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Original Research

Antimicrobial activity of garlic oil bonded to polypropylene

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ABSTRACT:

Food borne diseases are a serious global problem that need to be addressed. These diseases are usually caused by proliferation of micro-organism during storage of either raw, processed or cooked food. Synthetic food additives are usually incorporated to food to inhibit the growth of microorganisms. However, studies revealed that the use of synthetic food additives were linked to more serious ailments. In this study, garlic oil, a natural antimicrobial agent derived from plant was bonded to polypropylene for the development of antimicrobial food packaging. This was done by utilizing the method similar to the incorporation of dye to cellulose. The antimicrobial activity of the product was tested against gram negative bacteria (*Escherichia coli*), gram positive bacteria (*Staphylococcus aureus*) and fungi (*Candida albicans*) based on agar diffusion test to determine its sensitivity to different types of microorganisms. Results of instrumental analysis strongly justified the interaction of components of garlic oil and polypropylene. UV-Vis analysis showed observable differences in the absorbance of bonded and non-bonded samples. X-ray diffraction patterns revealed morphological changes of polypropylene upon interacting with garlic oil. Garlic oil bonded to polypropylene have susceptibility activity against all the microorganisms tested. Moreover, its antimicrobial activity is comparable to garlic oil as depicted by the statistical results. Thus, bonding of garlic oil to polypropylene has no effect on its antimicrobial activity. Hence, it can be further developed as an antimicrobial food packaging agent.

Keywords:
Antimicrobial activity, Garlic oil, Polypropylene, Antimicrobial packaging.

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INTRODUCTION

Foodborne illness continues to contribute large part of morbidity and mortality worldwide. Microbial proliferation and contamination is usually the main reason for food spoilage. Microbial growth occurs at astonishing rates every place where moisture, heat, and food source are present. Replication rates of million times per day lead to odors, discoloration, and the development of bio-slime. Thus, microbial infection is a frequent complication associated with food. The ability of these microorganisms to adhere to food is a key factor in the initiation and propagation of diseases caused by microbial infection.

Food are added with antimicrobial agents also known as additives in order to prevent biological deterioration caused by microorganisms. These additives can be synthetic such as the bicarbonates and benzoic acid or natural such as different plant extracts (Valencia-Chamorro *et al*., 2011). Synthetic antimicrobial additives used nowadays were linked to increase cases of multiple autoimmune diseases worldwide (Lerner and Matthias, 2015). Thus, much attention is needed to develop plant extracts as natural additives.

Garlic (*Allium sativum*) is a bulb crop that belongs to the family of onions, chive and shallots. It has long been known to have several uses such as diaphoretic, expectorant, antispasmodic, antiseptic, antihypertensive, anti-atherogenic, antithrombotic, and antimicrobial among others (Mathew and Biju, 2008) (Goncagul and Ayaz, 2010). It also has antifungal properties determined through *in vitro* and *in vivo* studies (Li *et al*. 2014). Aliin, a sulfur containing compounds present in garlic is determined to be responsible for its antimicrobial property. Alliin is converted to allicin by allinase enzyme upon crushing of the bulb (Londhe *et al.,* 2011; Farías-Campomanes *et al*., 2014; Casella *et al*., 2013). However, this compound is the same compound responsible for the strong odor and flavor of garlic. The strong odor and flavor of garlic hinder its application to other food as natural food additive since it affects greatly the taste of the food.

One way of preventing the direct incorporation of antimicrobial food additive to food is by using antimicrobial packaging technology. Through this technology, antimicrobial agent is bonded to polymer film in order to suppress the proliferation of microorganisms and extend the shelf-life of the food without interfering on its sensory attributes (Sung *et al*., 2013). Thus, by using this technology, food borne illnesses caused by microorganisms may be prevented and lessen.

In this study garlic oil was bonded to polypropylene, a usual plastic container used for food. It aimed to develop an antimicrobial food packaging. Bonding interaction of garlic oil and polypropylene was evaluated. The antimicrobial activity of the product against gram positive and gram negative bacteria, and fungi were also determined.

METHODOLOGY

Polypropylene plastic was purchased from Chemline Scientific Co. Other reagents that were used include garlic oil, sulfuric acid, chromic acid and perfluorooctanesulfonate were acquired from Belman laboratories.

Preparation of garlic oil bonded to polypropylene

Polypropylene plastic was subjected to several preparation steps such as abstraction, emersion and oven drying in order to graft the surface of the plastic for the garlic oil to chemically bond with it. The polymer had undergone β-scission in the presence of acetone for abstraction. Emerson process followed which include the preparation of bath consisting of 90% sulfuric acid, 8% distilled water, 2% chromic acid, 0.13% potassium permanganate and 0.025% perfluorooctanesulfonate. This method was based on the patent with modifications (Salvin, 1952), specially designed for incorporation of dyes to cellulose; thus, modifications were done. To the bath, polypropylene was immersed for 15-30 seconds

