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Starch as a potential fat replacer for application in cheese: Behaviour of different starches in casein/starch mixtures and in the casein matrix

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ABSTRACT

The behaviour of different types of native starch (regular, waxy, and high-amylose maize starch) and modified starch (acetylated/adipate maize starch with regular basis and hydroxypropylated/phosphate maize starch with waxy basis) in mixtures with calcium caseinate (CN) and embedded in casein matrix were studied to understand the potential use of starch as a fat replacer in fresh cheese. In mixtures with CN, the modified starches showed high viscosities (peak and final), indicating high potential for water retention in cheese, as well as low peak temperature and low variation of enthalpy (ΔH), requiring thereby low gelatinisation temperatures. They also showed low tendency for retrogradation, high swelling power, and good thermomechanical resistance. Furthermore, due to the very low loss in whey of both modified starches and their presence embedded in the protein matrix acting as inert fillers, they can be considered as promising fat replacers in cheese.

1. Introduction

Experts around the world recommend a reduced-fat intake to obtain health and aesthetic benefits. However, fat has a key role in food creaminess, appearance, aroma, tenderness and juiciness. In cheese, the removal of fat will mainly affect its texture, resulting in a product with a "rubbery" texture – more elastic and firm – besides lower yield of cheese production and atypical flavours (O'Connor & O'Brien, 2011; Rosenthal, 2003).

Fat replacers are ingredients that can be incorporated into food products, for total or partial replacement of fat, but with fewer calories. Among them, starch is largely used due to its low cost and high availability (Mistry, 2001; O'Connor & O'Brien, 2011).

Starches present a particularity when heated in an excess of water, they swell by binding water by the disruption of the molecular organisation within the granule. After cooling, intermolecular interactions involving amylose and amylopectin molecules occur, increasing the medium viscosity and forming a gel. Nevertheless, different behaviours during the heating and cooling processes are normally shown by differences in the composition of the different types of starch; such differences affect the characteristics of gels (Damodaran, Parkin, & Fennema, 2008; Kett et al., 2013; Tárrega, Vélez-Ruiz, & Costell, 2005).

Regular maize starches normally contain between 25% and 28% amylose and produce consistent gels after cooking. Waxy starches generally contain little or no amylose, and produce weak, clear and sticky gels with a high peak viscosity and low retrogradation tendency. Finally, high-amylose starches normally contain more than 50% amylose (reaching up to 70%), and present high gelatinisation temperatures and capacity of forming films (BeMiller & Whistler, 2009; Parker & Ring, 2001).

Some types of modifications can be made in native starches to improve the gel characteristics. Many of these modifications are applied to reduce the retrogradation tendency of pastes and to improve their resistance to heat, shear, and acid conditions. Starches that have been both crosslinked and stabilised (e.g., hydroxypropylated distarch phosphate, and acetylated distarch adipate) usually have lower gelatinisation and pasting temperatures, produce pastes with higher viscosity values and show increased thermomechanical resistance. Modified food starches are often both crosslinked and stabilised (Damodaran et al., 2008; Tárrega et al., 2005).

Due to their natural property of binding extra water, starches may be used as fat replacers as they can create a sense of lubricity

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similar to that of full-fat products (Sipahioglu, Alvarez, & Solano-Lopez, 1999). In reduced-fat fresh cheese, starches may act improving texture due to their high-water retention capacity (Brown, McMannus, & McMahon, 2012). Furthermore, starch is considered a permitted ingredient to be applied in fresh cheeses according to the Codex Standard 221–2001 (Codex Alimentarius, 2013). The concentrations of starch frequently used as fat replacer in cheese normally ranges from 0.5 to 1.5% (Aryana & Haque, 2001; Diamantino, Beraldo, Sunakozawa, & Penna, 2014; Kavas, Oysun, Kinik, & Uysal, 2004; Sipahioglu et al., 1999). Diamantino et al. (2014) reported that even though the addition of a modified waxy maize starch increased the moisture content and water holding capacity of a reduced-fat fresh cheese, its addition was insufficient to improve the yield of production and the texture parameters of a fresh cheese. The authors suggested as possible causes the loss of starch in whey or the amount of starch used (0.5%) that could be insufficient. On the other hand, other authors have suggested a possible interaction of starch with casein, either by the formation of cross-linkings between starch and casein molecules (Goel, Singhal, & Kulkarni, 1999) or by the simple mechanical retention of the swollen starch in the casein matrix, acting as a filler material (Noisuwan, Broland, Wilkinson, & Hemar, 2008; Ye & Hewitt, 2009; Zuo, Hemar, Hewitt, & Saunders, 2008).

Although starches and milk proteins, separately, have been extensively studied, there have been few studies on the microstructural characteristics, gelatinisation, pasting, rheological properties, and other physico-chemical properties of casein/starch mixtures (Considine et al., 2011; Goel et al., 1999; Kett et al., 2013; Noisuwan et al., 2008; Sun, Liang, Yu, Tan, & Cui, 2016). Furthermore, to the best of our knowledge, there are no studies on the properties of starches in the presence of casein, aiming to understand their behaviour and potential functionality as a fat replacer in cheese.

Therefore, our study investigated the behaviour of different types of native and modified starches in dispersions with calcium caseinate and in the casein matrix to evaluate their potential use as fat replacers in cheese.

2. Material and methods

2.1. Materials

Commercial maize starches were supplied by Cargill Starches and Sweeteners South America (São Paulo, SP, Brazil). Three types of pure native starch (regular maize starch – RMS, waxy maize starch – WMS, and high-amylose maize starch – HAMS) and two types of modified pure starch (acetylated/adipate maize starch with regular basis – AAMS, and hydroxypropylated/phosphate maize starch with waxy basis – HPMS) were used in this study. The calcium caseinate (CN) was Caseical™ (pure calcium caseinate) obtained from Danone, Medical Nutrition Division (São Paulo, SP, Brazil).

2.2. Study of starch properties in the presence of calcium caseinate

2.2.1. Preparation of calcium caseinate/starch mixtures

To evaluate the properties of the five different starches in the presence of casein, dispersions of CN/starch were produced. To simulate the concentrations of fat replacers frequently used in cheese (0.5, 1.0 and 1.5%), mixtures of CN and starch in the proportions of 2.5:0.5, 2.5:1.0, and 2.5:1.5 (CN:starch) were produced, taking into account that starch will be gelatinised in milk and the normal level of casein in 100 g milk is about 2.5 g. In addition, dispersions of each pure starch and pure CN were produced as controls, totalling 21 trials. Maintaining these proportions of CN:starch, the concentrations of the solutions varied for each test and are described in the subsequent sections below.

2.2.2. Pasting properties

The pasting properties (peak, breakdown, setback and final viscosities and pasting temperature) of CN/starch dispersions were determined using a Rapid Visco-Analyzer (RVA-4; Newport Scientific, Warriewood, Australia). CN/starch dispersions in deionised water (20%, w/w, i.e., 2.5 g sample per 12.5 g water) and pure CN and starch samples (10%, w/w, i.e., 2.5 g sample per 25 g water) were prepared in RVA aluminium canisters. Plastic stirring paddles were placed in the canisters containing the samples, and this system was attached to the RVA. A heating and cooling cycle method commonly used for starch pasting studies was applied. The samples were stirred at 960 rpm and heated to 50 °C, then the stirring was decreased to 160 rpm for the rest of the analysis. The samples were then heated from 50 to 95 °C at a rate of 6 °C min^{-1}, and the temperature kept at 95 °C for 5 min. Then, samples were cooled to 50 °C at a rate of 6 °C min^{-1}, totalling 23 min of analysis (adapted from Chaisawang & Suphantharika, 2006; Putseys, Lamberts, & Delcour, 2010; Tárrega et al., 2005). The results were analysed through Thermocline for Windows software (version 2.2, Newport Scientific) using the STD2 (Standard 2) thermal treatment profile. All the analyses were carried out in triplicate.

