

## ANTIBACTERIAL THERMOPLASTIC STARCH-CHITOSAN BASED MATERIALS PREPARED BY MELT-MIXING

Liliana C. Tomé,<sup>a</sup> Susana C. M. Fernandes,<sup>a</sup> Patrizia Sadocco,<sup>b</sup> Jessica Causio,<sup>b</sup> Armando J. D. Silvestre,<sup>a</sup> Carlos Pascoal Neto,<sup>a</sup> and Carmen S. R. Freire,<sup>a,\*</sup>

Antibacterial thermoplastic starch-chitosan based materials were successfully prepared by melt-mixing. The effect of chitosan modification (quaternization and grafting of long carbon chains) on the properties of the materials was also studied. All the ensuing materials were characterized in terms of thermal stability, crystalline structure, mechanical performance, and antibacterial activity. The incorporation of chitosan and its derivatives into the thermoplastic starch matrices resulted in an increment in tensile strengths (up to 85%); however for higher chitosan contents (5 and 7.5 wt.%), a decrease on the Young's modulus (around 50%) was observed, together with an increment in the elongation at break, which can be attributed to the prevention of the retrogradation process. Finally, the thermoplastic starch materials incorporated with 7.5 wt% of the unmodified chitosan and of the water-soluble chitosan derivative had partial (about 20% reduction of CFU) and total bactericidal effect against *S. aureus*.

*Keywords:* Thermoplastic starch; Chitosan; Amphiphilic and water-soluble chitosan derivatives; Compounding; Melt-mixing, Thermal stability; Mechanical properties; Antibacterial activity

*Contact information:* a: CICECO, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; b: INNOVHUB – Divisione Carta, Piazza Leonardo Da Vinci, 16 20133 Milano, Italy; \*Corresponding author: cfreire@ua.pt

### INTRODUCTION

In view of the predictable dwindling of fossil resources and of the severe environmental problems caused by the massive use of conventional packaging, there is a considerable and growing interest in biodegradable materials obtained from natural polymers. In particular, polysaccharides, such as cellulose, chitin (and its derivative chitosan), and starch, due to their abundance, are considered as key raw materials for the production of chemical and materials for the biorefineries of the future (Belgacem and Gandini 2008). For example, starch is one of the most commonly used raw materials for the preparation of biodegradable films because of its abundance, low cost, renewable nature, biocompatibility, and non-toxicity (Carvalho 2008). Moreover, starch is relatively easy to convert into a thermoplastic material, commonly known as thermoplastic starch (TPS), by the disruption of the polymeric chains interactions, under specific conditions of temperature and/or mechanical energy, in the presence of a plasticizer, such as water and glycerol (Chivrac *et al.* 2009).

In order to improve the physical and functional properties of starch films, which is an important aspect in some applications as in functional food packaging, blending with other biopolymers and substances with specific properties, in particular antioxidant and

antimicrobial activities, has been proposed. For example, several studies on the preparation and characterization of starch-chitosan films have been published so far (Bourtoom and Chinnan 2008; Chang *et al.* 2010; Chillo *et al.* 2008; Garcia *et al.* 2006; Liu *et al.* 2009; Shen *et al.* 2010; Vázquez *et al.* 2009; Xu *et al.* 2005; Zhong *et al.* 2011), because of their enormous potential in food preservation and packaging technology. This is mainly due to the fact that chitosan exhibits high antimicrobial activity against pathogenic and spoilage microorganisms, including both Gram-negative and Gram-positive bacteria and fungi (Mohammed 2010; No *et al.* 2002).

However, most studies that have reported on the preparation of starch-chitosan films involved the pre-gelatinization of starch followed by addition of chitosan and casting. This is a simple but extremely time-consuming methodology that is not suitable for production at an industrial scale. Thermoplastic starch (TPS) could also be obtained in common melt-mixing or extrusion machines; however only a few recent works reported the effect of temperature and screw speed on the mechanical and barrier properties of TPS (Pelissari *et al.* 2009, 2012; Pelissari *et al.* 2011), apparent opacity, and blow-up ratio of starch-chitosan-oregano essential oil films.

