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Effective Enhancement of Post-Harvest Tomato Storage through Wrapping Using Deacetylated Chitin Nanofibril-Plasticized Honey Films

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Abstract

As one of the most grown seasonal fruit, tomato is widely consumed for making food products, drugs and ornaments. However, poor post-harvest handling system, transport and storage facilities deteriorate the quality of tomato fruits resulting into post-harvest losses. These problems have been addressed by the use of nanotechnology in this study. The combination of a chitosan surface and the core shell chitin results into novel deacetylated chitin nanofibril (DeChNF) with good biological activity and mechanical strength. The effectiveness of the DeChNF films for food preservation as an alternative material to chitosan is investigated. The DeChNF with degree of acetylation (DA) = 60.2% and chitosan (DA = 7.1%) were obtained from crab shells. Two appraches were employed to store freshly matured tomatoes harvested at a breaker stage. The tomatoes were coated with the colloidal suspension and wrapped in the films. Flexible wrapping films were prepared from the colloidal suspension through solution casting using bees honey (35%) and compared to those with glycerol (25%) as plastisizers. The main output storage parameters analysed include colour development, surface spoilage, and weight loss over 20 days at a 73% relative humidity. Tomatoes covered with DeChNF-honey and chitosan-honey films remained in good condition after 20 days, while the coated samples lasted for up to 15 days, except those coated with DeChNF, and those covered with a polyethylene films or stored in a refrigerator at 9 °C. The samples covered with DeChNF films plasticized with honey showed the best resistance against weight loss. The tensile strength of the DeChNF-honey films (strength = 21.32 MPa) and the chitosan-honey films (strength = 21.86 MPa) were higher than the polyethylene films (10.9 MPa). The current work increases understanding on DeChNF as an alternative material, with additional of honey as a plasticizer, over the superior chitosan, or commercial films based on fossil resources.

Keywords: Deacetylated chitin nanofibrils, chitosan, bees honey, glycerol, shelf life, tomatoes.

Introduction

Tomato is a widely grown and consumed fruit for making food products such as tomato paste, sauce, jam, drugs, ornaments and other uses. The rich content of antioxidant substances such as carotenoids and vitamin C, lycopene and phenolic compounds in tomatoes is good for skin, prevent cancer, maintain strong bones and prevent non-communicable diseases (Loeb et al. 2022). The world tomato production reached 37.38 million metric tonnes of fresh fruits in 2019 (Colvine and Branthome 2020). The production is around 129,578 tons per annum in Tanzania (De Putter et al. 2007). The current data on tomatoes postharvest losses in Tanzania are not clear. Recent data, for example in Ghana, showed postharvest losses account for almost 43% of the harvests produced (Wongnaa et al. 2023); presumably a similar situation is reflected in Tanzania. First, the fruit suffers major losses of quantity and quality due to poor postharvest storage especially in the rural areas. Second, the average yield per hectare is ten times lower compared to the world data (Luzi-Kihupi et al. 2015). Third, economical similarities of these countries. The challenges encountered during the postharvest storage of tomatoes imply not only the loss of important nutritional values but also the extreme dependence on unreliable rains.

Tomato is a seasonal fruit and the postharvest storage starts from the harvest time until finally perished or consumed. The fruit is picked up when almost matured. The physiological processes such as ripening, respiration, transpiration and ethylene production after harvesting are controlled in order to extend tomatoes shelf life and conserve colour, firmness and nutritional values during storage. The ripen tomatoes is approximately 95% water and the variations of temperature and relative humidity accelerates loss of quality (Ayomide et al. 2019). The loss of water may reduce resistance to pests and diseases, induce change of appearance, and decrease weight. *Aspergillus niger* is an example of microbes that attack the tomato fruit within a storage period of 8 days (Reis et al. 2015). Among the tomato storage methods including canning, refrigeration, fresh storage and traditional storage methods such as smoking, drying, salting and pickling (Ajayi et al. 2003), refrigeration is the most common. The storage temperature of around 8–10 °C is appropriate to maintain qualities of ripe or firm tomatoes, and the temperature of 10–15 °C is good for green tomatoes (Ayomide et al. 2019).

Fresh tomatoes have a shelf life of 7 days at room temperature (Sinha et al., 2019) or 14 days in a refrigerator (Žnidarčič and Požrl 2006). Coating techniques using a film or solution has been an effective way to extend the postharvest life of tomatoes. Various edible films effectiveness to prolong tomatoes storage life have been reviewed (Duguma 2022). The coating films possess a range of preservative properties, often extending the storage life up to 15 days. For example, starch coating was used for fruits and vegetables, and provided barrier properties that reduced ethylene production and fruit respiration rate (Sapper and Chiralt 2018). Chitosan coating is a semi-permeable film on the fruit surface, responsible for delaying fruit ripening, microbial attack, decreasing weight loss, maintaining the quality, and prolong life in storage (Romanazzi et al. 2018). The therapeutic properties of chitosan strengthen the immune responses in the tissues and are of the advantages over many other films. The protonated amine groups in chitosan bind to the negative charge of the bacteria membrane, which disrupts the physiological functions and causes death (Eldin et al. 2008).

