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# Effects of cross-linked inulin with different polymerisation degrees on physicochemical and sensory properties of set-style yoghurt

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ABSTRACT

This work investigated effects of cross-linked inulin with different degrees of polymerisation (DP, average = 7 and 15) on physicochemical and sensory properties of set-style yoghurt. Compared with set-style yoghurt made with native inulin (average DP = 4 and 11), yoghurts with cross-linked inulin had higher acidity and lower syneresis values, with a shelf-life of 14 days. Supplementation of cross-linked inulin with higher DP resulted in enhanced firmness and adhesiveness of yoghurts. In addition, bacterial counts showed that yoghurts with cross-linked inulin exhibited longer retention of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* cell viability than that with native inulin. Sensory evaluation indicated that yoghurt with cross-linked long-chain inulin received higher scores for overall acceptability than other samples. However, different types of inulin did not significantly affect odour and colour of set-style yoghurt. Consequently, cross-linked inulin prepared can be exploited as a prebiotic to prolong shelf-life of yoghurt.



## 1. Introduction

Inulin is a polysaccharide composed of numerous fructose units and a terminal glucose unit. It can be exacted from various plants, such as onions, garlics, bananas and chicory roots. Native inulin has varying degrees of polymerisation (DP) ranging from 2 to 60 monosaccharide units, which depends on the source, harvest time and production process (Glibowski & Zielińska, 2015). Inulin preparations with different DP have varying physicochemical properties. Generally, long-chain inulin can act as a fat replacer or texture modifier due to its poor solubility and good viscosity stability, while short-chain inulin can contribute to improved mouthfeel due to good solubility and mild sweetness (Glibowski & Rybak, 2016). As a non-digestible dietary fibre and attractive prebiotic, inulin exhibits many beneficial physiological functions, such as antioxidant activity, ability to improve colonic microbiota, promoting mineral absorption, and regulating blood glucose and lipids (Balthazar et al., 2016).

Based on nutritional and physicochemical properties, inulin has been widely used in an increasing number of food applications. Inulin has proven to be a promising fat substitute and to improve the functional potential in sheep milk ice cream formulation (Balthazar et al., 2017, 2018). Inulin can also be added for stabilisation in the formulation of whey beverage (Guimarães et al., 2018a) and may enhance the viability of *Lactobacillus* in probiotic yoghurt during storage (Canbulat & Ozcan, 2015).

However, native inulin (a mixture of short-chain and long-chain inulin) has usually been employed without classification in most recent studies, and the lack of certainty of the DP of inulin leads to variable effects on food products since inulin with different DP has different effects. Luo et al. (2017) reported that inulin with lower DP displayed stronger suppression of the retrogradation of wheat starch compared with higher DP inulin. Guimarães et al. (2018b) showed that inulin with higher DP had better efficiency for beverage stabilisation. Nevertheless, there is still a lack of information about the effect of inulin with different DP on properties of other food products. Moreover, short-chain inulin may induce rapid renal excretion due to its low molecular weight. Cross-linking of inulin to increase its molecular weight is a strategy to avoid this disadvantage (Li et al., 2010). However, there is still no reported research exploring the influence of cross-linked inulin with different polymerisation degrees on quality attributes of food products, especially on such a popular dairy food as set-style yoghurt.

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Therefore, the present work was designed to prepare various set-style yoghurts containing native inulin or cross-linked inulin of different DP and aimed to evaluate the different effects of native inulin and cross-linked inulin on physiochemical properties and sensory properties of yoghurt, such as water retention, acidity, adhesiveness, firmness, colour, taste, and bacterial counts.

## 2. Materials and methods

### 2.1. Materials

Native inulins (average DP < 10) were supplied by Xi'an Ruiling Technology Co., Ltd. (Xi'an, China). Four types of inulin with different DP, i.e., native inulin S-In (average DP = 4), native inulin L-In (average DP = 11), cross-linked inulin CS-In (average DP = 7), and cross-linked inulin CL-In (average DP = 15), were prepared in our laboratory by the method described in Section 2.2. Commercial yoghurt starter culture (YFL-702, Chr. Hansen, Denmark) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was used. Milk powder was obtained from Nestle Co., Ltd. (Switzerland). Peptone water was purchased from Qingdao Jisskang biochemical Co., Ltd. (China). Anhydrous ethanol, sodium hexametaphosphate (SH), hydrochloric acid, phenolphthalein, sodium hydroxide, glutaraldehyde, potassium dihydrogen phosphate, tertiary butyl alcohol were purchased from Kelong Chemical Reagent Factory (Chengdu, China). All reagents were of analytical grade.

