RESEARCH ARTICLE

Starch
www.starch-journal.com

Antimicrobial Starch-Based Film for Food Packaging Application

Ain Nadirah Romainor, Suk Fun Chin, and Samuel Lihan*

Sago starch (*Metroxylan sago*) modified with polyvinyl alcohol (PVA) incorporated with citric acid as an antimicrobial agent at the optimized ratio of 5:8:15 (%, w v^{-1}) for starch: PVA: citric acid is synthesized. The starch-citrate film presents antimicrobial activity against the pathogenic food borne bacteria *Salmonella thypimurium* (*S. thypimurium*), *Escherichia coli* (*E. coli*) (O157:H7), and *Listeria monocytogenes* (*L. monocytogenes*) and food fungus *Aspergillus* spp. and *Rhizopus* spp. The studies showed that 98–99% of food borne bacteria growth and 87–99% of fungal growth can be inhibited. Qualitative assessment of food shelf-life reveals that the starch-citrate film is more efficient to inhibit the microbial growth in cake and bread samples compared to commercial food wrappers, as the film can extend the shelf-life of the food products (10 days for cake and 40 days for bread). The potential application of this starch-citrate film as antimicrobial and eco-friendly bio-based food packaging application is therefore demonstrated.

1. Introduction

Conventional plastic made from petrochemical resources provides enduring benefits in numerous industrial fields especially in food packaging due to their low cost, ease of processing, and good mechanical properties.[1] Unfortunately, the use of conventional plastic in food packaging industry has led to huge environmental damage since the current practices of disposable plastic waste are done through accumulation of plastic debris in landfill, which takes hundreds of years to degrade, in addition to incineration that releases hazardous gasses to the atmosphere.[2] Therefore, there is a pressing need to explore biodegradable and renewable resources such as cellulose and starch to replace the petrochemical-based food packaging materials.[3]

Among them, starch is one of the most promising precursor materials for food packaging since starch has inherent thermoplastic behavior, abundantly available and low in cost.[4] In addition, the demand for safe and efficient food packaging that can extend the shelf life of food has received great attention across food market worldwide, which is hoped to enhance the food quality and ultimately reduce food wastage. Native starch alone does not possess antimicrobial activity. Hence, antimicrobial agents need to be incorporated into starch polymer to prolong the shelf life of the food and prevent food spoilage.[5] Metal nanoparticles, essential oil, organic acid, natural antimicrobial polymer, and polyelectrolytes polymer are antimicrobial agents that could be incorporated into starch-based packaging films.[6]

Citric acid is a natural weak organic acid that exists widely from citric cycle of the citrus fruits, and is prized for their preservative quality to prevent food spoilage. It is worth to incorporate citric acid in starch-based packaging film in order to satisfy the pre-requisite of environmental friendly antimicrobial packaging films because citric acid has the ability as natural antimicrobial agent.[7] On the other hand, starch polymer suffers from moisture due to its hydrophilic nature. Herein, the effects of starch, citric acid, and PVA composition on the fabrication of starch-citrate film and their efficiency against bacterial and fungal inhibition have been evaluated to obtain films with optimum physicochemical, antimicrobial, and antifungal properties for potential food packaging applications.

2. Results and Discussion

2.1. FTIR Analysis

Starch-citrate film was prepared by incorporating PVA onto starch-citrate as shown in **Figure 1** Fourier transform infrared (FTIR) spectra of native starch, starch-citrate and starch-citrate film are shown in **Figure 2**(a), (b), and (c), respectively. The formation of new ester linkages in starch-citrate were evidenced by the appearance of carbonyl (C=O) and C-O-C stretching of the ester

A. N. Romainor
School of Liberal Arts, Science and Technology (PUScLST)
Perdana University
Suite 9.2, 9th Floor Wisma Chase Perdana, Jalan Semantan Damansara Heights, Kuala Lumpur 50490, Malaysia
S. F. Chin
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
Kota Samarahan, Sarawak 94300, Malaysia
E-mail: sfchin@unimas.my
S. Lihan
Institute of Biodiversity and Environmental Conservation
Universiti Malaysia Sarawak
Kota Samarahan, Sarawak 94300, Malaysia

