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Antimicrobial packaging based on linear low-density polyethylene compounded with potassium sorbate

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ABSTRACT

In recent years much attention has been devoted to active packaging technologies that offer new opportunities for the food industry and food preservation. In the present study, antimicrobial films were developed by compounding of a neat linear low density polyethylene (LLDPE) or its blend with ethylene vinyl acetate (EVA), with potassium sorbate (KS). A new approach for preservative incorporation into a polyolefin matrix was used to obtain uniform dispersions of the preservative in the films. This approach includes using glycerol monooleate (GMO) as a dispersant and preparation of GMO/KS concentrate by strong mechanical mixing. The antimicrobial activity of the films was studied using the yeast strain *Saccharomyces cerevisiae* S288C. All compositions of LLDPE or LLDPE/EVA containing GMO and KS demonstrated antimicrobial activity. Release tests showed that KS migrates from compression molded 300 μm films to an acidic food simulant and its diffusion is controlled by the Fickian diffusion rule. Thermal stability, rheological behavior, morphology and KS dispersion in the polymer matrices of the prepared blends and films were investigated. The results indicate that the presence of KS in the polymer matrix significantly improves the thermal stability of the blends compared with the neat matrices without a significant viscosity reduction.

1. Introduction

Preservation techniques for prevention of food spoilage have been practiced since ancient times. Food preservation is a process of treating and handling food to stop or slow down the process of spoilage thus allowing longer storage times. Nowadays, both traditional and modern preservatives are widely used to ensure the satisfactory maintenance of quality and safety of foods (Davidson, Sofos, & Branen, 2005). In most, but not all solid or semisolid foods, microbial growth occurs primarily on the surface and therefore preservatives that are mixed directly with the food may result in over-use (Dickson & Anderson, 1992). In order to avoid the over-use of food preservatives and at the same time to maintain the food quality, the concept of antimicrobial active packaging has been implemented. The main idea behind this concept is the incorporation of antimicrobial agents directly into packaging films. Such methods could provide advantages by releasing antimicrobials directly to the food surface where they may be most effective, and could be the answer to consumers' demand for healthy food without preservatives (Brody, Stupinsky, & Kline, 2002).

A wide selection of antimicrobial substances, e.g. organic acids and their salts, fatty acids, antibiotics, antimicrobial peptides, essential oils, bacteriocins, chelators, enzymes, parabens and metals, has been considered to have the potential for use in active food packaging (Suppakul, Miltz, Sonneveld, & Bigger, 2003). Traditional food preservatives' low cost, commercial availability, wide use in food industry and high thermal stability make them attractive antimicrobial agents for active packaging. According to the report "Food Preservatives Market by Types, Functions, & Applications, Trends & Global Forecasts (2011–2016)" (http://www.marketsandmarkets.com, October 2013) amongst all other antimicrobials, sorbates and benzoates hold the major share of the

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Nomenclature

C	Concentrate of potassium sorbate and GMO
D	Diffusion coefficient of active agent in the film (m^2/hr)
EDS	Energy-dispersive X-ray spectroscopy
EVA	Ethylene vinyl acetate (copolymer)
GMO	Glycerol monooleate
HRSEM	High resolution scanning electron microscopy
k	Slope of the linear regression of M_t/M_∞ vs. the square root of time
KS	Potassium sorbate
L	Thickness of the film, (μm)
LLDPE	Linear low density polyethylene
LLDPE-g-MA	Linear low density polyethylene grafted maleic anhydride
M∞	Total amount of diffusing substance released from the film at equilibrium, (g).
MFI	Melt flow index, (g/10 min)
MIC	Minimal inhibitory concentration
Mt	The amount of diffusing substance released from the film at time t, (g).
O.D.	Optical density in 600 nm
t	Time, (h)
TGA	Thermal gravimetric analysis
VA	Vinyl acetate

market among the antimicrobials, accounting for nearly 70% of the global food antimicrobial market.

Potassium sorbate (KS) is widely used for inhibiting mold, yeast and some bacterial strains in various foods, including cheese, baked goods, fruits and vegetables, jams and certain meat and fish products (Davidson et al., 2005). There are numerous, sometimes contradicting results published regarding KS incorporation into different polymer matrices. Weng and Hotchkiss (1993) reported that low-density polyethylene films (LDPE) (0.05 mm thick) containing 1.0% w/w sorbic acid were unable to suppress mold growth when brought into contact with inoculated medium. Contradictory results were reported by Han and Floros (1997) who studied the incorporation of 1.0% w/w KS in low-density polyethylene (0.4-mm thick) and found that KS lowered the growth rate of yeast, and lengthened the lag period before the mold growth became apparent. Research published by Devlieghere, Vermeiren, Jacobs, and Debevere (2000) revealed that ethylene vinyl alcohol/linear low-density polyethylene (EVOH/LLDPE) film (70 μm thick) compounded with 5.0% w/w KS is unable to inhibit the growth of microorganisms on cheese and thus to extend its shelf life probably due to limited migration of the antimicrobial agent from the polymer. Vartiainen, Skytta, Enqvist, and Ahvenainen (2003) studied antimicrobial properties of LDPE and other commercial polymers containing traditional food preservatives sodium benzoate, sodium nitrite, KS and sodium lactate at a concentration of 15% w/w. The authors used ground to micron size antimicrobial powders for compounding. None of the samples in that study showed any inhibition against *Escherichia coli*, but all films except sodium lactate-containing samples had high antifungal activity. Hauser & Wunderlich (2011) showed that films coated with a lacquer containing sorbic acid inhibit *E. coli*, *Listeria monocytogenes*, and *Saccharomyces cerevisiae*.

It is known that when organic acids or their salts are used as preservatives, antimicrobial activity is provided by undissociated molecules of the acid which penetrate the microbial membrane (Mani-López, García, & López-Malo, 2012). Subsequently, maximal antimicrobial activity is obtained at pH values below the acid's pKa. Therefore, KS-containing antimicrobial packaging is limited to products that are acidic by nature. The antimicrobial agent has to be free and able to migrate through the polymer matrix in order to be released from the packaging surface and penetrate through the microbial cell membrane. Thus, the release rate is an important parameter that has to be considered when designing the antimicrobial packaging. The present research focuses on the development and characterization of antimicrobial films, based on linear low-density polyethylene (LLDPE) and its blend with ethylene vinyl acetate (EVA) compounded with KS. The aim of the current study is to investigate the correlation between different compounding procedures, films' composition, their physical properties and antimicrobial activity.

