Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 3, 2023, 226
https://doi.org/10.33263/BRIAC133.226

Development and Characterization of Silk Fibroin-based Oral Films Containing Turmeric Extract as Dietary Supplement

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Scopus Author ID 55856374500
Received: 15.01.2022; Accepted: 13.02.2022; Published: 6.06.2022

Abstract: Dietary supplements have extreme importance. Due to the easily degradable nature of these supplements, the development and application of carrier systems in food technologies seem to be extremely important. Today, oral film technologies have gained importance due to their rapid and high absorption properties. This study chose silk fibroin (SF) as the main component due to its high biocompatibility. Turmeric extract has been added to the oral films as an active agent. The prepared films were analyzed with atomic force microscopy (AFM) and scanning electron microscopy (SEM) to reveal their morphological properties, and at the same time, the film thickness was measured. It has been found that the increase in extract amount is a factor that causes an increase in film roughness while causing a decrease in phase separation. It was observed that the film roughness increased twice with the addition of extract. The roughness of the films formed with 15% extract was measured as 28.5. The average roughness of the films formed without the use of extracts was observed as 15.7 mm. It was also observed that the Trolox equivalent antioxidant capacity (TEAC) was doubled for films containing 15% turmeric extract after one hour of release. The films exhibited different weight loss profiles after release as the amount of turmeric extract was changed. Disc diffusion experiments revealed that films containing turmeric extract exhibited antimicrobial effects.

Keywords: food supplements; oral film; biodegradable polymer; curcumin; turmeric extract.

1. Introduction

Dietary supplements have an extremely important place in today's world. Due to the easily degradable nature of these supplements, the development and application of carrier systems in the field of food technologies seems to be an extremely important field. Today's most commonly used oral delivery systems are pills, films, granules, and liquids, respectively. Oral film technologies have become an important research area among these systems due to their fast absorption and high bioavailability. Biopolymers such as chitosan, gelatin, silk fibroin, and zein are predominantly used to form oral films, as they easily degrade in the oral mucosa. Although most studies on oral films have been conducted with drug delivery systems, these studies can be used as preliminary data in food technology applications.

Oral administration, which patients mostly prefer among other drug delivery routes, has room for improvement due to many reasons. Oral drug delivery has progressed over the years in types of oral pills, granules, powders, liquids, and films. Oral film technology has gained

importance due to its fast dissolution and ease of administration. Since the mucosal surfaces have large veins and are rich in blood supply, rapid transport of drug molecules is provided [1-5]. Another advantage of oral film technology is avoiding being degraded by first-pass metabolism. These properties may lead to improved bioavailability of drugs [6-10]. Oral films also have the advantages of being thin, having them available in various sizes and shapes, and exhibiting excellent mucoadhesion, which may positively affect the frequency of their use [11-15].

Oral films are commonly originated from biopolymers such as corn zein, gelatin, collagen, casein, whey protein, fibrinogen, soy protein, and silk fibroin [16-19]. Adherence to hydrophilic surfaces and providing barriers for carbon dioxide and oxygen without resistance to water diffusion make them convenient to be used as oral films. Silk originated from different sources such as silkworms, spiders, scorpions, flies, and mites [20-23]. Most extensively characterized silks are produced by domesticated silkworm *Bombyx mori* (*B.mori*). Silk protein draws attention because of its high biocompatibility and mechanical properties [24-28]. Silk fibers are composed of two main proteins; sericin and fibroin. Silk fibroin is extensively studied in the field of biotechnology for enzyme immobilization, antithromboplastic materials, wound dressings, dialysis membranes, and drug-delivery carriers [29-34]. Aqueous silk fibroin solutions represent a good starting material for preparing film, powder, gel, and membranes. Blending with other substances may improve the processability of polymers and provide tailoring of the material according to demands. Sodium carboxymethylcellulose (NaCMC) is widely used for pharmaceutical applications. It has high viscosity in low concentrations and does not promote host response which makes its use favorable [20, 35].