In the present work, we describe the preparation of homogenous starch-chitosan materials by compounding in a melt-mixing equipment. The effect of the chemical modification of chitosan (quaternization and conversion into amphiphilic derivatives) on the final properties of starch-based materials was also investigated for the first time. All the obtained materials were characterized in terms of morphology, crystallinity, thermal and mechanical properties, and antibacterial activity.

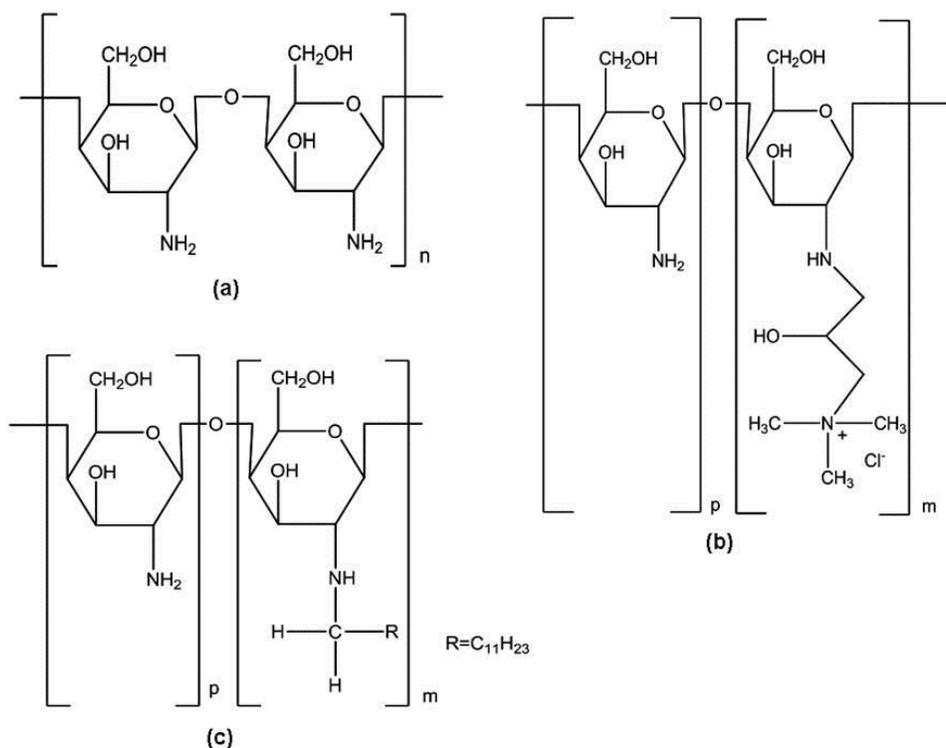
## EXPERIMENTAL

### Materials and Methods

Corn starch (27% amylose), glycerol (99.5%), and stearic acid (95%) were supplied by Sigma-Aldrich and used as received. Chitosan (CH, Fig. 1a) was purchased from Mahtani Chitosan PVT. Ltd. (India) in the powder form. Chitosan was purified by dissolution in a 1% (v/v) aqueous  $\text{CH}_3\text{CO}_2\text{H}$  solution, filtered, and precipitated by neutralizing with NaOH up to a pH of 8.5. The ensuing precipitate was washed with distilled water until a neutral pH. The degree of deacetylation was found to be 97% by  $^1\text{H}$  NMR (Desbrières *et al.* 1996), and the viscosity-average molar mass was  $350\,000\text{ g}\cdot\text{mol}^{-1}$ , obtained at  $25\text{ }^\circ\text{C}$  using the published Mark-Houwink constants (Rinaudo *et al.* 1993).

In order to synthesize a quaternary ammonium derivative of chitosan (water-soluble derivative - WSCH), following a described procedure (Seong *et al.* 2000), 5.0 g of purified CH were dissolved in 250 mL of 1% (v/v) aqueous  $\text{CH}_3\text{CO}_2\text{H}$  solution; glycidyltrimethylammonium chloride (GTMAC-purchased from Fluka, 90% purity), with a GTMAC/chitosan  $\text{NH}_2$  groups molar proportion of 4/1, was added with stirring. This mixture was kept at  $65\text{ }^\circ\text{C}$  for 24 h under a  $\text{N}_2$  atmosphere. The ensuing water-soluble chitosan derivative (WSCH, Fig. 1b) was precipitated and washed with ethanol. The substitution degree of the amino groups was around 30%, determined by  $^1\text{H}$  NMR (Desbrières *et al.* 1996).

