

Quality Assurance of Stored Pepper (*Piper guineense*) Using Controlled Processing Methods

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Abstract: The microflora of black and white pepper (*P. guineense*) during processing and storage were enumerated, isolated and identified. The fresh untreated pepper samples gave appreciable total aerobic mesophilic bacteria (TAMB) counts of 6.65 log₁₀ cfu/g in the white pepper and 7.04 log₁₀ cfu/g in the black pepper. Coliform counts ranged in number from 6.23 to 6.80 log₁₀ cfu/g while yeast and mould counts ranged from 2.00 to 3.74 log₁₀ cfu/g sample. The microflora associated with the untreated fresh pepper samples included species of *Staphylococcus*, *Micrococcus*, *Bacillus*, *Serratia*, *E. coli*, *Aspergillus*, *Fusarium*, *Itersonilia*, *Botrydiplodia*, *Penicillium*, *Mucor*, *Candida*, and *Brettanomyces*. Pretreatments involving steeping in boiling water for up to 20mins, surface disinfection with 2% formaldehyde solution and washing reduced the microbial load of the pepper samples to zero or less than 1.0 log₁₀ cfu/g; this was accompanied by the disappearance of yeast, coliforms and certain other species of bacteria. The low numbers were maintained during the drying period, whether in the oven or in the sun and later also during storage of the dried pepper samples. Our findings suggest that even in the traditional setting, simply washing and steeping, followed by controlled sun drying, could drastically and effectively decrease the associated microbial populations on pepper samples. The pretreatments of pepper samples described in this study kept the microbial load within the ICMSF acceptable limits throughout the storage period. This processing method has therefore been shown to be capable of elongating the shelf-life of the product and thus ensure the maintenance of good quality during storage for at least three months.

Key words: Black pepper, white pepper, microflora, fresh pepper

Introduction

Piper guineense (Schum and Thonn) commonly referred to as African black pepper or Ashanti pepper, is very similar to *Piper nigrum*, which is the true pepper of commerce from which 'black' and 'white' peppers are processed (Isawumi, 1984). *Piper guineense* as a plant has been fully described in literature (Hutchinson and Dalziel, 1954). Apart from its culinary uses, *Piper guineense* has been reported to have medicinal, cosmetic (Dalziel, 1955) and insecticidal (Fasakin and Aberejo, 2002) properties.

White and black pepper can be produced from *P. guineense* in much the same way as from *P. nigrum*. The fruit of *P. guineense*, called peppercorn, is the spice. 'Black' pepper is produced from unripe fully developed berries while 'white' pepper is from fully ripe decorticated and dried peppercorns (Purseglove *et al.*, 1981).

Spice plants are usually harvested and processed traditionally under variable sanitary conditions in many developing countries like Nigeria. This gives room for high levels of bacteria and fungal contamination. With the increasing awareness of hygiene and food spoilage, Microbiologists have acknowledged that traditional spices and herbs can increase the bacterial levels of some foods, which may result in their deterioration and possibly cause food poisoning. This paper is a report of the use of improved processing methods in the

production of black and white pepper from *P. guineense* purchased fresh from retailers and subjecting them to microbiological studies during storage for a period of three months.

Materials and Methods

Source of samples: Fresh fruits of fully developed unripe (for black pepper) and fully ripe (for white pepper) *P. guineense* seeds were purchased from local markets in Ile-Ife, Osun State, Nigeria.

Processing of samples

Black pepper: Fresh unripe berries of *P. guineense* were removed from the spikes and divided into three lots. One lot was left untreated, the second lot was steeped in boiling water for 15min as described by Parry (1969) while the third lot was steeped in boiling water and surface disinfected with 2% formaldehyde solution for 2 min as described by Christensen *et al.* (1967). Each of these lots was further divided into two portions, one of which was subjected to sun drying while the other was dried in the oven at 40°C. The drying continued until a moisture content of 10-12% was achieved (Purseglove *et al.*, 1981).

White pepper: White pepper was prepared from fresh fruits of fully ripe *Piper guineense* following the water

steeping and retting technique described by Pruthi (1980) with slight modification. Essentially, ripe berries were washed with tap water, packed in muslin bags and soaked in a basin of water for 7 days with daily changes of the steeping water. Retted berries were de-skinned by rubbing with hands and washed in running water. De-skinned berries were divided into two portions, one was surface-disinfected before the lots were dried as described above for black pepper.

Storage of samples: For storage, each of the dried sample lots was further divided into two portions. One set was ground into fine powder using the dry grinding compartment of a Moulinex mixer-blender mill 2 (sterilized by washing and rinsing with 80% ethanol solution) while the other set was left as dried whole fruits. Equal amounts of the samples were then distributed into screw-capped bottles, screwed tight and stored for three months at room temperature.