Active pharmaceutical ingredients are the main components of the drugs. Vitamin B12, chromium picolinate, melatonin, and coenzyme Q10, can be given as an example of active pharmaceutical ingredients used in film technology [36, 37]. There are also many natural products available to be used in oral films, such as guarana extract, lycopene, resveratrol, curcumin, caffeine, green tea extract, and ginseng extract [38-40]. Turmeric extract is one of the promising extracts approved for its pharmaceutical activity. Turmeric extract is obtained from Curcuma longa L. belonging to the Zingiberaceae family. This plant is a perennial plant that can grow up to a meter high, usually seen in tropical or subtropical regions. This plant, which is widely used in traditional medicine, is used as a home remedy for the treatment of many diseases. These diseases are sinusitis, rheumatism, cough, anorexia, diabetic wounds, and liver diseases [41-44]. Curcumin, which comprises 3-4% of turmeric extract, gives its yellowish color. A large number of *in vitro* and *in vivo* studies in both animals and humans have indicated that curcumin has strong antioxidant, anti-carcinogenic, anti-inflammatory, anti-angiogenic, antimicrobial, and anti-parasitic properties [45-53]. One disadvantage of curcumin is that its level in blood flow is low due to poor absorption, rapid metabolism, and rapid systemic elimination, which result in low bioavailability [54].

Oral drug delivery is comprised of digestion and absorption in the gastrointestinal tract. Compared to conventional administration, oral film technology provides an increase in bioavailability by first-pass metabolism. This study aims to prepare turmeric extract-loaded biodegradable polymer-based oral films to provide the release of natural compounds through mucosal delivery. NaCMC was used in polymer blend to tailor the mechanical properties of silk fibroin. Mechanical properties of the films affected release properties, which had an effect on antioxidant capacity, antimicrobial activity, and bioavailability of extract.

2. Materials and Methods

2.1. Preparation of aqueous silk protein fibroin solution.

Raw silk fibers (Bursa Institute for Silkworm Research) were kept in 50 times (v/w) of boiling aqueous 0.05% Na_2CO_3 for 30 minutes, and this treatment was repeated three times to remove sericin. After degumming, silk fibers were washed with deionized water and left to dry at room temperature. Dialysis tubings (MWCO 10 kD) (Thermo Scientific) were treated with 0.3% (w/v) sodium sulfide (Sigma) solution at 80°C for 1 minute, followed by rinsing for 2 minutes in deionized water at 60°C. Tubings were subjected to acidification in 0.2% (v/v) sulphuric acid (Merck) at room temperature for 1 minute and once again rinsed with deionized water at 60°C. To prepare aqueous silk fibroin (SF) solution, 1.2 g degummed silk was added to 20 times (v/w) of Ajisawa's reagent ($CaCl_2$/ethanol/water, 111/92/144 in weight). The mixture was stirred at 78°C at 150 rpm for 2 hours to form a clear solution. The resulting SF solution was dialyzed against deionized water for at least 3 days at 4°C in order to remove salts using cellulose tubing. Water was changed for half-an-hour intervals for the first 2 hours and then for 16-hour intervals for the rest of the 3 days. Dialyzed fibroin solution was filtered, and pure aqueous fibroin solution with a concentration of 2% (w/v) was obtained.

2.2. Preparation of NaCMC solution.

Reagent grade sodium carboxymethylcellulose (NaCMC) (Sigma) of 2% (w/v) was dissolved carefully and slowly in deionized water at room temperature and stirred for 24 h in a magnetic stirrer, followed by sonication for 1 h to remove the undesired air bubbles.

2.3. Turmeric extract preparation.

Powdered turmeric was extracted in 70% aqueous ethanol in an orbital shaker at 180 rpm overnight. The solid/liquid ratio was selected as 1/20. Centrifugation was performed afterward to remove solids. Ethanol in the supernatant was discarded by a rotary evaporator. The aqueous phase of the extract was used for further experiments.

2.4. Film casting.

Blends of SF/NaCMC and turmeric extract of various concentrations were prepared by dropwise addition of a required amount of aqueous SF and turmeric extract in NaCMC solution. Blends were mixed in an orbital shaker and cast on polypropylene Petri dishes at room temperature. Compositions of prepared films are presented in Table 1. Turmeric extract was loaded in 50:50 blends of SF:NaCMC in 5, 10 and 15% (w/w).

Table 1. Compositions of prepared films. The solid amount in polymer blends was kept constant as 2% (w/v).