An amphiphilic *N*-alkyl chitosan derivative, (ACH, Fig. 1c), was obtained by reaction of chitosan with dodecyl aldehyde, to generate the corresponding imine, which was then reduced using sodium cyanoborohydride ( $\text{NaBH}_3\text{CN}$ ), following the procedure described by Desbrières *et al.* (1996). The substitution degree was around 30%, as determined by  $^1\text{H}$  NMR.



**Fig. 1.** Chemical structures of (a) chitosan, (b) water-soluble chitosan and (c) amphiphilic chitosan

### Biocomposites Preparation

In preparation, the CH and ACH were dissolved in 1% (v/v) aqueous  $\text{CH}_3\text{CO}_2\text{H}$  solution, and WSCH was dissolved in water in order to avoid the formation of agglomerates. Then, starch (45%), glycerol (30%), water (25%), and chitosan (or its derivatives) solution in different percentages (1, 2.5, 5, and 7.5 wt.% with respect to the amount of starch, in a dry basis) were then pre-mixed in polyethylene bags until a homogeneous mixture was obtained. Stearic acid, used as demoulding agent, was also added at low concentration (0.5 wt.%). Then the blends were prepared using a Plastograph EC (Brabender) mixer. The mixtures (Table 1) were loaded into the mixer chamber working at  $120^\circ\text{C}$  and 60 rpm during 6 min for unfilled TPS and 15 to 30 min for the materials with chitosan and their derivatives, to allow for the release of the excess moisture, in order to reach comparable water contents of the final composites. Subsequently, the materials were molded in an injection moulding machine (Thermo-Haake Minijet II) for tensile tests (according to ISO 527-2-5A).

Before characterization, all samples were kept in a conditioning cabinet of 50% relative humidity at 25 °C to ensure the stabilization of their water content.

**Table 1.** Identification of the Starch-Chitosan Based Materials Prepared in this Study

Sample	Type of chitosan	wt. %
TPS	-	-
TPS-CH1	Chitosan	1
TPS-CH2.5		2.5
TPS-CH5		5
TPS-CH7.5		7.5
TPS-WSCH1	Water-soluble chitosan	1
TPS-WSCH2.5		2.5
TPS-WSCH5		5
TPS-WSCH7.5		7.5
TPS-ACH1	Amphiphilic chitosan	1
TPS-ACH2.5		2.5
TPS-ACH5		5

### Biocomposites Characterization

The prepared materials were characterized using thermogravimetry (TGA), X-ray diffraction (XRD), tensile tests, scanning electron microscopy (SEM), and antibacterial activity.

TGA essays were carried out with a Shimadzu TGA 50 analyzer equipped with a platinum cell. Samples were heated at a constant rate of 10 °C/min from room temperature to 800 °C under a nitrogen flow of 20 mL/min. The thermal decomposition temperature was taken as the onset of significant (*ca.* 0.5 %) weight loss, after the initial moisture loss.

X-ray diffraction (XRD) patterns were measured with a Phillips X'pert MPD diffractometer using Cu K $\alpha$  radiation. The scattered radiation was detected in the angular range from 5° to 40° ( $2\theta$ ).

Tensile tests were performed under ambient conditions on an EMIC DL-3000 device using a load cell of 50 Kg and a deformation rate of 0.5 mm/s. Tensile strength, tensile modulus, and elongation at break were calculated using the Tesc software (version 3.01). The results presented are an average of 5 determinations.

SEM micrographs of the composites' fractured surfaces were obtained using a SU-70 instrument. Samples were coated with evaporated carbon.