DOI: 10.1002/star.202100207

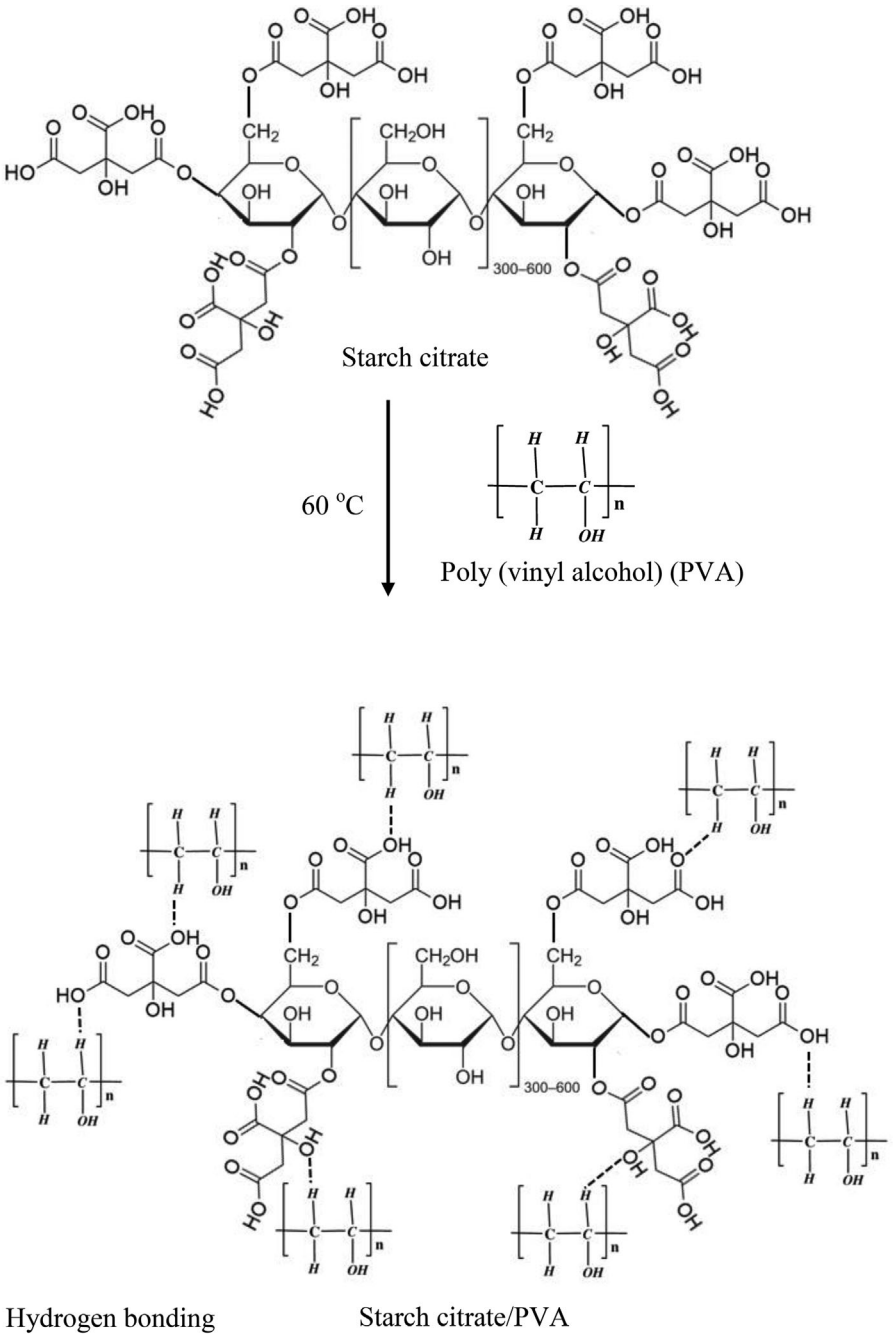

Figure 1. Schematic representation of the reaction between starch-citrate with PVA.

molecules absorbance peaks appeared at 1724 and 1156 cm^{-1} of FTIR spectrum of starch-citrate molecules (Figure 2(b)) after the esterification reaction between starch and citric acid molecules.[8] Ester bond was formed as the result of the hydrolysis of hydroxy group (OH) moieties on the starch backbones reacted with carboxylic acid (COOH) moieties from citric acid during esterification reaction.[9] After blended with PVA, the carbonyl of ester bond (C=O) and C-O stretching peaks of starch-citrate film were shifted to 1715 and 1290 cm^{-1}, respectively, as observed in Figure 2(c).[10]

The presence of C-O stretching from PVA in starch-citrate molecules was also supported by the absorbance peak at 1023 cm^{-1} in Figure 2(c).[11] It can be seen that the absorbance peak belonged to hydrogen bond of starch-citrate molecules has shifted from 3317 cm^{-1} to lower wavelength number of 3280 cm^{-1} after starch–citrate was blended with PVA in starch-citrate film as indicated in Figure 2(b) and (c), respectively. This indicated that hydrogen bonds were formed between PVA and starch-citrate in the starch-citrate/PVA system after oven drying to obtain starch-citrate film.[12]

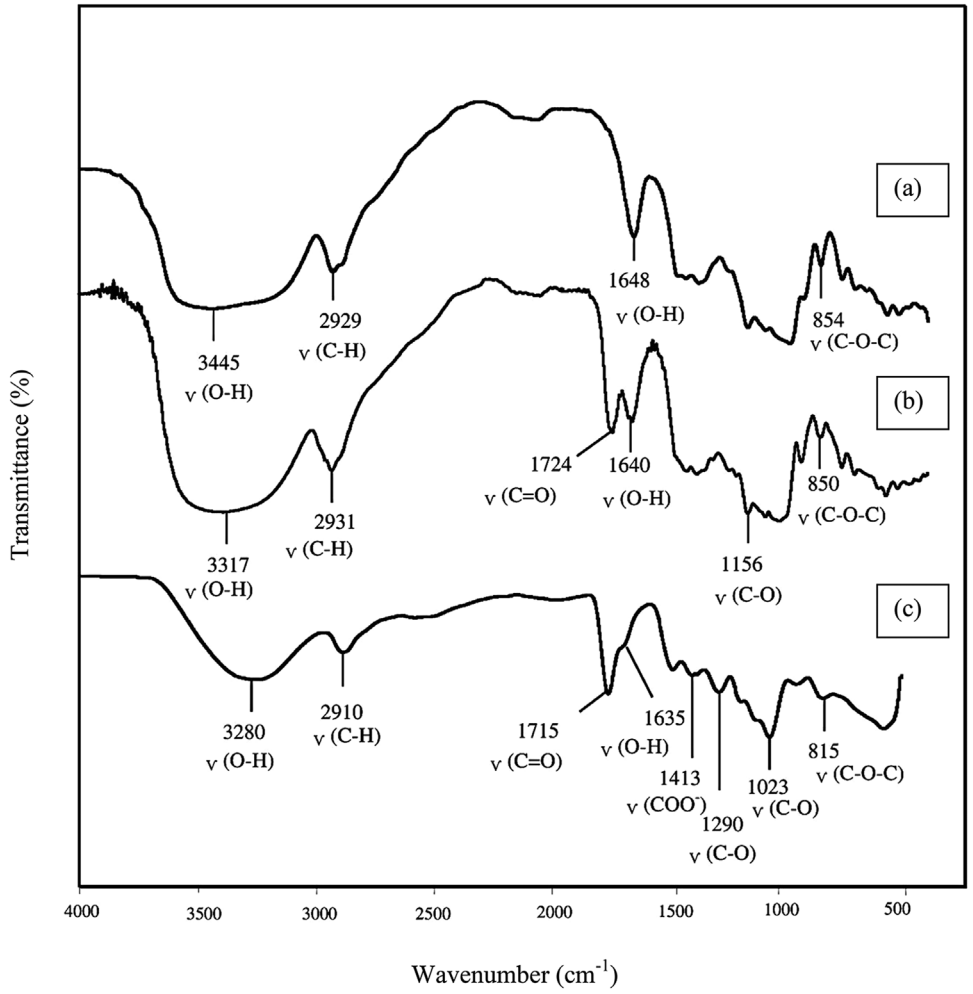

Figure 2. FTIR spectra of (a) native starch, (b) starch-citrate, and (c) starch-citrate film.

