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Storage Time: Influence of Nano-ZnO and Soft-Sterilization on Biophysical and Quality Attributes of Canned Cowpea (*Vigna unguiculata*, TN 5-78)

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Abstract

Cowpea seeds can be cooked in the dried form, sprouted, or ground into flour. This study is to investigate effect of soft-sterilization and nano-ZnO treatment on canned cowpea (TN 5-78) biophysical and quality attributes during 10 months of storage. Cowpea was blanched, ultrasonicated with nano-ZnO solution 0.025% (w/v) added prior to canning at 110 ºC for 15 min and analyzed every 2 months up to 10 month at ambient storage. Total mold and yeast count were below the limits of detection for nano-ZnO treated samples and control over the storage period though, some colonies of mesophilic bacteria were observed in the untreated samples at the 8th and 10th month of storage. There are significant differences ($P < 0.05$) between the treated samples and untreated one at the 10th month of storage for the pea's firmness. No significant differences was noticed between the samples from the initial analysis to the end of storage time for the leached solids percentage ($P < 0.05$). Moreover, slight change in protein content and pH values were also found. The overall acceptability score of nano-ZnO treated samples remained in a good range up to 10th month of storage whereas, untreated samples was under acceptation level. Therefore, ZnO nanoparticules combined with heat can be a possible alternative approach to can foods that the quality attributes are altered by conventional thermal sterilization.

Keywords: cowpea, soft sterilization, nano-ZnO, sensory, quality

1. Introduction

Cowpea (*Vigna unguiculata*) is a grain legume, with various ways of utilization in traditional and modern food processing across the world. Cowpea seeds can be cooked in the dried form, sprouted, or ground into flour (Odedeji & Oyeleke, 2011). Referred to as "poor man's meat" by West Africans cowpea is of considerable importance in many African countries as a nutritious leguminous crop providing an alternative source to animal protein (Afoakwa & Yenyi, 2006). Sefa-Dedeh et al. (2001) reported that cowpea contributes up to 80% of the total dietary protein intake in some parts of West Africa. In addition to its nutritional quality, cowpea presents also some health benefits. Gutiérrez-Uribe and Serna-Saldívar (2011) found that whole cowpeas are a good source of phytochemicals inhibiting in vitro cancer cell growth.

Depending on areas, cowpea is consumed in different ways. *Akara* (fried cowpea paste) and *moin-moin* (steam cowpea paste) are found to be the main cowpea-based foods in West African countries (Amonsou, Sakyi-Dawson, & Saalia, 2010). Previous studies (Odedeji & Oyeleke, 2011; Sefa-Dedeh, Sakyi-Dawson, & Afoakwa, 2001) have shown that the consumption of beans is curtailed because of the long cooking time needed to achieve the desired palatability and digestibility. Thus, it is important to develop new cowpea-based foods that are easy to prepare. Sefa-Dedeh et al. (2003) reported that canning is proven to give cowpea a longer shelf life. Cooking and industrial dehydration increases the nutritional value of legumes by inactivating most of the anti-nutritional factors present, but also alters the functional properties and microstructural characteristics of legume proteins (Antonia, Jiménez, & Martínez-Tomé, 2009; Foh, Wenshui, Amadou, & Jiang, 2012). Color and texture which are the most important quality attributes of processed foods change during thermal processing (Leadley, Tucker, & Fryer, 2008; Sankhon, Amadou, Yao, Wang, Qian, & Mlyuka, 2014). Thus, it's a challenge for food engineers to find the best processing condition to process food products with an acceptable quality.

Zinc oxide (ZnO) nanoparticules are increasingly been used in the food industry mainly because of their antimicrobial property (Jin, Sun, Su, Zhang, & Sue, 2009; Tayel, El-Tras, Moussa, El-Baz, Mahrous, Salem, et al., 2011; Xie, He, Irwin, Jin, & Shi, 2011). (Emamifar, Kadivar, Shahedi, & Soleimanian-Zad, 2010) found that low-density polyethylene nano composite packaging materials containing silver and ZnO nanoparticles is able to extend the shelf-life of fresh orange juice during cold storage (4 °C). Li et al. (2011) had developed a novel polyvinyl chloride film coated with nano-ZnO particles. Recently, Meng et al. (2014) found that combined application of ultrasound and nano-ZnO coating is capable of effectively delaying the senescence and significantly extending the storage life of fresh-cut kiwifruit. However, research by Wang et al. (2008) has reported nano-ZnO regulated low doze LD_{50} to be 20-nm ZnO is greater than 5-g/kg body weight and belongs to unclassified toxicity according to the Globally Harmonized Classification System (GHS) for the classification of chemicals. Therefore, it was concluded that 20- and 120-nm ZnO, to be relatively nontoxic.

