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Improved creaminess of low-fat yoghurt: The impact of amylomaltase-treated starch domains

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ABSTRACT

Amylomaltase-treated starch (ATS) is an excellent creaminess enhancer in yoghurt. Small amounts of ATS raised the creaminess perception of low-fat yoghurt (1.5%) to that of full-fat yoghurt (5%). In this way, a reduction in fat-related energy value could be achieved from 45 to 21.5 kcal/100 g product. The functionality of ATS in set yoghurt resulted from discrete domains of ATS that resemble the microstructural behaviour of fat particles. The microstructure of the yoghurt is dominated by the protein and the ATS domains are enclosed in or bound to this protein network. The perceived creaminess resulted from in-mouth melting of these ATS domains due to a combined effect of their physical melting and hydrolysis by amylase present in the saliva.

1. Introduction

Obesity is a fast growing global health problem and is connected to a broad spectrum of diseases such as cardiovascular diseases and type 2 diabetes. Replacement of fat in foods by ingredients having a lower caloric value is a strategy that can help in restoring the imbalance between caloric intake and exercise. However, fat is the main determinant for the creaminess perception in many diary products (de Wijk, Terpstra, Janssen, & Prinz, 2006 and references therein). In yoghurt, fat contributes in two ways to the perception. Fat in discrete particles (droplets) act as fillers and hence contribute to the thickness of the yoghurt and thereby to the overall smooth perception (Jonhøj, Petersen, Frøst, & Ipsen, 2006). Second, fat is essential for the creamy sensation of full-fat yoghurt, which is a combination of flavour and mouth-feel attributes (Bult, de Wijk, & Hummel, 2007).

Hydrocolloids are well-known ingredients to improve the mouth-feel of low-fat yoghurt (Everett & McLeod, 2005). Gelatine is a well-known ingredient in low-fat yoghurts, due to its melting behaviour at body temperature. Also polysaccharides are applied, for example, inulin, an oligosaccharide extracted from chicory *Cichorium intybus*, is described as creaminess-improving ingredient in yoghurt. Especially the variant with a relatively high degree of polymerisation (DP ~ 23) and applied in concentrations above 3% (m/m) showed high creaminess scores (Alexander, 1992). Already for decades starch-derived ingredients are applied to replace fat (in Alexander, 1992). In general these starches are either acid- or enzyme-degraded starches or derivatives thereof. The functionality of the products all derived from the fact that the starch derivatives form in the liquid food systems a continuous gel with specific particulate character. These particulates have a distinct disk-shape character as described by Reuther et al. (1984). The amorphous phase of the gel is weak and under shear in the mouth, the disks are loosened and perceived as fat droplets. However, these types of gel do not show thermoreversible melting. Another prerequisite for the function of the gel is that they are applied in concentrations where they do form gels. Especially maltodextrin is known for its functionality in low-fat products. Maltodextrin is obtained by partial enzymatic hydrolysis of starch (Belitz & Grosch, 1987). A major drawback of current starch-derived fat replacers is the relative high level that is needed to achieve the desired fat-mimicking properties. For example maltodextrin is typically used at concentrations above 3% (m/m) to show good fat-replacing behaviour. Thus more functional starch-based ingredients are needed. This publication describes the use of a new

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starch-type ingredient which is functional in yoghurt at lower concentrations.

Recent reports showed that enzymatic treatment of starch with amylomaltase (4-alpha-glucanotransferases; E.C. 2.4.1.25) results in a completely new starch functionality. This specific enzymatic treatment of starch results in a starch variant that showed thermoreversible gelation, which is a unique property for starches (van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). Moreover, the gel point (minimum level to obtain a self-supporting gel) in water was lowered after this treatment from typically 10% (m/m) to around 3% (m/m) (van der Maarel et al., 2005; Euverink & Binnema, 2003). As schematically illustrated in Fig. 1, treatment of starch with the enzyme led to disappearance of amylose and broadening of the side-chain length distribution of amylopectin. The linear side chains of amylopectin are longer in ATS than those of the parent starch. Starch from various botanical sources can be used for this enzymatic treatment. Moreover, different incubation times resulted in starch variants with different molecular characteristics and functional properties. Entanglement and helix formation of the elongated amylopectin side chains is supposed to be the mechanism behind the thermoreversible gelation of amylomaltase-treated starch (ATS).

This thermoreversible gelation and thus melting of ATS triggered the possible function as fat replacer. Melting of partial crystalline dairy fat contributes to the overall creamy perception of full-fat dairy products. Herein we describe our studies on the functionality of ATS in set-style yoghurt. Physical/chemical analyses of the yoghurts were combined with sensory description to understand the mechanism behind the functionality of ATS in low-fat yoghurt. Throughout our study, two benchmark products were included, maltodextrin and gelatine as these are the common texturising ingredients applied in low-fat yoghurt. Moreover, gelatine and maltodextrin were selected as benchmark products for their difference in functionality. Gelatine forms a thermoreversible gel, whereas maltodextrin forms a particle gel.

2. Material and methods

2.1. Materials

Amylomaltase-treated starch (ATS; Etenia), maltodextrin (Paselli SA2), and potato starch (Perfectamyl gel MB) were obtained from AVEBE (Veendam, The Netherlands). Gelatine (220 bloom 20 mesh) was from Gelita (Eberbach, Germany), α-amylase (from *Aspergillus oryzae* (A-6211)) from Sigma–Aldrich (Zwijndrecht, the Netherlands) and the EnzyPlus glucose kit from Diffchamb Biocontrol (EZS-781). Fresh milk (3.5% m/m protein) with various fat levels was used to prepare milk gels and yoghurt variants.