Furthermore, the formation of hydrogen bonding had shifted and reduced the intensity of COOH of starch-citrate at 1640 cm^{-1} (Figure 2(b)) to a less intense peak at 1635 cm^{-1} in (Figure 2(c)). This is due to the hydrogen bonding interaction between OH and COOH of starch-citrate with OH moieties of PVA in starch-citrate PVA blend film.[13] The absorbance peak at 1413 cm^{-1} indicated the presence of carboxylate moieties (COO^-) in the starch-citrate blend PVA film.[11]

Various studies attempted to prepare antimicrobial starch film including synthesis of silver nanoparticle in starch gelatinized medium suspension to fabricate silver nanoparticles coated with starch layer[14] and casting method of chitosan and starch to obtain active starch film's[15] due to its simplicity and promising active starch film against microbial attack. However, silver nanoparticles could lead to toxicity issues due to hazardous by product during the synthesis,[16] while casting method involved the use of concentrated antimicrobial agent due to possibility loss of the agent functionalities during the reaction.[17] Therefore, a synthesis of starch-citrate through esterification reaction for the fabrication of antimicrobial films is a promising approach to overcome the aforementioned drawbacks. This is due to the fact that esterification provides desirable amount of antimicrobial agent by integrated citric acid onto starch by the formation of ester bonds without altering the chemical functionalities of the citric acid. Moreover, citric acid is a natural-based antimicrobial agent and the starch-citrate esterification reaction is a green process which only produced water as a by-product.

2.2. Morphological Studies

The morphology of the starch-citrate film was observed to correlate with the starch solution concentration. The change in starch concentrations (from 1–7% (w v^{-1})) led to significant effect of brittleness and cracking on the films after drying as depicted in **Figure 3**(a), (b), and (c), respectively. However, 5% (w v^{-1}) of starch concentration was shown to be the optimum concentration for starch-citrate film formation since the films were uniform, less cracks, and easy to peel off from the cast (Figure 3(b)) compared to starch film prepared from 1% and 7% (w v^{-1}) starch concentrations (Figure 3(a) and (c)). This is because lower starch concentration (1% (w v^{-1})) would lead to lower viscosity, higher surface tension (due to excessive amount of water), and lower surface energy in the starch solution. Low surface energy makes

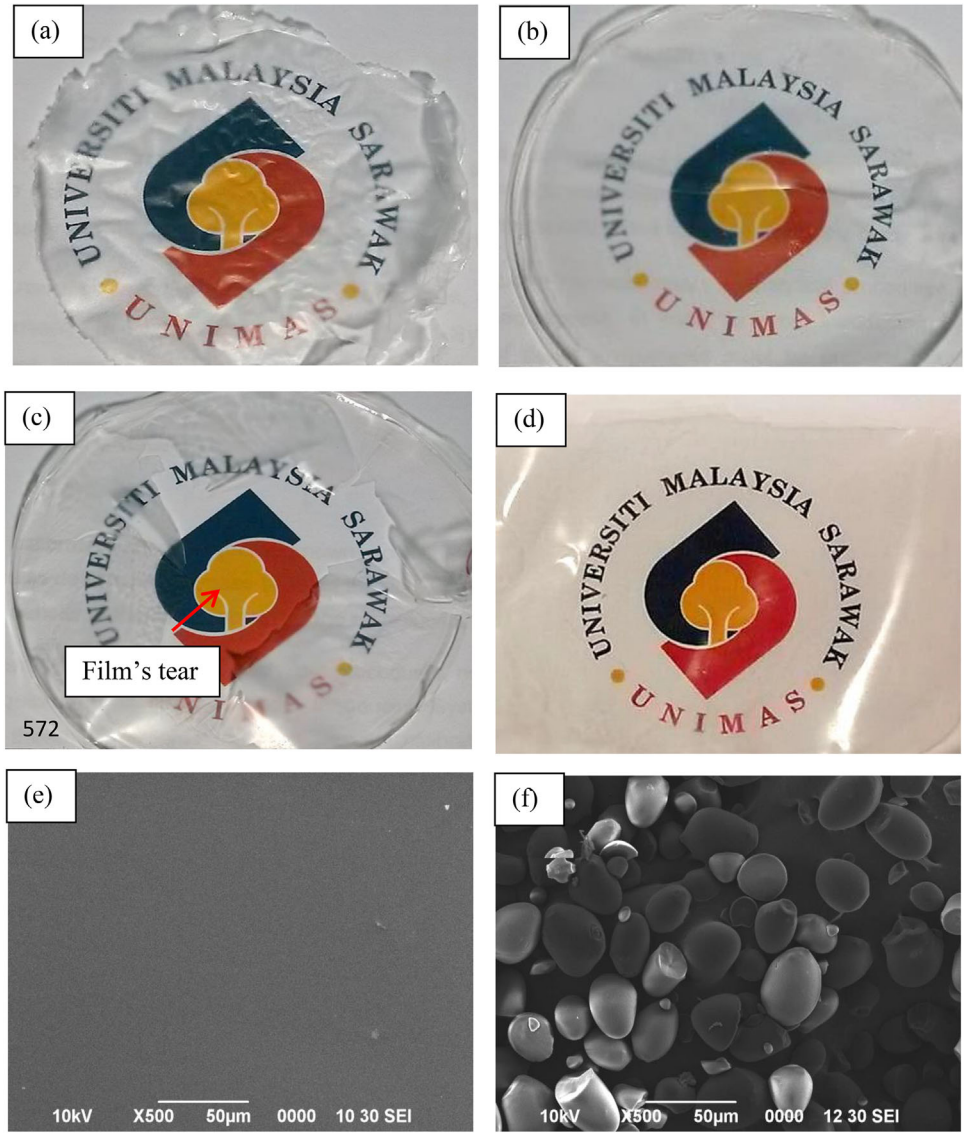

Figure 3. Photographs of starch films fabricated at (a) 1%, (b) 5%, and (c) 7% (w. v^{-1}) of starch concentration, (d) photograph of transparent starch-citrate blend PVA film, SEM micrographs of (e) surface of starch-citrate blend PVA film, and (f) native starch.

the peeling process harder, since there is a strong adhesion between the film and casting surface during solvent drying process. On the other hand, 7% (w v^{-1}) of starch solution was noticeable to be more brittle and ruptured on the film's surface after drying (Figure 3(c)). High starch concentration was undesirable due to high viscosity that would lead to higher surface energy in the starch solution.[18] The film formed was structurally compact and underwent a greater shear.[19] Therefore, in this study, 5% (w v^{-1}) of starch solution was chosen for the fabrication of starch-citrate films.

