Extraction and Evaluation of Chitosan Enhanced by *Lippia Multiflora* Oil Essential on Postharvest of Tomato

Cissé Mohamed[1*], Tia Vama Etienne[2], N'guessan Amoin Elise[3]

Université Peleforo Gon coulibaly de Korhogo (Côte d'Ivoire)
*****Corresponding author at: BP 1328 Korhogo, Côte d'Ivoire.

***Abstract*—** *Influence of chitosan and Lippia multiflora (Lm) essential oil used singly or combined was studied on postharvest tomato. Chitosan with 89.31% of DDA and solubility in acetic acid at 97.15 % was extracted from shrimp exoskeletons. Three concentrations of chitosan extracted (0.25; 0.5 and 1%) containing or not L. multiflora oil were used on Rhizopus stolonifer growth in vitro and in situ condition. In vitro condition, antifungal activity of the chitosan and Lm oil against R. stolonifer was conducted on agar media inoculated with fungal spores. Coating containing 1% chitosan incorporated with Lm efficiently inhibited fungal proliferation at 100% after 10 days. The antifungal effect of two molecules was effective when they were associated. In situ condition tomatos were coated with different solution. Antifungal effect and chemical parameters (pH and titrable acidity) were evaluated. Combination of Lm and 1% chitosan delayed efficiently R. stolonifer radial growth (2.1 mm) compared to uncoated fruit (70.37 mm) after 10 days of storage. Chitosan at 1% with or not Lm significantly reduced weight loss. Though, pH and total acidity (TA) were not influenced by coating solution.*

***Keywords*— Chitosan, Lippia multiflora, essential oil, antifungal, Rhizopus stolonifer, tomato.**

I. INTRODUCTION

Tomato (*Lycopersicon esculentum)* is the one of most popular consumed vegetables in Côte d'Ivoire because it is use in the composition of many dishes. However, due to its high-water content, intrinsically is likely to deteriorate rapidly during the postharvest handling. Rot disease caused by *Rhizopus stolonifer* is the most destructive disease of tomato [1, 2]. *R. stolonifer* is a good colonizer of plant debris and infects harvest fruits, often destroying the entire contents of a box within a few days by hydrolysis with tissue-macerating ability [3]. Over the past years, synthetic fungicides have been used to control this microorganism. However, it has been shown that some compounds used in these fungicides have caused strain resistance, representing a potential risk for the environment and human health [4]. Thus, there is a worldwide trend to explore natural products in order to reduce the use of synthetic fungicides, and options such as chitosan and plant extract have been evaluated.

Chitosan is a natural nontoxic biopolymer derived from partial or total deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp, and crawfish. In recent years, applications of chitosan to the fields of agriculture have received considerable attention [5-9]. The antifungal effect of chitosan has been observed against several fungi and its activity depends on its deacetylation degree, molecular weight and concentration [10-13]. Chitosan coating maintained the physico-chemical properties of fruits during conservation [14, 15]. By cons, chitosan is not a fungicide but rather a fungistatic [10]. Its effectiveness against fungi can be improved by adding natural antimicrobial substances vegetable. Essential oil of *Lippia. multiflora* can be incorporated in chitosan solution in order to strengthen the coating formulation. Indeed, essential oil of *L. multiflora* has been reported to exhibit a fungicidal [16, 17], a bactericidal and an insecticidal activity [18]. It has also been used to protect many fruits against fungi [19].

Use of *L. multiflora* to strengthen chitosan action against *Rhizopus stolonifer* can be an alternative way to inhibit this strains development and reduce the chemical substances use in food preservation. The purpose of the present work was designed to evaluate the effect of chitosan and *L. multiflora* singly or incombined treatments on the growth of tomato rot pathogens as well as their effect on physicochemical quality of tomato during its postharvest conservation.

II. MATERIALS AND METHODS

2.1 Extraction of Essential oil (EO) of *Lippia multiflora*

Leaves of *Lippia multiflora* were collected in Dikodougou northern of Côte d'ivoire. Leaves were dried for 7 days protected away from the sun. After drying, 10 kg of leaves were used for the extraction of essential oil by steam distillation using a

hydro-distillation. The extraction lasted 3 hours. After extraction, the volume of EO was stored in hermetically sealed glass bottle with screw lid cover under refrigeration at 4°C.