However, up to date, there are no publications reporting the combination of nano-ZnO and heat to process soft sterilized foods, therefore the objective of this study is to investigate the influence of combined effect of nano-ZnO and soft sterilization upon long period of storage on the quality factors such as microbial growth, leached solid, firmness, pH, proximate composition and sensory attributes of soft sterilized TN 5-78 cowpea variety.

2. Material and Methods

2.1 Materials

Zinc oxide (ZnO) nano-particules were purchased from Sigma-Aldrich and the cowpea variety TN 5-78 was obtained from a seed production company "EntrepriseSemencièreAlheri" (Niamey, Niger). All other reagents were of analytical grade.

2.2 Samples Treatments

Cowpea seeds were firstly sorted to remove under quality peas and washed with deionized water then soaked in sodium hexametaphosphate $[(NaPO_3)_6]$ 0.5% (w/v) solution for 12 h. The peas were then blanched in deionized water for 5 min at 90 °C afterwards; the blanched peas were versed in glass containers. The nano-ZnO solution 0.025% (w/v) used to fill the glass containers in a ratio of 1:3 (solid-liquid) was previously ultrasonicated for 20 min. The sealed containers were canned in a vertical rotor using optimized temperature-time (110 ºC and 15 min). Canned samples were cooled at room temperature (25 ºC) and one batch of six cans (3 cans for both treated and untreated samples) was selected randomly and analyzed. The remained cans were stored at room temperature (25 ºC) and one batch was randomly selected to perform the analysis each 2 months over 10 months.

2.3 Microbiological Analysis

The canned cowpeas were analyzed for mesophilic bacteria and for yeast and molds each 2 months. Ten grams of canned peas were removed aseptically from each container and diluted with 90 mL of sterile peptone water. For aerobic plate counts, after homogenization, samples were serially diluted (10^{-1}-10^{-6}) with sterile peptone water then enumerated using Nutrient Agar incubated at 35 °C during 72 h. Yeasts and molds were assessed on rose Bengal after 7 days of incubation at 28 °C. Each test was made in duplicate and results were expressed as colony-forming units (CFU) per gram.

2.4 Seed Firmness

Soft sterilized cowpea's hardness was determined after canning and each 2 months using texture analyzer TA-XT2i, Stable Micro Systems Ltd. (Godalming, UK). Seven canned peas were selected to undergo the firmness test. The tests were realized until a deformation of 20 mm, pretest speed of 1.0 mm/s, and test speed of 0.5 mm/s using a cylindrical shape probe(fat as probe base) of 35 mm end diameter. Hardness value was considered as mean peak compression force and express in g.

2.5 pH Measurement

Glass electrode pH meter was used to determine the pH of samples. The measurement was done on the drained liquid poured from the can into a beaker.

2.6 Leached Solids

Soluble solids was evaluated throughout the method proposed by Yeung et al. (2009). This method was to determine °Brix using a refractometerWAY-2W, Precision and Scientific Instrument co., Ltd (Shanghai, China). Briefly, broth was swirled to disperse solids settling at the bottom then a drop was placed on the prism surface for measurement. Soluble solid loss was calculated with the following formula:

$$\text{Soluble solids loss} = (°\text{Brix})\ (\text{final broth weight}) \times 100/\text{initial seed weight} \quad (1)$$

The final broth weight was determined by taking the drained weight after drainage throughout a sieve.

2.7 Proximate Composition

Crude protein, fat ash, and moisture percentages were determined using (AOAC, 1990) standard methods. Crude carbohydrate was obtained by the following formula:

$$\text{Crude carbohydrate} = 100 - (\text{Protein\%} + \text{Fat\%} + \text{Ash\%} + \text{Moisture\%}) \quad (2)$$

2.8 Sensory Evaluation

Twelve trained panelists had evaluated the sensory attributes of canned cowpea. Each 60 days one batch of canned cowpea was chosen randomly for sensory evaluation. Canned cowpea was evaluated for appearance and color, taste, texture and overall acceptability. Panelists used hedonic scale of 9 points to evaluate the canned peas (1-extremely dislike, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neitheir like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, and 9-extremely like). Canned cowpeas were considered acceptable when their overall mean value was equal or above 5 (neither like nor dislike).

2.9 Statistical Analysis

SPSS Inc. software (version 17.0) was used to perfume statistical analysis. One-way analysis of variance (ANOVA) was used to determine significant differences among the mean values, with the significance level taken at ($P < 0.05$) using Tukey test.

