Chitosan-based Multi-layer Coating to Maintain the Soybean Seed Quality During Storage

Christina Winarti[1]*[1]*, *Anna* Sulistyaningrum[1], *Eka* Rahayu[2], *and Mulyana* Hadipernata[1]

[1] Research Center for Agroindustry – National Research and Innovation Agency, Jln. Raya Bogor, KM 47, Cibinong, Bogor

[2] Research Center for Food Technology and Processing - National Research and Innovation Agency, Playen, Gunung Kidul, Yogyakarta

Abstract. Soybean seeds deteriorate quickly in storage due to their morphological characteristics and chemical composition. One technique needed to extend the shelf life of seeds is coating. The research aims to determine the effect of chitosan-based multi-layer coating formula by adding Zn nanoparticles to soybean seeds during storage. The treatments were control, chitosan nano, and non-nano coating, with and without wax-coated seed samples packed in plastic and stored at room temperature for five months. The three times replicates were applied; the observation was conducted every month. The parameter observed were moisture content, malondialdehyde, Electrical Conductivity (EC), vigor, and germination rate. The results showed that the chitosan-based multi-layer coating treatment gave good seed quality results during storage. The water content could be maintained low, while the MDA levels and Electrical Conductivity were lower than the control. Observation on vigor and germination of coated seed after the fourth month of storage indicated that the vigor and germination rate was slightly decreasing. Still, after the fifth month, the vigor starts to increase again. Implementing nano seed coating enriched with ZnONP and zeolite might help the seed grow healthy while planted in the field.

1 Introduction

In Indonesia, soybean is one of the strategic agricultural commodities; however, soybean is still mostly imported. Data showed that about 86.95% of soybean consumed in Indonesia is imported [1], mostly from the USA. One factor that hampers the soybean production is the availability of quality seeds. The quality of the seeds greatly affects the yield harvested. Soybean seed also has rapid deterioration during storage. Due to its high lipid content and polyunsaturated linolenic and linoleic acids, soybean seed has a short shelf life [2]. Moreover, the biochemical changes of soybean seeds will affect the seed quality and viability. The changes in physicochemical properties during storage are as follows, the content of carbohydrates, protein, lipids, amino acids, and free fatty acids [3]. Indonesia's tropical climate conditions, with high temperatures and humidity, can also trigger the rate of deterioration of soybean seeds in storage. The quality of seeds cannot be improved during

[*]Corresponding author : christina.winarti@yahoo.com

storage, but due to controlled temperature and relative humidity the quality may be preserved [4],[5]

Seed coating is one of the efforts that can be carried out to maintain seed vigor during storage. Seed coating is a seed coating technology using certain substances such as growth regulators, micronutrients, microbes, fungicides, or antioxidants. Developing seed coating technology has been widely carried out, especially on soybean seeds, to protect plants from pests and diseases and prevent considerable economic losses [6]. Seed coating can control and increase germination, protect seeds from pests and plant diseases that attack during seedbeds and early growing seasons, increase seedling vigor, and reduce the use of pesticides when planting. One important natural polymer coating is chitosan, the second largest biopolymer after cellulose. Chitosan (CS) is a carbohydrate biopolymer derived from chitin's deacetylation, mostly found in crustacean shells. Although CS is an excellent plant growth accelerator, but it has limitation in agricultural application due to its insolubility in water and soluble only in acidic solutions. The formulation into a nanosuspension is one of good and promising alternative to solve the poor aqueous solubility.

Chitosan nanoparticle (CSNPs) adsorption on the seed surface is significantly higher than CS at the same concentration [7]. Other research stated that combining the properties of hydrophilic biopolymers such as chitosan and hydrophobic characteristics such as lipids and beeswax to be composite bilayer or emulsion-based coatings and films is also promising alternative seed coating [8]. The application of beeswax and 2% chitosan significantly reduced physiological weight loss and disease incidence, while maintaining fruit firmness and extended shelf life compared to untreated controls [9].