2.2.3. Thermal properties

The thermal properties of the CN/starch dispersions were determined using a differential scanning calorimeter (DSC; Pyris1, Perkin Elmer Co., Waltham, MA, USA). CN/starch dispersions were prepared following the concentrations described above in section 2.2.1. Each sample (i.e., pure CN and starch, and their mixtures) was weighed (2 mg in dry basis) into aluminium pans and 6 mL deionised water was added. The pans were hermetically sealed and kept at room temperature for 24 h to equilibrate the distribution of water. The samples were scanned at a rate of 10 °C min^{-1} at temperatures ranging from 25 °C to 125 °C. An empty pan was used as reference. The gelatinisation endotherm as well as the gelatinisation temperatures: onset (To), peak (Tp) and conclusion (Tc), and the gelatinisation enthalpy (ΔH_{gel}) were obtained in triplicate using Pyris 1 software (Perking Elmer) (Villas-Boas & Franco, 2016). After the analysis, the pans were stored at 5 °C for 7 d and the thermal properties related with starch retrogradation were evaluated using the same equipment and parameters. The percentage of retrogradation (%R) was calculated as the ratio between the retrogradation enthalpy (ΔH_{ret}) and the gelatinisation enthalpy (ΔH_{gel}) multiplied by 100 (Sandhu & Singh, 2007).

2.2.4. Swelling power

Swelling power of starches, CN and CN/starch dispersions were determined in duplicate following a modification of the methods of Mandala and Bayas (2004) and Chaisawang and Suphantharika (2006). Starch, CN and CN/starch dispersions (1%, w/w, i.e., 0.2 g sample in 20 g distilled water) were put into centrifuge tubes with closed screw caps and heated in a shaking water bath for 30 min, at different temperatures: 55, 65, 75, 85 and 95 °C. After heating, the tubes were kept at room temperature until they reached approximately 25 °C and were then centrifuged at 3000× *g* at 25 °C for 15 min. The precipitated gel was weighed, and the swelling power was calculated as the ratio of the precipitated gel weight to the weight of its sample (dry basis). The analyses were carried out in duplicate.

2.3. Study of starch interaction with casein matrix

2.3.1. Preparation of curd samples

Curd samples were prepared using the modified starch samples as they presented high viscosity values (peak and final), indicating high potential for water retention in cheese, as well as lower peak

temperatures and lower variation of enthalpy (ΔH), requiring thereby lower gelatinisation temperatures, which is important for the adequate coagulation of cheese. Curd samples were produced in centrifuge tubes (50 mL), according to an adaption of the method of Brown et al. (2012), using the AAMS and HPMS samples at 1.5%. Starch samples (1.5 g 100 mL^{-1}) were dispersed in cold standardised milk (1.5% fat) and then heated to 70 °C for 1 min to allow the starch samples to become gel, without hardly affecting their whey proteins.

The milk with gelatinised starch was cooled to 35 °C. A control sample without starch was also prepared. After reaching 35 °C, the following ingredients were added: 0.025 g 100 mL^{-1} calcium chloride (Synth, Labsynth Lab Products, Diadema, SP, Brazil), 0.2 mL 100 mL^{-1} starter culture (R-704, 50 U; Christian Hansen, Hørsholm, Denmark) composed of *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* strains, diluted in ultra high temperature (UHT) skim milk, and 2.5 g 100 L^{-1} chymosin (Chy-max; Christian Hansen) previously diluted in water. The ingredients were stirred for 2 min, and coagulation was allowed to proceed for 45 min. Then, the gel was cut with a spatula vertically with three cuts in each of two perpendicular directions. The tubes containing the gels were centrifuged at 500× *g* at 25 °C for 15 min, followed by another centrifugation at 1000× *g* at 25 °C for 15 min. The whey released was collected and stored in freezer (−80 °C) for its later use in the starch content analysis, while the centrifuged curd (pellet) was maintained at 5 °C for its later use in the microstructural analysis.

2.3.2. Microstructural analysis

The curd samples obtained after centrifugation (pellets) were used for microstructural analysis using laser scanning confocal microscopy (LSCM), according to the methods of Van de Velde, Weinbreck, Edelman, Van der Linden, and Tromp (2003) and Brown et al. (2012) with some modifications. The curd was diced, measuring approximately 0.5 cm on each side, and frozen in liquid nitrogen for 10 min. The frozen curd samples were cut (1 mm-thick slices) at −8 °C with a cryostat equipment (Leica CM1850, Leica Biosystems, Oberkochen, BW, Germany) and directly mounted on glass slides. The samples were fixed in a solution of 4% paraformaldehyde and 4% glutaraldehyde in 0.1 mM phosphate buffer for 5 min, and subsequently washed with water. Then, the samples were stained with 0.1% eosin for 2 min, washed with 95% ethanol (twice), and subsequently stained with 0.1% fluorescein 5-isothiocyanate (FITC) for 10 min, in absence of light. A droplet of water was added to each sample and they were covered with a coverslip. The slides were maintained protected from light, until their visualisation through the microscope (Zeiss LSM 710 confocal microscope; Carl Zeiss AG, Oberkochen, BW, Germany). The observations were carried out in 10× and 40×, and the images were analysed using Zen 2010 software (Carl Zeiss AG).

2.3.3. Starch content analysis

Starch content in whey was measured in duplicate by liquid chromatography quantification of glucose using a high-performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD) system (ICS 3000, Dionex Corporation, Sunnyvale, PA, USA) equipped with an AS40 automatic sampler (adapted from Casarotti, Monteiro, Moretti, & Penna, 2014). The samples preparations were as follows: HCl was added to 25 mL of whey released from each curd sample (with AAMS, with HPMS, and without starch) to a final concentration of 10% and heat-treated at 120 °C for 20 min in autoclave to disrupt glycosidic linkages. The samples were then centrifuged (10,000× *g* for 15 min) and their supernatant was collected.

The supernatant from each sample was filtered through membrane filters with 0.22 μm pore diameters (Millipore®, Darmstadt, Germany) and injected into a HPAEC-PAD system (20 mL sample loop). Separation was achieved using a Dionex CarboPac™ PA1 guard column (250 × 2 mm) and a Dionex CarboPac™ PA1 column (250 × 2 mm). The flow rate was 1.0 mL min^{-1} at 30 °C. All eluents were prepared with ultrapure water (18 MΩ cm) with N_2 sparging. Eluent A was 200 mM NaOH, and eluent B was 1.0 M sodium acetate and 100 mM NaOH. The gradient elution was as follows: linear gradient from 0 to 24 mM NaOH (0–14 min), linear gradient from 24 to 107 mM NaOH and 0–70 mM sodium acetate (14–25 min), and constant concentration of 24 mM sodium hydroxide (25–30 min).

The quantification of starch in the samples was carried out by multiplying the glucose content by 0.9 (conversion factor for glucose to starch). Previously, the glucose content of AAMS and HPMS whey samples had been deducted from the glucose content of the control sample to eliminate the quantification of glucose resulting from lactose hydrolysis. The starch retention in curd was calculated by the ratio between the amount of starch that remained in cheese and the total starch added in curd, multiplied by 100.

2.4. Statistical analysis

Analyses of variance (ANOVA) were performed to evaluate the significant differences among data. Tukey's multiple comparison test was used to compare the mean values. A significance of 5% was used to establish statistical difference. The statistical analyses were conducted using Statistica 7.0 program (StatSoft, Inc., 2004, Tulsa, OK, USA).

3. Results and discussion

3.1. Study of starch properties in the presence of calcium caseinate

3.1.1. Pasting properties

In general, the properties of starch gels were affected by the concentrations of starch in the dispersions (Fig. 1, Table 1). In most of the samples, low concentrations of starch in the mixture resulted in lower viscosity values (peak, breakdown, final and setback), probably due to the dilution effect of starch. However, some differences were also observed, mainly for pasting temperatures and peak viscosities, which may be attributed to an interaction between the different types of starch and CN.