Antibacterial activity of biocomposites, in the powdered form, was tested by the Dynamic Shake Flask Test Method (ASTM E2149-10) towards *Staphylococcus aureus*. 30 mg of sample were analysed in 5 mL phosphate buffer + 5% Nutrient Broth, inoculated by an initial concentration of bacteria of  $1 \times 10^5$  CFU/mL. The samples were incubated for 24 h at room temperature, under continuous shaking. The analysed samples were subjected to sterilization (by autoclave). The initial and final concentration of CFU were determined by bacteria plate counting on plate count agar medium, by plating dilutions of the bacteria suspension, at T0 (zero contact time) sampling from blank flask (inoculated buffer), and at time 24 hours sampling the bacteria suspension from all the flasks.

## RESULTS AND DISCUSSION

The TPS-chitosan materials prepared in this study were very homogeneous, without formation of visible agglomerates, indicating that the presence of chitosan had not affected the gelatinization process of starch granules and it was well incorporated into the TPS matrix; therefore the preparation of TPS-chitosan materials could be successfully achieved by simple melting–mixing. The absence of chitosan agglomerates or unplasticized starch granules was confirmed by SEM as shown in Fig. 2 for the TPS materials with 7.5 % of CH and WSCH derivative. The surface of the TPS-chitosan materials (obtained by injection moulding) was very uniform and flat.

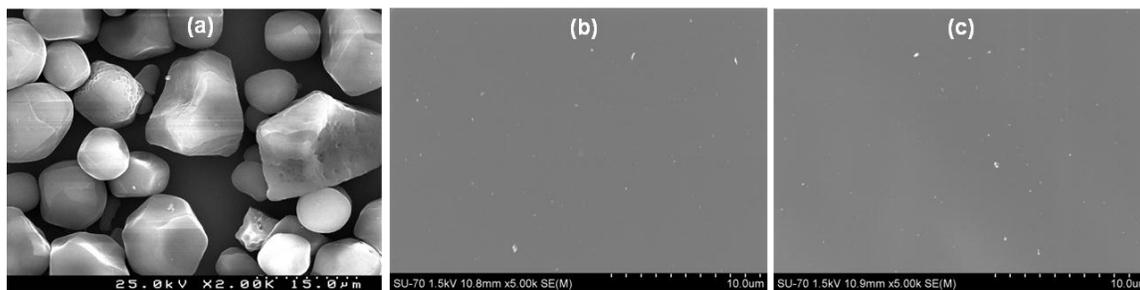


Fig. 2. SEM micrographs of (a) native starch and the fractured surfaces of (b) TPS-WSCH7.5 and (c) TPS-ACH5

