The impact of the mulberry (Morus nigra L.) leaf extract on the physicochemical properties of poly(vinyl alcohol) blend films

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Abstract. In this work, mulberry (Morus nigra L.) leaf extract was added in poly(vinyl alcohol) (PVA)-based films and its impact on the film’s properties was evaluated. In addition, HCl and glycerol were studied for their use as additives to prepare PVA-based films. The results showed that HCl and glycerol have minimal impacts on the films’ appearance, while mulberry leaf extract imparted green colour to the films produced, mainly due to the presence of green pigments. Moreover, the results suggested that a significant interaction has occurred between the polymer matrix and leaf extract, contributing to a more compact and uniform film morphology. The tensile strengths of the films increased from 21.38 to 28.28 MPa after the addition of mulberry leaf extract. Additionally, the films were tested for their application as food wrapping films. Overall, the results showed that PVA-based films incorporated with mulberry leaf extract have higher capability to preserve the freshness of food when compared to commercial cling wraps from brands such as Diamond and Glad. Appropriate proportions of additives (mulberry leaf extract, HCl and glycerol) used in the formulation of P-GH-M20 films showed improvement in its mechanical properties and food preservation capability.

1. Introduction

Food packaging plays an essential role in preserving the quality of food by providing a barrier that avoids excessive contact between food and external environmental factors such as microorganism, moisture, and water vapor. Following the industrial revolution, plastics made from petroleum-based polymer have been introduced into the food packaging industry. However, from environmental perspective, biopolymer is gaining more attention as a sustainable alternative to replace traditional plastic produced from petroleum derivatives. Poly(vinyl alcohol) (PVA) is found to be a promising material owing to its biodegradability, marginal toxicity and outstanding flexibility. Despite its remarkable characteristics, several weaknesses of PVA exist and thus hindering it from being commercialized for industry use. PVA is primarily comprised of hydroxyl bonds with a general formula of [CH\(_2\)CH (OH)]\(_n\), and therefore PVA-based film is highly hydrophilic. Besides, PVA-based film generally has weaker mechanical properties compared to plastic polymers. In view of these constraints, there were numerous studies carried out to improve the properties of PVA-based film by incorporating...
potential additives into its polymer matrices. The natural extracts obtained from various terrestrial sources such as fruits, leaves and roots of plants and herbs could be a good option. Examples of the several extracts from plants and fruit such as, mint, pomegranate [1], seaweed [2], Syzygium cumini [3] and Azadirachta indica (neem) extract [4] possess excellent antioxidant activities, good antimicrobial properties, and improve mechanical properties.

In this study, mulberry leaf extract was chosen to be incorporated into PVA-based film. To the best knowledge of authors, there is still limited study on the use of mulberry leaf extract in preparing PVA-based film. Mulberry leaf, or known as Morus nigra L., is highly recognized to be used as a herb in traditional Chinese medicine (TCM) to treat several diseases [5]. It was reported that mulberry leaf extract consists of substantial amount of total carotenoids (e.g. β-carotene and lutein) and polyunsaturated fatty acids (e.g. α-linolenic acid and linoleic acid), and therefore contributing to its outstanding antioxidant and cytotoxic activities [6–9]. In this work, mulberry leaf extract was used to prepare PVA-based films, and its impact on the film’s appearance, morphology, and mechanical properties, was evaluated. The produced films were also applied as food wrapping films, and their food preservation capability was studied and compared to commercial cling wraps, namely Diamond and Glad cling wraps, which were purchased from the market.

2. Materials and Methods

2.1. Materials

PVA (99.8% - 100% hydrolysed) was purchased from Sigma-Aldrich. Glycerol, methanol and HCl were obtained from Merck. All chemicals and solvents were of analytical grade and used without further purification. Commercial food wrapping films, namely Diamond Cling Wrap (Reynolds Consumers Products LLC, USA) and Glad Cling Wrap (The Glad Product Company, USA) were purchased from a local distributor and used directly. The main purpose of using low density polyethylene (LDPE) cling wrap plastic as a reference is to compare the characteristic of both polymer materials in plastic wrapping. The chosen conventional cling wraps are made of Polyethylene (PE). Mulberry leaves were picked from the garden in Putrajaya, Malaysia.