The incorporation of other materials might increase its effectiveness, including the use of nano zinc [10],[11]. ZnO nanoparticles (ZnO NPs) exhibit extensive antifungal and antibacterial action and can be applied to control the spread and infection caused by various plant pathogens [12]. Applying ZnO NP 500 mg/L increases protection from cadmium toxicity in corn (*Zea mays* L.) [13]. String bean plants (*Cyamopsis tetragonoloba* L.) fed with ZnO NPs showed significant increases in biomass, shoot and root length, root area, and chlorophyll content. Research stated that regardless of the particle size, ZnO based treatments increased the ratio of soybean seed germination, seedling root growth and shoots development, whereas zinc O4 suppressed them [11]. The layer-by-layer coating using zeolite between other coating materials might improve the coating performance. Zeolite application positively affects soybean's seed growth rate and improves soybean's agronomic attributes [14]. Applying several combinations of material in chitosan is expected to maintain soybean seed quality during storage.

2. Material and Methods

2.1 Material

The raw material used was soybean seeds of the Detap variety originating from UPBS Balai Penelitian Kacang dan Umbi/Indonesian Legume and Tuber Institute, Malang, East java, as certified seeds. The chemicals were coating materials (chitosan, beeswax, and carnauba wax), emulsifiers (Tween 80, triethanolamine), oleic acid, ascorbic acid, sodium tripolyphosphate, glacial acetic acid, commercial ZnO NPs (colloidal dispersion of zinc oxide nanoparticles) and zeolite powder. Almost all material were analytical grade, except ascorbic acid, ZnO and zeolite powder. and other chemicals for analysis. The equipment used included process equipment and analytical equipment. Process equipment included a magnetic stirrer, high-speed blender, and Ultra-turrax homogenizer, while analytical equipment included a particle size analyzer (PSA), SEM, glassware, and others.

2.2 Seed coating formulation

The coating formula that will be used was a formula that has been produced by ICAPRD and modified the coating formula, namely nano-zinc oxide (ZnO NP) as micro fertilizer enrichment. The coating material consisted of nano and non-nano structures, either chitosan or beeswax base. The non-nanostructure form of chitosan was prepared by dissolving chitosan (1%) with acetic acid (1%), then stirred using a magnetic stirrer until homogenously. Nano-chitosan structures were obtained through the ionic gelation process of chitosan with sodium tripolyphosphate (Na-TPP). Chitosan with concentration of 0.3% was dissolved in 1% of acetic acid, followed by ionic gelation process that was carried out by adding with 0.2% of Na-TPP and stirred thoroughly using a magnetic stirrer. The nano-chitosan particles synthetized; it was separated by centrifugation and stored for further use as a coating formula. Enrichment materials were zeolite and ZnO NPs to improve the performance of coating materials. The treated ZnO NPs were added to the chitosan dispersion formula. The percentage of ZnO NPs added is about 2 % to the weight of chitosan. The incorporation of ZnO NPs followed the previously developed methods [15]. After the chitosan dispersed well, glycerol and ZnO NPs 2 % w/w of the chitosan) were incorporated, followed by subsequent stirring for 5 min agitation with Ultra-Turrax (15,000 rpm).

The nano-emulsification process to prepare the nano wax emulsion was carried a using an Ultra-turrax homogenizer. Beeswax (1.5%) and carnauba wax (1.5%) were melted, then emulsified into hot water (~90°C) with the addition of Tween 80 (3%) emulsifier and oleic acid (1%). Emulsification was established while homogenised using an Ultra-turrax homogenizer at a 11,000 rpm for 5 min. Furthermore, the wax emulsion coating consisted of several materials such as beeswax (6%), triethanolamine (TEA, 2%), oleic acid (1%). All materials were prepared using a high-speed blender at the highest speed level until homogenous. The coating materials were characterized using a particle size analyser to determine the emulsion size and poly-dispersity index.

2.3 Seed coating application

Seed coating was carried out using a seed coater equipment through a spraying process. The coating was conducted layer-by-layer (multi-layers) techniques. The soybean seed samples used was 1 kg each and every treatment were done in triplicate. The treatment in this study was as follows: (1) control (no coating); (2) chitosan; (3) nano chitosan; (4) chitosan + wax; (5) nano chitosan + nano wax.