Components	NaCMC	Silk fibroin
Concentrations (%) (v/v)	100	0
	75	25
	50	50
	75	25
	0	100

2.5. Morphological characterization of films.

The surface morphology of the films was determined by scanning electron microscopy (SEM) (Philips XL 30S FEG, FEI Company) and atomic force microscopy (AFM) (Digital

Instrument). SEM micrographs were taken under high vacuum mode at an operating voltage of 5 kV after gold sputtering. The surface roughness of extract-loaded films was determined by AFM, operated in tapping mode. Both height and phase images were collected and analyzed by Nanoscope software. The image scan size was 10 μm × 10 μm.

2.6. Weight loss.

Each film was cut into discs of 1 cm diameter. Discs were immersed in 2 ml PBS (pH 7.2) at 37°C at 80 rpm after recording dry weight. At the end of determined time intervals, excess liquid was soaked on filter paper, and discs were weighed. Weight loss was measured using the following equation:

$$\text{Weight loss (\%)} = (W_i - W_f)/W_f * 100$$

W_i and W_f denote the weight of samples before and after immersion in PBS, respectively.

2.7. Soluble phenolic content and antioxidant activity determination.

Total phenol content and antioxidant activity were determined by batch release studies performed at 37°C and 80 rpm. Briefly, discs of 1 cm diameter containing different concentrations of turmeric extract were immersed in distilled water. Soluble phenolic content of turmeric extract at different time intervals was determined by the Folin-Ciocalteau method in Gallic acid equivalent (GAE). ABTS assay was employed to determine Trolox equivalent antioxidant activity (TEAC). Both of the methods were performed by spectrophotometric measurement at 725 and 734 nm, respectively.

2.8. Antimicrobial Activity Determination with Disc Diffusion.

Escherichia coli (*E. coli*) and *Candida albicans* (*C. Albicans*) were used for antimicrobial activity. Microbial strains were resuscitated by transferring one loop of stock on the agar surface. Potato agar was used for *C.albicans*, and nutrient agar was prepared for *E. coli*. Cultures were incubated overnight, and 0.5 McFarland unit for concentration was confirmed by measuring absorbance values. Strains were spread on agar surfaces, and films cut in discs of 10 mm, were placed on the agar surface. Cultures were incubated at 37°C for 24 hours. Iodonitrotetrazolium chloride (Merck), dissolved in ethanol at a concentration of 8 mg/ml, was sprayed on cultures. After 5 minutes, viable microorganisms changed color to purple while microorganism-free zones remained colorless.

3. Results and Discussion

3.1. Surface morphology.

SF:NaCMC blend films exhibited a heterogeneous appearance because solutions were not completely miscible. Phase separation was significant in blends composed of a high amount of NaCMC. SEM micrographs also revealed differences in surface morphology based on composition. An increase in NaCMC content enhanced heterogeneity on the film surface. Polymer films having 50:50 ratio of SF:NaCMC was chosen due to higher homogeneity and lower phase separation for further studies comprised of extract incorporation. Extract loading also affected phase separation by enhancing the homogeneity of films. SF-NaCMC blends exhibit phase separation, which results in heterogeneity and non-transparent appearance. It also caused grainy structures on the surface, as observed in SEM micrographs in this study. The

film composition was related to surface morphology. An increase in the amount of silk fibroin smoothened the surface, whereas an increase in NaCMC content increased surface roughness. High silk fibroin content caused a decrease in flexibility of the films due to improved mechanical properties of silk fibroin, which may affect processability. Films with a 50:50 ratio of SF:NaCMC was used for further studies, comprising extract loading. Turmeric extract loading also enhanced homogeneity by avoiding phase separation.