2. Materials

Commercial LLDPE, type FP120C, MFI = 1 g/10 min (Nova Chemicals, Calgary, Canada), and its blend with EVA, MFI = 2.5 g/10 min, VA content = 19% (Enimont, Milan, Italy) 70/30 by weight, served as polymer matrices. KS, 99% purity (Sigma Aldrich, Steinheim, Germany), served as the antimicrobial agent in this study. KS was grounded by hand prior to compounding with polymers. For better dispersion of KS in the polymer matrices, a food grade compatibilizer LLDPE grafted maleic anhydride (LLDPE-g-MA) MFI = 1.5 g/10 min, (DuPont Company, Wilmington, USA), and a food grade dispersant commercial glycerol monooleate (GMO), as received, were used.

YPD medium, served as a growth medium for yeasts, was prepared by dissolving 10 g yeast extract (Becton, Dickinson and Company, Sparks, USA), 20 g peptone (Becton, Dickinson and Company, Sparks, USA) and 20 g glucose (Sigma Aldrich, Rehovot, Israel) in 1 L of distillated water. YPD agar plates were prepared by adding 20 g agar (Acumedia, Neogen Corporation, Lansing, MI, USA) to the broth prior to sterilization. Acetate buffer pH 4.2, 9 mmol/L was prepared from ammonium acetate (Merck KGaA, Darmstadt, Germany) and acetic acid (Merck KGaA, Darmstadt, Germany) was used to adjust the pH of the medium. Xylene was used as a solvent

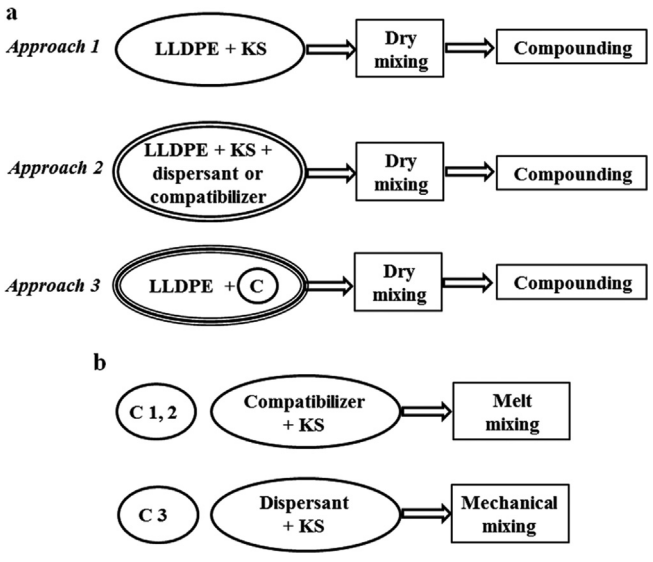

Fig. 1. Scheme of the different approaches used for preparation of the antimicrobial blends. a) Three general approaches comprising dry mixing and compounding, b) Preparation of concentrates containing the antimicrobial agent with the compatibilizer or dispersant. The composition of the concentrates is detailed in Table 1.

Table 1
List of the prepared concentrates and their compositions.

Concentrate code	Compatibilizer (LLDPE-g-MA) g/100 g	Dispersant (GMO) g/100 g	Antimicrobial agent (KS) g/100 g
C1	60	–	40
C2	91.5	–	8.5
C3	–	60	40

LLDPE-g-MA – Linear low density polyethylene grafted maleic anhydride, GMO – Glycerol Monooleate, KS- Potassium Sorbate.

for determination of crosslinking extent (Gadot, Haifa, Israel). Acetonitrile and water, HPLC grade (Spectrum Chemical Manufacturing Corporation, New Brunswick, USA), were used for the mobile phase in HPLC analyses.

3. Methods

3.1. Compounding procedures

LLDPE or 70/30 LLDPE/EVA was compounded with KS using a Brabender Plastograph batch mixer equipped with 50 cm^3 mixing cell and torque recorder, at 180 °C, 50 rpm, during 10 min. All compositions in the present study are expressed in unit g/100 g of matrix. Three different approaches for the incorporation of KS into the matrices were explored as shown in Fig. 1a:

1) Dry mixing of 50% of the polymer quantity with KS, followed by compounding of the dry mixture and the rest of the polymer to obtain a final KS concentration 2 or 5.3 g/100 g in the ready blends;
2) Dry mixing of 50% of the polymer quantity with a compatibilizer (LLDPE-g-MA) or a dispersant (GMO), and KS at a ratio of 1:1, followed by compounding of the dry mixture with the rest of the polymer to obtain a final KS concentration 5.5 g/100 g in the ready blends;
3) Two-step compounding procedure: a) preparation of concentrates as described in Fig. 1b, and b) final compounding of the polymer matrices with the prepared concentrates to obtain a final KS concentration 2 or 5.3 g/100 g in the blends.

Three concentrates were prepared as listed in Table 1. LLDPE-g-MA/KS concentrates were prepared by melt mixing of LLDPE-g-MA and KS in a Brabender Plastograph for 5 min, at 180 °C, 50 rpm. A GMO/KS concentrate was prepared by mechanical mixing of GMO and KS applying ultrasonic energy for 10 min (750 W Ultrasonic Processor, Sonics & Materials, New town, USA).

All prepared blends were compression molded at 180 °C to obtain films with an average thickness of 300 μm.

3.2. Rheological measurements

The AR 1000-N parallel plate rheometer (TA Instruments, New Castle, USA) was used for measuring the steady-state apparent viscosity of the samples. Measurement conditions were: 180 °C, 0.01–100 sec^{-1} shear rate range. 20 mm diameter plate was used and a maximal gap was set at 0.7 mm.

3.3. Thermal stability

Thermal stability of the prepared samples was analyzed using a TA 2050 Thermal Gravimetric Analyzer (TGA) (TA Instruments, New Castle, USA). Samples were heated under air, at a heating rate of 20 °C/min up to 800 °C, monitoring their weight loss as function of temperature.

