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Preparation and characterization of polyvinyl alcohol films modified with essential oils

Lucica Pop[1], Anca Mihaly-Cozmuta[1], Patrizia Fava[2], Andrea Pulvirenti[2], Leonard Mihaly-Cozmuta[1], Anca Peter[1]

[1]*Technical University Cluj Napoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victoriei 76, 430122 Baia Mare, Romania*
[2]*University of Modena and Reggio Emilia, Department of Life Sciences, Via Amendola 2, 42122 Reggio Emilia, Italy*

Abstract

Antimicrobial packaging is an active system used to prevent the development of surface microflora, obtained by adding active agents into the packaging film. The active packaging systems that can extend the shelf life and food safety of products by antimicrobial protection gained interest. The aim of this paper was to obtain modified films based on polyvinyl alcohol (PVA), by adding carvacrol, nutmeg and oregano essential oils, respectively and characterize them by evaluating the tensile strength, the elongation at break and the antimicrobial activity. Moreover, the wettability of the PVA modified with carvacrol onto three types of cheese was also determined. The results have showed that the tensile strength and the elongation at break decrease as the essential oil concentration rises up to 0.75% (mass percentage). No microbial cultures developed at the contact between the film and the culture medium showing that no microorganisms would develop even at the contact of the film with food. The wettability results demonstrated that PVA modified with carvacrol 0.25 % and 0.50 %, respectively are appropriate for the storage of cheese with different moisture and fat content.

Keywords*:* polyvinyl alcohol, antimicrobial activity, oregano essential oils, carvacrol

Introduction

Food supply is done globally, which implies new risks for food safety. Consequently, the contaminated food can reach different geographical areas and the foodborne pathogens present in food are much more resistant to antibiotics [3].

The food industry generally needs to make significant efforts in order to reduce costs, to avoid food contamination by using sensors into the processing and distribution chain, for the early discovery of pathogens, which could lead to default, to reduce treatment cost of health problems caused by consumption of potentially contaminated food products [3].

Over the past two decades, there has been a growing interest in antimicrobial packaging, along with increased consumer interest in food safety, due to the food deterioration under the action of microorganisms, light, oxygen and water vapor leading to significant losses for both, consumers and food industry.

Particular attention is also paid to the materials from which the packaging is obtain. The recent studies are focused on biodegradable and/or recyclable packaging that protects the environment as much as possible by reducing the waste.

Polyvinyl alcohol is a non-toxic, synthetic, biodegradable polymer with excellent film forming abilities that does not require expensive equipment for their preparation. It shows good mechanical strength and it is resistant in alkaline and acidic environments [13].

Youssef et al. [2019] (26), in their study aimed to obtain an antimicrobial packaging based on carboxymethylcellulose (CMC) and polyvinyl alcohol (PVA) modified with zeolite and doped with noble metals (gold and silver) in different concentrations. The obtained results showed an excellent antimicrobial activity of the films, considering that the studied film can be used as active food packaging.

Corresponding authors: lucica_pop74@yahoo.it

Narasagoudr et al. (2020) [18], obtained to obtain an active packaging based on chitosan (CS) and polyvinyl alcohol (PVA) with the addition of boswellic acid. The results of this study indicate that CS / PVA film prevents the penetration of UV rays due to the incorporation of boswellic acid in the film, improving the morphology, water solubility, mechanical properties, hydrophilicity of the films as well as the barrier properties of the film. The authors of this study concluded that the film obtained can be a promising material for food packaging.

Similar studies based on the including of PVA in the film matrix have been done by Haghighi et al. (2020) [11] who developed films based on chitosan - polyvinyl alcohol enriched with different concentrations of ethyl lauroyl arginate (LAE) and the results suggest this films could be considered potential antimicrobial food packaging to extend the shelf life of food.

Liu et al. (2018) [13] prepared films based on PVA and chitosan (CS) which has been analyzed in terms of film structures, morphology, thermal properties, mechanical properties, oxygen and water vapor permeability and antimicrobial activity against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. The results indicated these films as very promising material for food antimicrobial packaging.

According to the best of our knowledge there are only a few studies related to the antimicrobial films based on polyvinyl alcohol modified with essential oils. Many active ingredients of essential oils are considered GRAS (Generally Recognized As Safe) by the US Food and Drug Administration [8], being non-toxic, environmentally friendly and easily decomposed.