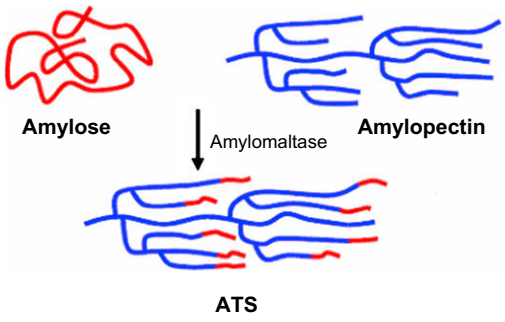

Fig. 1. Schematic representation of the enzymatic conversion of potato-starch-derived amylose and amylopectin into ATS by amylomaltase.

2.2. Rheological characterization in water

2.2.1. Gel setting measurements

Gel formation was followed by measuring the dynamic moduli (storage modulus, G' and loss modulus, G'') as function of time at a strain of 1% and a frequency of 1 Hz using an AR2000 rheometer (TA Instruments, Etten-Leur, The Netherlands). ATS (5% m/m) was dissolved in tap water at 70 °C and allowed to hydrate for 15 min. After pouring the sample in the heated cup of the rheometer, the temperature was increased to 92 °C and held at this temperature for 30 min. Next the temperature was decreased to 10 °C within 30 min. The sample was held at a temperature of 10 °C for 36 h and subsequently the temperature was increased to 70 °C within 30 min. After a holding time of 8 h at 70 °C, the temperature was decreased to 10 °C and finally held at 10 °C for again 36 h.

2.2.2. Thermoreversibility measurements

Identically to gel setting measurements, the sample was heated in the rheometer to 92 °C and cooled to 10 °C. A holding period of 8 h followed by a heating cycle to 70 °C and back to 10 °C within 60 min was applied to the sample for 5 cycles.

2.3. Rheological behaviour in milk

2.3.1. Continuous shear viscosity

The viscosity of the ATS solutions was measured by a continuous-flow measurement (shear rate from 0 to 100 s^{-1} in 15 min at 10 °C; forward and backward). The viscosity at a shear rate of 50 s^{-1} was used to compare the variants (AR2000, TA Instruments, Ettenleur, The Netherlands).

2.3.2. Gel-setting

Gel formation in milk was followed by measurement of the dynamic moduli (storage modulus, G' and loss modulus, G'') as a function of time at a strain of 1% and a frequency of 1 Hz (AR-2000, TA Instruments). ATS (various concentrations) was dissolved in skim milk at 70 °C and allowed to hydrate for 15 min. After pouring the sample in the heated cup of the rheometer, the temperature was increased to 92 °C and held at this temperature for 5 min. Next the temperature was decreased to 10 °C within 30 min. The sample was held at 10 °C for 24 h. After reaching a steady value for the storage modulus (G'), a strain sweep was applied at 10 °C. Therefore, the strain was increased from 0.1 to 1000% at constant frequency of 1 Hz.

2.4. Rheological characterization in yoghurt serum

2.4.1. Preparation of yoghurt serum

Freshly prepared reference yoghurt (3% fat) (see Section 2.5) was centrifuged at 10,240×g for 30 min to obtain yoghurt serum. The serum was filtrated trough paper filter (Schleicher & Schüll 1573½, Selecta, diameter 18.5 cm) prior to storage in portions of 30 g at −32 °C. Directly before analysis, the serum was thawed at 25 °C.

2.4.2. Viscosity

Samples were prepared by adding 1, 2.5, 3.5 or 5% ATS or 3% maltodextrin to the yoghurt serum at 70 °C under stirring and allowed to hydrate for 15 min at 70 °C. In 8 min they were heated to 92 °C and held at this temperature for 30 min. Subsequently the samples were cooled in running tap water and stored for 24 h at 7 °C prior to measurements. The samples with an ATS concentration of 2.5% and higher were stirred before the measurements as these solutions formed a gel. The viscosity of the sera was measured by a continuous-flow measurement (shear rate from 0 to 100 s^{-1} in 15 min at 10 °C; forward and backward). The viscosity at a shear

rate of 50 s^{-1} was used to compare the variants (AR2000, TA Instruments, Ettenleur, The Netherlands).

2.4.3. Gel-setting

Samples were prepared by adding 2.5, 3.5 or 5% ATS to yoghurt serum at 70 °C and allowed to hydrate for 15 min at 70 °C. The sample was poured in a pre-heated cup (70 °C) and directly heated to 92 °C (at 2.7 °C/min) and held at this temperature for 30 min. Next, the temperature was decreased to 10 °C within 30 min. The sample was held at 10 °C for 72 h. Gelation of yoghurt serums with ATS was followed by measurement of the dynamic moduli (storage modulus (G') and loss modulus (G'')) as a function of time at a strain of 1% and at an angular frequency of 1 Hz (AR-2000, TA Instruments).