The high amylose starch (HAMS) did not present viscosity over the experiment; neither for any of the mixtures with CN, nor for the pure starch sample (10%, w/w) (Fig. 1E). The high amylose content of the HAMS (more than 50%) generates a densely packed molecular structure, resulting in low molecular mobility and, therefore, in high gelatinisation temperatures, usually close to 100 °C (see DSC results in Table 2) (Copeland, Blazek, Salman, & Tang, 2009; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005). Such high temperatures are impossible to be achieved when using the RVA equipment, which reaches a maximum temperature of 95 °C. Consequently, the HAMS was certainly not gelatinised under the heating conditions of the RVA, generating very low viscosities; additionally, it was not affected by the presence of CN in the mixtures.

Among the studied starch samples, with exception of the HAMS, the regular maize starch (RMS) presented the highest pasting temperatures (Table 1). Higher pasting temperatures indicate higher starch gelatinisation resistance caused by high structural stability. The lower the temperature, the easier the cooking, and subsequently, the gelatinisation of starch (Copeland et al., 2009; Tester & Morrison, 1990). A high structural stability is expected for regular maize starches due to their higher amylose content (when compared with waxy starches) and high lipid content. The natural presence of lysophospholipids in maize starch forms

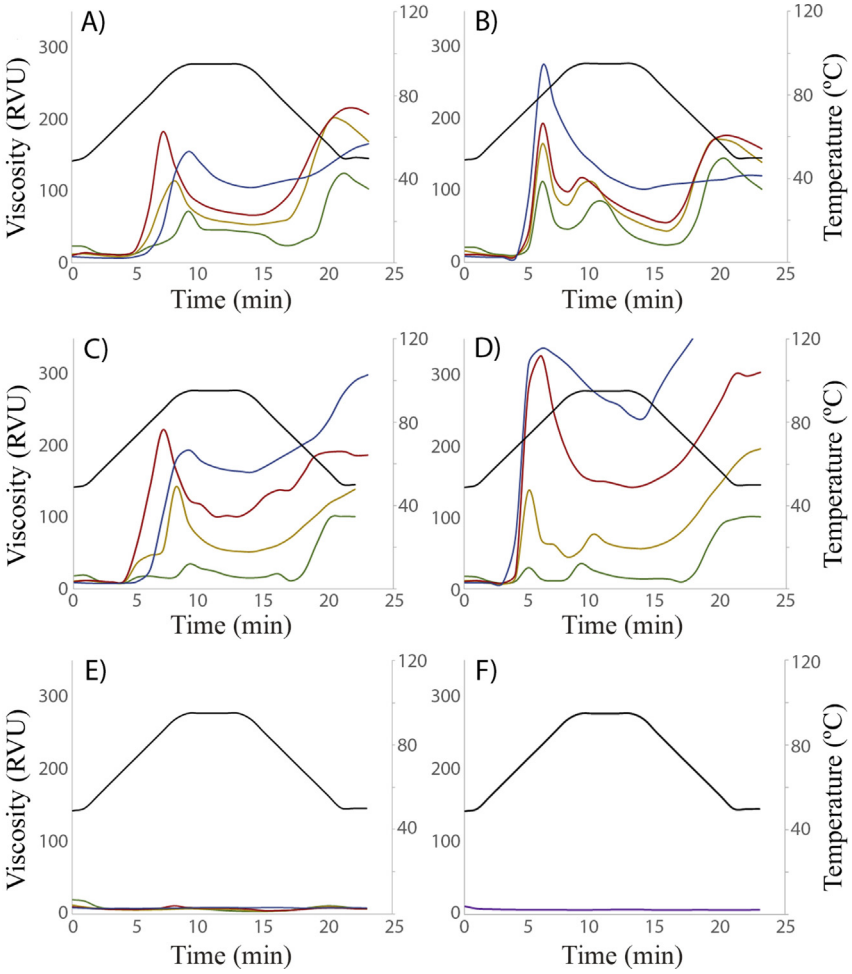

Fig. 1. Pasting profile of calcium caseinate (CN)/starch dispersions. Samples are CN in mixtures with: regular maize starch – RMS (A), waxy maize starch – WMS (B), acetylated/adipate maize starch with regular basis – AAMS (C), hydroxypropylated/phosphate maize starch with waxy basis – HPMS (D), and high-amylose maize starch – HAMS (E), in the proportions of 2.5:0.5 (—), 2.5:1.0 (—), 2.5:1.5 (—) CN:starch in 20%, w/w (2.5 g sample/12.5 g water). Samples are also each pure starch (—) and (F) pure CN (—) in 10%, w/w (2.5 g sample/25 g water); Temperature (—).

complexes with amylose and the very long branched chains of amylopectin, resulting in lower swelling power and lower heat paste viscosity (Putseys et al., 2010; Singh, Singh, Kaur, Sodhi, & Gill, 2003), as observed in this study by the low peak viscosity values of the RMS starch samples.

However, for the mixtures whose starch concentrations are very low when compared with pure starch dispersion, the pasting temperatures were very close to the pure starch sample (at 10%, w/w, solids in water) for 1% of starch addition (2.5:1.0 CN:starch at 20%, w/w, solids in water). For 1.5% starch addition (2.5:1.5 CN:starch at 20%, w/w, solids in water), the pasting temperatures were even lower, overcoming the dilution effect of starch that normally increases pasting temperature. This effect may be attributed to the presence of CN. According to Goel et al. (1999), starch pastes are suspensions of swollen particles dispersed in a macromolecular medium. It can be considered that proteins are located within the continuous phase, and thus the volume of the phase accessible to the proteins is reduced; this causes an increase in the continuous medium concentration, which results in a high viscosity. The swollen particles are mainly composed of amylopectin, while the continuous medium consists of amylose. It is probably the amylose interaction with casein that dominates the system. This theory may explain the highest peak viscosity of the sample with 1.5% starch addition, as well as its lowest pasting temperatures, when compared with the other RMS samples in the present study.

Although the acetylated/adipate maize starch (AAMS) has a regular maize starch as its base, its properties significantly changed due to its modifications. The AAMS is simultaneously a crosslinked and a stabilised starch and presents properties of these two types of modification. This type of starch generally has lower pasting temperatures, higher peak viscosity, and a reduced tendency of retrogradation, compared with a native regular starch (Damodaran et al., 2008). These properties corroborate with the results found for the AAMS samples (Table 1; Fig. 1C) and are important characteristics for cheese production. Lower pasting temperatures are desirable, since high thermal treatments negatively affect the coagulation process. At temperatures above 75 °C at the pH of milk (~6.5), whey proteins can participate in sulphydryl-disulphide interchange reactions that lead to the formation of disulphide-linked complexes with casein fractions, thus causing profound effects on the functionality of the milk protein system, disturbing rennet coagulation (Fox & McSweeney, 1998). Additionally, the same tendency observed for RMS of increase in peak viscosity and reduction in pasting temperature was observed for the sample with 1.5% starch addition, probably due to the interaction of CN with amylose leached from the starch granules.

Table 1
Pasting properties of starch, CN and CN/starch dispersions.