2.2 Extraction of chitosan

The shrimp were obtained from Azaguiéon center of Côte d'ivoire. Samples were washed with distilled water before oven-dried for 24h at 40°C. Shrimps exoskeletons were then crushed using a grinder. For extraction of chitin and chitosan, the conventional chemical method was followed. Extraction was done following three major steps, i.e., demineralization, deproteination, and deacetylation.

2.2.1 Demineralization

Dry powdered carapaces were soaked in HCl (1N) for 5h with magnetic stirring to remove the minerals (mainly calciumcarbonate). The ratio of solid to solvent is 1:10 (w / v).The product obtained is washed with water distilled several times at pH neutral, then oven-dried at 35°C overnight.

2.2.2 Deproteinization

Proteins were removed by a basic treatment with sodium hydroxide. Product obtained after demineralization was treated with NaOH (2.5 N) at a ratio of 1: 10 (w / v) for 3 h at 100 °C. The mixture was then filtered and washed several times until neutrality. The chitin thus obtained is dried in an oven at 35 °C for 24 hours.

2.2.3 Deacetylation

The deacetylation process was carried out by adding 60% NaOH to sample according to a ratio of 1:10(W / V)and then boiled at 100°C for 2 h on a hot plate. The samples were then placed under the hood and cooled for 30 min at room temperature. Afterwards the samples were washed continuously with the 60% NaOH and filtered in order to retain the solid matter, which is the chitosan. The produced chitosan was then filtered and washed to remove residual soda until the pH of the wash water reaches neutrality and then baked at 35°C overnight.

2.3 Properties of Chitosan

2.3.1 Degree of deacetylation (DD)

It refers to the removal of acetyl group from the chain which is determined by potentiometric titration (Homogenous solution of chitosan was prepared using diluted HCl (0.010 mol/L) which was titrated against 0.1M NaOH (w/v). The end point is determined by the inflections of the pH values. Two inflections were mainly considered out of which first one corresponds to neutralization of HCl and second one neutralization of ammonium ions from chitosan. The difference between two points gives the amount of amino groups in the chitosan it was also referred as degree of deacetylation [20].

$$DD(\%) = 100 - \mathrm{DA}(\%)$$

DD represents Degree of Deacetylation and DA degree of Acetylation.

The **pH** measurement of chitosan solutions was carried out using pH meter with microprocessor

2.3.2 Loss on drying

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water mass loss was determined by drying the sample to constant weight and measuring the sample before and after drying. The water mass was the difference between the weights of the wet and oven dry samples ([21]. Loss on drying (%) = $((wet\ weight - dry\ weight)/\ dry\ weight)$ 100.

2.3.3 The solubility of chitosan

The solubility of chitosan extracted was determined according to Premasudha[21]. About 0.1g of chitosan powder sample was taken in centrifuge tube and dissolved in 10ml of 1% acetic acid and kept in incubated shaker (250 rpm) at 25ºC for 30 minutes. The solution was immersed in boiling water bath for 15 minutes and cooled to room temperature followed by centrifuge at 12,000 rpm for 7 minutes and the supernatant was discarded. The undissolved particles were thoroughly washed using distilled water by centrifuging the contents at 10,000rpm for 10 minutes and the supernatant was discarded. The

undissolved pellets were dried at 70ºC for 24hours. At the end the dried particles were weighed and the solubility percentage was calculated as:

Solubility (%) = (initial weight of tube + chitosan) - (final weight of tube + chitosan) X 100

2.4 Preparation of the chitosan-Essential oil emulsion

Chitosan solutions were prepared by dissolving chitosan(0.25, 0.5 and 1g) in distilled water (80 mL) containing of acetic acidat 1% (w/v) under agitation using a magnetic stirrer, incubated for 5h at room temperature. The pH of the solution was adjusted to 5.5 with NaOH (2%) and the solution was made up to 100mL with distilled water *L. multiflora* oil (at 0% and 0.5%) mixed with Tween 80(0.2%) was added to the different chitosan solutions. The mixture was homogenized using a mixer for 5 minutes to have an emulsifying solution. Solution with oils (Lm) in water were prepared and homogenized under the same conditions.

