Research Article

Micro and nanoparticles of native and modified cassava starches as carriers of the antimicrobial potassium sorbate†

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List of abbreviations:
acetylated cassava starch (ACS)
acetylated cassava starch cross-linked with STMP (PCACS)
analysis of variance (ANOVA)
degree of phosphorylation (DP)
degree of substitution (DS)
dynamic light scattering (DLS)
generally recognized as safe (GRAS)
limit of detection (LOD)
native cassava starch (NCS)
native cassava starch cross-linked with STMP (PCNCS)
phosphorus (P)
potassium sorbate (KS)
sodium trimetaphosphate (STMP)
static light scattering (SLS)
zeta potential (z-pot)

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Abstract

Cross-linked and/or acetylated cassava starches were synthesized and characterized. The acetylation increased the water retention capacity and the solubility in water while the higher level of cross-linking produced the opposite effect on starch. Native (NCS) and acetylated cassava starches (ACS) were used to generate starch micro and nanoparticles by the dialysis technique. The nanoparticle fraction was around 1.8 g 100 g^{-1} and 12 g 100 g^{-1} (starch dry basis) for NCS and ACS respectively. The nanoparticle sizes were around 23-255 nm with zeta potential extending from -4 to -44 mV, while the microscopic fractions ranged 5-87 μm. In addition, the capacity of particles to support potassium sorbate (KS) was tested. NCS and ACS particles supported a similar quantity of KS (≈1400 ppm) and the presence of antimicrobial decreased the particle size for NCS. The precipitation in ethanol technique was also used to generate microparticles where the particles generated from acetylated starches were smaller (8-58 μm) than those from native ones (30-227 μm). The KS content that these particles could incorporate was around 2020 ppm. The applied technique modulated the average dimension of the particles obtained as well as the antimicrobial retention capacity. These innovative materials could be potentially helpful for shelf life extension by the contribution to the KS stabilization to be incorporated in the bulk of food products.

Keywords: native and modified cassava starch, micro and nanoparticles, potassium sorbate.

1. INTRODUCTION

Food grade polymers, such as proteins and polysaccharides, can be used to create a wide range of micro and nanostructured particles to support, stabilize and control the release of functional food components such as ω-3 fatty acids, vitamins, flavors, colors and nutraceuticals [1-3]. These structures have a high area/volume ratio that affects the interaction with food matrix, modulates the release of active compounds, and has the ability to modify the food structure and rheology. Among the polysaccharides, starches have the advantages of low cost, nontoxicity, renewability, biodegradability and compatibility with many other food materials [4]. The chemical or physical modification of starch is usually carried out to enhance the positive attributes and eliminate the shortcomings of native starches [5].

Recently, the use of starch micro/nanoparticles has been reported for the development of new packaging materials, for the stabilization of emulsions and as additives to modify the texture and flavor of foods [6]. Furthermore, these structures allow the compartmentalization of active substances, e.g. antimicrobials or antioxidants, which can be dissolved, trapped, absorbed or encapsulated in the matrix of the particles, decreasing or suppressing their interaction with other food components and contributing to their effectiveness [7]. Depending on the preparation method, the micro/nanoparticles may be obtained with different properties and release characteristics for the supported agents [8].

The osmosis and the precipitation methods were described as strategies to obtain starch micro/nanoparticles. The osmosis method was reported as simple and versatile for controlling both the size and morphology of polymeric material on the sub-micrometer scale [9]. It is based on the use of a physical barrier, specifically dialysis membranes that allow the passive transport of solvents to slow down the mixing of a polymer

solution (inside of dialysis membrane) with a non-solvent. The gradual mixing of the solvent and the non-solvent inside a bag causes the mixture to be progressively less able to dissolve the polymer. The consequent increase in interfacial tension drives the molecules of the polymer to aggregate into colloidal particles [9, 10]. On the other hand, the precipitation method involves a slow addition of a biopolymer solution into the non-solvent phase under stirring. The particles, with a well-defined size, are characterized by a narrow distribution formed instantaneously during the rapid diffusion of the polymer solution in the non-solvent phase [8].

Sorbic acid and its potassium salt (KS) are classified as generally recognized as safe (GRAS) additives and are active against yeast, molds, and many bacteria. These preservatives are unstable due to their chemical structure. They also suffer oxidative degradation and can interact with food components, thereby diminishing their antimicrobial potential [11]. Therefore, the addition of sorbates to biopolymer based micro/nano-structures can be thought of as a strategy to compartmentalize the antimicrobial when added to a food formulation and could help to minimize the preservative loss [12].

The objectives of this work were: i) to synthesize and characterize modified starches obtained from cassava starch, ii) to obtain micro and nanoparticles by the dialysis and the precipitation in ethanol techniques, using native and acetylated starches, and iii) to evaluate the particle size distribution, z-potential and ability to support the antimicrobial KS by these structures.