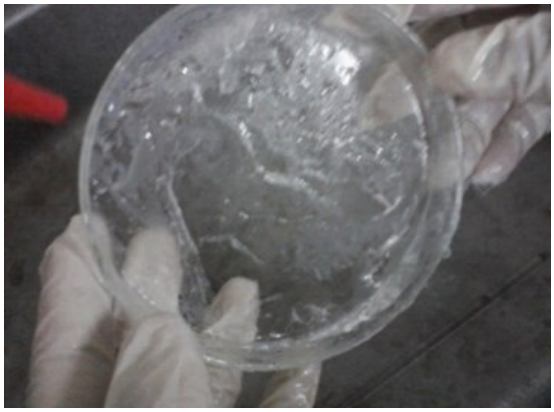

Figure 1.Polypropylene bonded with garlic oil

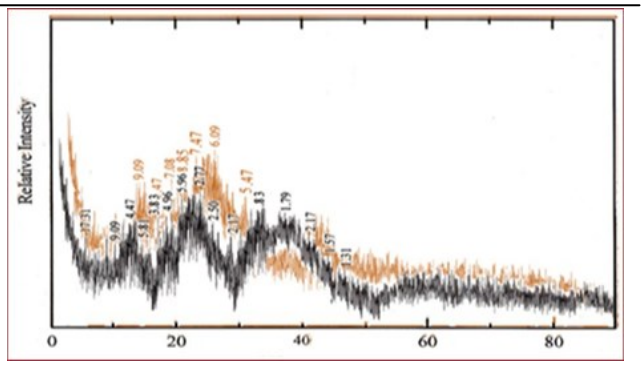

Figure 3. Overlapped peak intensities of the bonded sample (yellow) and polypropylene (black) on X-ray diffraction analysis.

followed by immersion to garlic oil. Three different treatments were prepared with varying ratio of polymer and garlic oil. A 0.1%, 0.3% and 0.5% weight of garlic oil per weight of the polymer were prepared. The polymer bonded with garlic oil was then rinsed using distilled water and oven dried at 105ºC for 1 hour.

The bonded polypropylene with garlic oil was then subjected to different instrumentation procedure for confirmatory analysis of the bonding that occurred. UV-Vis spectroscopy, Fourier transform-infrared spectroscopy and X-ray diffraction analyses were done to determine the change in functionality and possible bonding interaction.

Antimicrobial activity studies

Parallel with it was the microbial analysis which consisted of testing the developed product to different representative microorganisms that usually infect food. This was done using Kirby-Bauer disk diffusion method (Goncagul and Ayaz, 2010). *Staphylococcus aureus, Escherichia coli* and *Candida* spp. were used as representative microbes for gram positive, gram negative and fungi. A 6 mm disk is impregnated with a known concentration of an antimicrobial compound. The agar plate is inoculated with a suspension of the pathogen tested prior to placing of disks on the agar surface, simultaneous growth of the bacteria and diffusion of the antimicrobial compounds occurred. Comparative analysis of the inhibition of microbial growth using pure garlic oil and the developed product was also done using statistical tool Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

The bonded product obtained appeared clear and elastic similar to the untreated polypropylene (Figure 1). Polypropylene undergone several steps in order to modify the surface prior to bonding with garlic oil. Abstrac-

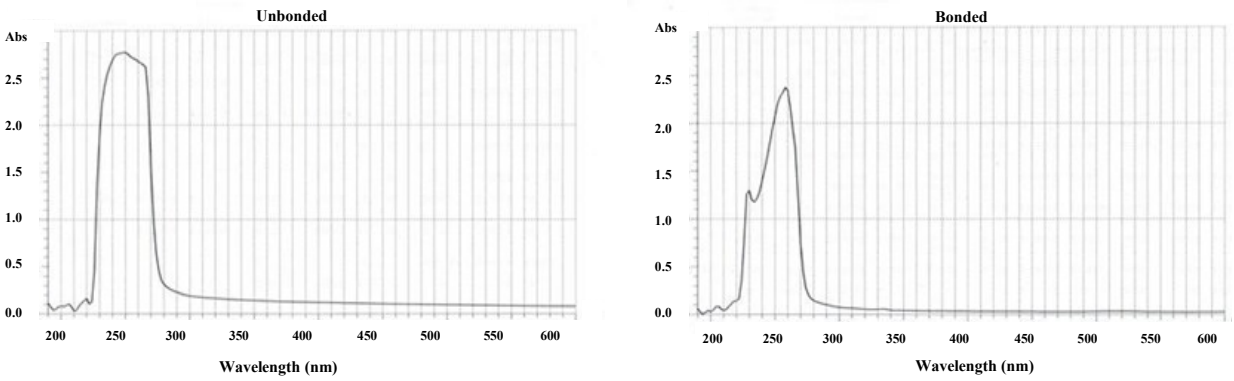

Figure 2. UV-Vis spectrum of polypropylene showing maximum absorbance peak at 250 nm for the unbonded sample (left) and shift in maximum absorbance peak at 255 nm and appearance of shoulder peak at 245 nm for the bonded sample (right).

