

EFFECTIVENESS OF CHITOSAN AS ANTIMICROBIAL AGENT IN LDPE/CS COMPOSITE FILMS AS MINCED POULTRY MEAT PACKAGING MATERIALS

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Low density polyethylene (LDPE)/chitosan (CS) composites and stratified composites have been tested for minced poultry meat packaging according to well-known standard methods used in food industry. It has been established that the composites containing 3-6 wt% CS or nanocomposites containing chitosan/nanoclay and 0.5 wt% vitamin E show superior properties (with respect to appearance, smell, bouillon after boiling and sedimentation, pH, reaction with H₂S and total number of germs), compared with commercially available foils, when applied to meat products. It was found that the number of germs decreased for meat packed in the obtained compositions by 10⁴-10⁵ times. The best values have been obtained with stratified composites obtained by covalent bonding and covered with chitosan by electrospaying.

The major characteristics of the LDPE foils, such as rheological behaviour, variation in crystallinity and permeability by treatment with chitosan and vitamin E, were studied, as they are important for packaging applications. The sensory methods, XRD, rheological and oxygen transmission rate analyses proved that the films accomplished all the requirements for minced poultry meat packaging.

INTRODUCTION

The intent of packaging is to maintain the cleanliness and sterility of the product from the manufacturing plant through transport, shelf-life and storage and to prevent undesirable impacts on quality, including microbiological and physio-chemical alteration. So, depending on the product, the marketer has to select the packaging material to protect the product.¹

Packaging protects foodstuffs during processing, storage and distribution from contamination by dirt (by contact with surfaces and hands), contamination by microorganisms (bacteria, moulds, yeasts), contamination by parasites (mainly insects), contamination by toxic substances (chemicals), influences affecting colour, smell and taste (off-odour, light, oxygen and other gases), loss or uptake of moisture

(evaporation or water absorption). However, the growth of microorganisms, which are already present in meat and meat products, cannot be interrupted through packaging only. To halt or reduce microbial growth, packaging has to be combined with other treatments, such as refrigeration, which will slow down or stop the further growth of microorganisms, or heating/sterilization, which will reduce or completely eliminate contaminating microorganisms, or by using antimicrobial and antioxidant agents.²

A range of synthetic materials suitable for meat packaging are available mainly in the form of plastic films or foils.

Packaging films must be flexible, light weight, odourless, hygienic (clean and toxicologically

harmless), easy to recycle, must have mechanical strength, resistance to hot and cold temperatures, and to oils and fats, good barrier properties against gases, sealing capability and low cost.³

Polyethylene packaging as sheets or films is suitable to wrap, seal, and protect consumer goods. Polyethylene, which is obtained from petroleum polymers, is ideal for accomplishing these tasks, since the material is both durable and resistant to environmental hazards. Polyethylene packaging is considered superior to any other material for this purpose and its use is widespread in the flexible packaging market.⁴ Polyethylene packaging is resistant to damage when coming into contact with chemicals or solvents, and is able to withstand friction.

Food package testing consists of several distinct testing streams, seal strength testing and package integrity (leak) testing, resistance to gas transmission and also under simulated conditions for commercial use.

Meat and meat products alteration depends on their nature, microorganisms' concentration, meat type, storage relative humidity and temperature. Meat spoilage starts with the growth of aerobic bacteria on the surface (*Pseudomonas*, *Bacillus*, *Proteus*, *Streptococcus*), then the muscular fibre is affected by protease action (mainly *Proteus*) and continues with anaerobic spoilage mediated by anaerobic bacteria (*Escherichia*, *Staphylococcus*, *Clostridium*). Meat spoilage is accompanied by organoleptic modifications related to aspect, colour, consistence and smell. There are the following types of meat alterations: (i) at the surface, occurring very slowly at 0-10 °C, and (ii) at high humidity, of 80-90%, favouring the growth of aerobic bacteria, as psychrophiles or cryophiles and psychrotrophs, such as *Pseudomonas* and *Psihrobacter*, on the meat surface. At a concentration of 10^7 colony-forming units (CFU) of bacteria/cm², the putrefaction smell can be detected, while at a concentration of 10^8 CFU/cm², this smell is associated with the formation of a characteristic mucus, because of the microbial colonies (*Pseudomonas fluorescens*, *Pseudomonas ambigua*, *Pseudomonas fragi*, *Pseudomonas putida*, *Aeromonas*, *Streptococcus*, *Bacillus*, *Lactobacillus*, *Leuconostoc*) and with the modification of the meat colour by oxidation of the oxyhemoglobin (red) to methemoglobin (brown).⁵ Some microorganisms can produce pigments on the meat surface: red spots produced by *Serratia* bacteria species and blue spots

produced by *Pseudomonas synsylenea*. If the meat is stored at humidity lower than 75%, it will get mouldy by the action of some moulds and yeasts. It is visible after 1-2 weeks when water activity (a_w) is low. The moulds that are present and develop on the meat surface under refrigeration conditions are: *Cladosporium herbarum*, *Sporotrichum carnis*, *Thanidium elegans* and *Penicillium* species. The yeasts are the psychrotrophs: *Candida*, *Rhodotorula*, *Debaryomyces*.

Another step is a fermentative process by autolytic bacteria, which occurs when the meat is piled up before cooling. It is produced by bacteria of the *Bacillus* genus (*B. megatherium*, *B. subtilis* - *mesentericus*), when their concentration is higher than 10^3 CFU/g and the meat smells sour. Deep putrefaction is due to the internal contamination when the meat is kept in the temperature range 20-45 °C, or the cooling is not applied immediately after slaughter, and because of unsuitable climatization of the storage spaces. In the first step of this process *Clostridium perfringens* anaerobic bacteria act, while in the second step, anaerobic bacteria of putrefaction *C. sporogenes* and *C. putreficus* come into play.