2. MATERIALS AND METHODS

2.1 Materials

The food grade native cassava starch (NCS) (92 – 98 g 100 g^{-1} purity grade, contents of

amylose 19 g 100 g^{-1} and amylopectin 81 g 100 g^{-1} (ISO 6647-1:2007(E)), molecular weights of $68x10^6$ g mol^{-1} (amylopectin) and $0.8x10^6$ g mol^{-1} (amylose)) was obtained from Bernesa S.A. (Argentina). Acetic anhydride (99.7 g 100 g^{-1} purity grade, Sintorgan S.A., Argentina, Code: SIN-032003-01), iodine (98.9 g 100 g^{-1} purity grade, Anedra, Argentina, Code: 6816), sodium trimetaphosphate (≥95 g 100 g^{-1} purity grade, STMP, Sigma, USA, Code: T5508), DMSO (Anedra, Argentina, Code: 6646), sodium thiosulphate ($Na_2S_2O_3$, ≥99.5 g 100 g^{-1} purity grade, Anedra, Argentina, Code: 7259) and potassium sorbate (≥99 g 100 g^{-1} purity grade, Sigma, USA, Code: 359769) were of reagent grade. Ethanol was pharmacopeia grade (98º, Fradealco S.A., Argentina, Code: 1133188).

2.2 Synthesis of modified starches

2.2.1 Acetylation process using microwave radiation

The acetylation of native cassava starch was performed according to Shogren and Biswas [13]. Briefly, starch, acetic anhydride and iodine as a catalyst were mixed in a mass relationship of 1:1.9:0.1 into a Teflon reactor. The synthesis was carried out by means of a microwave device (Ethos Plus, Milestone S.R.L., Italy) and with the following conditions: maximum power of 450W, irradiation time of 3.5 min and maximum temperature of 90°C. After reaction, the mixture was cooled to room temperature and $Na_2S_2O_3$ was added to reduce the remaining iodine (I_2) to iodide (I^-). The product was washed in four steps with different volumes of ethanol or water, filtered and the mass obtained was dried in a vacuum oven at 60° C overnight. The dry product (ACS) was ground in a mortar and sieved (100 μm mesh).

2.2.1.2 Degree of substitution (DS) of the acetylated starch

According to Bello-Pérez et al. [14], 1 g of ACS was weighed at the precision of 0.1 mg, placed in a 250 mL Erlenmeyer flask, added with 50 mL of an aqueous solution of ethanol 75 mL 100 mL^{-1}, tightly capped and stirred at 50°C for 30 min. After cooling to room temperature, 20 mL of 0.5N NaOH was added and the flasks were stirred for 24 hours at room temperature to proceed with the saponification of the acetyl groups. The excess of alkali was titrated with 0.5N HCl using phenolphthalein as an indicator. A blank sample was also prepared from NCS. The percentage of acetyl groups (g 100 g^{-1}) was calculated according to equation 1 [14]:

$$\%\ acetylation = \frac{(mL_{blank} - mL_{sample}) \times Molarity\ HCl \times 0.043}{g_{sample}} \times 100 \qquad \text{eq. (1)}$$

where mL_{blank} and mL_{sample} are the volumes of 0.5N HCl consumed for the native and acetylated starch samples in the titration. The DS of acetyl groups was determined according to equation 2 [14]:

$$DS = \frac{162 \times \%\ acetylation}{4300 - (42 \times \%\ acetylation)} \qquad \text{eq. (2)}$$

2.2.2 Cross-linking process with sodium trimetaphosphate (STMP)

For cross-linking of NCS or ACS, 5 g of sample was suspended in 10 mL of 10^{-4} N NaOH and STMP (cross-linker) was added to obtain concentrations of 0.5 g 100 g^{-1} and 1 g 100 g^{-1}. The pH of the suspensions was adjusted to 10.5 by the addition of NaOH (2 N) and subjected to heating in a water bath at 50ºC for 3 h [15]. Then, the pH was reduced to 7.0 by adding 0.5 N HCl. A washing step was performed to remove the

STMP in excess as follows: the samples were centrifuged at 5000 rpm for 5 min, the supernatant was removed and the pellet was resuspended in distilled water. The process was repeated twice. The drying of the samples was performed in a vacuum oven at 60°C overnight. The dry products, NCS or ACS cross-linked with STMP (PCNCS or PCACS), were ground in a mortar and sieved (100 μm mesh).

2.2.2.1 Degree of phosphorylation

PCNCS and PCACS were characterized by inductively coupled plasma atomic emission spectroscopy (Spectrometer Baird, USA), determining the total phosphorus content. The NCS and ACS were analyzed as controls. The samples were digested at reflux in a mixture of nitric acid/perchloric acid until total dissolution of the organic matter. Appropriate dilutions were made before injection. According to Atichokudomchai and Varavinit [15], the degree of phosphorylation (DP) can be determined employing the following equation:

$$DP = \frac{162P}{3100 - 102P} \qquad \text{eq. (3)}$$

where P is the phosphorus content (g 100 g^{-1}) of starches.

