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Full Length Research Paper

EFFECTS OF PACKAGING MATERIALS, PICKLING AGENTS AND SPICES ON THE MICROBIAL COUNTS OF PICKLED OKRA

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Abstract

Okra samples classified as variety 47-4 according to National Horticultural Research Institute (NIHORT), Ibadan, Nigeria were separately pickled in olive oil, groundnut oil and vinegar (5% acetic acid) spiced with ginger, garlic and mixture of ginger and garlic preserved in stainless steel, glass and plastic jars (12 jars each) for 7days at room temperature. For the purpose of comparison, the spices were controlled for using a parallel experiment with no spices added to the pickled okra. The storage environment temperature and relative humidity ranges from 26^0C to 29^0C and 66% to 78% respectively. Various microbial growth or count (bacteria and fungi) such as Total Viable Count, Coliform Count, Fecal Coliform Count and Fungal Count of each treatment combination (packaging materials, the pickling agents and spices) were observed at the end of the experiment and the result was evaluated using ANOVA at $p \leq 0.05$. From the results of the research, vinegar in glass jars shows an absent of some microorganisms as well as the least microbial counts (1.0×10^5 cfu/ml) irrespective of the spice used. Mixture of spices in any pickling agents irrespective of the packaging materials shows great increase in microbial load beyond value gotten when individual spices were used. In general, highest microbial load were observed in okra pickled with olive oil, spiced with ginger and preserved in stainless steel jar while okra pickled in vinegar shows comparatively very low microbial count. Generally, the TVC ranges within 8.6×10^5 to 1.0×10^5 cfu/ml, CC range from 6.9×10^5 to 0.00 cfu/ml, FCC ranges from 3.7×10^5 to 0.00 cfu/ml while FC ranges from 3.2×10^3 to 1.0×10^3 cfu/ml.

Keywords: Pickling, microbial, coliform count, fungal count, microorganisms

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Introduction

There are different methods of food preservation; these include drying (spray drying, freeze drying), freezing, canning, vacuum-packing, sugar crystallization, pickling, food irradiation, etc. (Abdulmumeen *et al.,* 2012). Pickling is a method of preserving foods in an edible anti-microbial liquid or in a high-acid solution, either by adding vinegar or naturally by means of fermentation (Abdulmumeen *et al.,* 2012). It can be broadly categorized as chemical pickling (fermentation) and fresh-pack pickling (quick). Both types of pickles are preserved by acid. The acid may be: "acetic acid" from commercial vinegar (5 percent acetic acid) used in fresh pack or quick process pickles and relishes or "lactic acid" produced by bacteria in fermented or crock pickles (Barbara, 2008).

In chemical pickling, the food is placed in an edible liquid that inhibits or kills harmful bacteria and other micro-organisms. Many chemical pickling processes also involve heating or boiling so that the food being preserved becomes saturated with the pickling agent. Common chemically pickled foods include cucumbers, carrot, peppers, corned beef, herring, and eggs, as well as mixed vegetables such as piccalilli etc. In fermentation pickling, the food itself produces the preservative agent, typically by a process that produces lactic acid (Abdulmumeen *et al.,* 2012).

Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite, sulfites (sulfur dioxide, sodium bisulfite, potassium hydrogen sulfite, etc.) and disodium. Natural substances such as salt, sugar, vinegar, alcohol, and diatomaceous earth are also used as traditional preservatives (Abdulmumeen *et al*., 2012).

For food products, U.S. Code of Federal Regulations (21 CFR Part 114) states that only acid or acid ingredients which will be acetic acid (vinegar) must be added so that the pH is maintained at or below 4.6; a heat treatment must be included in the process, if necessary, to prevent the growth of microbial pathogens. These regulations were designed to control the growth and toxin production by Botulism *Clostridium botulinum*. The regulations do not take into account the amount or type of organic acid present in acidified foods (Breidt *et al.,* 2004).