The coating process used seed coater equipment. The chamber containing the seeds was rotated on its axis. Then, the coating materials were sprayed on the surface of the seeds using a syringe with the order of coating material chitosan or nano chitosan formula (8 ml), followed by zeolite powder (4 g), for treatments (4) and (5) followed by wax formula (8 ml) for each kg of soybean seeds, so that a multi-layer coating was formed on the surface of the seeds. The chitosan-based formula without wax was carried out in the same procedure, with only two layers, namely chitosan and zeolite. Each spraying was carried out for 15 seconds, and then the seeds were allowed to rotate for 30 seconds with drying using compressed air. Drying with compressed air was carried out at the end of the coating for 2 minutes. The coated seeds were dried in an open room for 30 minutes, packed with airtight PP plastic, and stored at room temperature (28 - 30°C) for five months. Coated seeds were observed in moisture content, Electrical Conductivity, MDA, vigor, germination, and surface structure (SEM).

2.4 Coated seed observation

The germination and vigor index were observed by planting soybean seeds in rolled paper planting media through the rolled paper test method. The paper was moistened with distilled water, and then 50 seeds per repetition (3 repetitions each) were sown (seeds were arranged in a zigzag position). Pre-sown seeds, covered with moistened paper and neatly rolled up. The roll of paper containing the seeds was put in a plastic bag and given a repeat name or code. The rolls of paper were stored at room temperature; then, observations were made. Observations were carried out according to the schedule set by ISTA [16], namely for soybean seeds, the first count was carried out on the 5th day (I), and the final count was carried out on the 8th day (II). The measurement of seed germination (%) was calculated based on the percentage of the number of normal sprouts at the first count (5 HST) and the second count (7 HST) compared to the total number of seeds planted [16]. Germination of seeds was calculated using the following formula:

$$Germination = \frac{\text{Number of normal sprout I+II}}{\text{Number of total seed}} \tag{1}$$

The vigor index was calculated based on the percentage of normal sprouts at first count divided by the number of seeds planted in the germination test using the following formula:

$$\text{Vigor index: } = \frac{\sum \quad Normals\,prout\ I}{\sum No.of\ planted\ seed} \tag{2}$$

2.4.1 Malondialdehyde analysis

Fine ground samples were added with 5.0 mL of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 20 minutes at 4 °C. Then, 2 ml of supernatant was mixed with 0.67% thiobarbituric acid (TBA) in a ratio of 1: 1 (v/v). The mixture was heated to 100 °C for 20 min and then cooled immediately. After centrifugation at 10,000 rpm for 10 minutes, the absorbance of the supernatant was measured at 450, 532, and 600 nm using a UV-2600 spectrophotometer. The MDA concentration was calculated using the formula:

$$\text{MDA} = 6.45 \times (\text{A } 532 - \text{A } 600) - 0.56 \times \text{A } 450. \tag{3}$$

2.4.2 Electrical Conductivity (EC)

Soybean seeds were ground, then soaked in 100 ml aqua bides in an Erlenmeyer and left for 24 hours. The electrical conductivity of soaked samples was measured using an electrical conductivity meter. The EC was calculated by subtracting EC samples and EC aqua bides (blank).

2.4.3 Scanning Electron Microscope

Sampel of soybean coated seed were peeled its outer skin and cut cross sectionally using sharp cutter and put at the specimen holder, and then put at the sample holder of the FESEM (Field Emission SEM) Thermo Scientific Quattro S completed with EDS Detector. Samples were scanned its cross section and surface part with magnification of 1000x.

3 Result and Discussion

3.1 Particle size of coating material

The results of measuring the emulsion droplet using a Particle Size Analyzer (PSA) showed that the particle size of the emulsion gives a small value, ranging from 505.6 – 839.6 nm for chitosan emulsion, while the wax emulsion is between 121.9 and 126.42 nm. Chitosan is biopolymer derived from crustacean chitin. The chitosan polymeric dispersion consists of several active material namely ZnO NPs. By definition, polymeric nanoparticles (NPs) are particles having the particle size range from 1 to 1000 nm; and it can be incorporated with active compounds entrapped within or surface-adsorbed onto the polymeric core [17].