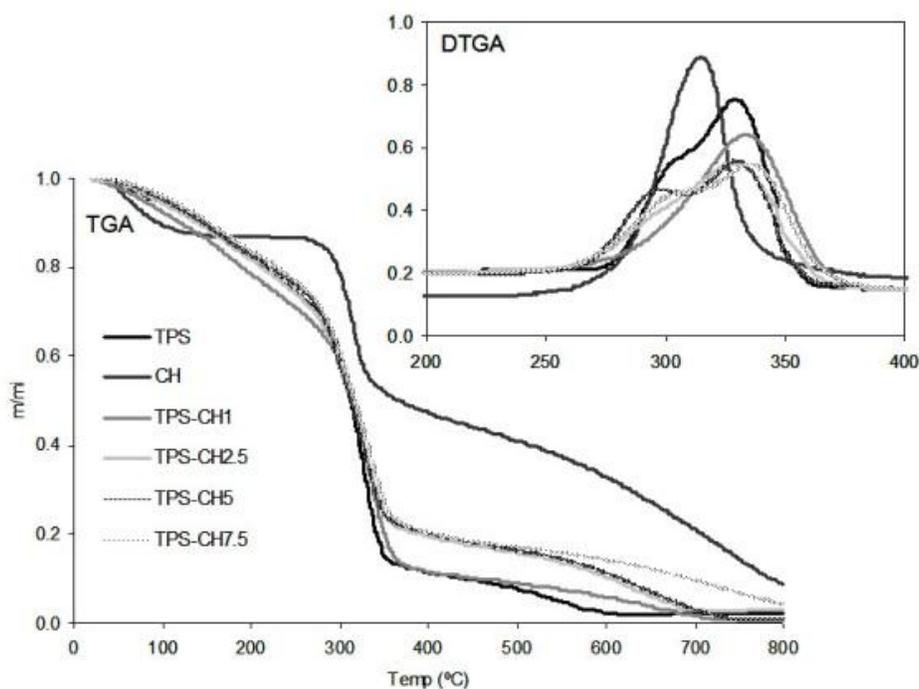
### Thermal Properties

The thermal stability and degradation profile of the TPS-chitosan materials were studied by TGA (Table 2, Fig. 3). The TGA tracings of all materials are a combination of those of the TPS matrix and of the corresponding chitosan filler used. The initial mass losses, at around 100 and 200 °C, before the onset degradation temperature, were associated with the volatilization of the plasticizers, water, and glycerol, respectively. The maximum degradation step at 299 to 338 °C is attributed to the degradation of the polysaccharides. The incorporation of chitosan fillers into the TPS matrix resulted in a slight decrease of the initial degradation temperature of the prepared materials (up to 17 °C for 7.5 wt.% of CH), which could be explained by the lower stability of chitosan and

in particular of its derivatives. Contrarily, the maximum degradation temperature was in most cases displaced for higher temperatures, which is obviously a result of the good compatibility between the two polysaccharide components of the composites.

**Table 2.** Thermal Data of All Starch-Chitosan Based Materials Prepared in This Study

Sample	$T_d$ (°C)	$T_{d_{max}}$ (°C)
TPS	273.4	330.4
CH	261.3	316.8
WSCH	215.1	269.4
ACH	222.3	317.9
TPS-CH1	259.3	336.6
TPS-CH2.5	263.9	330.2
TPS-CH5	261.6	331.6
TPS-CH7.5	255.9	338.1
TPS-WSCH1	269.7	331.6
TPS-WSCH2.5	263.5	306.2
TPS-WSCH5	257.7	300.6
TPS-WSCH7.5	256.8	299.0
TPS-ACH1	263.2	338.2
TPS-ACH2.5	265.6	334.4
TPS-ACH5	259.3	336.1



**Fig. 3.** Thermogravimetric curves for unfilled TPS, CH, and corresponding TPS-chitosan materials

## X-Ray Diffraction

The X-ray diffraction patterns of native corn starch, thermoplastic starch, chitosan and its derivatives, and corresponding materials are shown in Fig. 4. Native corn starch showed a typical A-type X-ray diffraction pattern with strong peaks at  $2\theta$  15.4°, 17.4°, 18.7°, and 23.4°. Retrograded thermoplastic starch gives both B and V-type diffraction patterns (Buléon *et al.* 1998), with the main peaks at  $2\theta$  12.9°, 16.8°, and 19.8°, respectively. The characteristic X-ray diffractogram of chitosan powder displayed two main peaks at around  $2\theta$  12° and 19°, which were assigned to the crystal forms I and II, respectively. Both chemical modifications of chitosan, but particularly the quaternization, led to an extensive decline in the crystallinity, since both derivatives have shown typical X-ray diffractograms of amorphous materials with a broad band centered at around  $2\theta$  20°.

The X-ray diffractograms of the TPS-chitosan materials showed typical diffraction peaks of both retrograded thermoplastic starch matrix and of chitosan (in the case of the unmodified chitosan). However, the increase in the chitosan contents seemed to disservice the retrogradation process, *i.e.* to promote the decrease of the TPS matrix crystallinity, since the peaks at  $2\theta$  12.9°, 16.8°, and 19.8° became less pronounced. This behavior is probably due to the fact that the chitosan chains are homogeneously distributed between the amylose and amylopectin chains, as indicated by the SEM analysis of the films, preventing their reorganization and therefore the retrogradation process. This phenomenon is particularly interesting because it will play an important role with respect to the mechanical properties of the materials, as will be discussed below.