In their study, Luzi et al. (2017) [14], used cellulose nanocrystals obtained from the residues of the plant Kiwi *(Actinidia deliciosa)* as reinforced material for PVA film mixed with natural chitosan, modified, for the first time, with carvacrol as active agent. The morphological and optical characteristics of the obtained films, the mechanical and thermal properties and the antimicrobial and antioxidant activity of the films and the migration in food were studied. The results obtained indicated that the films did not have significant changes in terms of their color and transparency.

The films demonstrated their antioxidant activity preventing migration within the permitted limits and inhibited the growth of bacteria (*Pectobcterium carotovorum susp. odoriferum, Erwinia amylovora, Xanthomonas axonopodis pv. vesicatoria and Xanthomonas arboricola pv. pruni*) by preventing food contamination with these microorganisms and being recommended as possible materials for future active food packaging.

Oregano is extracted by steam distillation from the leaves of *Origanum vulgare*, and its disinfectant and microbial properties have been known since ancient times and have been demonstrated in numerous studies [3]. Carvacrol, thymol, p-cymene, γ-terpinene are the main compounds responsible for the antifungal, antioxidant, insecticidal and antimicrobial activity of oregano [16].

Olasupo et al. (2003) [19] demonstrated in their study that although carvacrol and thymol have a similar structure show diverse antimicrobial efficacy in the agar medium and Dorman and Deans (2000) [6] concluded that this difference is due to the different localization of the -OH groups.

Oregano inhibits the enzymatic activity of pathogens [24] and the properties of p-cymene on the cytoplasmic membrane are sometimes higher than those of carvacrol [23].

Carvacrol is a phenolic monoterpen that belongs to the *Labiatae* family, which includes *Origanum, Satureja, Thymbra, Thymus* and *Coridothymus* and has antioxidant, antifungal, antiparasitic, antitumor and antimicrobial activity on many species of microorganisms, which is why it has been studied by many researchers around the world [3].

The decision to study the antimicrobial activity of carvacrol and oregano is due to the fact that oregano contains other active compounds besides carvacrol that can diversify the antimicrobial effect in the food matrix. In this study carvacrol was used in a concentration of ≥ 98% and oregano as well.

Nutmeg is part of the Myristicaceae family and is the seed of the fruit of an evergreen tropical tree. Due to its antimicrobial and antioxidant properties, nutmeg essential oil is considered a promising biopreservative. The main component of nutmeg essential oil is sabinene, the other components are limonene, α-pinene, β-pinene, myristicin, sabinene and safrol [22].

The antimicrobial and antioxidant potential of nutmeg has also been demonstrated by Gupta et al. (2013) [10].

Against this background, our study is focused on the obtaining and characterization of polyvinyl alcohol - based films modified with of carvacrol, nutmeg and oregano essential oils, respectively aiming to be used as active packaging materials.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) Mowiol® 10-99, M_w = 145,000 $g \cdot mol^{-1}$ and citric acid monohydrate RPE (assay 98%) was purchased from Sigma-Aldrich® (Milan, Italy). Glycerol RE 90% was purchased from Carlo Erba Reagents S.p.a (Milan, Italy). Essentials oils (oregano, carvacrol, nutmeg) ≥98% were provided by Sigma-Aldrich® (Milan, Italy).

2.2. Obtaining films based on PVA

The study of this paper started from the production of a favorable matrix from the strength and elasticity point of view, in which the essential oils are introduced in order to obtain a possible active packaging.

The films were prepared by mixing distilled water and granules of polyvinyl alcohol (PVA) into a covered Berzelius beaker, followed by heating in a water bath, on a magnetic stirrer, at 90°C, for about 4 hours, until the granules were completely dissolved and a homogeneous mixture was formed.

After mixing, the resulting solution was allowed to cool, under continuous stirring, until it reaches the room temperature. Then, citric acid (0.57 wt %) was added to the mixture and was allowed for homogenization for another 5 minutes.

A volume of 10 mL obtained mixture was introduced in every Petri dishe with a diameter of 90 mm and the cans were placed on a straight plane and left to dry at room temperature for two days, thus obtaining the film of polyvinyl alcohol with the addition of citric acid. The film was labeled PVA + Citric acid.

Polyvinyl alcohol film with citric acid and glycerol was obtained using the above described procedure, but the glycerol (1.5 wt %) was introduced after passing the 5 minutes from the citric acid addition, leaving to be homogenized on the magnetic stirrer for another 5 minutes.

In order to obtain the film, Petri dishes were also used, and their drying was done under the same conditions. The film thus obtained was labeled PVA + Citric acid + Gly.

The polyvinyl alcohol and glycerol film was obtained by adding glycerol (1.5 wt %) in the cooled mixture of polyvinyl alcohol and distilled water, followed by homogenization for another 5 minutes and drying under the conditions mentioned above. The film thus obtained was labeled PVA + Gly.