Samples	Pasting temperature (°C)	Viscosity (RVU)			
		Peak	Breakdown	Final	Setback
RMS	85.85^{b} ± 0.26	151.47^{b} ± 1.18	51.11^{d} ± 2.39	161.53^{b} ± 0.12	161.17^{a} ± 2.24
CN + 0.5%RMS	92.83^{a} ± 0.02	82.69^{d} ± 2.43	61.05^{c} ± 0.85	98.59^{c} ± 1.90	76.95^{d} ± 0.32
CN + 1.0%RMS	84.48^{b} ± 0.04	138.12^{c} ± 3.16	87.59^{b} ± 2.14	160.86^{b} ± 3.80	110.33^{c} ± 2.79
CN + 1.5%RMS	74.40^{c} ± 0.00	180.96^{a} ± 1.50	116.56^{a} ± 1.58	196.42^{a} ± 5.38	132.01^{b} ± 5.29
WMS	71.58^{c} ± 0.02	261.27^{a} ± 0.57	166.76^{b} ± 0.08	116.11^{c} ± 0.00	21.60^{c} ± 0.49
CN + 0.5%WMS	75.00^{a} ± 0.20	110.97^{d} ± 0.45	86.70^{d} ± 0.61	103.04^{d} ± 1.98	78.77^{b} ± 2.14
CN + 1.0%WMS	73.20^{a,b} ± 0.00	196.54^{c} ± 1.13	150.50^{c} ± 1.29	140.83^{b} ± 1.82	94.79^{a} ± 1.66
CN + 1.5%WMS	72.35^{b} ± 0.00	232.30^{b} ± 2.18	178.01^{a} ± 2.35	154.14^{a} ± 0.40	99.85^{a} ± 0.57
AAMS	74.80^{c} ± 0.00	187.76^{b} ± 3.36	31.44^{c} ± 1.42	286.68^{a} ± 4.04	130.35^{a} ± 2.10
CN + 0.5%AAMS	90.75^{a} ± 0.05	34.23^{d} ± 0.65	23.14^{d} ± 0.32	95.11^{d} ± 1.42	84.03^{c} ± 1.09
CN + 1.0%AAMS	88.28^{b} ± 1.03	138.56^{c} ± 3.28	88.72^{b} ± 2.14	148.64^{c} ± 4.21	98.80^{b} ± 5.34
CN + 1.5%AAMS	70.73^{c} ± 0.02	236.96^{a} ± 2.95	140.51^{a} ± 2.55	170.73^{b} ± 1.94	74.28^{d} ± 2.35
HPMS	65.55^{b} ± 0.00	327.94^{b} ± 0.89	93.78^{b} ± 5.17	483.82^{a} ± 2.14	249.66^{a} ± 2.14
CN + 0.5%HPMS	63.58^{c} ± 0.04	37.58^{d} ± 0.04	28.85^{d} ± 0.45	100.62^{d} ± 4.57	91.88^{d} ± 4.17
CN + 1.0%HPMS	68.35^{a} ± 0.00	136.58^{c} ± 4.29	83.62^{c} ± 2.47	194.19^{c} ± 4.53	141.24^{c} ± 2.71
CN + 1.5%HPMS	67.55^{a,b} ± 0.00	342.91^{a} ± 3.16	204.02^{a} ± 4.33	296.91^{b} ± 1.09	158.02^{b} ± 2.27
HAMS	–	7.98^{c} ± 0.45	0.65^{c} ± 0.29	7.71^{a} ± 0.23	0.38^{c} ± 0.17
CN + 0.5%HAMS	–	9.01^{b} ± 0.17	6.04^{a} ± 0.36	6.8^{b} ± 0.43	3.83^{a} ± 0.28
CN + 1.0%HAMS	–	7.31^{c} ± 0.66	3.99^{b} ± 0.57	6.53^{b} ± 0.45	3.21^{a,b} ± 0.09
CN + 1.5%HAMS	–	10.80^{a} ± 0.04	6.72^{a} ± 0.08	6.92^{b} ± 0.20	2.83^{b} ± 0.08
CN	–	8.34 ± 1.02	1.24 ± 0.36	7.55 ± 0.59	0.40 ± 0.14

Values are the mean of three replications ± standard deviation; different superscript letters in the same column for each sample differ significantly ($p \leq 0.05$). Samples are calcium caseinate (CN) in mixtures with: regular maize starch (RMS), waxy maize starch (WMS), acetylated/adipate maize starch with regular basis (AAMS), hydroxypropylated/phosphate maize starch with waxy basis (HPMS), and high-amylose maize starch (HAMS), in the proportions of 2.5:0.5, 2.5:1.0, and 2.5:1.5 CN:starch (20%, w/w; 2.5 g sample/12.5 g water). Samples are also pure starch dispersions of each starch as well as pure CN dispersion (10%, w/w; 2.5 g sample/25 g water).

Table 2
Gelatinisation temperatures: onset (T_o), peak (T_p), and conclusion (T_c), and gelatinisation enthalpy (ΔH_{gel}) of starch samples, CN and CN/starch dispersions.

Samples	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J g^{-1})
RMS	65.61^{c} ± 0.23	70.05^{c} ± 0.29	73.63^{b} ± 0.51	14.41^{a} ± 0.41
CN + 0.5%RMS	67.43^{a} ± 0.00	71.39^{a} ± 0.00	75.02^{a} ± 0.13	3.04^{d} ± 0.03
CN + 1.0%RMS	67.11^{a} ± 0.21	71.06^{a,b} ± 0.29	75.00^{a} ± 0.03	3.89^{c} ± 0.19
CN + 1.5%RMS	66.60^{b} ± 0.09	70.81^{b} ± 0.09	75.70^{a} ± 0.10	4.77^{b} ± 0.28
WMS	65.66^{b} ± 0.30	71.11^{b} ± 0.26	76.13^{b} ± 0.34	17.04^{a,b} ± 0.88
CN + 0.5%WMS	67.88^{a} ± 0.53	72.65^{a} ± 0.09	76.98^{a,b} ± 0.15	3.51^{b,c} ± 0.13
CN + 1.0%WMS	67.79^{a} ± 0.65	72.72^{a} ± 0.01	77.85^{b} ± 1.06	4.41^{c} ± 0.25
CN + 1.5%WMS	67.33^{a} ± 0.27	72.55^{a} ± 0.17	76.95^{a,b} ± 0.61	5.60^{d} ± 0.14
AAMS	61.92^{a} ± 0.67	67.81^{a} ± 0.25	72.46^{b} ± 0.26	14.04^{a} ± 0.20
CN + 0.5%AAMS	61.58^{a} ± 0.29	68.04^{a} ± 0.16	72.15^{a,b} ± 0.62	2.58^{d} ± 0.22
CN + 1.0%AAMS	61.87^{a} ± 0.31	68.15^{a} ± 0.19	73.23^{a,b} ± 0.41	3.81^{c} ± 0.51
CN + 1.5%AAMS	62.74^{a} ± 0.90	68.37^{a} ± 0.33	73.56^{a} ± 0.61	6.17^{b} ± 0.02
HPMS	61.65^{b} ± 0.10	66.73^{b} ± 0.01	71.53^{a} ± 0.02	13.78^{a} ± 0.20
CN + 0.5%HPMS	62.66^{a} ± 0.05	67.30^{a} ± 0.08	71.70^{a} ± 0.68	2.70^{d} ± 0.08
CN + 1.0%HPMS	62.54^{a} ± 0.12	67.48^{a} ± 0.26	72.09^{a} ± 0.30	3.45^{c} ± 0.25
CN + 1.5%HPMS	62.54^{a} ± 0.07	67.38^{a} ± 0.17	72.49^{a} ± 0.45	4.68^{b} ± 0.14
HAMS	67.46^{a} ± 0.96	89.45^{a} ± 0.00	102.68^{a} ± 0.02	10.73^{a} ± 0.35
CN + 0.5%HAMS	68.13^{a} ± 0.19	89.36^{a} ± 0.09	95.38^{c} ± 0.20	0.75^{d} ± 0.04
CN + 1.0%HAMS	68.90^{a} ± 1.40	89.46^{a} ± 0.00	102.36^{a} ± 1.07	2.30^{c} ± 0.12
CN + 1.5%HAMS	69.39^{a} ± 0.50	89.01^{a} ± 0.35	98.56^{b} ± 1.76	3.29^{b} ± 0.33
CN	–	–	–	–

Values are the mean of three replications ± standard deviation; different superscript letters in the same column for each sample differ significantly ($p \leq 0.05$). Samples (2 mg sample/6 mL deionised water) are calcium caseinate (CN) in mixtures with: regular maize starch (RMS), waxy maize starch (WMS), acetylated/adipate maize starch with regular basis (AAMS), hydroxypropylated/phosphate maize starch with waxy basis (HPMS), and high-amylose maize starch (HAMS) in the proportions of 2.5:0.5, 2.5:1.0, and 2.5:1.5 CN:starch. Samples are also pure starch dispersions of each starch as well as pure CN dispersion.