### 2.2. Preparation of cross-linked inulin

Native inulin (16 g) was added to 80 mL distilled water at room temperature and gently stirred until dissolved. Exactly 20 mL of anhydrous ethanol was added into the solution. The mixture solution was stored at 4 °C for 24 h, after which sediment and liquid supernatant were separated by centrifugation at 8000× *g*, 10 min. The sediment was lyophilised with vacuum freeze-drying equipment (Sihuan LGJ-25C, Beijing, China) at 5 Pa and −50 °C for 12 h; the product obtained was long-chain inulin (L-In) in native inulin. The liquid supernatant was condensed by rotary evaporation (Rongsheng RE-52CS, Shanghai, China) followed by lyophilising for 24 h (5 Pa, −50 °C); the product obtained was short-chain inulin (S-In). Cross-linked inulin was prepared by a method similar to that of starch modification (Ren, Jiang, Wang, Zhou, & Tong, 2012). Typically, L-In (10 g) was dispersed in water (200 mL) with constant stirring for 1 h at 25 °C and then 1.1 g of cross-linker SH was added followed by the addition of $Na_2CO_3$ (2 M) to attain pH 10. Then the pre-polymerisation solution was polymerised with continuous stirring at 45 °C for 3.5 h. Upon completion of the reaction, the pH of the suspension was adjusted to 7 with 2 M HCl. The cross-linked inulin was then precipitated with 95% ethanol, followed by centrifugation and washing with anhydrous ethanol. Finally, after lyophilising, cross-linked long-chain inulin (CL-In) was obtained. Cross-linked short-chain inulin (CS-In) was prepared in the same manner but with the S-In in place of L-In. The molecular structural formula of cross-linked inulin is illustrated in Fig. 1 (Li, Ma, & Liu, 2018).

### 2.3. Preparation of yoghurt

Non-supplemented (inulin-free) control yoghurt was prepared by dispersing 16.0% (w/w) milk powder and 8% sugar in deionised water. Inulin-supplemented yoghurts were made from 16.0% (w/w) milk powder, 6% sugar and 2% corresponding inulin (respectively, S-In, L-In, CS-In, and CL-In) and deionised water. All ingredients were weighed in a flask and mixed with continuous agitation (Sile HD2004W, Shanghai, China) at 60 °C until completely dissolved. The reconstituted milk mixtures were homogenised (20 MPa, 60 °C) in a homogeniser (Nuoni GJJ-0.06/40, Zhejiang, China) and then flasks with samples were heated in a water bath at 95 °C for 15 min and then rapidly cooled in chilled water to 45 °C. After that, all the mixes were each inoculated with 1 g $L^{-1}$ of yoghurt culture YFL-702, followed by incubation at 42 °C for 5 h. When acidity reached 0.8% lactic acid, the yoghurt was cooled to 4 °C.

### 2.4. Physicochemical determinations

Acidity, expressed as g of lactic acid per 100 g of yoghurt, was determined on days 1, 4, 7, 11 and 14 according to the titration method of the Association of Official Analytical Chemists (AOAC, 2002).

To measure syneresis (g per 100 g), a batch of prepared set-style yoghurt (25 g) was weighed in a centrifuge tube and then stored at 4 °C for 1, 4, 7, 11, and 14 days. Each sample was centrifuged at 3000× *g* (Centrifuge 5810, Eppendorf, Germany) at 4 °C for 20 min. The whey was expelled from the sample by inverting the sample on a fine mesh screen placed on top of a funnel. The clear supernatant was poured off, then weighed and expressed as percentage weight relative to the original weight of yoghurt (Srisuvor, Chinprahast, Prakitchaiwattana, & Subhimaros, 2013). All analyses were carried out in triplicate.

Textural characteristics such as hardness, cohesiveness, and adhesiveness were evaluated using a texture analyser (CT-3; Brookfield, USA) equipped with a TA4/1000 cylindrical probe (38.1 mm diameter). The yoghurt samples prepared were stored at 4 °C for 24 h before analysis. The texture analysis circumstances were as follows: penetration depth, 30 mm; force, 5 g; probe speed during penetration, 1 mm $s^{-1}$ (Tiwari, Sharma, Kumar, & Kaur, 2015). The samples, kept in original containers with a diameter of

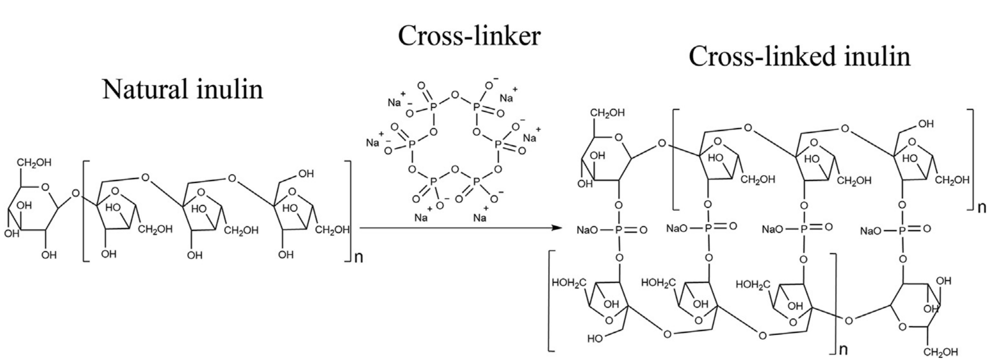


**Fig. 1.** The molecular structural formula of cross-linked inulin (from: Li et al., 2018).

70 mm, were penetrated at ambient temperature (10 ± 1 °C). Two cycles were applied; the values for texture attributes were obtained from the resulting curve using the Texture Pro CT equipment software. The following parameters were quantified: firmness as defined as the maximal peak value recorded in the first immersion into the sample; cohesiveness defined as the ratio of positive force area during the second immersion cycle to that of the first immersion cycle; and adhesiveness, or the negative area for the first cycle, representing the work necessary to pull the probe away from the sample.