3.4. Morphological characterization of the blends

The dispersion of the antimicrobial agent within the matrices was examined by a scanning electron microscope (Zeiss Ultra-Plus FEG-SEM) (Zeiss, Jena, Germany), equipped with a high-resolution field emission gun. Samples were freeze-fractured and the fracture surface was carbon sputtered prior to observation. AsB detector of backscattered electrons, which clearly shows phase contrast upon the observed samples, was used to obtain micrographs. Energy-dispersive X-ray spectroscopy (EDS; Link Isis, Oxford Instruments) was used to confirm the identity of the phases appeared on the fractured surfaces.

3.5. Release tests of KS from the films

A specimen of 20 mm in diameter of each sample was placed in a 28 ml vial filled with 25 ml acetate buffer, pH = 4.2, 9 mmol/L. All vials were heated to 70 °C, under agitation. At predetermined intervals, 0.4 ml of the buffer were withdrawn from the vials and replaced with an equal volume of fresh buffer. The KS concentration in the buffer samples was quantified using high-performance liquid chromatography (HPLC) with an Agilent 1100-series instrument (Agilent Technologies, Santa Clara, CA, USA). An Eclipse XDB C18, 5 μm, 4.6 × 150 mm column (Agilent Technologies, Santa Clara, CA, USA) was used. An isocratic mobile phase comprising acetate buffer (pH 4.2): acetonitrile (80:20) was used at a flow rate of 0.8 ml/min. Detection was performed at 255 nm. Under these conditions, KS was eluted at 9.6 min.

3.6. KS diffusion mathematical analysis

The KS release kinetic is described according to a particular solution of the Fick's second law of diffusion in the case of a sheet of 2 Lthickness immersed in an infinite volume of a stirred liquid. Assuming that the initial KS concentration is uniform, the equation describing the total kinetics of the diffusion of KS from the film is (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\frac{(2n+1)^2\pi^2}{4L^2}Dt\right] \quad (1)$$

where M_t is the amount of diffusing substance released at time t, and M_∞ is a corresponding amount in equilibrium (released in infinite time).

For short-term migration (M_t/M_∞) < 0.6:

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{0.5} \quad (2)$$

where D is the diffusion coefficient and L is the thickness of the film. A plot of (Mt/M_∞) versus $t^{0.5}$ should yield a straight line from which the diffusion coefficient can be calculated.

3.7. Antimicrobial activity

3.7.1. Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) is defined as the lowest antimicrobial concentration that under certain test conditions inhibits the visible growth of the bacterium being investigated (Andrews, 2001). The MIC of KS was determined by the broth dilution method (Wiegand, Hilpert, & Hancock, 2008) in YPD medium at three different pH levels: neutral, pH 4.2 and 5.2. YPD was adjusted to appropriate pH levels (4.2 and 5.2) with previously prepared ammonium acetate buffer (pH = 4.2). MIC was

determined by measuring the turbidity in 600 nm (O.D.) using a multi-plate reader (OPTImax tunable microplate reader, Molecular Devices, Sunnyvale, CA, USA).

3.7.2. Investigation of antimicrobial activity

Yeast strain *S. cerevisiae* S288C (ATCC 204508, Biological Industries, Beit Haemek, Israel) was used in this study as a model strain for determination of antimicrobial activity. Growth conditions were 30 °C in YPD broth medium. The quantitative antimicrobial activity was evaluated by inhibition of microbial development in a liquid medium (viable cell count assay). Compression molded 300 μm films were cut to 20 mm diameter specimens. Before the tests, specimens were wiped with ethanol, dried and stored at room temperature. *S. cerevisiae* cells were seeded from a −80 °C stock onto a YPD agar plate and incubated overnight at 30 °C. Then, 5 ml of fresh YPD were inoculated with a single yeast colony and incubated overnight under agitation of 250 rpm in an incubator shaker (TU-400 Orbital Shaker Incubator, MRC, Holon, Israel) (starter). The starter culture was diluted with fresh YPD to obtain an inoculum containing 105 cfu/ml of yeasts (cfu – colony forming units). The prepared specimens were placed in sterilized test tubes, to which 3 ml of inoculum were added aseptically, so as to cover the entire disk. Tubes were incubated under agitation at 250 rpm overnight. Afterwards, a live count was performed by transferring 200 μl from each tube with the tested samples into wells of a 96 well plate, performing 8 ten-fold dilutions with YPD and then performing a drop test. The drop test was carried out by transferring 10 μl from each sample (including the non-diluted samples) onto YPD agar plates and incubating overnight at 30 °C. Yeast colonies in the drops were counted.

3.8. Statistical analysis

MIC test was performed in 4 replicates. Antimicrobial activity test was performed in triplicate, in two separate experimental runs. Obtained data is expressed as mean value ± standard deviation. An analysis of variance (ANOVA) was performed on the reported results, which were an average of individual measures.

4. Results and discussion

4.1. Mixing process characteristics

Mixing torque generated during the compounding process may serve a preliminary indication of the melt rheological behavior.

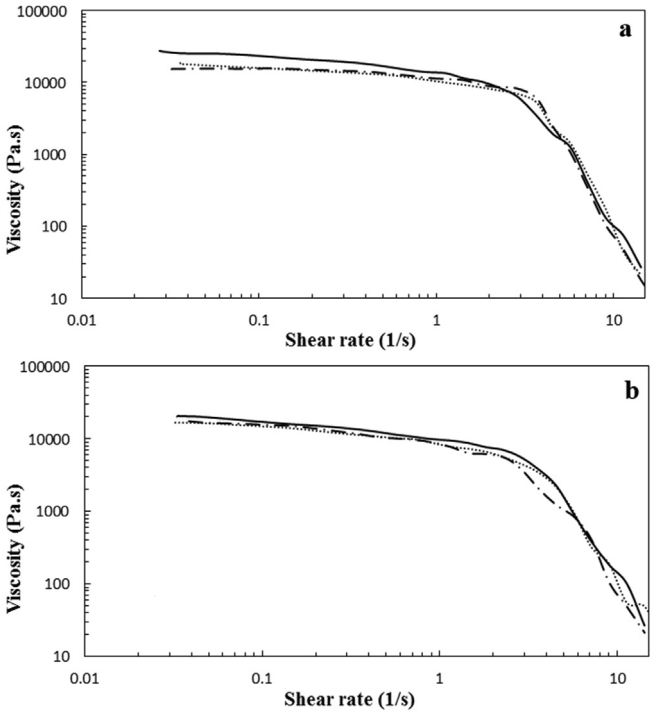

Fig. 2. The relationship between apparent viscosity and shear rate for: a) LLDPE compounded with KS applying approach 1. (—) L1, (•••••) L4, (– • –) L5; b) LLDPE/EVA compounded with KS applying approach 1. (—) LE1, (•••••) LE3, (– • –) LE4. Measurements were done with a parallel plate rheometer with the following conditions: 180 °C, 0.01–100 sec^{-1} shear rate range. A 20 mm diameter plate was used and maximal gap was set at 0.7 mm (n = 3).