Waxy maize starch (WMS) samples presented low pasting temperatures, higher peak viscosity and lower setback viscosity values when compared with RMS samples (Fig. 1B), since waxy starch is almost entirely composed of amylopectin molecules (Jane, 2007). Amylopectin promotes a higher swelling of the starch and, consequently, higher peak viscosity values due to the high quantity of available hydroxyl groups in its branched chains. In addition, lower setback viscosity values are observed because they are related to the amylose retrogradation, which provides the cold paste firmness characteristic. Amylose molecules are almost inexistent in waxy starches, thereby promoting the weak and sticky gels characteristic of waxy starch. However, this type of starch presented a fast decline of the curve between peak and minimum viscosity values (Fig. 1B). This is an undesirable feature for cheese production because it indicates low shear-resistance and low stability under processing conditions. In addition, for the mixtures, the same tendency observed for regular starches of increase in peak viscosity and reduction in pasting temperatures were not observed for WMS, and this may be due to the lack of amylose in waxy starch.

The waxy hydroxypropylated/phosphate maize starch (HPMS), also a crosslinked/stabilised starch, presented a slower decline in the curve between time and viscosity (i.e., a larger peak base),

which indicates more resistance to processing conditions (Fig. 1D) compared with the fast decline (i.e., sharp peak base) observed for WMS (Fig. 1B). This increase in starch resistance is hardly associated with the crosslinking by distarch phosphate esters that are responsible for the higher stability of this starch when compared with native waxy maize starches (Damodaran et al., 2008). The HPMS samples also showed the lowest pasting temperatures of the study (Table 1), possibly because hydroxypropylation generally reduces the starch pasting temperatures, which is relevant to cheese production as they reduce the energy to cook and accelerate the gel formation (Damodaran et al., 2008). On the other hand, the HPMS samples presented higher setback values, even for the pure dispersion. Their high setback values may be associated with their very high final viscosity value, which directly influences setback values and increases the consistency of gels. The characteristic high final viscosity of this type of starch is probably caused by the combination of modifications applied on it, by increasing the water binding capacity of the waxy starch through stabilisation, but maintaining granules resistance, avoiding breakdown and subsequent decreasing in viscosity, through the crosslinking process.

Therefore, the modified starches presented more promising results to be used as fat replacers in cheese. In the mixtures, the AAMS presented low pasting temperatures, high peak viscosity values, and a high resistance to process conditions, compared with RMS, while HPMS presented the lowest pasting temperatures and the highest peak viscosity values as well as a considerable resistance to process conditions (Table 1).

3.1.2. Thermal properties

In general, the presence of CN did not affect the thermal properties of the starch samples (Fig. 2). For onset, peak, and conclusion temperatures, there were small changes in the values among the different concentrations of CN/starch mixtures for a single type of starch (Table 2). However, the gelatinisation enthalpy values (ΔH) were higher in pure starch dispersions compared with those in the CN/starch mixtures, since they present a larger proportion of starch than the mixtures. For the same reason, there was a small increase in ΔH, as the concentrations of starch increased in the mixtures (Table 2). Therefore, the presence of CN did not influence significantly the thermal properties of the starch dispersions probably because pure CN did not dissociate at the temperatures applied in the study (25–125 °C), and no endotherm was formed (Fig. 2F). Additionally, the CN thermal resistance was so high that no endotherm peak was observed even under high temperature conditions, e.g., 25–200 °C, using a stainless-steel pan (data not shown).

The gelatinisation enthalpy (ΔH) was higher for the WMS than that of the other starch samples (Table 2). ΔH is the energy needed for dissociating the double helices from the crystalline regions. In these regions, starch molecules are found to be highly packed. The presence of amylose interrupts the compaction of the structure, decreasing the energy necessary for the occurrence of gelatinisation (Jane, 2007). Since waxy starches are almost totally composed of amylopectin, their ΔH is normally higher than that of regular starch. However, the modified waxy starch (HPMS) presented lower ΔH values than the WMS (Table 2). It may possibly occur due to the presence of hydroxypropyl groups that interact with amylopectin chains avoiding their self-association and the formation of double helices, promoting a less dense structure requiring less energy for the crystalline regions dissociation. In addition, the HAMS dispersions presented the lowest ΔH values (Table 2) because they have more amorphous regions, therefore, less energy is required for the crystals fusion (Singh et al., 2003).

High gelatinisation temperatures were observed for RMS and WMS (Table 2) samples. High gelatinisation temperatures are associated to more densely packed crystalline structures and a higher molecular order (Srichuwong et al., 2005), which is characteristic of the native maize starch structure, since it presents a type A crystalline pattern (BeMiller & Whistler, 2009). Considerably lower gelatinisation temperatures were observed for the AAMS and the HPMS (Table 2), possibly due to the modification process of both starch samples, which improves their stabilisation. Acetyl and hydroxypropyl radicals interact with amylopectin chains, promoting repulsion, which opens their structure and facilitates water penetration; consequently, lower temperatures are required for gelatinisation. On the other hand, the highest gelatinisation temperatures of the HAMS dispersions (Table 2) are due to their densely packed molecular structure, which in turn is due to the double helices formation by the long amylose chains (BeMiller & Whistler, 2009).

In the storage period after starch gelatinisation, an aggregation of starch molecules tends to occur; such phenomenon is known as retrogradation (Srichuwong et al., 2005). In general, retrograded starches showed lower onset, peak, and conclusion temperatures (Table 3) than non-retrograded starches (Table 2). This reduction is due to an improper alignment of the amylopectin chains during reassociation, causing the formation of less organised and less stable crystalline structures compared with those of native starches (Srichuwong et al., 2005). Consequently, less energy is required to melt the restructured crystals, and therefore, ΔH of retrograded starches is also lower than that of the non-retrograded starches.

As expected, the HAMS presented the lowest retrogradation rate (%R) due to the formation of double helices between amylose chains, which promotes higher stability. In addition, low %R values were observed for the waxy maize starch (WMS) and for the modified starch samples (AAMS and HPMS) than for the RMS (Table 3), which is expected, respectively, due to the low percentage of amylose in waxy starches (only traces), and the stabilisation process which can reduces the tendency of a regular starch to undergo retrogradation. A low retrogradation tendency is desirable since water exudation is considered a problem in fresh cheese – especially reduced-fat ones – since it is a refrigerated product and therefore more susceptible to retrogradation during its storage period.

Therefore, as observed for pasting properties, in most cases, the modified starch samples presented better results with respect to being used as fat replacers in cheese. They present lower peak temperatures (T_p) and lower ΔH values than the other starch samples, an important characteristic for the industry, since they are easier to cook, require low energy for gelatinisation, and produce high pasta viscosity values, as seen in the pasting properties results (Table 1). In addition, they present lower retrogradation rates than the regular starch, as well as the waxy starch, which is an important characteristic since fresh cheeses are maintained under refrigeration during storage.

3.1.3. Swelling power

As expected, CN did not present considerable swelling power values at any of the tested temperatures (55–95 °C), which contributed to a non-relevant effect of CN on CN/starch mixtures of any of the five starch samples tested at any concentrations (Table 4). Generally, the CN/starch mixtures presented the same profile of pure starch dispersions, but with lower values due to the dilution effect of the starch in the presence of CN.

The swelling power values increased as temperature increased for almost all the samples. It occurs because as the temperature of a starch suspension increases, starch hydrogen bonds gradually break, water molecules bind with free hydroxyl groups, and granules expand (Rickard, Asaoka, & Blanshard, 1991). Additionally, different swelling power values were observed among the different types of starch. This occurs mainly because of the inherent characteristics of each starch (Table 4).