2.2.3 Characterization of modified starches

2.2.3.1 Solubility and water retention capacity

The solubility in water of the starch particles was determined as follows [16]: 0.1 g of sample and 9 g of distilled water were added in a pre-weighed 15 mL Falcon tube. The samples were placed in a bath at 85ºC for 30 min with shaking every 5 min, then

centrifuged (Centrifuge Eppendorf, Germany) at 25°C. at 5000 rpm for 10 min. The supernatant volume was measured and an aliquot of 5 mL was transferred to a pre-weighed Petri dish. The withdrawn aliquot was dried overnight at 100°C. The proportion of the solubilized mass was calculated for the total volume of supernatant. The solubility (S, g 100 g^{-1}) is reported as the percentage ratio between the dry weight obtained from the supernatant and the initial weight of starch used.

In addition, Falcon tubes were allowed to drain, weighed and the gain in weight of the swollen starch (pellet) was used to calculate the water retention capacity (WRC, g 100 g^{-1}) as the percentage ratio between the gain in weight of the pellet and the initial weight of starch used.

2.2.3.2 Microstructure of starches

The microstructure analysis was carried out by SEM (Zeiss Supra 40, Germany). The samples, previously dehydrated in a desiccator containing $CaCl_2$, were fixed to the sample holder with the help of conductive double-sided adhesive tape and metallized by gold sputter coating (Cressington Scientific Instruments, UK).

2.3 Obtaining starch micro/nanoparticles to support KS

2.3.1 Dialysis technique

NCS, ACS and the corresponding cross-linked samples were used to obtain micro/nanoparticles according to Simi and Abraham [17] with slight modifications. The samples (2 g) were dissolved in 60 mL of DMSO (solvent) with constant stirring at room temperature for 24 h. This solution was introduced into a dialysis bag (Cut-off 12000D, Spectra/Por, USA) and dialyzed for 48 h in a beaker containing 1L of ultrapure water (non-solvent) and with constant stirring. The water was renewed periodically.

After the dialysis time, the bag was opened and an aliquot of the precipitate was separated for a particle size distribution analysis.

For starch particles containing KS, the same procedure was performed but 0.18 g of the preservative was added to DMSO previously to starch dissolution. After the dialysis process, the precipitate was submitted to centrifugation at 15ºC at 10000 rpm for 10 min, the supernatant was drained and the pellet was resuspended in ultrapure water. The washed step was performed in duplicate. Then, an aliquot of the resuspended precipitate was separated for the particle size distribution analysis. In **Figure 1**, a flowchart of containing KS particles preparation is shown for a better understanding.

2.3.2 Precipitation in ethanol technique

NCS or ACS (2.5 g) was suspended in 100 mL of distilled water, heated to 90°C (solvent) and maintained at 90-100ºC under constant stirring for 1 h in order to gelatinize the starch. The resulting viscous solution was homogenized at 21500 rpm for 2 min (Ultra-Turrax, IKA, Germany). Subsequently, 50 mL of ethanol (nonsolvent) was slowly added dropwise from a buret to the starch solution [18]. The precipitation was completed by spraying the previous solution (35 mL) with energetic stirring on 100 mL of ethanol that had previously been added with ethyl cellulose (0.1 g 100 mL^{-1}) as a stabilizer. For the atomization (spraying), a spray gun connected to a dry piston compressor device (San Up 3033, Argentina) was used. An aliquot of the produced suspension was separated for particle size determinations.

For starch particles containing KS, the antimicrobial (0.3 g) was added to the starch gelatinized solution previously to homogenization. Then, the precipitation of particles was performed as described above. The particles were centrifuged at 15°C at 1000 rpm for 20 min, the supernatant was drained and the pellet was resuspended in ethanol. The

washed step was performed in duplicate. Then, an aliquot of the suspension was separated for particle size determinations. In order to facilitate the understanding, a scheme of the precipitation in ethanol technique is also displayed in **Figure 1**.

2.3.3 Characterization of micro/nanoparticles

2.3.3.1 Particle size distribution and Zeta potential (z-pot)

The size distribution of microparticles was determined at room temperature by static light scattering (SLS) (Mastersizer 2000, Malvern Instruments, England) provided with He-Ne laser (λ: 633 nm). The samples were properly diluted with ultrapure water and the size distribution and the Sauter diameter (D [3,2], which is the diameter of a sphere having the same volume/surface ratio as the particle of interest), were evaluated. Determinations were performed in duplicate.

The size distribution of nanoparticles and the z-pot were characterized by dynamic light scattering (DLS) at 25°C (Zetasizer Nano Zs, Malvern Instruments, England) provided with a He-Ne laser (λ: 633 nm). The measurement of the hydrodynamic diameters was performed at a fixed scattering angle of 173°. The samples were properly diluted, filtered through a 0.450 μm pore filter (MSI-Osmonics, USA) and placed in disposable polystyrene cuvettes of 1 cm path length. For the surface net charge determination of the starch nanoparticles, the diluted samples were injected into a cuvette for the measurement of the electrophoretic mobility. The determinations were performed in triplicate.

In addition, the nanoparticle mass fraction was evaluated as the ratio between the dry mass of an aliquot of the precipitate that was previously filtered through a 0.45 μm pore filter and the dry mass from an equivalent aliquot of the entire precipitate.