The colours of set-style yoghurts stored at 4 °C for 24 h were measured with a colorimeter (Minolta, Spectrophotometer CM-3500d, Japan). The colorimeter used $L^*$ (lightness), $a^*$ (redness) and $b^*$ (yellowness) scales, and operating conditions were illuminant D65 and 2° sample-observer. Colour was measured in triplicate for each sample (Akbari, Eskandari, Niakosari, & Bedeltavana, 2016).

### 2.5. Determination of bacterial counts

A representative sample of yoghurt (1 g) was suspended in 9 mL of sterile 0.1% (w/v) peptone water (Jisskang, Qingdao, China) and subsequently serially diluted to $10^{-7}$. Counts of *S. thermophilus* (ST) were enumerated using the pour plate technique after aerobic incubation at 37 °C for 72 h on M17 agar (Merck, USA) and enumeration of *L. delbrueckii* ssp. *bulgaricus* (LB) was performed using MRS agar (Merck, USA) incubated at 37 °C for 72 h under anaerobic conditions, according to the technique for yoghurt enumeration of characteristic microorganism colony count described by International Organisation for Standardisation (ISO, 2003).

### 2.6. Sensory evaluation

Sensory evaluations were carried out 3 days after production and by a group of 20 panellists (10 females and 10 males, aged between 17 and 38 years old) who were trained in sensory analysis of dairy products. Panellists evaluated the samples and recorded their perceptions by making marks on a ten-score grades as described by Glibowski and Kowalska (2012). The properties evaluated included: colour (1 –unnatural colour, 10 – no criticism), taste (1 – bad, 10 – no criticism), odour (0 – undetectable, 10 – very intensive), texture and consistency (0 – liquid, 10 – very thick), and overall acceptability (0 – dislike extremely, 10 – like extremely).

Definitions for the descriptors describing the colour, taste, odour, texture and consistency of set-style yoghurt were developed by panel consensus using reference samples. Sample defects and descriptors such as acid, sweet, bitter, acetaldehyde taste, milky, creamy, grainy, off-flavour, ropy and gritty texture, whey syneresis, and lumpiness were recorded. Each sample (30 mL) was scored individually, and samples were presented to the panellists in random and balanced order in white plastic cups coded with three-digit random numbers. Mineral water was provided for mouth rinsing in between samples.

### 2.7. Statistical analysis

All experiments were conducted in triplicate for each sample, and results were expressed as mean ± standard deviation. All data were statistically compared between groups using one-way analysis of variance. Significant differences among the mean values were determined by Duncan's multiple range test with $p < 0.05$.

## 3. Results and discussion

### 3.1. Analysis of acidity

The titratable acidity values of the stored set-style yoghurt are presented in Table 1. An increase in titratable acidity was observed over storage time, due to more lactic acid production and the higher acidity in the yoghurt caused by continuous fermentation and stimulated growth of lactic bacteria, which often results in greater contraction of the casein micelle matrix and increased expulsion of whey (Achanta, Aryana, & Boeneke, 2007). Thus, the shelf-life of set-style yoghurt is often limited by excessive acidification during storage (Akalin, Gönç, Unal, & Fenderya, 2007).

Compared with yoghurt containing inulin, the control yoghurt had the lowest acidity value, which agrees well with that reported by Aryana, Plauche, Rao, Mcgrew, and Shah (2007) and Guven, Yasar, Karaca, and Hayaloglu (2005). According to research of Perrin, Fougnies, Grill, Jacobs, and Schneider (2002), the acidity of yoghurt containing lower DP inulin was higher than that of others, due to the faster consumption by the probiotic bacteria which could increase lactate production. However, data in Table 1 are not consistent with this trend, as these results showed that set-style yoghurt containing 2% cross-linked inulin (CS-In or CL-In) had significantly higher acidity than that with corresponding native inulin (S-In or L-In), while the CS-In yoghurt had a higher acidity than the CL-In yoghurt. Consequently, acidity values of yoghurts were mainly influenced by the introduction of acid phosphate groups and DP of inulin.

According to the result of a study of acid stability of inulin (Li et al., 2018), breakdown and hydrolysis would occur for the cross-linked inulin in acidic environment (pH < 6) and inulin with higher DP was more difficult to transform into reducing sugar. The pH value of yoghurt is about 4, so cross-linked inulin should partly break down to reducing sugar and phosphoric acid in this acidic condition, and the generated phosphoric acid would further increase the yoghurt acidity.