2.5. Set-yoghurt production

Set-style yoghurt variants (Table 1) were prepared from standardised (on fat and protein) and homogenised (200/20 bar) milk. Texturising ingredients, maltodextrin or ATS were added to the standardised and homogenised milk and allowed to hydrate during 15 min. The milk was pasteurised batch-wise (10 min at 90 °C), cooled to fermentation temperature (42 °C) and subsequently inoculated with 0.05% (m/m) ISt culture (CSK Food Enrichment, Leeuwarden). The inoculated milk was filled into 125 ml polypropylene cups for the QDA-panel and in 200 ml cups for the in vitro melting tests and serum preparation, sealed and allowed to ferment during 5–6 h at 42 °C. After reaching pH 4.5 the cups were cooled and stored at 4 °C until further analysis.

2.6. Determination of in vitro melting

In-mouth melting of model gel systems and set yoghurts was mimicked in vitro using α-amylase in combination with temperature-controlled torque measurements in a rheometer (AR-2000, TA Instruments) equipped with a vane geometry.

An α-amylase activity comparable with human saliva was obtained using the following protocol. Fresh solutions of α-amylase (0.1 g/g) were prepared daily in a buffer containing 100 mM MOPS (3-(*N*-morpholino)-propane-sulfonic acid), 160 mM NaCl and 5 mM $CaCl_2$, adjusted to pH 7.0 with 1 N NaOH. This solution was centrifuged for 10 min at 17,000×*g* and the clear supernatant was used. The solution was stored on melting ice until use.

Table 1
Formulation of the set-yoghurt variants

Code	Ingredient (% m/m)			
	Protein	Fat	ATS	Maltodextrin
10% Fat reference	3.5	10	–	–
5% Fat reference	3.5	5	–	–
3% Fat reference	3.5	3	–	–
Sample 1	3.5	–	0.7	
Sample 2	3.5	–	1.0	–
Sample 3	3.5	–	2.0	–
Sample 4	3.5	–	2.5	–
Sample 5	3.5	1.5	1.0	–
Sample 6	3.5	1.5	2.0	–
Sample 7	3.5	3	0.7	–
Sample 8	3.5	3	1.5	–
Sample 9	3.5	3	2.0	–
Sample 10	3.5	3	3.5	–
Sample 11	3.5	3	5.0	–
Sample 12	3.5	5	1.0	–
Sample 13	3.5	5	1.5	–
Sample 14	3.5	3	–	3

2.6.1. Model systems

2.6.1.1. Sample preparation. Aqueous solutions of 10% ATS, 1% gelatine and starch (10% Perfectamyl gel MB or 3% maltodextrin) were prepared as follows:

(1) ATS was suspended in water at 70 °C and allowed to hydrate for 15 min at 70 °C. In 8 min the solution was heated to 92 °C and held at this temperature for 30 min. Subsequently the solution was cooled to 50 °C.
(2) Gelatine was suspended in water (20 °C), heated to 50 °C to dissolve the gelatine and subsequently stirred for 5 min.
(3) Starch was suspended in water at 20 °C and heated to 95 °C (while stirring at 60 rpm) and held at that temperature for 30 min (while stirring).

Directly after preparation, the aqueous solutions of ATS, gelatine and starch were filled out in 60 ml syringes, which were closed and quickly cooled to 7 °C to allow a gel to form. The gels were stored at 6 °C until further analysis.

2.6.1.2. Measuring method. The gels were pressed out of the syringes into the rheometer cup and the vane was inserted in the sample. The measurement started by measuring the torque for 5 min at an angular velocity of 6.5 rad/s at 10 °C.

The vane was raised until one-third of the blades were above gel level and 250 μl RO-water or α-amylase solutions were injected at the end of a blade. Subsequently the vane was lowered and the rotating was continued at the same speed. The torque was measured with increasing temperature (from 10 °C to 50 °C in 40 min).

2.6.2. Set-yoghurt

2.6.2.1. Sample preparation. Set-yoghurt variants were prepared in 200 ml cups and stored for 1 week at 7 °C. Just before measurement the yoghurt was brought to 25 °C and mounted into the rheometer.

2.6.2.2. Measuring method. In vitro melting of set yoghurt was determined with torque measurements using a rheometer (AR-2000, TA Instruments) equipped with a vane geometry. The vane was lowered until two-thirds of the blades were inserted into the yoghurt. Subsequently, 250 μl RO-water or α-amylase solution was injected at the end of a blade followed by lowering the vane until it was fully inserted into the yoghurt. The measurement was started directly. The torque was measured for 15 min while rotating the vane at an angular velocity of 6.5 rad/s at 25 °C.

2.7. Determination of microstructure

2.7.1. Confocal laser scanning microscopy

The microstructure of the set yoghurts was determined at room temperature using a Confocal Laser Scanning Microscope (CLSM), equipped with an inverted microscope (model Leica DM IRBE) used in the single-photon mode with an Ar/Kr visible-light laser (Leica Microsystems (CMS) GmbH, Mannheim, Germany). The protein phase was stained with 0.2% (m/m) Rhodamine B (Sigma, USA). The excitation wavelength was 568 nm and the corresponding emission maximum was at 583 nm. The objective used had a magnification of 63× (Leica HSX PL APO 63×/1.20 w cor). Digital image files were acquired in tagged image format and at 1024 × 1024 pixels resolution.