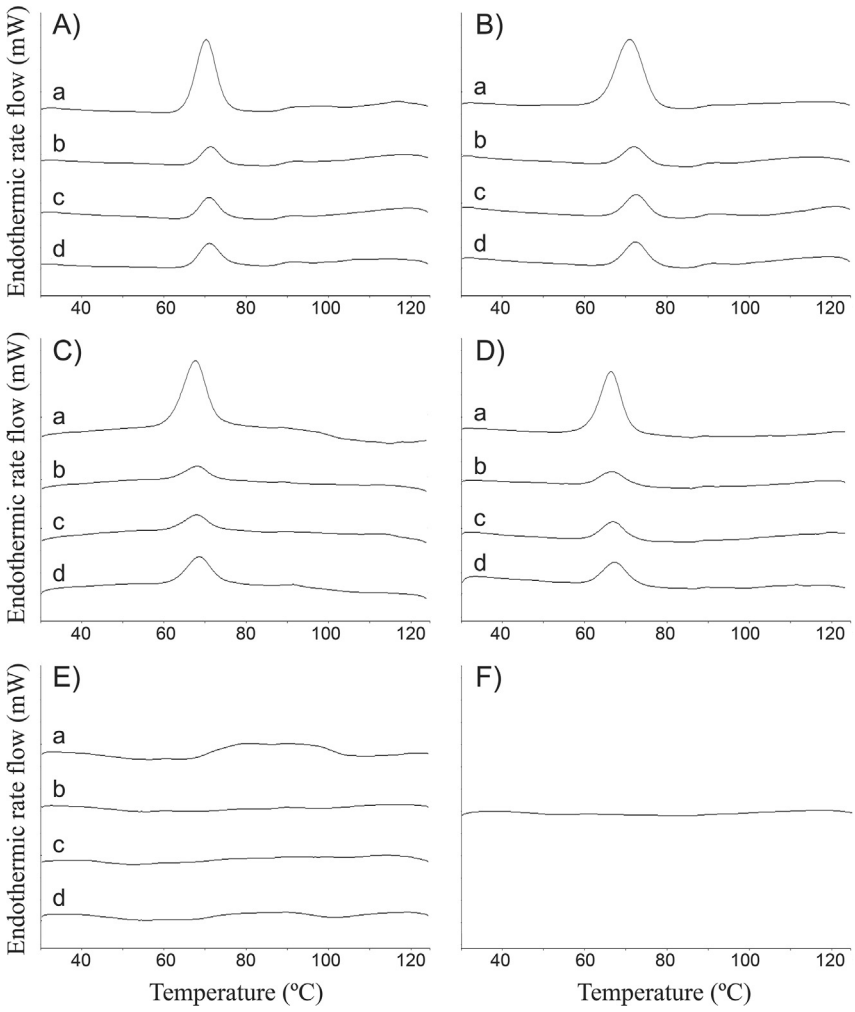

Fig. 2. Endothermic profile of calcium caseinate (CN)/starch mixtures. Samples (2 mg sample/6 mL deionised water) are CN in mixtures with: regular maize starch – RMS (A), waxy maize starch – WMS (B), acetylated/adipate maize starch with regular basis – AAMS (C), hydroxypropylated/phosphate maize starch with waxy basis – HPMS (D), and high-amylose maize starch – HAMS (E) in the proportions of 0.0:1.0 – pure starch (a), 2.5:0.5 (b), 2.5:1.0 (c), 2.5:1.5 (d), and 1.0:0.0 – pure CN (F) CN:starch.

High amylose starch dispersions presented the lowest swelling power values that did not significantly increase at the tested temperatures. This occurs because a considerable swelling of starch will only occur near their gelatinisation temperatures (when hydrogen bonds begin to dissociate), and high amylose starches normally have high gelatinisation temperatures, close to 100 °C, e.g., in this study, the conclusion temperatures of CN:HAMS dispersions ranged from 95 to 102 °C (Table 2). These high gelatinisation temperatures are due to the high amylose starches' characteristic structure of forming double helices by the interaction of amylose chains, creating a crystalline structure (Copeland et al., 2009; Jane, 2007).

The waxy maize starch samples presented high swelling power values, alone and in the mixtures with CN. Waxy starch is almost only composed of amylopectin, and its swelling power is most influenced by amylopectin content due to the high availability of hydroxyl groups in its branched chains (Singh et al., 2003). In general, the swelling power of the WMS dispersions increased as temperature increased, reaching a maximum at 85 °C, when most granules are swollen; then, their swelling power decreased due to the granules rupture, which occurs in consequence of the low intermolecular resistance of WMS granules, caused by their low amylose content.

In general, RMS presented lower swelling power when compared with WMS, AAMS, and HPMS in function of its considerable amylose content and it is well known that the presence of amylose is a swelling inhibitor (Singh et al., 2003). Another important characteristic is its higher thermal stability, without a decrease in the swelling power as temperature increases. A regular maize starch has considerable amylose and lipid contents, which restrict its swelling power, but increase its resistance to high temperatures.

The modified starch samples (AAMS and HPMS) presented, alone and in the mixtures with CN, considerable high swelling and great resistance to high temperatures, without decreasing their swelling power over the experiment, probably due to their cross-linking modification. The bifunctional ingredients bind two different starch chains, decreasing the starch swelling power and increasing stability (Damodaran et al., 2008). Consequently, even a starch with a waxy maize base, such as HPMS, presents a great resistance under high temperature conditions. In addition, both modified starches presented the highest swelling power at 65 °C. This is important because it is lower than the temperature at which milk is pasteurised (~72 °C) without negatively affecting the coagulation of milk for cheese production.

In most situations, the AAMS and HPMS samples presented high swelling power values and great resistance to high temperatures. In addition, the HPMS samples presented the highest swelling power at 65 °C (temperature of milk pasteurisation for cheese production) among the starch samples. This result corroborates with the pasting and thermal properties results that appoint the modified

Table 3
Retrogradation temperatures: onset (T_o), peak (T_p) and conclusion (T_c), retrogradation enthalpy (ΔH_{ret}) and retrogradation rate (% R) of starch, CN and CN/starch dispersions.