Poultry meat spoilage is produced by bacteria of the *Pseudomonas* genus and to a lower extent by *Aeromonas putrefaciens*, *Moraxella* and *Acinetobacter* genus.⁶ If the concentration of the *Pseudomonas* reaches 10^8 CFU/g, a mucus is formed. Frequently found pathogen microorganisms are: *Salmonella*, *Clostridium perfringens*, *Clostridium botulinum*, *Staphylococcus aureus*, *Yersinia enterocolitica*.⁷

Good barrier properties are essential features in order to ensure exclusion of oxygen and prevention of evaporation of product moisture. Oxygen negatively affects unpackaged meat and meat products during prolonged storage periods. It changes the red meat colour to grey or green and causes oxidation and rancidity of fats, resulting in an undesirable off-flavour. The oxygen permeability of films used for the packaging of meat products varies. The lower is the oxygen permeability, the more efficient the protection of product quality. The best protection will be achieved using oxygen-proof packaging films together with vacuum packaging of the product. This ensures that practically no oxygen remains in the package and no oxygen will penetrate from the air into the packaged product. While oxygen is generally undesirable in packages of meat and meat products, there is one

exception where oxygen-permeable foils are desirable, namely for fresh ready-to-sell meat portions in self-service outlets. In this specific case, the utilization of oxygen-permeable foils produces a desirable bright red meat colour. On the other hand, fresh meat or fresh sausages, cooked ham, etc. have relatively high moisture content and will suffer considerable weight and quality losses by evaporation and drying during storage if such products remain unpacked. The packaging material must therefore be sufficiently water-vapour-proof.³

There are only a few published reports on testing the feasibility of CS-based antimicrobial packaging film through incorporation of CS into polyethylene film fabricated either by *in situ* polymerization⁸ or via peroxide initiated melt-compounding process^{9,10} or crosslinking in the presence of vinyl triethoxysilane.^{11,12} S. Park *et al.*¹⁰ evaluated the antimicrobial activity of CS-incorporated LDPE films, measured their film properties and determined that significant extensions of red colour shelf-life were observed in sliced fresh red meats stored in LDPE/CS films.

In our previous studies, LDPE/chitosan composites and nanocomposites were prepared by melting compounding¹³ and stratified composites

were also obtained by grafting chitosan onto the LDPE surface.¹⁴ Both types of materials showed satisfactory mechanical and thermal properties and excellent antimicrobial action.

The objective of this study was the comparative analysis of some new food packagings based on low density polyethylene (LDPE), chitosan (CS) and vitamin E (VE) and those commonly used in specific conditions of the commercialization of food products, such as minced poultry meat. To demonstrate the efficiency of these new materials in their actual usage as minced poultry meat packaging materials, specific examinations have been carried out, such as sensory analysis, chemical and microbiological analyses, the changes during the shelf-life of products of 48 hours were monitored, and, in addition, XRD, rheological and oxygen transmission rate analyses have also been performed.

EXPERIMENTAL

The tested antimicrobial LDPE/chitosan or chitosan nanocomposites obtained by melt processing or as stratified composites are given in Table 1. Details on composite preparation and materials' characteristics have been previously given.^{13,14}

Table 1
Tested LDPE/CS composite foils

Sample symbols	Composition and method of preparation
LDPE	Low density polyethylene – melt mixing processed
LDPE/3 CS	97% LDPE/3% CS – melt mixing
LDPE/3 CS nano	97% LDPE/3% CSnano – melt mixing
LDPE/3 CS/VE	96.5% LDPE/3% CS/0.5% vitamin E – melt mixing
LDPE/3 CS/IRG	96.5% LDPE/3% CS/ 0.5% Irganox – melt mixing
LDPE/3 CSnano /VE	96.5% LDPE/3% CSnano/0.5% vitamin E – melt mixing
LDPE/6 CS	94% LDPE/6% CS – melt mixing
LDPE/6 CSnano	94% LDPE/6% CSnano – melt mixing
LDPE/6 CS/VE	93.5 % LDPE/6% CS/0.5% vitamin E – melt mixing
LDPE/6 CS/IRG	93.5% LDPE/6% CS/0.5% Irganox – melt mixing
LDPE/6 CS nano/VE	93.5% LDPE/6%CSnano/0.5% vitamin E – melt mixing
PEc, EDC+NHS, CHT+VE, spread – stratified composite	Stratified composite: PE corona activated and coated with CS and vitamin E by coupling reaction with chlorohydrate of 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide and N-hidroxsuccinimide (EDC+NHS) by spreading method
PEc, EDC+NHS, CHT+VE, ES – stratified composite	Stratified composite: PEc, EDC+NHS, coated by electrospaying with CS/vitamin E

Minced poultry meat commercialized as refrigerated product, packed in polyvinyl chloride (PVC) tray covered with alimentary foil (LDPE) with the average weight of 1050 g and shelf-life of 48 h from packaging was used.

Environmental conditions, simulating those from the marketing retail system, have been created.

The products are approved by the National Sanitary Veterinary and Food Safety Authority in Romania.

Such products are continuously monitored by measuring the microbiological and physico-chemical parameters, according to the legislation in force.

