat globules act like protein particles and thus increasing its ability to immobilise water (Keogh & O'Kennedy, 1998). Starches have been used to achieve fat mimetic properties by retaining substantial quantities of water into weak gel structures (Luo & Gao, 2011). Regarding set milk gels, E-1412 had an obvious negative effect, as it drastically increased syneresis. Among the other starches, only E-1422 equalled the syneresis values of the FFM gel for both days 2 and 9. Among the cross-linked starches with phosphate, only E-1442 was equal in syneresis to the one of FFM gel at day 9; meanwhile, for set gel at day 2 it presented slightly higher values.

Higher syneresis values were obtained for stirred milk gels than for set milk gels, probably due to breakdown of the protein tri-dimensional network. Addition of starches in stirred milk gels had a positive effect, as all starches reduced syneresis for day 2 compared with SM gel, although milk gels with PGE-1414 and E-1412 showed higher syneresis than the FFM gel. After 9 days of storage, only milk gels with E-1414, E-1422 and E-1442 reduced the syneresis compared with SM gel, and only the last two were able to equal FFM gel syneresis. For its part, E-1412 increased syneresis with respect to SM gel.

Among the studied starches, the one most effective in reducing syneresis was E-1422, which was the only starch cross-linked with adipate. Adipate is an organic molecule and therefore is less polar, while phosphate is inorganic and more polar. It seems that a lower polarity works better, so this may be related to repulsion between molecules. Among the starches cross-linked with phosphate, E-1442 presented the best results, while the starches with phosphate esterified with sodium trimetaphosphate, exhibited the poorest behaviour. As sodium trimetaphosphate is a bigger molecule than acetyl or hydroxpropyl, it appears that molecular weight can have an effect on the performance of modified starches in acidified milk products.

4. Conclusions

Starch addition, regardless of the type of starch, resulted in a reduction in the gelation time and an increase in the gelation pH. Starch increased final G′ values of acid milk gels, with exception of PG, and magnitude of increase was dependent on type of starch added to the milk. These parameters may not be relevant for final product quality, but they must be considered for the elaboration process.

For modified starches, the means by which both stabilisation and crosslinking are facilitated impacts their effectiveness as fat replacers. Among acetylated starches, ones cross-linked with adipate were superior for reducing gel syneresis and increasing both viscosity of stirred gels and firmness and consistency of set gels, when compared with the starches cross-linked with phosphate.

Focusing now on how stabilisation is achieved, the hydroxypropylated starch reduced syneresis to the further degree and gave higher viscosity/consistency, followed by the acetylated starch. For its part, starch esterified with sodium trimetaphosphate

gave the poorest results, presenting the acidified low-fat milk gels the highest syneresis when this starch was present.

In general, pregelatinised starches gave intermediate values, but they can be used as fat replacers when looking for ingredient products that do not present abrupt viscosity changes upon heating. Meanwhile, starch that is both pregelatinised and chemically modified is preferable if an increase in viscosity/firmness of the resulting stirred/set gels is desired.

Acknowledgement

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