2.5 *In vitro* antifungal assay

3 uL of the inoculum of *R. stolonifer* containing 10^5 spores/mL were dropped at the center of Petri plates (9 cm diameter) containing PDA with different solution coating at $1mg \cdot mL^{-1}$. Plates were then incubated at 30°C and linear growth of tested fungi was measured when the control plates (PDA with distilled water) reached full growth and the average growth diameter was calculated. Each treatment was represented by 3 plates as replicates.

The fungicide index (%) was obtained by the formula:

Fungistatic index (%) = (1- (DS/DC)) x100, where Ds is the diameter of the growth zone in the test plates and Dc is the diameter of growth zone in the control plate.

2.6 *In situ* antifungal assay

Tomato fruit were collected from a regional market in Korhogo (Côte d'ivoire). Fruit were selected based on size and absence of physical injuries or disease infection. Fruit were disinfected with 1% (w/v) sodium hypochlorite for5 min then rinsed with distilled water and air-dried. The fruit were randomly distributed (10 per treatment). Identical lesions (4) were performed on the fruit with sterile nails before dipping individually in different coating solutions for 1 min and air-dried. The fruit were then sprayed with spore solutions of *R. stolonifer* (10^5 spores $/mL^{-1}$). Fruit were kept at room temperature. The mycelial growth was measured at 5 and 10 days after inoculation. Each treatment contained three replicates with 10 fruits per replicate and the experiment was repeated twice

2.7 Evaluation of the quality of Tomato

Weight loss was determined by daily weighing tomato with a balance (Precisa, Switzerland). Weight loss was expressed as a percentage of initial weight.

To determine chemical properties, thirty (30) grams of mango pulp were homogenized in 150 mL of distilled water using a blender for 2 min and then filtered. The pH was determined with a pH meter. Total acidity (TA) was determined on 10mL of homogenate pulp by automatic titration with 0.1N NaOH up to pH 8.1. The results were expressed as g citric acid equivalent per 100 g fresh weight.

III. STATISTICAL ANALYSIS

Experimental data were subjected to ANOVA analysis using Statistica 7. The overall least significant differences (Student's procedure, $p<0.05$) were calculated and used to detect significant differences among treatments. Each trial contained three replicates.

IV. RESULTS AND DISCUSSION

4.1 Properties of Chitosan

The properties of chitosan obtained from shrimp is showed in table1. The degree of deacetylation (DD) was 85.31%. DD of chitosan range from 30% to 95% [22]. It depends on the source of chitin, concentration of acid and alkaline used, time and temperature, etc but the concentration of NaOH influences the DD values the most [23]. The DD consider being an important parameter for the identification of chitosan stated that DD analysis was affected the type of analytical methods employed,

type of instruments used and the preparation of sample. DD denoted the removal of acetyl group from the long chain of chitin and it plays a substantial role in deciding the precise application of chitosan. It is an important parameter to be considered for physical and chemical properties of chitosan including solubility, adsorption, chemical reactivity covalent linking encapsulation and biodegradability.

TABLE 1
PROPERTIES OF CHITOSAN EXTRACTED FROM SHRIMP

Characteristic	Value
Degree of deacetylation (%)	85.31
pH	7.4
Loss on drying (%)	9.25
Solubility in acetic acid (%)	97.15

pH measured was 7.4. This value was in line with the earlier report of Premasudha [21] who reported the pH of chitosan obtained from shrimp. The pH value of chitosan plays a major role in functional properties of chitosan including antimicrobial, cytotoxicity and also indirectly influences the hydrophilicity and deacetylation ratio [21].

Present study reveals that, loss of moisture content (dry weight**)** in studied chitosan of shrimp was 9.25% of total weight. This result is near with the reports of Sneba et al.,[24], who explained the acceptable moisture content of chitosan powder should be <10% for commercial applications.