The cumulative particle size distribution at 10%, 50%, and 90% showed that the four coating formulas provide a normal distribution pattern (Table 1). Meanwhile, based on the polydispersity index showed that wax-based nano emulsion offers a better level of uniformity than chitosan emulsion.

Table 1. The average particle size distribution and polydispersity index of the emulsion of the coating formula

Formula	Average particle size (nm)	Particle size distribution cumulative (nm)			Polydispersity index
		10%	50%	90%	
Nano-chitosan 0,3%+ ZnONP	505.60	262.70	530.84	889.37	0.630
Chitosan 1% + ZnONP	639.60	365.1	622.14	911.21	0.659
Beeswax nano-emulsion 3%	121.90	47.90	161.9	565.9	0.400
Beeswax emulsion	126.42	61.70	115.00	213.000	0.445

3.2 Moisture, Protein, Lipid Content

During storage, there is a tendency of an increase of moisture content, but the protein and lipid is decreasing (Table 2). Some changes in physicochemical properties occurred, such as the carbohydrates, proteins, lipids, amino acids and, free fatty acids content [3]. The study uses zeolite as a coating material between the coating solution and soybean. Zeolite will slow down the process of absorbing moisture into the surface of the seeds because zeolite functions as a moisture absorber. Zeolite is a type of mineral rock widely used in agriculture because of its excellent cation exchange capacity; thus, it can be used to improve soil conditions and as a moisture trapper. Applying zeolite as a desiccant (water vapor trapper) has been investigated before in soybean storage, and it is known that its absorption capacity is equivalent to silica gel [18].

Table 2. Changes of moisture, protein and lipid contend before and after storage

Treatments	Moisture (%)		Protein (%)		Lipid (%)	
	Before	After	Before	After	Before	After
Control	8.64±0.02[a]	9.05±0.09[a]	40.15±0.26[a]	36.89±1.14[b]	18.91±0.16[b]	10.57±0.03[c]
Chitosan	8.49±0.13[b]	8.78±0.12[ab]	40.10±0.20[a]	36.67±0.66[c]	18.66±0.06[c]	10.44±0.48[bc]
Chitosan NPs	8.55±0.12[b]	8.63±0.06[ab]	39.95±0.14[a]	37.25±0.04[d]	18.29±0.16[d]	11.51±0.05[a]
Chitosan + wax	8.68±0.15[a]	8.43±0.32[b]	40.22±0.20[a]	38.44±0.28[a]	19.04±0.07[ab]	11.15±0.47[ab]
Nano chitosan + nano wax	8.32±0.03[c]	8.41±0.02[b]	40.80±0.45[a]	37.12±0.90[b]	19.16±0.04[a]	10.58±0.06[bc]

Note: *Mean values in each column with the same letter are not significantly different (p = 5%)*

3.3 Malondialdehyde (MDA)

From the treatment tried, at the end of storage, it turned out that the nano-sized coating formula, namely nano-chitosan + nano wax coating treatment, showed lower MDA levels than other treatments. The highest MDA level is control treatment. However, all samples are statistically not significant (Table 3). The result showed that after five months of storage, all samples experienced the breakdown of protein and lipids that will produce toxic components. Accumulating toxic compounds during storage with low moisture content, respiration, and reduced enzyme activity causes the accumulation of toxic compounds that reduce seed viability. At this stage, disruption of the mitochondrial membrane increases respiration due to decreased energy production per substrate, besides a reduction in electron transport efficiency [19].