2.2. Preparation of mulberry leaf extract

The extraction process was performed following the protocols described by Lee and Choi [9]. Fresh mulberry leaves were picked from plant and washed to remove impurities. Subsequently, the mulberry leaves were dried in an oven at 80°C for 1 h. The oven-dried leaves were grinded using a medicine blender (Golden Bull SY-04, Malaysia). Fine leaf powder which passed through a 100-mesh sieve was used for extraction. 20 g of fine leaf powder was mixed with a solvent which was comprised of 80:20 (v/v) of methanol and distilled water, respectively. The mixture was shaken at 150 rpm for 8 h at room temperature in an orbital shaker. Next, the mixture was filtered using a Whatman No.1 filter paper. The solid residue obtained from the filtration process was mixed with fresh solvent, and the extraction process was repeated for two times. The filtrate obtained was concentrated using a reduced pressure rotary evaporator (Heidolph Hei-VAP Value, Germany) operating at 45 °C. The concentrated extract was stored at 4°C until further use. 5 mL of the concentrated extract was dried at 100°C until a constant weight was obtained to calculate the extract concentration. The mulberry leaves extract concentration obtained was 25.92 ± 2.1 mg/mL.

2.3. Preparation of films

The polymer films were prepared through the casting method. 10 wt% of PVA solution was prepared by dissolving PVA in water and stirring the mixture at 6000 rpm by using a homogeniser (IKA ULTRATURRAX T 18 digital) at 80 °C for 2 h. The PVA-based films with varying compositions, as described in table 1, were prepared. For some films, 15 % glycerol (w/w, based on PVA), was added into the film.
forming solution, aiming to study the influence of glycerol as a plasticizer on the film’s mechanical properties. Besides, the impact of HCl on the film’s characteristics was investigated by adding 10 % HCl (v/v, based on the final volume of film making solution) into the film forming solution. Each addition of substance was followed by a constant stirring of the mixture at 6000 rpm for 5 min before the subsequent step.

Table 1. Compositions of PVA-based films studied in this work.

| Film sample | 10 wt% PVA solution (mL) | Glycerol (mL) | HCl (mL) | Concentrated leaf extract (mL) |
|-------------|--------------------------|---------------|----------|-----------------------------|
| P-0         | 50                       | -             | -        | -                           |
| P-G         | 50                       | 0.5952        | -        | -                           |
| P-H         | 50                       | -             | 0.5      | -                           |
| P-GH        | 50                       | 0.5952        | 0.5      | -                           |
| P-M10       | 45                       | -             | -        | 5                           |
| P-M20       | 40                       | -             | -        | 10                          |
| P-M30       | 35                       | -             | -        | 15                          |
| P-M40       | 30                       | -             | -        | 20                          |
| P-GH-M10    | 45                       | 0.5952        | 0.5      | 5                           |
| P-GH-M20    | 40                       | 0.5952        | 0.5      | 10                          |
| P-GH-M30    | 35                       | 0.5952        | 0.5      | 15                          |
| P-GH-M40    | 30                       | 0.5952        | 0.5      | 20                          |

All film forming solutions were left overnight so that they were completely degassed before film casting. The film forming solution was casted on a glass plate with a wet thickness of 0.25 mm [10]. The film sample was then dried for 24 h and was peeled and kept in a desiccator for further characterization and application tests.

2.4. Characterization of films

2.4.1. Morphology. Morphology of films was studied using scanning electron microscopy (SEM) (Hitachi, S-3400N, Japan) [11]. All films were sputter-coated with gold before SEM imaging.
2.4.2. Mechanical properties. Mechanical properties of films were acquired by a universal testing machine (Instron 5582Q4970, USA) with a grip separation of 15 mm and a cross-head speed of 20 mm/s. The specimen strips were prepared with a dimension of 25.4 mm × 100 mm and the thickness of specimen strips were measured first before mechanical measurement using a hand-held micrometre (Mitutoyo, Japan).