When the food is to be stored for a prolonged period, use of additives and preservatives is essential in order to maintain its quality and flavor (Winter, 1994). Excess water in the foods can cause growth of bacteria, fungi and yeasts. Use of additives and preservatives prevents spoiling of the foods due to the growth of bacteria and fungi. Additives and preservatives maintain the quality and consistency of the foods (Winter, 1994). They also maintain palatability and wholesomeness of the food, improve or maintain its nutritional value, control appropriate pH, provide leavening and colour, and enhance its flavour (Pandey and Upadhyay, 2014).Therefore, the main objective of this research was to determine the effect of different pickling agents on the microbial growth of okra. The specific objectives were to carry out pickling in different packaging materials (stainless steel, glass and plastic jars) using different

pickling agents (olive oil, groundnut oil and vinegar) and to compare the microbial response of okra when spices (ginger, garlic and mixture of ginger and garlic) were added and when spices were not added (control).

Material and Methods

Sample Collection

The materials used in this research were carefully selected based on relative availability, cost, and engineering properties. The okra was gotten from International Institute of Tropical Agriculture, Ibadan, Nigeria(I.I.T.A.) and classified as variety 47-4 (according to NIHORT classification) while spices, other pickling agents as well as packaging materials were obtained from Shoprite(a grocery store).

Preparation of Antimicrobial Liquids (Pickling Agents)

Preparation of Groundnut Oil, Olive oil and Vinegar

Groundnut oil was extracted from groundnut seeds by sun drying the groundnut seeds in order to aid the removal of the husk (seed coat) followed by seeds sorting/grading to remove extraneous materials (dirts) in the groundnut seeds. The seed coats were removed and separated from the seeds before size reduction. The naked seeds were milled and loaded into a hydraulic press where it was heated to 70^{o}C for 10mins before pressure was applied (Plate 1). As the pressure was applied the groundnut oil leached out and collected.

Olive oil is a natural preservative that prevents spoilage by isolating the food from air, providing a seal that can delay oxidation, deterioration and molding. Distilled vinegar (5% acetic acid) which is clear colourless vinegar with a mellow aroma and a tart acid flavour was used for this research.

Preparation of culture Media

The media to be used for this analysis are Nutrient Agar (NA) for total bacteria, MacConkey agar for enumeration of coliform bacteria, Eosin Methylene Blue agar for fecal coliform enumeration, Demann Rogossa Sharpe agar for enumeration of lactobacillus, Yeast Extraction agar for enumeration of yeast and Potato Dextrose Agar (PDA) for enumeration of fungi count. The said culture media were prepared in line with the manufacturer's instruction. The colonies were counted and associated microorganisms were isolated, characterized and identified according to the techniques described by Fawole and Oso, (2007) in the laboratory manual of microbiology.

Preparation of Spices

Preparation of Garlic and Ginger

The outer layer of the garlic was removed with the aid of sharp knife and then made to cloves before used. Fresh ginger with a root that is firm and smooth to the touch was chosen and ginger was peeled before use. This involves removing the thick outer skin with a sharp and sturdy knife. Rhizome of ginger of virtually uniform size of ginger was used for this research.

Figure 1: Groundnut oil extraction set up

Okra Pickling Process

Sterilization of Packaging Materials: The packaging materials were prepared for pickling process by sterilizing the packaging jars and lids. Steamer rack (clean dish towel) was placed at the bottom of water bath, and jars were placed on the steamer rack. The water bath was filled with water to the rim of the jars. Steamer rack was used to prevent the jars from touching the bottom of the water bath because they may break as a result of the heat (thermal shock). Then boil for 10 minutes.

To sterilize the lids, the lids were placed in a large bowl and boiling water was poured over them. Pickling agents were placed in a medium saucepan separately and boiled to keep warm. Okra and spices were sorted/graded based on their average lengths and physical appearances. Stem ends of the okra were trimmed to 1/4-inch and then rinsed. Garlic cloves and ginger rhizomes were placed into separate small bowls. Each jar was labelled based on initial set up.

Spices were measured and weighed as well as the okra. Weighed pickling spices were introduced into each required labelled jars and okra was packed into the jars alternating stem-side-up and stem-side-down to allow the okra to be well packed into the jars. The top of the okra should come between an inches to 1/2 an inch from the rim of the jar. Hot pickling agents (vinegar, olive oil, groundnut oil) were poured over the jars, up to 1/4-inch from the rim of the jars. A thin knife was run between the okra and the jars to dislodge any obvious air bubbles. Okra is filled with air, so while running the knife between the okra and the jars, air bubbles were released from the mixture and within the okra as well. The rim of the jars was wiped with clean damp towel.