Thus, all mixing torques were recorded and are presented in Table 2. All blends were characterized by similar torque values and constant melt temperature within the cell during compounding, except C1. For C1, the recorded torque value increased from 30 N•m to 70 N•m and the melt temperature rose from 180 °C to 220 °C within 3 min.

Due to the unexpected strong torque increase, mixing of C1 was stopped after 5 min. The mixture obtained formed a single block, which might indicate the occurrence of a crosslinking reaction. In order to examine, whether KS promotes the crosslinking of LLDPE-g-MA, another concentrate with a lower KS concentration (C2) was prepared. During mixing of C2 similar changes of the torque and

Table 2
Torque values recorded during different compounding processes (n = 2).

Blend/film code	Matrix	Antimicrobial agent (KS)	Dispersant (GMO)	Compatebilizer (LLDPE-g-MA)	Concentrate (C3)	Torque
		g/100 g of polymer				N × m
L1	LLDPE	–	–	–	–	40
L2	LLDPE	5.5	–	5.5	–	35
L3	LLDPE	–	5.3	–	–	30
L4	LLDPE	2	–	–	–	30
L5	LLDPE	5.3	–	–	–	30
L6	LLDPE	5.5	5.5	–	–	28
L7	LLDPE	–	–	–	5.3	30
L8	LLDPE	–	–	–	14.3	25
LE1	LLDPE/EVA	–	–	–	–	30
LE2	LLDPE/EVA	–	5.3	–	–	25
LE3	LLDPE/EVA	2	–	–	–	30
LE4	LLDPE/EVA	5.3	–	–	–	30
LE5	LLDPE/EVA	5.5	5.5	–	–	25
LE6	LLDPE/EVA	–	–	–	5.3	25
LE7	LLDPE/EVA	–	–	–	14.3	22

LLDPE – Linear low density polyethylene, GMO – Glycerol Monooleate, KS- Potassium Sorbate, EVA – Ethylene Vinyl Acetate, C3 – concentrate code 3 described in Table 1.

temperature were observed. Both concentrates were extracted in xylene heated to reflux, for 24 h, according to ASTM D 2765-68 (Standard method of test for degree of crosslinking in crosslinked ethylene plastics as determined by solvent extraction, 1972). The extraction results demonstrated that the extent of crosslinking in both concentrates was approximately 55% (w/w). Contrary to that, direct compounding of LLDPE with compatibilizer and KS (approach 2) revealed the usual behavior and the recorded torque value was similar to that of neat LLDPE 35 N•m. Since all molded films containing compatibilizer and KS were inactive (results not shown), a further study focused on the blends prepared by compounding with GMO addition (by all three approaches). As for the blends containing the dispersant, it is known that GMO acts as a plasticizer when added to a polymer matrix (Ash & Ash, 2007). Thereby the torque values of the LLDPE and LLDPE/EVA compounded with GMO were lower than those of the neat LLDPE and LLDPE/EVA matrices, respectively. In addition, increasing the C3 concentration in the blend (prepared according to the third approach), resulted in lower recorded torque values.

4.2. Melt rheology

In order to examine whether the antimicrobial agent influences the viscoelastic properties of the matrices, a rheological analysis was performed (Figs. 2 and 3). Fig. 2 presents the results of viscosity measurements of the neat matrices and their KS containing counterparts prepared by approach 1. The addition of KS to LLDPE (Fig. 2a) or LLDPE/EVA (Fig. 2b) leads to insignificant viscosity reduction compared to the analogous neat polymer matrices. Fig. 3 depicts results of viscosity measurements for the composites prepared according to approaches 2 and 3. These blends also showed insignificant viscosity decrease compared to the LLDPE and LLDPE/EVA matrices. The higher the C3 concentration in the matrix, the lower the apparent viscosity was. In general, there were insignificant differences in viscosity reduction of the blends prepared by the different approaches. These results are correlated with the torque values recorded during mixing.

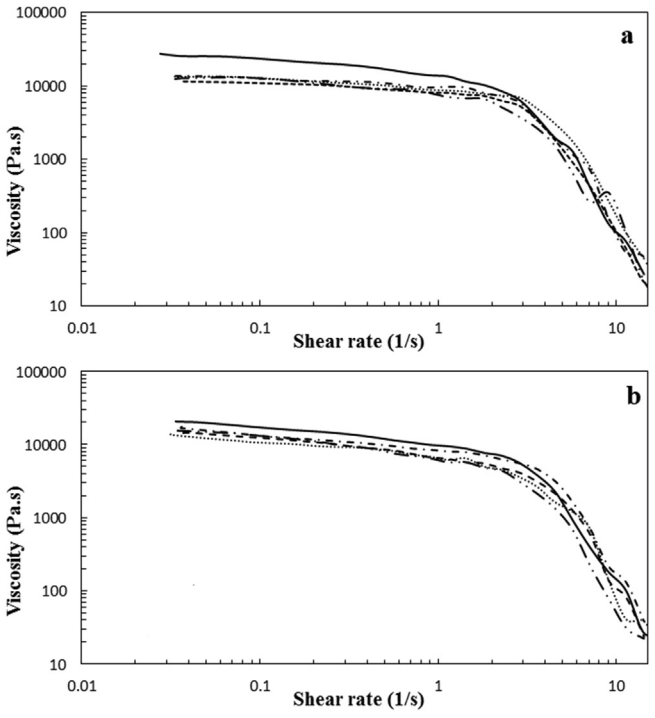

Fig. 3. The relationship between apparent viscosity and shear rate for: a) LLDPE compounded with KS applying approach 2 and 3. (—) L1, (•••••) L3, (– • – •) L7, (——) L8, (– •• –) L6 b) LLDPE/EVA compounded with KS applying approach 2 and 3. (—) LE1, (•••••) LE2, (– • – •) LE6, (——) LE7, (– •• –) LE5. Measurements were done with a parallel plate rheometer with the following conditions: 180 °C, 0.01–100 sec^{-1} shear rate range. A 20 mm diameter plate was used and maximal gap was set at 0.7 mm (n = 3).