The results of chitosan solubility shown in Table 1 (97.15%) clearly reveals the high solubility nature of chitosan in 1% acetic acid aqueous solution. The solubility of chitosan is one of the important parameters for quality of chitosan, where higher solubility will produce a better chitosan. There are several critical factors affecting chitosan solubility including temperature and time of deacetylation, alkali concentration and prior treatments applied to chitin isolation, ratio of chitin to alkali solution and particle size [21]. The solubility, however, is controlled by the degree of deacetylation and it is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility [25]. Proportionally increase in solubility was observed with increasing deacetylation degree. Brine and Austin, [26] suggested that the incomplete removal of protein and acetyl group leads to lower solubility. Since solubility of chitosan depends on the removal of acetyl group from chitin, therefore the lower DD value could adversely interfere with the results.

4.2 *In vitro* antifungal assay

The antifungal effects of the different treatments on tomato postharvest are summarized in Table 2. Significant difference between treatments effects on *Rhizopus* inhibition was observed at different days recorded. After 2 days of storage, *R. stolonifer* reacted differently toward coating solution. Chitosan at different concentrations without EO significantly decreased *R. stolonifer* growth. This decrease improved when chitosan concentration improved. *R. stolonifer* inhibition improved to 32.49 at 86.86% when chitosan concentration passed to 0.25 at 1%. The percentage of strain inhibition was improved with the increase of chitosan concentration. Similar results were obtained by other authors [8, 27]. EO of *L. multiflora* alone affected fungal growth. Its controlled *R. stolonifer* at 89.41%. When *L. multiflora* wad added to chitosan coating at different concentration, inhibitory effect improved to reach 100%. The antifungal effect of *L.multiflora* was reported in literature. [16, 18]. After 6 days of storage, only 1% Chi + Lm maintained its efficacy at 100% and this efficacy endured after 10 days when the others treatments decreased or lost their efficacy. Chitosan at 0.5% containing Lm nevertheless allowed to inhibited *R. stolonifer* at 66.67 % after 10 days of storage. In view of these results, we could affirm that chitosan had no fungicide activity against *R. stolonifer*. It had bacteriostatic effect against this fungus. But when chitosan at high concentration was associated with Lm EO, the formulation became effective against *R. stolonifer* during 10 days and more. Fungistatic activity of chitosan has been also demonstrated by several authors. [2829]. Also, the strengthening of the antifungal activity of chitosan by addition of essential oil has been reported [30].

TABLE 2
ANTIFUNGAL ASSAY *IN VITRO*

	Inhibition (%)		
	2 Days	**6 Days**	**10 Days**
0,25%Chi	32,49c	0d	0c
0,5%Chi	60,85b	0d	0c
1%Chi	86,86a	40cb	0c
0,25%Chi+Lm	100a	24,70c	0c
0,5%Chi+Lm	100a	66,67b	66,67b
1%Chi+Lm	100 a	100a	100a
Lm	89.41a	85ab	61b

Values within a column with the same letter are not significantly different (p > 0.05).

4.3 *In situ* antifungal assay

Results (Fig.1) shows that fungi strains reacted differently toward coating solutions. After 5 days of storage, radial growth measured (45.07 mm) on uncoated tomato was significantly high than others treatment. It was followed by chitosan at 0.25% (17.80 mm). On the other land, Lm and chitosan (0.5% and 1%) singly or combined protected effectiveness tomato against *R. stolonifer*. After 10 days, all radial measured on fruits improved. But chitosan at 1% mixing with Lm presented the lowest radial measured (2.1 mm) following by chitosan (at 0.25 and 0.5%) incorporated by Lm. Chitosan and Lm singly were effective to protect tomato against *R. stolonifer*. When they were associated, the combination becomes better than individually effect. As in Antifungal assay *in vitro*, chitosan concentration played an important role in Lm oil fixing and protection of tomato against *R. stolonifera*. The results were illustrated by the figure3. Antifungal activity of coating was better when chitosan improved. Chitosan action on fruit protect against fungi was been demonstrated. Hernández-Lauzardo *et al.*,[34] demonstrated that chitosan at 2 $mg \cdot mL^{-1}$ was effective in reducing the percentage of infection and the severity index on peach, papaya and tomato fruit compared with those of non-treated control. As for essential oils, their effectiveness with or without chitosan has been demonstrated by Sivakumar and Bautista-Banos [35].