The addition of PVA as a plasticizer in starch-citrate film led to the formation of smooth, flexible and transparent without cracks as observed on the final product of the film (Figure 3(d)) compared to the film without PVA (Figure 3(b)), which displayed cracks on the film's surface after drying. The OH group of PVA played a crucial role to decrease the intra-molecular forces of hydrogen bonding in the starch-citrate polymer chain by providing inter-molecular hydrogen bonds between the starch-citrate and PVA polymeric chains (Figure 1), increasing the molecular mobility of the polymer thus providing films with flexible property.[20] This fact can be confirmed by the occurrence of hydrogen bonding dominated at 3280 cm^{-1} in FTIR spectrum of starch-citrate blend PVA film as shown in Figure 2(c). In the same way, the smooth structure of the film was attributed to the great compatibility and homogeneous blending of PVA and starch-citrate mixture.[20] The scanning electron microscopy (SEM) images in Figure 3(e) and (f) shows the morphological changes of starch granules after transformation into films. SEM image in Figure 3(e) shows the surface of the starch-citrate/PVA film was homogenous and dense. SEM image in Figure 3(f) shows starch granules isolated from sago starches displayed an oval shape with average diameter of 20–40 µm.

Table 1. Antimicrobial activities of starch-citrate films against *S. thypimurium*, *L. monocytogenes*, and *E. coli* (starch: citric acid: PVA of 5: 8: 15% [w v^{-1}]).

Bacteria strains	Antimicrobial analysis		
	MIC [mg. L^{-1}]	MBC [mg. L^{-1}]	Inhibition growth [%]
S. thypimurium	3.62×10^4	1.45×10^5	98.46
L. monocytogenes	3.62×10^4	1.45×10^5	99.02
E. coli	1.81×10^4	1.45×10^5	98.73

2.3. Antibacterial Activities of Starch-citrate Films

Starch-citrate films were evaluated for their in vitro antimicrobial activities against *S. thypimurium*, *L. monocytogenes*, and *E. coli* as representative food borne bacteria strains. The results in **Table 1** indicated that unmodified starch polymer did not exhibit antimicrobial property of all the bacteria strains tested since there was no inhibition zone observed on the agar plate. Starch-citrate film completely inhibited the growth of both *S. thypimurium* and *L. monocytogenes* at lower concentration of minimum inhibition concentration (MIC) value of 3.62×10^4 mg L^{-1}, while *E.coli* was inhibited at 1.81×10^4 mg $L^{-1.}$ Meanwhile, starch-citrate film was able to suppress almost 98–99% of the *S. thypimurium*, *L. monocytogenes* and *E. coli* at the minimum bactericidal concentration (MBC)of 1.45×10^5 mg L^{-1} as shown in Table 1.

The COOH moieties of starch-citrate film penetrated through the bacteria cell wall and disrupted the integrity of the bacteria cell. During penetration, COOH molecules from starch-citrate dissociated their H^+, which led to the reduction of the internal pH of the bacteria, consequently hindered the proliferation of the bacteria cell.[21] **Figure 4**(a) shows the representative SEM micrograph of food borne bacteria of *L. monocytogenes* in a fresh culture with the size of the bacteria cells were approximately 1.5 μm long and 0.8 μm in diameter. It was observed that after treating with starch-citrate film as shown in Figure 4(b), the physical structure of the *L. monocytogenes* that existed in colonies of rod shape cells in fresh culture were distorted.

2.4. Antifungal Activities of Starch-citrate Film

SEM images of *Aspergillus* spp. and *Rhizopus* spp. spores in **Figure 5** showed that starch-citrate film is an effective antifungal film for suppression of the growth of fungal organism isolated from contaminated cake and bread product. The spore of the fungal was observed to be ruptured after being exposed to starch-citrate film as presented in Figure 5(a)-(f). In addition, the corresponding analyses of disk diffusion, MIC, and minimum fungicidal concentration (MFC) results further confirmed the effect of starch-citrate film to suppress the growth of *Aspergillus* spp. and *Rhizopus* spp. as shown in **Table 2**. *Aspergillus* spp. was observed to be more susceptible to starch-citrate film as compared to *Rhizopus* spp. The higher sensitivity of *Aspergillus* spp. to starch-citrate film was shown by the remarkable higher percentage of effective inhibition of black and green mold colonies growth (98.58% and 96.56%, respectively) and the huge inhibition zone diameter (1.5 cm for both black and green molds) compared to lower percentage of effective inhibition growth and smaller inhibition zone diameter for *Rhizopus* spp. (87.93% and 0.7 cm, respectively).[22]

The antifungal mechanism for *Aspergillus* spp. against starch-citrate film can be probably attributed to the charge interaction between negative charge of the spores (due to the spores possess carboxyl-rich component named melanin, which contributes to negative charge of carboxylate ion (COO^-) on the spores) and positive charges of proton (H^+) present on the starch-citrate film. It is noteworthy that H^+ from starch-citrate film bound to the carboxyl group of the spores to reduce negative charge on the spore walls. As a result, spore walls suffered from proton toxification (acidic environment), which caused fungal germination disrupted as shown in Figure 5(a)-(d).[23] However, the presence of melanin constituent on fungal cell wall varied widely between the types of fungal.[24] In this regard, *Rhizopus* spp. spore cell walls contained lower melanin distribution (major component of *Rhizopus* spp. is protein) than *Aspergillus* spp. spore cell walls.[25] As a result, *Rhizopus* spp. showed weaker charge interaction and was less sensitive to starch-citrate film.