### 3.2. Analysis of syneresis

Syneresis value could reflect the water-holding capability of yoghurt product during storage. The syneresis values of yoghurt samples over storage are presented in Table 2. Both inulin polymerisation degree and storage time had significant effects on

**Table 1**
Effect of polymerisation degree of inulin on acidity (g 100 $g^{-1}$) of set-style yoghurt.[a]

| Sample | Day 1 | Day 4 | Day 7 | Day 11 | Day 14 |
|---|---|---|---|---|---|
| Control | 0.86 ± 0.01$^{dC}$ | 0.86 ± 0.01$^{bC}$ | 0.87 ± 0.02$^{cC}$ | 0.90 ± 0.01$^{cB}$ | 0.93 ± 0.02$^{A}$ |
| S-In | 0.89 ± 0.01$^{bcB}$ | 0.87 ± 0.03$^{bB}$ | 0.89 ± 0.01$^{cB}$ | 0.98 ± 0.01$^{bA}$ | 0.99 ± 0.02$^{bA}$ |
| CS-In | 0.94 ± 0.01$^{aD}$ | 0.94 ± 0.01$^{aD}$ | 0.96 ± 0.01$^{aC}$ | 1.07 ± 0.01$^{aB}$ | 1.10 ± 0.01$^{aA}$ |
| L-In | 0.88 ± 0.01$^{cC}$ | 0.92 ± 0.01$^{aB}$ | 0.93 ± 0.01$^{bB}$ | 0.97 ± 0.01$^{bA}$ | 0.93 ± 0.01$^{cB}$ |
| CL-In | 0.90 ± 0.01$^{bC}$ | 0.94 ± 0.01$^{aB}$ | 0.95 ± 0.01$^{abB}$ | 0.98 ± 0.01$^{bA}$ | 0.97 ± 0.01$^{bA}$ |

[a] Treatments: Control, yoghurts made without inulin; S-In, yoghurts made with S-In; CS-In, yoghurts made with CS-In; L-In, yoghurts made with L-In; CL-In, yoghurts made with CL-In. Mean values followed by different superscript lowercase and uppercase letters in columns for different inulin types and in rows for time (day), respectively, are significantly different ($p \le 0.05$).

**Table 2**
Effect of polymerisation degree of inulin on syneresis (g 100 g$^{-1}$) of set-style yoghurt.[a]

| Sample | Day 1 | Day 4 | Day 7 | Day 11 | Day 14 |
|---|---|---|---|---|---|
| Control | 19.31 ± 0.90$^{aC}$ | 19.73 ± 0.81$^{aC}$ | 21.08 ± 0.84$^{aC}$ | 23.16 ± 1.16$^{aB}$ | 25.50 ± 1.25$^{aA}$ |
| S-In | 15.93 ± 0.51$^{bC}$ | 17.38 ± 0.76$^{bB}$ | 17.89 ± 0.49$^{bB}$ | 17.57 ± 0.70$^{bB}$ | 19.20 ± 0.88$^{bA}$ |
| CS-In | 14.01 ± 0.55$^{cD}$ | 14.43 ± 0.48$^{cD}$ | 16.29 ± 0.53$^{cC}$ | 17.91 ± 0.65$^{bB}$ | 18.98 ± 0.60$^{bA}$ |
| L-In | 13.67 ± 0.42$^{dD}$ | 14.79 ± 0.56$^{cC}$ | 14.87 ± 0.47$^{dC}$ | 17.28 ± 0.57$^{bB}$ | 18.46 ± 0.75$^{bA}$ |
| CL-In | 12.54 ± 0.81$^{dB}$ | 13.86 ± 0.72$^{cA}$ | 14.57 ± 0.53$^{dA}$ | 15.02 ± 0.82$^{cA}$ | 15.06 ± 0.45$^{cA}$ |

[a] Treatments: Control, yoghurts made without inulin; S-In, yoghurts made with S-In; CS-In, yoghurts made with CS-In; L-In, yoghurts made with L-In; CL-In, yoghurts made with CL-In. Mean values followed by different superscript lowercase and uppercase letters in columns for different inulin types and in rows for time (day), respectively, are significantly different ($p \leq 0.05$).

syneresis. There was a steady increase in syneresis with storage time for all yoghurt samples, including the control, which may be explained in terms of the increase in acidity during storage causing the casein micelle matrix to contract and extrude more whey (Canbulat & Ozcan, 2015). The extent of syneresis was affected by the addition of inulin. Yoghurt samples made with inulin underwent less syneresis than the control, which may be explained by the ability of inulin molecules to bind water, preventing free movement of water, and to complex with protein aggregates and provide stability to the protein network. In fact, many studies have shown decreased syneresis of yoghurt following the addition of inulin (Meyer, Bayarri, Tárrega, & Costell, 2011).