Samples	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH_{ret} (J g^{-1})	% R
RMS	$37.25^{a} \pm 0.32$	$50.48^{a} \pm 0.29$	$62.07^{a} \pm 0.14$	$8.46^{a} \pm 0.28$	58.71
CN + 0.5%RMS	$37.36^{a} \pm 0.56$	$50.56^{a} \pm 0.00$	$60.56^{a,b} \pm 0.10$	$3.75^{b} \pm 0.28$	123.36
CN + 1.0%RMS	$39.22^{a} \pm 1.37$	$51.31^{a} \pm 0.60$	60.66 ± 0.65	$3.72^{b} \pm 0.10$	95.63
CN + 1.5%RMS	$38.60^{a} \pm 0.28$	$51.03^{a} \pm 0.60$	60.29 ± 0.41	$4.34^{c} \pm 0.11$	90.99
WMS	$37.58^{c} \pm 0.27$	$53.09^{a} \pm 1.68$	$62.02^{a} \pm 0.69$	$3.99^{a} \pm 0.41$	23.42
CN + 0.5%WMS	$44.34^{a} \pm 0.48$	$51.23^{a} \pm 0.59$	$59.79^{a} \pm 1.72$	$0.59^{c} \pm 0.03$	16.81
CN + 1.0%WMS	$42.15^{b} \pm 0.14$	$51.24^{a} \pm 0.59$	$60.93^{a} \pm 0.95$	$1.40^{b} \pm 0.16$	31.75
CN + 1.5%WMS	$38.25^{c} \pm 0.36$	$51.40^{a} \pm 0.75$	$61.02^{a} \pm 0.75$	$0.88^{b,c} \pm 0.00$	15.71
AAMS	$37.30^{b} \pm 0.60$	$53.25^{a} \pm 0.26$	$62.44^{a} \pm 0.45$	$3.54^{a} \pm 0.08$	25.21
CN + 0.5%AAMS	$44.57^{a} \pm 0.15$	$53.32^{a} \pm 1.84$	$61.39^{a} \pm 0.18$	$0.92^{b} \pm 0.04$	35.66
CN + 1.0%AAMS	$44.69^{a} \pm 0.14$	$53.56^{a} \pm 0.09$	$61.11^{a} \pm 0.31$	$1.00^{b} \pm 0.27$	26.25
CN + 1.5%AAMS	$45.07^{a} \pm 0.44$	$54.85^{a} \pm 1.18$	$62.75^{a} \pm 2.92$	$1.04^{b} \pm 0.50$	16.86
HPMS	$40.47^{a} \pm 0.51$	$56.42^{a} \pm 0.26$	$74.91^{b} \pm 0.42$	$3.37^{a} \pm 0.08$	24.46
CN + 0.5%HPMS	$40.44^{a} \pm 0.03$	$55.91^{a} \pm 0.92$	$78.89^{a} \pm 1.25$	$1.27^{c} \pm 0.30$	47.04
CN + 1.0%HPMS	$39.47^{a} \pm 1.18$	$56.16^{a} \pm 0.50$	$79.00^{a} \pm 1.13$	$1.60^{b,c} \pm 0.17$	46.23
CN + 1.5%HPMS	$39.40^{a} \pm 0.50$	$55.83^{a} \pm 0.00$	$77.55^{a} \pm 0.34$	$1.89^{b} \pm 0.18$	40.38
HAMS	$84.65^{a} \pm 0.91$	$92.60^{a} \pm 0.00$	$102.08^{a} \pm 0.12$	$2.02^{a} \pm 0.01$	18.83
CN + 0.5%HAMS	$84.11^{a} \pm 0.19$	$87.47^{c} \pm 0.20$	$91.14^{d} \pm 0.16$	$0.20^{d} \pm 0.00$	26.67
CN + 1.0%HAMS	$84.16^{a} \pm 0.32$	$88.66^{b,c} \pm 0.75$	$94.12^{c} \pm 1.27$	$0.40^{c} \pm 0.00$	33.48
CN + 1.5%HAMS	$83.37^{a} \pm 0.34$	$88.74^{a} \pm 0.50$	$96.90^{b} \pm 0.22$	$0.73^{b} \pm 0.03$	22.19
CN	–	–	–	–	–

Values are the mean of three replications ± standard deviation; different superscript letters in the same column for each sample differ significantly ($p \leq 0.05$). Samples (2 mg sample/6 mL deionised water) are calcium caseinate (CN) in mixtures with: regular maize starch (RMS), waxy maize starch (WMS), acetylated/adipate maize starch with regular basis (AAMS), hydroxypropylated/phosphate maize starch with waxy basis (HPMS), and high-amylose maize starch (HAMS) in the proportions of 2.5:0.5, 2.5:1.0, and 2.5:1.5 CN:starch. Samples are also pure starch dispersions of each starch as well as pure CN dispersion. % R = (ΔHret/ΔHgel) × 100; ΔHgel = gelatinisation enthalpy.

Table 4
Swelling power of starch, CN, and CN/starch dispersions at different temperatures.

Samples	Temperature (°C)				
	55	65	75	85	95
RMS	$1.84^{D,a} \pm 0.02$	$4.45^{C,a} \pm 0.01$	$10.44^{B,a} \pm 0.03$	$12.97^{B,a} \pm 0.16$	$16.72^{A,a} \pm 0.91$
CN + 0.5%RMS	$1.38^{B,a} \pm 0.15$	$1.29^{B,d} \pm 0.03$	$3.11^{A,c} \pm 0.24$	$3.56^{A,c} \pm 0.03$	$3.02^{A,b} \pm 0.31$
CN + 1.0%RMS	$1.48^{C,a} \pm 0.14$	$1.60^{C,c} \pm 0.09$	$3.67^{B,b} \pm 0.05$	$4.69^{A,b} \pm 0.23$	$3.09^{B,b} \pm 0.38$
CN + 1.5%RMS	$1.56^{C,a} \pm 0.22$	$1.85^{C,b} \pm 0.05$	$4.15^{B,b} \pm 0.01$	$5.03^{A,b} \pm 0.03$	$4.00^{B,b} \pm 0.01$
WMS	$2.10^{E,a} \pm 0.03$	$5.58^{D,a} \pm 0.01$	$9.79^{C,a} \pm 0.41$	$25.76^{A,a} \pm 0.77$	$22.51^{B,a} \pm 0.46$
CN + 0.5%WMS	$0.90^{C,b} \pm 0.14$	$1.12^{C,b} \pm 0.13$	$3.87^{B,c} \pm 0.00$	$4.40^{A,b} \pm 0.11$	$4.08^{A,B,b} \pm 0.18$
CN + 1.0%WMS	$0.98^{D,b} \pm 0.07$	$1.59^{C,b} \pm 0.35$	$5.13^{B,b} \pm 0.03$	$7.32^{A,b} \pm 0.07$	$4.55^{B,b} \pm 0.36$
CN + 1.5%WMS	$0.97^{D,b} \pm 0.17$	$1.74^{C,b} \pm 0.13$	$5.96^{B,b} \pm 0.11$	$8.07^{A,b} \pm 0.09$	$5.70^{B,b} \pm 0.24$
AAMS	$2.81^{D,a} \pm 0.04$	$9.65^{C,a} \pm 0.07$	$12.94^{B,a} \pm 0.06$	$13.57^{A,a} \pm 0.98$	$12.90^{B,a} \pm 0.26$
CN + 0.5%AAMS	$2.37^{D,a} \pm 0.32$	$3.23^{C,c} \pm 0.05$	$3.63^{B,C,d} \pm 0.08$	$3.73^{B,b} \pm 0.16$	$4.71^{A,c} \pm 0.11$
CN + 1.0%AAMS	$1.68^{D,a} \pm 1.32$	$4.10^{C,c} \pm 0.15$	$4.64^{B,c} \pm 0.17$	$4.72^{B,b} \pm 0.00$	$5.79^{A,c} \pm 0.39$
CN + 1.5%AAMS	$2.61^{C,a} \pm 0.41$	$5.24^{B,b} \pm 0.42$	$5.47^{B,b} \pm 0.11$	$5.30^{B,b} \pm 0.25$	$7.45^{A,b} \pm 0.47$
HPMS	$7.02^{C,a} \pm 0.69$	$16.77^{A,a} \pm 0.64$	$16.67^{A,a} \pm 0.52$	$15.61^{B,a} \pm 0.35$	$16.50^{A,a} \pm 0.83$
CN + 0.5%HPMS	$2.36^{C,b} \pm 0.14$	$3.97^{B,c} \pm 0.13$	$4.65^{A,c} \pm 0.12$	$3.80^{B,d} \pm 0.11$	$4.55^{A,c} \pm 0.18$
CN + 1.0%HPMS	$2.68^{C,b} \pm 0.07$	$5.44^{A,B,b,c} \pm 0.35$	$5.75^{A,b,c} \pm 0.12$	$4.83^{B,c} \pm 0.07$	$5.73^{A,b,c} \pm 0.36$
CN + 1.5%HPMS	$2.77^{D,b} \pm 0.17$	$6.02^{B,C,b} \pm 0.13$	$6.49^{B,b} \pm 0.04$	$5.70^{C,b} \pm 0.09$	$7.26^{A,b} \pm 0.24$
HAMS	$2.25^{D,a} \pm 0.03$	$2.67^{C,D,a} \pm 0.06$	$3.18^{C,a} \pm 0.00$	$4.05^{B,a} \pm 0.01$	$5.65^{A,a} \pm 0.00$
CN + 0.5%HAMS	$1.00^{A,b} \pm 0.00$	$1.00^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.00^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$
CN + 1.0%HAMS	$1.00^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.02^{A,b} \pm 0.00$
CN + 1.5%HAMS	$1.00^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.01^{A,b} \pm 0.00$	$1.02^{A,b} \pm 0.00$
CN	$1.18^{A} \pm 0.11$	$0.99^{A} \pm 0.13$	$1.27^{A} \pm 0.06$	$1.65^{A} \pm 0.06$	$1.25^{A} \pm 0.04$

Values are the mean of three replications ± standard deviation; different superscript uppercase letters in the same row and different superscript lowercase letters in the same column differ significantly ($p \leq 0.05$). Samples (1% w/w; 0.2 g sample/20 g distilled water) are calcium caseinate (CN) in mixtures with: regular maize starch (RMS), waxy maize starch (WMS), acetylated/adipate maize starch with regular basis (AAMS), hydroxypropylated/phosphate maize starch with waxy basis (HPMS), and high-amylose maize starch (HAMS) in the proportions 2.5:0.5, 2.5:1.0, and 2.5:1.5 CN:starch. Samples are also pure starch dispersions of each starch as well as pure CN dispersion.

starches as more promising fat replacers in cheese. However, an in-depth study may be necessary to provide a better understanding of the behaviour of these starch samples within the casein matrix of cheeses. Therefore, the modified starches (AAMS and HPMS) were selected to continue the study of starch behaviour within the casein matrix.