In conformity with these findings, MIC and MFC values appeared to account the minimum concentration required for starch-citrate film to inhibit and kill the fungal, respectively. The results presented in Table 2 indicated that starch-citrate film showed good antifungal property and was able to inhibit and kill the *Aspergillus* spp. pathogen at 3.62×10^4 and 7.25×10^4 mg L^{-1} MIC and MFC concentration, respectively, for black mold colonies. While for green mold colonies the MIC and MFC concentration obtained were at 7.25×10^4 mg $L^{-1}$and 1.45×10^5 mg L^{-1}, respectively. However, starch-citrate film did

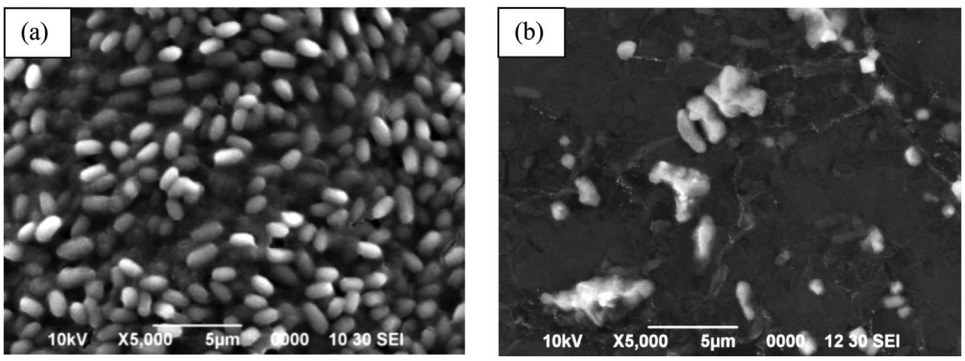

Figure 4. SEM micrographs of *L. monocytogenes* cells (a) in fresh culture and (b) after treated with starch-citrate film.

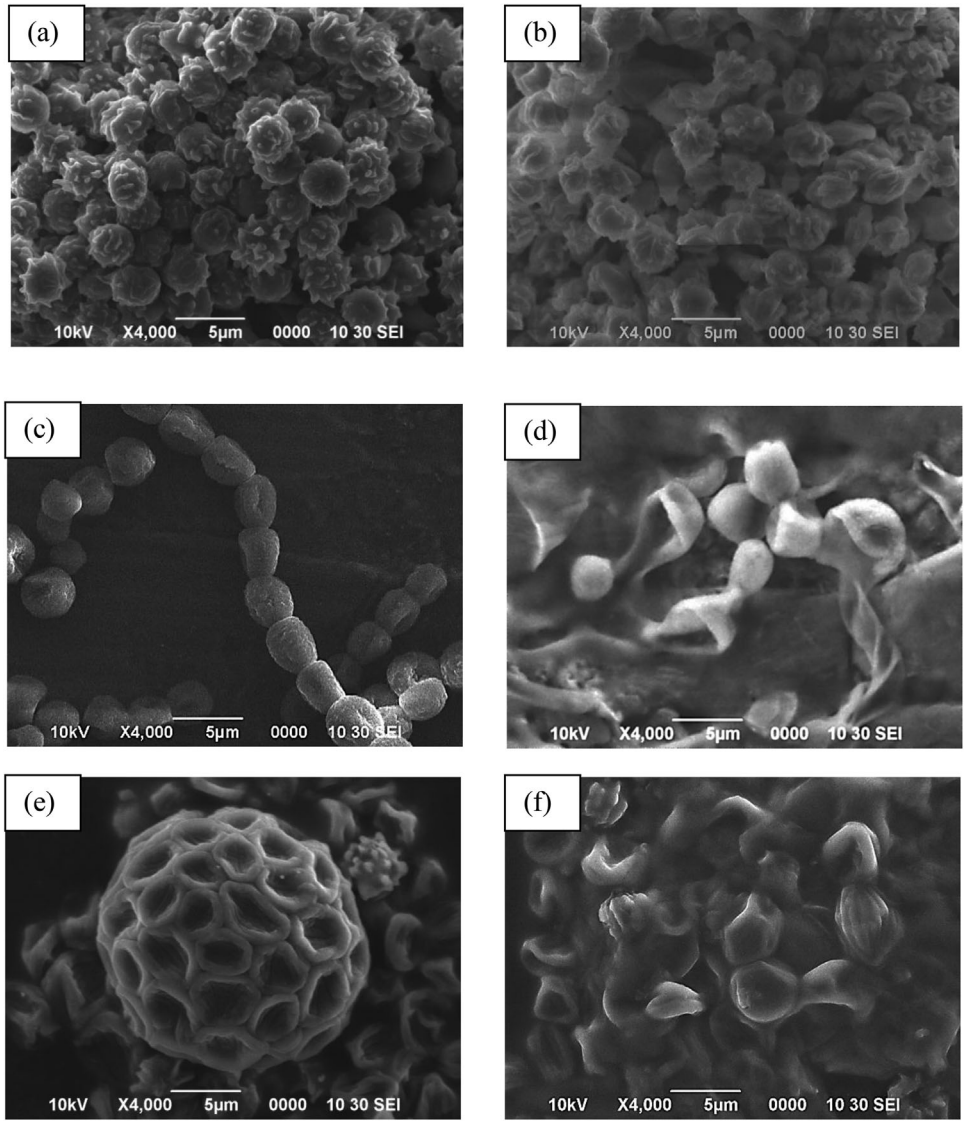

Figure 5. SEM images (a) and (b) spore of black mold *Aspergillus* spp. before and after treated with starch-citrate film, (c) and (d) spores of green mold *Aspergillus* spp. before and after treated with starch-citrate film, and (e) and (f) spores of *Rhizopus* spp. before and after treated with starch-citrate film.

Table 2. Disk diffusion, MIC, MFC, and percentage of effective inhibition growth of *Aspergillus* spp. and *Rhizopus* spp. colonies to starch-citrate film.

Genus	Aspergillus spp.		Rhizopus spp.
	Black mold	Green mold	
MIC [mg. L^{-1}]	3.62×10^4	7.25×10^4	7.25×10^4
MFC [mg. L^{-1}]	7.25×10^4	1.45×10^5	Colonies growth
Effective inhibition growth [%]	98.58	96.56	87.93

not show fungicidal activity against *Rhizopus* spp. since there was no MFC values due to the growth of *Rhizopus* spp. colonies on PDA plates observed after incubation compared to no noticeable recovery of *Aspergillus* spp. colonies on PDA plates after incubation period of 3 days. Therefore starch-citrate film was able to inhibit the growth of *Rhizopus* spp. colonies (MIC value was at 7.25×10^4 mg L^{-1}) but unable to completely prevent the colonies growth. This result was anticipated, due to the less effectiveness of starch-citrate film against *Rhizopus* spp. obtained from primary prediction of antifungal activity in screening disk diffusion analysis.