4.3. Thermal stability

The thermal stability of the studied blends was examined using TGA (Fig. 4). Compounding of LLDPE with KS according to approach 1 led to improved thermal stability compared with that of the neat polymer (Fig. 4a), while compounding with GMO resulted in reduction of thermal stability due to its plasticizing effect (Fig. 4c). Fig. 4c reveals that direct KS and GMO incorporation to the matrix or incorporation of the concentrate C3 (approaches 2 and 3) improves the compositions thermal stability. However, increasing of the C3 concentration leads to lower improvement in thermal stability due to a relatively higher content of GMO in the blend that is responsible for reducing of LLDPE thermal stability. Thus, the highest improvement in thermal stability in our study was observed for the blend L7 (LLPDE + 5.3 g/100 g C3).

Fig. 4b and c depict that thermal stability of LLDPE/EVA blends is markedly different from that of LLDPE blends due to the presence of the polar EVA. Fig. 4b shows that the thermo-oxidative degradation of the LLDPE/EVA polymer blend occurs in two consecutive steps. The first step (300–380 °C) corresponds to deacylation of the vinyl acetate group of EVA with elimination of acetic acid and formation of double bonds. The second step (380–450 °C) may be assigned to further degradation of polyethylene chains formed in the first step accompanied by degradation of LLDPE (Guo et al., 2004). KS slightly decreased the thermal stability of LLDPE/EVA blend during the first step, but at the same time partially improved it during the second step. Compounding of LLDPE/EVA with GMO manifested a similar effect on thermal stability as the presence of KS, i.e. revealed a small reduction in the temperature range 100–380 °C, while slightly increased it at higher temperatures, 380–450 °C (Fig. 4d). It is assumed that GMO acts as a compatibilizer between the non-polar LLDPE and the polar EVA. Fig. 4d reveals that compounding of LLDPE/EVA according to approaches 2 and 3 improves the composition's thermal stability. The difference between approach 2 and 3 is that in case of direct incorporation of GMO and KS to the matrix (approach 2), the polar EVA interacts strongly with both additives. In contrast, in approach 3 (concentrate (C3) incorporation), GMO and KS interact mostly between them during the concentrate preparation and are less involved in improving the matrix thermal stability. Similar to LLDPE blends, the higher the C3 concentration, the lower the improvement in thermal stability.

4.4. Morphological characterization of the blends

Film transparency is an important feature of food packaging. A rough comparative estimation of films' transparency was performed. In the antimicrobial films produced according to approach 1 and 2 visible white KS clusters appeared. In contrast, all films prepared by approach 3 looked uniform and no visible KS clusters were observed. In addition, a color change, i.e. light brown coloring of all films containing KS was observed. This brown coloring is caused by heating of KS (Han & Floros, 1998a, 1998b), a phenomenon which could not be prevented during the compounding process, unless using special additives.

To further investigate KS dispersion within the polymer matrices, a freeze fractured surface of the blends was thoroughly studied using HRSEM. KS dispersion in the blends was studied using an AsB detector (Figs. 5 and 6). The identity of the each phase

