# Design of a starter culture to produce a reduced-fat soft cheese with added bio-value

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**Summary**

The aim of the work was to optimize the combination of two strains of *Streptococcus thermophilus* used as a starter culture to develop a reduced-fat soft cheese with improved texture and added bio-value. Two strains, St 907 and St 399, were selected for the ability to produce exopolysaccharides (EPS) in milk. Biochemical characterization of EPS showed that the two biopolymers were similar in monosaccharide composition (glucose, galactose, rhamnose and mannose) but different in the relative molar ratio of the monomers and in molecular weight. St 907 produced a long chain and stringy EPS with very high water holding capacity, whereas St 399 produced a smaller-sized EPS, acting as texture enhancer. The strain St 399 was also selected as a major folic acid producer. The cheese obtained had 69.3% of moisture, 18.1% of proteins and 11.5% of fat. Despite the high level of hydration, the structure of the cheese was preserved and the low fat content was successfully compensated by EPS, which gave a positive effect on mouthfeel and cheese yield. The optimized formulation of the starter culture significantly increased the content of folates by up to 67% compared to control cheeses obtained using a commercial starter culture for soft cheeses.

**Keywords**

reduced-fat cheese; *Streptococcus thermophilus*; exopolysaccharide-producing strains; folates

The ability shown by some strains of lactic acid bacteria (LAB) to produce exopolysaccharides (EPS) has been extensively studied in the last years, given the technological relevance that EPS play in food processing, especially in the dairy field. Due to their hydrophilic nature, EPS exert a high water retention in the food matrix, minimizing syneresis phenomena and acting as thickening agents. Another aspect that is rapidly emerging concerns the health-promoting properties of some EPS, as reported by a widely documented evidence that supports, for instance, their involvement in antitumor and anti-oxidant activities [1], immune modulation capability [2] and therapeutic effects on chronic gastritis [3]. Among the LAB species, *Streptococcus thermophilus* is a major starter culture that, together with other important technological properties, is often involved in EPS production. In addition, it is also known that *S. thermophilus* has a strain-specific ability to synthetize folate, in higher quantities compared to other LAB [4], because the majority of folate is excreted extracellularly, hence increasing the vitamin bioavailability when cultivated in milk. These characteristics can be exploited to produce dairy products naturally fortified by nutritionally relevant compounds. LAB producing EPS have been used to produce yoghurts, fermented milks and some cheeses with improved rheological properties [5]. Although numerous papers report on the use of EPS in mozzarella, limited literature is available about the application of EPS-producing cultures in soft cheeses [6]. Crescenza, an Italian rindless soft cheese characterized by low acidity, limited proteolysis and a creamy paste [7], was chosen as a suitable soft cheese model to investigate the use of EPS-producing cultures.

Fat plays a crucial role in the flavour and

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smooth texture of cheeses [8]. On the other hand, consumers are becoming more aware of the health concerns related to increasing the risks associated with obesity, atherosclerosis and coronary heart disease, due to the relatively high amount of saturated fatty acids [9]. Therefore, the fat content in such products is still a matter of debate [10]. The expanding market demand for additive-free, low-calories and nutritionally valuable products prompted us to undertake this work. The aim was to formulate a starter culture made of two strains able to produce EPS and folic acid for the production of a reduced-fat soft cheese similar to Crescenza. The synthesis of EPS was aimed to mask the lack of fat in the cheese, whereas the increased content of folates improved the nutritional value of the product.

## MATERIALS AND METHODS

### Strains employed

The EPS-producing strains used in this work were: *Streptococcus thermophilus* St 907, kindly provided by Sacco (Cadorago, Italia); and *Streptococcus thermophilus* St 399, belonging to the Fodder and Dairy Productions Research Centre (CREA-FLC, Lodi, Italy) collection, previously selected for the capacity to produce both EPS and folates [11]. The strain *Streptococcus thermophilus* St B01 (Christian Hansen, Parma, Italy), EPS-negative and weak folate producer, was used as a control starter in cheese experiments. A freshly spreading culture in M17 (Merck, Darmstadt, Germany) broth was used to inoculate (1% v/v) sterile skim milk (Oxoid, Basingstoke, United Kingdom) and it was then incubated at 42 °C for 16 h. The milk cultures were properly combined and employed as a starter (3% inoculum) for cheese manufacture.

