TITLE

TRADITIONAL FERMENTATION AND THE QUALITY OF BALANCOTTA VARIETY OF BLACK PEPPER (*PIPER NIGRUM*)

BY

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DECLARATION

I conducted this research work under the supervision of Professor Samuel Sefa-Dedeh of The Department of Nutrition & Food Science, University of Ghana, Legon.

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JOHN ESIAPE PROF. S. SEFA-DEDEH

DEDICATION

I dedicate this work to my Father in Heaven, for His Great Plan for Mankind, and in Thanksgiving for The Gospel Restored. This research work is also dedicated to Sedinam, Marian, our forebears, and to our future.

ABSTRACT

A study was made of the effect on pepper quality of the traditional method of processing the "Balancotta" variety of *Piper nigrum* (black pepper). The study comparatively considered the microbiological quality, drying method, drying rate, colour and appearance, washing or cleaning method, prior to fermentation or heaping as well as an alternative method - heat treatment processing of the commodity.

Using a solar dryer as a drying apparatus resulted in 9.8% moisture content of the sample as opposed to 12.5% obtained for the traditional method of sun/open air-drying. The drying was more effective using a solar dryer and hence provided a better-dried product giving an assurance of better keeping/storage qualities. Air-drying and solar drying were carried out in the same area/locality with solar drying yielding a more hygienic product, giving 1.2×10^2cfu/g of microorganisms compared to 8.0×10^4cfu/g enumerated in the sample dried by the traditional open air method.

Chlorine disinfection prior to drying was, comparatively, a potent cleaning mechanism, yielding samples with microbial counts in the region of 0.1×10cfu/g immediately after its application. Sanitation of the processing/drying environment had a significant bearing on the

microbiological quality of the dried products after the chlorine washing hence the 1.2 $\times 10^2$cfu/g and 8.0 $\times$ 10^4cfu/g microbial counts obtained for the solar and air-dried samples respectively.

Product Colour and appearance were studied by comparing traditionally heaped/fermented samples with samples, which were given some heat treatment by immersion in hot water prior to drying, and that, which was not given any treatment at all. The heat-treated sample tended to be more brightly coloured (black), followed by the untreated, but solar dried sample and then the traditionally fermented sample, which had a brownish (dull) black colour. The highest L values for colour intensity for both the heat-treated and traditionally fermented samples were 54.8 and 51.7 respectively.

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CHAPTER ONE

Introduction

Spices have always been treasured for their ability to flavour foods. Spices evoke images of exotic splendour and a myriad of taste sensations. Spices provoked trade wars and voyages of exploration by Europeans like Columbus and Magellan. Spices are aromatic vegetable substances used to provide flavour and aroma. A convenient classification for black pepper spice is that:

1. The plant is a perennial woody climber growing to about 10 metres high.
2. The mature plant bears flowers, which can be either unisexual or hermaphrodite.
3. Flowers are protogynous - with stigmas exserted 3 - 8 days before the dehiscence of the anthers in a hermaphrodite flower.
4. The fruits are sessile globose drupes of 4 - 6 mm in diameter. They have a pulpy mesocarp, with the exocarp turning red when ripe.
5. It fruits in spikes, and the entire fruit spike must be harvested when mature. The fruit is small and berry-like, and contains a single seed. It is sessile, nearly globular and at first green, then yellowish and finally it turns red when ripe (Parry, 1969).

6. Black pepper has a characteristic, penetrating, aromatic odour and a hot biting and a very pungent taste. These attributes are due to the presence in the pepper of the chemical compound piperine and its stereoisomer chavicine.

Piper nigrum (Black Pepper) is one of the oldest and most important of all spices. It is the whole dried fruit and used in treating ailments such as cholera, dyspepsia, fevers etc., and is believed to enhance metabolism by affecting thyroid function. It is most widely used as a condiment, its flavour and pungency blending well with most savoury dishes. Its stimulating action on the digestive organs produces increased flow of saliva and gastric juices. It is used in pickles, ketchup, sauces, and for seasoning dishes. (Akromah, 1991).

Black pepper enters international trade in the form of the whole seed (dried unripe fruit of the plant). In recent years black pepper has represented around 80% of the world's pepper exports. Recently, the Ghana Export Promotion Council (GEPC), the Crops Division of the Ministry of Agriculture and other Non-governmental Organizations (NGO's) have intensified the promotion of the spice as a non-traditional export produce. In Ghana, black pepper is called "Sorowisa" by the Akans, the West African or Ashanti pepper (*Piper guineense* - Schum and Thonn). This variety is not high yielding and lacks the competitive quality characteristics of the other varieties being currently propagated by the University of Ghana's Agricultural Research Stations at Kade and Bunso (Nyamekye-Boamah, 1991). In the tropics and many of the developing countries including Ghana, the staple diet consists mainly of starchy foodstuffs of cereals and root crops, which are supplemented by dried meat and fish. Much of the food lacks natural flavour and so spices are used liberally to flavour dishes and disguise unpleasant taste thereby increasing their palatability.

Because spices come from every area in the world and are grown and harvested on many small farms, or even found growing wild, quality is difficult to control. Sanitary conditions are often inadequate. Hence, cleanliness, insect and rodent infestation, and microbiological quality are important concerns for both the spice processor and product developer. Food technologists working with and specifying spices and seasonings need to be aware of the major quality attributes and microbiological concerns associated with spices. Spice processors consider essential oil and ash contents, percent moisture, and heavy and light filth levels as major spice quality attributes. Heat/burning sensation and colour units are also important quality indicators (Tainter, 1992). Spices can contain high levels of microorganisms. Total counts in black pepper can be as high as 40 million per gram (Tainter, 1993). To reduce bacterial loads, majority of spices is treated with ethylene oxide. Irradiation is sometimes used where applications demand even lower counts such as seasonings for salad dressings. Other bacterial reduction techniques have been developed for spices, even though none are as effective as ethylene oxide. For instance, steam sterilization has been used as a cleaning step for spices before grinding. The process has limitations because the moisture and heat generated may cause a loss of flavour.

A critical problem of spices is their susceptibility to microbial contamination. Post-harvest cleaning and handling methods can greatly influence the level of contamination. Various levels of chlorine concentrated (3 - 5 ppm) water have been used to clean/wash raw foods such as vegetables, prior to processing, and this was found to be effective in reducing the microbial population in fresh agricultural produce before processing (Foegeding, 1983). As a result of in-plant chlorination, bacterial and microbial slimes were practically eliminated from processing equipment and bacterial counts in the end products were significantly lowered (Mercer and Somers, 1957).

The use of chlorine prevents or greatly reduces the accumulation of microbial slimes on equipment that is constantly washed with chlorinated water. It also prevents development of off odours from fermentation or decay. The total bacterial counts on finished products are reduced when raw products are washed in chlorinated water.

1.1 Objective

Black pepper is being promoted in Ghana for export. Therefore, there is the need to identify the peculiar characteristics of the spice as found in Ghana so as to provide adequate information for its trade. This should assist processors and farmers to take the necessary measures to turn out the approved commercial spice.

In view of the high microbial counts associated with black pepper processing and marketing, this project would seek, under broad outlines, to investigate the quality of traditionally processed black pepper (balancotta variety).

The following specific objectives are to be carried out:

1. A study into the drying characteristics of the heaped, and heat-treated samples.

2. Investigating the effect of heaping and heat treatment on the physical and chemical attributes of the black pepper variety.
3. A study into the effect of chlorine disinfection on the microbial quality of black pepper.
4. A study into the effect of chlorine disinfection on the microbial quality of the food-contact surfaces.
5. A study of the characteristics of the stored products.
6. Establishing optimum levels of heaping, heat treatment and chlorine disinfection and
7. Developing suitable cleaning (disinfection) and heaping/heat treatment programme for primary processing of black pepper.