The microbiological criteria for food products of the "minced poultry meat" category are the following: total number of germs varies between 1×10^2 - 1×10^5 (1×10^6) CFU/g, of which *Salmonella* germs/25 g: absent; *Aerobe colonies*: 5×10^5 CFU/g- 5×10^6 CFU/g; *Escherichia coli*: 50 CFU/g – 500 CFU/g.¹⁵ The microorganisms content of the minced poultry meat varies as a function of external contamination of a carcass or edible offal. At least five determinations have been performed on each set and only two could meet the admissible maximum limits.

Methods

Sensory analysis

The freshness exams of the samples are organoleptic, chemical and bacteriologic. Firstly, the organoleptic exam was done, because when the modifications at this level are evident, the other exams are not necessary anymore. Organoleptic properties are the aspect of food as experienced by the senses, including taste, sight, smell, and touch, in cases where dryness, moisture, and stale-fresh factors are to be considered.

The following parameters have been monitored by using standardized methods:

- SR EN ISO 5495:2007 – Sensory analysis. Methodology, testing by pairs comparison;
- SR ISO 6658:2007 – Sensory analysis. Methodology. This standard method presents the general principles for methodology of the sensory analysis;
- SR ISO 2917:2007 – Meat and meat products. pH measurement. Reference method;
- SR 9065-11:2007 – Meat and meat products. H₂S identification;
- SR ISO 7031:2008 – Poultry meat. Technical conditions of quality. Analysis method.

14 samples of minced poultry meat packed in LDPE/CS composite foils as active food packaging have been examined comparatively with the reference sample by sensory analysis, pH determination, reaction with H₂S and the total number of aerobic mesophyle germs (*Staphylococcus aureus*, *Salmonella sp.*, *Proteus vulgaris*, and *Yersinia enterocolitica*) before packaging and after 48 hours of shelf-life.

The general characteristics of the poultry meat at different stages of spoilage up to 48 h are mentioned below.

Fresh minced poultry meat has a bright light red surface colour, elastic consistence and characteristic taste and smell; deeper inside the colour is the same as on the surface, the meat is glossy and slightly humid; the muscular juice released is clear and in a small amount; the meat is elastic, compact in cross-section and does not leave an impression upon pressing with a finger; the smell is pleasant and barely detectable;

upon boiling the smell is also pleasant, a white colour foam is slightly observed and the broth is clear without flakes.

Relatively fresh poultry meat shows a thin film partially covered with sticky mucus, matte aspect in comparison with fresh meat, low consistence; the colour is darker, in cross-section it is humid and the serum is turbid; the meat is soft but elastic both at the surface and in section; the smell is slightly acidic or similar to that of pickled cabbage; on the surface, discoloured grey zones are remarked, mainly in the areas that come into contact with packaging materials, which tend to extend deeper; upon boiling the smell is acidic/fermentative, the foam of opalescent white-grey colour is formed in appreciable amount without flakes.

Spoiled poultry meat exhibits a humid sticky surface covered with a continuous layer of grey-green colour, the weak consistence, intense smell of putrefaction; in cross-section the meat is humid and very sticky, has a grey green colour, the serum released is significant, of reddish colour and slimy; after pressing and it exhibits persistent fingerprint; the smell of putrefaction is characteristic and is detectable both at the surface and in bulk; upon boiling an intense bad smell is evolved, the foam is consistent, of dark grey colour, the broth is turbid and with many flakes.

pH determination

Immediately after butchering, the pH of the meat is alkaline, but becomes very soon acidic, due to the carbon dioxide accumulation and because the muscular glucose and glycogen are enzymatically degraded to lactic and pyruvic acid. pH decrease favours the activity of the tissue cathepsins, which can hydrolyze a part of the proteins, various products resulting (ammonia, amines), which shift pH from neutral to alkaline. This phenomenon is favoured by increasing temperature and high microbial loading. With respect to pH, the meat categories are the following: fresh meat has a pH < 6; relatively fresh meat with pH = 6.2-6.6; spoiled meat with pH > 6.6. The pH was determined with a multi-parameter analyzer C835 from CONSORT (Belgium).

Hydrogen sulphide determination: Hydrogen sulphide results from thio-aminoacids decomposition during meat alteration. It is formed by the reaction with lead acetate, lead sulphide of black colour whose intensity is directly proportional with the degree of meat alteration. For fresh meat, the reaction is negative, no colour is formed after 15 minutes; for relatively fresh meat, a dark brown colour appears after 10 minutes, while for spoiled meat the colour formed is black.

Determination of the total number of mezophile aerobic bacteria: the number of the mezophile aerobic bacteria is indirectly assessed on the basis of the number of colonies generated by the cells of these microorganisms. By its values, the quality and stability during storage of the food products are appreciated.

The techniques consist in the use of two cultures Petri plates on agar (PCA) and 1 ml sample diluted ten times. Then the plates are incubated in aerobiose conditions at 30 °C for 72 h.

The assessment of the number of microorganisms per gram of sample from the number of colonies on selected plates was performed according to ISO 7218/2009. The total number of mezofile aerobic bacteria was determined by the relation:

$$CFU/g = \frac{\sum c}{V(n_1 + 0.1 \times n_2) \times d} \quad (1)$$

where: $\sum c$ = sum of the number of colonies on all used Petri plates; n_1 = number of Petri plates after the first dilution; n_2 = number of colonies after the second dilution, a.s.o; d is dilution at which the first counting was obtained; V = inoculum volume on each plate (1 ml). The required values are: fresh meat max. 5×10^5 CFU/g; relatively fresh meat = 5×10^6 - 5×10^8 CFU/g; spoiled meat $> 5 \times 10^8$ CFU/g.

Before experiments all samples were sterilized at 121 °C in an autoclave with electronic control.

The colonies have been counted by an Aco Lyte automatic counter.