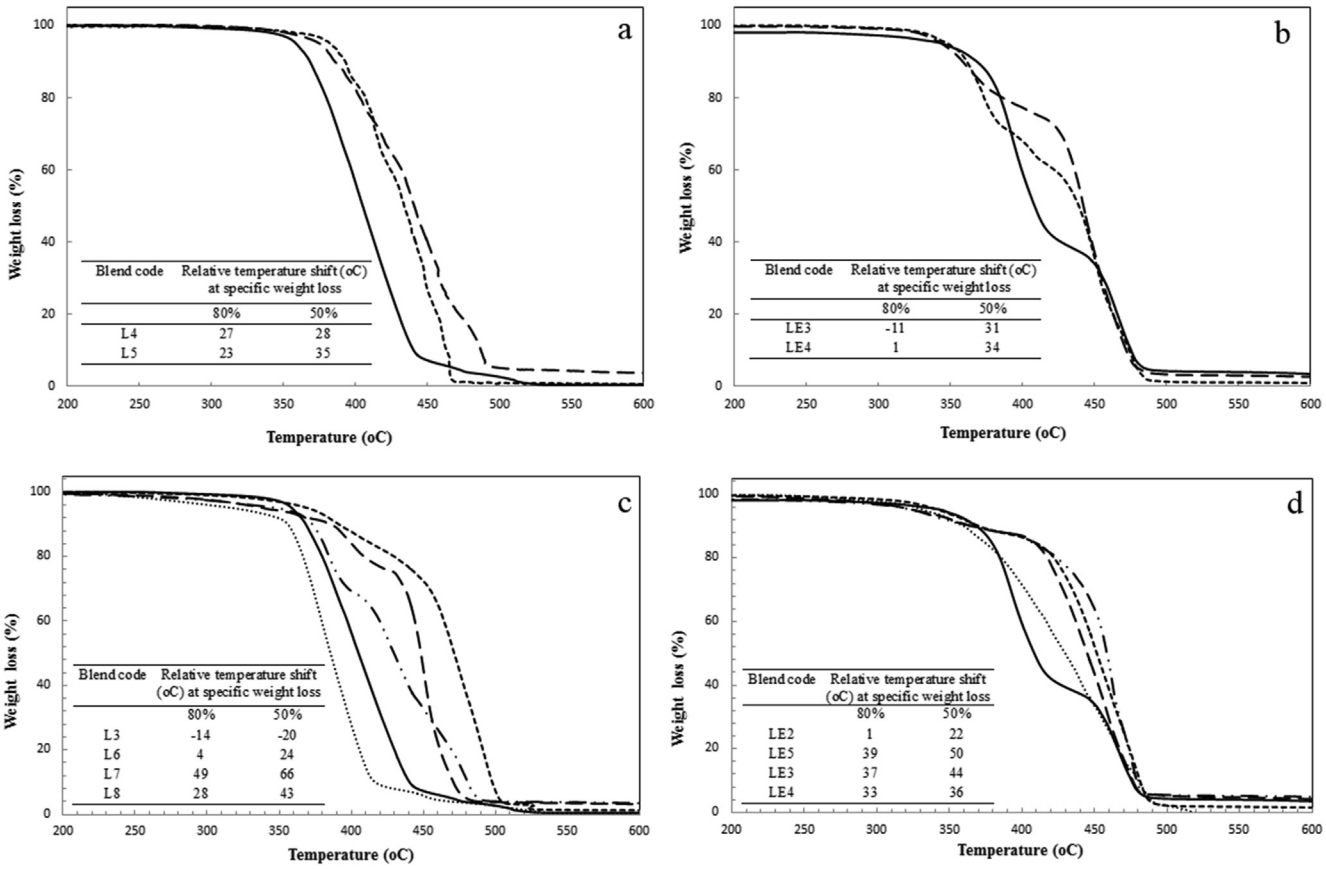

Fig. 4. TGA thermograms of: a) LLDPE compounded with KS applying approach 1. (—) L1, (——) L4, (– –) L5; b) LLDPE/EVA compounded with KS applying approach 1. (—) LE1, (——) LE3, (—) LE4; c) LLDPE compounded with KS applying approach 2 and 3. (—) L1, (——) L7, (—) L8, (•••••) L3, (– •• –) L6; d) LLDPE/EVA compounded with KS applying approach 2 and 3. (—) LE1, (——) LE6, (– –) LE7, (•••••) LE2, (– •• –) LE5 Samples were heated under air, at a heating rate of 20 °C/min up to 800 °C, monitoring their weight loss as function of temperature (n = 3).

appearing in the studied blends was confirmed by performing energy dispersive spectroscopy (EDS). The lighter phase is the KS phase, and the darker one is the polymer matrix phase (see also Supplementary Material).

Fig. 6a reveals that the LLDPE blends prepared by approaches 1 and 2 are characterized by a non-uniform KS dispersion. KS agglomerates of ~100 μm in diameter can be seen upon the surface of the LLDPE compounded with 5.3 g/100 g KS (Fig. 5a). In the LLDPE +5.5 g/100 g GMO +5.5 g/100 g KS blend, slightly smaller KS agglomerates ranging from 20 to 60 μm appear (Fig. 5b). Moreover, in both blends KS crystals on the fractured surface were observed. It was found that a crystallization process takes place when the local KS concentration reaches super saturation (Uz & Altınkaya, 2011). Thus, due to the rough dispersion of KS in these two blends, large KS agglomerates induced crystal growth. Contrary to these two blends, the blend prepared according to approach 3 (5.3 g/100 g KS) has shown a fine and uniform KS dispersion (Fig. 5c). LLDPE/EVA blends prepared according to approach 1 are also characterized by a rough, non-uniform KS dispersion. In these blends, the KS phase agglomerates were distributed from 2 to 25 μm by size. In the larger KS agglomerates, KS crystals were also observed (Fig. 6a). In contrast to the LLDPE blend, a uniform and fine KS dispersion was observed in the LLDPE/EVA blend prepared by approach 2 (Fig. 6b). The difference in KS dispersion within the two compositions prepared by approach 2, LLDPE (Fig. 5b) and LLDPE/EVA (Fig. 6b), is attributed to the difference in the matrices' polarities. It is evident that the matrix containing EVA is characterized by a higher polarity compared with the nonpolar LLDPE matrix. Therefore, polar additives such as GMO and KS interact with EVA and hence are dispersed more homogenously in the LLDPE/EVA matrix. The average KS phase size, about 2 μm, was similar to that of the same composition prepared by approach 3 (Fig. 6c).

4.5. KS release rate from the films

The efficiency of antimicrobial films is based on the migration of active components to the food, thus knowledge of the diffusivity of these components from the film is an important factor in the development of an antimicrobial food packaging system. In order to investigate the diffusion rate of KS from the different composite films, release tests were performed. The amount of KS released (M_t) to the acidic medium was measured as a function of time during 96 h. The calculated values of KS fractional mass released, defined as M_t/M_∞ were then plotted versus the square root of release time. Parameters k and D were calculated according to the model presented in Eq. (2), and together with correlation coefficient R^2, are presented in Table 3.

The high values of the correlation coefficient presented in Table 3 express a good correspondence of the experimental and the theoretical data, which indicates that the diffusion process is governed by the Fick's law (see also Supplementary Material). All films had similar values of the diffusion coefficient, $1.9–3.9 \times 10^{-10}$ m^2h^{-1}. To our best knowledge, there is no published calculated diffusion coefficient of KS from LLDPE. Han &