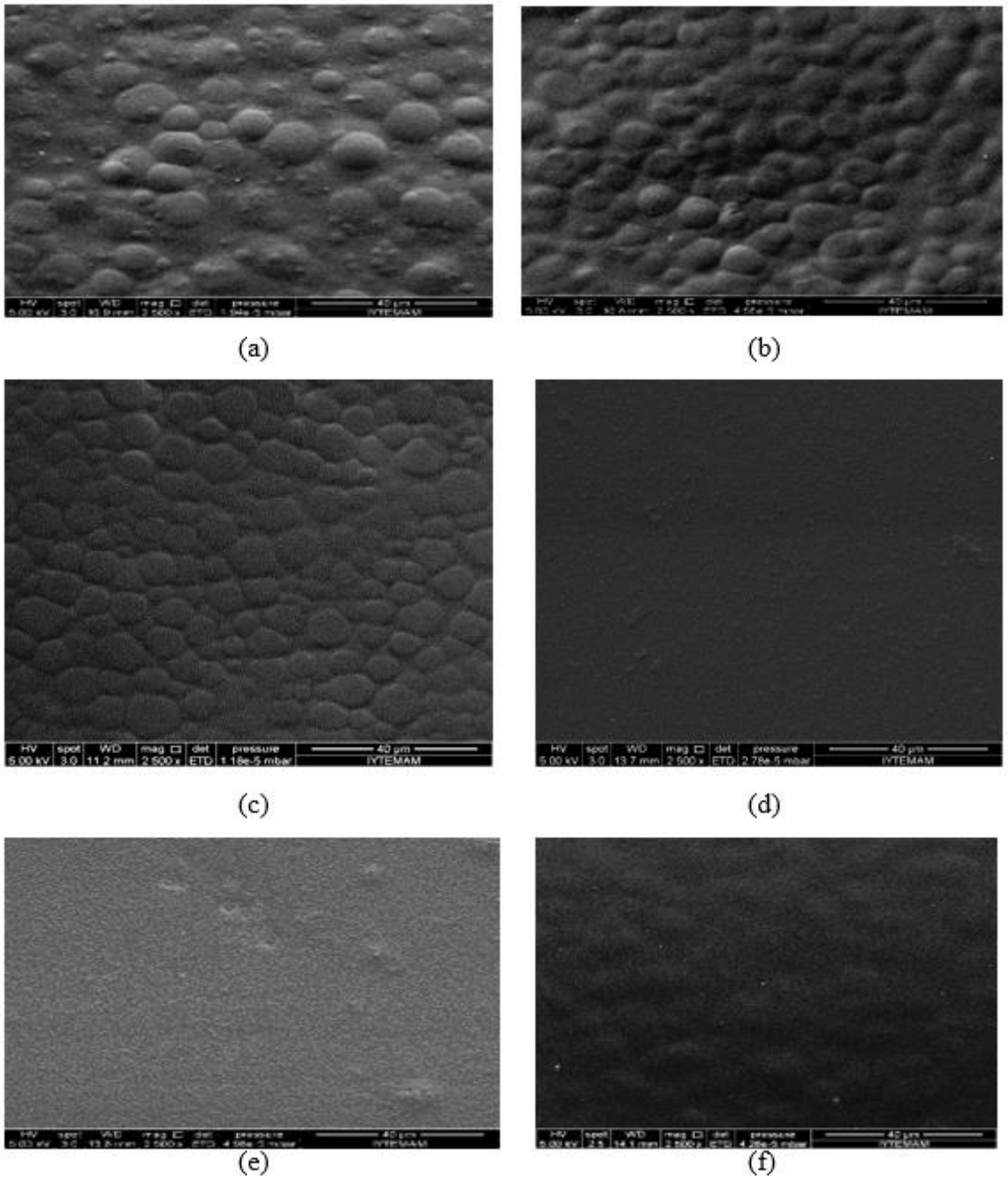

Figure 1. SEM micrographs of upper surface of the SF:NaCMC blend films at different ratios: (**a**) 25:75; (**b**) 50:50; (**c**) 75:25; (**d**) 0:100; (**e**) 100:0; (**f**) 50:50 with 15% (w/w) turmeric extract. Scale bar is 40 µm.

AFM was employed to determine the surface roughness of extract-loaded films. Although the primal effect of extract incorporation was to decrease surface roughness, further extract addition caused an increase in roughness, as seen in Figure 2. An increase in surface roughness may indicate successful extract incorporation. Table 2 depicts surface roughness values depending on the extract amount in films. AFM results indicated that extract loading affected surface roughness in a decremental manner at initial concentrations. Further extract

loading led to an increase in surface roughness, indicating successful incorporation and saturation point of extract concentration.