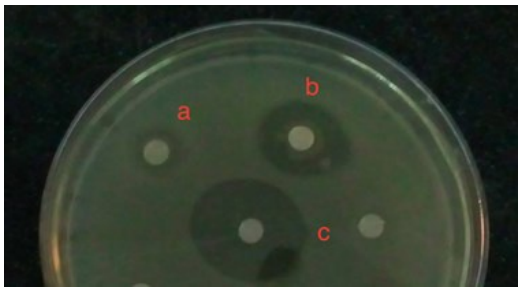

Figure 4. Zone of inhibition of bonded garlic against (a) *S. aureus* (b) *E. coli* (c) *Candida albicans*

Table 1. Antimicrobial activity of garlic oil bonded to polypropylene.

S. No	Microorganism	Average zone of inhibition (mm)	Description
1	*Escherichia coli*	32.4632	Susceptible
2	*Staphylococcus aureus*	25.1328	Susceptible
3	*Candida albicans*	54.4544	Susceptible

tion of a hydrogen atom led the formation of a tert-carbon radical. This radical is unstable and further formed upon application of heat. Sulfone component of the garlic oil then formed bonding on the primary layer of the activated polypropylene. The bonding of the sulfone components of garlic oil which includes allicin was confirmed by the appearance of peak at around 245 nm and shift of the maximum peak from 250 to 255 nm in the UV-Vis spectrum of the bonded sample (Figure 2). Allicin has absorption peaks at 245 and 255 nm (Farías-Campomanes *et al*., 2014).

The incorporation of garlic oil to polypropylene caused a more crystalline structure as depicted by the increase in the relative intensity of the peaks of the bonded sample x-ray diffraction pattern (Figure 3). Increase in the relative intensity of the peaks corresponds to changes to the morphology of the samples. These changes in morphology may be due to particle size changes as well as to texture of the samples. The uniformed crystalline structure created a uniform strain termed as a macrostrain which is a permanent change in the crystal lattice of polypropylene. This can be observed as a shift in the diffraction peak pattern at around 40 to 45.

The product obtained was then subjected to microbial analysis against representative microorganisms of gram positive, gram negative and fungi that usually caused food borne diseases. Garlic oil was used as the control group since garlic oil has been proven to have microbial inhibiting activity. Moreover, this will determine whether bonding of garlic oil to polypropylene affects its antimicrobial activity since this property is crucial in developing an antimicrobial packaging. Based on the average zone of inhibition, it was observed that the polypropylene bonded garlic oil have antimicrobial activity that falls on susceptible range against *E. coli, S. aureus* and *Candida albicans* (Table 1). The average zone of inhibition determined against *E. coli* was 32.4632 mm while for *S. aureus* and *Candida albcans* were 25.1328 mm and 54.4544 mm, respectively. This antimicrobial activity is mainly due to allicin component of garlic oil that is known to inhibit *S. aureus* (Wu *et al*., 2015; Marchese *et al*., 2016; Li *et al*., 2015; Fratianni *et al*., 2016; Pérez-Köhler *et al*., 2015), *E. coli* (Horita *et al*., 2016; Müller *et al*., 2016; Yang *et al*., 2016) and *Candida albicans* (Li *et al*., 2015; Thomas *et al*., 2015; Müller *et al*., 2016; Vikrant *et al*., 2015). Thus, this implies that growth of these microorganisms is inhibited by the product due to incorporation of garlic oil. The zones of inhibition observed were shown in

Table 2. Comparison of antimicrobial activity of garlic oil and garlic oil bonded to polypropylene

S. No	Microorganism	Zone of inhibition (mm)		t-calculated	t-critical
		Garlic oil	Garlic oil bonded to polypropylene		
1	*Escherichia coli*	33.5104	32.4632	1	2.920
2	*Staphylococcus aureus*	27.2272	25.1328	2	2.920
3	*Candida albicans*	55.5016	54.4544	0.5	2.920

Figure 4.

Statistical evaluation of the results further suggests that the t-calculated do not exceed the t-critical for comparison of the zone of inhibition of garlic oil with the product against the different microorganisms (Table 2). Zone of inhibition of garlic oil against *E. coli* was determined to be 33.51 mm which was slightly larger as compared to that of the product which is 32.46 mm. Zone of inhibition of garlic oil against *S. aureus* was determined to be 27.22 mm while that of the bonded product was 25.13 mm. Zone of inhibition of garlic oil against *Candida albicans* was determined to be 55.5016 mm while that of the bonded product was 54.4544 mm. The t-calculated values for *E. coli, S. aureus* and *Candida albicans* were 1, 2 and 0.5 respectively.

Thus, the antimicrobial activity of the bonded sample is comparable with that of the pure garlic oil. These results suggests that bonding garlic oil to polypropylene did not affect its antimicrobial activity. Hence, the objectives of the study were satisfied.

CONCLUSION

Garlic oil was bonded to polypropylene following the method of incorporating dye to cellulose. Bonding interaction of garlic oil and polypropylene was confirmed by UV-Vis spectra and X-ray diffraction pattern of the product. Antimicrobial activity of garlic oil against gram positive, gram negative and fungi was not altered by its bonding to polypropylene. Thus, the product obtained may be further developed as an antimicrobial food packaging agent.

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