Pure chitosan based films are inherently brittle in nature, which is a drawback in food packaging (Van den Broek et al. 2015). Therefore, glycerol is used as a plasticizer in chitosan films to improve the mechanical as well as barrier properties. The film becomes flexible. Moreover, the plasticized films with glycerol enhance the shelf life of tomatoes (Leceta et al. 2013). Other plasticizer types include ethylene glycol (EG), poly (ethylene glycol) (PEG), and propylene glycol (PG) (Suyatma et al. 2005). The potential plasticization properties of honey to chitosan, including chitin deacetylated chitin nanofibrils (DeChNF) have not being investigated. Honey is very hydrophilic with the moisture content of 17–18%, attributed to the content of sugars, protein, vitamins, minerals, and antioxidants (Chen 2019), making the blending with the chitosan or DeChNF attractive. Apart from being a good source of food, honey shows anti-inflammatory, antioxidant and antimicrobial properties, essential for food storage (Szweda 2017).

Even though, the commercial food packaging film is still dominated by materials based on synthetic polymers such as polyethylene, polyvinyl chloride, polyvinyl alcohol, and nylon, the advantage of chitosan and chitin is that they are biodegradable and renewable. Recently, poly lactic acid and polycaprolactone, originated from bio- or fossil resources, have replaced the poly olefins in the commercial scale because of their biodegradability. But, the performance of synthetic based films maybe difficult, and often depends on the successful incorporation of active components such as nanoparticles or extracts to the matrix or by tailoring of a conducive storage environment. Films based on chitin nanofibrils (ChNF) have not been extensively investigated as wrapping films for food storage. For example, recently a nanocomposite of ChNF was reported containing thermoplastic starch, gelatin, and poly (vinyl alcohol) (Heidari et al. 2021). The properties of the nanocomposite films were good but no data on food postharvest storage life. So far, chitosan have shown the most promising properties because of their antimicrobial properties and good mechanical characteristics.

The literature on ChNF provide a fundamental understanding of nanofibril structure, mechanical potential (Mushi et al. 2014a), gas barrier properties (Satam and Meredith 2021), and biomedical properties (Azuma et al. 2014). Chitin polymer is a predominantly long chain of amine polysaccharide with a repeating β-(1,4)-linked 2-acetamido-2-deoxy-D-glucopyranose monomers. A ChNF possesses a crystalline structure (Mushi et al. 2014a). A native ChNF is highly acetylated. A well preserved native structure demonstrates impressive mechanical properties (Mushi et al. 2014a). A deacetylated ChNF (DeChNF) has a chitosan topochemical surface and a chitin crystalline core shell (Fan et al. 2010). An advantage of the nanofibril structure is the nanoscale effect such as it forms strong, transparent and flexible films (Mushi et al. 2014a). In addition, the components in nanometre scale express some therapeutic effect against pathogens (Wang et al. 2017). Moreover, the long range crystallinity is good for gas barrier properties (Liu et al. 2011). DeChNFs requires less chemicals to prepare as compared to chitiosan and this is an added advantage in green technologies. The ChNF-chitosan films of high strength and toughness have been reported (Mushi et al. 2014b).

As the surface spoilage, weight loss and colour deterioration are the dominant causes of tomato postharvest losses, the main objective of this study was to understand the perfomance of the DeChNF against chitosan as a potential biopolymer for effective improvement of tomato storage. The pubication discussed two aspects–first, the tomato postharvest storage life, second and most important the mechanical property aspects of the films based on pure chitiosan, and DeChNF and after plasticization. DeChNF is a novel nanocomponent with unique biological activity and mechanical properties but also suffers from brittleness as chitosan films. Moreover, the use of this material for effective storage of tomato is not well reported in the literature. Employment of honey to mitigate mechanical properties is a new concept. Despite the inherent potential of honey for food and drug, little is known on their potential as ChNF or chitosan plasticizer. As such, the DeChNF was plasticized using bees honey, and glycerol was used for comparison, to increase the mechanical ductility without impairing the biological activity of the DeChNF films. Both glycerol and honey possess chemical compatibility to chitosan and chitin because of the presence of hydroxyl groups. The current study offers a possibility for the development of new environmentally friendly films as well as sustainable technologies for food, cosmetics, paper as well as pharmaceuticals industries. A future study may elaborate more on the concept of mechanical plasticization using honey.

Materials and Methods

Materials

Fresh matured tomatoes (*Lycopersicum esculentum* Mill) of pinkish-red colour were picked from a local farm in Tanzania at a breaker stage. DeChNF and chitosan were prepared from crab shells purchased from the Dar es Salaam fish market in Tanzania along the coast of East Africa. The chemicals such as hydrochloric acid, sodium hydroxide, ethanol, and acetic acid were purchased from Loba Chemie PVT LTD in India. The glycerol and bees honey harvested from *Apis mellifera scutellata* located in Kigosi-Moyowosi game reserve were purchased from local vendors in Dar-es-Salaam.

Preparation of DeChNF and chitosan

The raw crushed crab shells were treated with 2 M of HCl for demineralization at room temperature (Mushi et al. 2014a). Then, the shells were washed with deionized water to neutral pH. The sample was decolourized by treating it with ethanol under constant stirring overnight. Figure 1 shows the deacetylation mechanism for preparing chitosan and forming surface amino groups in DeChNF from raw crab shells. First, deproteinization and deacetylation processes were performed by treating the sample with 50% NaOH w/v at 100 °C for 36 h. Then, the sample was washed several times with deionized water to neutral pH. Next, the DeChNFs were prepared based on the method developed by Fan et al. (2010). First, the samples were deproteinized and deacetylated by treatment with 33% NaOH at 100 °C for 6 h. Last, the sample was washed several times with deionized water until it reached neutral pH.