CHAPTER TWO

Literature Review

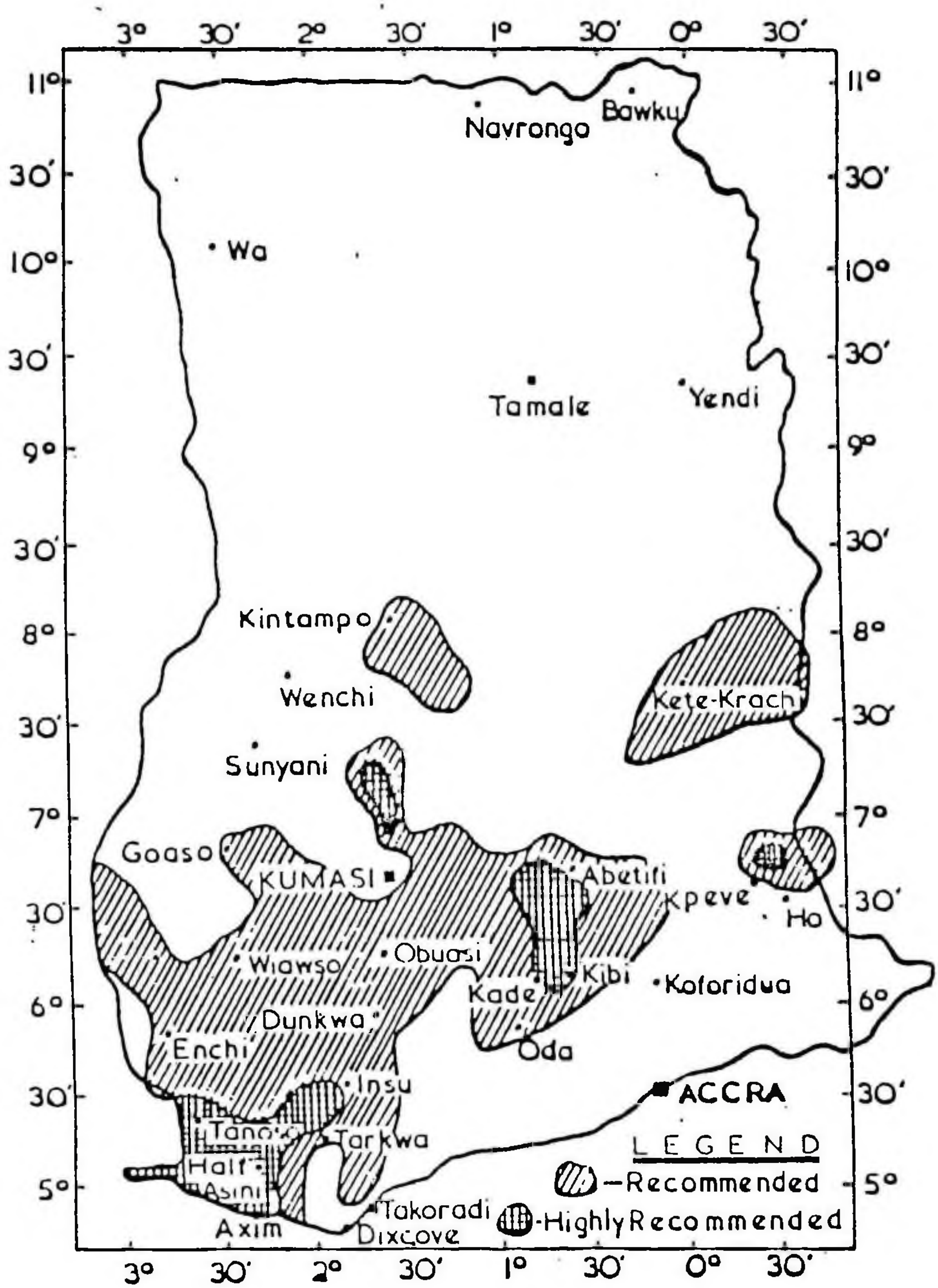
2.1 Growth Requirements of Black Pepper

Black pepper is a member of the Piperaceae or pepper family. It is grown mainly in forest areas with moderate to heavy rainfall, usually 1800-3000 mm of rain per year. In effect the crop will grow well in all the cocoa growing areas in Ghana. It requires a hot, wet tropical climate, and is grown at low altitudes. It cannot stand water logging and is usually planted on mounds and a temperature of about 25°C-30°C is quite ideal. The ideal soil is a well-drained soil, rich in humus and having a pH above 5.5 (Akromah, 1991). Black pepper is cultivated in many tropical and subtropical parts of the world including India, Sri Lanka, Malaysia, Indonesia, Thailand, Malagasy Republic, and Brazil (Parry, 1969). In Ghana, black pepper is potentially suitable for cultivation in areas indicated on the sketch map of Ghana (figure 1). Pepper growing is highly recommended in areas where the rainfall ranges from 1700-2250 mm per annum; Axim, Half-Assini, Tanoso, Abetifi, Kibi, Kade and Kpeve, and only recommended in areas of annual rainfall between 1400-1700 mm; Enchi, Dunkwa, Wiawso, Obuasi, Tarkwa, Insu, Goaso, Oda, Ho, Kete-Krachi and Kintampo (Whatley, 1962); forest areas of the Central Region are currently supporting black pepper production. These areas fall within the high and low rain forests of Ghana (Lane, 1962). Incidentally, these are the areas suited for cocoa cultivation. Cocoa does not do well in areas where the soils are predominantly limestone. Also, some forest belts experience long dry season, which can affect cocoa, yields adversely. Pepper appears to tolerate a long dry season as well as limestone soils (Popsil and Co., 1976)

Figure 1: Potential Black Pepper Growing Areas in Ghana.

Source: Whatley, 1962.

...PPER GROWING AREAS
...HANA



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SOURCE : WHATLEY [1962]

2.2 Nomenclature and varieties of black pepper

Black peppercorn and black pepper berry are the terms used to refer to the dried kernels or caryopsis and the raw/undried fruits respectively. The various kinds of pepper known to spice trade are named for the districts in which they are produced or for neighbouring ports through which they are exported. Lampong black pepper is from Lampong, a district of southern Sumatra, Indonesia. Sarawak black pepper from former British colony of Sarawak, now part of Malaysia. Ceylon black pepper takes its name from the island of Ceylon now Sri Lanka. Alleppey black pepper is cultivated in the southern Malabar Coast region of India and is named for the shipping port of Alleppey. Today, this pepper is generally known as "Malabar Alleppey". Tellicherry black pepper is cultivated in the northern Malabar Coast region of India and is named for the city of Tellicherry in Cannanore District of Kerala. Today the pepper is generally referred to as "Malabar Tellicherry". Brazilian black pepper takes its name from Brazil. In Ghana the local variety (*P. guineense*) is a poor commercial variety. The plant is very unproductive and has a low yield compared to others (Table 1). Its piperine content of about 10% exceeds normal, thus making it extremely hot and pungent. A small quantity in food makes one feel quite unpleasant and perspire so much.

It also fails as a commercial variety by not being uniform in ripening. The entire fruit spike must be harvested and only fully ripe berries produce top-quality pepper. Its only advantage is its resistance to pyphytophthora root rot and tolerance to nematodes and drought. The United States Operations Mission to Ghana brought into this country the following commercial varieties (Whatley, 1962): Balancotta, Killuvalli, Karinotta, Kalbalacotta and Cheriakodi.

Table 1: Yield Records of *Piper nigrum* - 3 years after planting

Variety	Plants Bearing	Spikes harvested	No. Of Berries	Total wt. (Ib) fresh
Balancotta	16	366	12,469	1.27
Cheriakodi	13	143	4,684	0.47
Kalluvalli	7	64	1,809	0.02

Source: The Ghana Farmer, Vol. xvl, No. 2

These varieties were first established at the Bunso Research Station from where planting materials have been supplied to farmers. The Kade Material was obtained from this source. Wild *P. nigrum* is mostly dioecious but man has selected cultivars, which are mainly hermaphrodite, and are maintained by cuttings.

1. "Balancotta": most widely grown in India. Have large leaves, and high and regular yields.
2. "Kalluvalli": second most popular cultivar in India. Have small leaves and large fruits.
3. "Kuching": have large leaves, popular in Sarawak. Very susceptible to root-rot.
4. "Sarikei": small leafed and found in Sarawak.
5. "Belantung": the most popular cultivar in Sumatra. Has some resistance to root-rot, and is fast growing.
6. "Lampong" and "Muntok": grown mainly for white pepper in Bangka.

2.3 The Black pepper plant

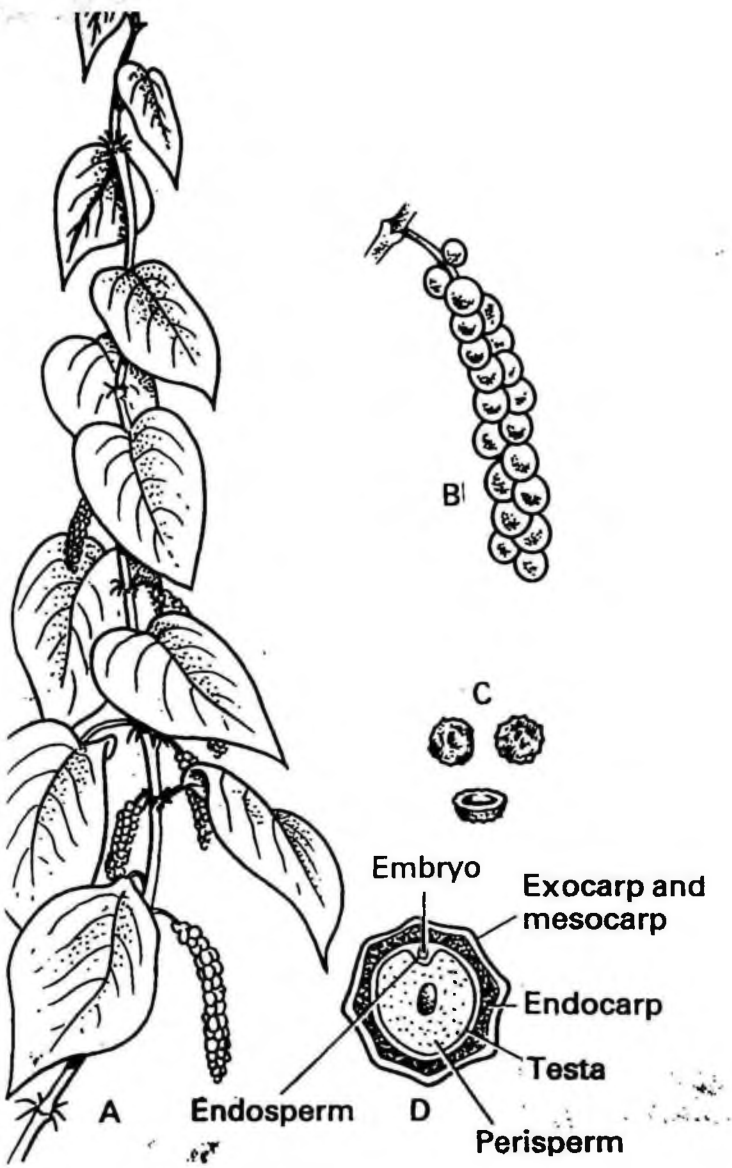
Black pepper is a perennial woody climber to 10 metres in height. The mature vine has a bushy columnar appearance, 4 m high and 1.5-m in diameter under best cultivation practices. It has dimorphic branching - orthotropic vegetative branch and plagiotropic fruiting branch. Both types of stems branch but only orthotropic stems produce further climbing shoots, a factor considered in selecting material for propagation. The flowers are unisexual (monoecious/dioecious) or hermaphrodite. Most improved cultivars are hermaphrodite. Flowers are protogynous - stigmas exserted 3-8 days before the dehiscence of the anthers in a hermaphrodite flower and remains receptive for 10 days. Hermaphrodite flowers are self-sterile. "Balancotta" and "Kalluvalli" are hermaphrodites. The fruits are sessile globose drupes, 4-6 mm in diameter with pulpy mesocarp, and exocarp turning red when ripe, and drying black. 100 peppercorns usually weigh 4.5g (Akromah, 1991). The fruit is small berry-like, containing a single seed, sessile, nearly globular; at first green, then yellowish, and finally red when ripe (Parry, 1969).

Figure 2: A) A Fruiting Branch of *Piper nigrum*

B) A Pendulous dense spike

C) Whole and split peppercorn

D) Peppercorn cut longitudinally



B
C
Embryo
Exocarp and
mesocarp
Endocarp
Testa
A
Endosperm
D
Perisperm

2.4 Gross structure and variation in black peppercorn

Black pepper berries or peppercorns are dark brown to black in colour; nearly globular in shape; around 5mm or less in diameter; with deep-set wrinkles forming a characteristic network. The pericarp is thin and encloses a single seed with a hollow centre (fig. 2). The perisperm of the seed is horny in the outer part, floury around the central cavity. The embryo is embedded in a small endosperm at the apex of the seed. Black pepper has a characteristic, penetrating, aromatic odour; and a hot, biting, very pungent taste.

The pericarp comprises, among other tissues, an outer mesocarp of parenchyma tissue in which many oleoresin cells occur; a central mesocarp of about seven layers of tangentially elongated parenchyma cells traversed by vascular tissues; an inner mesocarp of parenchymatous tissue with numerous large oil cells; and a parenchymatous endocarp. There are no stone cells in the pericarp. The chief features of the seed are the seed coat of three layers of compressed parenchyma cells with the outer walls of the outer layer being thickly cutinized; a perisperm of polygonal-shaped cells containing masses of starch; a small, parenchymatous endosperm in which is embedded a minute embryo composed of tiny parenchyma cells with a number of small oil cells occurring toward the radicle.