X-ray diffraction measurements

X-ray diffraction measurements were performed using a 3003 TT Rich. Seifert Diffractometer (Ni filtered, with Cu Ka irradiation, $\lambda = 1.54 \text{ \AA}$). The 2θ scan range was varied from 1° to 50° with a step of 0.01 and a measuring time of 15 s per step. The crystallinity index has been evaluated as the ratio between crystalline peaks area (A_{cryst}) to total area under diffractogram (A_{total}):

$$CrI = \frac{A_{\text{cryst}}}{A_{\text{total}}} \quad (2)$$

Dynamic rheological measurements

The frequency dependent storage modulus (G'), loss modulus (G'') and complex viscosity (η^*) were determined by using an Anton Paar rheometer equipped with CTD450 parallel plate geometry, having a plate-plate geometry diameter of 25 mm. The specimens from the compression molded sheets were cut according to the diameter of the plate. The experiments were performed at 115 °C over the frequency range of 0.01-100 rad/s in the linear domain of viscoelasticity. The gap between the plates was set to 1 mm. The samples were placed onto the plate for 3 min to eliminate residual shear history, and then experiments were carried out immediately. The measuring device was equipped with a temperature unit that gave good temperature control ($\pm 0.05 \text{ °C}$). The viscoelastic behaviour was isothermally tested by oscillatory conditions of amplitude (AS) and frequency sweep (FS) at 115 °C.

Permeability tests

Permeability tests were performed with a Lyssy L100-5000 Manometric Gas Permeability Tester (ASTM D1434, DIN53380, ISO2556), using nitrogen, carbon dioxide and oxygen as test gases at 0% relative humidity (RH) and temperature = 23 °C.

All tests have been performed at least three times and the results are the average of these determinations.

RESULTS AND DISCUSSION

A summary of the results obtained by all the tests carried out by the above-mentioned sensory and microbiological methods is given in Table 2.

It can be remarked that all the characteristics of the minced meat packed in the LDPE/CS are superior to those of the control sample and of the sample packed with LDPE foil. The best results are obtained in the case of packaging using LDPE/CS/Vitamin E composites. Comparing LDPE/CS composites with LDPE/CS nanocomposites, the latter are superior. As regards the preparation method, the samples deposited by electrospraying are superior to those prepared by melt mixing.

Some images of the samples after 48 hours from expiration time are given in Figure 1, the effect of antimicrobial packaging on the minced meat quality being evident.

XRD results

Semicrystalline polyethylene contains Bragg reflections from the crystalline regions (orthorhombic). The most intense of them are the 110 ($2\theta = 21$ degrees) and 200 ($2\theta = 23.5$ degrees) reflections and at lower angles a halo characteristic of the amorphous structure.^{16,17} The diffractogram of chitosan consists of two typical crystalline peaks at $2\theta = 11.8^\circ$ and 22.9° .¹⁸

The X-ray diffractograms of LDPE/chitosan composites are similar to those of LDPE matrix – Figure 2, variations appearing in the intensity and shape of the peaks, and also the displacement of crystallographic planes can be seen – Table 3. By introducing nanoclays in the LDPE/CS composites, one can observe that the values for the (200) crystallographic plane are shifted to higher values, in comparison with the LDPE/CS composites values. The modification is very evident for composites containing 6 wt% CSnano and it is shifted to high 2θ in the composites containing vitamin E, probably because of some interactions between these components of the composites.

Table 2
 Characteristics of the minced poultry meat during examination

Parameters	Reference sample (fresh meat)	Characteristics after 48 h (entire shelf-life = 48 h) under normal conditions													
		Control*	LDPE/3 CS	LDPE/3 CSnano	LDPE/3 CS/VE	LDPE/3 CS/IRG	LDPE/3 CS nano/VE	LDPE/6 CS	LDPE/6 CS nano	LDPE/6 CS/VE	LDPE/6 CS/IRG.	LDPE/6 CSnano/VE	PEc, EDC+NHS, CHT+VE, spread	PEc, EDC+NHS, CHT+VE, electrospraying	LDPE
Aspect	Surface aspect: homogenous, light pink, bright	spoiled	spoiled	relatively fresh	fresh	spoiled	fresh	spoiled	relatively fresh	fresh	relatively fresh	relatively fresh	spoiled	fresh	spoiled
	Elastic consistence	spoiled	relatively fresh	relatively fresh	fresh	relatively fresh	fresh	relatively fresh	relatively fresh	fresh	relatively fresh	relatively fresh	relatively fresh	fresh	relatively fresh
Smell	In-depth humid and bright aspect	relatively fresh	relatively fresh	fresh	fresh	relatively fresh	fresh	relatively fresh	fresh	fresh	relatively fresh	fresh	fresh	fresh	relatively fresh
	Pleasant, species specific	spoiled	relatively fresh	relatively fresh	relatively fresh	relatively fresh	fresh	relatively fresh	relatively fresh	fresh	relatively fresh	relatively fresh	relatively fresh	fresh	spoiled
Bouillon after boiling and sedimentation	Transparent, clear, aromatic; On the surface, there separates a compact foam and fat islands; pleasant taste and smell	spoiled	relatively fresh	relatively fresh	relatively fresh	spoiled	relatively fresh	spoiled	relatively fresh	relatively fresh	spoiled	relatively fresh	relatively fresh	relatively fresh	spoiled
pH	5.7	6.9	6.7	6.3	6.1	6.6	6.0	6.8	6.3	6.2	6.6	5.9	6.3	5.8	6.5
Reaction with H ₂ S	negative	relatively fresh	relatively fresh	relatively fresh	fresh	relatively fresh	relatively fresh	relatively fresh	fresh	fresh	relatively fresh	fresh	relatively fresh	fresh	relatively fresh
Total number of germs, CFU/g	1.9×10^3	8.3×10^9	2.9×10^7	2.6×10^5	6.7×10^4	1.5×10^8	1.9×10^7	8.7×10^6	9.6×10^4	1.7×10^5	4.8×10^8	2.4×10^5	8.2×10^7	9.4×10^4	5.6×10^8