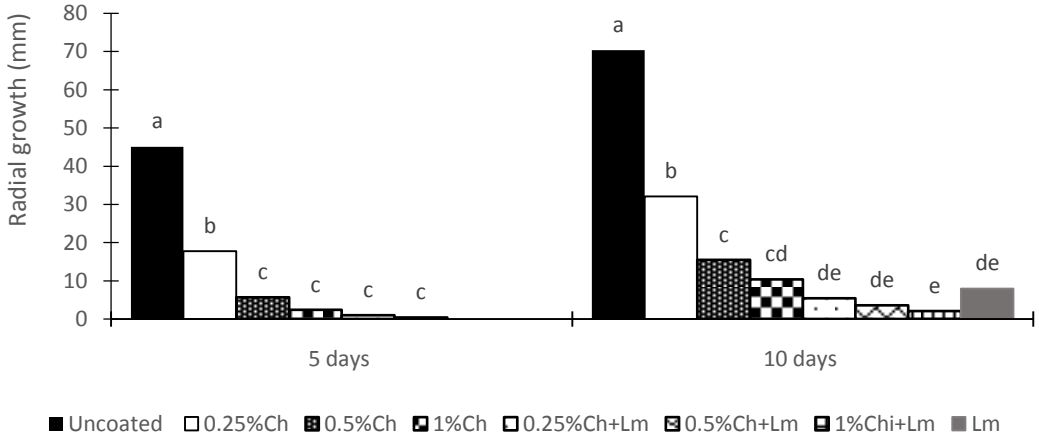

FIGURE 1: Effect of chitosan and Lm essential oil on *R. stolonifer* growth on tomato during storage

4.4 Evaluation of the quality of Tomato

Figure 2 shows weight loss during storage of uncoated tomato compared to coated fruit after 10 days of storage. Loss of weight of uncoated (90.58%) fruit was significantly greater than that of coated fruit. Coated fruits with chitosan presented low weight loss compared to fruits coated with Lm and uncoated. Low lowest was noticed with chitosan at 1% with or not EO. These results highlight a protective action of coating against moisture loss, which has also been reported by several authors [5, 8]. The reduction in water loss can be attributed to an additional barrier against diffusion through the stomata Incorporation of Lm EO into the coating solution did not have any significant effect on weight loss reduction.

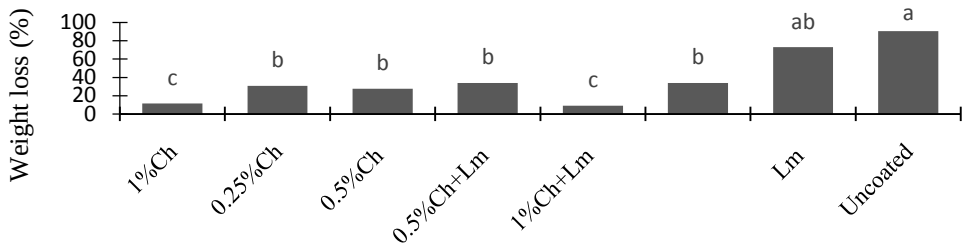

FIGURE 2: Weight loss of fruit during storage

4.5 Chemical composition change in fruit

The chemical composition of the fruit pulp is an important criteria needed for the evaluation of fruit quality. Normally, biochemical changes of tomato during ripening include aincrease of pH and Total acidity (TA). The changes in the chemical composition of tomato after 10 days of storage were studied (Table 3). There was not a significantly different change in pH observed between control fruit and coated fruit with different coating. Though, uncoated and fruit coated with Lm showed lowest pH values (4.46). Regarding TA, its is an important factor to be considered with respect to consumer acceptance. It is expected to increase during ripening [36]. Ours results showed that no significant difference between values of coated and uncoated tomato (Table 3). However fruit uncoated showed lesser change in acidity (0.44%) while 1%Chi + Lm indicated a lowest value (0.37%) after 10 days of storage.