2.5 Microbiological concerns

Spices and herbs, like most raw agricultural products, are subject to microbial contamination after harvesting. The microbial flora of whole spices, herbs, and spice blends is dominated by members of the genus *Bacillus*, such as *B. subtiles*, *B. licheniformis* and *B. megaterium.* Anaerobic spore-forming bacteria within the genus *Clostridium* are also found in small numbers. Thermophilic anaerobes and aerobes are found occasionally, sometimes in moderate numbers. *Enterococci* and gram negative bacteria, including members of the family *Enterobacteriaceae,* occur occasionally. A variety of molds may be found; spore density range from insignificant levels to many millions. Yeasts are found infrequently. Pathogens such as *Shigella, Salmonella*, and coagulase-positive *staphylococci* are found rarely in spices. *Escherichia coli*, an indicator of faecal contamination, only occasionally is found. *Clostridium perfringes* spores are present usually at low levels, i.e., from ten to several hundred per gram. The most frequently used spices, black and white pepper, usually contain very high microbial populations. Plate counts of 10-100 million per gram have been obtained. Members of the genus *Bacillus* usually predominate, generally as spores rather than as vegetative cells.

Some spices, because of the bactericidal effect of their essential oil content, harbour few bacteria. The antibacterial action of cloves, for example, is due to the presence of eugenol. In mustard seed, the essential oil, allyl isothiocyanate, is inhibitory to bacteria. Onion and garlic inhibit bacterial growth because of their sulphur containing compounds. Oregano and many other herbs are bactericidal also.

In suspensions with relatively high concentrations of these spices it is not uncommon to find low colony counts on the low dilution plates because of the carryover of inhibitory substances with the inoculum, while moderately high counts may be obtained on the higher dilution plates.

Plate counts of spices containing antibacterial compounds or oils seldom exceed several thousand organisms per gram and only a few microbial types are present. Pathogens or organisms of sanitary significance rarely are found in such spices. While aerobic plate count values have no public health significance, they do have a bearing on the degree to which a spice may contribute toward the spoilage of a product in which that spice is used.

2.5.1 Methods to reduce microbial load in spices

Cleaned spice materials show variable but moderately low incidence of sanitary indicator microorganisms. Root items, berries, and herbs, generally carry a slightly greater microbiological load than do bark and seeds, but organisms of sanitary significance occur only occasionally while pathogens rarely occur.

Spices are grown and harvested in areas of the world where often, sanitary practices are poor. Moreover, many of the spices are grown in warm, humid areas where a wide variety of fungi and bacteria are supported readily, and are easily distributed during the handling of the commodities. Consequently, uncleaned spices may be grossly contaminated and occasionally carry organisms of sanitary or public health significance. For these reasons, spices are generally not used in the form in which they are imported. They usually undergo extensive cleaning by a variety of means including sifting, aspiration and other methods that take advantage of the shape or density of the spice particles.

Fumigation of spices with ethylene oxide or propylene oxide can effectively reduce the microbial population as much as 99.9%. This includes organisms, which are public health hazards. The majority of residual bacteria are spore-forming bacilli. Current FDA regulations limit the permissible residue of ethylene oxide to 50 ppm and of propylene oxide to 300 ppm in spices. Irradiation with gamma rays has been attempted also in an effort to rid spices of undesirable bacteria, or to reduce total microbial levels. However, at this time FDA does not permit irradiation of any spices.

Aqueous chlorine is used extensively in the food industry to sanitize food processing equipment and food containers (100-200 ppm), to rinse and convey raw fruits and vegetables (1-5 ppm), and to cool heat sterilized canned foods (1-2 ppm) (Foegeding, 1983). Chlorine is also widely used in the fishing industry (Lane, 1974); in washing nutmeats (Smith and Arends, 1976), poultry (Ranken et al., 1965), and red meats (Kotula et al., 1974). Chlorine gas is used in the flour industry as an oxidizing and bleaching agent to improve the quality of flours (Johnson et al., 1980)

The destruction of bacterial spores by chlorine is thought to be due to the disruption of spore coats and underlying layers, resulting in an increase in spore permeability and a concomitant increase in susceptibility to destruction (Foegeding, 1983). Chlorination may also inactivate the germination/growth mechanism of spores. Wyatt and Waites (1975) reported a reduced rate of germination in spores that were treated with chlorine.

2.6 Chlorine disinfection

2.6.1 Mechanism

Several theories have been proposed to explain the antimicrobial action of chlorine, but none have been completely substantiated experimentally (Dychdala, 1977). One of the first theories advanced was that chlorine kills bacteria by reacting with cell membrane proteins, resulting in the formation of N-chloro compounds that interfere with cell metabolism (Baker, 1926). Rudolph and Levine (1961) suggested that the chlorine penetrates the bacterial cell and then reacts with cellular protoplasm to form toxic N-chloro compounds.

Because very low levels were sufficient for bactericidal action, Green and Stumpf (1946) postulated that chlorine acts by inhibiting some key enzymatic reactions within the cell. They found out that the rate of inhibition of glucose oxidation in the cell correlated with the extent of bacterial destruction. The inhibition of enzymes was due to chlorine oxidation of sulfhydryl groups (Knox, 1948)

Using radio-labeled $^{36}Cl_2$ and ^{32}P, Friberg (1957) showed that low concentration of Cl_2 could kill bacteria prior to the formation of N-chloro compounds in the protoplasm. The exposure to very small amounts of chlorine resulted in a destructive permeability change in bacterial cell membrane, as evidenced by leakage of ^{32}P out of the cell. More recently, Campers and McFeters (1979) reported that chlorine impaired the function of the bacterial membrane in transporting extra cellular nutrients. Cells exposed to chlorine were not able to take up radiolabelled carbohydrates and amino acids. The destruction of bacterial spores by chlorine is thought to be due to the disruption of spore coats and underlying layers, resulting in an increase in spore permeability and a concomitant increase in susceptibility to destruction (Foegeding, 1983). Chlorination may also inactivate the germination mechanism of spores. Wyatt and Waites (1975) reported a reduced rate of germination in spores that were treated with chlorine.

2.6.2 Chemistry

The form of chlorine in aqueous solution influences the antimicrobial properties of chlorine compounds. When elemental chlorine or hypo chlorites are added to water, they undergo the following reactions:

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

$$NaOCl + H_2O = HOCl + NaOH$$

$$HOCl = H^+ + OCl^-$$

Hydrolysis of chlorine to form hypochlorous acid (HOCl) and the chloride ion (Eigen and Kustin, 1962) is a very rapid and almost complete process. Hypochlorous acid is a weak acid with dissociation constant at 0° to 25°C of 1.6 to 3.2 (10^{-8}) and a pKa of 7.8 to 7.5 (Morris, 1966). The basis for the reactivity of HOCl is the electrophilic nature of the molecule at either the oxygen or chlorine atom.

2.7 Quality Attributes of Black Pepper

Several specialized tests, such as Scoville heat units for red peppers and colour units for paprika are important quality indicators. Essential oils vary for each spice and within each lot of spices depending on growing and handling conditions. Essential oils are lost with age even in intact spices, and when spices are ground these compounds are rapidly lost because the oil-cell structures are ruptured. Spices usually contain 0.5 to 3.0% volatile oils. With levels in seed spices being higher than in herbs (Tainter, 1992).

Moisture content is an important quality attribute since high levels of moisture may indicate mold and microbial growth problems. Vacuum oven drying and toluene distillation are two tests used in determining moisture content. Spices are also tested for foreign material, such as insect parts, small stones, metal fragments, and glass. Filth testing may be done, by separating spices with an organic solvent. Acid-insoluble ash is also used as an indicator of foreign material in spices.

For spice processors, heat in peppers is a critical factor. Hot peppers are brought and sold on the basis of their heat value.

2.7.1 Spice Storage

Storage life of spices depends not only on the storage conditions, but also on the age, type, and source of the spice. The American Spice Trade Association (ASTA) (1990) recommends the following general guidelines for storing spices and seasonings to maximize flavour and colour:

Spices and spice extractives should be stored in closed containers under cool and dry conditions (Table 2). Recommended storage temperature is 68°F (20°C) with 50% relative humidity. Light-sensitive materials such as paprika, parsley, chives, and other green herbs should be protected against direct exposure to sunlight and fluorescent lights. Storage under these conditions can prevent caking and fading of colour, and keeps essential oils from volatilizing. The same general storage recommendations would also apply to seasoning blends. Storage under refrigeration temperatures may help to slow microbial growth. If spices are taken from a bulk container, the container should be tightly sealed after each use to prevent loss of flavour and aroma volatiles as well as avoiding insect and rodent infestation.

Table 2: Storage Guidelines for spices, seasonings, tenderizers, & patty mixes

Product	Normal Attributes a	Critical temperature(°F)	Storage shelf-life
Seasonings	1, 2	below 60	12months
Spices	1, 3	"	12 "
Dry tenderizers	2, 4	"	6 "
Liquid tenderizers	4	"	6 "
Patty mixes	1, 3	"	6 "
Seasonings with flours	1, 3	"	6 "

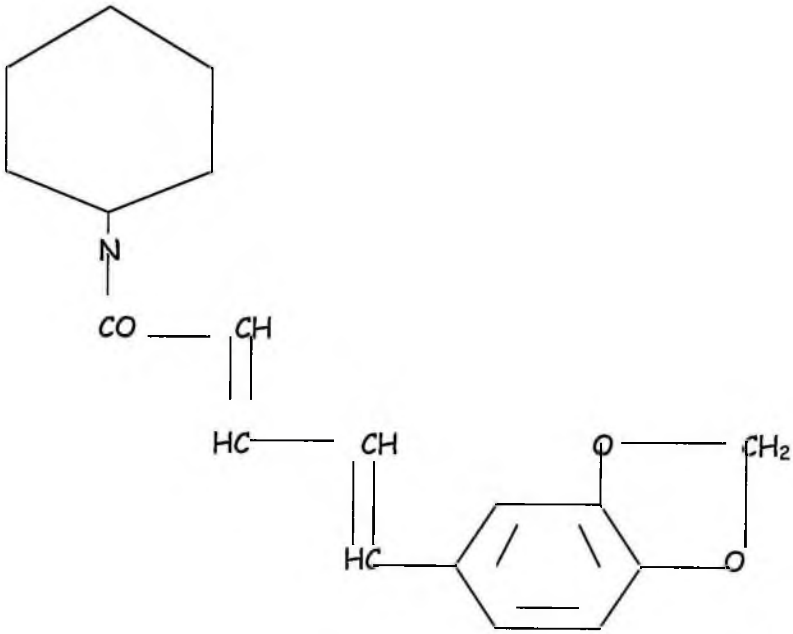
a= (1) flavour (2) caking (3) infestation and (4) enzyme activity
From Chaudry (1993)

2.8 Isolation of Piperine from black pepper

Piperine occurs in the unripe fruit (black pepper) as well as in the kernel of the ripe fruit (white pepper) of *Piper nigrum*. The piperine content of black pepper varies from 6 to 9%. Piperine is tasteless, so some investigators have suggested that the stereo isomeric chavicine, which accompanies piperine in the pepper, is possibly responsible for the particular taste of piperine

Piperine is extracted from black pepper with ethanol and may be converted to a red TNB (1,3,5-trinitrobenzene) complex. Although at first tasteless, piperine does ultimately produce a burning sensation and sharp aftertaste. The initial tastelessness of piperine may be a consequence of its extremely low solubility in water. The crude ethanol extract contains, in addition to piperine and chavicine. Its geometric isomer), some acidic, resinous material. In order to prevent co-precipitation of piperine and the resin acids, dilute ethanol KOH solution is added to the concentrated extract to keep acidic material in solution and/or as solid gummy material (Ikan, 1991)

Figure 3: Chemical Structure of Piperine


N
CO
CH
HC
CH
HC
O
CH2
O

2.9 The Colour of Black Pepper

Two main grades of pepper are recognized in the spice trade; black pepper and white pepper. For black pepper, the berries are picked while still immature (green), or at least when only a few on the spike are red, piled in heaps and left in the sun for several days to dry. Sometimes the berries are artificially dried in smokehouses. As they dry the pericarp becomes tough and wrinkled and develops a dark brown or black colour owing to natural fermentation. Occasionally the freshly harvested unripe spikes are dipped in boiling water to quicken the blackening and drying processes (Kochlar, 1989).