2.5. Qualitative Assessment of Food Shelf-life

Figure 6 demonstrates the application of starch-citrate film (starch: citric acid: PVA ratio 5:8:15% (w v^{-1})) as antimicrobial food packaging films. There was absence of mold that caused food spoilage observed on the piece of cake wrapped with starch-citrate film even after 10 days, as shown in Figure 6(a). On the other hand, mold was observed on the cake that was wrapped with commercial food wrapper even after 4 days, as seen in

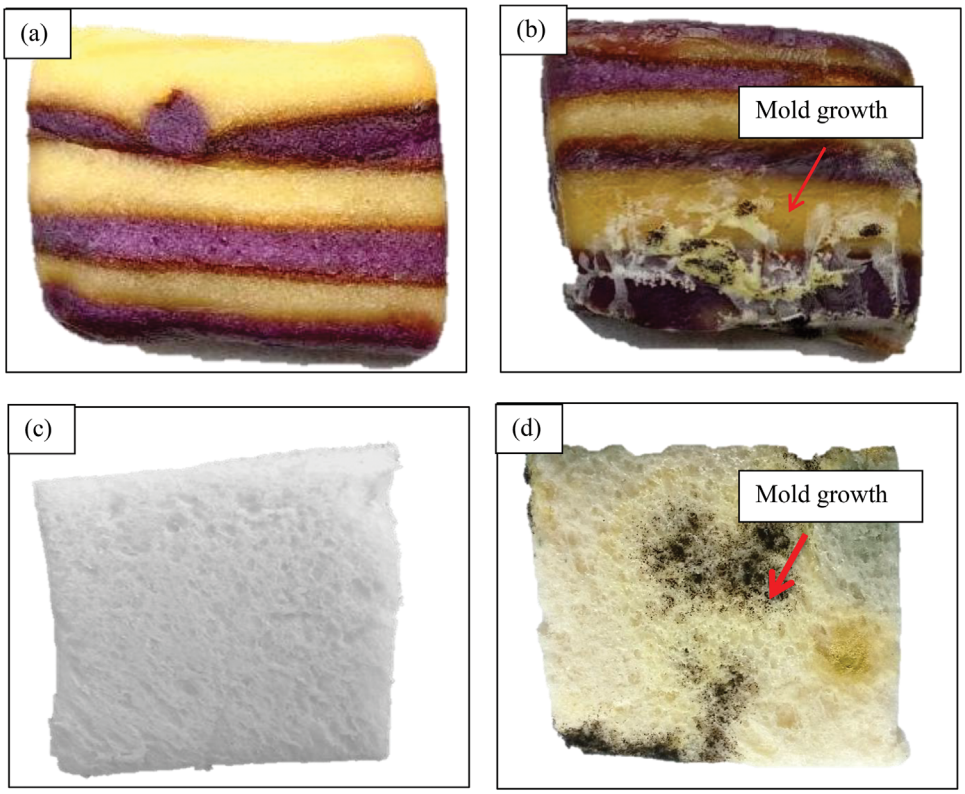

Figure 6. Photographs of (a) a piece of cake wrapped in starch-citrate film and (b) a piece of cake wrapped in commercial food wrapper after 10 days, (c) a piece of bread wrapped in starch-citrate film and (d) a piece of bread wrapped in commercial food wrapper after 40 days.

Figure 6(b). Besides, starch-citrate film was observed to extend the shelf life of bread up to 40 days compared to a week of the expiring date of the bread. After day 40th, the bread still remained odorless and mold-free, thus prevent food from perish (Figure 6(c)).

Furthermore, the texture of the bread packed in commercial food wrapper became soft and fragile after the expiring date, with black and green mold grew on the surface of the bread (Figure 6(d)). Starch-citrate film has proven to prolong the shelf life of the food. The main mechanism of antimicrobial starch-citrate food packaging film is associated with the diffusion of H^+ from starch-citrate film packaging surface towards food product.[26] The gradual release of H^+ increased the food shelf life by hindering the growth of mold and bacteria on the food due to slightly acidic pH of the starch-citrate (pH 4.0–5.0), thus increasing the food quality.[27]

3. Conclusion

The potential application of starch-citrate film as antimicrobial and antifungal packaging film has been evaluated. The starch-citrate film could suppress 98–99% of food borne bacteria growth and 87–99% of fungal growth. Furthermore, the starch-citrate film exhibited transparency, homogeneous, flexible and easy to handle properties. It can be observed that starch-citrate film was able to extend the shelf-life of food longer (10 days longer for cake and 40 days longer for bread) than shelf-life of food wrapped with commercial food wrapper. Therefore, it is envisaged that starch-citrate film has great potential to be used as the promising antimicrobial food packaging film.

4. Experimental Section

Materials: Native starch powder (OPAC brand) and commercial cling wrap of food freshness wrapper made of low-density polyethylene (LDPE) (GLAD brand) were purchased from local grocery store at Kuching, Sarawak, Malaysia. A freshly baked layer cake (ingredients: flour, eggs, butter, sugar, and ovalette) was purchased from Asma Cake House and sandwich bread (ingredients: flour, yeast, finetex, water, sugar, monofresh, salt, and shortening) was purchased from TAKA Cake House, Kuching, Sarawak, Malaysia. Citric acid monohydrate was supplied from Merck, Germany. Sodium hypophosphate monohydrate crystal was obtained from J.T Baker, United State. Polyvinyl alcohol (PVA) 99 +% hydrolyzed was purchased from Steinheim, Germany. Muller Hinton agar (MHA), potato dextrose agar (PDA), and malt extract broth were supplied from Oxoid, Hampshire, England. Luria Bertani (LB) broth was purchased from Sentmenat, Spain. Ultrapure water (UPW) with resistivity of 18.2 MΩ.cm was used throughout the experiments.

Preparation of Starch-citrate Film: About 100 mL of 1%, 5%, and 7% (w. v^{-1}) starch solution was gelatinized at 90 °C for 2 hours with continuous stirring. Then, citric acid (1%, 2%, 4%, 6%, 8%, and 10% (w. v^{-1})) and sodium hypophosphate monohydrate (1% (w. v^{-1})) were added into the gelatinized starch solution. The mixture was refluxed at 100 °C for 30 minutes to obtain starch-citrate solution. After that, PVA solution (0.5%, 2%, 4%, 6%, 10%, and 15% (w. v^{-1})) was added into the starch-citrate solution as plasticizer and the solution was magnetically stirred for 30 minutes. The solution was casted on the mold and heated at 60 °C for 4 hours to allow the formation of starch-citrate film. Finally, the starch-citrate film was cooled down to room temperature and peeled off from the mold.