* Represents the reference sample after 48 h in the absence of polymeric composites

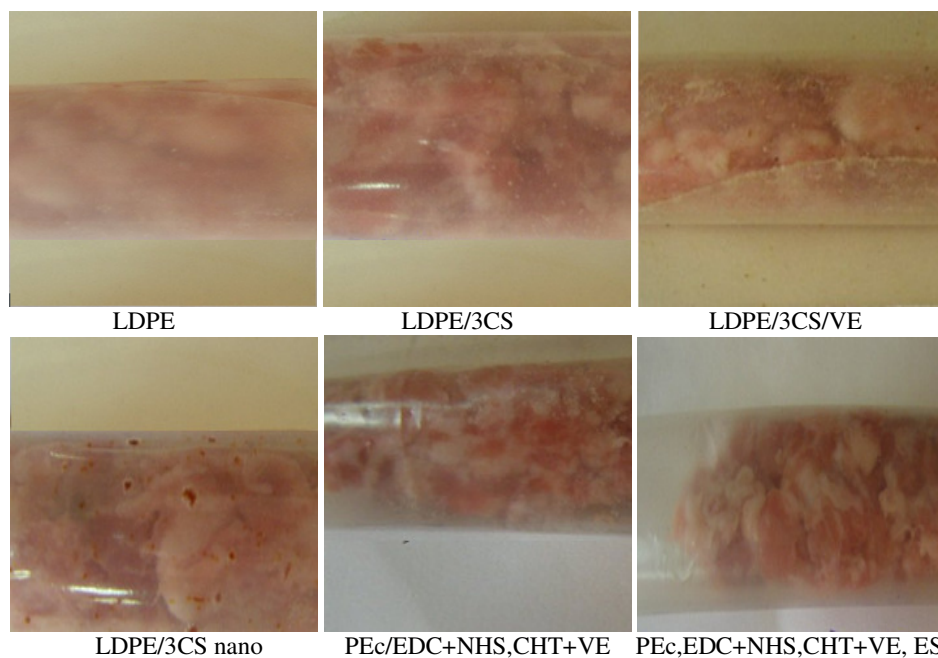


Figure 1: Microscopical aspects of the minced poultry meat packed in foils of LDPE/chitosan composites

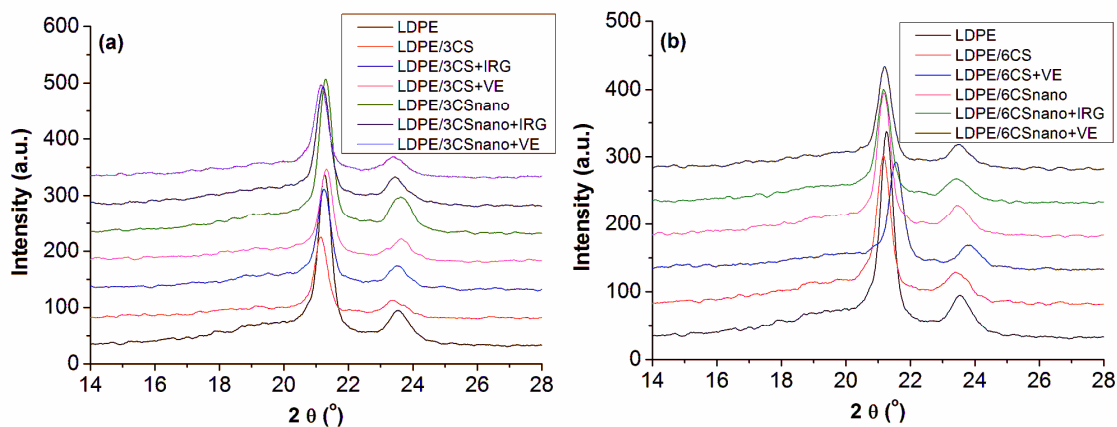


Figure 2: X-ray diffractograms of LDPE/chitosan composites with 3 CS (a) and 6 CS (b)

Table 3

Positions of the crystalline peaks (cr) and amorphous peak (am) of LDPE/CS composites

Sample	Peaks positions (2θ)		
	am	cr (110)	cr (200)
LDPE	20.36	21.26	23.58
LDPE/3CS	20.01	21.14	23.46
LDPE/3CS+IRG	20.23	21.24	23.54
LDPE/3CS+VE	20.18	21.31	23.63
LDPE/3CSnano	20.34	21.29	23.64
LDPE/3CSnano+IRG	20.22	21.21	23.50
LDPE/3CSnano+VE	20.14	21.15	23.44
LDPE/6CS	20.27	21.16	23.47
LDPE/6CS+VE	20.43	21.54	23.84
LDPE/6CSnano	20.31	21.18	23.51
LDPE/6CSnano+IRG	20.13	21.17	23.49
LDPE/6CSnano+VE	20.12	21.20	23.54

The crystallinity index of each sample was evaluated and the results are given in Figure 3.

A slight decrease in the crystallinity of all the samples can be remarked, which explains the slightly inferior mechanical properties to those of LDPE. Even so, they were maintained in satisfactory limits for utilizing the composites as food packaging foils.