2.9.1 Colour and Gloss

When light strikes an object, it is reflected, absorbed, or transmitted. Because reflected light determines the colour of a material, the appearance changes are based on the amount of light, the light source, and the observer's angle of view, size of the object and any background differences. Depending on how light acts, food products can be classified as opaque, translucent, or transparent (Mabon, 1993). The Commission Internationale de l'Eclariage or CIE developed the most influential system for the description of colour. This system is based on using standard sources of illumination and a standard observer. It is used to obtain CIE tristimulus values, based on visible spectrum. Any colour is thus uniquely specified by this set of three imaginary red (X), green (Y) and blue (Z) primaries. To make colour data more intuitive and easier to interpret, these tristimulus values are usually converted to another colour scale. The Hunter L, a, and b and the CIE L* a* b* colour scales are opponent type systems that are often used in the food industry. These measure the degree of redness or greenness (+/- a), and the degree of yellowness or blueness (+/- b). Colour can be measured with either colorimeter or spectrophotometer. tristimulus filter colorimeters are designed to reproduce the "psych-physical" sensation of the human eye's perception of colour (Mabon, 1993; Anonymous, 1993). In practice, a set of red, green, and blue filters with transmission curves shaped like those of the standard observer are used to stimulate the eye's sensitivity to colour. A photocell and meter take reading of the light reflected from an object through each of these filters and X, Y, and Z tristimulus values are obtained. These values can be converted to other colour spaces.

2.10 Harvesting and processing

Two types of commercial pepper can be prepared from the berries:

(1) Black pepper from fully mature but unripe (green) berries.

(2) White pepper from the fully ripe (red) berries

The correct stage of harvesting for the preparation of black pepper is when a few of the berries in the spikes are ripe or when the last berries in the spike (distal end from the stalk) cannot be chopped easily with two fingers. During harvesting, each spike is picked off the fruiting branches taking care to avoid damage to tender branches.

2.10.1 Heaping

The harvested pepper spikes are heaped for 24-48 hours and thereafter the berries are separated from the stalk them by trampling. During heaping heat is generated and retained. At the same time air is allowed to pass through the mass. Heaping induces fermentation, which normally involves the conversion of sugar to alcohol and to acetic acid. However in the case of black pepper other changes also take place within the seed. The death of the seed results in the breakdown of the internal cell structure, allowing various enzyme reactions to occur. The proteins in the cotyledon are hydrolyzed to amino acids and converted to insoluble forms by reactions with polyphenols. These changes are reflected in the flavour, aroma and colour of the seed (Asiedu, 1990). The berries are then spread in a thin layer on mats and dried in the sun for 5-6 days. The green colour of the skin of pepper changes to black during drying. If there is no bright sunshine, pepper will not dry uniformly and mold may grow on the surface giving uneven coloured product. (Bavappa and Ruettiman, 1990).

2.10.2 Heat treatment

A better method used sometimes is to steep the pepper in hot water (80°-90°C) for 2-3 minutes and then drying in the sun or preferably hot dryers. This treatment helps to get rid of molds and other microorganisms from the surface of the berries and quicker drying. Also, a uniform glossy black colour is imparted to the product. A longer shelf life and better price is obtained for the pepper produced following this method. (Bavappa and Ruettiman, 1990).

2.10.3 Post-harvest handling

Pepper dealers evaluate the product according to its appearance, pungency level and the extent of microbiological contamination.

With a given cultivar, these quality attributes are affected greatly by stage of maturity of berries at harvest and preparation methods. The volatile oil content of immature green pepper reaches its maximum $4\frac{1}{2}$ months after fruit setting, and then diminishes while piperine continues to increase for a period. Premature harvesting results in decortication problems as well as shriveling in the core of white pepper. The initial appearance, level of microbial and mould contamination and the susceptibility to insect infestation are dependent upon the care taken during the drying and preparation of the spice. Moisture level of 11% is considered safe for storage. (Nymekye-Boamah, 1995)

2.10.3.1 Black pepper preparation methods

Whole, unripe but fully developed tree clusters of berry in a heap.

↓ initiation of browning fermentation

(i) Spread berry out on a suitable drying floor in the sun (Traditional approach)

(ii) Blanch berry clusters in boiling water for 10 min. (Indonesia & Sri Lanka)

↓ accelerates browning and drying process. Sun dry or artificial dry (Brazil). Two periods of 4 ½ hours drying, with a 6-hour delay in between.

Grade black pepper. (Nyamekye-Boamah, 1995).

Alternatively, the berries are picked when they are just mature but before they ripen. The picked berries are heaped together for a day or two to enable fermentation to take place. The seeds are then removed from the spikes and steamed in boiling water for 5 to 10 minutes and then spread out in the sun for about 7 days. After sun drying, the seeds are put in the oven at a temperature of about 60°C for 4 - 5 days to oven dry. (The Ghana Farmer, Vol. XVI No. 2, 1962).

2.10.4 Grades of Black Pepper

Black pepper is graded as Grade I Special, Grade I, FAQ Grade, and Light berries. Light berries are defined as those that float in a solution of alcohol and water.

2.11.1 Standard specifications for black pepper

Colour: The colour should be black or brownish black.

Appearance: There should be solid deep-set wrinkles on the surface.

Cleanliness: The pepper should be free of dead or live insects, rat droppings, moulds, dirt, etc.

(i) **Mould berries:** 1% maximum for Grade I Special and Grade I, 2% maximum for Grade FAQ.

(ii) **Dirt:** 1% maximum for Grade I Special and Grade I, 2% maximum for FAQ Grade.

Light berries: 2% maximum is Grade I Special, 4% maximum is Grade I, and 10% maximum in Grade FAQ.

Moisture: Maximum content of 12% in Grade I Special and Grade I, 14% maximum in FAQ Grade and Light berries. (Bavappa and Ruettiman, 1990).

Extraneous matter: That form plant source should be 1.5% maximum, and that not coming from plant should be 1% maximum. (Nyamekye-Boamah, 1995).

CHAPTER THREE

Materials and Method

3.1 Source and Supply of Sample

The Balancotta variety of black pepper used was obtained from the Pilot farm of Crop Research Institute, Bunso, Eastern Region of Ghana, as well as from the biggest out-grower farm at Tanoso near Bunso. A third sample was obtained from an out-grower farm at Kade.

3.2 Physical Analysis

The raw samples to be given various treatments were sorted, leaving out the damaged prior to treatment and analysis.

3.2.1 Dimensional Studies

One hundred (100) peppercorns of the fresh, and the differentially fermented and blanched samples were randomly picked and measured by use of The Vernier caliper (Type: Rabon Chesterman Vernier calliper No. 600). Each Peppercorn was measured both at the shortest and longest circumference sites. The longer side was the length and the shorter, width.

3.2.2 Determination of 1000 peppercorn weight

One hundred (100) peppercorns of the raw and dried samples were randomly selected from the bulk of samples and weighed using The Metler Balance (Type H16 cap.80.g No. 181023). Each sample weight, was multiplied by 10, to obtain the 1000 peppercorn weight.

3.2.3 Estimation of suitable heat treatment temperature

100 grams samples were weighed and blanched as outlined in **2.3.4** but at the following temperatures: 60°C, 70°C, 80°C, 85°C, 90°C, 95°C, and 100°C, for 10 minutes, a period which enables samples blanched at 60°C to turn black in an hour (Parry, 1969). The samples were left in trays and the time taken for each sample to turn black was noted and recorded. Other physical changes such as damage were also noted.

3.2.4 Heat treatment of samples

About 1.0 Kg of the raw sample was weighed into small-sized baskets and immersed in bowl containing water kept at a temperature of 95°C by an electric stove (Type: Electro thermal Cat. No. EME60250/CE MK1, Serial No. 10068739). The immersion was done for specific duration and then the basket was lifted for any trapped water to drain and the samples spread on aluminium trays and dried. This procedure was followed to obtain samples blanched for 0, 1, 2, 3, 5, 7, and 10 minutes respectively. The appearance and condition of samples after blanching were noted.

3.2.5 Fermentation of samples

Approximately 1.0 Kg of the raw sample was weighed and heaped on a previously sterilized yarn sack. Heap height was determined to be 18cm using a calibrated ruler. Six (6) lots of samples of 1.0 Kg each were heaped and designated ferment for 0, 1, 2, 3, 4, and 5 days respectively. Organoleptic evaluation as well as the temperature and pH were determined for each sample after the designated period of fermentation. Thermometer (Type: 76 mm Immersion Fisher brand 14-997) and pH-meter (Type: TOA pH meter HM-30S) were used in each case.

3.2.5.1 Determination of fermentation pH

The pH meter (TOA pH meter HM-30S) was set using buffer solutions of pH 9 and 4. The electrode was washed well and placed in the wash water and made to remain till the pH meter gave a steady reading. Upon elapse of each heap period, 20g of the sample was weighed, in duplicate, into 250ml Erlenmeyer flasks and 50ml-distilled water added. These were allowed to stand for about an hour while stirring. The pH of the wash water was then read and the average computed.

3.2.5.2 Determination of Heap temperature

The temperature of the heaped samples was determined by thermometer measurements three times within a 24-hour fermentation period using the mercury-in-glass thermometer (Type: 76mm Immersion Fischer Brand 14-997), and the average computed as daily fermentation/heap temperature. The measurement was taken by dipping the thermometer into the heap from the top and four other sides.

3.2.5.3 Organoleptic evaluation of samples

The colour, odour, appearance (microbial growth/spoilage) as well as ease of de-stalking, by rubbing between the palms, were carried out on each sample. The colour was observed to determine those that were bright black and those, which appeared brownish (dull) black. Also, deep-set wrinkled seed coats were noted.

Odour was determined by smelling samples to find out those with a more pungent smell. Microbial/mold growth resulted in whitish coloration on the seed coat. About ten lots of 50 peppercorns were sampled and peppercorns with whitish patches were separated from whole black peppercorns. Their ratio was then expressed in percentage mold formed.