3. Results and Discussion

3.1 Microbiological Analysis

Table 1 shows that after 10 months of storage, no detectable counts were found in both treated and untreated samples for mold and yeast, however, some colonies of mesophilic bacteria were detected in the control at the 8th and 10th month of storage (2.05 log CFU/g and 4.12 log CFU/grespectively). From these results, it can be concluded that nano-ZnO and heat have acted synergistically to prevent the growth of microorganisms that could have been in the samples. The results are in agreement with previous studies on antimicrobial efficacy of nano-ZnO (Jin, Sun, Su, Zhang, & Sue, 2009; Tayel et al., 2011; Xie, He, Irwin, Jin, & Shi, 2011). Nano-ZnO can therefore be combined with soft sterilization to process commercially ambient stable low foods especially those that the quality characteristics are altered by normal sterilization conditions. However, Leadley et al. (2008) had reported that the absence of detectable counts after processing cannot assure safety from a process establishment perspective, in fact, authors have suggested that a challenge testing with pathogens or a suitable indicator organism would be required to demonstrate a known level of microbial reduction.

Table 1. Microbial analysis of processed cowpea

Parameters	Treatment	Storage time (Month)					
		0	2	4	6	8	10
Mesophilic bacteria (log CFU/g)	Control	Nil	Nil	Nil	Nil	2.05 ±0.2	4.12±0.97
	Treated samples	Nil	Nil	Nil	Nil	Nil	Nil
Mold and yeast (log CFU/g)	Control	Nil	Nil	Nil	Nil	Nil	Nil
	Treated samples	Nil	Nil	Nil	Nil	Nil	Nil

Values are means and standard deviation of duplicate; Values are significantly different ($P < 0.05$) from each other.

3.2 Firmness

From Figure 1, it can be observed that some softening was observed over the storage time, similar results were reported by Leadley et al. (2008) when they compared the effect of high pressure sterilization and conventional thermal sterilization on the quality of green beans. As shown in Figure 1 the initial hardness values were similar for both control and treated sample. However, on the 6th month of storage the untreated peas started to loss their firmness. This difference in the firmness between the treated peas and untreated became significant ($P < 0.05$) at the 10th month of storage. This findings corroborate with the results reported by Meng et al. (2014) who found that the firmness of fresh-cut Kiwifruit can be kept while coated with nano-ZnO. Firmness was maintained likely due to the antimicrobial effect of nano-ZnO. Indeed, Hong et al. (2012) had reported that maintenance of firmness in fruits treated with chitosan coatings could be due to their higher anti-fungal activity,however, in the present study no molds had grown in control samples and covering of the cuticle and lenticels. As firmness is one of important quality attributes of canned foods judged by consumers, it can highly affect the product overall acceptance. These results support the fact that the combination between soft sterilization and nano-ZnO can be used as an alternative to the conventional sterilization to process this cowpea variety (TN 5-78).

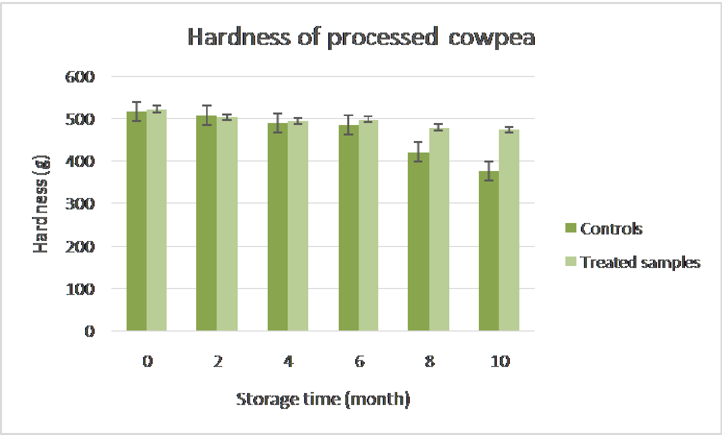

Figure 1. Combined effect of soft sterilization and nano-ZnO on the hardness of processed cowpea

3.3 Leached Solids

Solids leaching is a key parameter in canned food quality keeping during the storage time. Afoakwa et al. (2006) had reported that too high amount of leached solids can bring about low quality and fast deterioration of the canned cowpea. The combination effect of soft sterilization and nano-ZnO of the solids leaching is shown in Figure 2. From this study, no significant ($P < 0.05$) differences were observed between both treated and untreated samples for leached solids percentage throughout the storage time.