2.5. Evaluation of film antifungal activity on pumpkin slice. In this test, 40 mm × 20 mm of pumpkin cut was wrapped using the prepared sample film and stored in a refrigerator at 6 °C. The food sample was monitored daily for any growth of mould. The time it took before the pumpkin cut wrapped using the respective film started to grow mould was recorded for each film.

3. Results and discussion

3.1. Physical Appearance

Figure 1 displays the physical appearance of all films studied in this work. Diamond and Glad cling wraps were included for comparison purpose. From figure 1, all PVA-based films (P-0, P-G, P-H and P-GH films) without mulberry leaf extract appeared clear and transparent. This indicates that both glycerol and HCl in P-G, P-H and P-GH did not affect the films’ appearance. Whereas, for films which uses mulberry leaf extract as an additive, it was observed that the greenish hue turned darker (from pale to olive green) with increasing amount of mulberry leaf extract. Besides, a brownish green colour was observed for films containing the leaf extract, glycerol and HCl, namely P-GH-M10, P-GH-M20, P-GH-M30 and P-GH-M40. This might be attributed to the colour change of green pigments in the leaf extract under acidic environment when HCl was added in the film making solution. It was frequently reported that chlorophyll molecules change colour from bright green to a dull brown colour in response to the acidity of the water [12]. The colour change of chlorophyll is due to denaturation which takes place when the magnesium ion in the centre of the chlorophyll molecule is replaced with a hydrogen atom [13,14]. On the other hand, both commercial cling wraps by Diamond and Glad showed clear transparency.
Figure 1. Films studied in this work, (a) P-0; (b) P-G; (c) P-H; (d) P-GH; (e) P-M10; (f) P-M20; (g) P-M30; (h) P-M40; (i) P-GH-M10; (j) P-GH-M20; (k) P-GH-M30; (l) P-GH-M40; (m) Diamond cling wrap; and (n) Glad cling wrap.

3.2. Morphology of films

Figure 2 shows the SEM results of the commercial grade cling wraps and films prepared using different compositions. From figure 2, P-0 surface morphology was found to be more homogeneous than other films with additives. Some droplets and embedded particles were found in the films containing leaf extract, particularly P-M10, P-M20, P-M30 and P-M40. Similar findings were obtained by Ma et al. [15] in their study on an intelligent film composed of PVA, chitosan nanoparticle and mulberry extract. The authors observed the presence and formation of droplets in the cross-sections of films incorporated with mulberry extract. This phenomenon was a result of a phase separation or chemical segregation in the chlorophyll pigment found in the leaf extract, where polysaccharide molecules were isolated from the film forming mixture resulting in the formation of droplets [15]. Likewise, Bashir et al. [16] reported that many uniformly distributed pores were found in blended tetraethoxysilane-crosslinked films with the addition of mint and grapefruit peel extracts.
On the other hand, there were fewer white particles observed in films incorporated with leaf extract, glycerol and HCl. This might be due to the action of HCl which alters the solubility and nature of the bio-compounds in leaf extract, resulting in smoother cross sections compared to other films with only leaf extract. Commercial Diamond and Glad cling wraps showed slightly non-homogeneous surface morphology compared to P-0, however neither pores nor embedded particle was observed. The non-uniform morphology could be caused by a certain degree of phase separation in films.

![Figure 2. Morphology of films studied in this work, (a) P-0; (b) P-G; (c) P-H; (d) P-GH; (e) P-M10; (f) P-M20; (g) P-M30; (h) P-M40; (i) P-GH-M10; (j) P-GH-M20; (k) P-GH-M30; (l) P-GH-M40; (m) Diamond cling wrap; and (n) Glad cling wrap.](image)

3.3. Mechanical properties of films

It is important to determine the film’s mechanical properties since a packaging material will be exposed to various kind of stress during its use. A good food packaging film should not only possess high tensile strength (TS) but also good ductility. Ductility is measured based on fracture strain or also known as elongation at break (E%). It is closely related to the ability of a plastic specimen to resist change in shape without cracking or breaking [17,18]. In this study, the thickness of film strips was measured first before mechanical measurement. P-0, P-G and P-H films’ thickness were 0.0250±0.0029 mm, 0.0233±0.0029 mm and 0.015±0 mm, respectively. On the other hand, the thickness of P-GH and P-M_X resulted in
stable thickness of 0.0200±0 mm. The thickness of sample PVA added with glycerol, HCl and leaf extract (P-GH-MX) varied from 0.0130±0.0029 mm to 0.0220±0.0058 mm with increasing among of leaf extract. The conventional cling wrap thickness was 0.0117±0.0029 mm for Glad and 0.0100±0 mm for diamond. The mechanical properties of the films studied were presented in figure 3.

