Journal of Food Technology 3(2): 113-117, 2005

Microbial Profile of Alligator pepper (*Aframomum melegueta*) and Negro pepper (*Xylopia aethiopica*) During processing

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Abstract: The microbial profile of fresh Alligator pepper (*Afromomum melegueta*) and Negro pepper (*Xylopia aethiopica*) was studied. The efficacy of the traditional method of processing in reducing the microbial load of the pepper samples was also investigated. The total aerobic mesophilic bacteria count of the fresh pepper ranged from 5.43 $\log_{10}$ cfu/g to 6.88 $\log_{10}$ cfu/g in Alligator pepper and 6.43$\log_{10}$ cfu/g to 6.54$\log_{10}$ cfu/g in Negro pepper.Coliform counts ranged from 5.23 $\log_{10}$ cfu/g in Negro pepper to 6.11 $\log_{10}$ cfu/g in Alligator pepper. The fungal counts showed that yeasts were predominant in the fresh pepper samples. The microflora associated with the fresh pepper samples included species of *Staphylococcus, Micrococcus, Bacillus, Serratia, Escherichia, Enterobacter, Aspergillus, Fusarium, Curvularia, Itersonilia, Botrydiplodia, Mucor, Penicillium, Brettanomyces* and *Candida*.Traditional processing methods involving washing, steeping or cooking in boiling water reduced the numbers and types of microorganisms in the pepper samples. This is reflected in the disappearance of yeast and coliforms.The low microbial level of the treated pepper was sustained during sun drying. Our data suggest that although the fresh pepper samples contain appreciable microbial load, their numbers and types are reduced by the traditional methods employed in their processing. Thus improving the quality of the processed pepper

Key words: Alligator pepper, Negro pepper, Microorganisms, Processing

Introduction

Alligator pepper (*Aframomum melegueta* (Roskoe) K. Schum) and Negro pepper (*Xylopia aethiopica* (Dun.) A. Rich) belong to the family Zingiberaceae and Annonaceae respectively. Alligator and Negro pepper also known as Grains of paradise and Ethiopian pepper respectively falls among the plants with promising anti-infective activity described by Iwu *et al.* (1999). These peppers are fully described in literature (Dalziel, 1955; Lock *et al.,* 1977 and Isawumi, 1984). Their medicinal uses are well documented (Addae-Mensah and Aryee, 1992 and Iwu, 1993) and include carminative, diuretic, aphrodisiac and lactation aid. They are also used in cosmetic products and perfumes (Burkill, 1985). The use of Negro and Alligator pepper as spices is generally confined to West African cookery. Negro pepper is used in the preparation of local soups in the Southwest and Southeastern parts of Nigeria (Tairu *et al.,* 1999) while Alligator pepper is used as flavoring in alcoholic beverages such as beer, ale, wine, gin and vinegar (Hesser, 2000).

Information on the medicinal, antimicrobial and chemical composition of these peppers is enormous (Iwu, 1993; Lajide *et al.,* 1995; Tairu *et al.,* 1999 and Konning *et al.,* 2004) while there is paucity of information on their microbial contaminants. Commercially available fresh herbs and whole spices have been reported to carry various microorganisms that include aerobic spoilage bacteria, fungi and potential pathogens (Kneifel and Berger, 1994 and McKee, 1995). The use of such unclean herbs and spices without an appropriate intervention step to eliminate microflora may result in contamination and spoilage of food or survival of pathogens with the potential for the product to cause foodborne illness (Krishnaswamy *et al.,* 1971; Powers *et al.,* 1976 and Andress *et al.,* 2001). This study is designed to investigate the microbial profile of fresh Alligator and Negro pepper, and to determine the efficacy of the traditional processing methods in reducing the microflora of the spices.

Materials and Methods

Source of Samples: Fresh fruits of Alligator pepper and Negro pepper were procured from retail markets in Ile-Ife, Osun state, Nigeria.