Figure 2. Combined effect of soft sterilization and nano-ZnO on the soluble solids loss

3.4 pH

Canned food's shelf life is strongly affected by their pH stability because its variation may cause a denaturation of nutrients or bioactive compounds or sometimes promote microbial growth. Minguez and Rojas (1994) reported that carotenoids are quite sensitive to acids. Later, Andrés-Bello et al. (2013) found that pectin shows a high stability in aqueous solutions at pH 3.0-4.0 Pigments (e.g., chlorophyll, carotenoids, anthocyanins, myoglobin, etc.) responsible for the color of fruits, vegetables and meats can also be affected by pH as reported by Andrés-Bello et al. (2013). Therefore, pH can affect texture and color which are important sensory attributes of processed foods. Table 2 shows that there were significant ($P < 0.05$) difference in the pH values between treated and untreated samples from the initial time to the 6th month of storage. From the 8th to 10th month of storage, significant differences were observed between nano-ZnO treated sample and the reference for the pH values. This may be explained by the microbial growth observed at these periods. The maintaining of pH in the treated samples can be imputed to the antimicrobial property of nano-ZnO. The results support the fact that nano-ZnO can be combined to heat in the aim to softly sterilized and extend the shelf life of this cowpea variety.

3.5 Effect on Proximate Composition

The combined effect of soft sterilization and nano-ZnO treatment on the proximate composition of cowpeas is presented in Table 2. The difference between the reference and treated samples were not statistically significant ($P > 0.05$) for crude carbohydrate ash and fat content from the initial time to the end of storage period. Initially, the protein percentage of treated samples and reference was significantly ($P < 0.05$) difference. It is shown that the treated sample have 6.07% as protein content which is lower than that of the control sample (6.25%). The reduction of treated sample's protein content may be attributed to their adsorption onto Zn nano-particules surfaces. The study by Dembereldorj et al. (2012) had revealed that the conformational changes of proteins adsorbed onto ZnO nanoparticle surfaces were adsorbed onto ZnO surfaces through both PO_3H_2 and CO_2H head groups. However, from Table 2 it can be noticed that the protein content of treated samples remained stable during the storage time whereas, a significant reduction in the protein content of untreated samples was observed. This result shows the usefulness of nano-ZnO for the stability of soft sterilized cowpea's proximate composition during storage.

Table 2. Proximate composition and pH of processed cowpea

Parameters (%)	samples Treatment	Storage time (month)					
		0	2	4	6	8	10
Protein	Control	$6.25^{f}\pm0.15$	$6.20^{f}\pm0.14$	$5.12^{c}\pm0.19$	$5.75^{d}\pm0.36$	$5.10^{bc}\pm0.17$	$5.04^{abc}\pm98$
	Treated	$6.07^{e}\pm0.34$	$6.04^{e}\pm0.11$	$6.05^{e}\pm0.17$	$6.10^{e}\pm1.01$	$6.08^{e}\pm1.10$	$6.03^{e\cdot}\pm1.31$
Ash	Control	$0.52^{a}\pm0.01$	$0.51^{a}\pm0.04$	$0.50^{a}\pm0.07$	$0.51^{a}\pm0.02$	$0.48^{a}\pm0.15$	$0.49^{a}\pm0.11$
	Treated	$0.48^{a}\pm0.08$	$0.49^{a}\pm0.05$	$0.51^{a}\pm0.01$	$0.49^{a}\pm0.01$	$0.52^{a}\pm0.07$	$0.50^{a}\pm0.01$
Carbohydrate	Control	$18.19^{a}\pm1.23$	$18.65^{ab}\pm1.18$	$19.57^{ab}\pm1.98$	$19.25^{ab}\pm2.03$	$19.21^{ab}\pm3.90$	$19.45^{ab}\pm2.01$
	Treated	$19.10^{ab}\pm1.91$	$18.57^{ab}\pm0.97$	$18.74^{ab}\pm1.22$	$20.27^{ab}\pm1.12$	$20.93^{ab}\pm1.86$	$19.98^{ab}\pm1.23$
Fat	Control	$0.53^{b}\pm0.03$	$0.49^{ab}\pm0.01$	$0.53^{b}\pm0.10$	$0.49^{ab}\pm0.02$	$0.49^{ab}\pm0.03$	$0.49^{ab}\pm0.02$
	Treated	$0.55^{b}\pm0.07$	$0.45^{a}\pm0.03$	$0.47^{ab}\pm0.02$	$0.47^{ab}\pm0.05$	$0.44^{a}\pm0.01$	$0.46^{a}\pm0.03$
Moisture	Control	$74.54^{a}\pm2.35$	$74.05^{a}\pm3.18$	$74.28^{a}\pm2.03$	$74.00^{a}\pm1.98$	$74.72^{a}\pm0.98$	$74.53^{a}\pm3.14$
	Treated	$73.80^{a}\pm1.79$	$74.41^{a}\pm0.93$	$74.23^{a}\pm3.64$	$72.67^{a}\pm2.14$	$72.03^{a}\pm2.45$	$73.03^{a}\pm2.13$
pH	Control	$5.95^{bc}\pm0.01$	$5.96^{c}\pm0.1$	$5.92^{bc}\pm0.02$	$5.94^{bc}\pm0.13$	$5.86^{ab}\pm0.11$	$5.82^{a}\pm0.07$
	Treated	$6.54^{de}\pm0.43$	$6.55^{de}\pm0.04$	$6.47^{d}\pm0.08$	$6.56^{e}\pm0.21$	$6.48^{de}\pm0.12$	$6.50^{de}\pm0.31$