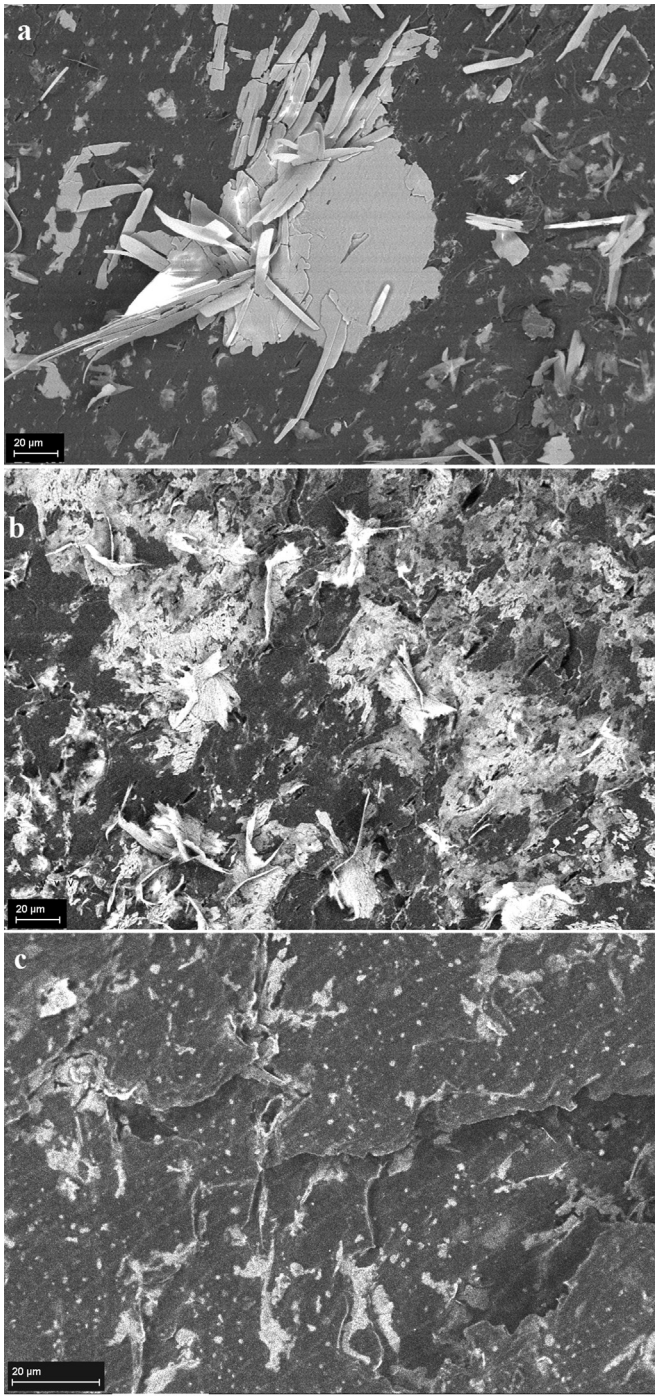

Fig. 5. HRSEM micrographs of freeze fractured surface: a) LLDPE + 5.3 g/100 g KS (approach 1); b) LLDPE + 5.5 g/100 g GMO + 5.5 g/100 g KS (approach 2); c) LLDPE + 14.3 g/100 g C3 (approach 3).

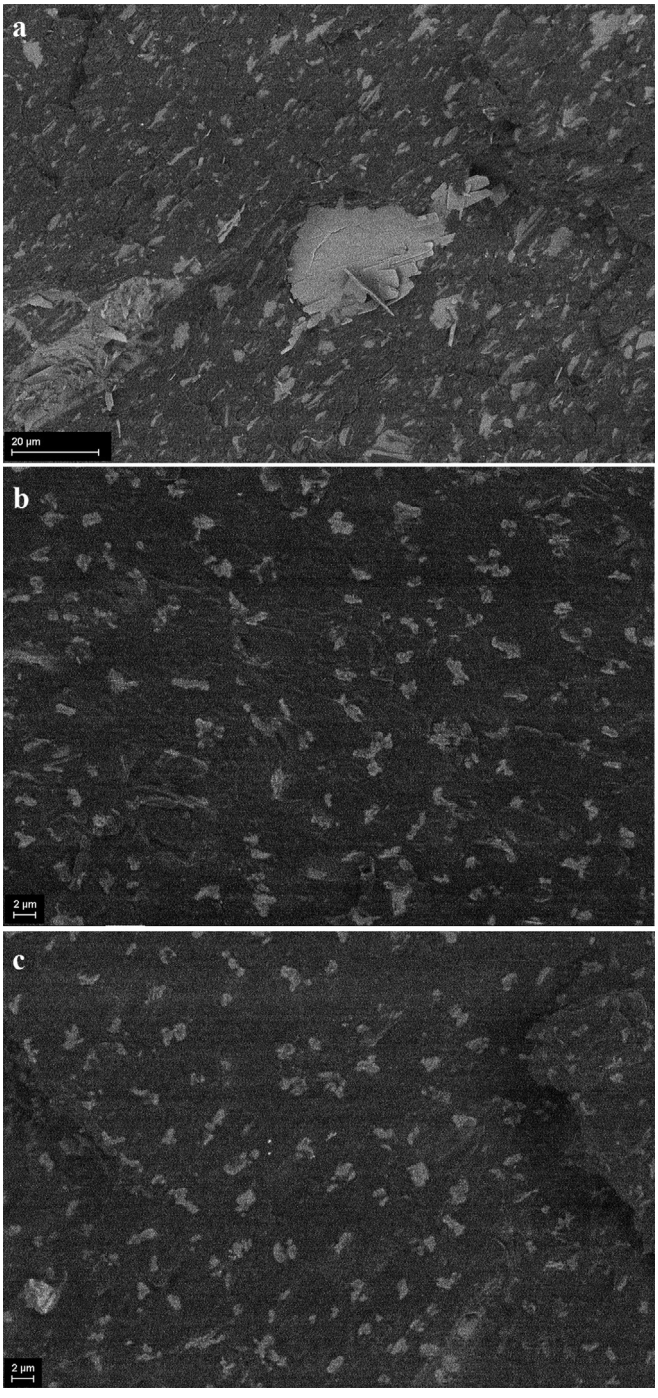

Fig. 6. HRSEM micrographs of freeze fractured surface: a) LLDPE/EVA + 5.3 g/100 g KS (approach 1); b) 5.5 g/100 g GMO + 5.5 g/100 g KS (approach 2); c) LLDPE/EVA + 14.3 g/100 g C3 (approach 3).

Floros (1998a, 1998b) investigated the diffusivity of KS through various commercial plastic films by a lag time method. The authors concluded that low density polyethylene, LDPE had the highest (fast) diffusivity compared with the other tested films. They also showed that the diffusivity of KS through an LDPE film did not depend on the KS concentration and followed a typical behavior of a Fickian diffusion. The diffusivity of KS through the LDPE films was determined as 1.83×10^{-12} m^2 s^{-1} at 25 °C. Calculated diffusion coefficients in this study are 20 times smaller than this value. Although LDPE and LLDPE have a similar chemical structure, they differ in the degree of crystallinity and density, which have a direct impact on diffusion of molecules dispersed within the polymer matrix. It is possible that the KS release is limited by interactions between GMO and KS, in the films prepared by approach 2 and 3, or by poor KS dispersion in the films prepared by approach 1.

GMO is defined as GRAS (generally recognized as safe) by the FDA legislation and it is also approved for use as an additive in

Table 3
Diffusion parameters and correlation coefficients for the release of KS from the studied films into the acidic medium.