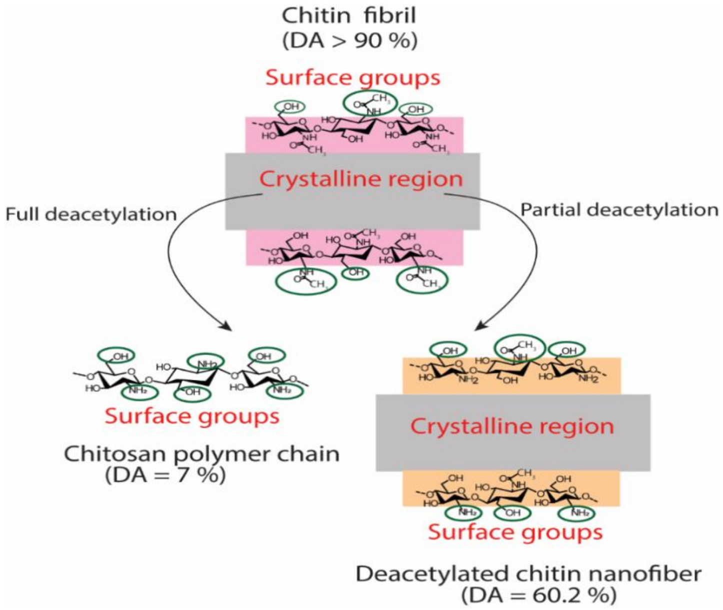

Figure 1: Schematic diagram of raw crab shells conversion from chitin fibrils to chitosan and DeChNF.

Determination of degree of acetylation

Conductometric titration was done to obtain the degree of acetylation (DA) of DeChNF and chitosan. About 0.2 g of the dry sample was suspended in 40 mL of 0.05 M HCl under constant stirring and titrated against 0.5 mL of 0.165 M of NaOH for 60 seconds. The values of conductance (mS/cm) with corresponding titration volumes at the initial stage v_1 until complete neutralization step v_2 of the ammonium cations were recorded and plotted to find the stoichiometric points. The DA value was calculated using Eq. (1), which represents the acid consumed by amine groups (Czechowska-Biskup et al. 2012).

$$DA = 1 - \left[\frac{[base](v_2 - v_1)161}{m}\right] \times 100 \qquad 1$$

Where *base* is a constant = 0.165 and *m* is the mass of the titrated sample.

Fourier Transform Infra-Red (FTIR) Spectroscopy

The surface functional groups were studied through IR spectra recorded using a Spectrum 2000 FTIR spectrophotometer (Perkin-Elmer Inc., USA) equipped with an attenuated total reflectance crystal accessory (Golden Gate). All samples were scanned between 400 and 4000 cm^{-1} wavenumber.

X-ray Powder Diffraction (XRD)

Pure chitosan and surface DeChNF films were prepared to analyse the chitin crystalline pattern. The X-ray Powder Diffraction (XRD) patterns were collected in the 2θ range of 5−45° using an X-ray diffractometer (Rigaku Co., Ltd., South Africa) with Cu Kα radiation (λ = 1.5418 Å) at 40 kV and 40 mA.

Procedure for evaluation of tomato conditions in storage

The tomatoes' weight loss, colour development, and surface spoilage parameters evaluated the post-harvest shelf life. The freshly prepared tomatoes (twenty-five pieces) were cleaned with running water, disinfected with 4% hypochlorite acid for 5 min (Sucharitha et al. 2018), and then rinsed several times in clean water. One batch of tomatoes was covered using the suspended solution and another in a wrapping film. The chitosan solution (0.7 g) was dissolved in 2% acetic acid, and 0.7 g of DeChNF was suspended in 4% acetic acid (Mushi et al. 2014b). First, three coating samples (control, DeChNF, and Chitosan) of tomatoes were prepared, each experiment with three numbers of tomatoes. A 1 wt.% sample of the chitosan solution and DeChNF suspension with pH 5 were prepared, and 25% glycerol (w/w) was added as a plasticizer**.** The samples were blended for 10 minutes to form a homogenous solution. The colloidal suspension was degassed for one hour. The tomatoes were dipped into the plasticized solution of chitosan and DeChNF suspension, respectively, and removed immediately. The tomato samples without coating was kept one at room temperature and another in the refrigeration conditions (9 °C) in a fridge as controls. A mercury thermometer recorded the temperature. The experiment for coated tomatoes was conducted at room temperature (29 °C) and refrigeration conditions (9 °C). The relative humidity at ambient conditions was 73%. The relative humidity was measured using a hygrometer (Clas Olson, Sweden). The tomatoes were weighed daily for eight days. Second, Pure DeChNF, DeChNF-glycerol, DeChNF-honey films, pure Chitosan, Chitosan-glycerol films, and Chitosan-honey-films were prepared by mixing the colloidal suspension with a 25 wt % glycerol (Ifuku et al. 2014) and 35 wt % bees honey (moisture content 17%), respectively. The DeChNF and honey was used for the first time and the 35% concentration was possible to cast the film. The suspensions were solution cast on an aluminum tray of 220 mm diameter covered with a Teflon film and then dried in an oven at 37 °C. Sixteen tomatoes were manually wrapped completely in the DeChNF films and the honey and glycerol plasticized films. The unwrapped tomatoes at room temperature were control1. The tomatoes wrapped in a polyethylene film at room temperature were control2. The unwrapped tomatoes at refrigeration temperature were control3. The relative humidity of the refrigerator was ranged between 45–75%. The tomatoes were weighed at the interval of one day for twenty days. The weight losses of coated tomatoes under refrigeration condition were not investigated. The colour development was evaluated through visual observations using the United States Department of Agriculture colour chart according to maturity and ripeness classes for fresh market tomatoes (Cantwell 2000). See colour scores in Table 1. The surface spoilage on the tomatoes' outer skin was observed manually through visual inspection. The images were captured using an ordinary camera. The weight of tomatoes was measured using an electronic weighing balance. A statistical method was used to analyse the weight loss results.