The films modified with essential oils were obtained by adding oregano, carvacrol, and nutmeg essential oil, respectively (0.25 wt %, 0.50 wt %, respectively 0.75 wt %) in the mixture polyvinyl alcohol and glycerol. The essential oils`s addition of was made at ambient temperature, after which the obtained solutions were left on the magnetic stirrer until homogenization and drying was done as for the above described films.

The films thus obtained were labeled PVA + Gly + O 0.25 %, PVA + Gly + O 0.50 %, PVA + Gly + O 0.75 %, PVA + Gly + C 0.25 %, PVA + Gly + C 0.50 %, PVA + Gly + C 0.75 %, PVA + Gly + N 0.25 %, PVA + Gly + N 0.50 %, PVA + Gly + N 0.75 %.

2.3. Characterization of the PVA - based films

The tensile strength (TS, MPa) and the elongation at break (E, %) were performed by using a Zwick/Roell Z1.0 dynamometer testing machine (Ulm, Germany), according to the stardardized method D882-12 of the American Society of testing and materials (ASTM) [2].

Before testing, four rectangular samples with a length of 90 mm and a width of 20 mm were cut from each film and the thickness of each sample was measured in three random points around the film, by using a Syntek micrometer having a measurement range of 0-12.7 mm and a sensitivity of 0.001 mm. The initial grip distance was 50.0 mm, the load cell was 1kN and the crosshead speed was 50.0 mm / min.

The measurements were influenced by the film`s thickness and width and by the cross-sectional area. The TestXpert ® II (V3.31) (Zwick/Roell, Ulm, Germany) was the software program used to obtain the stress-strain curve.

2.4. Testing the antimicrobial activity of PVA - based films

Circles with a diameter of 23 mm were cut from each film and were used for the microbiological tests.

Microbiological tests were carried out using extract of commercial minced meat, that was prepared by placing 1 g of minced meat in 9 mL of sterile saline in a test tube, followed by centrifugation at 6000 rpm for 10 minutes. A volume of 1 mL of aliquot was inoculated on the agar infusion culture medium (BHIA) and the PVA - based film samples were placed on the inoculated medium. The test samples were incubated at 37°C for 48 hours.

The antimicrobial effect of the active agents was verified by control samples which were carried out by applying glycerol and essential oils to absorbent paper circles with a diameter of 23 mm after which were tested under the same conditions. The samples thus obtained have been labeled as ”Control”.

2.5. Determining the compatibility of PVA - based films with food

The compatibility of PVA - based films modified with carvacrol essential oil was tested on three types of cheese, namely cow cheese, raw cheese and parmesan, by determining the contact angle formed by dropping the liquid film onto the cheese`s surface, having different moisture and grease contents.

One drop of liquid film was applied on the cheese by using a disposable Pasteur pipette. The drop was photographed in the profile and the contact angle formed by the drop on the surface of the cheese was subsequently measured using the Golden Ratio software. Six samples were prepared for each determination and the average and the standard deviation were calculated.

The statistical significance of the differences between the contact angle values was statistically analyzed by using the ANOVA ONE - WAY software by applying the TUKEY statistical model [17].

This analysis was performed on polyvinyl alcohol films modified with carvacrol in different mass proportions (0.25 wt %, 0.50 wt %, 0.75 wt %).

3. Results and discussions

3.1 The characteristics of the PVA – based films

Tensile strength (TS, MPa) means the maximum stress point that material can resist, which may or may not be the break point, expressed in force per unit area and elongation at break (E, %) means deformation at maximum stress, expressed in percent.

Firstly, PVA and PVA + Citric acid film were prepared, but their high tensile strength (126,6 MPa and 133.5 MPa, respectively) (Figure 1) and low elongation at break (13.5% and 10.1%, respectively) (Figure 2) showed, as a stress-strain behavior, they are strong, but not tough. Therefore, glycerol were introduced as plasticizer.

PVA + Citric acid + Gly and PVA + Gly films were tested and it was concluded that the films increased their elasticity by introducing glycerol. The difference between these two films is not significant (16.8 MPa as tensile strength and 41.2 % as elongation at break), therefore, it was decided to use the PVA + Gly film as mold for the essential oils modified films.