2.7.2. Light microscopy

The starch present in the ATS or maltodextrin containing yoghurt variants was visualised at room temperature using a light microscope (Reichert-Jung, type Polyvar). The starch phase of the yoghurt was stained with 0.01% iodine/potassium iodine

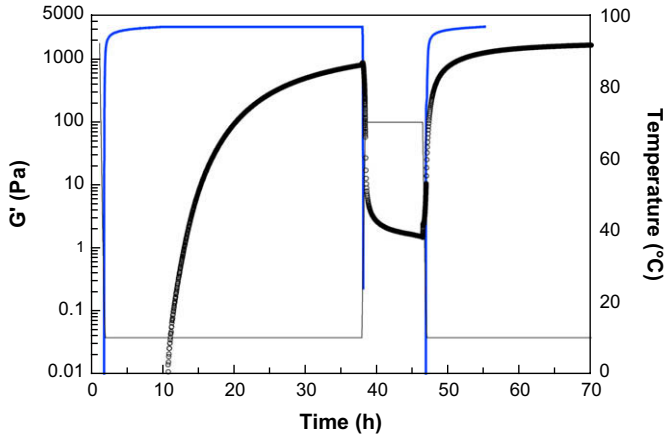

Fig. 2. Storage modulus (G') and temperature (thin line) as functions of time for ATS (circles) and gelatine (bold blue line).

(Aldrich–Merck, Darmstadt, Germany). The objective used had a magnification of 40×.

2.8. Determination of the amount of ATS in yoghurt and yoghurt serum

Set-yoghurt variants (samples 7–11) were centrifuged for 10 min at 4500×*g* and at 20 °C to obtain serum. Additionally, the samples 9 and 10 were centrifuged for 18 h at 2880×*g* and at 20 °C. The serum was filtered (paper filter of Schleicher & Schüll 1573½, Selecta, diameter 18.5 cm). The starch concentrations (ATS and maltodextrin) within these sera were determined by first degrading the starch into glucose by the enzymes α-amylase and amyloglucosidase. In short, to approximately 35 mg starch or product containing this amount of starch 3 ml of water was added, subsequently 100 ml boiling water was added to gelatinize the starch. Subsequently, 100 ml cold water was added. To the starch-dispersion, 2.5 ml acetate buffer (pH 4.6: 136.0 g sodium acetate.3aq, 60 ml glacial acetic acid in a total volume of 500 ml water) and 0.20 ml 1:1 mixture of the enzymes α-amylase (Megazyme, cat. no. E-BLAAM) and amyloglucosidase (Megazyme, cat. no. E-AMGDF) were added. The dispersion was subsequently homogenised and incubated for 2 h at 60 °C. After cooling to ambient temperature the volume was brought to 250 ml.

Subsequently, the amount of glucose was determined using the Glucose kit, EnzyPlus of Diffchamb Biocontrol according to the manufacturer's protocol. Starch concentrations determined were compared to the total applied ATS concentration in the yoghurts.

2.9. Sensory evaluation

The yoghurt variants were evaluated by a panel consisting of professionally trained and paid sensory graders. In two instruction sessions the panel was trained and a list of sensory attributes was generated and discussed to reach consensus. The next sessions were used to evaluate the yoghurt variants. Each session included the 3% fat reference to monitor the panel performance and reproducibility. Samples tested were coded by arbitrary numbers (three digits) and were presented in random order. The taste panel ($N = 10$–12) scored each individual sample on the attributes agreed. All assessors were provided with their own individual yoghurt-cup taken directly from the refrigerator operating at approximately 10 °C. The temperature of the sensory evaluation room was constant at 20 °C.

The sensory software package FIZZ (Biosystems) was used to generate an acquisition session for the sensory trials. Using the computer, each panellist was able to score consecutive sensory attributes and could also provide free remarks for each individual sample. The FIZZ software was used to statistically analyse and export the data obtained. Typically, the sample sets were first analysed qualitatively by descriptive techniques like principle component analysis. In order to obtain quantitative data, analysis of variance statistics were executed using the calculation routines of the FIZZ software.

3. Results and discussion

3.1. Thermoreversibility of ATS

Composition and processing conditions may affect the gelation and melting properties of ATS and thereby its functionality. Therefore, the gel setting and thermoreversible melting of ATS was studied at different conditions. Fig. 2 shows that the gelation of a 5% ATS solution in water started after 12 h at 10 °C. Table 2 shows the results of the effect of calcium on gelation of a 5% (m/m) ATS solution in water at 5 °C. Increasing the calcium level decreased gelation time and reduced the occurrence of syneresis (from a volume fraction of 1–2% to almost no visually observable syneresis) of the gel formed.

Based on the above-described results it is postulated that the gelation of ATS is driven by the formation of crystalline regions and depends on the amount of crystalline regions still present after the dissolution step. These crystalline regions are likely formed by the elongated linear side chains of the amylopectin in ATS. ATS forms a particle gel with a small yield stress (results not shown). Gel setting time can thus be controlled by the efficacy of the dissolution step of ATS and is further dependent on the calcium concentration. The latter is of importance in dairy-based products that are relatively rich in calcium, such as yoghurt.

Gel setting and melting characteristics of ATS were compared with those of maltodextrin and gelatine as these are the common texturising ingredients applied in low-fat yoghurt. Moreover, gelatine and maltodextrin were selected as benchmark products for their difference in functionality. Gelatine forms a thermoreversible gel, whereas maltodextrin forms a particle gel. Clear differences between these three ingredients were observed (Fig. 2). At the concentration applied (5% m/m for all ingredients) maltodextrin did not form a gel (the storage modulus (G') of <0.01 Pa felt within the noise of the measuring equipment) and is therefore not included in Fig. 2. Both gelatine and ATS showed

Table 2
Appearance of 5% ATS solution in time as function of calcium concentration

Calcium concentration (mM)	Appearance (h)			
	0	16	24	40
0	Clear liquid	Opaque liquid	White gel with syneresis ++	White gel with syneresis +++
5	Clear liquid	White gel with syneresis ++	White gel with syneresis ++	White gel with syneresis ++
10	Clear liquid	White gel with syneresis +	White gel with syneresis +	White gel with syneresis +
20	Clear liquid	White gel with syneresis ±	White gel with syneresis +	White gel with syneresis +

Syneresis level ± (low), + (medium), ++ (high) and +++ (very high).