Sterilized lids were placed on their corresponding jars. The lids were screwed firmly, but not too tight. Picked jars were placed back in the water bath with water used

to sterilize the jars and pasteurized at 74^{0}C for were removed and allow to cool. As the jars cool, a popping sound was heard as the vacuum created by the cooling air in the jars. The jars was completely sealed and stored at room temperature. Plate 2 shows okra pickling 48hours using pH meter.

15 minutes. After the pasteurization, the jars set up. The temperature (^{0}C)and relative humidity (%) of the storage environment were taken three times daily at 8:00am, 12noon and 6:00pm while pH of the samples was measured and recorded consecutively at every

Figure 2: Okra Pickling set up

Microbial Analysis

The effect of pickling agents and spices on the microbial growth of okra in different packaging materials were investigated using ANOVA at $p \leq 0.05$ and the significant means were further evaluated using

Duncan's New Multiple Range Test (DNMRT) as shown in Table 1.

Table 1: Design Layout for Possible Treatment Combinations

				Materials		
				M1	M2	M3
Pickling Agent	P1	Spices	S1	M1P1S1	M2P1S1	M3P1S1
			S2	M1P1S2	M2P1S2	M3P1S2
			S3	M1P1S3	M2P1S3	M3P1S3
			S4	M1P1S4	M2P1S4	M3P1S4
	P2		S1	M1P2S1	M2P2S1	M3P2S1
			S2	M1P2S2	M2P2S2	M3P2S2
			S3	M1P2S3	M2P2S3	M3P2S3
			S4	M1P2S4	M2P2S4	M3P2S4
	P3		S1	M1P3S1	M2P3S1	M3P3S1
			S2	M1P3S2	M2P3S2	M3P3S2
			S3	M1P3S3	M2P3S3	M3P3S3
			S4	M1P3S4	M2P3S4	M3P3S4

Materials (M1=Stainless Steel, M2= Plastics, M3= Glass Jar), Pickling Agent (P1= Olive Oil, P2= Groundnut oil, P3= Vinegar), and Spices (S1=Ginger, S2=Garlic, S3=Mixture, S4=No Spice)

Results and Discussion

Results

From Table 2, it was observed that depending on the material, pickling agent and the spice employed in the storage process, there are variations in total viable count of pickled okra. Similarly, variances were also observed in coliform count of the pickled okra from different spices along the various pickling agents and materials. Similar observation also exists for fecal coliform count and fungi count. These may imply that the behaviors of microbial count in pickled okra under varied storage condition are not the same with regard to the parameters used.

Table 3shows the effects of the measured parameters on behavior of microbial count. The results revealed that stored okra using the three selected materials (stainless, glass and plastic), the three selected pickling agents and the two spices had significantly different total viable count, coliform count, fecal coliform count, and fungal count at 5% level. Also the interactions between study parameters were also significant at 5%.Table 4 shows the different mean values of the microbial counts in the materials assuming all other parameters were fixed.

Table 2: Summary Statistics of the Measured Data

Materials	Pickling Agents	Spices	TVC	CC	FCC	FC
Stainless steel	Olive Oil	Ginger	8.567	5.700	1.200	3.300
		Garlic	5.900	4.767	0.000	2.367
		Mixture	8.233	6.500	0.000	3.000
		No Spice	5.300	3.600	1.767	2.267
	Groundnut Oil	Ginger	3.400	2.467	1.033	1.267
		Garlic	3.567	2.800	0.000	1.633
		Mixture	3.267	2.900	2.167	1.400
		No Spice	6.700	5.100	3.233	2.667
	Vinegar	Ginger	1.267	0.000	0.000	1.067
		Garlic	1.567	1.167	1.000	1.033
		Mixture	1.267	0.000	0.000	1.133
		No Spice	2.367	1.667	0.000	1.167
Glass	Olive Oil	Ginger	4.933	4.067	0.000	2.067
		Garlic	7.833	6.900	3.600	2.867
		Mixture	3.000	2.433	0.000	1.467
		No Spice	4.467	4.000	0.000	2.333
	Groundnut Oil	Ginger	8.000	6.967	0.000	2.767
		Garlic	8.300	7.167	0.000	3.167
		Mixture	7.767	6.100	2.400	2.600
		No Spice	3.967	3.200	0.000	1.667
	Vinegar	Ginger	1.167	0.000	0.000	1.000
		Garlic	1.133	0.000	0.000	1.000
		Mixture	1.600	0.000	0.000	1.033
		No Spice	1.000	0.000	0.000	1.200
Plastics	Olive Oil	Ginger	3.900	3.067	0.000	1.367
		Garlic	4.900	3.633	0.000	2.267
		Mixture	2.167	1.767	1.433	1.167