2.3.3.2 Potassium sorbate dosage

The starch particles containing KS were freeze dried (Christ, Germany) at 1.1 Pa and 25ºC for 24 h. The dry product was ground to powder and the KS concentration was evaluated through the oxidation technique that involved distillation and a colorimetric reaction using thiobarbituric acid, as proposed by the AOAC International (1990) [19]. The determination was performed at least in duplicate.

2.4 Statistical analysis

The significant differences between the systems were established by analysis of variance (ANOVA) with a significance level of 0.05 and applying an "*a posteriori*" test (Least Statistical Difference). The software Statgraphics Centurion XV (V2.15.06, 2007, StatPoint Technologies, Inc., USA) was used for the processing and analysis of data. The results are reported based on their average and standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of modified starches

Modification of cassava starch rendered whitish powders similar in appearance to native polysaccharide. The results indicated that the DS of acetylated starches (cross-linked or not) averaged 0.23±0.03, which corresponds to a content of acetyl groups of (5.8±0.9) g 100 g^{-1}. These starch acetates with relative high DS had been suggested for food applications such as food packaging or as a material for the support and controlled release of active agents [14].

The DP for NCS and ACS was around 0.0015 mainly due to endogenous P from phospholipids [20]. Cross-linked starches increased the DP to values ranging from 0.0045 to 0.0151 depending on the type of starch and the amount of STMP used, as the

DP values for PCACS were significantly higher than those corresponding to PCNCS (**Table 1**).

The WRC of acetylated starches, cross-linked or not, was around 92% higher than the values for native ones. According to Mirmoghtadaie et al. [21], the introduction of acetyl groups in the granule increased the swelling degree of acetylated oat starch ([27.5±0.1] g 100 g^{-1}), DS 0.11) in comparison with the native one ([19.6±0.2] g 100 g^{-1}). These authors suggested that acetyl groups could facilitate the water access to the amorphous areas, due to a granular structural disruption caused by steric effects and the interruption of hydrogen bonds in the granules.

Interestingly, levels of cross-linking (0.5 or 1 g 100 g^{-1} STMP) had a differential effect on the WRC. For 0.5 g 100 g^{-1} STMP, an increase in WRC in native or acetylated starches was observed. Contrarily, the 1 g 100 g^{-1} STMP level produced a decrease of the WRC for both starches, even below the values observed for NCS and ACS, respectively. It could be inferred that the lightly cross-linked starch retained granule integrity but absorbed more water rendering higher swelling. Indeed, at low DP levels, the presence of negatively charged phosphate groups could promote the repulsion between adjacent starch molecules and collaborate in the swelling. Conversely, in the presence of a greater number of phosphodiester groups the granular structure might have become more rigid, limiting swelling. Wongsagonsup et al. [22] reported that the swelling of cross-linked cassava starch increased significantly when starch was treated with a mixture of 1.0% STMP/STPP (sodium tripolyphosphate) while concentrations of cross-linkers from 1.5 to 6.0%, decreased the water uptake.

As observed in **Table 1,** the S values for native starches were 400% to 615% lower than the corresponding S for the acetylated ones. On the other hand, native and acetylated starches showed lower S as the DP increased due to a higher number of phosphodiester

bridges that prevented the molecules from leaching [23].

3.2 Microstructure of starches

The SEM images of the starches studied are shown in **Figure 2**. The microstructure analysis revealed that NCS was constituted by spheroidal shaped granules with smooth surfaces and edges (**Figure 2, panel a**). PCNCS showed a similar aspect to NCS but granules with superficial damage were observed, especially in PCNCS 1.0 g 100 g^{-1} STMP (**Figure 2, panels b and c**). For ACS (**Figure 2, panel d**), deformation was detected due to granule damage as a consequence of the incorporation of acetyl groups [24]. In addition, the microwave-assisted synthesis at a high temperature used in the present study could have promoted the observed structural damage of the granules. The PCACS granules exhibited increased and noticeable surface erosion as the cross-linking level increased (0.5 and 1.0 g 100 g^{-1} STMP) (**Figure 2, panels e and f**). Such granule alterations could explain the WRC, S and DP increase observed for acetylated starches. Granule damage might have produced a higher exposition of hydroxyl groups to interact with water or react as a nucleophile with STMP allowing a greater phosphorylation in acetylated than in native starches (**Table 1**).