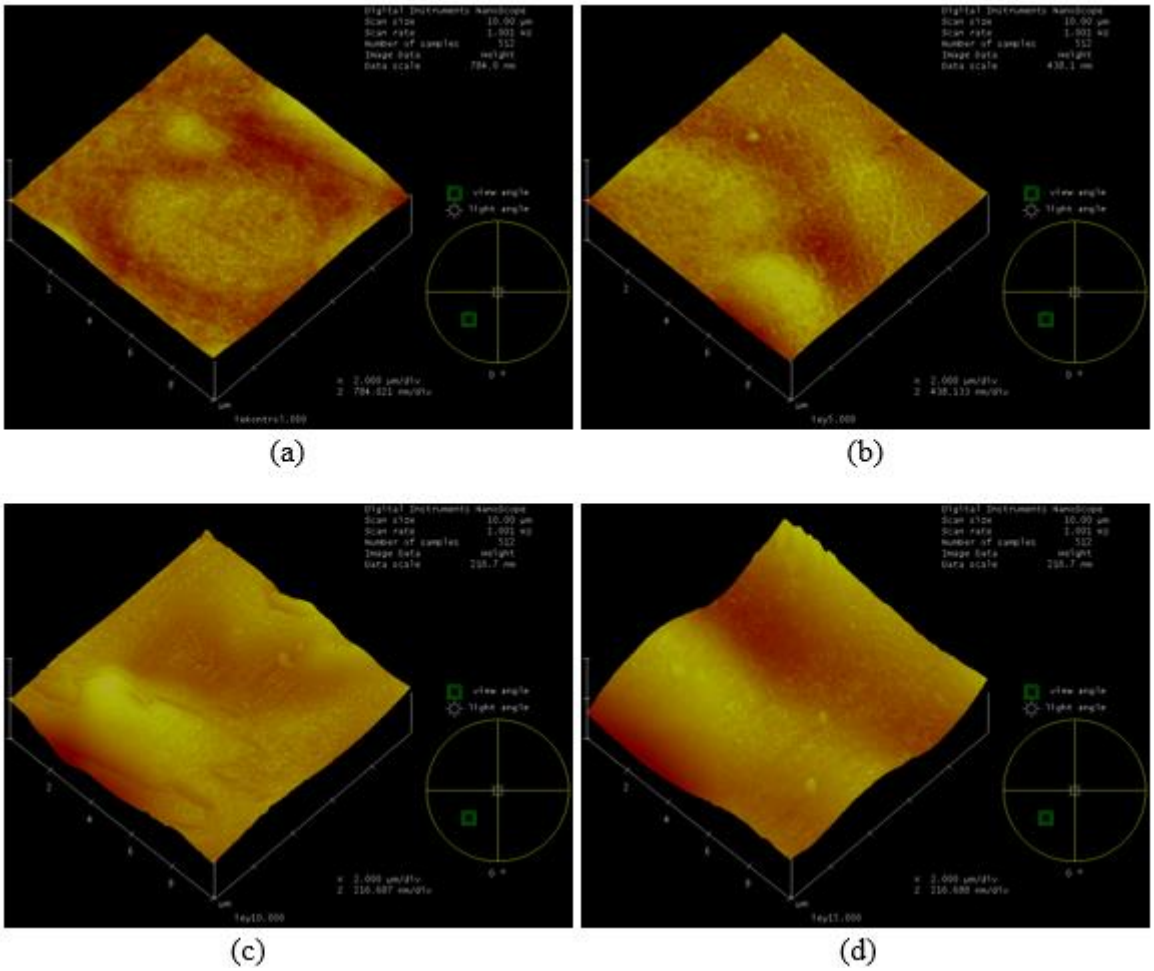

Figure 2. AFM images of SF:CMC films having ratio of 50:50 with extract amount of (**a**) 0%; (**b**) 5%; (**c**)10%; (**d**) 15%.

Table 2. Mean roughness depending on extract content of films.