		No Spice	3.200	2.400	2.000	1.067
	Groundnut Oil	Ginger	1.567	1.067	0.000	1.200
		Garlic	7.533	6.000	2.000	2.733
		Mixture	1.600	1.067	0.000	1.100
	Vinegar	No Spice	4.767	3.233	2.367	2.100
		Ginger	1.233	0.000	0.000	1.033
		Garlic	1.433	1.100	1.000	1.200
		Mixture	1.800	0.000	0.000	1.000
		No Spice	1.100	0.000	0.000	1.400

TVC: Total Viable Counts (cfu/ml), **CC:** Coliform Counts (cfu/ml), **FCC:** Fecal Coliform Counts (cfu/ml), **FC:** Fungi Counts (cfu/ml)

Table 3: Multivariate Analysis of Variance for Measured Parameters

Source	Dependent Variable	Sum of Squares	Df	Mean Square	F	Sig.
M	TVC	49.030	2	24.515	2522.000	0.000*
	CC	41.782	2	20.891	3178.000	0.000*
	FCC	2.480	2	1.240	405.818	0.000*
	FC	4.429	2	2.214	451.245	0.000*
P	TVC	330.278	2	165.139	16990.000	0.000*
	CC	330.362	2	165.181	25130.000	0.000*
	FCC	16.640	2	8.320	2723.000	0.000*
	FC	22.811	2	11.405	2324.000	0.000*
S	TVC	25.108	3	8.369	860.835	0.000*
	CC	32.194	3	10.731	1632.000	0.000*
	FCC	9.241	3	3.080	1008.000	0.000*
	FC	3.411	3	1.137	231.717	0.000*
MP	TVC	94.839	4	23.710	2439.000	0.000*
	CC	61.123	4	15.281	2324.000	0.000*
	FCC	4.283	4	1.071	350.455	0.000*
	FC	10.286	4	2.571	523.981	0.000*
MS	TVC	53.450	6	8.908	916.283	0.000*

	CC	33.851	6	5.642	858.192	0.000*
	FCC	19.884	6	3.314	1085.000	0.000*
	FC	4.727	6	0.788	160.528	0.000*
PS	TVC	28.918	6	4.820	495.730	0.000*
	CC	10.893	6	1.816	276.164	0.000*
	FCC	13.124	6	2.187	715.838	0.000*
	FC	3.227	6	0.538	109.604	0.000*
MPS	TVC	105.434	12	8.786	903.721	0.000*
	CC	75.751	12	6.313	960.221	0.000*
	FCC	52.889	12	4.407	1442.000	0.000*
	FC	9.726	12	0.810	165.151	0.000*
Error	TVC	0.700	72	0.010		
	CC	0.473	72	0.007		
	FCC	0.220	72	0.003		
	FC	0.353	72	0.005		
Total	TVC	2315.650	108			
	CC	1433.710	108			
	FCC	171.680	108			
	FC	390.770	108			

*Significant at 5% level

Table 4: Multiple Comparison using the New Duncan range Test

Factor	Levels	TVC	CC	FCC	FC
Material	Stainless steel	4.283a	3.056a	0.867a	1.858a
	Glass	4.431b	3.403b	0.500b	1.931b
	Plastic	2.933c	1.944c	0.733c	1.469c
Pickling Agent	Olive Oil	5.200a	4.069a	0.833a	2.128a
	Groundnut Oil	5.036b	4.006b	1.100b	2.025b
	Vinegar	1.411c	0.328c	0.167c	1.106c
Spice	Ginger	3.782a	2.593a	0.248a	1.674a
	Garlic	4.685b	3.726b	0.844b	2.030b
	Mixture	3.411c	2.307c	0.667c	1.544c
	No Spice	3.652a	2.578d	1.041d	1.763d