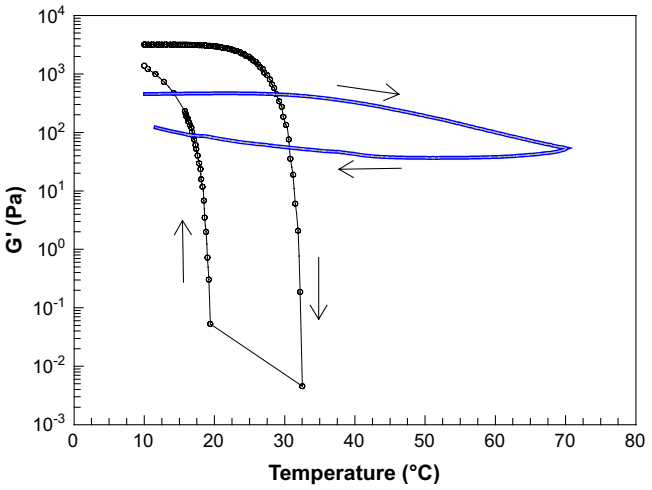

Fig. 3. Melting and gel setting behaviour of 5% m/m ATS (bold line) compared to 5% m/m gelatine (black symbols); heating and cooling rate: 1 °C/min (arrows indicate time course).

thermoreversible gelling behaviour, although clear differences were observed. The gel setting of gelatine is faster compared to that of ATS, instantaneous and 12 h, respectively. Moreover, the maximum gel strength (plateau value of G') for gelatine was reached within hours, whereas that of ATS was reached in days. This clearly indicates the difference in the gel network of these two gelling agents. Gelatine forms a polymer gel, whereas ATS forms a particle gel.

From a rheological point of view, a gel is defined by two criteria: (1) a storage modulus above 1 Pa and (2) the loss tangent below 1 ($\tan\delta = G''/G' < 1$) (Almdal, Dyre, Hvidt, & Kramer, 1993). Thus, a liquid is formed as either the $G' < 1$ Pa or the $\tan\delta > 1$. From this rheological point of view ATS did not form a liquid during the heating step, whereas gelatine did. The difference between the melting behaviour of gelatine and ATS was visualised in more detail by the G' vs. temperature curves (Fig. 3). Gelatine showed a sharp transition to a liquid upon heating, whereas the decrease in gel strength for ATS is gradual and much smaller. The decrease in gel strength of ATS started around 32 °C and continued to decrease at higher temperatures. Although, gelatine and ATS behave differently, they both show a lowering of the gel strength at a temperature that is relevant for the perception in the mouth. This decrease in gel strength is a factor for the perception of the yoghurts prepared with ATS. The difference in decrease in gel strength between gelatine and ATS is partly compensated by the degradation of ATS by amylase (see Section 3.3).

3.2. Microstructure of ATS-containing yoghurt

ATS was successfully incorporated into set-style yoghurts. Starch concentrations up to 5% (m/m) did not result in product flaws, such as syneresis. Thus, macroscopically homogeneous yoghurt variants were produced. The microstructure of these variants was investigated by confocal laser scanning microscopy (CLSM) and light microscopy (LM). Addition of ATS at different concentrations to milk prior to set-yoghurt production resulted in different microstructures of the set yoghurts as observed with CLSM (Fig. 4). In these CLSM images the protein phase is visible as bright areas, whereas the ATS cannot be visualised under these conditions. The microstructure of set yoghurt with maltodextrin (3% m/m) was similar to that of the reference, a homogeneous structure without phase separation (images not shown). In other words, no phase separation occurred during the preparation of the yoghurt in the presence of maltodextrin. With increasing concentration of ATS, coarsening of the protein microstructure was clearly observed (up to a concentration of 3.5%) showing the occurrence of a phase-separation process. Above 3.5% the microstructure is less coarse. Probably the phase-separation process is arrested in an earlier stage due to gelation.

Polymer physics theory predicts that any two types of polymers demix, either in bulk or in solution, unless the two polymer types show specific interactions. Phase separation also occurs in mixtures of protein and polysaccharide (Loren et al., 2001; de Jong & van de Velde, 2007). Therefore phase separation alone already induces formation of discrete domains of ATS in a protein phase. However, locally, within a domain, the concentration of ATS is higher than the minimum level to form a self-supporting gel (in serum >2.5%, see below). Hence these domains act as gel particles with the same melting characteristics as are found for a macroscopic gel of this starch. The gel is caused by small crystalline regions formed by the long branches of the amylopectin molecules that were elongated through the enzymatic treatment. These crystalline regions act as physical cross-links between the starch molecules. Melting is caused by thermal dissolution of these small crystalline domains. Possibly the formation of the small crystalline domains stimulates the phase separation: starch molecules are thermodynamically driven to form associates.