3.2.5.4 Estimation of damaged peppercorns due to heat treatment

Twenty lots of 10 peppercorns were selected at random from the raw and each of the treatment samples and the ratio of damaged to undamaged peppercorns noted and the result reported in percent average. Damage was taken as any visible break in the peppercorn seed coat.

3.2.5.5 Estimation of ripeness due to Heaping

After each fermentation period, 50 peppercorns in lots of 10 were sampled at random and the ripe (red) ones separated from the unripe (green/yellowish). The ratio of ripe to the unripe was determined as a percentage.

3.3 Chlorine disinfection/cleaning of samples

The incoming municipal water was initially tested for its pH and for its free residual content and these were used as standards. 100 grams each of the raw-washed, raw-unwashed, dried blanched- and fermented-washed, dried blanched-and fermented-unwashed, as well as air-dried washed and unwashed samples were weighed into 500ml beakers and washed/disinfected with 200ml aliquots of gas-chlorinated water to a concentration of 3ppm free residual chlorine. (A washed sample here refers to use of municipal water). The samples were left to stand for 30 minutes while stirring and swirling at 10-minute intervals after which the wash-water was tested for free residual chlorine. Where there was no free residual chlorine the washing process was repeated until some free residual chlorine was obtained. The pH of the water before and after washing was also noted in each case. Part of the washed sample was solar-dried. The freshly washed and solar dried samples were then taken through microbiological analysis.

3.3.1 Determination of wash-water free residual chlorine

The wash/disinfection water is poured in duplicate into calibrated test tubes. Into one test tube is poured a sachet of chlorine reagent and placed in the sample cell of a pH and Chlorine Test Kit (Type: Cat. No. 15-396). The colour developed was compared to colours of standards using the chlorine/pH slide comparator. Each colour standard corresponded to a known free chlorine concentration. (Hach kit).

3.3.2 Determination of wash-water pH

The procedure used for free chlorine determination (3.5.1) applied to wash-water pH determination but in this system instead of the chlorine reagent, four drops of Hach Phenol Red Indication solution, standardized for colorimetric pH determination (Type: cat. 211-32), was added to the water sample before placing in the sampler. The pH slide comparator was used to compare sample colour with colours of standards. (Hach Test Kit for pH)

3.4 Drying of samples

In order to select the most suitable drying method two sample lots were washed and spread on aluminium trays. One was placed in the open air and dried directly under the sun (sun drying). The other was placed in a wooden Solar-drier that had net-covered holes at all sides to allow for ventilation, and the top was covered with polythene film. The solar-drier had the following size dimensions: Length 4feet, Width 2feet and height 7inches. The solar dryer is a locally manufactured one for use in research work at the Nutrition and Food Science Department, University of Ghana.

3.4.1 Solar drying of samples

After each blanched sample had been allowed to drain for 5 minutes they were spread on aluminium trays and placed in the solar-drier. On the other hand each fermented sample was spread on aluminium trays and placed in separate solar-drier. The weight of all the samples were noted prior to drying and after each 24-hour drying period the weights were again measured until they stabilized. The average daily temperature was estimated by taking temperature readings of the solar drier environment 5 times during the drying period (0800 - 1600 Hours).

3.4.2 Estimation of colour (Hunter lab)

Each of the dry samples was milled using the hammer mill (Type: Size 8 inch Laboratory mill Serial No. 41076) and about 60-gram portions in triplicate analyzed for colour using the Hunter Lab (Type: Colour Minolta Chroma meter CR 310)

3.5 Determination of extraneous material

200 grams of fresh peppercorn was weighed and thoroughly washed with water to remove all debris and unwanted material. The washed sample was then left to dry under the fan and then re-weighed (r) to determine the amount of filth originally present in the sample. The process was repeated for five randomly weighed samples for each pepper lot/source.

$$\% \text{ Extran. mat.} = \frac{(200 - r)\,100}{200}$$

3.5.1 Determination of extraneous material (plant source)

This determination was carried out on the dry samples. Due to the method of de-stalking, stalk pieces get stuck to the peppercorn. 50-gram sample weights (a) were randomly taken and any pieces of attached stalk were removed with a blade, after which the sample was re-weighed (b). The difference in weight was computed as:

$$\% \text{Extran. mat. (Plant source)} = \frac{(50 - b)\,100}{50}$$

3.6 Chemical Analysis

The blanched, fermented and disinfected samples were milled using the laboratory Disc Attrition Mill (Straub Model 4E Grinding Mill) prior to carrying out the chemical analysis.

3.6.1 Moisture content - by distillation with toluene

The distillation tube receiver and condenser were initially cleaned with $K_2Cr_2O_7/H_2SO_4$ mixture and thoroughly rinsed with water and then with 0.5N alcoholic KOH and drained for about 10 minutes. 20g of sample were weighed into distillation flask and the apparatus connected. The receiving tube was filled with toluene by pouring through top of condenser. The sample was brought to boiling to first distill slowly by use of the electric burner (Type: Electrothermal Cat. No. EME60250/CE MK1 Serial No. 10068739) at 80 amp, until most of the water had distilled over; the rate of distillation was then increased by setting the burner to 100. When all the water had apparently distilled over after 45 minutes, the condenser was washed down by pouring of toluene via the top with distillation continuing for a further 15 minutes. The washing was done for 2-3 times until no more water distilled over. Remaining water in the condenser was brushed down with tube brush attached to copper wire and which had been saturated with toluene, while the system was being washed down with toluene at the same time. Drops of water, stuck to sides of graduated water-receiving tube, were forced down using copper wire with the end wrapped in rubber band. The volume of water was recorded and the percent moisture calculated. (A.O.A.C, 1984).

3.6.2 Determination of Ash

About 2g of sample was weighed into a flat-bottom asbestos dish and placed in the entrance of an ashing furnace and ignited for 30 minutes at 550°C. The samples were transferred into a desiccator containing fresh desiccant, and allowed to cool to room temperature after about 45 minutes and then weighed using the Metler Balance (Type: Mettler Toledo Serial No. 1115341812) (A.O.A.C, 1984)

3.6.3 Determination of volatile and non-volatile ether extract

2 grams ground sample was weighed into an asbestos vial and extracted for 20 hours in a continuous extraction apparatus with anhydrous ether. The ethereal solution was collected in a flask of known weight and left to evaporate for 2 hours at room temperature before being stored over H_2SO_4

in a desiccator for 18 hours. The ethereal sample was weighed and the extract heated gradually, with weighing and re-weighing to a minimum weight at 110°C. Loss was determined as volatile ether extract whereas residue was weighed as non-volatile ether extract. (A.O.A.C, 1984). The chaff/waste in the asbestos vial was kept for crude fibre analysis.

3.6.4 Determination of volatile oil

The ground sample was passed through N°. 20 sieve, stirred thoroughly and 2g of it weighed and transferred to a 1litre flask. Distilled water was added to half-fill the flask. A stirring-bar was inserted and the flask placed on a hot-plate/heating mantle set over a magnetic stirrer. Trap and condenser, were previously cleaned with chromic acid solution just before use and trap was filled with water. The apparatus was set up so that the condensate would not drop directly on surface of the liquid in the trap, but would run down the side. The stirrer was started and the heat set through a variable transformer at 90volts (3amp.). Oil clung to walls of the graduated receiver was washed once with about 8 drops of saturated aqueous detergent through the top of the condenser. Distillation continued for 10 minutes after addition of detergent to wash it out of the trap. The distillation was carried out for 1 1/2 hours and the volume of oil noted. An hour later another reading was taken. The reading was noted after every hour until after 5 1/2 hours when two consecutive readings taken at the hourly intervals showed no change in oil constant. The oil was left to cool for 90 minutes and the volume of collected oil read and reported as percent oil. (A.O.A.C, 1984)

3.6.5 Determination of crude fibre

The 2g of extracted sample from 3.6.3 were transferred to 600ml beaker. 1g prepared asbestos powder and 200ml boiling 1.25-% H_2SO_4 was added. The beaker was placed on a digestion apparatus and the sample boiled for exactly 30 minutes, during which period the beaker was rotated periodically to dislodge adhering solids at the sides. The beaker was removed and its contents filtered through a buchner funnel, which had been pre-coated with asbestos. The beaker was rinsed 3 times with 50ml aliquots of boiling water, and each wash was sucked dry through the buchner funnel. The residue was scraped from the funnel into a beaker, 200ml boiling 1.25-% NaOH was added and boiled for exactly 30 minutes. The beaker was removed and filtered as before, while washing with 25ml boiling 1.25% H_2SO_4, followed by three 50ml portions of boiling water and then 25ml alcohol. The residue was scraped and transferred into ashing dish. The residue was dried for 2 hours in an oven (Type: Compenstat Brit. Pat. No. 882942) at 130+/-2°C and then cooled in a desiccator for 8 hours and weighed. The sample was then ignited for 30 minutes at 600+/-15°C, cooled in a desiccator and re-weighed. A blank made of 2g asbestos was run alongside.

%Crude Fibre, C=(Wt. loss on ignition - Wt. loss in asbestos blank) 100/sample wt.

Crude fibre (desired moisture basis)=C (100-%Moisture desired)/(100-%Moisture in sample). (A.O.A.C., 1984)

3.6.6 Determination of starch

The direct acid hydrolysis methods (A.O.A.C, 1984) were used to determine the starch content of sample. Four (4) grams milled sample was stirred in a beaker containing 50ml distilled water for 30 minutes. The contents of the beaker were filtered and washed with 200ml-distilled water. The insoluble residue was heated for 2 hours using 200ml distilled water and 20ml hydrochloric reflux condenser. The contents were cooled and neutralized with NaOH solution and then filtered. The dextrose in the aliquot filtrate was determined by the Lane-Eynon method.

% Starch = (% Dextrose obtained) 0.9

3.6.7 Determination of piperine

10g of finely ground sample was extracted with 150ml 95% ethanol in a soxhlet extractor for 2 hours. The solution was filtered and concentrated in vacuo on a water bath at 60°C for 1 hour. 10ml 10% alcoholic KOH was added to the filtrate residue and left for 30 minutes and then decanted from the insoluble residue. The alcoholic solution was left overnight. Yellowish needles were deposited. Drops of 1,3,5-trinitrobenzene were added to 5ml aliquots of the alcoholic solution in known drops and in excess, and the contents swirled. The red precipitates formed were filtered, dried and weighed. The weight of piperine was thus computed from the concentration (drops) of TNB used for optimum precipitation in 1:1 ratio. (R. Ikan, 1991).

3.7 Microbiological Analysis

The following microbiological quality tests were carried out on the variously treated samples to ascertain the most suitable treatment.

3.7.1 Treatment (preparation) of samples

Aseptically, 10 gram sample was weighed into thio-bags containing 90-ml sterile phosphate buffer. The mouth of the bag was secured and shaken for 5 minutes at 200 strokes per minute with mechanical shaker. The appropriate media were then inoculated within 15 minutes. (Speck, 1976).