Dynamic rheological measurements

Melt rheology of thermoplastic blends is vital to understand the structural property relationship and their processibility.¹² The presence of a stable viscoelastic region was confirmed by the time and strain sweep experiment before the frequency sweep. The results show that the samples were within the linear viscoelastic region.

The rheological behaviour of the LDPE/CS samples was investigated in the melt state for two different amounts of incorporated chitosan. Rheological evaluation started with the amplitude sweep test in order to establish the amplitude γ , which was determined as 5% for the studied systems. The viscoelastic parameters – storage modulus (G'), loss modulus (G'') and complex viscosity (η^*) – were followed as a function of the angular frequency, ω , – Figures 4-7. The results show that the composition in the blend affected the values of G' , G'' and η^* . The variation in G' and G'' corresponded to the energy change occurring during the dynamic shear process and is strongly dependent on the interaction between the polymers interphase in the blend.¹⁹

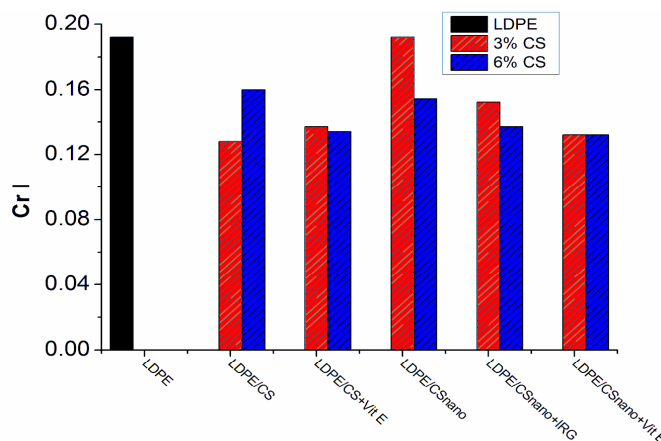


Figure 3: Crystallinity index from XRD data for LDPE/CS composites with 3% and 6% CS

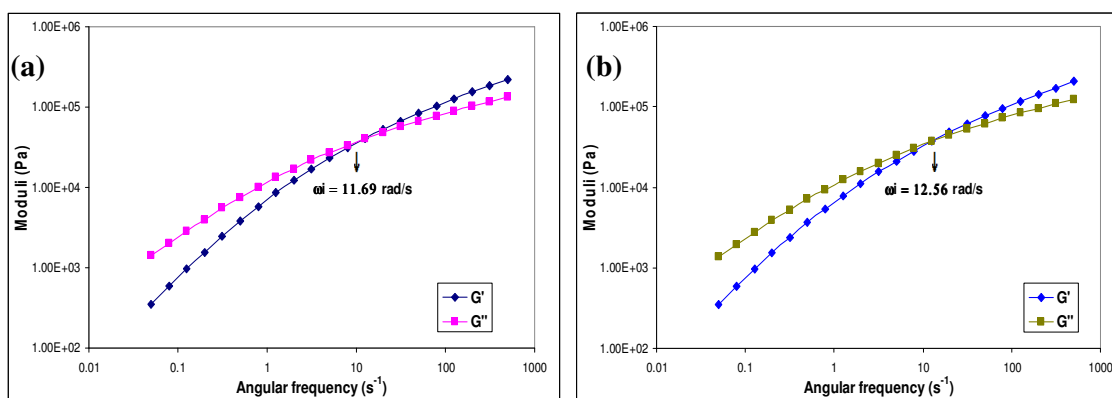


Figure 4: G' and G'' vs angular frequency for LDPE (a) and LDPE/6CS (b) samples

An increase in G' and G'' values with the increase in frequency was observed. This is due to the fact that at low frequency there is enough time

for the chains to unfold and they relax slowly. This high relaxation tends to reduce the G' and G'' values. However, when the polymer chains were

deformed at higher frequency, the entangled chain had less time for re-orientation, which increased the moduli.²⁰ Figure 4 also revealed that the G'' curves were well above the G' curves. This shows that chitosan interacted poorly with LDPE and the components were weakly associated with each other. As chitosan is hydrophilic and polyethylene is hydrophobic in nature, the resultant blend of these two components was immiscible. It was also observed that G'' values increased by chitosan addition. The complex viscosity (η^*) increased with an increasing chitosan amount in the blend and decreased with increasing frequency – Figure 7. This was due to the strong shear thinning behaviour of the blend in molten state. A greater amount of chitosan loading will cause greater entanglement of the chains and thus gradually increase the η^* .

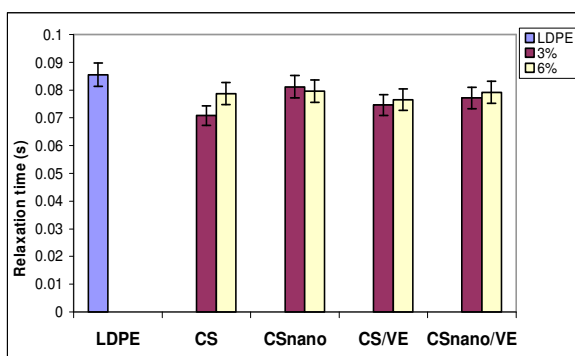


Figure 5: Relaxation time for studied materials

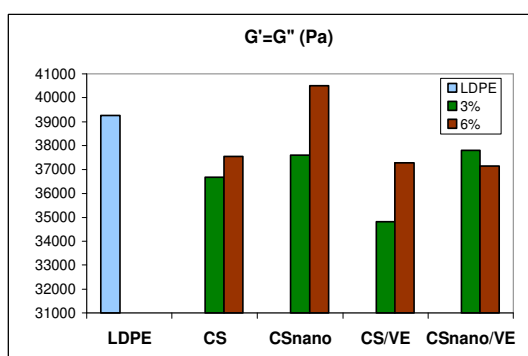


Figure 6: Values of cross-over points of the dynamic moduli ($G'=G''$) for LDPE/CS composites