To prove this gelation hypothesis, the viscosity of different concentrations of ATS in yoghurt serum was measured. Below 2.5% (m/m) ATS the viscosity increased with increasing ATS concentration, whereas above 2.5% (m/m) ATS gelation will occur in the yoghurt. The microstructure observed is the result of the balance between phase separation and gelation. Most likely ATS forms a gel or gelled regions/domains within the yoghurt microstructure. If the presence of ATS during set-yoghurt production results in phase separation, the presence of ATS domains can be expected in addition to the observed coarser protein network.

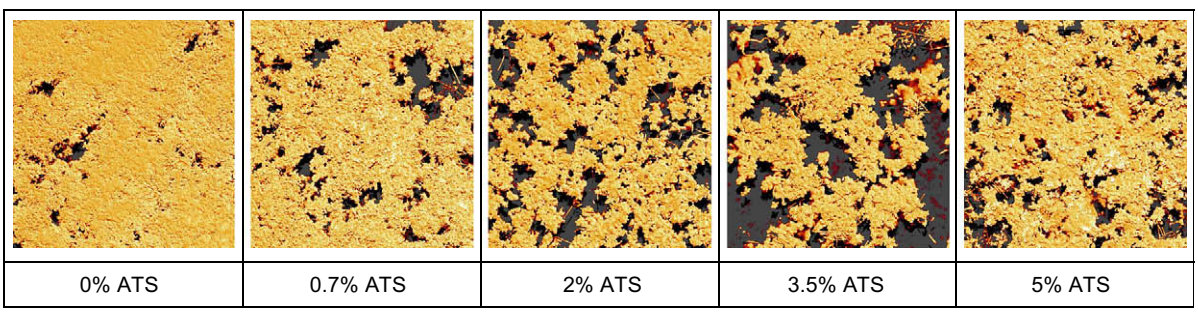

Fig. 4. CLSM images of set yoghurts (3.5% protein; 3% fat) with an increasing amount of ATS. Picture size 160 μm × 160 μm.

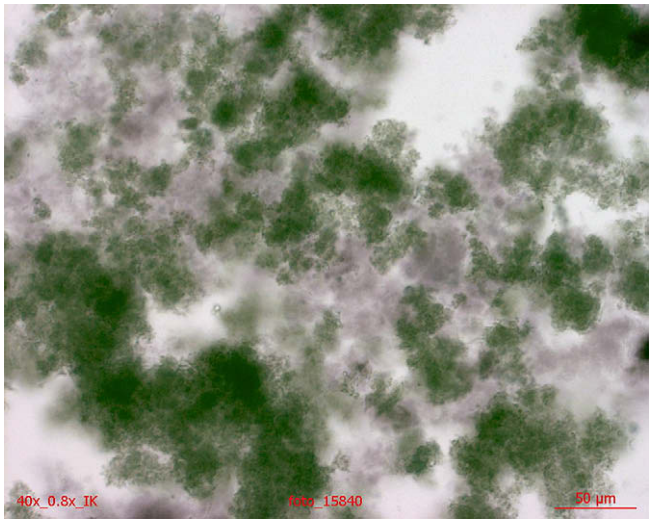

Fig. 5. Light microscopic image of set yoghurt with 0.7% ATS. ATS is stained with iodine (purple). The green areas correspond to the protein network.

Light microscopy in combination with starch staining was used to visualise and localise the ATS domains. Fig. 5 shows a microscopic image of a set yoghurt prepared of milk with 0.7% added ATS. It can be clearly observed that the ATS is localised in distinct areas (purple-stained with iodine). The protein phase appeared in green in these images. It was further observed that the size of these domains increased with increasing ATS concentration (images not shown) (5–25 µm). The ATS domains were enclosed in the protein network and did not move upon compression of the samples between the cover slips. In contrast, maltodextrin (purple-stained with iodine) flowed out with the serum. This indicated that the ATS domains are entrapped within the continuous protein phase, analogous to the protein–fat network in, for example, full-fat yoghurt and cheese, whereas the maltodextrin did not.

This entrapment of ATS domains in the protein network of set yoghurts was confirmed by the determination of the concentrations ATS in set yoghurt before and after centrifugation. Maltodextrin was used as a reference. ATS in set yoghurt was not found in the serum, irrespective of the concentration and time of centrifugation (Fig. 6). In other words, ATS is retained within the protein network. This is in contrast to maltodextrin, which ends up in the serum phase after centrifugation. In addition, the viscosity of the yoghurt serum was independent of the ATS concentration added to the milk prior to the yoghurt preparation (close to that of water).

Summarizing, microscopic images showed the presence of ATS domains entrapped in the continuous protein phase. This was confirmed by the results of the relatively low amounts of ATS in the yoghurt serum and the serum viscosity results (independent of initial ATS concentration). The results strongly indicate the presence of ATS domains. Moreover, it is likely that the ATS present in these domains is in a gelled state since in yoghurt serum already at 2.5% (m/m) gelation occurs. Gelled ATS domains entrapped in the continuous protein phase could behave like fat particles and therefore result in interesting sensory properties of yoghurt containing ATS. Therefore, set yoghurt with added ATS was investigated on its melting characteristics and sensory properties.

It is important to note that the observed ATS-induced phase-separation can result in large protein aggregates in case of higher ATS concentrations. As is discussed below, these protein aggregates can affect the sensory perception of ATS-containing food products such as set yoghurt.