### Water-holding capacity

The water-holding capacity (*WHC*) of fermented milk cultures containing EPS was determined as described by Zhang et al. [11]. Briefly, 10 g of fermented skim milk ($W_0$) was centrifuged at 1610 ×*g* for 10 min at 5 °C. The supernatant was carefully removed, the resulting residue was weighed ($W_1$) and the *WHC* was determined as follows:

$$WHC = \frac{W_1}{W_0} \times 100 \quad (1)$$

Reported data are means of triplicate fermented milk batches and analyses.

### Cheese production

Preliminary micro-scale cheese making tests were performed in laboratory using 3 l of half-skimmed milk (1.8% of fat content) of retail to optimize the design of the starter culture for cheese making. Cheeses were manufactured at the pilot plant of CREA-FLC Centre, using ~150 l (for each batch) of milk, provided by Baroncina farm (Lodi, Italy). Fat milk was standardized at 1.8% by means of a creamer (Seital separator SE 05X, Seital Separation Technology, Vicenza, Italy) at 3000 ×*g*. The same manufacturing process (in triplicate) was applied to both the experimental and control cheeses, as described above. Crescenza cheese making was performed as previously described [7]. Breifly, pasteurized milk was inoculated with the starter, salted (7 g·l$^{-1}$ of NaCl) and maintained at 40 °C until pH decreased to a value of 6.2. Then, 0.4 ml·l$^{-1}$ of liquid calf rennet (strength 130000 International Milk Clotting Units, IMCU, per kilogram) was added. In 20 min, clotting occurred and the curd was cut to obtain cubes of approx. 3 cm$^3$. The cheese samples were shaped and incubated at 37 °C until pH decreased to a value of 5.4, then kept at 4 °C for 7 days and weighed daily. After 7 days, the visual and sensory properties were estimated by an internal panel composed of frequent consumers of Crescenza cheese, evaluating the overall taste, structure and mouthfeel perception.

### Composition analysis

For milk and whey, fat, proteins, lactose and total solids were determined by infrared spectroscopy (IR; MilkoScan FT2 Foss, Padova, Italia). For cheeses, fat and proteins were determined by IR, after an internal validation (over 30 samples tested in parallel with official methods). Dry matter was determined according to a standard method [12]. Apparent yield was calculated as the net weight (in kilograms) of cheese from 100 kg of milk [13].

### Folate content

Extraction of total folates from milk samples and cheeses was performed according to a modified method described by Lin et al. [14]. The extracted samples were diluted (1:5) in sodium phosphate buffer (0.05 mol·l$^{-1}$) containing 1% of ascorbic acid, pH adjusted to 7.2, and then added (2.5%, v/v) to the substrate devoid of folic acid (Folic acid assay medium; Becton Dickinson Difco, Milan, Italy). This medium was inoculated with the indicator strain (*Lactobacillus rhamnosus* ATCC 7469, LMG bacterial collection, Ghent, Belgium), auxotrophic for folic acid, and folate content was quantified by means of the microbio-

logical assay in agreement with official method AOAC 944.12/45.2.03 [15].

#### Isolation of exopolysaccharides

For the EPS extraction, milk cultures were added to an equal volume (1:1) of 10% (v/v) trichloroacetic acid, mixed for 30 min in a magnetic stirrer, and centrifuged at 8150 ×*g* for 20 min (Avanti J-26 XP; Beckman Coulter, Mississauga, Canada) according to a modified protocol of PAULO et al. [16]. To the collected supernatant, a double volume (1:2) of concentrated ethanol was added to sediment EPS at 4 °C during 24 h. The EPS precipitates were separated by centrifugation at 21000 ×*g* at 4 °C for 30 min, the supernatant was discarded while the pellet was dissolved in distilled water and re-precipitated with concentrated ethanol (1:2) as described above. Finally, the pellet obtained by centrifugation was dialysed against distilled water using molecular porous membranes with 6–8 kDa cut-off (Spectra/Por, Spectrum Labs, Breda, The Netherlands) and recovered by centrifugation (Sorvall RC-5C Plus, Mandel Scientific, Guelph, Canada) at 3619 ×*g*. Retentate containing EPS was freeze-dried and stored for analysis of composition and molecular size.