Table 3. Average MDA level of soybean-coated seed during storage

Treatment	MDA					
	0	1	2	3	4	5
Control	0.41±0.0^{a}	0.48±0.02^{a}	0.55±0.0^{a}	0.62±0.01^{a}	0.63±0.0^{a}	0.64±0.29^{a}
Chitosan	0.410.0±^{a}	0.43±0.03^{b}	0.48±0.04^{b}	0.53±0.06^{b}	0.58±0.04^{ab}	0.60±0.23^{ab}
Chitosan NPs	0.41±0.0^{a}	0.41±0.02^{b}	0.46±0.03^{b}	0.49±0.02^{b}	0.59±0.03^{ab}	0.60±0.25^{ab}
Chitosan + wax	0.41±0.0^{a}	0.44±0.01^{b}	0.47±0.03^{b}	0.50±0.01^{b}	0.60±0.03^{ab}	0.60±0.26^{ab}
Nano-chitosan + nano wax	0.41±0.0^{a}	0.41±0.02^{b}	0.45±0.05^{b}	0.48±0.04^{b}	0.56±0.05^{b}	0.57±0.24^{b}

Note: *Mean values in each column with the same letter are not significantly different (p = 5%)*

3.4 Electrical Conductivity (EC)

Table 4. Average of electrical conductivity level of soybean coated seed during storage

Treatment	Electrical Conductivity (µS $cm^{-1}g^{-1}$)					
	0	1	2	3	4	5
Control	39.41±0.0^{a}	45.38±5.50^{a}	47.11±5.50^{a}	48.83±4.34^{a}	50.27±1.30^{a}	51.47±1.40^{a}
Chitosan	39.41±0.0^{a}	42.75±2.53^{a}	44.26±2.53^{a}	45.79±1.03^{a}	47.79±3.32^{a}	50.09±1.34^{a}
Chitosan NPs	39.41±0.0^{a}	41.76±5.01^{a}	44.57±5.01^{a}	47.38±3.41^{a}	46.81±$^{a1.48b}$	50.71±1.70^{a}
Chitosan + wax	39.41±0.0^{a}	42.67±2.95^{a}	43.75±2.95^{a}	44.71±1.96^{a}	44.0±31.96^{b}	51.33±2.06^{a}
Nano-chitosan + nano wax	39.41±0.0^{a}	43.54±2.71^{a}	44.97±2.71^{a}	46.40±2.74^{a}	48.76±0.68^{a}	51.83±1.37^{a}

Note: *Mean values in each column with the same letter are not significantly different (p = 5%)*

Measurement of EC showed that the longer the storage period, the higher the EC (Table 4). The EC of control tends to be higher than that of coated seeds. However, it is statistically insignificant, especially at the end of storage, indicating seed compounds' leakage from the seed membrane. Decreased cell membrane integrity resulted in the release of compounds from the seed, such as sugar, amino acid, fatty acid, etc., which was observed based on EC. The results differ slightly from the previous research in which the EC of control is significantly higher than that of coated seed [20]. The different soybean varieties, treatments, and coating formulas might affect the performance of coated seeds. Previous research reported that the value of EC and free fatty acids in seeds would increase during storage [3]. Soybean seeds in the group have a high vigor since they have an EC value of < 80 µS $cm^{-1}g^{-}$

[1] [21]. The fewer leaks in the seed bath solution indicated that the seeds have a high vigor [22].

3.5 Vigor and germination

The vigor and germination of soybean seeds have decreased after being stored for five months at room temperature (Figure 1 and figure 2). There is a gradual decrease in control (no coating) until the end of storage. However, the coated seed indicates a different pattern, especially for nano-coating, in which after five months the vigor increases (Figure 1). The coating formula can have a negative impact, which causes radicular to be challenging to penetrate the coating material so that the vigor index of the seed decreases, and the time to grow becomes more extended than that without the coating. The Detap seed variety used in this research showed that after five months (150 days) of storage, the vigor ranges from 66 – 76.67%, and the germination is 76.66 – 84.44%. These results are agreed to other research showing that soybean seeds' germination stored ambient temperature, with uncontrolled environment condition at 180 and 240 days of storage was 69 and 55%, respectively [23]. However, compared to other research with different local soybean varieties, namely the Biosoy variety, it is much lower. Chitosan-based coating formula only without wax tends to have better vigor and germination. Moreover, the addition of ZnONPs seems do not have significant effect on vigor and germination. Nanoparticles of ZnO at a concentration exhibited neutral or beneficial effects on seed germination in soybean seeds and inhibitory effects with higher concentrations, highlighting the dose dependent pattern [24]. Several studies reported that in the form of nanoparticles, chitosan coatings could better maintain chili seed quality [25] and wheat [7] compared to original/non-nano, in which CSNPs, in lower concentrations, promote seed germination and seedling growth of wheat than CS. Treatment of chitosan-based nano-coating is considered to be able to maintain the germination of soybean seeds during the storage period if the initial conditions of soybean seeds are of good and optimum quality [20].