Table 1: Tomato colour score chart from Cantwell (2000)

Colour class	Colour score	Remarks
Mature green	1	Internal red colour
Breaker	2	First external pink or yellow
Turning	3	More than 10% but not more than 50% of the surface tannish-red, pink or red or the combination of both
Pink	4	More than 30% but not more than 60% of the surface shows pink or red
Light red	5	More than 60% shows pink-red or red but not more than 90% of the surface shows a red colour
Red	6	More than 90% of the fruit surface is red

Tensile test of the films

Tensile test was conducted according to the previous method ((Mushi et al. 2014a, b). The tensile properties of the DeChNF and the plasticized films were studied from 5 specimens. The specimens were cut into a width = 5 mm and length = 20 mm, then conditioned at 20 °C overnight. The uniaxial deformation test was performed using a Universal tensile testing machine (Instron, UK) with a crosshead speed of 2 mm/min and a load cell of 10 kN to obtain tensile modulus, tensile strength and tensile strain to failure. The statistical analysis was performed on the tensile data and comparison made to the properties from commercial films based on polyethylene.

Results and Discussion

Physical properties of DeChNF and chitosan

Figure 2 (a) presented a colloidal suspension of chitosan and DeChNF. Glass vials displayed an excellent dispersion of DeChNF and chitosan solution as expected. The chitosan solution is brownish, and the chitin fibril vial indicates a milky suspension. The degree of acetylation of the chitosan (7.02%) and DeChNF (60.12%) by conductometric titration are presented in Figure 2 (b). The DA of around 60–70% distinguishes chitin from chitosan polymer (Hajji et al. 2014). The chitosan was highly deacetylated, facilitating chitosan solubility in 2% acetic acid. The DA of DeChNF is close to the results from DeChNF of DA, around 70–74% prepared through a similar protocol (Fan et al. 2010). The FTIR spectral image of DeChNF and chitosan is presented in Figure 2 (c). The amide spectral peaks at 1626 cm^{-1} decreased to 1646 and 1655 cm^{-1} as the DA is correspondingly reduced by the removal of the acetyl groups. The spectral peaks around 1660–1620 cm^{-1} originate from the presence of an amide group in chitin (Ahlafi et al. 2013). The structure of DeChNF or chitosan may be distinguished based on the crystalline data. The XRD curves in Figure 2 (d) complement the FTIR data and confirm the presence of crystalline chitin and an amorphous chitosan. A sharp peak at 2theta = 16.9° originated from DeChNF due to α–chitin allomorph (Mushi et al. 2014a). The chitosan curve is characterized by a weak and broad peak around 2theta = 16.9°.

Performance of the coated and wrapped tomatoes in storage

The evaluation results of surface changes, colour deterioration, and weight loss data for tomatoes are presented in Figure 3 (a, b, c) for coated samples, and Figure 4 (a, b) are samples wrapped in a film. Figures 3 (a, b) and 4 (a) present the post-harvest appearance of the tomatoes after storage, used to evaluate surface spoilage and colour development. Figure 3 (c) shows data for weight loss of coated tomatoes and the control samples after 8 days of storage. Figure 4 (b) presents the weight loss of wrapped tomatoes and three control samples observed for up to 20 days.

