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PRESERVATIVE EFFECT OF GELATIN COATINGS ON CARROT

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ABSTRACT

Edible coating extends the life span of fresh fruits and vegetables. It is used to preserve food and prevent spoilage of fruits stored at room temperature. Preservative effect of gelatin coatings on the preservation of carrots was analyzed. Gelatin was prepared in four concentrations (0.5 g/ml, 1.0 g/ml, 1.5 g/ml and 2.0 g/ml). It was applied using dipping technique and allowed to dry before storage in sterile containers. Physiochemical parameters, weight loss and microbiological qualities of the coated and uncoated carrots were analyzed for a period of 7 days using standard procedures. Isolation and identification of bacteria was carried out using pour plate method and biochemical tests. Gelatin concentration at 0.5 g/ml attained nutrient retention with moisture content, total soluble solids and protein content of coated carrots ranging from 90.50 ± 0.01 – 85.40 ± 0.00 %, 12.20 ± 0.01 – 10.60 ± 0.00 Brix^{o} and 1.50 ± 0.01 – 0.25 ± 0.01 % respectively. Weight loss of coated carrots at 0.5 g/ml concentration ranged from 0.35 ± 0.01 – 0.25 ± 0.02 g while 1.0 g/ml concentration ranged from 0.35 ± 0.00 – 0.28 ± 0.03 g. The bacterial and fungal counts of carrots coated with gelatin ranged between 0.20 ± 0.01 x 10^4 - 7.50 ± 0.05 x 10^4 Cfu/g and 0.20 ±0.01 x 10^4 – 18 ± 0.01 x 10^5 Cfu/g respectively. Eight bacterial and five fungal isolates namely *Proteus* sp., *Staphylococcus* sp., *Enterobacter* sp., *Escherichia coli*, *Pseudomonas* sp., *Aeromonas* sp, *Bacillus* sp, *Streptococcus* sp, *Fusarium* sp., *Aspergillus niger*, *Aspergillus fumigatus, Rhizopus stolonifera*, *Penicillum* sp. were the most occurring bacterial and fungal species respectively. Coating with gelatin extends life span, reduce water and helps maintain the phytochemical properties of the carrot.

KEYWORDS: Gelatin, Carrot, Coating, Preservation and Microorganism.

INTRODUCTION

Carrot is known to have originated from Asia and is frequently cultivated in many countries. Carrot is largely consumed in nearly all parts of the world and it provides adequate and essential nutrients needed for the growth of the body. It is usually orange in color, though some may be pink, purple or different shades of yellow, these add beauty to foods on a plate, and it is rich in vitamin K and calcium which helps to strengthen the bone. It lowers risk of having diabetes and helps maintain blood sugar level. Its helps protect the skin from intense sun rays and also helps repair worn out skin tissues. (Ahmad *et al*., 2005)

Edible coatings can be used as impediments to inhibit the growth of microorganisms while also reducing the damaging effects of fresh fruits and vegetables (Moreira *et al*., 2011; Correa-Betanzo *et al*., 2013). There is wide use of edible coatings in the industries and in the world at large, due to their numerous uses for prolonging fruit life span and also as conveyor for different food additives (Mastromatteo *et al*., 2011).

Edible coatings also act as impediments for moisture and gases throughout the preservation process. It lowers food spoilage and promotes safety by their activity and by incorporating it on fresh fruits. Other benefit of using edible coating is to reduce litters, to extend the life span of fresh and perishable processed product and protect it from harmful environment conditions by maintaining the transfer of oxygen, carbon dioxide, moisture and aroma as reported by Mastromatteo *et al*., 2011.

Gelatin is a group of peptides and proteins that is yielded by partial hydrolysis of collagen removed from the skin, bones, and connective tissues of animals such as domesticated animals. Results of different studies has shown the effectiveness of gelatin in preservation of fruits and it has also showed good impediments characteristics against oxygen and aroma transfer at low and intermediate relative humidity (Andrade *et al*., 2014).

The study aimed to determine the preservative effect of gelatin coatings on preservation of carrot under the following objectives: determine the physiochemical

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properties of carrot samples before and after carrot coating, to determine the effect of gelatin coating on weight loss on carrot samples before and during the days of preservation, determine the microbial load on carrot samples and to identify microorganisms isolated from the carrot samples.