Interestingly, yoghurts made with CL-In had significantly lower syneresis values than those of yoghurt made with other inulins, which suggests that CL-In possibly had a better water-holding capacity than L-In, S-In, and S-In, due to the higher DP. This result was consistent with that of Aryana and Mcgrew (2007) who reported that long-chain prebiotic had a better water-holding capacity than that of short-chain prebiotic in yoghurt. A good-quality yoghurt should hold water without syneresis. Therefore, the utility of CL-In in this study to act as a food stabiliser could contribute to the yoghurt gel structure, preventing fracture and whey-off.

### *3.3. Analysis of textural characteristics*

Table 3 shows the effect of adding different types of inulin at a level of 2% on textural parameters of set-style yoghurts. Some significant differences in textural parameters were observed among different yoghurt samples. The addition of S-In and CS-In, composed of short-chain inulin, decreased the firmness of control yoghurt compared to L-In and CL-In with higher DP. Set yoghurt made with cross-linked inulin (CS-In and CL-In) had higher firmness than that with corresponding native inulin (S-In and L-In), which can be explained by the fact that the viscosity of inulin increases with the degree of polymerisation and cross-linked inulin had a higher viscosity and water-binding capacity than native inulin, which caused enhanced molecular flow resistance and decreased molecular mobility (Akalin, Unal, Dinkci, & Hayaloglu, 2012; Meyer et al., 2011). Addition of S-In with low DP resulted in lower firmness of yoghurt. Some studies have shown decreased firmness of yoghurt with added inulin (Kusuma, Paseephol, & Sherkat, 2009), and the lower firmness of inulin-supplemented yoghurts may be attributed to short-chain inulin not generating strong viscosity and possibly interfering with the formation of the protein network, resulting in softening of yoghurt. Therefore, the DP of added inulin had an important influence on firmness of yoghurt, a result consistent with that of Glibowski and Kowalska (2012).

The same observations were made for adhesiveness, which is defined as the work required to overcoming the attraction forces between the surface of yoghurt and the probe surface (Chiavaro, Vittadini, & Corradini, 2007). Yoghurt containing inulin with higher DP had greater adhesiveness. The presence of CL-In significantly increased the work required to remove yoghurt that adheres to the probe. This result is in agreement with that of Villegas and Costell (2007), who showed that addition of inulin with higher DP resulted in significantly enhanced adhesiveness of whole milk. Values of firmness and adhesiveness of yoghurt made with CL-In were higher than those of the yoghurts made with other inulin types, which could be attributed to that inulin of higher DP generating a higher viscosity and water-binding capacity.

Cohesiveness ratio, expressed as values between 0 and 1, show the ability for rebuilding the structure of the sample. The highest value of cohesiveness was shown by control yoghurt, with slightly lower cohesiveness by yoghurt with inulin. A lower cohesiveness ratio was observed in yoghurt with CL-In, which could be due to inulin of higher DP being dispersed within the casein micelles, interfering with protein matrix formation (Meyer et al., 2011).

**Table 3**
Effect of polymerisation degree of inulin texture characteristics of set-style yoghurt.[a]

| Time (days) | Control | S-In | CS-In | L-In | CL-In |
|---|---|---|---|---|---|
| Firmness (g) | | | | | |
| 1 | 106.00 ± 6.00$^{bB}$ | 99.67 ± 3.51$^{bB}$ | 104.52 ± 4.26$^{bB}$ | 108.00 ± 5.00$^{aB}$ | 121.67 ± 5.51$^{aA}$ |
| 7 | 102.33 ± 0.58$^{bB}$ | 103.67 ± 3.51$^{abB}$ | 107.33 ± 3.92$^{abAB}$ | 113.67 ± 9.50$^{aB}$ | 130.33 ± 10.50$^{aA}$ |
| 14 | 123.67 ± 4.51$^{aAB}$ | 113.67 ± 8.50$^{aB}$ | 115.57 ± 5.58$^{aB}$ | 118.33 ± 4.5$^{aB}$ | 133.67 ± 5.51$^{aA}$ |
| Cohesiveness ratio | | | | | |
| 1 | 0.41 ± 0.03$^{aB}$ | 0.40 ± 0.05$^{aAB}$ | 0.38 ± 0.04$^{aAB}$ | 0.38 ± 0.02$^{aAB}$ | 0.34 ± 0.02$^{aA}$ |
| 7 | 0.42 ± 0.07$^{aA}$ | 0.42 ± 0.02$^{aA}$ | 0.41 ± 0.02$^{aA}$ | 0.41 ± 0.02$^{aA}$ | 0.38 ± 0.02$^{aA}$ |
| 14 | 0.35 ± 0.04$^{aA}$ | 0.38 ± 0.02$^{aA}$ | 0.37 ± 0.04$^{aA}$ | 0.38 ± 0.03$^{aA}$ | 0.35 ± 0.03$^{aA}$ |
| Adhesiveness (mJ) | | | | | |
| 1 | 3.67 ± 0.25$^{bC}$ | 4.33 ± 0.15$^{bB}$ | 4.52 ± 0.17$^{bB}$ | 4.43 ± 0.15$^{bB}$ | 6.63 ± 0.35$^{aA}$ |
| 7 | 4.63 ± 0.06$^{aC}$ | 4.63 ± 0.15$^{aC}$ | 4.94 ± 0.24$^{abBC}$ | 5.60 ± 0.20$^{aB}$ | 7.03 ± 0.75$^{aA}$ |
| 14 | 4.80 ± 0.20$^{aC}$ | 4.87 ± 0.12$^{aBC}$ | 5.17 ± 0.26$^{aBC}$ | 5.63 ± 0.25$^{aB}$ | 7.27 ± 0.80$^{aA}$ |