Processing of Samples: Fresh matured green fruits of Negro pepper were divided into 2 portions. One portion was left untreated and designated as "fresh samples". The second portion was processed following the traditional post harvest treatment practiced locally in the localities where they are utilized in Nigeria. Essentially, the fruits were washed, cooked in boiling water for 20 minutes on a Gallenkamp hot plate. Excess cooking water was drained off and the cooked seeds were designated as "treated samples". The fresh and treated samples were then spread out in clean enamel trays and subjected to sun drying. Drying of samples continued until a moisture content of about 10-12% was achieved (Purseglove *et al.,* 1981).

Ripe Alligator pepper fruits were divided into two portions. One portion was left as whole fruits while the second portion was treated. The treatment involved the removal of the seeds from the rind manually and division into two portions. One portion was washed and steeped in boiling water for 5min. while the other portion was left and designated as "untreated seeds". The whole fruits and seeds were then sun dried as described for Negro pepper samples.

Microbiological analysis: The microbial load on the surface and homogenate of the untreated fresh and treated samples of Alligator and Negro pepper were estimated before drying and during drying as previously described (Omafuvbe and Kolawole, 2004). Nutrient agar (oxoid) and Eosin methylene blue agar were used for the isolation of total aerobic mesophilic bacteria (TAMB) and coliforms respectively. Malt extract agar containing 50lg streptomycin per ml. was used for the isolation of yeast and mould. The bacteria isolates were identified according to the scheme of Harrigan and McCance (1976) while mould and yeast were identified as described by Collins and Lyne (1970), Barnett and Hunter (1972) and Lodder (1971).

Moisture Content Determination: The moisture content of fresh, treated and sun dried samples was determined in accordance with the method of the Association of Official Analytical Chemists (AOAC, 1990).

Results and Discussion

The microbial load of fresh and treated samples of Alligator and Negro pepper is presented in Table 1. The total aerobic mesophilic bacteria count (TAMB) in fresh Alligator pepper ranged from 5.43 $\log_{10}$ cfu/g in the whole fruit to 6.88 $\log_{10}$ cfu/g on the surface of the seeds, while coliform counts ranged from 5.67 $\log_{10}$ cfu/g to 6.11 $\log_{10}$ cfu/g respectively. Negro pepper on the other hand had a TAMB count which ranged from 6.43 $\log_{10}$ cfu/g in the fruit homogenate to 6.54 $\log_{10}$ cfu/g on the fruit surface, while coliform ranged from 5.23 $\log_{10}$ cfu/g to 5.99 $\log_{10}$ cfu/g respectively. It is significant to note that the fresh samples of Alligator and Negro pepper have higher numbers of TAMB and coliforms than mould and yeast (Table 1). The fungal (mould and yeast) counts demonstrated that yeasts were the predominant fungi in the fresh pepper samples (Table 1). Similar result has been reported in other spices (Andress *et al.*, 2001). Also, the removal of Alligator seeds from the rind of the fruits increased the microbial load of the seeds that should otherwise contain very low numbers if any, of microorganisms. Although, there is no available literature on the microbial load of fresh Alligator and Negro pepper, our result falls within the range reported for other spices (Beckmann *et al.*, 1996; Garcia *et al.*, 2001; Andress *et al.*, 2001 and Omafuvbe and Kolawole, 2004). The growing and harvesting conditions of the pepper samples and post-processing environmental exposure may have contributed to their microbial load. According to Julseth and Deibel (1974), spices are grown and harvested in areas of the world where sanitary practices are often poor. In addition, the humid and warm environment supports the growth of a wide variety of fungi and bacteria.

Table 1: Microbial load* and moisture content of fresh and treated ** Alligator and Negro pepper