Extract amount in SF: Na-CMC films (50:50) (%)	Mean Roughness (Ra) (nm)
0	15.7
5	6.4
10	16.3
15	28.5

3.2. Weight loss.

Weight loss was determined by immersing disc-shaped films into PBS (pH 7.4) at 37°C at 80 rpm and measuring their weights at defined time intervals. Films of pure NaCMC and blends of SF:NaCMC in various ratios became soluble within 1 hour. Films of 100% SF and 5%, 10%, and 15% turmeric extract-loaded SF:NaCMC films having a ratio of 50:50 maintained their form for 4 hours. As seen in Figure 3, pure SF films gained weight due to swelling while extract-loaded films exhibited different weight profiles depending on extract amount. An increase in extract amount caused a decrease in weight, as observed in 15% extract-loaded films. Enhanced weight loss may point out the possible crosslinking effect of turmeric extract when blended with the polymer solution. Weight loss studies also indicated the effects of extract amount on swelling and degradation. SF:NaCMC blended films were completely dissolved due to the solubility of NaCMC in an aqueous environment, while pure SF and

extract-loaded SF:NaCMC films maintained their forms during the elapsed time. The weight of SF films also increased, which may refer to swelling behavior. Weight loss of films loaded with 15% turmeric extract exhibited decreased profile, indicating degradation. Possible crosslinking of the extract with polymer blend may also decrease weight and prevent swelling. Degradation also affected release properties, as depicted in antioxidant activity and phenolic content profile.

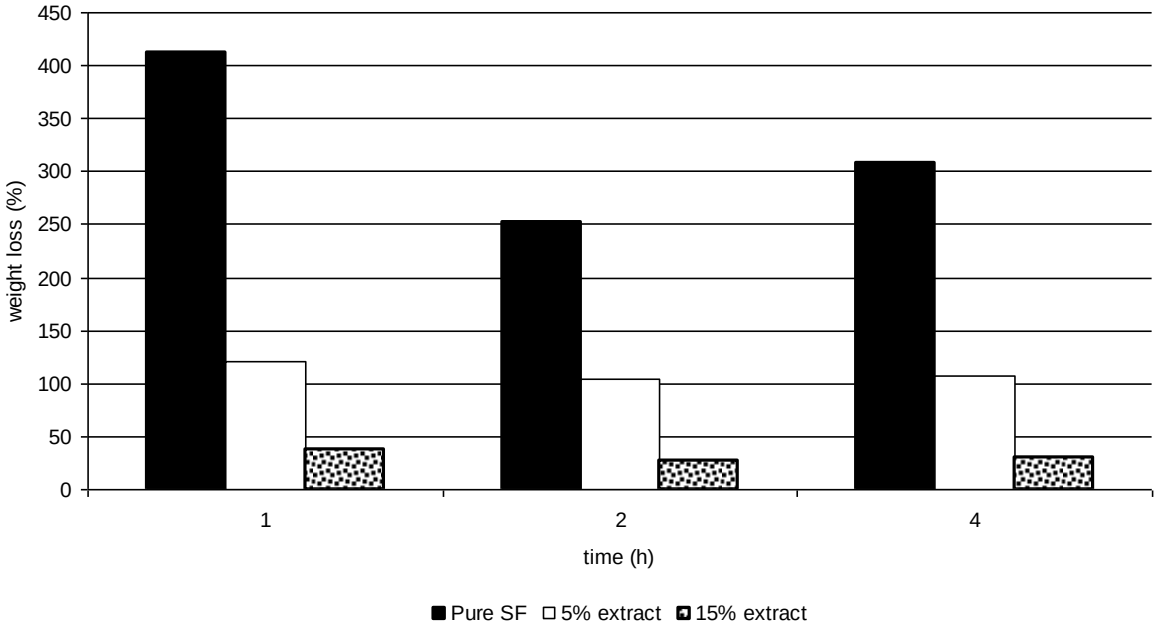

Figure 3. Weight change in pure SF and extract-loaded films.

3.3. Determination of antioxidant activity and soluble phenolic content.

Trolox equivalent antioxidant capacity of SF-NaCMC films with 5% and 15% turmeric extract was determined at different time points. Films without extract were used as controls. Values obtained from controls were subtracted from extract-loaded samples. Figure 4 indicates that, after one hour, a sample including 5% turmeric extract exhibited the highest antioxidant capacity in release media.