Microbiological analysis: The microbial load on the surface of black and white pepper samples collected immediately before drying, during drying and storage and their respective homogenates were estimated. Five grams of each sample was aseptically transferred into sterile 45ml of 0.1% peptone water diluent in a conical flask, the mixture was vigorously shaken for 5min and the wash-water kept. The washed sample was transferred into a sterile stomacher bag and homogenized in 45ml of fresh 0.1% peptone water with the aid of a Colworth Stomacher (Model 400). The wash water and sample homogenates were then diluted serially and appropriate dilutions plated out in triplicates. One set was plated out on Nutrient agar (NA Oxoid) and incubated at 30°C for up to 48h for total aerobic mesophilic bacteria (TAMB), the second on Eosin methylene blue agar (EMB), incubated at 37°C for 24h for coliform, while the third set was plated out on Malt Extract agar (containing 50µg streptomycin per ml) and incubated at 28°C for up to 7 days for yeast and mould. The bacterial isolates were identified according to the schemes of Harrigan and McCance (1976) and Buchanan and Gibbons (1974). Yeast and moulds were identified as described by Collins and Lyne (1970), Barnett and Hunter (1972) and Lodder (1971).

Moisture content determination: The moisture content of the Samples was determined at each sampling time in accordance with AOAC (1990).

Results

The microbial load and moisture content of *Piper guineense* samples before drying are shown in Table 1. The total aerobic mesophilic bacteria counts of both fresh untreated pepper sample surface and their respective homogenates were 6.65 log₁₀ cfu/g in white

pepper and 7.04 log₁₀ cfu/g in black pepper. Coliform counts ranged from 6.23 log₁₀ to 6.80 log₁₀ cfu/g sample while yeast and mould counts were generally low, ranging from 2.00 to 3.74 log₁₀ cfu/g. The moisture content of black pepper was 64.20% while that of white pepper was 55.40%.

Pretreatments such as steeping alone or steeping and surface disinfection (before drying) of fresh *P. guineense* samples reduced their microbial load to <1.0 log₁₀ cfu/g or even zero except for white pepper (Table 1). Steeped white pepper had TAMB counts of 2.86 log₁₀ cfu/g and coliforms count of 1.48 log₁₀ cfu/g on the surface, while the homogenate had a TAMB count of 3.99 log₁₀ cfu/g and a coliform count of 3.08 log₁₀ cfu/g sample. Pretreatments caused a general increase in the moisture content of black pepper (from 64.20% in the untreated sample to 66.70% in steeped and surface-disinfected sample) while it decreased in white pepper (from 55.40% in the untreated sample to 49.50% in the steeped and surface-disinfected sample).

The effect of sun-and oven-drying on the microbial load and moisture content of pretreated and untreated *P. guineense* samples is shown in Table 2. The desired moisture content of pepper samples was achieved within 4 to 7 days of oven-drying and 7 to 14 days of sun-drying. In general, the desired moisture content was achieved earlier in white pepper than in black pepper samples.

In the untreated black pepper that was sun-dried for 14 days, the TAMB counts dropped from 7.04 log₁₀ cfu/g to 5.11 log₁₀ cfu/g on the surface and from 6.78 log₁₀ cfu/g to 3.54 log₁₀ cfu/g in the homogenate. In comparison, untreated black pepper showed a drop in TAMB counts from 7.04 log₁₀ cfu/g to 4.08 log₁₀ cfu/g on the surface and from 6.78 log₁₀ cfu/g to 3.32 log₁₀ cfu/g in the homogenate after oven-drying for 7 days (Table 2). The microbial load of the black pepper samples pretreated by steeping alone or steeping and surface disinfection increased slightly during oven and sun drying. On the other hand, the microbial load of white pepper dropped slightly or was maintained at the low or negligible levels (<1.0 log₁₀ or zero cfu/g) during drying.

The microbial loads of all dried pretreated samples stored as whole fruits or in powdered form remained either at zero or negligible levels throughout the 3 months of storage. Untreated sun-dried whole black pepper samples however still had appreciable numbers of microorganisms ranging from 4.00 to 4.48 log₁₀ cfu/g while the oven-dried portions gave approximately 3.88 log₁₀ cfu/g. Powdered samples gave comparable numbers.