Mean with the same alphabet are not significantly different from each other

Discussion

From Table 2, it was observed that olive oil spiced with ginger as well as the mixture of spices stored in stainless steel jar has higher TVC value of 8.6×10^5 cfu/ml and 8.2×10^5 cfu/ml respectively while almost the same trend was observed in groundnut oil spiced with garlic, ginger and the mixture of garlic and ginger with 8.3×10^5 cfu/ml, 8.0×10^5cfu/ml and 7.7×10^5 cfu/ml TVC respectively, but least TVC ranging from 2.3×10^5 cfu/ml to 1.0×10^5 cfu/ml was observed in okra pickled with vinegar irrespective of the spice and the container used (Figure 1).

In Figure 2, higher CC value of 7.1×10^5 cfu/ml, 6.9×10^5 cfu/ml and 6.1×10^5 cfu/ml was respectively observed in groundnut oil spiced with garlic, ginger and mixture of garlic and ginger preserved in glass jar while CC of okra pickled in olive oil spiced with ginger and mixture of garlic and ginger preserved in stainless steel have values of 6.5×10^5 cfu/ml and 5.7×10^5 cfu/ml respectively as well as garlic in groundnut oil preserved in plastic jar with CC value of 6.0×10^5 cfu/ml.

In Figure 3, No trace of CC was observed in vinegar preserved in glass jars irrespective of the spice while the least CC value was also observed in other vinegar regardless of the

spice and container ranging from 1.0×10^5 cfu/ml to 1.6×10^5 cfu/ml. Figure 4 show a FCC values ranging from 1.0×10^5 cfu/ml to 3.6×10^5 cfu/ml in some of the preserved okra while FCC was observed to be absent in most of the samples especially in vinegar. Most samples especially vinegar (irrespective of the spice and the container) have FC values ranging from 1.0×10^5 cfu/ml to 1.4×10^5 cfu/ml while other samples have FC value ranging from 1.6×10^5 cfu/ml to 3.3×10^5 cfu/ml.

It can be inferred from Table3 that samples pickled in stainless steel using olive oil, groundnut oil and vinegar respectively have TVC of 54.47%, 32.94%, 12.56%, CC of 56.09%, 36.18%, 7.73%, FCC of 28.53%, 61.86%, 9.62% and FC of 49.03%, 31.24%, 19.73%. Samples pickled in glass jars using olive oil, groundnut oil and vinegar respectively have TVC of 38.06%, 52.92%, 9.22%, CC of 42.61%, 57.39%, 0.00%, FCC of 60.00%, 40.00%, 0.00% and FC of 37.70%, 44.03%, 18. 27%.Also samples pickled in plastic jars using olive oil, groundnut oil and vinegar respectively have TVC of 40.26%, 43.95%, 15.79%, CC of 46.57%, 48.71%, 4.71%, FCC of 39.01%, 49.63%, 11.36% and FC of 33.28%, 40.45%, 26.27%.

The variation in the microbial load may be as a result of host of environmental factors, such as heat and the presence of microorganisms which acts to change foodstuffs in ways that may harm the food product as suggested by Muhammed and Kayode (2014).Low microbial counts observed on samples pickled in vinegar is in line with the U.S. Code of Federal Regulations (21 CFR Part 114).