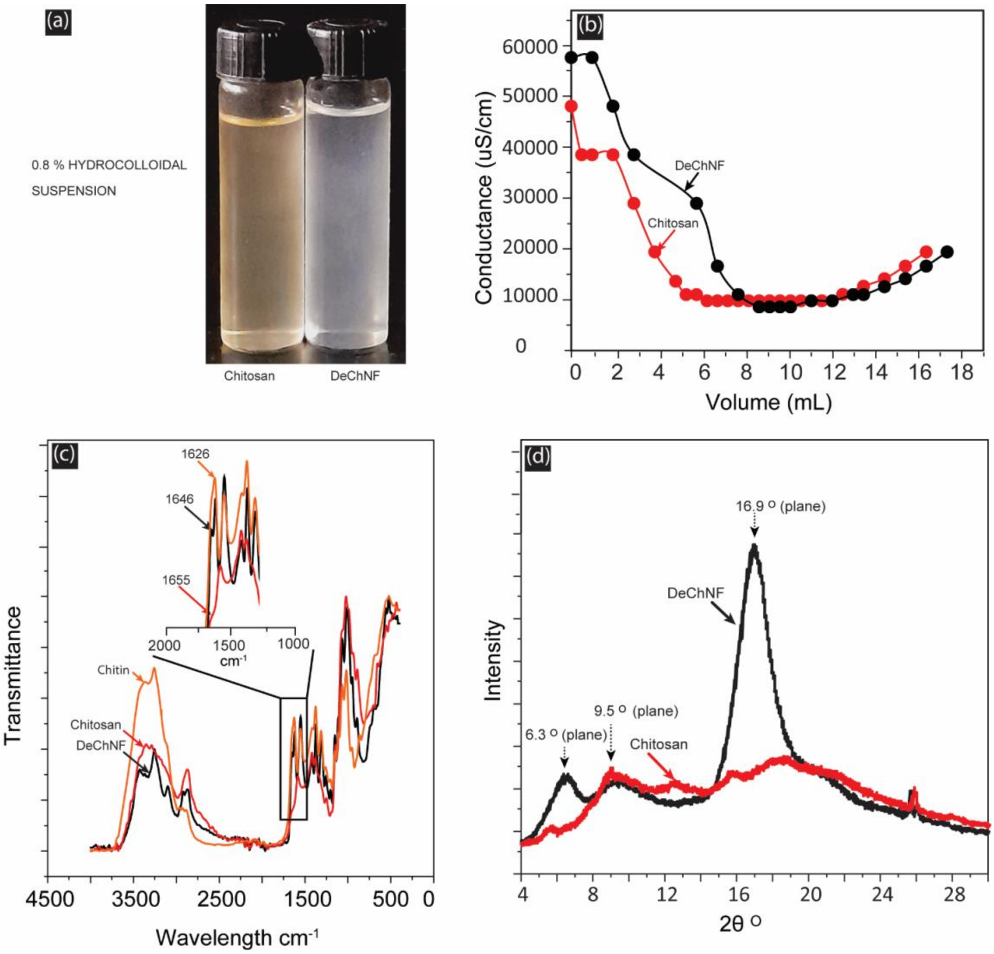

Figure 2: (a) Hydrocolloidal suspension of chitosan and DeChNF in a glass vials. (b) Conductometric titration of chitosan and DeChNF. (c) FTIR peaks of DeChNF and chitosan powder. (d) XRD of chitosan and DeChNF films.

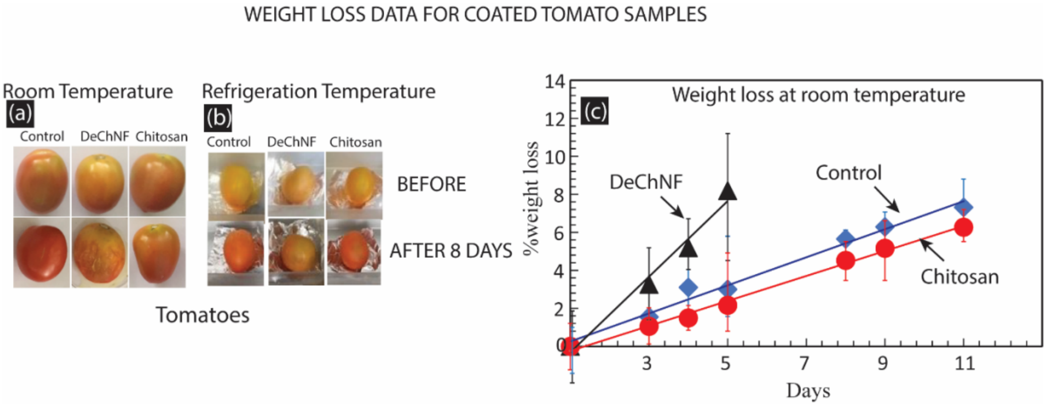

Figure 3: Visual appearance of coated tomatoes at (a) room and (b) refrigerated temperatures showing colour development and surface spoilage (c) Weight loss data of the coated tomatoes.

a) Coated tomatoes

Figures 3 (a, b) are images showing colour development from pinkish-red at the breaker stage to deep red after 8 days. The tomatoes were pinkish-red after 5 days (image not shown). According to the colour score chart (Cantwell 2000), tomato undergoes several colour changes to ripening. Initially, the colour score was 2–3 implies that at least 10% of the fruits were tannish-yellow, pink or red, or a combination of both. Finally, the tomato colour score for both coated samples at room temperature was 5 after 8 days, which means no appreciable colour preservation occurred. The colour score for the control sample at room temperature was 6. For the case of refrigerated samples, the final colour score of DeChNF coated sample was 4, and the chitosan-coated sample was 3. The initial green colour is due to the presence of chlorophyll and turns red from the increased formation of lycopene (Soares et al. 2019).

Through visual inspection, the black stains can be observed on the surface of DeChNF-coated tomatoes after 8 days (see Figure 3 (a)). The wrinkles appear on the tomato's surface. The black stains were associated with a surface spoilage since the freshly prepared tomatoes were clear. As stated, the most appropriate storage time for tomatoes is around 7 days (Žnidarčič and Požrl 2006, Sinha et al. 2019). The DeChNF-coated tomatoes started to disintegrate after 8 days (see image Figure 3 (a)). Perhaps, there was a poorly formed coating, accelerating the surface spoilage after drying over the surface of the tomatoes.

The chitosan-coated tomatoes with a colour score of 5 showed significant weight preservation after 8 days of storage at room temperature; see Figure 3 (c), and the tomato coated with DeChNF shrunk after 8 days. Since the observations of weight loss at room temperatures were not good, the coated tomatoes (chitosan and DeChNF) were not evaluated against weight loss under refrigeration condition. With the coating technique, it is probably difficult to fully cover the tomato surface using the ChNFs at the concentration used (1 wt. %). The control samples at room temperature had a weight loss of around 7.5% compared to 5.5% of the chitosan-coated samples. The control tomatoes stored at a low temperature (9 ℃) for 9 days had a weight loss of 5.5%, while that of chitosan-coated was 4.1% (image not shown). A review study reported weight losses around the same range using edible coatings from chitosan, e.g., a 16.7% weight loss in tomatoes stored at 4 ℃ (Duguma 2022). The current data on chitosan-coated tomatoes are comparable to the results reported after 10 days (Sree et al. 2020). The enhanced storage conditions of the glycerol plasticized samples match with the literature data (Leceta et al. 2013). A higher water loss observed from the DeChNF sample compared to the control samples is difficult to understand. The handling problem of the spoiled tomatoes, which become very soft, was experienced, limiting the weight loss measurements to 5 days. The higher weight loss could be accelerated due to the surface spoilage or from the effect of the acidic pH of the suspension, which was maintained at pH 5 to keep the nanofibrils dispersed.