#### Composition of exopolysaccharides

Approx. 10 mg of EPS were hydrolysed with 2 mol·l$^{-1}$ of $H_2SO_4$ at 100 °C for 2 h. Monosaccharides were determined according to GUO et al. [17] using a Dionex high performance anion-exchange chromatography (HPAEC) system (DX-500; Shimadzu Scientific Instruments, Columbia, South Carolina, USA) equipped with pulsed amperometric detection (PAD) (Thermo Fisher Scientific, Sunnyvale, California, USA) and eluted by isocratic eluent (100 mmol·l$^{-1}$ NaOH) on a CarboPac PA1 column (4 mm × 250 mm, Dionex). A guard column (3 mm × 25 mm, Dionex) was utilized. The flow rate was 1.0 ml·min$^{-1}$.

#### Molecular mass analysis of exopolysaccharides

The molecular weight and intrinsic viscosity were determined using high performance size exclusion chromatography (HPSEC) on Viscotek TDAmax system (Malvern Instruments, Malvern, United Kingdom) using multiple detectors: a differential pressure (DP) viscometer for viscosity determination; a refractive index (RI) detector and a UV detector for concentration determination; a right angle laser light scattering (RALLS) detector and a low angle laser light scattering (LALLS) detector for direct molecular weight determination. Two columns in series were used: a Shodex Ohpak KB-806M (Showa Denko, Tokyo, Japan) and a Ultrahydrogel linear (Waters, Milford, Connecticut, USA). The columns, viscometer and RI detector were maintained at 40 °C. The eluent was 0.1 mol·l$^{-1}$ of $NaNO_3$ containing 30 g·kg$^{-1}$ $NaN_3$ at a flow rate of 0.6 ml·min$^{-1}$. Data were obtained and analysed using OmniSEC 4.6.1 software (Viscotek, Malvern Instruments). For calculations, 0.146 l·kg$^{-1}$ was adopted as the refractive index increment (dn/dc) value in aqueous solution.

#### Statistical analysis

Student's t-test (Paleontological statistics software, PAST, Natural History Museum, Oslo, Norway) was applied to determine significant differences between the cheeses produced with the functional strains (St 399 and St 907) and the control cheeses obtained with the commercial starter culture (St B01). Pairwise averages of composition parameters, folate content and yield were the main factors investigated. The level of significance was determined at $p < 0.05$.

## RESULTS

#### Properties of the starter culture strains

The ability of the two *S. thermophilus* strains to produce EPS and folates was preliminarily confirmed in milk cultures incubated at 42 °C for 10 h (Tab. 1). The strain St 907 produced high amount of ropy EPS having a marked water-holding capacity (Tab. 1), whereas the strain St 399 synthetized less EPS that acted rather as a texture enhancer. The latter strain was selected for its ability to produce either a different type of EPS or a higher amount of folates compared to the strain St 907.

#### Analysis of exopolysaccharides

The EPS synthesized by the two strains (St 399 and St 907) were both hetero-polysaccharides composed of the same monomers, with galactose

**Tab. 1.** Exopolysaccharides, folate production and water-holding capacity of starter culture strains.

| Strains | Folates [ng·ml$^{-1}$] | EPS yield [mg·l$^{-1}$] | WHC [%] |
|---|---|---|---|
| St 399 | 46.65 ± 10.79 | 92.50 ± 12.81 | 32.1 ± 2.8 |
| St 907 | 9.96 ± 7.30 | 236.40 ± 40.30 | 38.2 ± 0.1 |
| St B01 | 10.30 ± 4.65 | nd | 29.7 ± 1.7 |

Folates, exopolysaccharides and water-holding capacity were determined after 10 h of growth in skim milk. Limit of quantification for folates was 0.1 ng·ml$^{-1}$, for EPS was 0.1 mg·l$^{-1}$, whereas for *WHC* was 0.1%.
EPS – exopolysaccharides, *WHC* – water-holding capacity, nd – not detected.