It can be inferred from Table 4 that the mean total viable count (TVC) of pickled okra in glass was significantly higher (4.431×10^5 cfu/ml) than those of stainless steel (4.238×10^5 cfu/ml) and plastics (2.933×10^5 cfu/ml) irrespective of pickling agent and/or spice used for preservation of the okra. Similarly, the same trend was observed in the mean coliform count (CC) of the okra pickled in glass (3.403×10^5 cfu/ml) which was significantly higher than the coliform count of 3.056×10^5 cfu/ml and 1.944×10^5 cfu/ml observed in stainless steel and plastic respectively, as well as the mean fungal count (FC) in glass material (1.931×10^3 cfu/ml) which was significantly higher than those preserved in stainless steel (1.85×10^3 cfu/ml) and plastic (1.469×10^3 cfu/ml). Lastly, mean fecal coliform count (FCC) was significantly higher in stainless steel (0.867×10^5 cfu/ml)

than in glass (0.5×10^5 cfu/ml) and plastic (0.733×10^5 cfu/ml) respectively.

The mean total viable count (TVC) of pickled okra using olive oil (5.200×10^5 cfu/ml) was significantly higher than the total viable count of pickled okra using groundnut oil (5.036×10^5 cfu/ml). Okra pickled using vinegar has the least TVC of (1.411×10^5 cfu/ml). This value is about one fifth of the TVC observed for olive and groundnut oil respectively. Similar trend was observed for mean coliform count (CC) and mean fungal count (FC) of the preserved okra where olive oil had significantly higher coliform count (4.069×10^5 cfu/ml) and fungal count (2.128×10^3cfu/ml) followed by groundnut oil (4.006×10^5 cfu/ml, 2.025×10^3 cfu/ml) respectively. Fecal coliform count was significantly higher in okra preserved using groundnut oil as pickling agent (1.100×10^5 cfu/ml) than all other pickling agent investigated.

Spice was seen to play some role in microbial growth or behavior as observed in the experiment. Table 4 imply that mean TVC of pickled okra spiced with garlic was statistically higher (4.685×10^5 cfu/ml) compared to pickled okra spiced with ginger (3.782×10^5 cfu/ml) and the mixture (3.411×10^5 cfu/ml) of the two spices. Consequently ginger did not significantly altered the TVC of pickled okra since it has almost the same TVC with pickled okra with no spice additive (3.652×10^5 cfu/ml). Similarly, all microbial growth such as CC, FCC, and FC, were significantly altered by the addition of garlic as a spicing agent. It was also observed that ginger does not change the coliform count and fungal count of stored okra irrespective of the material or pickling agent used. However, garlic significantly decreases the FCC of pickled okra.