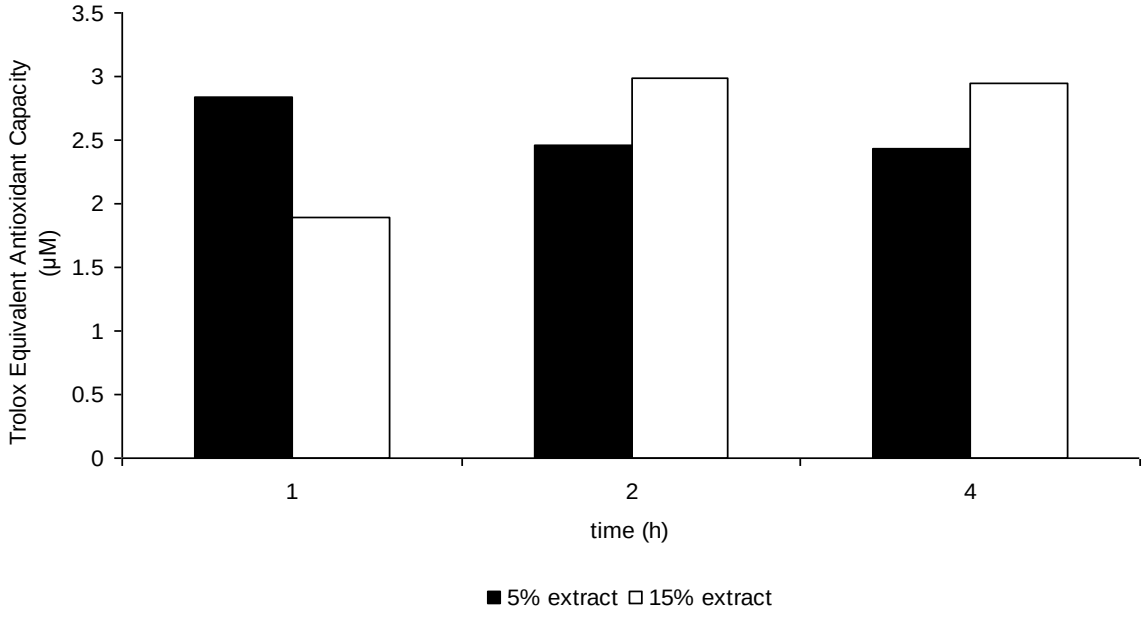

Figure 4. Trolox equivalent antioxidant capacity of turmeric extract-loaded SF-NaCMC films.

This behavior was followed by 15% extract-loaded films after 4 hours of release, which showed the highest antioxidant compound release during a total period of time. Antioxidant capacity profiles were similar to total phenolic compound release. Figure 4 shows that 15% of extract-loaded films had the highest phenolic content in release media over time, as the phenolic content of 5% of extract-loaded films showed a decrease as time passed. Swelling properties and weight differences of films during release study can be suggested as a reason for fluctuations in release media composition. Antioxidant activity and amount of soluble phenolics were increased gradually in 15% turmeric extract loaded films, referring to the possibility of sustained release.