**Tab. 2.** Chemical characterization of exopolysaccharides.

| EPS | Gal [%] | Glu [%] | Rha [%] | Man [%] | $M_w$ [g·mol$^{-1}$] | *IV* [dl·kg$^{-1}$] |
|---|---|---|---|---|---|---|
| St 399 | 45 ± 1 | 16 ± 1 | 32 ± 3 | 7 ± 1 | (4.05 ± 0.79) × $10^5$ | (2.03 ± 0.08) × $10^{-4}$ |
| St 907 | 56 ± 2 | 16 ± 1 | 27 ± 1 | 1 ± 2 | (3.92 ± 1.53) × $10^6$ | (2.75 ± 0.82) × $10^{-3}$ |

EPS – exopolysaccharides, Gal – galactose, Glu – glucose, Rha – rhamnose, Man – mannose, $M_w$ – molecular weight, *IV* – intrinsic viscosity of EPS synthesized by the two EPS$^+$ strains.

**Tab. 3.** Chemical composition of cheese samples after 7 days of maturation.

| Sample | pH | Moisture [%] | Proteins [%] | Fat [%] | Folates [μg·kg$^{-1}$] | Cheese yield [%] | |
|---|---|---|---|---|---|---|---|
| | | | | | | After 1 day | After 7 days |
| Cheese EPS$^+$ | 5.21 ± 0.10 | 69.3 ± 0.7 | 18.1 ± 0.3 | 11.5 ± 0.3 | 170.42 ± 25.06 | 16.7 ± 0.4 | 14.1 ± 0.3 |
| Cheese EPS$^-$ | 5.16 ± 0.06 | 64.3 ± 1.5 | 20.7 ± 1.0 | 12.7 ± 0.5 | 100.45 ± 10.45 | 11.1 ± 0.7 | 10.8 ± 0.7 |
| Student's t-test | ns | $p < 0.05$ | ns | ns | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |

EPS$^+$ – cheeses containing exopolysaccharides, EPS$^-$ – control cheeses, ns – not significant.

as the most abundant monosaccharide, followed by rhamnose, glucose and mannose. However, the relative molar ratio of EPS was a little different as reported in Tab. 2. The molecular weight of EPS produced by St 907 was 10 times higher than that produced by St 399. Accordingly, the intrinsic viscosity calculated for EPS of St 907 was also higher compared to EPS of St 399 (Tab. 2).

### Optimization of the starter culture

In the preliminary micro-scale cheese making tests, the employment of St 907 as a starter culture, based on its highest EPS production, allowed to obtain cheeses with a very high moisture content (approx. 74%), but showing problems of structure (poor body) and a strong tendency to release whey during storage. In particular, this cheese exhibited a defect, due to the type of EPS produced by this strain, which conferred a mucoid appearance on the surface cut and a stringy aspect of the released whey. Conversely, when St 399 was used as a single strain starter, the obtained cheeses had a good structure, although with a lower moisture content (67.8%) than the ones from St 907, but they showed an excessively sticky texture. Taking into account the cheese characteristics observed in experiments with the single strain, and based on the need to enhance both the structure and to increase the content of folic acid in the final product, the two strains were employed in association, at different mutual ratios (50:50, 75:25, 90:10, 95:5). The optimal combination of the two strains resulted to be 95% of St 399 and 5% of St 907, thus the experimental cheeses were manufactured with this starter formulation (EPS$^+$) and compared to control cheeses, using the commercial St B01 starter (EPS$^-$).

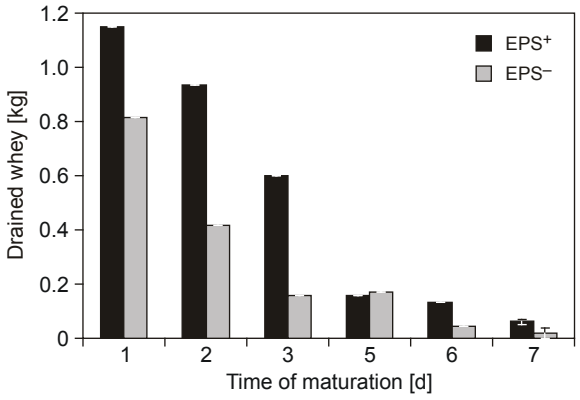


**Fig. 1.** Whey drained from the cheese samples during maturation.

Cheeses were kept at 4 °C. EPS$^+$ – cheeses containing exopolysaccharides, EPS$^-$ – control cheeses.