Figure 1. Tensile strength of PVA - based films (Gly – Glycerol, C - carvacrol, N - nutmeg, O - oregano)

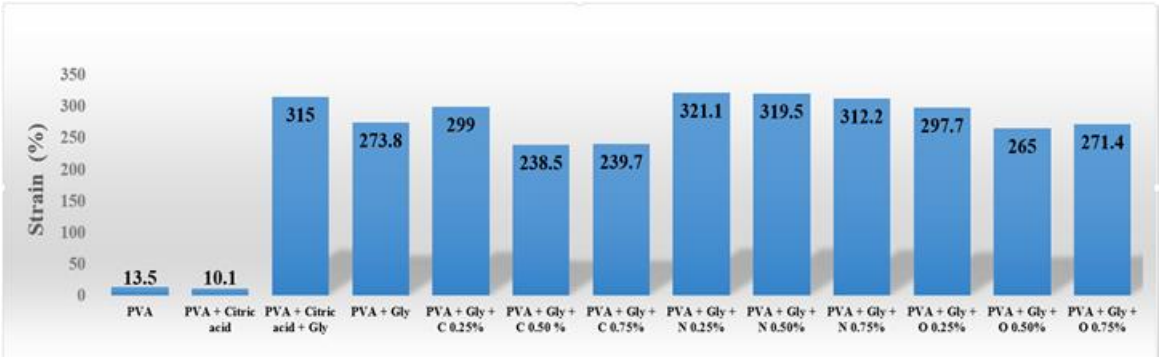

Figure 2. Elongation at break of PVA - based films (Gly – Glycerol, C - carvacrol, N - nutmeg, O - oregano)

The tensile strength of the films modified with carvacrol is higher than of the films with the other essential oils. The tensile strength decreases in order: PVA + C 0.25 % (27.6 MPa) > PVA + C 0.50 % (21.9 MPa) > PVA + C 0.75 % (20.7 MPa). As for the elongation at break testing, the films modified with nutmeg had the best results – that is the PVA + N 0.25 % film proved to have the highest elongation at break (321.1 %), being followed by the PVA + N 0.50 % film (319.5 %) and by the PVA + N 0.75 % film (312.2 %).

The tensile strength of the films modified with essential oils decreases as the concentration of the essential oils increases and the percentage of elongation at break is higher than that of films without essential oils, thus becoming lower with the increase of the essential oils concentration.

Depending on how essential oils interacts with the film-formation biopolymer, essential oils being able to modify the physical properties of the films, different trends have been registered [3].

Andrade et al. (2020) [1] studied the effect of carvacrol in PVA films with different molecular weights (M_w) and found different trends. Films with a high level of M_w provided better mechanical performance, tensile strength and elongation at break, decreasing with increasing carvacrol concentration and partially acetylated PVA films with lower M_w obtained higher tensile and elongation at break than films without carvacrol, decreasing with increasing carvacrol concentration

Chen et al. (2018) [4] have characterized active poly(vinyl alcohol) packaging films incorporated with clove oil si it was found that tensile strength decreases and elongation at break increases with increasing concentration of clove oil.

Debiagi et al. (2014) [7] prepared biodegradable trays based on cassava bagasse, polyvinyl alcohol and essential oils clove si oregano and found that tensile strength decreases with the addition of oils and elongation at break increases. The same trend was established by Wu et al. (2014) [25] by the addition of oregano essential oil in the gelatin-chitosan film

Kong et al. (2020) [12], developed and characterized active corn starch / PVA films incorporated with carvacrol nanoemulsions and were concluded that the incorporation of CNE significantly increased tensile strength and elongation at break.

Pelissari et al. (2009) [21] studied the effect of oregano essential oil on cassava-chitosan starch films and demonstrated that the addition of oregano influences the mechanical strength of the films, decreasing the tensile strength and increasing the tensile strength.

3.2. Testing the antimicrobial activity of PVA - based films

The microbiological analyzes showed an inhibition zone surrounding the control films with carvacrol C 0.25 wt % and C 0.75 wt % and the inhibition area is narrower around the Control C 0.50 % (Figure 3). In the case of carvacrol modified PVA - based films, it has been found that the inhibition area is larger as the concentration of the carvacrol raised from 0.25 wt % to 0.75 wt % (Figure 3). Thus, the presence of carvacrol oil inhibits the development of microorganisms when it comes in contact with the culture medium. The antimicrobial effect of the film increases as the carvacrol oil concentration increases (Figure 3).