3.7.2 Total bacteria count: thinning out method

Four (4) Total Count tubes (Hach kit) were taken and marked A, B, C, and D. Their caps were loosen. 1 ml of the sample was dispensed into tube A, the contents was thoroughly mixed by inverting the tube 4 times. Using a new dropper, 1 ml of A was transferred to B and mixed as before. The process continued by transfers from B to C and then to D. In each case, fresh droppers were used. The tubes were incubated at 35°C for 24 - 48 hours, after which period the tubes were observed for cloudiness/bacterial growth and the results compared to MPN Tables (Appendix 1).

3.7.3 Presumptive coliform test: Lauryl tryptose MPN method

By pipeting 1 ml of sample into each of three (3) volumetric flasks containing 0.1% peptone water, diluted samples of 1:10, 1:100, and 1:1000 were prepared and used to inoculate three (3) replicate tubes of lauryl tryptose containing Durham fermentation tubes. A set of test tubes was incubated at 37°C (for total coliforms) and another at 44.5°C (for faecal coliforms) for 24 - 48 hours. Tubes were observed for gas production either in the Durham tubes or by effervescence when the tube was gently shaken and recorded as positive. The observation was done after 24 hours and negative tubes re-incubated for additional 24 hours. By reference to MPN Tables the results were reported as presumptive MPN coliform per gram or ml of sample.

3.7.4 Confirmed coliform test: brilliant green bile (BGB) MPN method

Positive Lauryl tryptose tubes from 3.7.3 were sub-cultured into BGB broth tubes using a 3mm pre-sterilized loop, and the tubes incubated at 35°C for 48 hours. All BGB broth tubes showing gas production were reported as confirmed MPN of coliform per gram or ml of sample.

3.7.5 Confirmed E. coli test: EC broth method

All positive Lauryl tryptose broth tubes (3.7.3) were sub-cultured into EC broth by means of sterilized 3 mm loop and incubated for 48 hours at 45.5°C. Growth or cloudiness was reported as confirmed E. coli.

3.7.6 Microbial quality of food contact surfaces: Millipore Swab Test

The inside of the solar drier and trays used for drying were thoroughly cleaned and microbiologically tested using the Millipore swab test kit method. The swab was removed the buffer solution case by an end-to-end rocking motion. The tip of the swab was rolled on the inside of the case to wring out excess buffer. Five areas of the surface to be monitored were randomly selected and the swab used to draw the letter "M", rotating the swab tip in the process. Each straight section of "M" is of about 2 inches in length (i.e. distance between swab tip and base of swab handle). The process was repeated for the four remaining areas selected. The swab was firmly reinserted into the case and shaken about 30 times to dislodge organisms into the buffer. The sampler paddle was carefully removed and firmly inserted into the buffer. The unit was carefully laid horizontally on a flat surface with the membrane facing down and uniformly wetted. After 30 seconds the paddle was removed and vigorously shaken twice to remove excess buffer. The sampler was reinserted into the dry sampler case, labeled and, with the gritted side facing down, incubated at 35°C for 22 - 24 hours (Coliform count) and 48 - 72 hours (total count). The diagram and colony colours (Appendix 2) were used to estimate results. (User Guide - Millipore Samplers, Dilution Kits, and Swab Test Kits)

3.8 Storage studies

The treated samples were stored under warehouse condition of about 45 - 60% RH and a temperature range of 25 - 38°C. Ventilation was relatively static and samples drawn at 4-week intervals, prepared and analyzed for moisture, volatile and non-volatile ether extract, starch, crude fibre, ash, piperine, weight/size of peppercorn, as well as insect/fungal growth.

CHAPTER FOUR

Results and Discussions

Table 4.1 Characteristics of The Black Pepper Variety (control)

	Commercial indices	Experimental
1000 peppercorn weight (grams)	*	77.0±1.0
Starch (%)	30 approx.	29.0±0.8
Ash (%)	6 maximum	3.72±0.04
Non-volatile ether extract (%)	6 maximum	5.49±0.60
Volatile ether extract (%)	1-2.5	1.3±0.4
Crude fibre (%)	15 maximum	14.4±2.0
Piperine (%)	5-9	6.0±1.0
Light berry (%)	10 maximum	7.5±0.5
Foreign matter (%)	1.5 maximum	2.1±1.0
Moisture content (%)	12 maximum	9.8±0.9
Dimensions - Length (mm)	*	3.2±0.3
- Width (mm)	*	2.9±0.1

(Results are mean of 3 determinations ± standard deviation)
** =Not available Source of commercial indices: Nyamekye-Boamah,1995.*

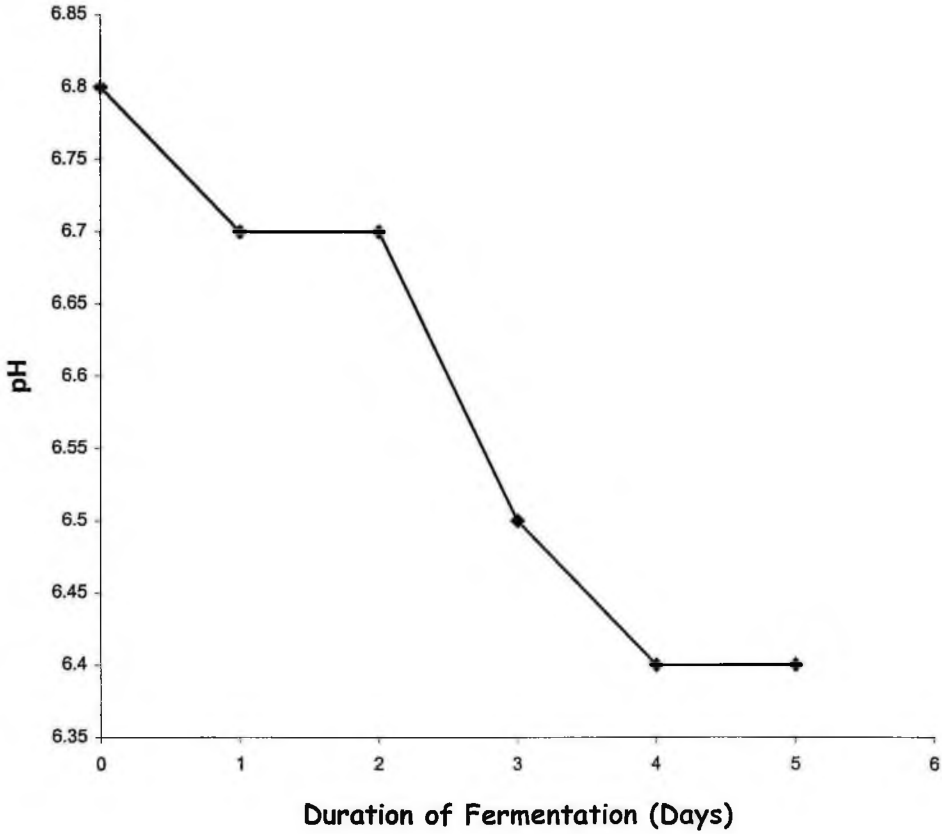
The final results due to treatment are dependent on the initial raw material quality, therefore the need to ascertain the untreated sample quality and characteristics. One thousand peppercorn weight of 77.0 grams far exceeds 45.0 grams quoted by Akromah (1991). This may be due, generally, to cultivation and climatic conditions, which affect yield (Akromah, 1991 and Parry, 1969). Starch content of 29.0% compares with the commercially acceptable value of about 30%. The ash of 3.72% falls within the expected commercial figure of 6.0% (maximum). Whereas the volatile oil/ether extract, 1.3%, falls within the quoted range of 1-2.5%, the non-volatile ether extract obtained (5.49%) was just about the same as quoted for commercial acceptance, about 6.0%. Crude fibre of 14.4% compares with the 15% maximum expected for commercial Balancotta black pepper. Piperine, 6.0%, also falls within the acceptable range of 5-9%. Light berry and foreign matter were determined to be 7.5% and 2.1% respectively and their corresponding expected values are 10% maximum and not more than 2.0% respectively. Moisture content of commercial black pepper (Balancotta) is pegged at 11.0% maximum, but the experimental sample reported a 9.8% after 5 days of drying, in a solar drier, at an average daily solar drier environment temperature of 39°C, (range 28°-50°C). The length (the longer circumference of the peppercorn) and the width (the shorter circumference) were estimated to be 3.2 mm and 2.9 mm respectively.

4.1.1 Effect of treatment on sample characteristics

4.1.1.1 Changes in pH during heaping/fermentation

During heaping, browning fermentation is induced. Microorganisms in the air, which were capable of thriving due to the drippings from the seeds and the elevated temperatures due to the heap, carry out various metabolic activities, which result in metabolites that tend to cause pH variations. The longer the period of heaping, the more the population of microorganisms for the five-day heap studies. This increase in microbial population with time can be attributed to the release of food from the cells due to increasing rot of the seeds with time, hence the more the metabolites formed. Also, enzymatic and other metabolic activities within the

Figure 4: pH Variations in Black Pepper Sample Heaps Kept At Different Time Intervals.

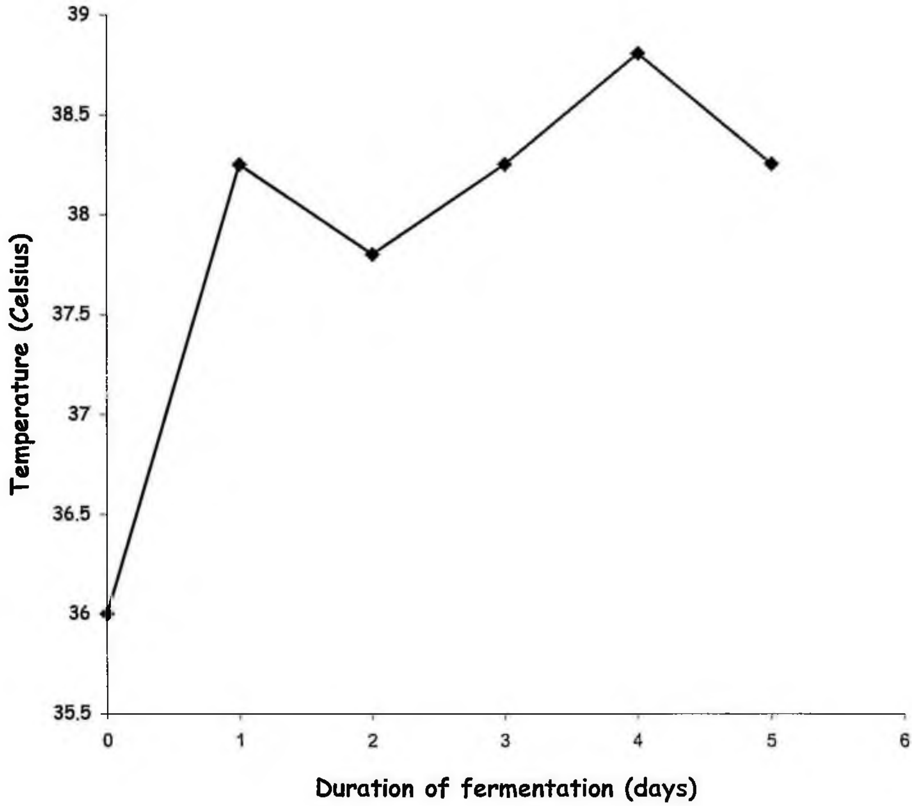

6.85
6.8
6.75
6.7
6.65
6.6
6.55
6.5
6.45
6.4
6.35
pH
0
1
2
3
4
5
6
Duration of Fermentation (Days)

Peppercorns were likely to result in secretions across the seed coat. Such secretions may also cause the observed trend of pH changes with increasing period of heap/fermentation (Figure 4). Thus from an initial pH of 6.8, the pH dropped after day one of fermentation to 6.7, staying stable till the third day when it dropped again to 6.5. The downward trend continued to pH 6.4 and again remained stable till observation was discontinued on the fifth day.