MATERIALS AND METHODS

Collection of Samples

Carrot samples were purchased from Owode market, Offa, Kwara State. They were carefully sorted out and thoroughly washed and kept under room temperature for further use.

Preparation of Gelatin

Gelatin was procured and four (4) concentrations were used for the study. 50 g of gelatin was dissolved in 100 ml of distilled water to give 0.5 g/ml and it was labeled as treatment 2, 100 g of gelatin was dissolved in 100 ml of distilled water to give 1.0 g/ml and it was labeled as treatment 3, 150 g of gelatin was dissolved in 100 ml of distilled water to give 1.5 g/ml and it was labeled as treatment 4 and 200 g of gelatin was dissolved in 100 ml of distilled to give 2.0 g/ml and labeled as treatment 5.

Coating of Carrot Samples

The carrot samples were divided into five different treatments which included control and different concentrations of gelatin, these were coated using dipping techniques. The coated carrot samples were stored in different sterile containers at 28±2 ^{o}C for seven days.

Physicochemical Analysis

The carrot samples were analyzed for some physicochemical compositions such as moisture content, crude protein, crude fat, total soluble solids, total titratable acid and pH. Weight loss was also determined during the seven days period of storage (AOAC, 2000)

Microbiological Analysis

Standard method was used for the microbiological analysis as explained by Fawole and Oso, 2007. Pour plate method of isolation was used. Biochemical test such as catalase test, coagulase test, starch hydrolysis, citrate test, sugar fermentation test, indole test and gram's reaction were all done for characterization and identification of the isolates

Statistical Analysis

Results from the study were analyzed and interpreted as mean ± standard deviation of three replicate determinations. Statistical analysis was performed on the data using one way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) IBM software version 16. Significance was accepted at $P < 0.05$

RESULTS

The physiochemical properties of the carrot samples are shown from Table 1. The moisture content ranged (81.5-90.5 %), pH (5.10-5.50), Protein (0.20-1.50 %), Fat (0.12-1.34 %), Total Titratable acidity (0.06-0.18 %) and Total soluble solid (9.20-12.20 Brix^{o}). The weight loss in carrot samples is shown in Table 2. It ranged from 0.25-0.35 % across the treatment's groups. Figures 1&2 shows the microbial count of samples and it comparison among each treatment group across the seven days of storage.

The biochemical parameters carried out on the bacterial isolates and probable identity of the organisms is shown in table 3. Isolates B2 and B9 tested positive to catalase, coagulase, citrate and starch hydrolysis while B1 and B10 tested positive to catalase, citrate and starch hydrolysis. Ten bacteria were isolated and the probable organisms were of the genus *Proteus*, *Staphylococcus*, *Enterobacter*, *Escherichia*, *Pseudomonas*, *Aeromonas*, *Bacillus* and *Streptococcus*. Three isolates were gram positive while five were negative. Table 4 shows the colonial morphology and microscopy of the fungal isolates. Probable organisms include *Aspergillus* sp., *Rhizopus* sp., *Penicillum* sp. and *Fusarium* sp.

Table 1: Physicochemical Characteristics of Carrot Samples

		Treatments				
Physicochemical Parameters	**BC**	**T1**	**T2**	**T3**	**T4**	**T5**
MC (%)	91.40±0.01^{a}	88.60±0.01^{a}	85.40±0.00^{b}	88.50±0.00^{a}	86.40±0.01^{a}	90.50±0.01^{a}
pH	5.20±0.00^{a}	5.26±0.02^{a}	5.32±0.01^{b}	5.32±0.01^{b}	5.50±0.00^{c}	5.55±0.01^{c}
TTA (%)	0.08±0.00^{a}	0.06±0.00^{a}	0.10±0.00^{a}	0.12±0.00^{b}	0.14±0.00^{b}	0.16±0.01^{b}
TSS (Brix^{o})	9.20±0.00^{c}	10.20±0.00^{b}	12.00±0.01^{a}	12.20±0.00^{a}	12.02±0.00^{a}	10.60±0.00^{b}
Protein (%)	0.85±0.01^{b}	0.90±0.01^{b}	0.80±0.01^{b}	1.50±0.01^{b}	1.20±0.01^{b}	0.25±0.01^{b}
Fat (%)	0.20±0.01^{a}	0.15±0.01^{c}	0.20±0.02^{a}	1.15±0.00^{b}	1.00±0.00^{b}	0.23±0.00^{a}