3.2. Starch behaviour within casein matrix

3.2.1. Microstructural analysis

The micrographs of gels containing HPMS and AAMS showed swollen starch granules (stained in green) embedded in a protein matrix (stained in red) (Fig. 3), indicating that the starch added, or at least a great part of it, remained in the casein matrix, not being released in whey.

The gel with AAMS (Fig. 3A,B) showed a considerable interruption of the casein matrix by the starch; however, its swollen granules were smaller and not well distributed in the matrix, compared with those of HPMS, forming agglomerates. The gels with HPMS (Fig. 3C,D) presented a more open casein structure than the gels with AAMS. The protein network of HPMS gels appeared to be less compact due to their larger swollen particles and their more homogenous distribution over the matrix. Such larger HPMS swollen particles are probably due to the higher swelling power of this type

of starch when compared with AAMS (Table 4), which is also an important characteristic, since a high-water binding capacity may improve the texture of reduced-fat cheese. In addition, a more open protein matrix is desirable to improve textural characteristics of reduced-fat cheese. This occurs because fat acts as fracture points in the matrix, interrupting the protein-protein interactions and decreasing hardness. Such protein-protein interactions result in a harder reduced-fat cheese (Sipahioglu et al., 1999).

Therefore, the presence of both starches embedded in the casein matrix, acting as filler materials and causing its interruption, suggests a potential use of both starches as fat replacers in cheese. Nonetheless, HPMS promoted a more open and homogenous structure for the gel and may be considered the most promising fat replacer among the studied starches. On the other hand, its highest interruption in the casein matrix may be so intense that it would promote a softer texture than the ideal for a fresh cheese. A complementary texture analysis of fresh cheese samples produced with each starch, would contribute to this knowledge.

3.2.2. Starch content analysis

The presence of starch embedded in the casein matrix of gels was qualitatively shown by the microstructure analysis (Fig. 3). However, it is important to quantify the real content of starch that remained in the matrix, as its functionality as a fat replacer will only be achieved if a considerable amount of starch remains in the casein matrix.

The whey released from the curd samples presented very low starch contents, and consequently, both starches showed high retention in curd (Table 5). However, HPMS showed a slightly higher retention in curd, possibly due to its larger swollen granules (when compared with those of AAMS) which may have contributed to a more mechanical entrapment of the starch within the protein matrix.

Brown et al. (2012) observed a considerable lower value of starch retention in curd for a normal waxy corn starch (70%), when compared with the retention found for a waxy corn starch in this present study (96.9%). This difference, however, may be associated with the modifications of the HPMS, which improved starch retention, probably by increasing its water binding capacity through stabilisation, and maintaining its granules resistance, avoiding their breakdown, through the crosslinking process, and consequently, improving their behaviour as a filler material. The same authors also observed similar values of starch retention in curd (90%) for waxy rice and instant tapioca starch samples.

Table 5
Starch content in whey and starch retention in curds produced with modified starches.

Attribute	AAMS	HPMS
Total starch in whey (g 100 g^{-1})	$0.210^{a} \pm 0.39$	$0.049^{b} \pm 0.04$
Starch retention in curd (%)	86.0	96.9

Values are the mean of three replications ± standard deviation; different superscript letters in the same line differ significantly ($p \leq 0.05$). Samples are whey released from curd produced with the regular acetylated/adipate maize starch (AAMS) and waxy hydroxypropylated/phosphate maize starch (HPMS).

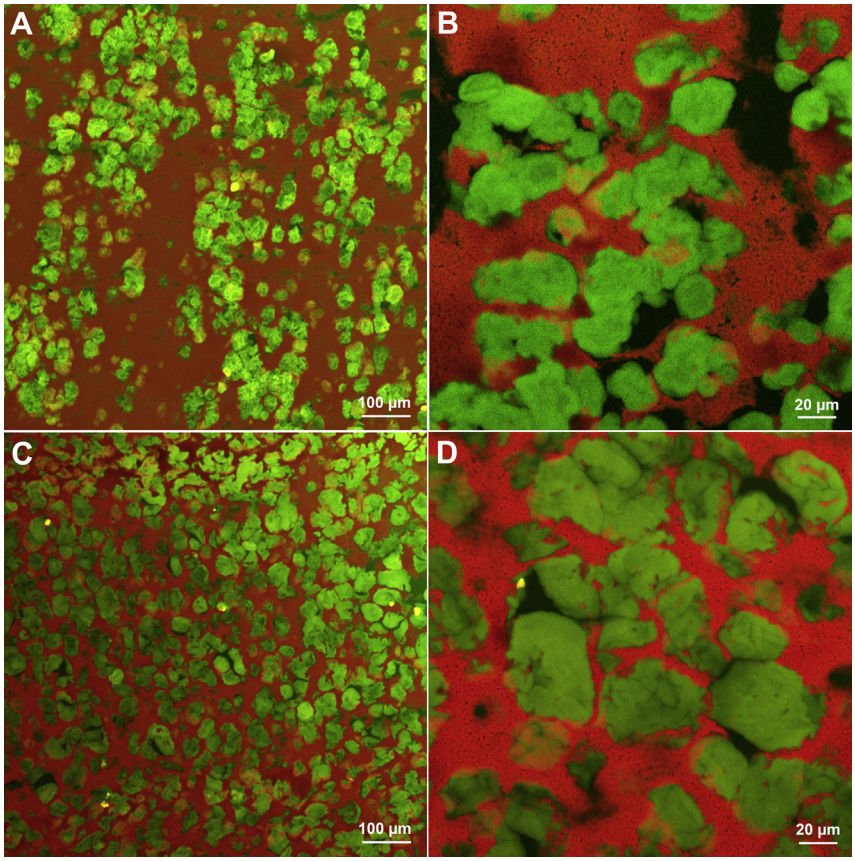

Fig. 3. Laser scanning confocal micrographs of curds with 1.5% of the following starches: (A) AAMS, magnification: 10×; (B) AAMS, magnification: 40×; (C) HPMS, magnification: 10×; (D) HPMS, magnification: 40×. Starch: swollen granules stained in green; Casein: continuous phase stained in red. AAMS: normal acetylated/adipate maize starch; HPMS: waxy hydroxypropylated/phosphate maize starch. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusions

In general, the modified starches presented the best results for being used as fat replacers in reduced-fat cheese. They produced high pasta viscosity values, which indicate high water binding capacity and a potential moisture increase in cheese. They also presented lower peak temperatures and lower ΔH values, thus they were considered easy to cook, requiring lower gelatinisation temperatures. Additionally, they presented low retrogradation rates, which is important since fresh cheese is a refrigerated product. They also presented a considerably high swelling power at 65 °C and a great resistance under the cheese processing conditions. Furthermore, the AAMS and HPMS appeared embedded in the casein matrix and presented a very low content of starch released in whey, thus being considered promising fat replacers in cheese.

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