Characterization of Starch-citrate Film: The functional groups of starch-citrate films were determined using FTIR spectroscopy (Thermo Scientific Nicole iS10). The pellets of starch-citrate films were ground with potassium bromide (KBr) and scanned in the range 400–4000 cm^{-1}. The morphology of starch-citrate films, bacteria strains and fungi were observed using SEM (JOEL, JSM-6390 LA). Starch-citrate film was mounted on the aluminum plate and sputtered with a layer of gold to avoid charging during scanning. 1 cm x 1 cm of agar contained bacteria colonies and fungus were cut off, placed on the aluminum plate, and dried in an incubator at 37 °C before sputtered with a gold layer. Optimization of antimicrobial activity associated with the ratios of citric acid and PVA in starch-citrate films was assessed through disk diffusion assay analysis by measuring the inhibition zones.

Antibacterial Properties of Starch-citrate Films: Antimicrobial susceptibility test of starch-citrate films was performed against foodborne pathogenic bacteria of *Salmonella thypimurium* (S. *thypimurium*), *Escherichia coli* (*E. coli*) (O157:H7), and *Listeria monocytogenes* (*L. monocytogenes*). All the bacteria strains were cultured in LB broth at 37 °C for 24 hours before being further used for disk diffusion assay,MIC, and MBC) analysis. All the procedures performed were according to methods approved by Clinical and Laboratory Standards Institute (CLSI) protocol.[28]

Disk Diffusion Assay: The inoculated bacteria in Luria Bertani (LB) broth were adjusted to 0.5 McFarland standard concentrations. Twenty microliter of the bacteria suspension was spread on the MHA agar plate using cotton swab. Starch-citrate film disks (0.5 cm diameter and 0.34 mm width) were placed on the Mueller Hinton Agar (MHA) plate containing *S. thypimurium*, *E. coli*, and *L. monocytogenes* before being incubated at 37 °C, for 24 hours to promote bacterial growth. After 24 hours, the antimicrobial activity of the starch-citrate films was evaluated by measuring the diameter of clear zone (inhibition zone) surrounding the starch-citrate film disks.

Minimum Inhibition Concentration (MIC): One milliliter of LB broth was prepared in 10 test tubes. Two-fold dilutions of starch-citrate solutions were prepared in 10 test tubes containing broth solution through serial dilution method. One milliliter of starch-citrate solution (2.90×10^5 mg. L^{-1}) was added into the first test tube containing 1 mL LB broth. One milliliter of the mixture from the first test tube was transferred to the second test tubes and the same procedure was repeated until the tenth test tube to obtain two-fold dilution series concentration of samples. Then, 1 mL of bacteria suspensions (1×10^8 CFU. mL^{-1}) were added into every test tube and incubated at 37 °C for 24 hours. The turbidity of the mixture was observed as indication of the growth of bacteria in the mixture. MIC was determined as the lowest concentration of starch-citrate samples to inhibit the growth of bacteria.

Minimum Bactericidal Concentration (MBC): Twenty microliter of an aliquot (transparent suspensions from MIC) were spread uniformly on the MHA agar plates and placed in an incubator for 24 hours at 37 °C to allow bacteria colonies to grow on the plates. MBC is the minimum concentration of starch-citrate required to obtain bactericidal effect, where almost 99% of the bacteria were killed. The percentage of bacteria reduction was calculated by the difference in numbers of colonies counting from the control plates and number of colonies from MBC assay plates as expressed in Equation (1). The colonies were counted using a hemocytometer.

$$\text{Inhibition growth } (\%) = \frac{A_1 - A_2}{A_1} X\ 100 \quad (1)$$

where A_1 is the number of colonies in control plates (1×10^8 CFU. mL^{-1}) and A_2 is the number of colonies in assay plates.

Antifungal Activity of Starch-citrate Film: The standard protocol for antifungal susceptibility test was applied in accordance to Clinical and Laboratory Standard Institute (CLSI) document A38-A2.[28]

Disk Diffusion Assay: Fungi spore suspension was prepared by adding 0.1% Tween 80 ($v.v^{-1}$) to the culture plates and the surface of the medium was scrabbled with loop to separate spore from fungus mycelium. Then, the suspension was filtered and centrifuged for 5 minutes. The resulting spore was resuspended in sterile distilled water. The spore solution containing 1×10^7 spore. mL^{-1} of fungi was determined using a hemocytometer.[29] A total of 0.5 mL fungi suspension was inoculated on the PDA agar before starch-citrate film disk (0.5 cm diameter, 0.34 mm width) was placed on the plate. Finally, the plate was incubated at 28 °C for 7 days.

Minimum Inhibition Concentration: Two-fold serial dilutions of starch-citrate film was performed by diluting the concentration of the starch-citrate film solution (2.90×10^5 mg. L^{-1}) to half of its original concentration with malt extract broth as diluent solution. One milliliter malt extract broth diluent was dispensed to 10 test tubes labelled as 1×10^{-1} to 1×10^{-10}. Then, 1 mL of the starch-citrate solution was transferred to the first test tube (1×10^{-1}) and mixed. This was the first two-fold dilution. Next, 1 mL solution from the first two-fold dilution was transferred to the test tube labeled 1×10^{-2} to obtain second two-fold dilution. The following two-fold dilution series was continued until the tenth test tube labeled 1×10^{-10}. Subsequently, 1 mL of spore suspension (1×10^7 spore. mL^{-1}) was added to each test tubes and incubated at 28 °C for 7 days. MIC value for starch-citrate film was recorded as the lowest concentration that completely inhibited the growth of white mycelium during 7 days of incubation period.[30]

Minimum Fungicidal Concentration (MFC): After 7 days of incubation, 20 µL aliquot of serial dilution concentration that showed no sign of mycelium growth was inoculated on PDA agar plate. The plates were then incubated at 28 °C for 3 days. MFC was determined as the lowest concentration that showed no growth of fungus after incubation period. The percentage of fungi's spore reduction due to fungistatic effect was calculated based on the spore counting using a hemocytometer as shown in the Equation (2)

$$\text{Fungi's spore reduction } (\%) = \frac{F_1 - F_2}{F_1} \times 100 \quad (2)$$

where F_1 is the concentration of fungi's spore control plates (1×10^7 spore. mL^{-1}) and F_2 is the concentration of spore in assay plates.