Moreover, the degree and speed of decline in seed quality during storage depend strongly, besides storage conditions, on plant species stored and initial seed quality [26]. The Detap seed variety used in this research is certified soybean seed released (84% germination) from Source Seed Management Unit (UPBS) ILETRI, with the expired seed certification last on December 2022. Meanwhile, the research starts on October 2022 to April 2023.

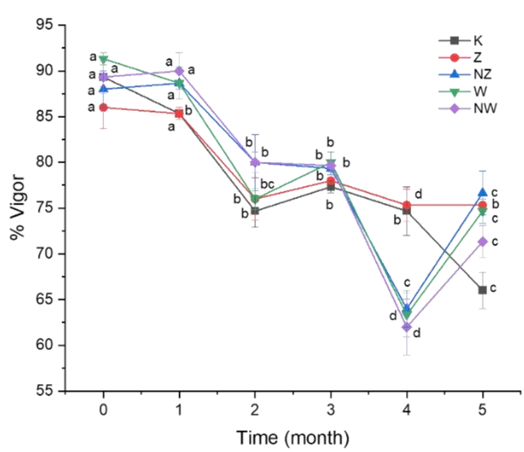

Fig 1. Effect of coating on the vigor of soybean seed during storage

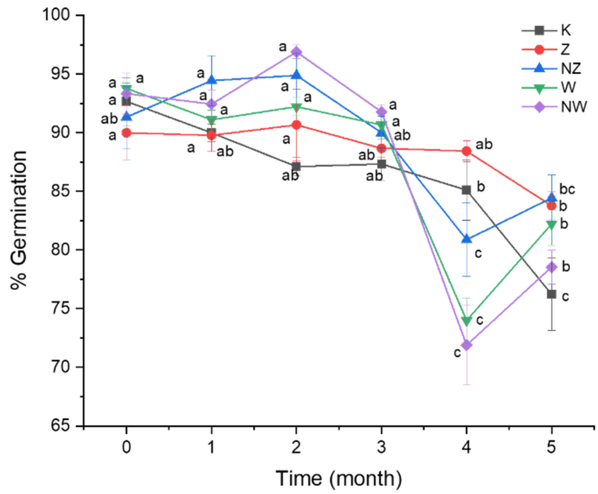

Fig2. Effect of coating on the germination of soybean seed during storage

It is expected that when applied in the field, the nano seed coating enriched with ZnO NPs and zeolite might help the seed grow healthy. Coating of soybean seeds had a different effect depending on the study site (laboratory, greenhouse, field) [27]. The bulk forms of NPs may penetrate the seed coat in smaller qualities and impact the seed quality. The retention of NPs on seed coat is likely more in the case of bulk forms and coating; thus, the form may be more effective in protection against a pathogen in storage and the field; similar findings were reported in the previous study [27].

3.5. Surface morphology

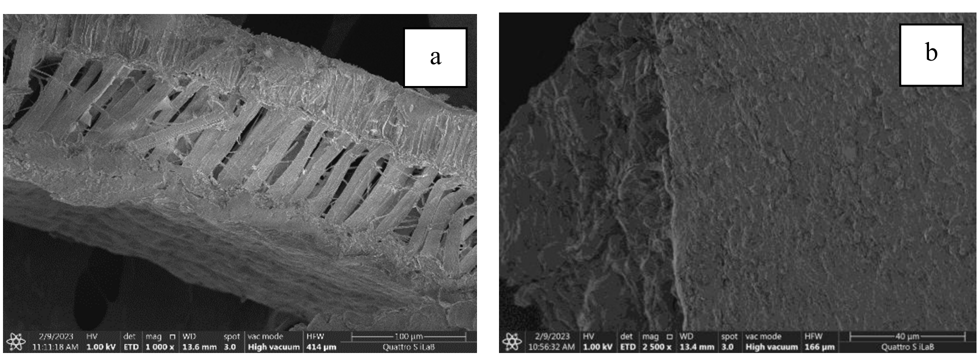

Fig 3. Morphology of chitosan base coated soybean seed; (a) cross section; and (b) outer surface