[a] Treatments: Control, yoghurts made without inulin; S-In, yoghurts made with S-In; CS-In, yoghurts made with CS-In; L-In, yoghurts made with L-In; CL-In, yoghurts made with CL-In. Mean values followed by different superscript lowercase and uppercase letters in columns for different inulin types and in rows for time (day), respectively, are significantly different ($p \leq 0.05$).

**Table 4**
Bacterial counts (log cfu g-1) in various yoghurts with different types of inulin over a storage period of 14 days.[a]

| Sampling (day) | Control | S-In | CS-In | L-In | CL-In |
|---|---|---|---|---|---|
| *Streptococcus thermophilus* | | | | | |
| 1 | 7.82 ± 0.28$^{bA}$ | 7.76 ± 0.28$^{bA}$ | 7.64 ± 0.19$^{bA}$ | 7.60 ± 0.21$^{bA}$ | 7.46 ± 0.38$^{cA}$ |
| 4 | 8.69 ± 0.23$^{aA}$ | 8.81 ± 0.19$^{aA}$ | 8.72 ± 0.15$^{aA}$ | 8.67 ± 0.28$^{aA}$ | 8.84 ± 0.26$^{bA}$ |
| 7 | 8.77 ± 0.15$^{aA}$ | 8.88 ± 0.25$^{aA}$ | 9.11 ± 0.30$^{aA}$ | 8.90 ± 0.37$^{aA}$ | 9.33 ± 0.37$^{abA}$ |
| 11 | 8.74 ± 0.13$^{aB}$ | 8.86 ± 0.12$^{aB}$ | 8.94 ± 0.17$^{aB}$ | 8.95 ± 0.28$^{aB}$ | 9.62 ± 0.36$^{aA}$ |
| 14 | 8.66 ± 0.32$^{aA}$ | 8.79 ± 0.55$^{aA}$ | 8.85 ± 0.25$^{aA}$ | 8.75 ± 0.31$^{aA}$ | 9.08 ± 0.18$^{bA}$ |
| *Lactobacillus delbrueckii* ssp. *bulgaricus* | | | | | |
| 1 | 7.81 ± 0.31$^{bA}$ | 7.76 ± 0.15$^{bA}$ | 7.76 ± 0.18$^{bA}$ | 7.64 ± 0.24$^{bA}$ | 7.58 ± 0.19$^{cA}$ |
| 4 | 8.67 ± 0.25$^{aA}$ | 8.81 ± 0.42$^{aA}$ | 8.72 ± 0.34$^{aA}$ | 8.67 ± 0.21$^{aA}$ | 8.74 ± 0.28$^{bA}$ |
| 7 | 8.87 ± 0.17$^{aA}$ | 8.89 ± 0.25$^{aA}$ | 9.11 ± 0.27$^{aA}$ | 8.80 ± 0.26$^{aA}$ | 9.33 ± 0.33$^{abA}$ |
| 11 | 8.66 ± 0.26$^{aB}$ | 8.86 ± 0.19$^{aB}$ | 8.94 ± 0.31$^{aB}$ | 8.95 ± 0.18$^{aB}$ | 9.62 ± 0.50$^{aA}$ |
| 14 | 8.59 ± 0.11$^{aA}$ | 8.79 ± 0.24$^{aA}$ | 8.84 ± 0.34$^{aA}$ | 8.75 ± 0.22$^{aA}$ | 9.08 ± 0.32$^{abA}$ |

[a] Treatments: Control, yoghurts made without inulin; S-In, yoghurts made with S-In; CS-In, yoghurts made with CS-In; L-In, yoghurts made with L-In; CL-In, yoghurts made with CL-In. Mean values followed by different superscript lowercase and uppercase letters in columns for different inulin types and in rows for time (day), respectively, are significantly different ($p \leq 0.05$).