Values are means and standard deviation of triplicate; Values in columnwith different superscripts are significantly different ($P < 0.05$) from each other *($n = 3$)*

3.6 Sensory Qualities

As shown in Table 3, there were no significant ($P < 0.05$) differences in the score of appearance, color, and taste, between treated and untreated samples over the storage time. However, significant ($P < 0.05$) differences were recorded for texture score between the two groups of samples from the 6th to the 10th month. The texture scores were decreased by about 28% and 12.46% for control and treated sample respectively. The high depreciation of control's texture may be the consequence of the significant loss of firmness observed at that period. Sefa-Dedeh et al. (2001) had reported that for acceptable cowpea product formulation, a firmness level of 500 g is ideal. Counter to the control, the treated samples were acceptable after 10 month of storage because their overall acceptability score remained in a good range. Similar results were reported by Kumar et al. (2011) on the effect of combination processing on the microbial, chemical and sensory quality of ready-to-eat vegetable *pulav*. These results show that nano-ZnO can be combined to heat to produce good quality shelf-stable low-acid foods accepted by consumers.

Table 3. Sensory analysis of combination processed cowpea

Parameters	Samples	Storage time (month)					
	Treatment	0	2	4	6	8	10
Appearance	Control	7.05^{c}±0.11	7.03^{c}±0.03	6.46^{b}±0.41	6.19^{b}±0.18	6.28^{b}±0.45	5.75^{a}±0.17
	Treated	7.05^{c}±1.02	6.93^{c}±0.49	6.31^{b}±0.12	6.30^{b}±0.35	6.22^{b}±0.07	5.65^{a}±0.23
Texture	Control	7.25^{d}±0.64	7.13^{d}±1.23	7.14^{d}±1.74	6.38^{c}±0.32	5.25^{a}±0.13	5.15^{a}±0.21
	Treated	7.14^{d}±1.01	7.18^{d}±0.67	7.17^{d}±0.17	7.04^{d}±1.21	6.37^{c}±1.11	6.25^{c}±1.23
Taste	Control	7.51^{b}±0.15	7.28^{b}±0.48	6.84^{ab}±0.23	6.24^{ab}±0.14	5.64^{ab}±3.51	5.09^{ab}±0.42
	Treated	7.16^{b}±0.23	7.18^{b}±0.23	6.76^{ab}±1.17	4.08^{a}±1.83	5.33^{ab}±0.33	5.47^{ab}±2.07
Overall acceptability	Control	7.82^{e}±0.03	7.60^{cde}±0.18	7.16^{bcd}±0.23	6.57^{b}±0.97	5.34^{a}±0.12	4.95^{a}±0.54
	Treated	7.75^{cde}±0.19	7.71^{cde}±0.09	7.54^{cde}±0.31	7.40^{cde}±0.38	7.21^{cde}±1.25	7.09^{bc}±0.13

Values expressed are mean ± standard deviation. Values in column with different superscripts are significantly different ($P < 0.05$) from each other.

4. Conclusion

This study showed that ZnO nano-particules can be used in combination with heat as an alternative for processing and preserving low-acid foods especially for raw foods that the quality parameters are negatively affected during conventional thermal sterilization. The microbiological results obtained in this study suggest that sterilization is possible using nano-ZnO even when F_0 value is lower than required. The sensory evaluation revealed that the quality attributes were not affected by nano-ZnO, thus, thetaste and appearance declines strongly, they were maintained in good range over the storage. Thus, this cowpea variety (TN 5-78) can be canned by applying this treatmentthat is best before ten month (shelf live). Further studies are needed to evaluate its efficiency by using specific pathogens in order to reveal whether or not a commercial sterility is reached for a further industrial use of this cowpea variety.

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