Mazarei et al. (2019) [13] obtained a stable carvacrol - rich *Satureja khuzestanica* essential oil (SKEO) nanoemulsion and evaluated the antimicrobial activity of carvacrol, pure SKEO and their nanoemulsion against three food bacteria, including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica*. A serial dilution method was used. The final concentration of microorganisms 5 × 10^6 CFU / mL, the Mueller Hinton Broth (MHB) medium and plates were incubated at 37 °C for 24 hours. The aim was to determine the minimum inhibitory concentrations (MIC) and bactericide (MBC). The results obtain for Carvacrol was: *Escherichia coli* 0.25 % (MIC, MBC), *Staphylococcus aureus* 0.125 % MIC and 2% MBC, *Salmonella enterica* 0.5 % (MIC, MBC). For carvacrol nanoemulsion, various results compared to carvacrol were obtained for *Staphylococcus aureus* 1% (MBC) and *Salmonella enterica* 0.125 % (MIC) si 0.25 % (MBC). For SKEO and SKEO nanoemulsion the concentration obtained for *Escherichia coli* and *Salmonella enterica* is 0.25 % (MIC, MBC) and for *Staphylococcus aureus* 0.25 % (MIC) and 2% (MBC) for SKEO and 0.125 % (MIC) and 1% for SKEO nanoemulsion.

Churklam et al. (2020) [5] have studied the effect of carvacrol against *Listeria monocytogenes* to explain possible mechanisms of action. The results obtained showed that carvacrol inactivates the bacterium inhibiting respiratory activity and affects the cell membrane leading to cell lysis. Furthermore, the synergistic effect of nisin and carvacrol on *Listeria monocytogenes* was studied of sliced bologna sausages during 4 °C storage, resulted in a significant reduction in *Listeria monocytogenes* growth rate for 7 days.

The authors of the study recommending the combination of nisin - carvacrol for a potential preservation method against *Listeria monocytogenes*.

By analyzing the results when the oregano modified PVA – based films, in different mass percentages (0.25 %, 0.50 %, 0.75 %) are used, it can be seen that the antimicrobial effect of the film at the contact with the culture medium has been proven, and, once the concentration percentage increased, the microorganisms population decreased (Figure 4).

For the three concentrations of oregano oil, the Control samples show visible areas of inhibition that increase with increasing the essential oil's concentration. Antimicrobial activity of essential oils of oregano and clove has been demonstrated against several microorganisms strains by Debiagi et al. (2014) [7] who prepared biodegradable active packaging based on cassava bagasse, polyvinyl alcohol. Starch and PVA trays were obtained by a baking process and the essential oils were incorporated either directly into the matrix (in percentage between 6.5 – 10 %) or by surface coating method (2.5 – 7.5 %). Petri dishes were inoculated with 1.5 × 10^8 CFU mL^{-1} of bacterial cultures for *Candida albicans* and incubated for 18 – 24 h at 37°C and for *Aspergillus niger* and *Penicillium* citrinum 10^6 spores mL^{-1}, incubated at 37°C for five days and for evaluation, the inhibition zone test was performed. The stability of antimicrobial activities was tested on trays with the addition of carvacrol 5 % every 3 days for 15 days.

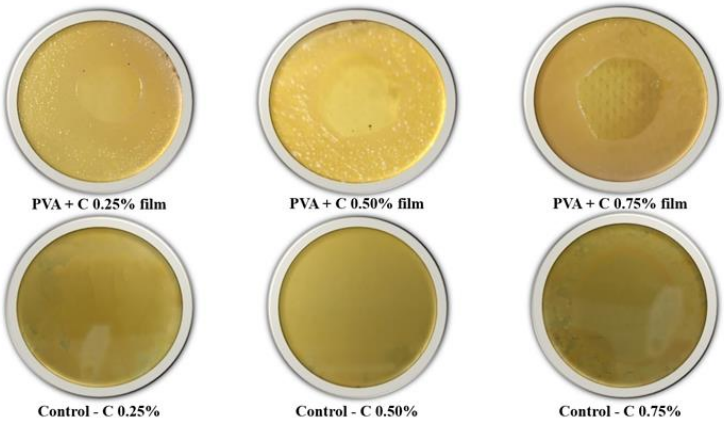

Figure 3. Image of samples of PVA-based films modified with carvacrol essential oil and Control on culture medium with microorganisms from minced meat extract (Control - was obtained from glycerol, absorbent paper and carvacrol essential oil).