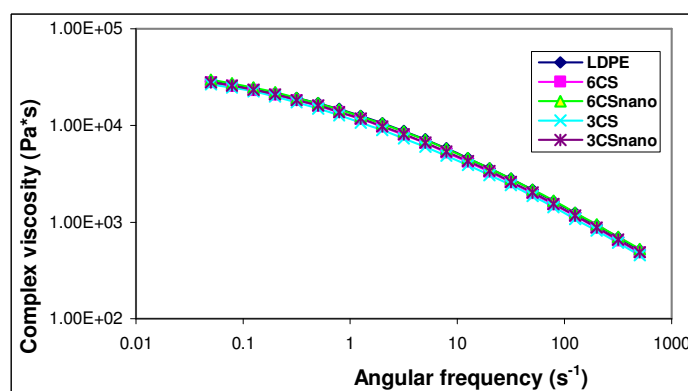


Figure 7: Variations of complex viscosity as a function of angular frequency for neat LDPE and studied composites

The obtained results show that all the samples behave as viscoelastic materials, G' and G'' crossing function of the angular frequency (ω). At low ω values, up to the crossover point (ω_i), most of the materials have a predominant viscous behaviour, $G'' > G'$, while over ω_i , this changes to an elastic one ($G' > G''$).

The crossover frequencies take higher values for the composites, compared to LDPE, and the composites containing vitamin E show the highest values. The crossover of G' and G'' at high frequency should indicate that the PE melt should relax in a much shorter time in the presence of chitosan and vitamin E. The rate at which the global structure changes in response to the change in flow is measured by the characteristic relaxation time – Figure 5. From crossover frequency (ω_i), the relaxation time (θ) can be calculated as $\theta = 1/\omega_i$.

A general observation is that the samples containing 6 wt% CS and those with CSnano

require a slightly increased relaxation time (θ_i), respectively a decreased crossover point for melt

state relaxation, although the difference between composites containing different amounts of chitosan on the material is not high. The most obvious modification in rheological behaviour is that related to the dynamic moduli, highlighted also through changes in the values of crossover points of the dynamic moduli, where $G' = G''$, which are plotted in Figure 6.

Regarding the differences in complex viscosity for the studied materials, one could observe a

slight increase of the complex viscosity in melting state for the system containing 6CSnano – Figure 7.

It can be concluded that, from the rheological point of view, there are no important differences between LDPE as matrix and nanocomposites, which should mean that the processing behaviour remains unchanged in the existing technology of obtaining antimicrobial foils for food packaging.

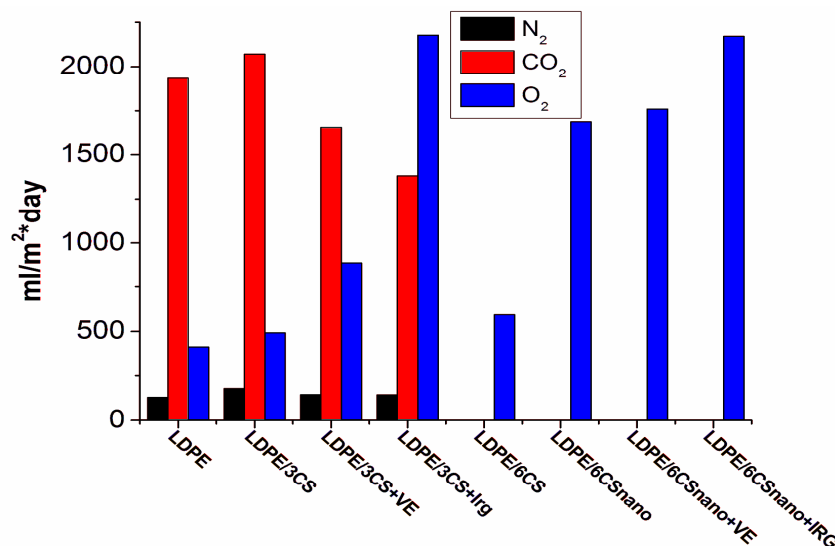


Figure 8: Gas transmission rate for LDPE/3CS composites and nanocomposites

Table 4
Oxygen permeability for chitosan+vitamin E-coated PE films

Samples	CHT/VE layer thickness [μm]	O ₂ transmission rate (mL/m ² *day)
PE	0	3833.36
PEc, EDC+NHS, CHT+VE, spread	10-20	2142.52
PEc, EDC+NHS, CHT+VE, ES	8.5×10^{-3}	2952.43

Barrier against gases

In the case of LDPE/chitosan composites, the permeability values for nitrogen gas are similar, differences being observed for carbon dioxide and oxygen as permeability gases – Figure 8.

For LDPE and LDPE/chitosan, the permeability values are similar for both carbon dioxide and oxygen, but by introducing the antioxidants in the matrix we can observe that the carbon dioxide permeability decreases, while the oxygen permeability increases. As oxygen is the main gas that influences the shelf-life of minced

poultry meat, we further investigated mainly the composites permeability to this gas.

In the case of stratified composites, the oxygen barrier properties are influenced by the thickness of the applied chitosan layer – Table 4. The deposition by the spreading (S) method creates a layer of 10-20 μm , which decreases the oxygen transmission rate up to 2142 mL/m²*day, when compared with native PE, while when electrospinning was applied, the thickness of the layer was thinner and the decrease in oxygen transmission rate was lower (2952 mL/m²*day).