3.2 Obtaining and characterization of starch micro/nanoparticles

3.2.1 Dialysis technique

The osmosis method performed in the present study, involved the dissolution of starch granules in DMSO and, subsequently, the precipitation of starch particles inside the dialysis bag by slow interchange of the solvent (DMSO) and the non-solvent (ultrapure water). It has been reported in previous researches, that the morphology and size of the particles were determined by kinetic and thermodynamic factors, obtaining dimensions

ranging from microns to nanometers depending on the experimental setup [9]. Accordingly, the speed of the solvent/non-solvent interchange, the selected pair of solvent/non-solvent, the cut-off dialysis membrane, the polymer concentration and the temperature were among the factors that modulated the product characteristics

3.2.1.1 Capacity of supporting KS

When the NCS particles were obtained in the presence of KS (NCS+KS), the antimicrobial content was 1427±117 ppm, demonstrating their capacity for retaining the preservative. Additionally, the nanoparticle fraction (a size lower than 450 nm) was analyzed in relation to the KS content. It was determined that the retention capacity of NCS+KS nanoparticles was 1372±116 ppm, which was similar ($p>0.05$) to the microparticles fraction. On the other hand, PCNCS`s samples rendered particles with a level of KS inferior to the limit of detection (LOD≈0.1 ppm in distillate) of the oxidative method applied for antimicrobial quantification. Such a result could be related with two aspects of the cross-linked starches: i) the presence of phosphate groups that increased the negative charge density of the particle determining the repulsion of the negatively charged sorbate molecule (pH≈7.0 in aqueous media) and ii) the reduction of HO- group that decrease the hydrogen bond interactions between starch and sorbates due to cross-linking unions.

Similarly, when acetylated starches were tested for KS retention capacity, it was observed that the ACS particles obtained in the presence of the antimicrobial (ACS+KS) had a KS content of 1416±105 ppm without significant differences ($p>0.05$) in comparison with NCS+KS. In addition, the results for the KS content of a nanoparticle fraction (a size lower than 450 nm) indicated that ACS+KS nanoparticles tended ($p>0.05$) to carry a higher amount of antimicrobial (≈2070±450 ppm) than the

microparticle sample. With regard to cross-linked samples (PCACS), these samples retained very low quantities of sorbates molecules (lower than LOD) in their structures. Therefore, cross-linked starches were not appropriate for a significant KS support.

3.2.1.2 Dimension and z-pot of particles

According to our results, the precipitate presented two main particle size fractions. The yield of the nanoparticle fraction with a size lower than 450 nm was approximately 1.8 g 100 g^{-1} dry basis (d.b.) for native and 12.5 g 100 g^{-1} d.b. for acetylated starches. Such a difference could be attributed to the higher granule erosion produced by acetylation as was observed through the SEM images (**Figure 2**).

Table 2 summarizes the results of nanoparticle size distribution and z-pot for the studied starches. As regards the native starch, a monomodal profile with a hydrodynamic diameter of 36 nm was observed. Previous investigations [25] reported two peaks around 40 and 300 nm for corn starch nanoparticles dissolved in water and measured by DLS and the higher size was ascribed to nanoparticle aggregation. On the other hand, the NCS samples tested in the present work had a low and negative z-pot of -4.3 mV. These results were in agreement with those of Song et al. [26] who reported z-pot values ranging from -4 to -12 mV for corn starch based nanoparticles obtained by reactive extrusion. It is important to remark that particles with a module of z-pot lower than 30 mV could be unstable since the electrostatic repulsion forces are not sufficiently high to avoid aggregation [27].

The ACS particles present a bimodal size distribution with peaks of 29 and 205 nm. The z-pot presented more negative values than NCS (**Table 2**), probably because of the presence of acetate groups with a higher and a negative charge density.

The particle size distribution of the microscopic fraction analyzed by SLS is shown in

Table 3. The NCS presented a wide distribution with particle sizes ranging between 5 and 56 μm while the ACS particles were significantly bigger (20 to 87 μm).

In addition, it was observed that the size of NCS+KS nanoparticle fraction was similar to that of NCS (**Table 2**). However, the z-pot was significantly more negative ($p<0.05$) for NCS+KS respect to NCS, indicating that the antimicrobial with an anionic group (carboxylate) increased the negative surface charge of the particle. Moreover, the micrometric fraction of NCS+KS showed a reduced dispersion of size distribution in comparison with NCS rendering particles between 7.9 and 27 μm **(Figure 3, panel a**).

As shown in **Table 2**, the ACS+KS nanoparticle fraction presented a bimodal profile in the DLS analysis where the main peak, (23±5) nm, corresponded to a smaller particle than that of NCS+KS, probably as a consequence of the important degradation of acetylated granules as previously mentioned. In addition, a second main peak appeared at 255 nm, which involved 10% of the volume distribution. As was previously mentioned, the presence of acetate groups and the incorporation of sorbate promoted the occurrence of a more negatively charged starch than the native one (-8.3 and -44 mV). With reference to the microparticle size distribution (**Table 3**), the ACS+KS particles ranged between 22 and 84 μm. It can be seen that modified starch tended to form bigger microparticles than NCS+KS that showed sizes ranging from 8 to 27 μm. This increase in size is the result of the introduction of acetyl groups to the starch chain [28] because the presence of KS did not affect, in general, the size distribution (**Figure 3, panel a**) for acetylated samples.

3.2.2 Precipitation in ethanol technique

For this procedure, the starch granules were gelatinized in hot water and particle precipitation was carried out by the contact of disrupted granules with a non-solvent

media (ethanol). According to previous studies, the particle morphology, size and its reproducibility depended on some process parameters such as the extent of gelatinization, concentration of starch slurry, final water: ethanol ratio, temperature, rate of precipitation, as well as were influenced by amylose and amylopectin content or the use of surfactants [29, 30].