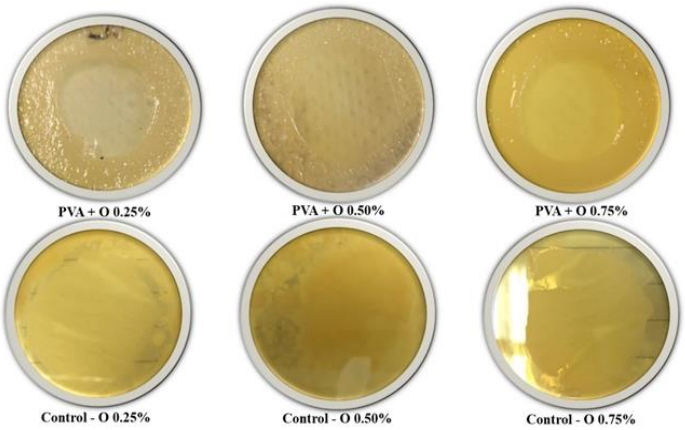

Figure 4. Image of PVA-based films samples modified with oregano essential oil and Control on culture medium with microorganisms from minced meat extract (Control - was obtained from glycerol, absorbent paper and oregano essential oil).

The trays contening oregano obtained the best results regardless of the incorporation method having a largest inhibition area, but those obtained by surface coating with 5 and 7.5% were the most effective. The trays obtained were more efficient against Gram - positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), yeast (*Candida albicans*), molds (*Aspergillus niger* and *Penicillium* citrinum) less effective against Gram - negative bacteria (*Escherichia coli* and *Salmonella Typhimurium*).

Wu et al. (2014) [25] prepared gelatin-chitosan films modified with oregano essential oil (OEO), cinnamon essential oil (CEO) and anise essential oil (AEO) and studied their antimicrobial effect in preservation of fish muscle against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella enteritidis, and Shigabacillus.* OEO was added in different mass proportions: 1%, 2%, 3%, 4%. Filter paper disc diffusion method was used to measure the inhibition zone and the Petri dishes was inoculated with 100 µL of these bacteria 24 h grown (108 CFU/mL) incubated for 24 h at 37°C.
OEO and CEO inhibited the development of all microorganisms studied, but OEO recorded the largest areas of inhibition except *Salmonella enteritidis*. Therefore, the study's authors recommend OEO incorporated gelatin-chitosan films as possible active packaging for fish preservation.

In the case of PVA films modified with nutmeg essential oil, PVA + N 0.25 % and PVA + N 0.50 % films did not inhibit the microorganisms`s growth. Moreover, additional molds were formed on their surface. The inhibition in Control 0.5 wt % and Control 0.75 wt % is clearly seen as well as in PVA + N 0.75 % film. The more reduced inhibition of the microorganism observed in Control 0.25 wt % revealed the lower antimicrobial activity of this essential oil at small concentration. Thus, only the PVA - based film with 0.75 wt % nutmeg oil has antimicrobial activity (Figure 5).

Firouzi et al. (2007) [9] studied the effect of oregano and nutmeg essential oils on *Yersinia enterocolitica* and *Listeria monocytogenes* in broth culture and in Iranian barbecued chicken. The samples were inoculated with *Yersinia enterocolitica* and *Listeria monocytogenes* of 6 to 7 log CFU/g and were stored at 3, 8, and 20°C. The counting of microorganisms was done at 24, 48 and 72 h.

Nutmeg essential oil had a higher inhibition rate on *Listeria monocytogenes* (MIC = 0.20 microl/ml) than did the oregano essential oil (MIC = 0.26 microl/ml) in the broth culture and oregano essential oil had a higher inhibition rate on *Yersinia enterocolitica* (MIC = 0.16 microl/ml) than did the nutmeg essential oil (MIC = 0.25 microl/ml). The antimicrobial effect of the studied oils was not demonstrated in contact with the Iranian chicken.

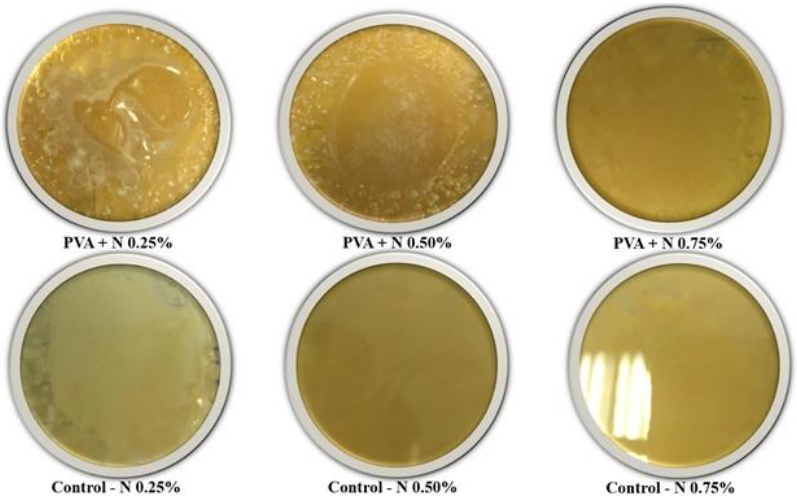

Figure 5. Image of PVA-based films samples modified with nutmeg essential oil and Control on culture medium with microorganisms from minced meat extract (Control - was obtained from glycerol, absorbent paper and oregano essential oil