The morphological structures of coated and non-coated soybean seeds with cross-sections and outer surfaces are shown in Figure 3 and Figure 4. From the picture, it can be seen that the surface structure of soybeans without coating (control) looks more compact. In contrast, coated soybeans look more tenuous due to coating treatment using chitosan emulsion (8 ml/kg) that hydrates the soybean skin's surface. The chunky surface of chitosan-coated soybean seed is due to its excellent film-forming property of chitosan, making it easy to form a semipermeable film on the seed surface, which can maintain the seed moisture and absorb the soil moisture. Thus it can promote seed germination [28]. The surface seed will affect the imbibition process and the capacity of water absorption by the embryonic axis.

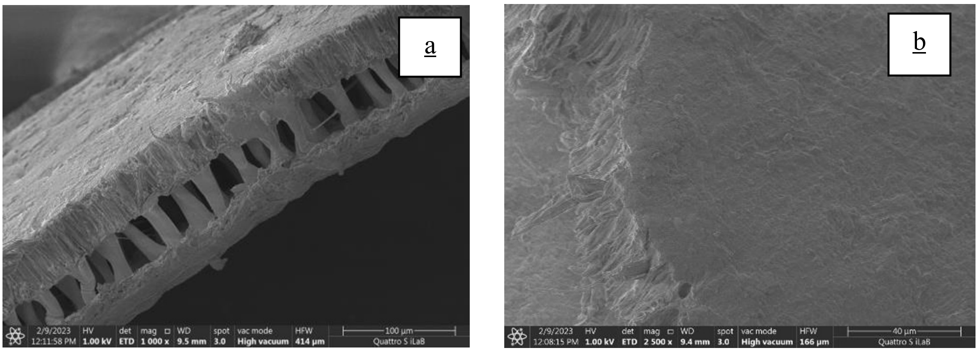

Fig 4. Morphology of control (non-coating) soybean seed; (a) cross section, and (b) outer surface

4 Conclusion

The multi-layer coating treatment with the addition of ZnO-NPs incorporated into the chitosan emulsion and the addition of the zeolite layer gave quite good results on seed quality during storage, but statistically insignificant. The water content could be maintained low and decreased, while the MDA levels of and electrical Conductivity were also lower than the control. Observation on vigor and germination of coated seed after four months of storage indicated that the vigor and germination rate is decreasing, but after the fifth month, the vigor increase again. Implementing nano seed coating enriched with ZnONP and zeolite might help the seed grow healthy while planted in the field.

Acknowlegments. The author would like to thank the Research Organization of Agriculture and Food, The National Research and Innovation Agency (BRIN) through the Research Program of Superior Seeds 2022.

References

1. Masa'ud and S. Wahyuningsih, *Statistik Konsumsi Pangan 2022* (Jakarta, 2022).
2. N. Bachleda, T. Grey, and Z. Li, Plant Breed. **136**, 539 (2017).
3. A. Begum, R. Jerlin, and M. Jayanthi, Int. J. Sci. Res. **2**, 1 (2013).
4. P.C. Coradi, R.E. Lima, C.L. Padia, C.Z. Alves, P.E. Teodoro, and A.C. da S. Candido, J. Stored Prod. Res. **89**, 1 (2020).
5. S.B. Zhang, H.C. Zhai, S.X. Huang, and J.P. Cai, J. Stored Prod. Res. **59**, 146 (2014).
6. M. Rahman, M. Hossain, and R.. Bell, Asian J. Plant Sci. **10**, 278 (2011).
7. R. Li, J. He, H. Xie, W. Wang, S.K. Bose, Y. Sun, J. Hu, and H. Yin, Int. J. Biol. Macromol. **126**, 91 (2019).
8. M.M. Gutiérrez-Pacheco, L.A. Ortega-Ramírez, B.A. Silva-Espinoza, M.R. Cruz-Valenzuela, G.A. González-Aguilar, J. Lizardi-Mendoza, R. Miranda, and J.F. Ayala-Zavala, Coatings **10**, 1 (2020).
9. A. Eshetu, A.M. Ibrahim, S.F. Forsido, and C.G. Kuyu, Heliyon **5**, e01116 (2019).