b) Wrapped tomatoes

Figure 4 (a) presents the wrapped tomatoes for up to 20 days. The initial tomato ripening stage was ranked at a 3–4 colour score. The wrapped tomatoes using the plasticized films from chitosan and DeChNF have already shown a full-colour development of deep red with a colour score of 5–6 after 5 days of storage. For control samples and the unplasticized DeChNF films, the samples degraded before the storage life of 20 days. The surface spoilage and associated weight loss were more critical after the full-colour development. The room temperature (control1) and refrigerated tomatoes (control3) were surface spoiled after 5 days; the spoilage was severe after 15 days. Black stains on the tomatoes can spot the spoiled area. The tomatoes wrapped in the polyethylene film (control2) were spoiled on the surface after 20 days of storage. Polyethylene plastic films are good for food storage because of their gas barrier properties (Ge et al. 2021). For example, low density polyethylene has been used as laminates in packaging films (Ge et al. 2021). But, polyethylene films possess poor antimicrobial properties compared to chitosan or deacetylated chitin (Butchosa et al. 2013, Muñoz-Núñez et al. 2022). The tomatoes covered in DeChNF films spoiled after 15 days, probably due to film cracking during folding. Pure chitosan was not possible to form a dried film because of inherent brittleness (Van den Broek et al. 2015). The tomatoes wrapped in the plasticized films were in good shape throughout the experiment. The plasticized films formed from an excellent colloidal dispersion, and with low moisture content (around 12%), provided the favourable storage conditions.

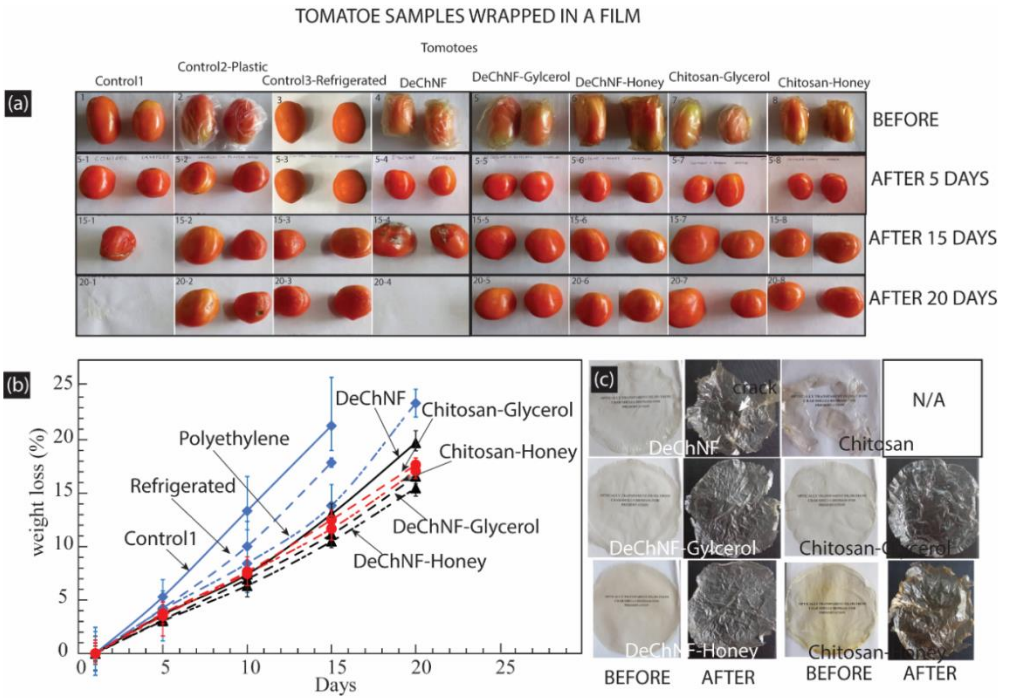

Figure 4: (a) Wrapped tomatoes samples visual appearance showing the surface spoilage and colour development, (b) Weight loss data of the wrapped tomatoes (c) Used film after 20 days of storage. Scale 1: 9 mm.