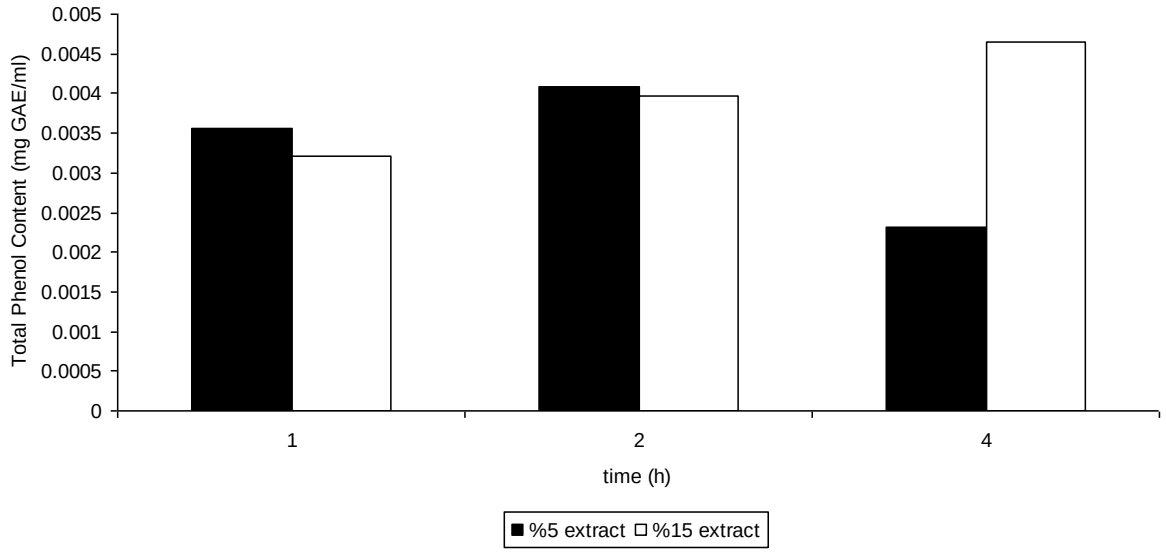

Figure 5. Gallic acid equivalent total phenol concentration of turmeric extract-loaded SF-NaCMC films.

3.4. Antimicrobial activity.

Two microorganism species were chosen to determine the antimicrobial activities of SF-NaCMC films in disc diffusion assays. *Escherichia coli* was used as gram-negative bacteria, and *Candida albicans* was used as fungus. Microorganism-free regions were determined by spraying iodonitrotetrazolium chloride (INT) on agar surfaces since it worked as a dye indicating the viability of microorganisms. The samples used during the experiment were respectively: (50:50%) SF:CMC films without extract, (50:50%) SF:NaCMC film with 5% turmeric extract,(50:50) SF:NaCMC film with 10% turmeric extract , (50:50) SF:NaCMC film with 15% turmeric extract, Antibiotic control, Pure SF film without extract, Pure CMC film without extract. When we examined the antimicrobial activity against *E. coli*, it was observed that the films made with only 15% turmeric extract stopped the bacterial spread. In the disc diffusion method, antibiotic discs formed a clear zone with 24.8 ± 0.8 mm diameter against *E. Coli*. It was calculated that the films made with 15% turmeric extract formed an average of 16.4 ± 0.5 mm against *E. Coli*. It was observed that other concentrations and samples did not form a zone against *E. coli*. Table 3 shows the oral film disk diffusion results on *E. Coli* with standard deviations. None of the films exhibited antifungal activity against *C. albicans*. Antioxidant and antimicrobial properties were found to be related to release properties which were also related to degradation, and further studies will be conducted to optimize polymer blend composition in this aspect.

Table 3. Disc diffusion results of different films on *E. coli*. **a.** (50:50%) SF:CMC films without extract, **b.** (50:50%) SF:NaCMC film with 5% turmeric extract, **c.** (50:50) SF:NaCMC film with 10% turmeric extract , **d.** (50:50) SF:NaCMC film with 15% turmeric extract, **e.** antibiotic control, **f.** pure SF film without extract, **g.** pure CMC film without extract.

a	**b**	**c**	**d**	**e**	**f**	**g**
0 mm	0 mm	0 mm	16.4 ± 0.5 mm	24.8 ± 0.8 mm	0 mm	0 mm

4. Conclusions

The results of this study firstly indicate that increase in the amount of silk fibroin smoothened the surface, whereas an increase in NaCMC content increased surface roughness. On the other hand, high silk fibroin content caused a decrease in the flexibility of the films. This resulted from improved mechanical properties of silk fibroin, which may affect processability. Among the study's findings, it was observed that loading an extract such as Turmeric extract into the mixture during the formation of the oral film increased homogeneity by preventing phase separation. Also, AFM results indicated that extract loading affected surface roughness in a decremental manner at initial concentrations. Weight loss studies also indicated the effects of extract amount on swelling and degradation. Lastly, antioxidant activity and the number of soluble phenolics gradually increased in 15% turmeric extract-loaded films. This increase has been observed to alter the antimicrobial effects of films to a similar extent. This study has pointed out the potential of extract-loaded biopolymer films as therapeutics. These films may also be utilized in food packaging and coating materials due to their water-barrier property as an effect of crosslinking.

Funding

This research has no funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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