Qualitative Assessment of Food Shelf-life: The samples of food, which included a piece of fresh layer cake and bread, were wrapped in a starch-citrate film and stored at room temperature. The shelf-life of the food wrapped in starch-citrate films was compared with a piece of food sample wrapped in commercial food wrapper.[7]

Acknowledgements

Financial support from Ministry of Education, Malaysia via the award of the Fundamental Research Grant Scheme [FRGS/STG02(02)/1116/2014(02)], UNIMAS Postgraduate Research Grant F07(DP11)/1167/2014(11), and MyBrain15 (MyPhD) for graduate scholarship are gratefully acknowledged.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords

antimicrobial, bio-based, food packaging, starch-based films

Received: August 11, 2021
Revised: November 3, 2021
Published online: December 23, 2021

[1] V. P. Romani, C. Prentice-Hernández, V. G. Martins, *Ind. Crops Prod.* **2017**, *97*, 268.
[2] A. K. Awasthi, M. Shivashankar, S. Majumder, *Inst. Physic Conf. Series Mater. Sci. Eng.* **2017**, *263*, 1.
[3] S. W. Chook, C. H. Chia, S. Zakaria, H. M. Neoh, R. Jamal, *New J. Chem.* **2017**, *41*, 5061.
[4] A. Khumkomgool, T. Saneluksana, N. Harnkarnsujarit, *Food Packag. Shelf Life* **2020**, *26*, 1.
[5] A. S. Abreu, M. Oliveira, A. de Sá, R. M. Rodrigues, M. A. Cerqueira, A. A. Vicente, A. V. Machado, *Carbohydr. Polym.* **2015**, *129*, 127.
[6] F. Sen, İ. Uzunsoy, E. Baştürk, M. V. Kahraman, *Carbohydr. Polym.* **2017**, *170*, 264.
[7] A. H. Ramírez, A. Aparicio-Saguilán, G. Reynoso-Meza, J. Carrillo-Ahumada, *Carbohydr. Polym.* **2017**, *157*, 1125.
[8] S. H. Classen, C. M. Müller, A. L. Parize, A. T. Pires, *Carbohydr. Polym.* **2018**, *180*, 348.
[9] M. A. Berube, D. Schorr, R. J. Ball, V. Landry, P. Blanchet, *J. Polym. Environ.* **2017**, *26*, 970.
[10] A. Ricci, K. J. Olejar, G. P. Parpinello, P. A. Kilmartin, A. Versari, *Appl. Spectrosc. Rev.* **2015**, *50*, 407.
[11] C. de Lima Barizão, M. I. Crepaldi, S. Oscar de Oliveira, A. C. de Oliveira, A. F. Martins, P. S. Garcia, E. G. Bonafé, *Int. J. Biol. Macromol.* **2020**, *165*, 582.
[12] A. Mittal, S. Garg, D. Kohli, M. Maiti, A. K. Jana, S. Bajpai, *Carbohydr. Polym.* **2016**, *151*, 926.
[13] S. C. Pang, S. F. Chin, S. H. Tay, F. M. Tchong, *Carbohydr. Polym.* **2011**, *84*, 424.
[14] F. Ortega, L. Giannuzzi, V. B. Arce, M. A. García, *Food Hydrocolloids* **2017**, *70*, 152.
[15] M. J. Bof, D. E. Locaso, M. A. Garcia, *Starch-Stärke* **2021**, *73*, 2000104.
[16] C. Zhang, R. Wohlhueter, H. Zhang, *Food Science and Human Wellness* **2016**, *5*, 116.
[17] A. N. Romainor, S. F. Chin, S. C. Pang, L. M. Bilung, *J. Nanomater.* **2014**, http://doi.org/10.1155/2014/710459.
[18] T. A. Nascimento, V. Calado, C. W. P. Carvalho, *Food Res. Int.* **2012**, *49*, 588.
[19] S. C. Pang, C. L. Tay, S. F. Chin, *Ionics* **2014**, *20*, 1455.
[20] C. A. Gómez-Aldapa, G. Velazquez, M. C. Gutierrez, E. Rangel-Vargas, J. Castro-Rosas, R. Y. Aguirre-Loredo, *Mater. Chem. Phys.* **2020**, *239*, 1.
[21] S. F. Chin, A. N. Romainor, S. C. Pang, B. K. Lee, S. S. Hwang, *Starch-Stärke* **2019**, *71*, 1.
[22] D. Nithyadevi, P. S. Kumar, D. Mangalaraj, N. Ponpandian, C. Viswanathan, P. Meena, *Appl. Surf. Sci.* **2015**, *327*, 504.
[23] L. Zhang, J. Zhang, *Crit. Rev. Biotechnol.* **2016**, *36*, 1066.
[24] J. D. Nosanchuk, R. E. Stark, A. Casadevall, *Frontiers in Microbiology* **2015**, *6*, 1.
[25] N. Chookhongkha, T. Sopondilok, S. Photchanachai, *Acta Hortic* **2012**, *973*, 231.
[26] L. A. Castillo, S. Farenzena, E. Pintos, M. S. Rodríguez, M. A. Villar, M. A. García, O. V. López, *Food Packag. Shelf Life* **2017**, *14*, 128.
[27] V. H. Campos-Requena, B. L. Rivas, M. A. Pérez, C. R. Figueroa, N. E. Figueroa, E. A. Sanfuentes, *Postharvest Biol. Technol.* **2017**, *129*, 29.
[28] Clinical and Laboratory Standard Institute (CLSI). *CLSI document M100-S22. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*, Clinical and Laboratory Standard Institute, Pennsylvania **2012**, pp. 70–114.
[29] M. Nikkhah, M. Hashemi, M. B. H. Najafi, R. Farhoosh, *Int. J. Food Microbiol.* **2017**, *257*, 285.
[30] Clinical and Laboratory Standard Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard*, 2nd ed., Clinical and Laboratory Standard Institute, Pennsylvania **2008**. pp. 5–11.