The tomatoes were unwrapped and then wrapped back every time for weighing. As such, it explained the cracking of the unplasticized pure DeChNF film. The control1 tomato samples (presented by blue lines in Figure 4b) had the highest weight loss compared to the rest of the samples because the direct exposure to the environment, leading to surface spoilage and subsequently accelerated water loss. The spoilage by bacteria or fungus on tomatoes after 5 days of storage is also possible, fastening weight loss since the tomato's outer protective waxy skin was damaged. The tomatoes (control3) kept in the fridge at 9 °C showed a higher weight loss after 10 days of storage. The tomatoes (control2) wrapped in a polyethylene film were surface spoiled after 20 days, see Figure 4 (a). Since polyethylene films are not antimicrobial, the surface spoilages observed in Figure 4 (a) after the twentieth day (images of samples) were responsible for increased weight loss. The tomatoes covered with the plasticized chitosan films (red lines) had almost the same weight loss percentage. The films plasticized with honey showed lower weight loss than glycerol-plasticized films. The tomatoes covered with DeChNF lost much higher weight after 15 days, as evident in Figure 4 (a). As stated, the DeChNF films were brittle and folding may affect the films (see Figure 4 (a) and the corresponding film photographic images in Figure 4 (c)). Figure 4 (c) shows the photographic images of the films before and after being used to wrap the tomatoes. The chitosan and DeChNF films with plasticizers were in good shape after being used several times. The lowest weight loss was observed from the DeChNF films plasticized with glycerol and honey (the black lines). The weight loss data shows a linear increase with time, similar to the observation from the chitosan-plasticized films. The DeChNF films plasticized with honey provided better results than those plasticized with glycerol. The tomatoes wrapped with a DeChNF plasticized with honey extended the useful postharvest life of the tomatoes by at least 5 days, maintaining their colour and weight. Honey has a broader range of biological activity, such as antimicrobial properties, compared to glycerol (Szweda 2017). This property of honey is attractive for DeChNF since the control samples, and the pure DeChNF showed a sharp increase in weight loss after 5 and 15 days of storage, respectively. This is revealed by exhibiting a steep slope in the control1 and DeChNF curves after 5 and 15 days, respectively. Apart from being defective, leading to surface spoilage and the associated weight loss, the DeChNF films showed better performance for tomatoes storage. The amine groups in the chitosan and DeChNF are the primary reasons for the good tomato condition in storage. Investigation on the therapeutic (or antibiotic) properties of the nanofibril nanocomponent will be interesting in the future. The microbial activity in relationship to surface spoilage, and weight loss will also be attractive. The DeChNF with preserved crystallinity and nanofibril diameter in the range of 3 to 5 nm resulted in thin films which is an advantage. A well-dispersed DeChNF hydrocolloid, the hydrophilic properties from side groups (hydroxyl and amine groups), and the nanofibril structure contributed to the capillary condensation during drying, and formation of a good network structure. Apart from flexibility, probably from the effect of plasticization, the films based on DeChNF and honey perhaps provided a good gas barrier network structure.

Four aspects may be highlighted as the limitations to this study. First, the test was carried out and monitored throughout from the beginning to the end. But, only the best results were selected for presentation in the manuscript. Second, the image processing results could also alter the actual size of the tomatoes. Third, even though colour was monitored throughout the entire fruit surface and a colour chart was used to decide on colour development, the dominating colour in the fruit (sample) was matched to the chart qualitatively rather than quantitatively based on rather intuitive judgment. Fourth, the microbiological performance of the films is essential but not reported here. Further research may be required to understand the effectiveness of the DeChNF compared to chitosan against various post-harvest metabolic activities but the study is expecting to prompt more researches along this line including microbial analysis.

Data from the previous study compared the shelf life of wrapped and unwrapped tomatoes using chitosan films (El Ghaouth et al. 1992) and polyethylene (Sinha et al. 2019). The unwrapped tomatoes were spoiled after 7 days of storage at room temperature (Sinha et al. 2019), similar to the current data. We have observed an extended storage life of up to 15 days by storage at 9 °C temperature, which is also according to the previous data of 14 days (Žnidarčič and Požrl 2006). The tomatoes wrapped in a polyethylene film reported a storage life of 6 days at ambient temperature (Sinha et al. 2019), which is shorter than our control data (15 days) from control2.

Colour change, surface spoilage, and weight loss are triggered by an ethylene gas, a gaseous plant hormone, enzymatically produced during ripening (Alexander et al. 2002, Hertog et al. 2004, Greco et al. 2012). As such, the tomatoes postharvest life span may be linked to dehydration, transpiration, and respiration rate (Žnidarčič and Požrl 2006), besides microbial attacks, physical damages or seasonal fluctuations. The chitosan coating reduces gaseous exchange (El Ghaouth et al. 1992), which disturbs such physiological processes such as ethylene production. At maturity, tomatoes perish due to ethylene biosynthesis, synthesis of lycopene, and degradation of chlorophyll, a purely biochemical processes, that in turn allows the development of a deep red colour (Hatami et al. 2012). According to Hatami et al. (2012), weight losses may be associated with the degradation of polysaccharides in the cell wall. Even though the physical appearance, such as colour and physical damage or surface defects or spoilage, and weight loss of tomatoes presented here are essential criteria, they give limited information for tomatoes' storage evaluation. For commercial applications of the films, a total viable bacterial counts (TVBC) and total yeast and mould counts (TYMC) will be assessed according to CODEX and Tanzanian Bureau of Standards (TBS) requirements. Currently, our data may be comparable to those of edible films in the literature. For example, Trigo et al. (2012) coated papayas with 3% rice starch, 0.5% sodium alginate, and 0.25% carboxymethyl cellulose and stored them at 5 °C and 90% relative humidity. The shelf life of the papaya was extended by 15 days. Various other types of films have been developed in combination with chitosan, cellulose and other additives for food storage. For example, chitosan film with ethanolic propolis extract (1 and 2%) and cellulose nanoparticle (1 and 2%) was used for minced beef meat storage, extending the shelf life up to 14 days (Shahbazi and Shavisi 2018).