Figure 3. Mechanical properties, including (a) tensile strength and (b) elongation at break of films studied in this work.

In general, all films showed improved in tensile strength when additive were added in the films, except for P-GH-M40 films exhibited exceptionally poor tensile strength. Besides, it was observed that most of the films with glycerol as a plasticizer, namely P-G, P-GH-M20, P-GH-M30 and P-GH-M40, revealed better ductility (higher E%). Several works have highlighted that the addition of glycerol in PVA polymer matrices promotes great flexibility in films by attracting more water into the structure of films [19–21]. On the other hand, the dispersion of lipids glycerol might introduce discontinuities in the polymer matrices which contribute to a reduction in the polymer cohesion forces and thus, the films’ have low resistance to break.

On the flip side, the addition of HCl in the PVA polymer film improved TS of films by 10.11% when comparing TS values of P-H and P-0. This might be due to the action of HCl which facilitates the crosslinking process of PVA polymer [22]. However, it was found that the presence of mulberry leaf extract in high amounts hindered the effect of HCl in enhancing the TS of films, as displayed by P-GH-M30 and P-GH-M40 films. An appropriate amount of mulberry leaf extract together with HCl and glycerol could be a good formulation for film composition to produce a film with high TS. Both P-GH-MN10 and P-GH-MN20 have relatively high TS than the Diamond cling wrap. Nevertheless, all films still showed lower TS compared to those reported earlier by Kanatt et al. and Qianyun Ma et al.[1,15].

3.4. Evaluation of film antifungal activity on pumpkin slice

Food packaging analysis is necessary to evaluate the preservation capability of films towards the freshness of food. The results were expressed in the terms of the time it took for mold to grow on the wrapped pumpkin slice, as summarised in figure 4.

The final results proved that the films with additives such as HCl, glycerol and/or mulberry leaf extract have better food preservation power to resist the growth of mold on the pumpkin compared to pure PVA film, which is P-0. Several films showed promising results, particularly, P-GH-M20 and P-GH-M40. These films were able to preserve the pumpkin cut for at least 29 days before mold started to grow, compared to the commercial Glad cling wrap which was able to preserve the pumpkin for up to 21 days. This might be due to the presence of additives, which consist of antimicrobial behaviour, which
helped to extend the freshness of the pumpkin. It was frequently reported that mulberry leaf extract has an antimicrobial effect to inhibit the growth of microorganisms such as *Streptococcus mutans* [23] and *Helicobacter pylori* [24]. P-GH-M20 displayed the highest food preservation power by keeping the food sample away from mould for up to 34 days. This indicated that the optimum amount of mulberry leaf extract to be incorporated into the PVA-based film was 20% (v/v).

![Figure 4. Evaluation of film antifungal activity on pumpkin slice](image)

### 4. Conclusion

By blending mulberry leaf extract into PVA-based film, developed films have enhanced mechanical properties and their food preservation. Besides, the addition of glycerol and HCl in PVA-based films might affect the film properties. With optimum formulation, it is possible to produce a film with advantageous properties for food packaging application. Among all films, P-GH-M20 demonstrated the highest TS of 28.38 ± 0.07 MPa with a thickness of 0.0130 mm and food freshness preservation of up to 34 days. Besides, a compact and smooth surface section was observed compared to conventional and other developed films. All the films were cast at 0.0250 mm wet thickness and result showed that the dry thickness of sample films varied between 0.0250 and 0.0130 mm, depending on the addition of glycerol, HCL and amount of leaf extract in the PVA solution. Soil biodegradation test will be required in future studies to determine the degradation time of produced films in soil while an antimicrobial study can be done to determine the antimicrobial performance of leaf extract towards *E. coli* bacteria.

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