The bacteria associated with black and white peppers during processing and storage are shown in Table 3. They included *Staphylococcus*, *Micrococcus*, *Bacillus*, *Serratia*, and *Escherichia*. Of these bacteria species, only *Micrococcus* and *Bacillus* were detected by the end

Omafuvbe and Kolawole: Quality Assurance of Stored Pepper Using Controlled Processing Methods

Table 1: The microbial load* and moisture content of untreated and treated black and white pepper samples before drying

Treatment/analysis	Pepper type			
	Black		White	
	Surface	Homogenate	Surface	Homogenate
Untreated Samples:				
** Moisture		64.20		55.40
Bacteria (TAMB)	7.04	6.79	6.88	6.65
Coliform	6.80	6.23	6.43	6.38
Yeast	3.67	3.36	3.74	3.74
Mould	3.64	3.40	2.00	2.00
Steeped Samples:				
Moisture		65.30		48.80 ^a
Bacteria (TAMB)	0.00	0.00	2.86	3.99
Coliform	0.00	0.00	1.48	3.08
Yeast	0.00	0.00	0.00	1.48
Mould	1.00	1.00	1.00	1.00
Steeped and surface disinfected:				
Moisture		66.70		49.50
Bacteria (TAMB)	0.00	0.00	0.00	0.00
Coliform	0.00	0.00	0.00	0.00
Yeast	0.00	0.00	0.00	0.00
Mould	1.00	0.00	0.00	0.00

Values are means of three determinations. *Microbial load expressed as log₁₀ of colony forming unit (cfu) / g sample. **Moisture content of pepper fruits expressed as percentage. ^a*P. guineense* fruits were retted in water in addition to steeping before moisture content determination. TAMB - Total aerobic mesophilic bacteria.

Table 2: The microbial load* and moisture content of untreated and treated black and white pepper samples during drying

Pepper Type	Pretreatment	Drying period (Days)	Sun-drying			Oven-drying		
			Microbial load			Microbial load		
			Mc	Surface	Homogenate	Mc	Surface	Homogenate
Black	Untreated	7	14.60	5.99	5.67	11.20	4.08	3.32
		14	10.50	5.11	3.54	ND	ND	ND
	Steeped only	7	15.20	2.41	2.08	10.40	<1.48	<1.48
		14	10.38	<1.48	<1.48	ND	ND	ND
Steeped & surface disinfected	7	16.02	<1.48	<1.48	11.00	<1.00	<1.48	
	14	10.50	<1.48	0.00	ND	ND	ND	
White ^b	Steeped only	7(4)	11.30	2.36	2.15	10.50	<1.00	<1.00
		7(4)	12.00	<1.00	0.00	11.30	<1.00	<1.00

Values are means of three determinations. *Microbial load expressed as log₁₀ of 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. Mc - Moisture content expressed as percentage. ND - Not determined since desired moisture content was already achieved within 7 days. ^bThe desired moisture content of white pepper was achieved within 4 and 7 days for oven and sun drying respectively.

of the drying period and during storage of the pepper samples.

The fungi isolated from white and black pepper during processing included mould species namely *Aspergillus*, *Fusarium*, *Itersonilia*, *Botrydiploia*, *Mucor* and

Penicillium and yeast species such as *Brettanomyces* and *Candida*. Most of the yeast and mould species disappeared during the drying period and were hence absent from the stored samples. Of significant note is the absence of *Aspergillus flavus*, *Fusarium solani* and

Omafuvbe and Kolawole: Quality Assurance of Stored Pepper Using Controlled Processing Methods

Table 3: The occurrence of bacteria isolated from white and black pepper during processing and storage

Bacteria Isolates	Pepper type/Treatment			
	Black pepper		White pepper	
	Processing	Storage	Processing	Storage
<i>Staphylococcus aureus</i>	+	-	+	-
<i>S. epidermidis</i>	+	-	+	-
<i>Micrococcus roseus</i>	+	-	+	-
<i>M. luteus</i>	+	-	+	-
<i>M. varians</i>	+	+	+	+
<i>Bacillus cereus</i>	+	+	+	-
<i>Bacillus subtilis</i>	+	+	+	+
<i>B. firmus</i>	+	-	-	-
<i>Serratia marcescens</i>	+	-	-	-
<i>Escherichia coli</i>	+	-	+	-

+ Present - Absent

Table 4: The occurrence of fungi isolated from white and black pepper during processing and storage