Values are means of triplicate readings and standard deviation. Values in the same rows having different superscript are significantly different at ($P \leq 0.05$)

Keys: **BC**= Before Coating: **T1**= Control: **T2**= 0.5 g/ml Gelatin Coating: **T3** =1.0 g/ml Gelatin Coating: **T4**=1.5 g/ml Gelatin Coating: **T5** = 2.0 g/ml Gelatin Coating: **MC**= Moisture Content: **TTA**= Total Titratable Acid, **TSS** = Total Soluble Solid

Table 2: Effect of weight loss (%) on the vegetative sample during storage

Samples	1	2	3	4	5	6	7
T1	0.35±0.02^{b}	0.34±0.02^{b}	0.35±0.01^{a}	0.32±0.03^{c}	0.30±0.01^{a}	ND	ND
T2	0.34±0.01^{a}	0.35±0.01^{a}	0.34±0.05^{c}	0.33±0.05^{c}	0.30±0.02^{b}	0.29±0.05^{c}	0.25±0.02^{b}
T3	0.35±0.00^{a}	0.34±0.03^{b}	0.32±0.04^{c}	0.31±0.02^{a}	0.29±0.05^{c}	0.28±0.03^{b}	ND
T4	0.35±0.03^{c}	0.33±0.02^{b}	0.31±0.01^{a}	0.30±0.02^{b}	0.30±0.04^{c}	ND	ND
T5	0.34±0.02^{a}	0.33±0.05^{c}	0.32±0.03^{b}	ND	ND	ND	ND

Values are means of triplicate readings and standard deviation. Values in the same rows having different superscript are significantly different at (P≤0.05)
Keys: **T1**= Control: **T2**= 0.5 g/ml Gelatin Coating: **T3** =1.0 g/ml Gelatin Coating: **T4**=1.5 g/ml Gelatin Coating: **T5** = 2.0 g/ml Gelatin Coating: **ND** = Not Determined