In the present work and for comparison with the dialysis technique, both NCS and ACS with or without KS were precipitated by means of ethanol.

3.2.2.1 Capacity of supporting KS

When the precipitation was performed in the presence of KS, the antimicrobial content of particles was 2010±424 and 2038±371 ppm for NCS+KS and ACS+KS respectively, without significant differences between them.

3.2.2.2 Dimension of particles

Table 4 shows the size distribution of microparticles obtained from NCS and ACS with ranges of 30-171 μm and 8-58 μm, respectively. These results indicate that acetylated starches had lower tendency to form clusters in ethanol media, rendering smaller particles than NCS. Similar results were reported by Patindol et al. [30] who obtained rice starch based particles of 42-75 μm in size when starch slurry was precipitated with ethanol (2.5 g 100 mL^{-1}).

With regard to the effect of KS addition on the size particle, it could be observed that the preservative presence gave origin to a wider and asymmetrical size distribution in the case of NCS+KS. However, the addition of KS to ACS produced the narrowing of the distribution curve (**Figure 3, panel b).**

3.3 Effect of the technique applied on the microparticle size and KS content

The Sauter diameter variation of the microparticles obtained by dialysis and precipitation in ethanol techniques is shown in **Figure 4** ilustrating the polarity of the non-solvent used. It could be observed that when NCS was precipitated in an aqueous media (dialysis technique), the resulting size was smaller than the corresponding microparticles obtained from ACS. However, the contrary was registered when NCS and ACS were precipitated in ethanol, independently of the presence of KS (**Figure 4**). Therefore, it could be suggested that when the starch conserved its highly polar groups (NCS with plenty of hydroxyls) and interacted with a non-solvent of high polarity (water), the size of microparticle was lower. Similarly, ACS with reduced polarity in comparison with NCS, because of the introduction of acetate groups, rendered smaller particles when it was precipitated in a less polar non-solvent like ethanol.

It can be concluded that the technique applied to obtain particles modulates the average dimension of the particles obtained as well as the capacity of supporting the antimicrobial. It is known that the introduction of compounds, such as aliphatic fatty acids into starch preparations, disrupts double helix conformations by forming stable single chain V-conformation helices [31]. In the case of sorbates, as they behave like short chain fatty acids due to their chemical structure, they probably also need to be fixed to the helices to be supported [32]. Under these considerations, it could be thought that the fact that the ethanol precipitation technique gave origin only to microparticles might be the reason for the easier interaction between starch and KS than when the dialysis technique was applied with the formation of nano and microparticles. However, since the determined nanoparticle mass fractions were low (1.8 or 12 g 100 g^{-1}), the nanoparticles presence had no significant effect on the NCS+KS or ACS+KS antimicrobial content of particles obtained by dialysis.

CONCLUSIONS

The acetylation process of cassava starch increased WRC and S of the granules in comparison to native starch, while cross-linking with 1 g 100 g^{-1} STMP reduced those parameters in both NCS and ACS.

The ability of the starch particles to act as carriers for KS was dependent on their chemical properties and the technique applied. The precipitation in the ethanol technique promoted the obtaining of NCS and ACS microparticles with the KS content approximately 40% higher than the corresponding particles obtained by dialysis. Cross-linked particles with STMP showed a reduced ability to retain the preservative.

The technique applied to obtain particles could modulate the average dimension. By applying the dialysis technique, two particle size fractions were obtained from native and acetylated starch. The nanoparticles were smaller than 255 nm with negative z-pot, while microparticles ranged between 5-87 μm. The precipitation in the ethanol technique rendered microparticles with sizes of 30-227 μm or 8-58 μm from native and acetylated starch, with or without KS.

In the present research, it was possible to produce particles from native and acetylated cassava starch with the ability to support the antimicrobial KS. Taking into account that sorbates are highly reactive in the presence of additives or other food components, these developed particles could probably be used to stabilize KS and minimize the quantities added to food products. Further studies are being performed in order to confirm the capacity of starch particles for stabilization and/or control release of KS as well as the bioavailability of the antimicrobial in the starch matrix.

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Conflict of Interest Statement

The authors have declared no conflict of interest in relation to this paper.

Novelty statement

It was possible to produce micro/nanoparticles from native and acetylated cassava starch with the ability to support the antimicrobial KS. Taking into account that sorbates are highly reactive in the presence of additives or other food components, these developed particles could probably be used to stabilize KS and minimize the quantities added to food products.

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FIGURE LEGENDS

Figure 1. Flowchart of nano/micro particles obtention process from native cassava starch (NCS) or acetylated cassava starch (ACS) to support KS by dyalisis technique and precipitation in ethanol technique.