Pepper Type	Parameters									
	Fresh sample					Treated samples				
	MC***	Microbial load				MC	Microbial load			
		TAMB[a]	Coliform	Mould	Yeast		TAMB	Coliform	Mould	Yeast
Alligator:										
Whole Fruits:	77.40 ± 0.72	NA	NA	NA	NA	NA	NA	NA	NA	NA
Homogenate	NA	5.43 ± 0.13	5.67 ± 0.30	2.30 ± 0.08	3.77 ± 0.15	NA	NA	NA	NA	NA
Seeds:	43.20 ± 0.50	NA	NA	NA	NA	44.50 ± 0.12	NA	NA	NA	NA
Surface	NA	6.88 ± 0.03	6.11 ± 0.18	2.30 ± 0.04	4.04 ± 0.09	NA	2.00 ± 0.07	0.00	2.40 ± 0.05	0.00
Homogenate	NA	5.97 ± 0.20	6.00 ± 0.03	2.00 ± 0.03	3.23 ± 0.07	NA	0.00	0.00	0.00	0.00
Negro:	65.00 ± 1.00	NA	NA	NA	NA	66.00 ±0.95	NA	NA	NA	NA
Surface	NA	6.54 ± 0.14	5.99 ± 0.18	3.41 ± 0.16	3.36 ± `0.12	NA	2.48 ± 0.08	0.00	1.78 ± 0.06	0.00
Homogenate	NA	6.43 ± 0.04	5.23 ± 0.05	2.78 ± 0.18	3.45 ± 0.04	NA	0.00	0.00	0.00	0.00

Values are means ± SE of three determinations.
* Microbial load expressed as $\log_{10}$ colony forming unit (cfu)/g sample.
** Treatment involved steeping in boiling water for 5min. (Alligator seeds) and Cooking in boiling water for 20min. (Negro pepper)
*** MC; Moisture content expressed as g/100g sample.
NA, Not applicable.
a, Total aerobic mesophilic bacteria (TAMB).

Table 2: Microbial load* and moisture content of fresh and treated Alligator and Negro pepper during drying.

Pepper type	Length of drying (days)	Fresh samples MC**	Fresh samples Microbial load Surface	Fresh samples Microbial load Homogenate	Treated samples MC	Treated samples Microbial load Surface	Treated samples Microbial load Homogenate
Alligator:							
Whole Fruits	7	21.00±0.20	NA	4.26± 0.12	NA	NA	NA
	14	11.80±0.14	NA	2.58±0.06	NA	NA	NA
Seeds:	7	10.30±0.04	4.18±0.12	3.99±0.14	10.20±0.05	2.00±0.02	2.00±0.03
	14	Na	Na	Na	Na	Na	Na
Negro:	7	16.20±0.07	4.89± 0.16	4.32±0.20	18.60±0.05	2.00±0.02	0.00
	14	11.50±0.06	3.30±0.14	2.00±0.03	10.80±0.03	0.00	0.00

Values are means ± SE of three determinations.
* Microbial load expressed as $\log_{10}$cfu/g sample and represents total aerobic mesophilic bacteria count only. Coliform, mould and yeast were either not detected or their counts were not significant.
** Moisture content (MC) expressed as g/100g sample.
NA, Not applicable.
Na, not available since desired moisture content was already achieved within 7 days of sun drying.

Table 3: The occurrence of microorganisms isolated from Alligator and Negro pepper.

Microbial isolates	Alligator Fresh	Alligator Dried	Negro Fresh	Negro Dried
Bacteria:				
Staphylococcus aureus	+	-	+	-
S. epidermidis	+	-	+	-
Micrococcus roseus	+	-	+	-
M. luteus	+	-	+	-
M. varians	+	+	+	+
Bacillus cereus	+	-	-	-
B. subtilis	+	+	+	-
B. firmus	+	-	-	-
Serratia marcescens	+	-	-	-
Escherichia coli	+	-	-	-
Enterobacter sp.	+	-	+	-
Moulds:				
Aspergillus niger	+	+	+	-
A. flavus	+	+	-	-
A. fumigatus	+	+	+	+
A. ochraceous	+	-	+	-
Fusarium solani	+	-	+	-
Curvularia lunata	+	+	-	-
Itersonilia sp.	+	+	-	-
Botrydiplodia theobromae	+	-	-	-
Mucor hiemalis	+	+	-	-
Penicillium verrucosum	+	+	+	+
P. sclerotigenum	+	+	+	-
Yeasts:				
Brettanomyces claussenii	+	-	+	-
B. intermedius	+	-	-	-
Candida guilliermondii	+	-	+	-
C. lusitaniae	+	-	-	-