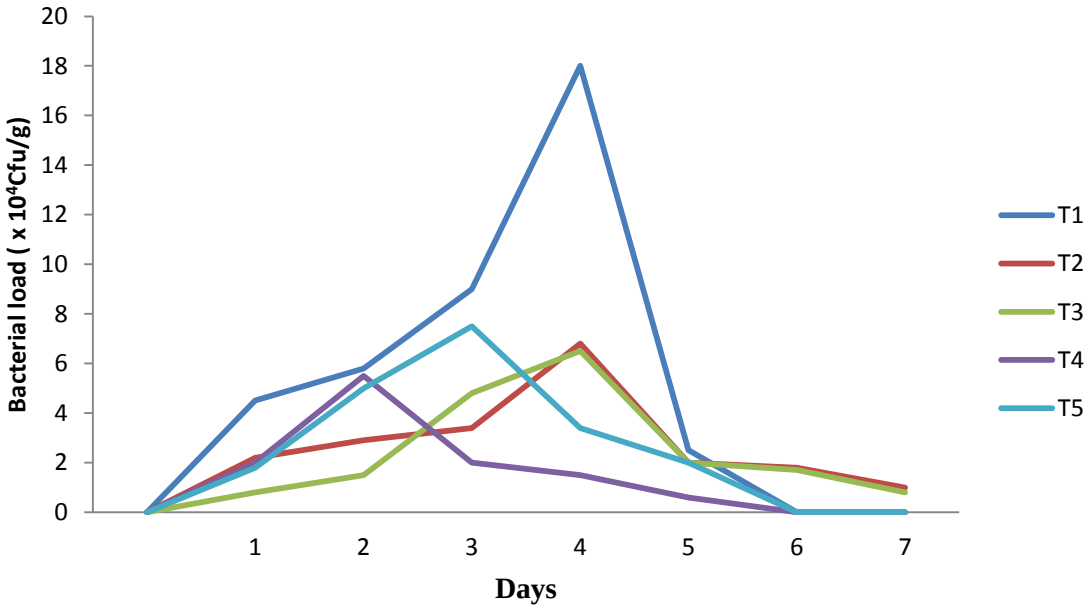

Figure 1: Bacteria Load (Cfu/g) of Carrot Samples
Keys: **T1**= Control: **T2**= 0.5 g/ml Gelatin Coating: **T3** =1.0 g/ml Gelatin Coating: **T4**=1.5 g/ml Gelatin Coating: **T5** = 2.0 g/ml Gelatin Coating

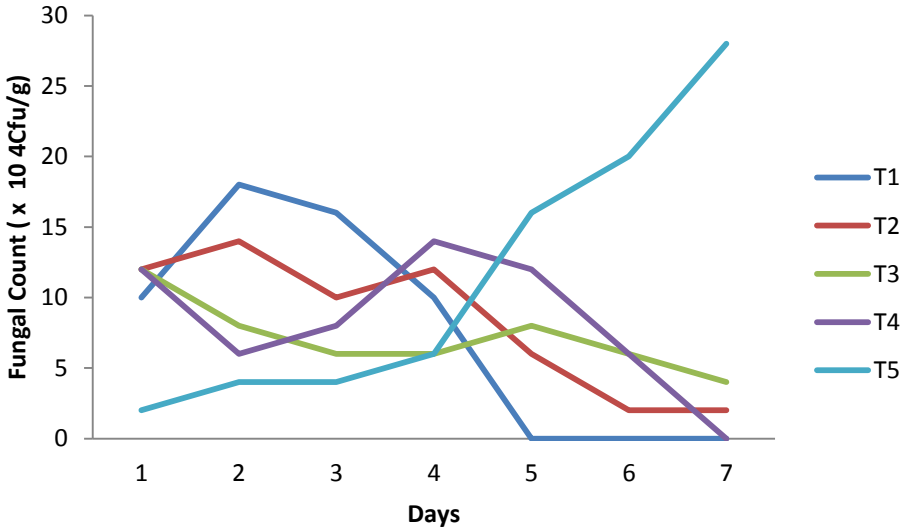

Figure 2: Fungi load (Cfu/g) of carrot Samples
Keys: **T1**= Control: **T2**= 0.5 g/ml Gelatin Coating: **T3** =1.0 g/ml Gelatin Coating: **T4**=1.5 g/ml Gelatin Coating: **T5** = 2.0 g/ml Gelatin Coating

Table 3: Biochemical Test Bacterial Isolates

Isolates	CA	CO	CIT	IND	OX	SH	Sugar Fermentation			Oxygen Relationship	Probable organisms
							Sucrose	Glucose	Lactose		
B1	+	-	+	-	-	+	-	+	-	FA	*Proteus* sp.
B2	+	+	+	-	-	+	+	+	+	FA	*Staphylococcus* sp.
B3	+	-	+	-	-	+	+	+	-	FA	*Enterobacter* sp.
B4	-	-	-	+	-	+	+	+	+	FA	*Streptococcus* sp.
B5	+	-	-	+	-	+	+	+	+	FA	*Escherichia coli*
B6	+	-	+	-	-	-	-	-	-	Ae	*Pseudomonas* sp.
B7	+	-	-	-	-	-	+	+	VAR	Ae	*Aeromonas* sp.
B8	+	-	+	-	-	+	+	+	VAR	FA	*Bacillus* sp.
B9	+	+	-	-	-	-	+	+	+	FA	*Staphylococcus* sp.
B10	+	-	+	-	-	+	-	+	-	FA	*Proteus* sp.