## Mechanical Properties

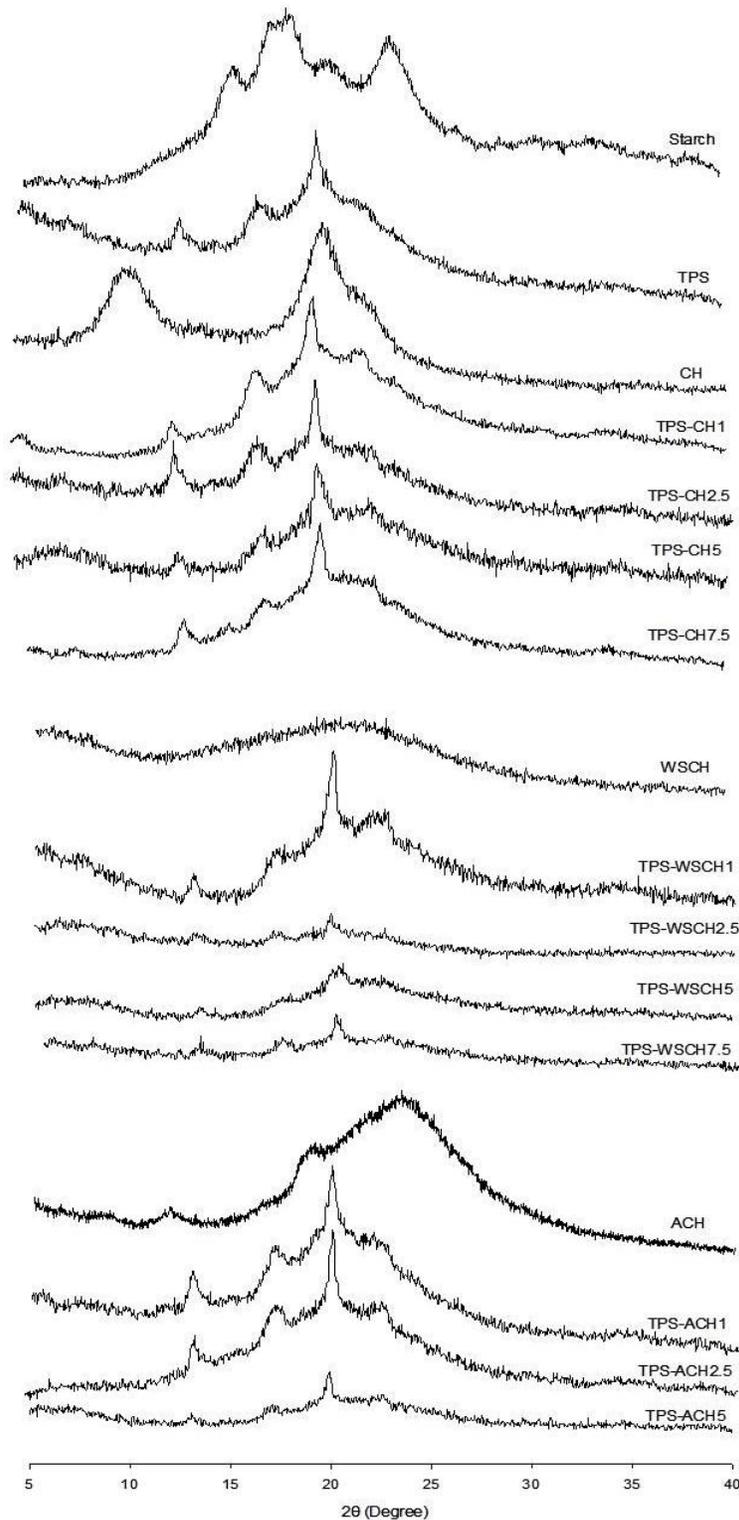
Although the addition of chitosan (and derivatives) to TPS was aimed at imparting antimicrobial properties, the effect of the incorporation of chitosan (and its derivatives) on the large strain behaviour of TPS-based materials was also investigated.

The tensile strength, Young's modulus, and elongation at break determined from the typical stress–strain curves are displayed in Fig. 5.