3.3. Determining the compatibility of PVA - based films with food

The PVA films modified with carvacrol essential oil in different mass percentages (0.25 %, 0.50 %, 0.75 %) were tested on three types of cheese: cow's cheese, raw cheese and parmesan cheese, by measuring the contact angle formed by dripping film forming onto a piece of cheese. The obtained results are presented in Figure 6..

The results obtained have been analysed by means of Anova One - Way statistics , the TUKEY model, in order to establish if the differences are statistically significant. The influence of film types for the same type of cheese was evaluated and the results are presented in Table 1.

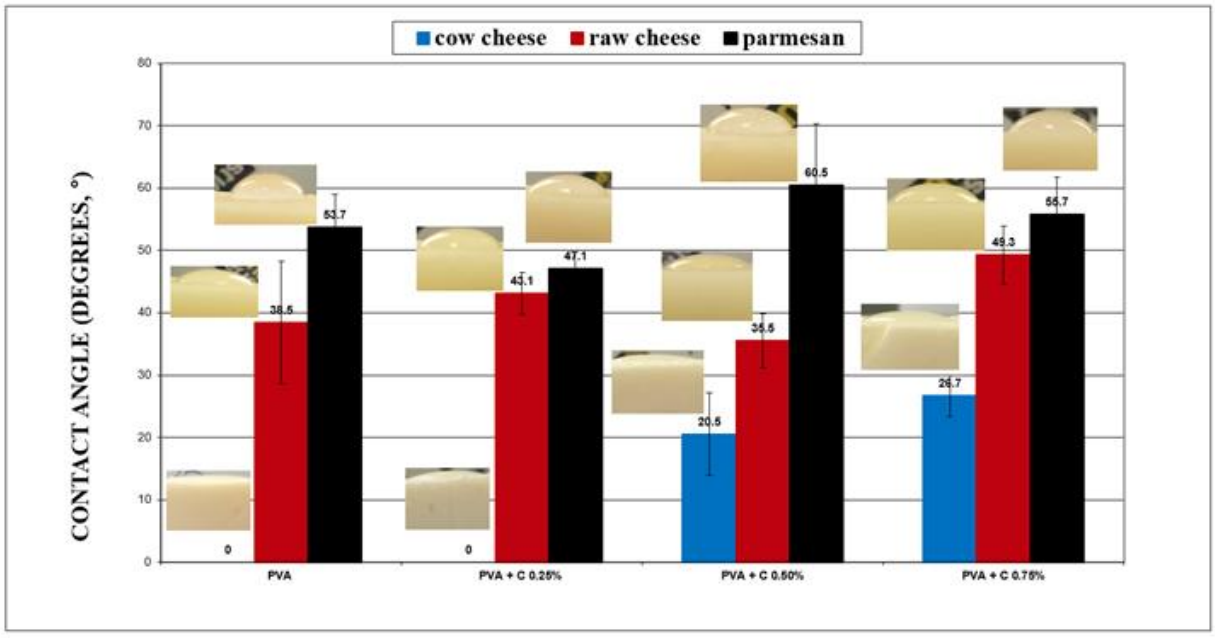

Figure 6. The values of the contact angle formed by dripping film forming on different types of cheeses

Table 1. Statistics analysis (TUKEY model) of the difference between the two types of carvacrol modified PVA films on the three types of cheese

Group 1	Group 2	Cow cheese	Raw cheese	Parmesan
PVA	**PVA 0,25**	NS	NS	NS
PVA	**PVA 0,50**	p<0.01	NS	NS
PVA	**PVA 0,75**	p<0.01	p<0.05	NS
PVA 0,25	**PVA 0,50**	p<0.01	NS	p<0.05
PVA 0,25	**PVA 0,75**	p<0.01	NS	NS
PVA 0,50	**PVA 0,75**	NS	p<0.01	NS

Legend:
NS – the difference between the contact angles according to the two types of group pack is insignificant
eg.: for cow cheese, the difference between the values of the contact angles obtained on PVA and PVA + C 0.25 is not significant (as if the values were identical).
p<0.01 – the difference between the contact angles according to the two types of group pack is 99% significant
p<0.05 - the difference between the contact angles according to the two types of group pack is 95% significant

In the case of cow cheese, the significant differences in terms of statistics (p<0.01) have been noticed between the values of the contact angles obtained for PVA and PVA + C 0.50 % films, between PVA and PVA + C 0.75 % films, the values of the angles formed by PVA + C 0.50 % film and by the PVA + C 0.75 % film being higher than the angle formed by the PVA film, which means that the PVA film has a better adherence on cow cheese than the PVA + C 0.50 % film or the PVA + C 0.75 % film.