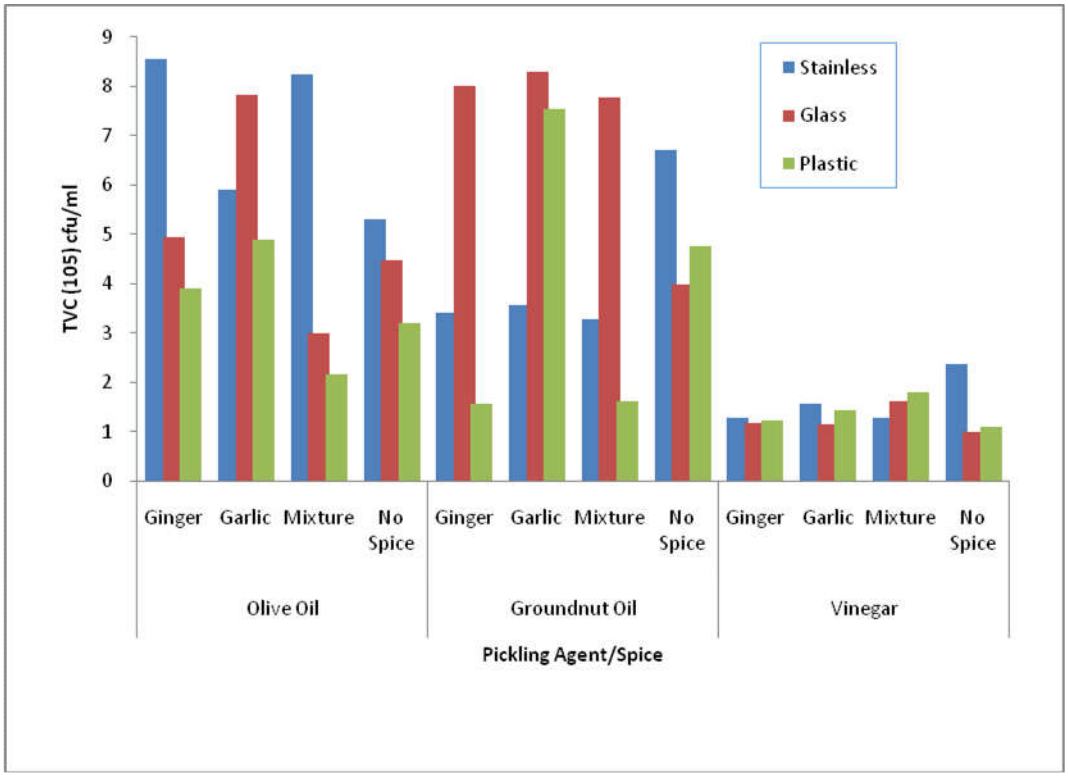

Figure 1: Total Viable Count in Various Pickling Agent and Spice

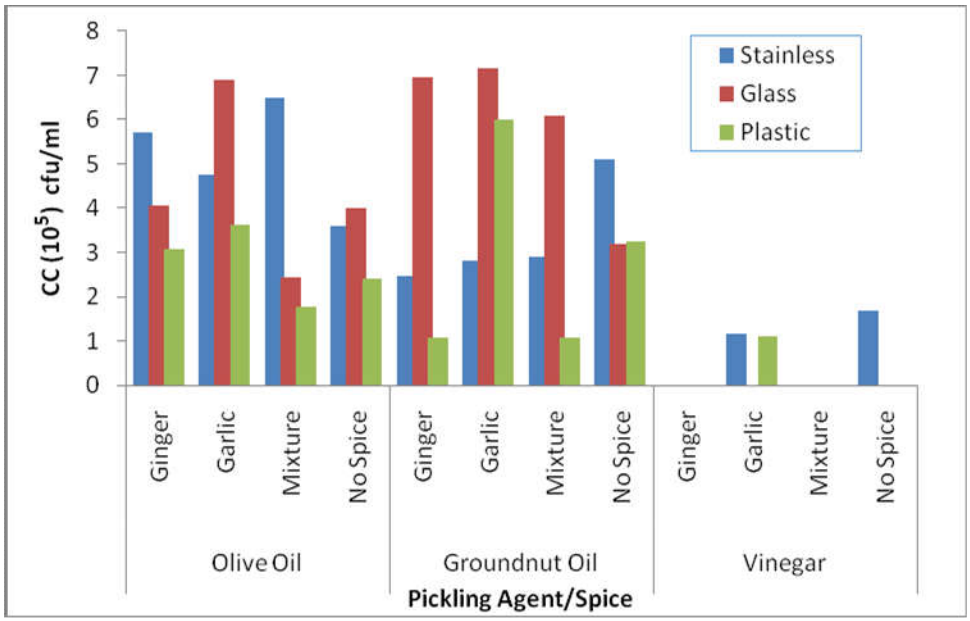

Figure 2: Coliform Count in Various Pickling Agent and Spice

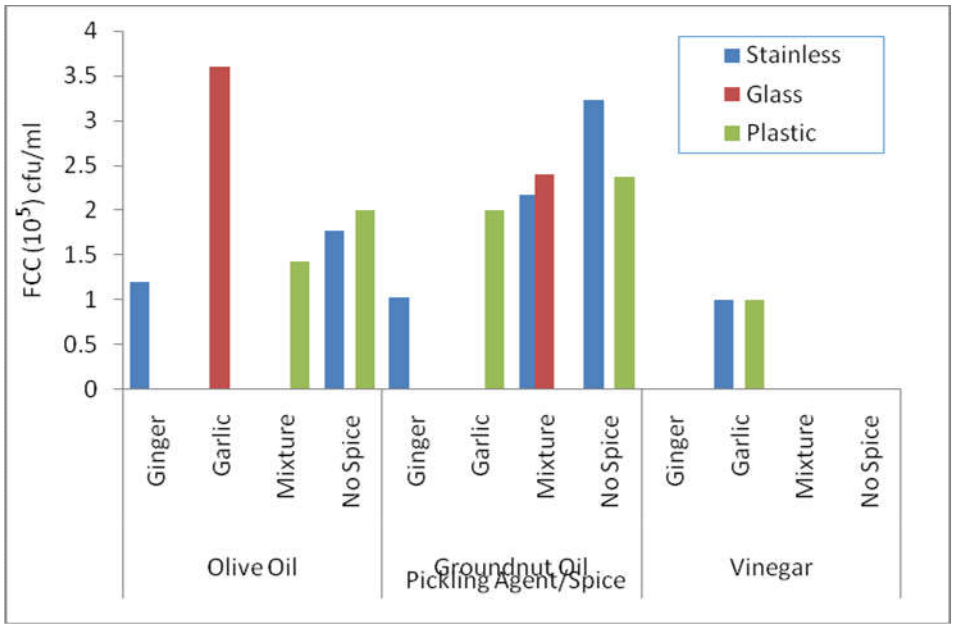

Figure 3: Fecal Coliform Count in Various Pickling Agent and Spice

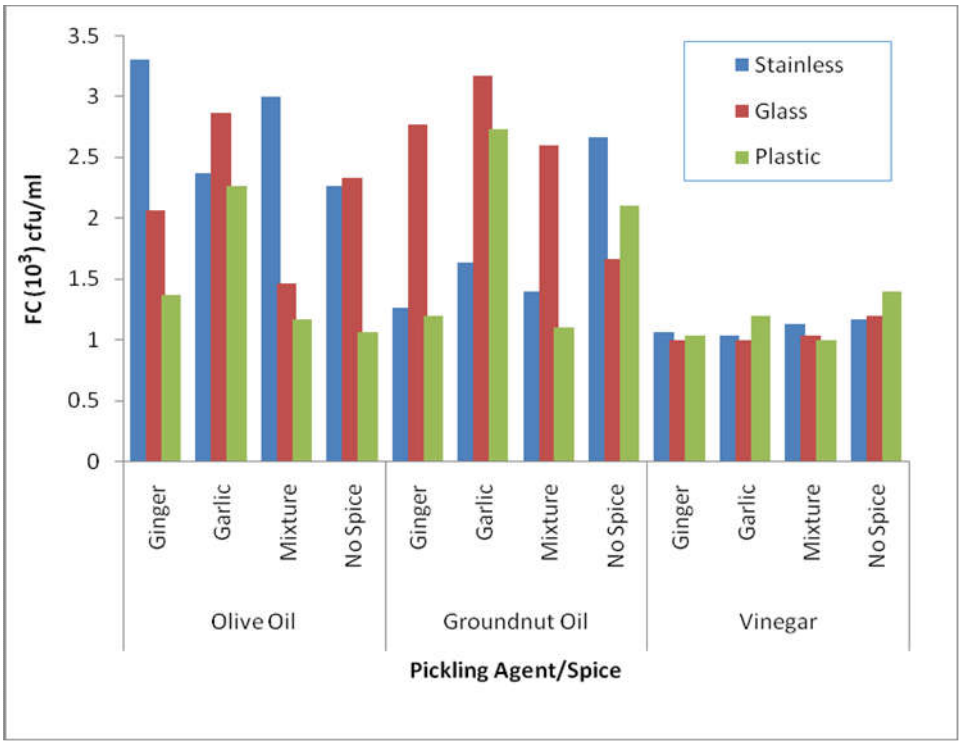

Figure 4: Fungal Count in Various Pickling Agent and Spice

Conclusion

It can be concluded that all pickling agents spiced with garlic and preserved in stainless steel jars have low microbial count compare to no spice, ginger and mixture of spices. Garlic also significantly shows an increase in the microbial loads in glass and plastic packaging materials irrespective of the pickling agent used except for FCC in olive oil and groundnut oil. The low microbial count result may be due to opaque properties of the stainless steel jar while the increase microbial load may be as a result of the transparency of both the plastic and the glass jars which may influence the growth of the organisms present as well as sensitivity of the organisms to light. This means that garlic as a spice have significant effect on microbial growth when used in different packaging materials with different pickling agents. Also, microbial load is significantly increased when pickled with olive oil in stainless steel and spiced with ginger as compared to others, but reduced microbial load is observed when groundnut oil and vinegar were used as pickling agents in stainless steel jars.

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