Figure 2. SEM pictures : a) native cassava starch (NCS), magnifications: 800X, bar: 20 μm; b) native cassava starch cross-linked with 0.5 g 100 g^{-1} STMP (PCNCS 0.5 STMP), magnifications: 1500X, bar: 10 μm; c) native cassava starch cross-linked with 1.0 g 100 g^{-1} STMP (PCNCS 1.0 STMP), magnifications: 1500X, bar: 10 μm; d) acetylated cassava starch (ACS), magnifications: 800X, bar: 20 μm; e) acetylated cassava starch cross-linked with 0.5 g 100 g^{-1} STMP (PCACS 0.5 STMP), magnifications: 1500X, bar: 20 μm; and f) acetylated cassava starch cross-linked with 1.0 g 100 g^{-1} STMP (PCACS 1.0 STMP), magnifications: 1500X, bar: 10 μm.

Figure 3: Particle size distribution for native cassava starch (NCS), native cassava starch + potassium sorbate (NCS+KS), acetylated cassava starch (ACS) and acetylated cassava starch + potassium sorbate (ACS+KS) starch microparticles obtained by a) dialysis technique, and b) precipitation in ethanol technique.

Figure 4: The Sauter diameter (D [3,2]) of microparticles obtained by dialysis (polarity index of non-solvent: 9.0) and precipitation in ethanol (polarity index of non-solvent: 5.2) techniques. Equal letters on the columns indicate no significant differences between systems ($p>0.05$).

Table 1. Degree of phosphorylation (DP), water retention capacity (WRC) and solubility in water (S) of native and modified starches.

Starch	DP[1]	WRC[2] (g 100 g^{-1})	S[2] (g 100 g^{-1})
NCS	0.0015[a]	324±36[f]	1.6±0.8[l]
PCNCS 0.5 STMP	0.0045[b]	436±20[g]	1.1±0.2[l]
PCNCS 1.0 STMP	0.0109[c]	272±6[h]	0.6±0.2[m]
ACS	0.0015[a]	630±58[i]	8.8±0.8[n]
PCACS 0.5 STMP	0.0085[d]	834±57[j]	5.5±0.2[o]
PCACS 1.0 STMP	0.0151[e]	516±3[k]	4.3±0.3[p]

[1]Coefficient of variation <10%
[2]Averages of at least two replicates and the corresponding standard deviation are reported.
Equal letters in the same column indicate no significant differences between systems ($p>0.05$).
Native cassava starch (NCS), native cassava starch cross-linked with 0.5 g 100 g^{-1} STMP (PCNCS 0.5 STMP), native cassava starch cross-linked with 1.0 g 100 g^{-1} STMP (PCNCS 1.0 STMP), acetylated cassava starch (ACS), acetylated cassava starch cross-linked with 0.5 g 100 g^{-1} STMP (PCACS 0.5 STMP), acetylated cassava starch cross-linked with 1.0 g 100 g^{-1} STMP (PCACS 1.0 STMP).

Table 2. Particle size distribution and z-pot for starch nanoparticles obtained by dialysis technique.

Starch	Size distribution			z-pot[3] (mV)
	Peak 1 (d, nm)[1]	Peak 2 (d, nm)	PdI[2]	
NCS	36±4 (100)[a]	N.O.	0.367	-4.3±0.4 (100)[d]
NCS+KS	37±3 (100)[a]	N.O.	0.346	-12±3 (100)[e]
ACS	29±2 (90)[b]	205±18 (10)[c]	0.437	-7±2 (90)[f] -34±7 (10)[g]
ACS+KS	23±5 (90)[b]	255±51 (10)[c]	0.408	-8.3±0.5 (90)[f] -44±4 (10)[g]

[1] d: hyrodynamic diameter of the particle. N.O.: not observed.
[2] PdI: polydispersity index, represents the heterogeneity of sample sizes. Values lower than 0.5 are recommended.
[3] z-pot: zeta-potential
Values in brackets represent the % volume (size) or % area (z-pot) of the distribution. Averages of at least three replicates and the corresponding standard deviation are reported.
Equal letters in the same column indicate no significant differences between systems ($p>0.05$).

Table 3. Particle size distribution for microparticles obtained by the dialysis technique.

Starch	Size distribution[1]		
	d(0.1) (μm)	**d(0.5)** (μm)	**d(0.9)** (μm)
NCS	5.0±0.5[a]	15.6±0.3[d]	56±3[g]
NCS+KS	7.9±0.5[b]	14.6±0.3[e]	27±3[h]
ACS	20.8±0.2[c]	46±1[f]	87.3±0.4[i]
ACS+KS	22±1[c]	46.3±0.5[f]	84±5[i]

[1] d(0.1), d(0.5) and d(0.9): Diameter 10, 50 or 90% of the cumulative population.

Equal letters in the same column indicate no significant differences between systems ($p>0.05$).

Table 4. Particle size distribution for starch microparticles obtained by precipitation in ethanol technique.

Starch	Size distribution[1]		
	d(0.1) (μm)	**d(0.5)** (μm)	**d(0.9)** (μm)
NCS	30±7[a]	71±13[d]	171±38[f]
NCS+KS	31±5[a]	85±19[d]	227±47[f]
ACS	8±1[b]	20±3[e]	58±6[g]
ACS+KS	14±2[c]	26±4[e]	48±8[g]

[1] d(0.1), d(0.5) and d(0.9): Diameter 10, 50 or 90% of the cumulative population.
Equal letters in the same column indicate no significant differences between systems ($p>0.05$).