All the results presented confirm the antioxidative and antimicrobial properties of the LDPE/CS composites, their quality depending on

the composition and preparation methods. The composites could be classified as presented in Table 5.

Table 5

Classification of the studied LDPE/CS composite films as to their minced poultry meat antimicrobial and antioxidative protection properties

Property	Superior characteristics to those required for reference/fresh meat	Characteristics comparable with reference/fresh meat	Characteristics comparable with altered meat
Surface aspect	PEc,EDC+NHS,CHT+VE,ES; LDPE/6 CS/VE; LDPE/3 CSnano/VE; LDPE/3 CS/VE; LDPE/3 CS/VE	LDPE/6CSnano/VE; LDPE/6 CS/IRG; LDPE/6 CSnano; LDPE/3 CSnano	LDPE/3 CS; LDPE/3 CS/IRG; LDPE/6 CS; PEc,EDC+NHS, CHT+VE, spread; LDPE
In-depth aspect	PEc, EDC+NHS, CHT+VE, ES; PEc, EDC+NHS, CHT+VE, spread; LDPE/6 CSnano/VE; LDPE/6 CS/VE; LDPE/6 CSnano; LDPE/3 CSnano/VE; LDPE/3 CS/VE; LDPE/3 CS/VE; LDPE/3 CSnano	LDPE; LDPE/6 CS/IRG; LDPE/6 CS; LDPE/3 CS/IRG; LDPE/3 CS	
Consistence	LDPE/3 CS/VE; LDPE/3 CSnano/VE; LDPE/6 CS/VE; PEc, EDC+NHS, CHT+VE, ES	LDPE/3 CS; LDPE/3 CSnano; LDPE/3 CS/IRG; LDPE/3 CS/VE; LDPE/6 CS;LDPE/6 CSnano; LDPE/6 CS/IRG; LDPE/6 CSnano/VE; PEc, EDC+NHS, CHT+VE, spread; LDPE	
Smell	PEc, EDC+NHS, CHT+VE, ES; LDPE/6 CS/VE; LDPE/3 CSnano/VE	LDPE/3 CS; LDPE/3 CSnano; LDPE/3 CS/VE; LDPE/3 CS/IRG; LDPE/3 CS/VE; LDPE/6 CS; LDPE/6 CSnano; LDPE/6 CS/IRG; LDPE/6 CSnano/VE; PEc, EDC+NHS, CHT+VE, spread PEc, EDC+NHS, CHT+VE, ES; PEc, EDC+NHS,CHT+VE, spread; LDPE/6 CSnano/VE; LDPE/6 CS/VE; LDPE/6 CSnano; LDPE/3 CSnano/VE; LDPE/3 CS/VE; LDPE/3 CS/VE; LDPE/3 CSnano; LDPE/3 CS	LDPE
Boiling testing			LDPE; LDPE/6 CS/IRG; LDPE/6 CS; LDPE/3 CS/IRG
pH	LDPE/3 CS/VE; LDPE/3 CS/VE; LDPE/3 CSnano/VE; LDPE/6 CS/VE; LDPE/6 CSnano/VE; PEc, EDC+NHS, CHT+VE, ES	LDPE/3 CSnano; LDPE/6 CSnano; PEc, EDC+NHS, CHT+VE, spread; LDPE	LDPE/3 CS; LDPE/3 CS/IRG; LDPE/6 CS; LDPE/6 CS/IRG
H ₂ S	LDPE/3 CS/VE; LDPE/3 CS/VE; LDPE/6 CSnano; LDPE/6 CS/VE ; LDPE/6 CSnano/VE; PEc,EDC+NHS, CHT+VE, ES	LDPE/3 CS; LDPE/3 CSnano ; LDPE/3 CS/IRG ; LDPE/3 CSnano/VE; LDPE/6 CS ; LDPE/6 CS/IRG ; PEc,EDC+NHS,CHT+VE, spread; LDPE	
Total number of germs, CFU/g	LDPE/3 CS NANO; LDPE/3 CS/VE; LDPE/3 CS/VE; LDPE/6 CSnano; LDPE/6 CS/VE; LDPE/6 CSnano/VE; PEc,EDC+NHS, CHT+VE, ES	LDPE/3 CS; LDPE/3 CSnano/VE extrudat; LDPE/6 CS; PEc,EDC+NHS,CHT+VE, spread	LDPE/3 CS/IRG; LDPE/6 CS/IRG; LDPE

CONCLUSION

LDPE/CS composites, LDPE/CS nanocomposites, LDPE/CS/Vitamin E composites, LDPE/CS nanocomposites/Vitamin E and stratified LDPE/CS/Vitamin E composites have been tested as packaging foils for minced poultry meat.

Chitosan incorporation in a LDPE matrix or applied by coating improved the oxygen barrier properties of PE and also conferred antimicrobial characteristics, which makes it very promising as food packaging material. All the characteristics of the minced meat packed in the LDPE/CS are superior to those of meat packed in reference foil and LDPE foil. The best results are obtained in the case of packaging using LDPE/CS/Vitamin E composites. As regards the preparation method, the samples deposited by electrospraying are superior to those prepared by melt mixing.

Processing characteristics were not affected by chitosan or chitosan nanocomposites incorporation, while the techniques of coating LDPE with chitosan gave better results than the incorporation method. Permeability to gases was also improved. The XRD, rheological and oxygen transmission rate analyses proved that the films accomplished all the requirements for minced poultry meat packaging.

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