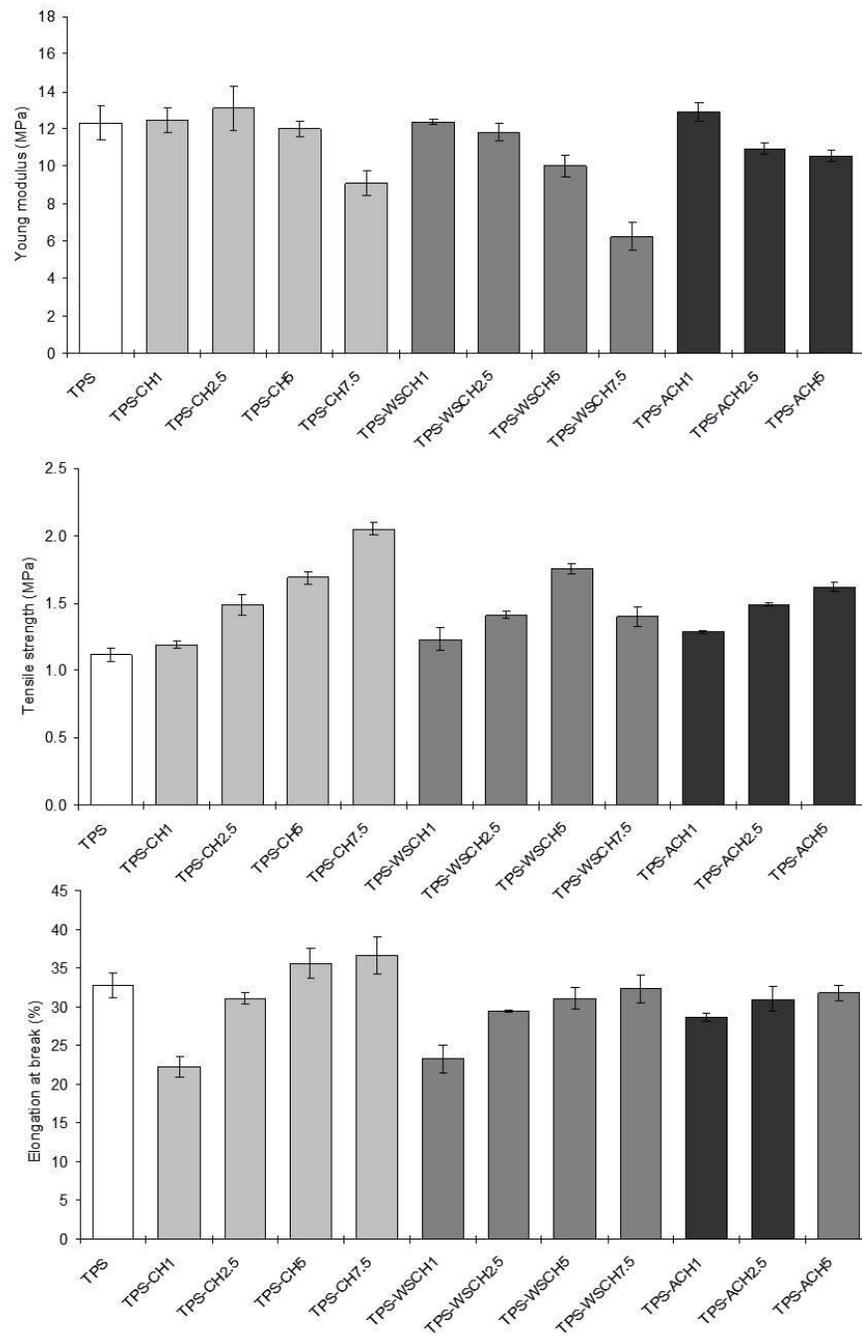
The presence of chitosan (or its derivatives) promoted a considerable increment of the tensile strength of the materials, when compared to TPS, increasing with the chitosan (or derivatives) content. This increment was more pronounced for the materials with pure chitosan (TPS-CH), where it was around 85% higher for TPS-CH7.5. The materials filled with the amphiphilic chitosan (TPS-ACH) showed the lowest increment of tensile strength, reaching around 45% for TPS-ACH5. TPS-WSCH materials showed an intermediate behaviour in terms of the tensile strength increments, reaching about 55% for TPS-WSCH5.0, although for TPS-WSCH7.5 the increment was only of 25%.