Fungi isolates	Pepper type /Treatment			
	Black pepper		White pepper	
	Processing	Storage	Processing	Storage
Moulds				
<i>Aspergillus niger</i>	+	+	+	+
<i>A. flavus</i>	+	-	-	-
<i>A. fumigatus</i>	+	+	+	+
<i>A. ochraceous</i>	-	-	+	+
<i>Fusarium solani</i>	+	-	+	-
<i>Itersonilia sp</i>	+	+	+	+
<i>Botrydiplodia theobromae</i>	+	-	+	-
<i>Mucor hiemalis</i>	+	+	+	+
<i>Penicillium verrucosum</i>	+	+	-	+
Yeasts				
<i>Brettanomyces claussenii</i>	+	-	+	-
<i>B. intermedius</i>	+	-	-	-
<i>Candida tropicalis</i>	+	-	+	-
<i>C. guilliermondii</i>	-	-	+	-
<i>C. lusitaniae</i>	-	-	+	-

+ Present - Absent

Botrydiplodia theobromae in the dried stored pepper samples (Table 4).

Discussion

The microbial counts of fresh untreated black and white pepper in this study appear to conform with earlier reports on black and white pepper prepared from *P. nigrum* in other parts of the world (Krishnaswamy *et al.*, 1971; Beckmann *et al.*, 1996; Garcia *et al.*, 2001). The growing and harvesting conditions of the pepper samples and post-processing environmental exposure may have contributed to their high microbial load. The microbial load of the untreated pepper samples dropped with drying whether in the sun or in the oven and was

generally accompanied with the disappearance of the coliforms and the yeasts leaving *Micrococcus*, *Staphylococcus* and *Bacillus* species and a variety of moulds dominated by *Aspergillus* sp. Hence the aerobic plate counts on the dried samples still showed the presence of a few hundred organisms per gram of each of the samples and these were carried into storage whether as whole berries or ground spices.

Pretreatments such as steeping in boiling water alone or steeping and surface disinfection of fresh pepper fruits before drying drastically reduced their microbial load. This was reflected in the elimination of coliforms, yeasts, *Staphylococcus* species and *Serratia* sp. The low numbers resulting from the pretreatment

manipulations were sustained by both drying methods. This trend agrees broadly with earlier reports (Christensen *et al.*, 1967). These investigators reported the reduction in microbial load of spices to negligible numbers following treatment involving steeping in boiling water for up to 20mins, surface disinfection with 2% formaldehyde and drying. Also, Andress *et al.* (2001) reported a significant reduction in the aerobic microflora of spices when preliminary treatments such as washing with water and dipping in chlorine were employed. These pretreatments caused a general increase in the moisture content of black pepper but a decrease in that of white pepper samples. The black pepper samples may have absorbed moisture during the steeping treatment while the decrease in white pepper may be a result of the retting and de-skinning process.

This study showed that drying in the oven generally achieved the required moisture content earlier and more uniformly than sun-drying. 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. Sun-drying could be affected by annual seasonal changes, for example effective drying could be achieved in only a few days in the dry season while it could take weeks during the raining season. In the latter case, the samples also stand the risk of further recontamination before they are dry enough to withstand microbial growth (Ayres *et al.*, 1980). Once the required moisture content of 10-12% was achieved however, both drying methods effectively kept down the numbers of microorganisms, since the level of moisture essential for microbial growth was not available (Purseglove *et al.*, 1981).

The predominant microorganisms isolated during processing and storage included *Micrococcus* sp, and the spore formers *Bacillus*, *Aspergillus*, and *Penicillium* species all of which are able to survive dry conditions. These microorganisms have been reported in dried spices from other parts of the world (Mckee, 1995; Garcia *et al.*, 2001; Banerjee and Sarkar, 2003). It is interesting to note, however, that the number of microorganisms isolated during drying and storage falls within the acceptable ICMSF (International Commission on Microbiological Specification for Foods) level ($>10^6$ cfu/g) reported by Banerjee and Sarkar (2003). Also, the numbers are not sufficient to cause the spoilage of the spices provided that they are kept at the required dryness. This finding does not conform to those in existing literature (King *et al.*, 1981; Seenappa and Kempton, 1981; Banerjee and Sarkar, 2003). These investigators reported high microbial counts on dried spices of export quality. This may be due to the preparation methods and handling of the spices by the producing countries, since production practices range from sanitary to unsanitary. If the aerobic mesophilic

count is high or allowed to increase, this may accelerate product spoilage while relatively significant fungal counts may be a problem, especially when such spices are used in long-term storage type products.

Most of the microorganisms associated with the fresh pepper samples were eliminated by the pretreatments and drying process. This finding suggests that even in the traditional setting, washing and steeping alone followed by controlled sun-drying, could go a long way in providing spices with minimal microbial load and which could therefore be stored for long periods of time without undergoing spoilage.

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Omafuvbe and Kolawole: Quality Assurance of Stored Pepper Using Controlled Processing Methods

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