Key: + = positive; - = negative; Ae = aerobe; FA= facultative anaerobe; IND = indole; OX = oxidase; CA = catalase; CO = coagulase; CIT = citrate; SH = Starch Hydrolysis; VAR= Variable

Table 4: Characterization and Probable Identification of Fungal Isolates

Fungal isolates	Cultural characteristics	Microscopic characteristics	Tentative identification
F1	Brown yellow colony with raised center and a flat white periphery followed by a yellow edge	Micro-conidial are ovoid in shape, borne on phialides on branded conidiophores with septate hyphae	*Fusarium* sp.
F2	Presence of spores with gray tips around the apex. They have a smooth surface with small columbous globuse	Conidial head are strongly columnar. Conidiophores are smooth walled. Uncolored and terminate in a doomed shape vesicle	*Aspergillus fumigatus*
F3	Fast growing colonies in green color with dense felt conidiophores	Branded conidiophores with chains of conidial looks with brush-like appearance	*Penicillium sp.*
F4	Wooly white colony with orange spots rapidly filling the plate and produces spores	Non septate hyphae, sporangiospores are ovoid in shape and are directly opposite branched rhizoid	*Rhizopus*
F5	Yellow at first but quickly becoming brown to yellowish green with radial grooves, cottony and powdery colony	Conidial heads are large globose and dark brown hyaline hyphae and septate	*Aspergillus niger*

DISCUSSION

Moisture content of the coated carrot samples decreased significantly across the storage period. This was distinguished by reduction in size and weight with retained firmness and there was no colour change as compared to uncoated samples. (Udoh *et al*., 2005)

There was a slight increase in the total soluble solids of the samples with storage time. Coatings with lower concentration of gelatin (0.5 g/ml) had a high increase in TSS while the lowest increase was observed in before coating group with relative significant differences between all other groups. The lowest concentrations of gelatin were found to inhibit microorganisms and preserve the spoilage of the carrot (Ahmed *et al*., 2009). This is because it forms a thin film coat on top of the carrot acting as an additional barrier to moisture loss as reported by (Togrul and Arslan, 2004).

pH and total titratable acidity (TTA) increased significantly in all treatments during storage. These changes could be due to the presence of organic acid in the sample and the slower rate of respiration and metabolic activity as reported by Jitareerat (2007).

There was a rapid increase in weight loss in uncoated carrot than that of coated samples. The highest rate was observed in treatments 1, 3 and 4 at day 1 of storage. Inevitably, coated carrot samples with lower concentration of gelatin had lower weight loss compared to other treatment groups. Similar results were reported by Radi *et al*. (2017).

In figure 1 and 2, there was an increase in growth of microorganisms at day 3-5 of the storage period and afterwards a decrease at day 6 and 7. At day seven, treatment two (lower concentration) with the gelatin coat had a reduced microbial load compared to other treatments and the highest concentration (treatment five) was rotten. Fungal count was relatively high in uncoated sample and this could be due to presence of moisture (Udoh *et al*., 2005). Coatings with lower concentration of gelatin had controlled microbial growth compared to samples with higher concentrations.

There were eight bacterial isolates obtained from the samples. The isolated bacteria were of the genus *Aeromonas*, *Pseudomonas, Bacillus*, *Escherichia*, *Proteus*, *Enterobacter*, *Staphylococcus* and *Streptococcus*. *Staphylococcus* and *Escherichia* are the most naturally occurring organisms on the carrot samples. The presence of *E. coli* could be an indication of feacal contamination as reported by Al-Hindi and Al-Nagada, 2011. This can be as a result of the cultural practices carried out during the production process. The five fungal isolates obtained from the carrot samples agrees with the findings of Adebayo *et al*., 2012 who reported that the species of *Rhizopus* and *Aspergillus* are the most occurring fungi in carrot samples. The contamination of carrots by fungi could be as a result of unhygienic practices carried out and poor storage facilities. The occurrence of *Aspergillus* sp. were found to be relatively high and also one of the common fungi that is usually found on stored fruits after harvestings according to a report carried out by Frisvad and Samson, 1991.

CONCLUSION

The use of gelatin coatings to carrot was shown to be beneficial in preserving the quality of carrots in storage. Coating with gelatin slowed down the weight loss and the growth of microorganisms. The use of gelatin as edible coating materials showed great potential in expanding the life span of carrot. Gelatin (0.5 g/ml and 1.0 g/ml) coating preserved the fresh like quality of carrot thereby extending the shelf life of carrot to 7 days at room temperature (35 ^{0}C).

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