### Production of the cheeses

The in situ production of the EPS exerted a different technological impact on the physico-chemical characteristics of the cheese compared to the control, i.e. a commercial *S. thermophilus* starter (EPS-negative and weak folic acid producer). During cheese processing, a lower syneresis of the curds (after cutting) produced with the EPS$^+$ starter culture was observed, compared to the control ones (EPS$^-$). After 1 day from production, the cheese yield obtained from the EPS$^+$ cheeses was 16.8%, significantly ($p < 0.01$) higher than that (11.1%) shown by the control cheeses (Tab. 3). However, during the maturation period (7 days from production) the EPS$^+$ cheeses showed a tendency to drain whey higher than the EPS$^-$ cheeses (Fig. 1), although a cheese yield higher ($p < 0.01$)

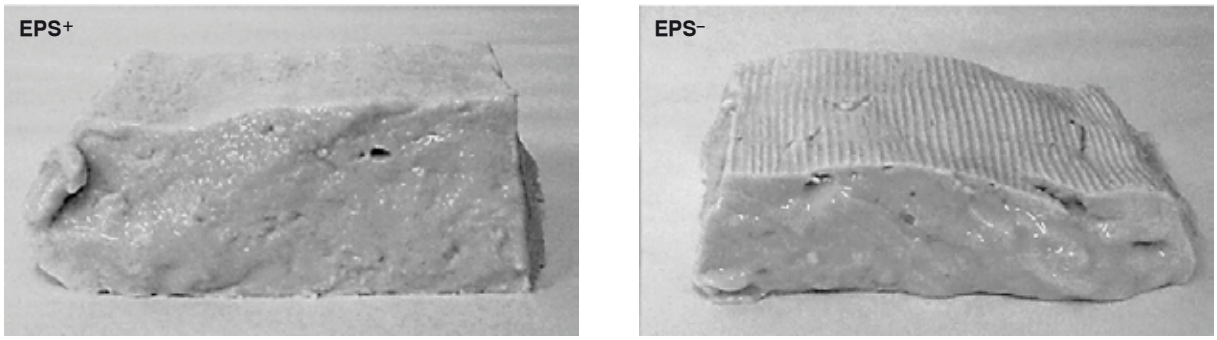


**Fig. 2**. Appearance of the cheese samples after maturation.

Images were acquired after 7 days of maturation, just before packaging.
EPS+ – cheese containing exopolysaccharides, EPS– – control cheese.

than the control was still obtained (Tab. 3). At the end of the maturation period, cheeses were packaged in plastic containers, sealed and stored for 15 days in refrigerated conditions. During storage, both the EPS+ and EPS– cheeses showed only negligible further syneresis, which was considered as normal for this type of product. The resulting cheeses were pleasant in taste and showed a typical slightly creamy structure (Fig. 2).

**Composition of cheeses and changes in folate content**

At the end of the maturation period, cheeses were ready for consumption. A significantly higher ($p < 0.05$) moisture content was determined in the cheeses containing EPS (69.3%) compared to the control cheeses (64.3%), confirming the role of EPS as hydrocolloids with important water-binding properties. The dry mass of the EPS+ cheeses was mainly composed of 18.1% of proteins and 11.5% of fat, values slightly lower than the control counterpart but the differences were not significant (Tab. 3). This was likely due to a dilution factor, suggesting that the yield increase was only related to the moisture content, with no effects on fat retention since the fat content of the EPS+ and EPS– whey drained was the same (Tab. 4). Similarly, final pH of the EPS+ and EPS– cheeses were not significantly different (Tab. 3). The whey drained from EPS+ cheeses appeared to be more viscous than that drained from EPS– cheeses, although the gross composition of the whey did not reveal any significant difference. Moreover, the optimized formulation of the starter, focused on the advantage of the high folate production by the strain St 399, allowed a significant increase of the folate content in EPS+ cheeses (170.42 ± 25.06 $\mu g \cdot kg^{-1}$) compared to the control cheeses (100.45 ± 10.45 $\mu g \cdot kg^{-1}$).