\+ Present - Absent

In this study, pretreatments such as washing of the fresh pepper samples followed by steeping or cooking in boiling water reduced their microbial load drastically (Table 1). For example, the TAMB count on the surface of Alligator pepper seed and Negro pepper reduced from 6.88 $\log_{10}$ cfu/g to 2.00 $\log_{10}$ cfu/g and 6.54 $\log_{10}$ cfu/g to 2.48 $\log_{10}$ cfu/g sample respectively. The pretreatment step completely eliminated coliform and yeast from the pepper samples. Similar reduction in microbial load following pretreatments involving washing and steeping of spices in boiling water has been reported earlier (Christensen *et al.*, 1967; Andress *et al.*, 2001 and Omafuvbe and Kolawole, 2004). The moisture content of fresh Alligator and Negro pepper increased slightly with pretreatment (Table 1). Similar pattern was reported in black and white pepper processing (Omafuvbe and Kolawole, 2004). The pepper samples may have absorbed some moisture during the steeping and cooking stage.

The microbial load and moisture content of fresh and treated Alligator and Negro pepper samples during sun drying are presented in Table 2. In general, drying reduced the microbial load of both fresh and treated pepper samples. This was reflected in the elimination of coliforms, yeasts and some bacteria species (Table 3). The TAMB on the surface of fresh Alligator and Negro pepper was reduced from 6.88 $\log_{10}$ cfu/g to 4.81 $\log_{10}$ cfu/g and 6.54 $\log_{10}$ cfu/g to 4.89 $\log_{10}$ cfu/g respectively with drying (Table 2). The low numbers resulting from the pretreatment of the peppers were sustained during the drying process. The reduction in microbial load with drying was not surprising since the level of moisture essential for microbial growth was not available (Purseglove *et al.*, 1981). In this study, the recommended moisture content (10-12%) for dried spices was achieved within 14 days of sun drying compared to 4 to 7 days reported for other spices with oven drying (Omafuvbe and Kolawole, 2004). This may be related to the fact that oven drying is free from the vagaries of the weather which could lead to a re-wetting of the samples as is the case with sun drying. However, once the required moisture content of the spices was achieved the numbers and types of microorganisms were reduced irrespective of the method of drying (Omafuvbe and Kolawole, 2004).

The occurrence of microorganisms in fresh and dried Alligator and Negro pepper are presented in Table 3. The microorganisms found in the fresh Alligator pepper included bacteria species namely *Staphylococcus, Micrococcus, Bacillus, Serratia, Escherichia* and *Enterobacter*; mould species such as *Aspergillus, Fusarium, Curvularia, Itersonilia, Botrydiplodia, Mucor* and *Penicillium* and *Brettanomyces* and *Candida* as yeast species. Fresh Negro pepper contains the above named microorganisms with the exception of *Serratia, Escherichia, Curvularia, Itersonilia, Mucor* and *Botrydiplodia* species. Drying of the peppers not only reduced the load but also the types of microorganism found in the samples. Some of the microorganisms found in the fresh and dried Alligator and Negro pepper have been reported as contaminants in other spices and medicinal plants (Aziz *et al.*, 1998; Garcia *et al.*, 2001; Banerjee and Sarkar, 2003 and Omafuvbe and Kolawole, 2004). Of significant note is the predominance of *Micrococcus luteus, Bacillus subtilis, Aspergillus fumigatus* and *Penicillium verrucosum* in both the fresh and dried pepper samples. These microorganisms are able to survive dry conditions. It is also interesting to note that Negro pepper contain fewer microbial species than Alligator pepper. This may be related to their antimicrobial properties. Although, the antimicrobial properties of the peppers on the isolated microorganisms were not tested in this study, some spices have been reported to harbor fewer microbial species because of the marked antimicrobial effect of their essential oil (Frazier and Westhoff, 1978).

On the basis of our data, we conclude that although fresh Alligator and Negro pepper contains appreciable microbial load, their numbers are still within the acceptable ICMSF (International Commission on Microbiological Specification for Food) limits. Traditional treatments involving washing, steeping in boiling water and drying reduced the microbial populations and therefore improved the quality of Alligator and Negro pepper.

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