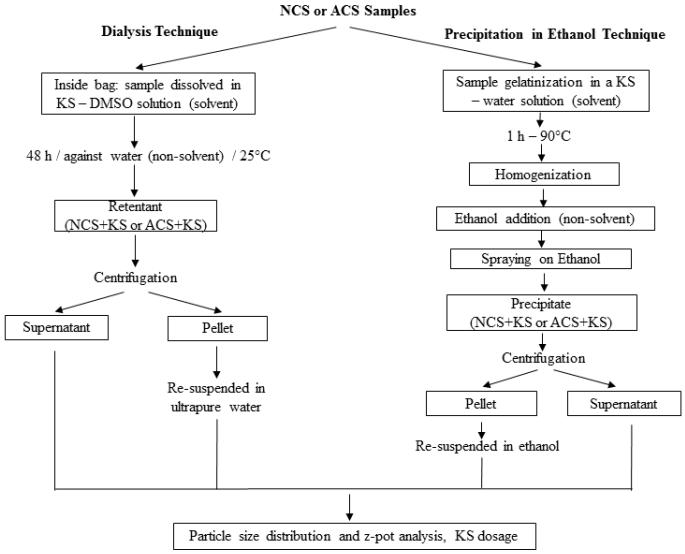

Figure 1

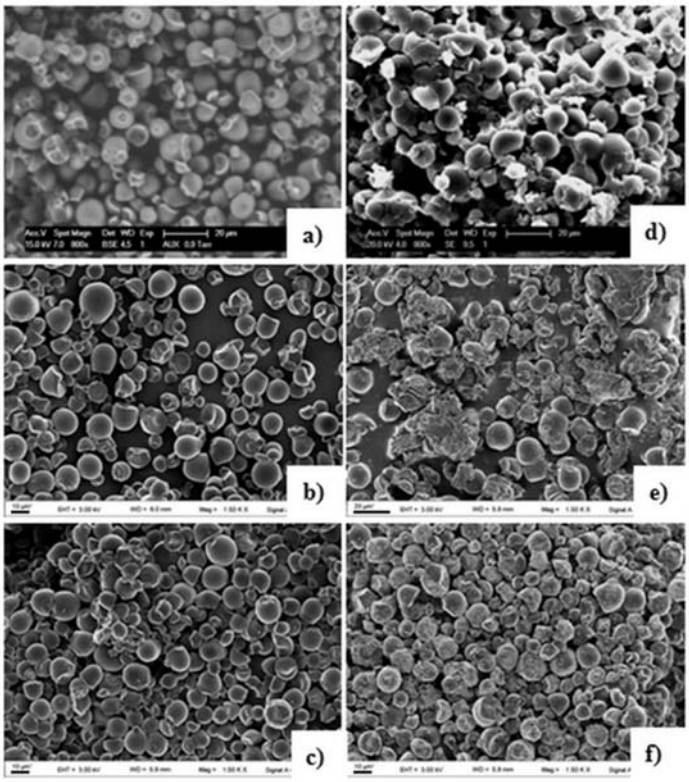

Figure 2

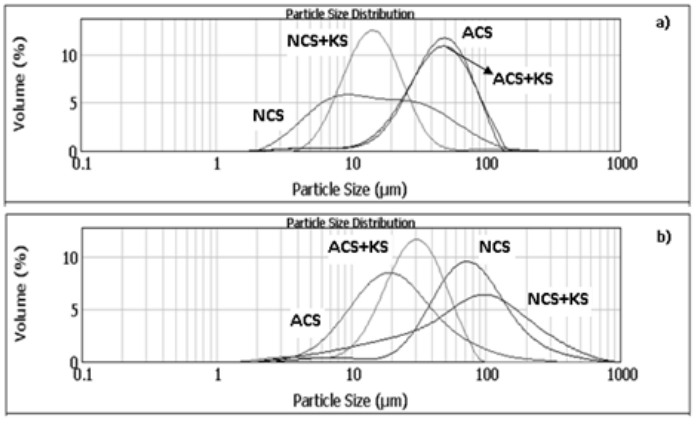

Figure 3

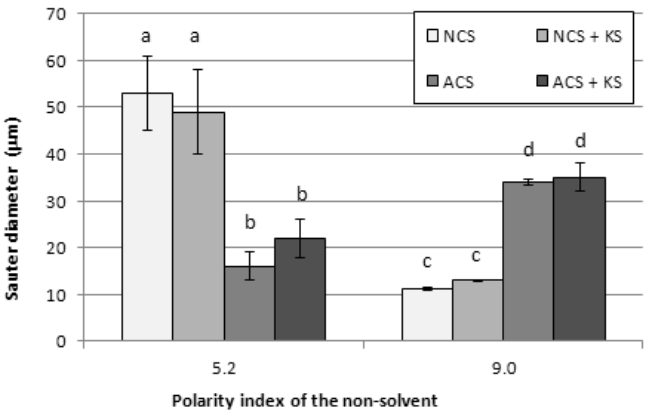

Figure 4