TABLE 3
CHEMICAL COMPOSITION CHANGE IN FRUIT

	Ph	TA (%)
Uncoated	4.45a	0.44a
0,25%Chi	4.39a	0.4a
0,5%Chi	4.40a	0.40a
1%Chi	4. 32a	0.38a
0,25%Chi+Lm	4.38a	0.4a
0,5%Chi+Lm	4.41a	0.39a
1%Chi+Lm	4.30a	0.37a
Lm	4.46a	0.4a

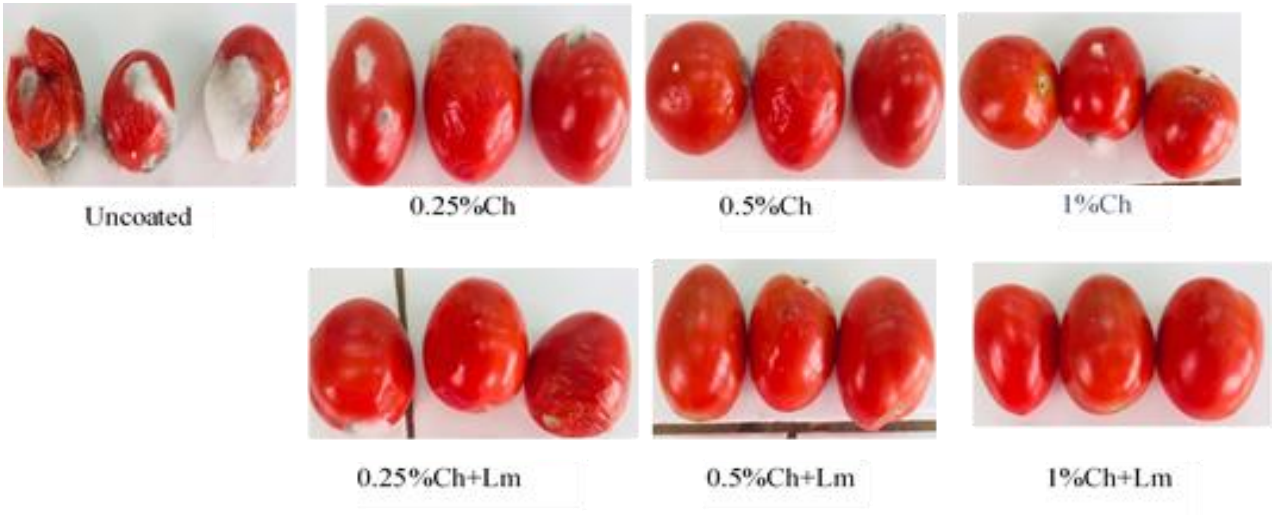

FIGURE 3: Antifungal activity of different coatings against *R. Stolonifer* on Tomato fruit.

V. CONCLUSION

This study demonstrated the effectiveness of chitosan coating containing Lm essential oil in postharvest conservation of tomato. Chitosan and *L. multiflora* essential oil singly used had antifungal activity which was been strengthened by mixing the two. A chitosan concentration at 1% containing *L. multiflora* essential oil was sufficiently effective against *R. stolonifer* contamination without altering fruit quality.Use of chitosan–Lm could thus be an effective approach in the preservation of tropical fruit, an alternative in limiting synthetic pesticide use.

REFERENCES

[1] Petrasch, S., Silva, C.J., Mesquida-Pesci, S.D., Gallegos, K., van den Abeele, C., Papin, V., Fernandez-Acero, F.J., Knapp, S.J., Blanco-Ulate, B Infection Strategies Deployed by Botrytis cinerea, Fusarium acuminatum, and Rhizopus stolonifer as a Function of Tomato Fruit Ripening Stage. Frontiers in Plant Science, 2019. **10**(223).

[2] Bautista-Baños, S., E. Bosquez-Molina, and L.L. Barrera-Necha, Chapter 1 - Rhizopus stolonifer (Soft Rot), in Postharvest Decay, S. Bautista-Baños, Editor. 2014, Academic Press: San Diego. p. 1-44.

[3] Bautista-Baños, S., Velázquez-del Valle, M.G., Hernández-Lauzardo, A.N., E., A.-B. The Rhizopus stolonifer-Tomato interaction, in: Plant-microbe interaction, Ait-Barka E., Clément C. (Eds.), . Res. Signpost, Kerala,India, 2008.

[4] Adaskaveg, J.E., H. Förster, and N.F. Sommer, Principles of postharvest pathology and management of decays of edible horticultural crops, in: Kader A. (Ed.). Postharvest Technology of Horticultural Crops, Univ. California, Oakland, USA, 2002.