4.1.1.2 Temperature Variations During heaping of Black Pepper

As metabolic activity increases, temperature also increases. Thus, as was observed in **4.1.1.1** for the causes for pH variation, the changing temperature profile was justified. Hence, as activity within the heap increased the temperature also increased, but when activity literally ceased (that is, stable pH), the temperature also dropped because of heat exchange between the heap and its surroundings (Figure 5).

Figure 5: A Graph Of Variation In Heaped Black Pepper Temperature With Increasing Time for Heaping.


39
38.5
38
37.5
37
36.5
36
35.5
Temperature (Celsius)
0
1
2
3
4
5
6
Duration of fermentation (days)

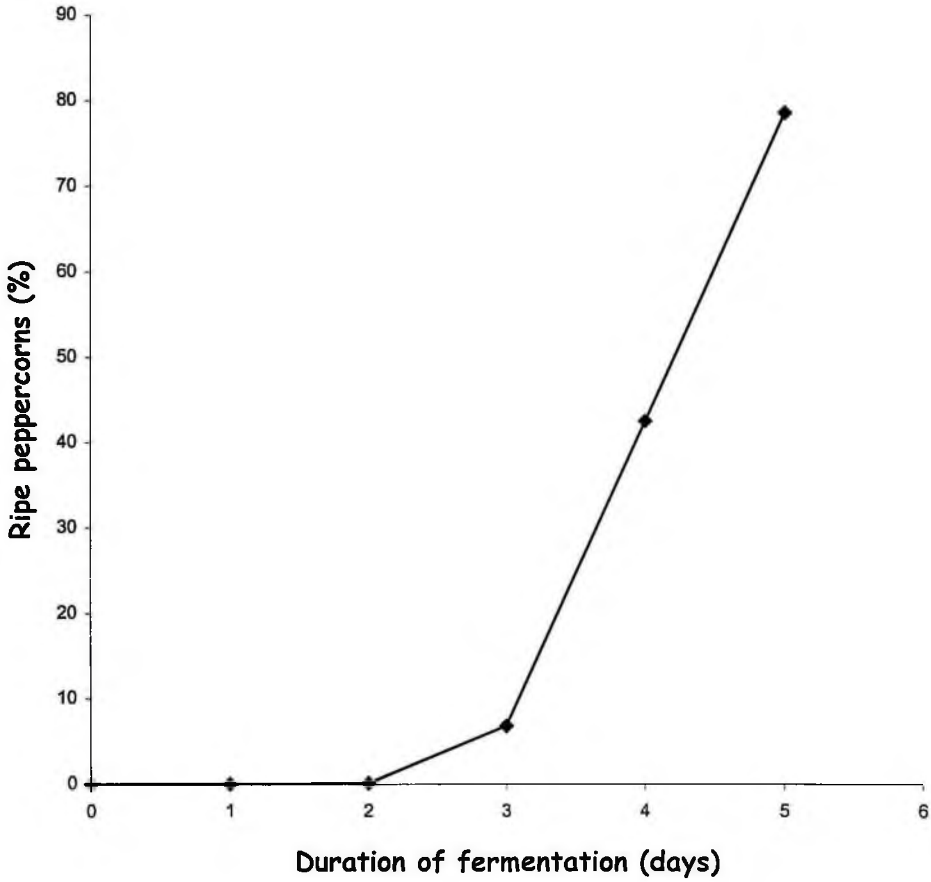
4.1.1.3 Ripening of heaped black pepper

The black pepper plant, from the time it fruits, sets a natural process in motion which was expected to result in maturation of fruits, ripening, wilting, rot, and finally death and decay. At the time of harvest, however, some metabolic and/or enzymatic activity continues until life ceases within the peppercorn. Coming with this is the process of ripening. Depending on the level of maturation of the fruits at harvest, ripening may set in early or late. When it starts, however, it proceeds rapidly. As shown in figure six (6), the amount of fruits that ripen began to increase after the heap of pepper was three days old, and from then the rate of ripening was faster. Such trend was expected because not all the fruits were borne at the same time. Those that were borne initially, take the lead in ripening. Thus the amount of ripe peppercorn depended on the amount of fruits that were borne by the plant at a given time during the fruiting period.

4.1.1.4 Mold infestation of heaped black pepper

As microorganisms began to settle and feed on the heaped samples, whitish mold growth was observed, and this whitish growth persisted even after drying. The extent to which this affected the black colour of the final product depended on the extent of infestation prior to drying. As the heap period extended beyond two days, figure 7, mold growth became more and more extensive. By the second day, mold

Figure 6: A Graph Showing The Amount of Black Pepper Fruits That Ripen When Heap Period Is Increased



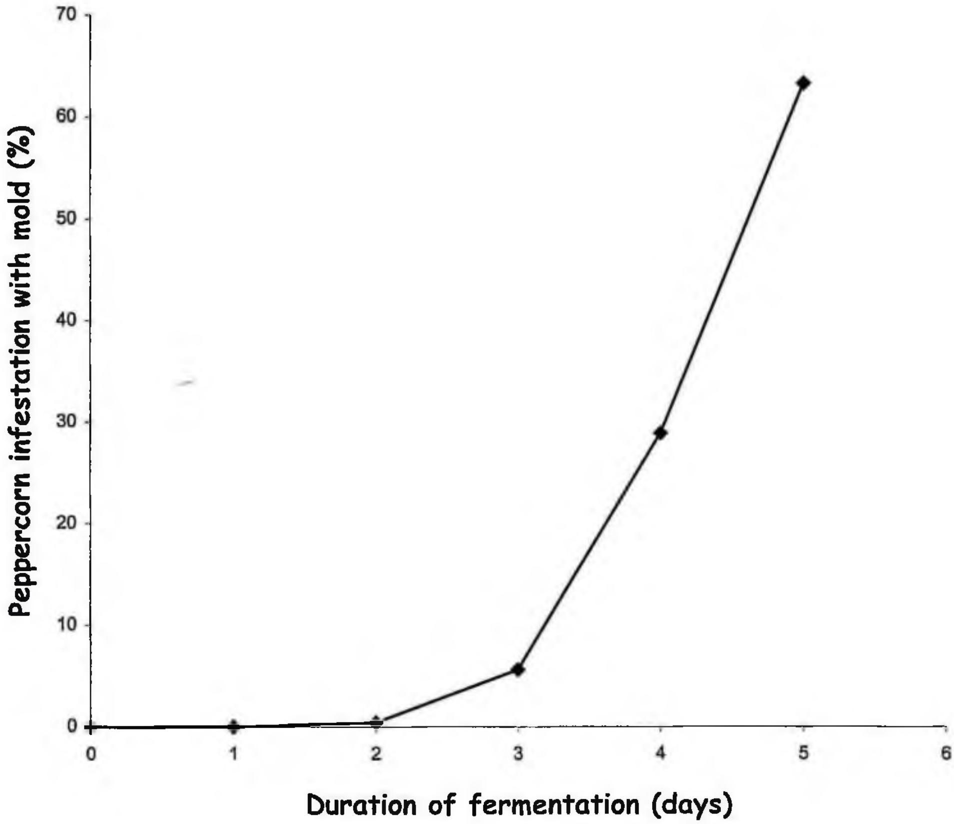
Ripe peppercorns (%)
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Duration of fermentation (days)

growth had just begun, yet the browning objective was appreciably attained. Hence two days heaping may just be the requirement for the desired browning of the fruits, as any further extension of the treatment would only result in an unacceptable product-colour with a high microbial load.

4.1.1.5 Ease of de-stalking and foreign material (plant source)

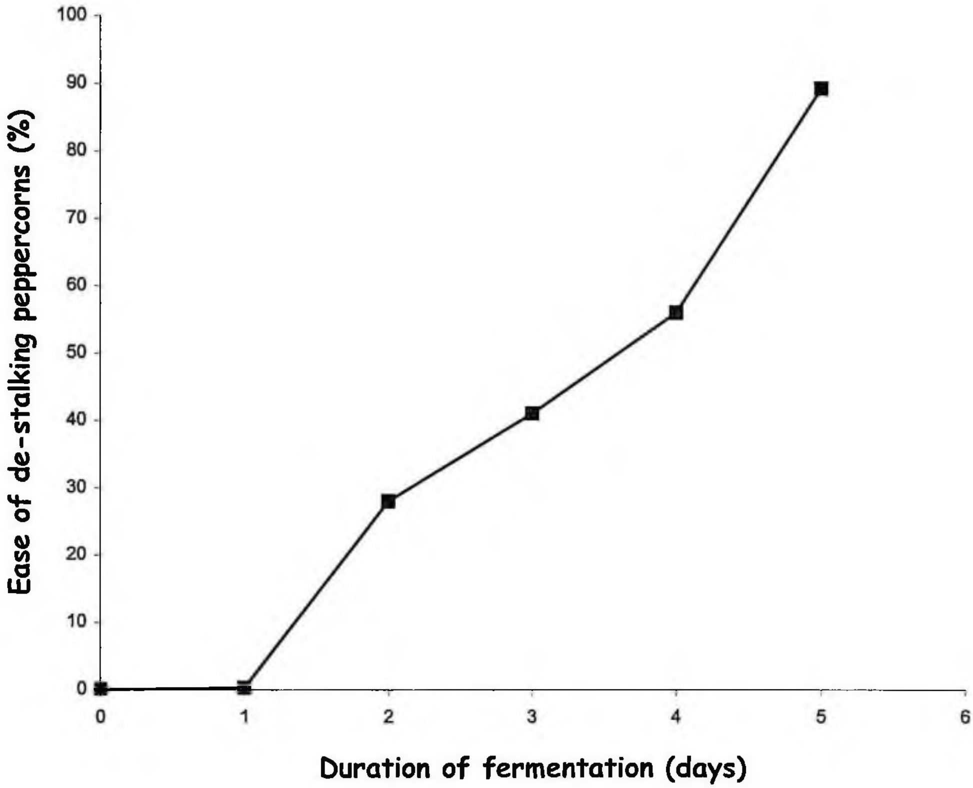
Immediately following harvest, detaching the peppercorns from the stalk was difficult and may result in damage to the seed coat. After some period of heaping, de-stalking is much easier. However, the seed coat of the peppercorns at this stage were fragile, hence they had to be dried for two to three days before de-stalking was carried out. As heap duration, prior to drying, was increased, the ease with which de-stalking was achieved also increased (Figure 8). However, looking at the trend of foreign material content of de-stalked peppercorns form F1 through to F5, figure 9, it was obvious that parts of the pedicel or fruit stalk, was attached to the peppercorn. Thus the longer the heap period, the more stuck the pedicel to the peppercorn, and the higher the foreign material (plant source). The levels decreased gradually from day one to day three, after which they increased above the commercial requirement of 1.5%. Thus the easier it was to de-stalk, with increasing heap duration, the higher the amount of foreign material.