### 3.4. Counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*

Changes in counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* over a storage period of 14 days are presented in Table 4. Storage time markedly influenced the levels of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in CL-In yoghurt throughout 14 days of storage, while statistical differences of counts for other sample groups (the control, CS, CS-In and CL) could be observed only between day 1 and day 4. This is in agreement with results of Balthazar et al. (2016), who reported significant changes occurred in *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* counts during the storage of yoghurt. Although very few statistical differences were observed between samples (control and different inulin types) for each day of storage, overall, the counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in yoghurts with inulin were slightly higher than that of the control after storage of day 4. The result was consistent with Akalin, Fenderya, and Akbulut (2004), who reported higher counts of two commercial strains of bifidobacteria in yoghurts containing fructo-oligosaccharide compared with yoghurts without this prebiotic. At the beginning of storage, all yoghurt samples showed counts more than 7 log cfu $g^{-1}$ of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, varying from 7.46 to 7.82 log cfu $g^{-1}$ for *S. thermophilus* and 7.58–7.81 log cfu $g^{-1}$ for *L. delbrueckii* ssp. *bulgaricus*. The numbers of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in control, S-In, CS-In treatments remained marginally increased up to day 7, but slowly declined over the following week, whereas the counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in L-In, CL-In yoghurts slightly rose up to day 11 and then decreased. By day 14, the populations of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in all yoghurt samples increased compared with the beginning, while the increase in yoghurts containing CL-In was most noticeable (1.48 log cfu $g^{-1}$ for *S. thermophilus* and 1.5 log cfu $g^{-1}$ for *L. delbrueckii* ssp. *bulgaricus*).

Aryana et al. (2007) also reported higher counts of *Lactobacillus acidophilus* in yoghurts supplemented with long-chain inulin than yoghurts with short-chain inulin at the end of storage. Yoghurts containing S-In and CS-In showed slightly higher counts in both *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* than that of L-In and CL-In on day 1 and day 4, suggesting that supplementation with inulin with an increased DP led to a decreased rate of consumption of inulin by *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. According to Akalin et al. (2007), the prebiotic activity of inulin was dependent on DP of inulin and shorter-chain inulin is more commonly used as a prebiotic. However, this trend was reversed at subsequent storage up to day 11 and day 14, as the *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* counts of the yoghurts with L-In and CL-In were higher than that of S-In, CS-In and the control. This may be explained by the high acidity of yoghurts with short-chain inulin, consistent with the findings of Lankaputhra, Shah, and Britz (1996) and Cruz, Antunes, Sousa, Faria, and Saad (2009) that high yoghurt acidity negatively affected the viability of probiotic bacteria. Overall, yoghurts with added CL-In exhibited longer retention of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* cell viability than yoghurts made with other DP inulin.

### 3.5. Colour characteristics

The colour values ($L^*$, $a^*$, $b^*$) of different yoghurts are summarised in Table 5. For different yoghurts, $a^*$ values varied between −1.69 and −1.17, and $b^*$ values varied from 7.17 to 11.13 while, overall, both $a^*$ and $b^*$ values increased with higher inulin DP. Addition of inulin gave slightly higher $L^*$, $b^*$ and $a^*$ values than the control. These changes in colour may be associated with inulin molecules interacting with casein micelles, which along with the fat globules, are responsible for the diffusion of incident light. In contrast, Aryana et al. (2007) reported that addition of 1.5% (w/w) inulin in yoghurts did not have any significant effect on $L^*$, $a^*$, or $b^*$

**Table 5**
Colour characteristics and sensory evaluation of set-style yoghurts with different inulin preparations.[a]

| Sample | $L^*$ | $a^*$ | $b^*$ | Colour | Odour | Taste | Texture and consistency | Overall acceptability |
|---|---|---|---|---|---|---|---|---|
| Control | 90.25 ± 6.70$^{a}$ | −1.69 ± 0.27$^{a}$ | 7.17 ± 1.64$^{a}$ | 8.64 ± 1.20$^{a}$ | 8.06 ± 1.14$^{a}$ | 7.47 ± 1.29$^{a}$ | 5.15 ± 1.28$^{a}$ | 6.98 ± 0.72$^{a}$ |
| S-In | 95.82 ± 0.35$^{b}$ | −1.51 ± 0.06$^{a}$ | 8.85 ± 0.17$^{b}$ | 8.77 ± 1.47$^{a}$ | 8.07 ± 1.64$^{a}$ | 7.69 ± 1.38$^{a}$ | 7.43 ± 1.35$^{b}$ | 7.85 ± 0.52$^{ab}$ |
| CS-In | 97.22 ± 0.12$^{b}$ | −1.50 ± 0.06$^{a}$ | 9.19 ± 0.25$^{b}$ | 7.78 ± 0.83$^{a}$ | 7.69 ± 1.01$^{a}$ | 7.02 ± 1.19$^{a}$ | 5.69 ± 1.30$^{ab}$ | 7.01 ± 0.81$^{ab}$ |
| L-In | 95.56 ± 0.26$^{b}$ | −1.53 ± 0.03$^{a}$ | 9.05 ± 0.31$^{b}$ | 8.37 ± 1.03$^{a}$ | 8.69 ± 1.13$^{a}$ | 7.64 ± 1.49$^{a}$ | 8.11 ± 1.49$^{b}$ | 8.34 ± 0.89$^{ab}$ |
| CL-In | 95.12 ± 0.14$^{b}$ | −1.17 ± 0.02$^{b}$ | 11.13 ± 0.08$^{c}$ | 8.52 ± 1.08$^{a}$ | 8.56 ± 1.35$^{a}$ | 7.73 ± 1.36$^{a}$ | 7.72 ± 1.60$^{ab}$ | 8.94 ± 0.75$^{b}$ |