3.3. Melting properties of ATS domains

The effect of temperature and α-amylase on firmness of ATS and gelatine gels is shown in Fig. 7. These force–temperature curves show that with increasing temperature a decrease in force was observed ("melting"). For the ATS gel, the addition of α-amylase led to a decrease of firmness. This effect can be explained by the degradation of the starch-based product by α-amylase. As expected, no effect of the addition of α-amylase was measured for gelatine (results not shown). This means that the ATS gel shows a "melting" behaviour, although with a different profile compared to gelatine. The gelled domains of ATS will melt in the mouth during oral processing and thereby may mimic the melting of fat globules.

3.4. Sensory properties of ATS-containing yoghurts

The sensory properties of ATS-containing yoghurt variants and references with different amounts of fat were analysed using a sensory panel. The focus of this analysis was on the mouth-feel or texture attributes. Therefore, plain yoghurts without added sugar

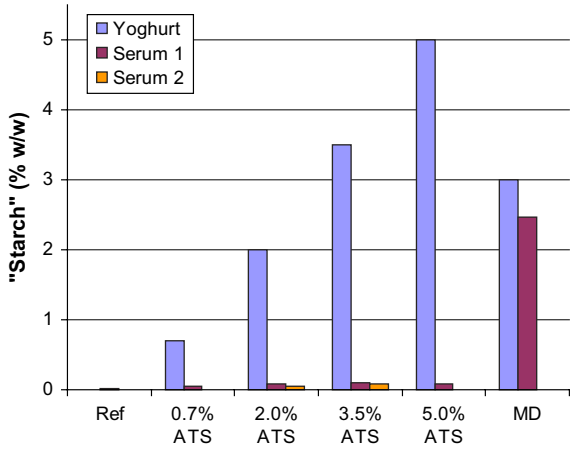

Fig. 6. Concentration ATS and maltodextrin (MD) in set yoghurt and in the serum after centrifugation for Serum 1: 10 min at 5000×*g* and for Serum 2: 18 h at 4000×*g*.

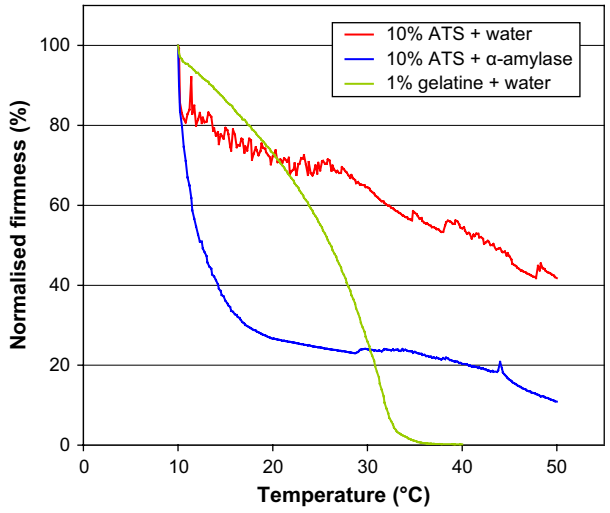

Fig. 7. The impact of shear, temperature and α-amylase on the firmness of the different gels.

Table 3
Sensory attributes and their definitions

English translation	Dutch term	Description
Jelly layer	Gelei laag	Occurrence of a jelly-like layer on top of the yoghurt
Serum layer	Water laag	Layer of serum on top the yoghurt
Shiny	Glimmend/glanzend	Shiny or glossy appearance of the yoghurt
Sour	Zuur	Sour taste
Creamy	Romig	Creamy texture
Thickness	Dikte	Thickness, firmness of the yoghurt
Smooth	Glad	Smooth
Bite	'Bite'	The tendency to bite the yoghurt (in contrast to palating)
Sticky	Plakkerig	The sample sticks to the oral cavity
Slippery	Glibberig	The sample easily slides through the oral cavity
Powdery	Poederig	Presence small particles, like flour
	Griezig	"Not to be translated" relates to the amount of small/medium sized particles present in the yoghurt
Mouth coating	Zalvig (film/sm)	A film of product is perceived in the oral cavity after swallowing
Dissolving	Oplosbaarheid	The speeds in which the samples disappear during mastication
Rough	Stroef	A rough feeling on the tongue and palate
Dry	Droog	A dry feeling in the mouth

The sensory panel is Dutch native speaking and for comprehensive understanding of sensory attribute names Dutch terms are preferably used. Translation of Dutch sensory attributes can be quite awkward; therefore we prefer to relate to Dutch terminology with respect to attribute names. Precise and exact English translation is sometimes rather difficult or even hardly possible.

and/or flavour were used. The list of attribute used throughout this study is given in Table 3.

Fig. 8 shows the result of the principal component analysis (PCA plot) of a series of yoghurt variants with a constant fat content (3% m/m) and an increasing amount of ATS. For comparison a sample containing 3% (m/m) maltodextrin (MD) was included. The two principal component axes can be distinguished. The first one ranging from "Thickness" to "Dissolving" is the dominant axis, which explains 82% of the variance in the sample set. The second axis (14% explained variance) ranged from "Powdery" to "Creaminess". With increasing ATS concentration the perceived thickness of the yoghurt increased, thus the samples moved from right to left in the PCA plot. This is the main shift in the sensory perception of the yoghurts. Next to the firmness of the yoghurts, also the creaminess increases with increasing ATS concentration showing an optimum at 2% (m/m) ATS. At higher concentrations the creaminess decreases and the roughness increases. This increase in roughness of the yoghurt samples coincided with the coarse microstructure as observed by CLSM (Fig. 4). Thus, the creaminess of the yoghurt variants containing 0.7 and 2% ATS is larger than the reference with 3% fat without added thickener. The benchmark sample containing 3% maltodextrin (MD) showed a mouth-feel comparable to that of the variant containing 0.7% (m/m) ATS. Thus, ATS is more efficient to improve the mouth-feel of set-style yoghurt.