[5] Betchem, G., N.A.N. Johnson, and Y. Wang, The application of chitosan in the control of post-harvest diseases: a review. Journal of Plant Diseases and Protection, 2019.

[6] Xoca-Orozco, L.-Á., Aguilera-Aguirre, S., Vega-Arreguín, J., Acevedo-Hernández, G., Tovar-Pérez, E., Stoll, A., Herrera-Estrella, L., Chacón-López, A. Activation of the phenylpropanoid biosynthesis pathway reveals a novel action mechanism of the elicitor effect of chitosan on avocado fruit epicarp. Food Research International, 2019. **121**: p. 586-592.

[7] Rendina, N., Nuzzaci, M., Scopa, A., Cuypers, A., Sofo, A.Chitosan-elicited defense responses in Cucumber mosaic virus (CMV)-infected tomato plants. Journal of Plant Physiology, 2019. **234-235**: p. 9-17.

[8] Cissé, M., Polidori, J., Montet, D., Loiseau, G., Ducamp-Collin, M.N. Preservation of mango quality by using functional chitosan-lactoperoxidase systems coatings. Postharvest Biology and Technology, 2015. **101**: p. 10-14.

[9] Shamshina, J.L., T. Oldham, and R.D. Rogers, Applications of Chitin in Agriculture, in Sustainable Agriculture Reviews 36: Chitin and Chitosan: Applications in Food, Agriculture, Pharmacy, Medicine and Wastewater Treatment, G. Crini and E. Lichtfouse, Editors. 2019, Springer International Publishing: Cham. p. 125-146.

[10] Mohamed, C., Clementine, K.A., Didier, M., Gérard, L., Marie Noëlle, D.-C.Antimicrobial and physical properties of edible chitosan films enhanced by lactoperoxidase system. Food Hydrocolloids, 2013. **30**(2): p. 576-580.

[11] Chang, A.K.T., Frias, R.R., Alvarez, L.V., Bigol, U.G., Guzman, J.P.M.D.Comparative antibacterial activity of commercial chitosan and chitosan extracted from Auricularia sp. Biocatalysis and Agricultural Biotechnology, 2019. **17**: p. 189-195.

[12] Zhuang, C., Y. Zhong, and Y. Zhao, Effect of deacetylation degree on properties of Chitosan films using electrostatic spraying technique. Food Control, 2019. **97**: p. 25-31.

[13] Hosseinnejad, M. and S.M. Jafari, Evaluation of different factors affecting antimicrobial properties of chitosan. International Journal of Biological Macromolecules, 2016. **85**: p. 467-475.

[14] Kerch, G., Chitosan films and coatings prevent losses of fresh fruit nutritional quality: A review. Trends in Food Science & Technology, 2015. **46**(2, Part A): p. 159-166.

[15] Vieira, J.M., Flores-López, M.L., de Rodríguez, D.J., Sousa, M.C., Vicente, A.A., Martins, J.T.Effect of chitosan–Aloe vera coating on postharvest quality of blueberry (Vaccinium corymbosum) fruit. Postharvest Biology and Technology, 2016. **116**: p. 88-97.

[16] Bassolé, I.H.N. and H.R. Juliani, Essential Oils in Combination and Their Antimicrobial Properties Molecules, 2012. **17**: p. 3989-4006.

[17] Goly, C., Soro, Y., Kassi, B., Dadié, A., Soro, S., Dje, M.Antifungal activities of the essential oil extracted from the tea of savanna (Lippia multiflora) in Côte d'Ivoire. Int. J. Biol. Chem. Sci., 2015. **9**(1): p. 24-34.

[18] Ilboudo, Z., et al., Optimizing the use of essential oils to protect stored cowpeas from Callosobruchus maculatus (Coleoptera: Bruchinae) damage. African Entomology, 2015. **23**(1): p. 94-100.

[19] Kunle, O., F and O. Egharevba H., Essential oil of Lippia multiflora Moldenke: A review Journal of Applied Pharmaceutical Science, 2012. **2**(1): p. 15-23.

[20] Yongqin, Z., et al., Determination of the degree of deacetylation of chitosan by potentiometric titration preceded by enzymatic pretreatment. Carbohydrate Polymers,2011. **83**(2): p. 813-817.