[a] Treatments: Control, yoghurts made without inulin; S-In, yoghurts made with S-In; CS-In, yoghurts made with CS-In; L-In, yoghurts made with L-In; CL-In, yoghurts made with CL-In. Mean values followed by different superscript lowercase and uppercase letters in columns for different inulin types and in rows for time (day), respectively, are significantly different ($p \leq 0.05$).

values. The different result can be explained by the higher concentration of casein and fat globules in this matrix and higher inulin amount used in this work. However, Balthazar et al. (2015) observed slightly higher $L^*$, $b^*$ and $a^*$ values for ovine milk yoghurt with inulin, in agreement with this work. In addition, acid hydrolysis of inulin can probably occur in yoghurt containing inulin due to the presence of lactic acid formed by the starter culture and adequate fermentation temperature (42 ± 1 °C), interfering with the colour of yoghurts. Schreiner (1950) reported a colorimetric method for the determination of inulin by means of resorcinol, due to the ability of inulin to change the medium colour after acid hydrolysis above 40 °C. Although all yoghurt samples appeared white to the naked eye, the instrument picked up slight green and yellow colours.

### 3.6. Sensory evaluation

Sensory evaluation to complement the physicochemical properties of the yoghurt samples were performed (Table 5). No significant effect of inulin on odour, colour and taste was observed. However, significant differences were observed between the sensory evaluations regarding the texture and consistency and overall acceptability of yoghurt samples. For overall acceptability, the sensory scores of the yoghurt containing CL-In and L-In were slightly higher than those of the control yoghurt and the yoghurt containing S-In and CS-In. In addition, the panellists deemed that S-In and CS-In yoghurts were slightly sweeter than CL-In and CL-In yoghurts, which tasted more smooth and creamy, this indicating that taste and texture of yoghurt, to a certain extent, was influenced by the presence of inulin. This is in agreement with the result of Canbulat and Ozcan (2015), who reported that short-chain inulin has 30%–50% of the sweetness of sucrose, while long-chain inulin is bland. The differences in chain length between short-chain inulin and long-chain inulin account for their distinctly different functionality attributes, short-chain inulin possesses functional qualities similar to sugar or glucose syrup, etc., while long-chain inulin has been used successfully to replace fat in dairy products and baked goods.

Several authors have studied the replacement of sugar and fat with different types of inulin in food products. Tarrega and Costell (2005) showed that adding inulin to fat-free dairy model desserts significantly increased sweetness compared with those with no addition. Rodríguez-García, Salvador, and Hernando (2014) reported the good functionality of short-chain inulin as replacers for sugar in low-sugar cakes and long-chain inulin as replacers for fat in low-fat cakes. Another interesting observation was that of worse texture and consistency scores given to CS-In in comparison with other types of inulin yoghurts. Similar results have been reported in some studies concerning yoghurts with inulin. Aryana et al. (2007) reported that inulin chain length had insignificant effect on colour and appearance of fat-free yoghurt, but that medium-chain and long-chain inulin gave yoghurt better sensory properties. Yi, Zhang, Hua, Sun, and Zhang (2010) found that inulin and its hydrolysate could partially replace sucrose in yoghurt. Kip, Meyer, and Jellema (2006) observed that moderately long-chain inulin could be used successfully to improve the creamy mouthfeel of low-fat yoghurt, whereas Guven et al. (2005) found that excessive addition of inulin in fat-free yoghurt negatively influenced some physical properties of yoghurt, i.e., whey separation, consistency and organoleptic scores.

## 4. Conclusions

Compared with set-style yoghurt made with native inulin (S-In and L-In), the yoghurt samples supplemented with cross-linked inulin (CS-In and CL-In) had higher acidity and lower syneresis values during the observed shelf-life of 14 days, indicating that inulin with higher DP had better water-holding capacity.

Supplementation of cross-linked inulin with higher DP resulted in enhanced firmness and adhesiveness of yoghurt. In addition, bacterial counts indicated that the addition of cross-linked inulin as a prebiotic to the yoghurt led to longer retention of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* cell viability during storage. Moreover, sensory evaluation of yoghurts indicated that the yoghurt with CL-In received higher scores for overall acceptability than yoghurt containing lower DP inulin, whereas the different types of inulin did not significantly affect odour and colour of set-style yoghurt. Therefore, exploited as an effective prebiotic ingredient, the cross-linked inulin prepared could have significant application potential for improving textural characteristics and prolonging shelf-life of set-style yoghurt.

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