Therefore, the PVA film is more compatible with cow cheese than the other films, thus the compatibility for moist cheeses increases as the carvacrol concentration drops.
Also, the value of the angle formed by the PVA + C 0.50 % film and by the PVA + C 0.75 % film is higher than the value of the angle formed by the PVA + C 0.25 %, which means that the PVA + C 0.25 % film is more adherent on cow cheese and more compatible with it.

For the raw cheese, the significant statistic differences (p<0.05) have been found between the values of the contact angles between the PVA and the PVA + C 0.75 % films, the PVA film being more adherent to raw cheese and more compatible with it. Other significant statistic differences (p<0.01) have been found between the values of the angles obtained by the PVA + C 0.50 % film and by the PVA + C 0.75 % film, the angle formed by the PVA + C 0.75 % film being bigger, therefore the PVA + C 0.50 % film is more compatible with raw cheese.

As for parmesan, the significant statistic differences (p<0.05) between the values of the contact angles have been noticed on the PVA + C 0.25 % film and the PVA + C 0.50 % film, the angle formed by the PVA 0.50 % film being bigger, which means that the PVA + C 0.25 % film has a better adherence to parmesan, thus revealing its compatibility with this type of cheese.

An evaluation of the influence and the type of cheese has also been made, the results being reported in Table 2.

Table 2. Statistic analysis (TUKEY model) of the difference between two and two types of cheese for the same type of film

Group 1	Group 2	PVA	PVA 0,25	PVA 0,50	PVA 0,75
Cow cheese	**Raw cheese**	p<0.01	p<0.01	p<0.01	p<0.01
Cow cheese	**Parmesan**	p<0.01	p<0.01	p<0.01	p<0.01
Raw cheese	**Parmesan**	p<0.01	p<0.05	p<0.01	NS

Legend:
NS – the difference between the contact angles is not significant
eg.: for the PVA + C 0,75% film, the difference between the values of the contact angles obtained on the raw cheese and on parmesan is not significant(the values are almost identical)
p<0.01 – the difference between the contact angles made by the types of cheese from a group on the same film is 99% significant
p<0.05 - the difference between the contact angles made by the types of cheese from a group on the same film is 95% significant

For all the films, the significant differences in terms of statistics ($p < 0.01$) have been obtained for the groups cow cheese-raw cheese and cow cheese-parmesan, respectively which means that all the films are more adherent to cow cheese, therefore the films are more compatible with the cow cheese. The same statistic differences ($p<0.01$) have established that PVA and PVA + C 0.50 % films are more compatible with raw cheese than with parmesan. PVA + C 0.25 % is more adherent to raw cheese ($p<0.05$), therefore it is highly compatible with it in comparison with the other cheeses. The results show that the contact angle formed between the film and the cheese varies inversely with the cheese`s moisture.

Pan et al. (2019) [20] obtained polyvinyl alcohol / β-cyclodextrin antimicrobial nanofibers with the addition of cinnamon essential oil in order to delay the rapid decomposition of mushrooms during storage. The contact angle of the films with water was analyzed and it was concluded that it increases with the increase of the concentration of essential oil, its values not being higher than 90°C.

The same trend of the water contact angle was obtained by Youssef et al. (2015) [27] who prepared bionanocomposites based on chitosan / poly (vinyl alcohol) / titanium nanoparticles for packaging of soft white cheese.

4. Conclusions

The tensile strength of the films decreased as the concentration of the essential oils raised up and the elongation at break percentage had the same tendency.

The antimicrobial activity of the films varied directly proportional with the concentration of the essential oil.

In the contact phase between the film and the culture medium, the activity of the microorganisms has been inhibited, suggesting that this film may be used as active food packaging, in particular if the film is tightly wrapped around the food products, such as hard and semi - hard cheeses and, probably also fish and meat.

The compatibility of the PVA - based film with cheese varies inversely proportional with the cheese's moisture. If the film is more adherent to the cheese, the air cannot generate oxidation processes on the surface of the foodstuff.

PVA – based films modified with carvacrol 0.25 wt % and 0.50 wt % are valuable candidates for the cheese preservation.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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