## DISCUSSION

The use of EPS-producing strains allowed to retain a high moisture content throughout the storage period (15 days) of the cheese and to stabilize the product. Consequently, the cheese yield was increased even if half-skimmed milk was processed, which is a crucial factor for the cheese manufacturing plants. Similar results were already reported for other types of cheese, such as mozzarella [18] and reduced-fat Cheddar cheese [19]. The comparison between EPS+ and EPS– starter cultures was also investigated in a reduced-fat Kasar cheese, which resulted in an improved cheese composition [20]. Differently

**Tab. 4.** Chemical composition of milk and whey.

| Sample | Fat [g·kg$^{-1}$] | Protein [g·kg$^{-1}$] | Lactose [g·kg$^{-1}$] | Total solids [g·kg$^{-1}$] |
|---|---|---|---|---|
| Milk | 18.67 ± 0.24 | 33.18 ± 0.15 | 48.12 ± 0.16 | 110.58 ± 0.14 |
| Whey EPS+ | 1.33 ± 0.11 | 9.48 ± 0.38 | 46.87 ± 2.63 | 68.73 ± 3.25 |
| Whey EPS- | 1.20 ± 0.24 | 9.40 ± 0.32 | 46.50 ± 1.65 | 67.80 ± 2.78 |
| Student's t-test | ns | ns | ns | ns |

EPS+ – cheeses containing exopolysaccharides, EPS– – control cheeses, ns – not significant.

from Kasar, which is aged and stored for 90 days, Crescenza is a fresh soft cheese that allowed to achieve extremely high moisture content and successfully proved to be a suitable model for the use of EPS$^+$ starter cultures. EPS-producing strains are available on the market although they do not always lead to improved texture attributes or firmness, which also depend on the specific EPS structures and on the interactions between EPS and proteins [21]. As shown in our preliminary cheese production, the use of single EPS$^+$ strains, which produced two types of EPS differing mainly in molecular weight, gave very different results on cheese texture and characteristics, with some disadvantages in each case. To this regard, visual and sensory properties should also be evaluated even for well performing strains since the employment of the single St 907 led to the formation of a cheese with an unpleasant filamentous serum. In a previously published study [22], it was reported that the use of EPS-producing strains alone improved the cheese yield but had a negative effect on the texture and flavour of Chihuahua cheese. Optimization of the addition of two strains in association enabled us to enhance the beneficial effects of the EPS produced and to limit the potential drawbacks. The strain St 907, which constituted only 5% of the starter formulation, contributed with its high water-holding ability until an acceptable level (~70%), at which the undesired characteristics of its EPS were no longer perceived. Despite the high level of hydration, the structure of the reduced-fat cheese was preserved and EPS proved to be effective fat replacers, exerting a positive impact on mouth-feel, apparently with no evident difference from the full-fat equivalent. Moreover, microbial EPS can be considered as non-digestible dietary fibres, which may also have beneficial effects on the gastrointestinal tract [23]. In addition, the bio-value of the experimental cheese was remarkably enhanced, increasing the folate content up to 66.7% compared to the control cheese. Bio-fortification by folates produced by LAB was reported for fermented milks [24] and yoghurts [25]. In this work, the increase of folates was achieved in a soft cheese, whose high moisture content likely favoured the retention of most of this water-soluble vitamin. Promising biotechnological applications may contribute to cover the daily required folic acid intake. Although the folate content of green vegetables and cereals is higher, dairy products represent an underestimated source of folates that proved to be highly bioavailable and stable due to the presence of hydro- and lipophilic antioxidants [26].

## CONCLUSION

The ability of lactic acid bacteria to produce nutritionally valuable metabolites, such as exopolysaccharides and vitamins, is an added value to be properly exploited, adapting the microbial fermentation to food processing. Results of this study clearly showed how a focused formulation of the starter culture enabled to achieve multiple benefits, producing a soft cheese with a higher cheese yield and improved texture and bio-value. To our knowledge, this work is one of the first attempts to increase the folate content in cheese. The manufacturing process of this cheese showed potential industrial perspectives and could meet the growing demands of consumers for low-fat and healthier products.

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