10. T. Adhikari, S. Kundu, and A.S. Rao, J. Plant Nutr. **4167**, (2015).
11. G.S. Montanha, E.S. Rodrigues, J.P.R. Marques, E. de Almeida, M. Colzato, and H.W.P. de Carvalho, SN Appl. Sci. **2**, 1 (2020).
12. S.V. Raskar and S.L. Laware, Int. J. Curr. Microbiol. Appl. Sci. **3**, 467 (2014).
13. S. Gowayed, J. Plant Prod. Sci. **6**, 1 (2017).
14. A.Z. Khan, S. Nigar, S.K. Khalil, S. Wahab, A. Rab, M.K. Khattak, and T. Henmi, Pakistan J. Bot. **45**, 1063 (2013).
15. V.G.L. Souza, C. Rodrigues, S. Valente, C. Pimenta, J.R.A. Pires, M.M. Alves, C.F. Santos, I. Coelhoso, and L.A. Fernando, Coatings **10**, 1 (2020).
16. I.S.T.A. (ISTA), *International Rules for Seed Testing 2016* (The International Seed Testing Association, Bassersdorf, Switzerland, 2016), pp. 1–40.
17. A. Zielinska, F. Carreiro, A.M. Oliviera, A. Neves, B. Pires, D.N. Venkatesh, A. Durazzo, M. Lucarini, P. Eder, A.M. Silva, A. Santini, and E.B. Souto, Molecules **25**, 3731 (2020).
18. N. Nethra, K.U. Rani, R. Gowda, S.R. Prasad, and S. Narayanaswamy, Seed Sci. Technol. **44**, 207 (2016).
19. X. Xin, Q. Tian, G. Yin, X. Chen, J. Zhang, S. Ng, and X. Lu, J. Plant Physiol. **171**, 140 (2014).
20. A. Bin Arif, S. Yuliani, Q. Hernani, I. Agustinisari, and C. Winarti, Emirates J. Food Agric. **35**, 232 (2023).
21. J.M.G. Neves, J.A. Oliveira, H.P. Silva, R.D.G.E. Reis, J. Zuchi, and A.R. Vieira, Rev. Bras. Eng. Agric. e Ambient. **20**, 1025 (2016).
22. L.F.D.E. Carvalho, C.S. Sediyama, and M.S. Reis, Rev. Bras. Sementes **31**, 009 (2009).
23. G.M. Conceição, A.D. Lúcio, L.M. Mertz-Henning, F.A. Henning, M. Beche, and F.F. De Andrade, Rev. Bras. Eng. Agric. e Ambient. **20**, 1020 (2016).
24. Z. Yang, J. Chen, R. Dou, X. Gao, C. Mao, and L. Wang, Int. J. Environ. Res. Public Health **12**, 15100-15109 (2015).
25. N. Chookhongkha, T. Sopondilok, and S. Photchanachai, Acta Hortic. **973**, 231 (2013).
26. P.K. Mishra, S. Paroha, and R.P. Mishra, Int. J. Curr. Res. Acad. Rev. **4**, 108 (2016).
27. V.M. Santos, T.C. Oliveira, M.G. Mendes, C.H. Yamanaka, and W.R. Macedo, Plant Physiol. Reports **26**, 247 (2021).
28. D. Zeng, X. Luo, and R. Tu, Int. J. Carbohydr. Chem. **2012**, 1 (2012).