Film code code	k	R^2	D^x10^{10}
	$h^{-0.5}$		m^2h^{-1}
L4	0.128	0.999	2.9
L5	0.113	0.999	2.3
L6	0.121	0.983	2.6
L8	0.120	0.990	2.6
LE3	0.092	0.989	1.5
LE4	0.084	0.984	1.2
LE5	0.143	0.974	3.6
LE7	0.125	0.994	2.7

k, slope of the linear regression of M_t/M_∞ vs. the square root of time; D, diffusion coefficient; R^2 correlation coefficients. Film codes are the same as in Table 2.

Table 4
Antimicrobial activity of studied films: a mean bacterial log reduction of *S. cereviciae* measured by a drop test.

Film code	*S. cereviciae*, YPD, pH = 4.2
	Log reduction
L1	0
L3	1 ± 0.30
L4	1.5 ± 0.44
L5	1.4 ± 0.31
L6	0.9 ± 0.41
L7	1.7 ± 0.70
L8	2.2 ± 0.14
LE1	0
LE2	2.3 ± 0.39
LE3	2.6 ± 0.14
LE4	1.4 ± 0.28
LE5	1.4 ± 0.19
LE6	2.2 ± 0.37
LE7	3 ± 0.60

Films were tested in duplicates; a drop test was performed in triplicates. Data expressed as means ± standard deviations.
Film codes are the same as in Table 2.

polymer packaging by European legislation. In prospect, overall migration from the films will be determined in the future.

4.5.1. Antimicrobial activity

S. cerevisiae was chosen as a model yeast strain for the antimicrobial activity tests in this study. Yeasts are known to be able to grow on the food surface, in foods with low pH values (5.0 and lower) (Kurtzman, 2006; Praphailong & Fleet, 1997). There are several yeast species that were isolated from different meat products and dairy products (Fleet, 1992).

In order to determine appropriate antimicrobial test conditions, MIC of KS against yeast strain was measured at 3 different pH levels. An inoculum culture diluted twice at a concentration of 10^5 cfu/ml without KS, served as a negative control in this test. As shown in Fig. 7, the MIC value of KS decrease with the decrease of pH: at a neutral pH KS concentration that inhibits yeasts growth is about 1250 mg/L (O.D. < 0.2), and at a pH level lower than pKa of sorbic acid, namely pH = 4.2 MIC falls to 156 mg/L. Thus, YPD at a pH 4.2 was chosen for further antimicrobial activity tests.

Antimicrobial tests were performed in liquid media, which allows the accurate estimation of reduction in yeast growth by a viable cell count.

Table 4 summarizes the measured antimicrobial activity tests results of the studied films. It was discovered that the reference films, i.e. LLDPE or LLDPE/EVA blended with 5% GMO decreased yeast growth by 1–2 log values, suggesting that GMO itself has a particular antimicrobial activity. The films prepared by approach 3 showed a slightly higher antimicrobial activity (2–3 log reduction) than those prepared by approaches 1 and 2 due to the fine KS dispersion obtained, as shown by HRSEM observations (see Figs. 5 and 6). Fine KS dispersion results in the presence of numerous KS nuclei upon the surface of the film, and therefore provides a relatively rapid release of KS during the test time.

5. Conclusions

Antimicrobial films based on LLDPE or 70/30 LLDPE/EVA blend and KS as an antimicrobial agent were explored. A new approach for incorporation of KS into a polymer matrix that leads to uniform and thin dispersions of a preservative within the polymer matrix was developed.

All studied films, except those containing concentrate of KS/LLDPE-g-MA, demonstrated an antimicrobial activity determined by yeasts' growth inhibition. It was found that the film references, namely LLDPE or LLDPE/EVA blended only with 5.3 g/100 g GMO, also inhibited yeasts' growth, indicating that GMO has antimicrobial properties. An antimicrobial activity test, performed for a longer duration, with an initial yeast load corresponding to food manufacturing conditions, may provide results reflecting the activity of the films during the storage of a food product (shelf life). By adjusting the test to the food storage conditions it will be possible

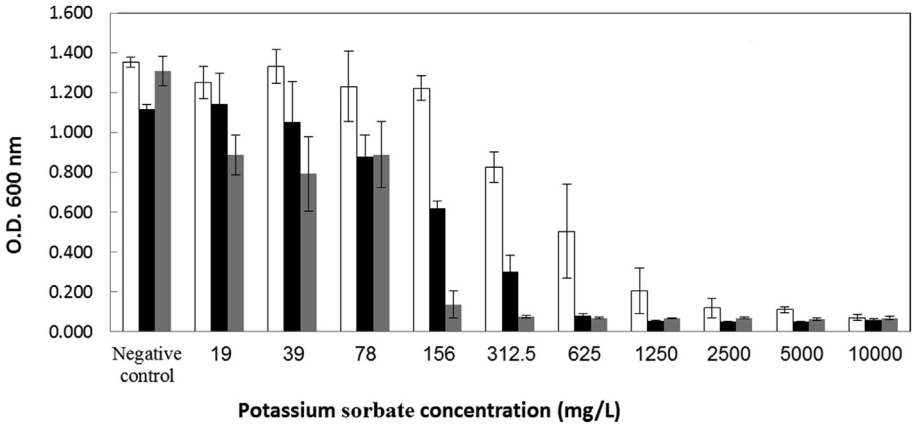

Fig. 7. MIC test results for inhibition of *S. cerevisiae*, performed at pH 7 (white), 5.2 (black), and 4.2 (grey). Growth was measured by turbidity at 600 nm. The negative control included diluted inoculum at initial concentration 10^5 cfu/ml, without KS (n = 4).

to determine the contribution of GMO to the antimicrobial activity of the films prepared by approach 3.

A thermal stability study performed by TGA has shown that incorporation of KS in the polymer matrices improves their thermal stability. In addition, the apparent viscosity of the blends is marginally affected by the presence of a dispersant and antimicrobial agent in the matrices.

It was found that the KS release from all films was controlled by the Fickian diffusion. The diffusion coefficient of KS released from the blends did not depend on the KS concentration and the presence of a GMO in the matrices.

Applying the concentrate (dispersant/KS) approach for compounding of antimicrobial films may be used for production of food packaging. Additional parameters of dispersant/KS concentrate, such as composition ratios and mixing conditions and their influence on antimicrobial film's properties will be further investigated to control the KS migration rate. The method applied in this study may be suitable as well for the incorporation of other organic salts.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2015.01.002

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