Mechanical properties of the chitosan and DeChNF films

Figure 5 presents the mechanical properties of the plasticized and unplasticized DeChNF films and reference materials from plasticized chitosan. Figure 5 (a) shows the nature of the stress-strain behaviour of the DeChNF films and those of the plasticized chitosan and DeChNF films. The initial elastic region shows a linear pattern for all the samples. The plastic region is poorly developed for chitosan-honey, chitosan-glycerol, and DeChNF-glycerol, probably because of defects associated with the film-forming process and sample preparation. When polymeric materials deform beyond the elastic limit, a permanent deformation occurs (Michler 1999). Chitosan or chitin nanofibril based films show the same deformation behaviour (Mushi et al. 2014b). The macroscopic deformation mechanism in the plastic region may be controlled by the presence of defects (such as cracks, pores, agglomerations, etc), molecular weight, and interfacial interactions. Ideally, glycerol-plasticized chitosan and chitin show a well-developed plastic region and more extended strain to failure than the pure sample due to its plasticizing effect (Ifuku et al. 2014). In the plasticized samples with honey, the strength is reduced more than in glycerol because of the higher content of honey (35% compared to 25%). A more prolonged strain to failure is demonstrated by the DeChNF-honey. The properties of the DeChNF-honey are exciting since the films showed the best data for tomatoes' storage against surface spoilage, colour development, and weight loss. Figure 5 (b) shows the correlation of strength and modulus of the DeChNF-honey films and compares it to the DeChNF and reference materials from chitosan and polyethylene films. The DeChNF-honey films showed the lowest modulus, and the strength is comparable to that of chitosan-honey. The mechanical properties of the films obtained correspond to the mechanical properties of polyethylene films for fruits packaging, i.e., the lower strength = 10.19 MPa as shown in Figure 5 (b), and the upper strength data may reach up to 29 MPa and 39.4 MPa (Sangroniz et al. 2019). But, a high strength is possible from the DeChNF cast films through a careful, slow drying process.

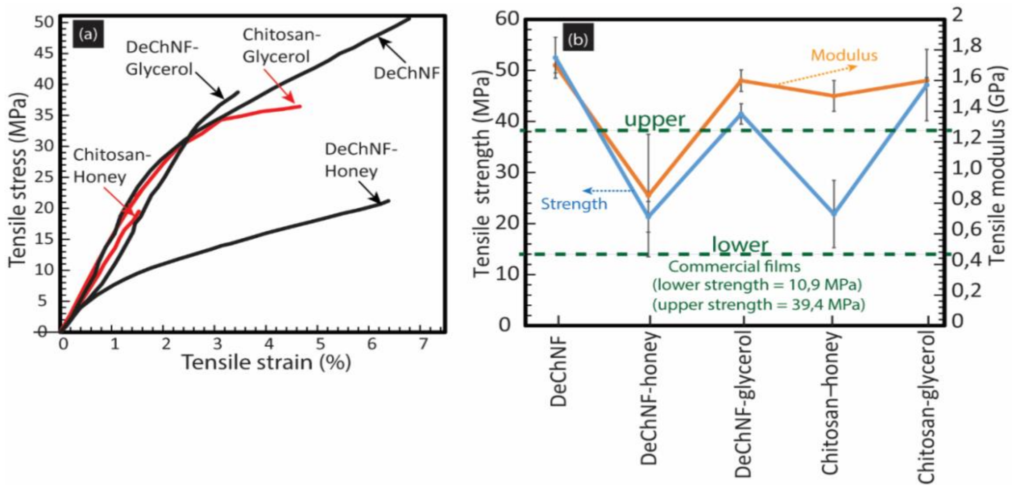

Figure 5: Mechanical properties of the DeChNF, DeChNF-glycerol, DeChNF-honey, Chitosan-Glycerol, Chitosan-honey (a) Stress strain curves (b) The comparison of the tensile strength (left) and modulus (right) of the films in this study. The strength of the commercial film from polyethylene is presented in a red dotted line.

Conclusions

The current study provides a comparison of the performance of a rather superior chitosan-based films against DeChNF-based films on the storage life of tomatoes. The intention was to establish the performance of the DeChNF as a potential cost effective and better material mechanically for packaging application. Individualized DeChNF (DA = 60%) and chitosan (DA = 7%) prepared from crab shells, resulted in a stable hydrocolloid suspension. Coating and wrapping techniques were used for tomato storage. First, the chitosan–coated tomatoes showed enhanced the storage life compared to DeChNF-coated samples. Second, the wrapping films were prepared from chitosan and DeChNF using honey and glycerol as plasticizers. The DeChNF-honey films demonstrated superior performance for tomato preservation at ambient temperature and 73% humidity, close to that of chitosan-based films. In addition, the surface spoilage and the weight loss was lower than that of chitosan-based films. Since the storage life of tomatoes at room temperature is 7 days, then the shelf life has been prolonged for more than 10 days using DeChNF-honey films. Interestingly, the tensile strength of the DeChNF films plasticized with honey and glycerol were also comparable to those of plasticized chitosan films (tensile strength = 21.86 MPa and 47.14 MPa for chitosan films plasticized with honey and glycerol, respectively) and polyethylene films for fruits packaging. Our data for DeChNF-honey plasticized films for tomato wrapping are presented for the first time. The results also shows that the performance of chitosan film is better than that of pure DeChNF films, and that treatment is required to improve its nanoscale performance. The limitation of the study on the effects of DeChNF-honey films on the tomato storage have been highlighted, and would needs more understanding in this area. For example, first the terms plasticization has to be sufficiently studied to understand the molecular mobility over various transition point preferably using thermal mechanical analysis. A further understanding will tune the mechanical properties of the DeChNF films using honey as a plasticizer. Second, the microbiological activity will complement the current data on colour evolution, surface spoilage, and weight loss. Since DeChNF are novel nanofibrils in the chitin field, the study may be of interest for the development of sustainable post-harvest materials.

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