The Young's modulus was poorly affected for lower chitosan contents; however it decreased more considerably for the addition of 5.0 and 7.5 wt.% of chitosan. In the case of elongation at break, although a reduction was observed for the addition of 1wt.% of chitosan in comparison with TPS, this parameter tended then to increase with increasing amounts of chitosan. The decrease on the Young's modulus (and corresponding increase on elongation at break) observed for higher chitosan contents is certainly associated with

the decreases of the crystallinity of the TPS matrix previously observed for these samples due to the decline of the retrogradation process.



**Fig. 4.** X-ray diffractograms of native starch, unfilled TPS, chitosan and its derivatives, and all the TPS-chitosan materials

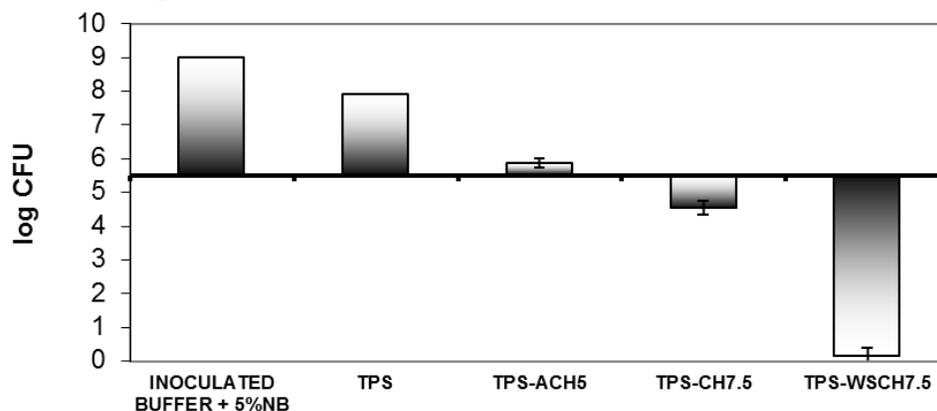


**Fig. 5.** The Young's modulus (top), tensile strength (middle), and elongation at breakage (bottom) of all the TPS-chitosan materials

In fact, chitosan samples appear to work more as functional fillers rather than as reinforcement elements. Moreover, the low amounts incorporated are probably not sufficient to induce substantial modifications of the mechanical performance of the TPS materials.

## Antibacterial Activity

The antibacterial activity of TPS-chitosan based composites against *S. aureus* is shown in Fig. 6.



**Fig. 6.** Antibacterial activity on *S. aureus* of thermoplastic starch-chitosan based materials. The obtained results are compared with reference sample (unfilled TPS) as well as inoculated broth added with 5% of Nutrient Broth (NB). The dark line refers to the initial inoculum (log CFU at time 0 = 5.5).

As expected, the control TPS sample did not show any inhibitory effect on *S. aureus*. However, the TPS films incorporating 5 wt.% of amphiphilic chitosan (TPC-ACH5) had a bacteriostatic effect, while those with 7.5 wt.% of unmodified chitosan (TPS-CH7.5) and of water-soluble chitosan derivative (TPS-WSCH7.5) had partial and total bactericidal effects, respectively, against this microorganism. The lower effect of the amphiphilic chitosan-based TPS is certainly due to the lower amount of ACH incorporated. However, the higher bactericidal effect of the TPS sample with the water soluble chitosan derivative, with respect to that with unmodified chitosan, is associated with the presence of quaternary ammonium groups in the chitosan backbone, which are well known to induce higher antibacterial activity (Seong *et al.* 2000).

## CONCLUSIONS

1. Thermoplastic starch-chitosan based materials were effectively prepared by melt-mixing.
2. The incorporation of chitosan and its derivatives (water soluble and amphiphilic chitosan derivatives) into the thermoplastic starch matrix had in general a poor effect on the mechanical properties of the materials. However for higher contents (5 and 7.5 wt%) the retrogradation process is prevented and a decrease in the Young's modulus was observed.
3. The thermoplastic starch materials loaded with 7.5 wt.% of the unmodified chitosan and of the water-soluble chitosan derivative had partial and total bactericidal effect against *S. aureus*.

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