As discussed in the introduction, nowadays, fat replacement is an important issue. Creaminess is the dominant attribute in the overall liking of low-fat yoghurt. Therefore, the impact of different concentration of ATS on this attribute was studied in more detail. Fig. 9 shows the relation between the ATS concentration and the creaminess perception of yoghurt variants containing different amounts of fat. Three reference yoghurts containing 3, 5 or 10% fat (m/m) are included in this graph. The impact of ATS on the creaminess perception of set-style yoghurt is clearly shown for yoghurts with different fat levels, although the impact of ATS is larger in yoghurt with a relatively small amount of fat (1.5–3%) than for zero-fat yoghurt. The optimum in the creaminess perception of the yoghurts containing 3% (m/m) fat is in agreement with the trend shown in the PCA plot (Fig. 8). A similar optimum is observed for the yoghurts containing 5% (m/m) fat. It seems that the concentration at which this optimum occurs depends on the fat concentration. Concluding, ATS is an efficient creaminess enhancer in a whole range of yoghurt variants containing different amounts of fat. However, a small amount of fat is profitable for an optimum creaminess perception, as ATS does not replace the lubricating properties of fat.

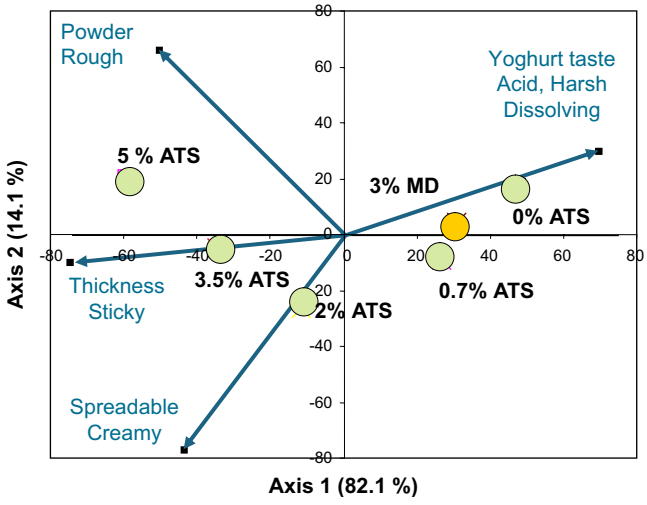

Fig. 8. PCA plot of the sensory properties of series of set-style yoghurt containing 3% fat and an increasing amount of ATS.

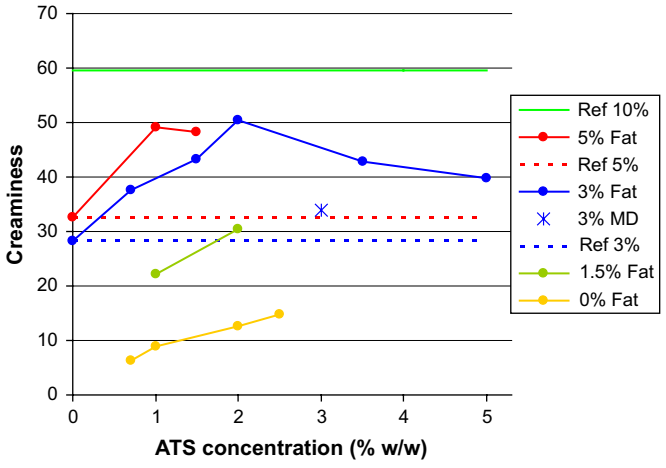

Fig. 9. Creaminess (expressed as the QDA-scores on a 0–100 scale) of set-style yoghurts having different fat and ATS contents (MD: maltodextrin).

4. Conclusions

Sensory analysis of different set-style yoghurt variants containing amylomaltase-treated starch (ATS) showed that ATS is a creaminess enhancer. Small amounts of ATS raised the creaminess perception of low-fat yoghurt to that of full-fat yoghurt. In yoghurts containing 3% (m/m) fat, ATS is four times as effective as maltodextrin, which is a current fat replacer in set-style yoghurt. The functionality of ATS in set-style yoghurt resulted from discrete domains of ATS as observed by light microscopy in combination with iodine staining. The microstructure of the yoghurt is dominated by the protein phase as observed by confocal microscopy. The ATS domains are enclosed in or bound to this protein network and did not appear in the serum phase after extensive centrifugation. In contrast, maltodextrin is not enclosed in the protein phase and was completely found in the yoghurt serum after centrifugation. The perceived creaminess resulted from in-mouth melting of these ATS domains due to a combined effect of their physical melting (thermoreversible behaviour of ATS) and hydrolysis by amylase present in the saliva during mastication. In this way the ATS domains resemble the microstructural behaviour of fat particles. Since polysaccharides have a lower energy value than fats (conversion factor of 9 for fat and 4 for polysaccharides, Kriketos, Peters, & Hill, 2000), replacing 3.5% fat in a 5% fat-yoghurt with 2% ATS will result in a reduction of the fat-related energy value from 45 to 21.5 kcal/100 g.

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