[21] Premasudha P., P. Vanathi, and M. Abirami, Extraction and Characterization of Chitosan from Crustacean Waste: A Constructive Waste Management Approac. . International Journal of Agriculture and Crop Sciences., 2017.

[22] Di Martino, A., Liliana, L., Alberto, R., Giuseppe, S., Marcella, T., Vincenzo, D. Electrospun scaffolds for bone tissue engineering. MUSCULOSKELETAL SURGERY, 2011. **95**(2): p. 69-80.

[23] To Oanh Truong, Hausler, R., Monette, F., Niquette, P.Valorisation des résidus industriels de pêche s pour la transformation de chitosane par technique hydrothermo-chimique. Journal of Water Science 2007. **20**(3): p. 253-262.

[24] Sneha, P., Jayan, A., Sasikumar, C.S., Cherian, S.M.Extraction and purification of chitosan from chitin isolated from sea prawn (Fenneropenaeus indicus)Asian Journal of Pharmaceutical and Clinical Research, 2014. **7**(4): p. 201 - 204.

[25] No, H.K. and S.P. Meyers, Preparation and characterization of chitin and chitosan. A reviewJournal of aquatic food product technology, 1995. **4**(2): p. 27- 52.

[26] Brine, C.J. and P.R. Austin, Chitin variability with species and method of preparation. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 1981. **69**(2): p. 283 - 286.

[27] García-Rincón, J., Vega-Pérez, J., Guerra-Sánchez, M.G., Hernández-Lauzardo, A.N., Peña-Díaz, A., Valle, M.G.V.-D. Effect of chitosan on growth and plasma membrane properties of Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. Pesticide Biochemistry and Physiology, 2010. **97**(3): p. 275-278.

[28] Plascencia-Jatomea, M., Viniegra, G., Olayo, R., Castillo-Ortega, M.M., Shirai, K.Effect of Chitosan and Temperature on Spore Germination of Aspergillus niger. Macromolecular Bioscience, 2003. **3**(10): p. 582-586.

[29] Muzzarelli, R.A.A., Muzzarelli, C., Tarsi, R., Miliani, M., Gabbanelli, F., Cartolari, M. Fungistatic Activity of Modified Chitosans against Saprolegnia parasitica. Biomacromolecules 2001. **2**(1): p. 165-169

[30] Perdones, Á., Escriche, I., Chiralt, A., Vargas, M. Effect of chitosan–lemon essential oil coatings on volatile profile of strawberries during storage. Food Chemistry, 2016. **197**: p. 979-986.

[31] Noshirvani, N., Ghanbarzadeh, B., Gardrat, C., Rezaei, M., R, Hashemi, M., Le Coz, C., Coma. Cinnamon and ginger essential oils to improve antifungal, physical and mechanical properties of chitosan-carboxymethyl cellulose films. Food Hydrocolloids, 2017. **70**: p. 36-45.

[32] Avila-Sosa, R., Palou, E., Munguia, M.T.J., Nevarez-Moorillon, G.V., Cruz, A.R.N., Lopez-Malo, A. Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films. International Journal of Food Microbiology, 2012. **153**(1-2): p. 66-72.

[33] Hafsa, J., Smach, M., Ben Khedher, M.R., Charfeddine, B., Limem, K., Majdoub, H., Rouatbi, S. Physical, antioxidant and antimicrobial properties of chitosan films containing Eucalyptus globulus essential oil. LWT- Food Science and Technology, 2016. **68**: p. 356-364.

[34] Hernández-Lauzardo, A., Velázquez-del Valle, M., Veranza-Castelán, L., Melo-Giorgana, G., Guerra-Sánchez, M.Effect of chitosan on three isolates of Rhizopus stolonifer obtained from peach, papaya and tomato. Fruits, 2010. **65**(4): p. 245-253.

[35] Sivakumar, D. and S. Bautista-Baños, Review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop Protection, 2014. **64**: p. 27-37.

[36] Taşdelen, Ö. and L. Bayindirli, controlled atmosphere storage and edible coating effects on storage life and quality of tomatoes. Journal of Food Processing and Preservation, 1998. **22**(4): p. 303-320.