Figure 7: A Graph Showing How Period of Heap Impacts The Extent of Mold Infestation of Heaped Black Pepper


70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Peppercorn infestation with mold (%)
Duration of fermentation (days)

This notwithstanding, by the fifth day most of the peppercorns had self-destalked, however, the remaining which had not self-destalked generated so much foreign material to offset an expected fall in the level of foreign contamination at day four and five. The acceptable heap period would, thus range from one to three days but factors such as mold growth; ease of de-stalking in relation to plant foreign material may have to be considered in order to make a treatment choice that would give optimum results.

Figure 8: A Graph Showing The Ease With Which Peppercorns Were De-stalked As Heap Period Increased



100
90
80
70
60
50
40
30
20
10
0
Ease of de-stalking peppercorns (%)
0
1
2
3
4
5
6
Duration of fermentation (days)

Figure 9: A Graph Relating The Plant Source of Foreign Material With Increasing Heap Period

Ctrl.: Control

B1: Sample given heat treatment at 95°C for 1 minute

B2: Sample given heat treatment at 95°C for 2 minutes

B3: Sample given heat treatment at 95°C for 3 minutes

B5: Sample given heat treatment at 95°C for 5 minutes

B7: Sample given heat treatment at 95°C for 7 minutes

B10: Sample given heat treatment at 95°C for 10 minutes

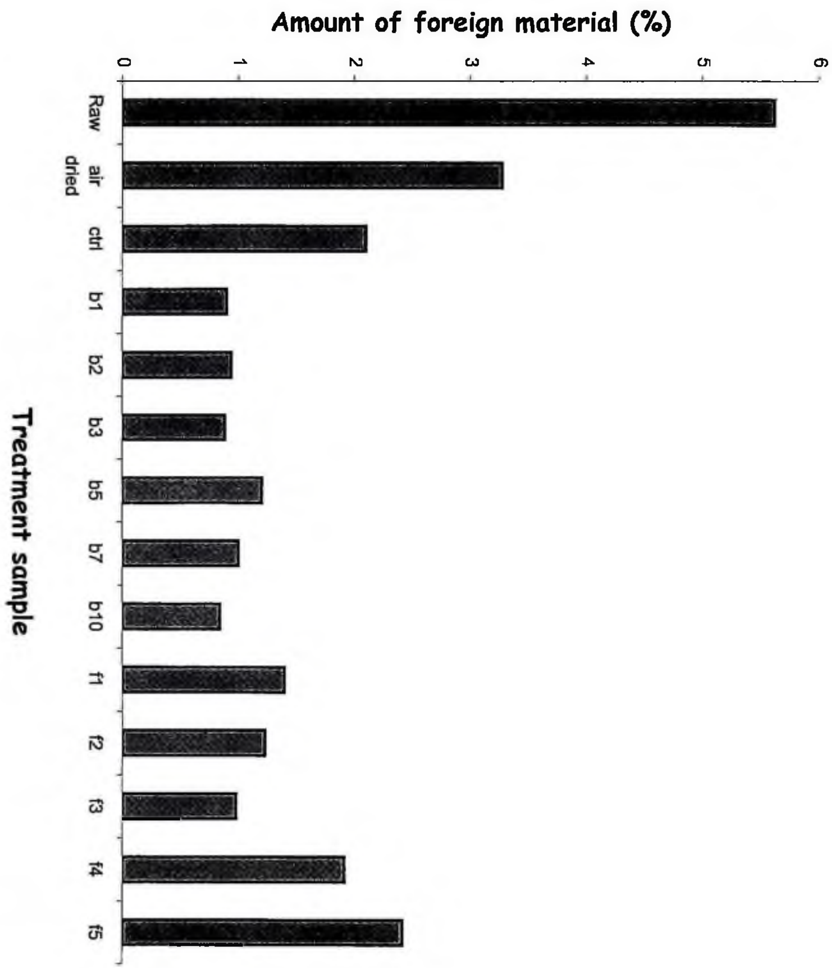
F1: Sample heaped at room temperature for 1 day

F2: Sample heaped at room temperature for 2 days

F3: Sample heaped at room temperature for 3 days

F4: Sample heaped at room temperature for 4 days

F5: Sample heaped at room temperature for 5 days

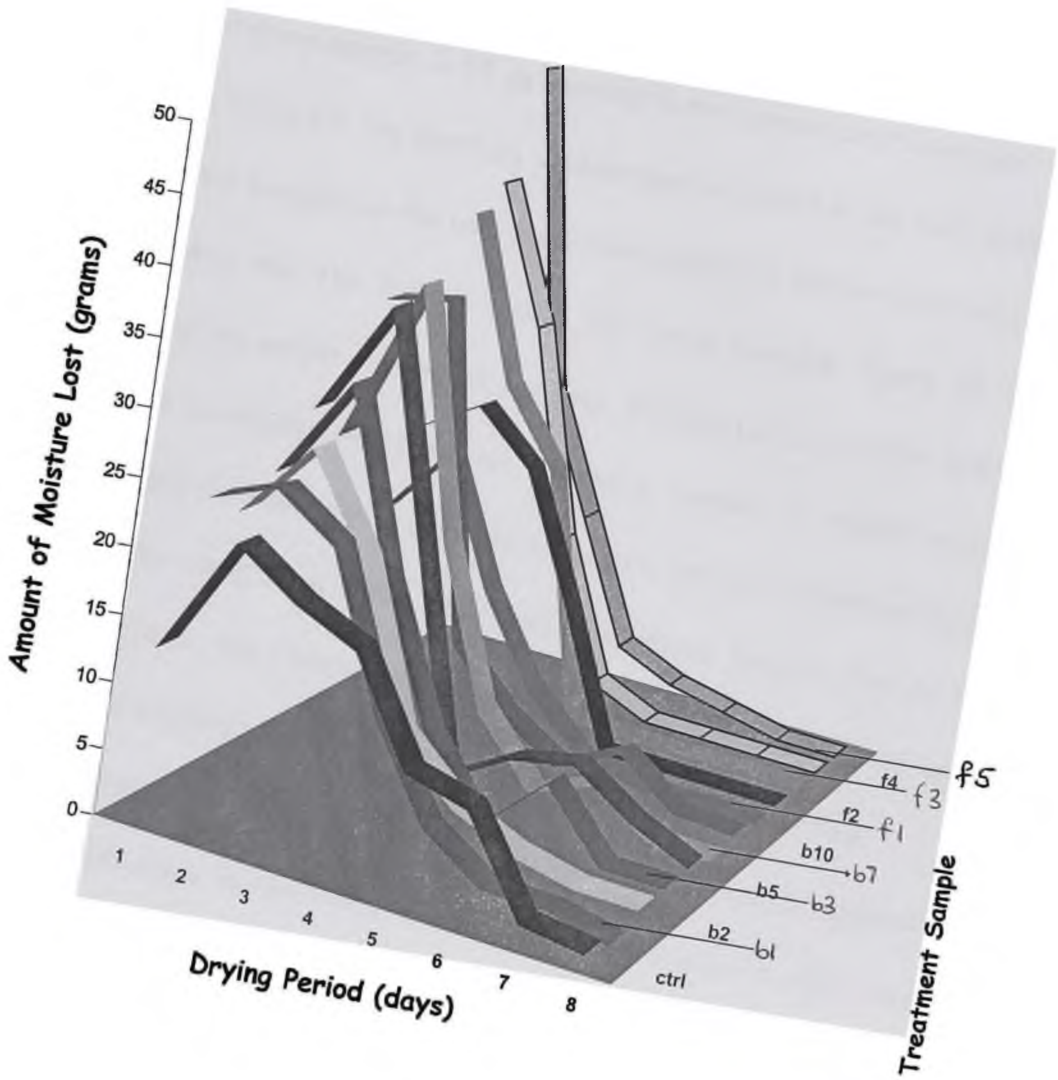


Amount of foreign material (%)
0
1
2
3
4
5
6
Raw
air dried
ctrl
b1
b2
b3
b5
b7
b10
f1
f2
f3
f4
f5
Treatment sample

4.1.2 Weight loss during drying; Moisture content and Light berry

During the drying process, moisture was lost from the treatment samples at varying rates. Thus, the observed differences may be attributed to treatment, since all other conditions were the same. Figure ten (10) presents the scenario of the drying process. Apart from the control and F3 samples, which continued to lose differentially less amount of moisture with increasing drying period, the other samples did not show such a normal definitive drying pattern, especially during the second and sixth day of drying. B5, in addition, exhibited an abnormal trend on the fifth day. As drying proceeds it becomes more difficult for moisture to be lost as more energy may be required to get the most tightly bound water. The F3 sample dried fastest, since after the fourth day of drying it lost very little or no moisture again. After the seventh day of drying no significant moisture loss was observed for all the samples.

Figure 10: A Graph of Weight Differential of The Treatment Samples During Drying



Amount of Moisture Lost (grams)
50
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
Drying Period (days)
ctrl
b2
b1
b5
b3
b10
b7
f2
f1
f4
f3
f5
Treatment Sample

Considering the moisture content graph (Figure 11), there is evidence that the solar drying technique was much more efficient compared to open, air-drying. The final moisture content of all the samples was acceptable, except for the air-dried sample, 12.5% as opposed to the commercial requirement of 12%. The trend for the moisture content was reflected in the final weight of the dried samples, as the unit peppercorn weight of the air-dried sample (0.92 grams) was the highest among the dried samples, figure 12. In considering the weight of samples, however, another factor of yield, due to production practices, soil type and nutrients, amount of rainfall among others needs consideration in order to arrive at a justified conclusion. Since the samples came from known plantations and these factors tend to be largely uniform, the close relationship between moisture content and unit peppercorn weight as depicted by their respective graphs is justified.

The same reasons of yield and moisture loss, used to explain unit peppercorn weight would apply to the trend observed for the variation in light berry with treatment (Figure 13). In this case, the samples that had relatively higher moisture content had lower levels of light berry. The heaped/fermented samples had higher amounts of light berry compared to the treatment samples. This may be due to the utilization/loss of nutrients during fermentation.

Figure 11: Moisture Content of Raw & Treated Black Pepper

Ctrl.: Control

B1: Sample given heat treatment at 95°C for 1 minute

B2: Sample given heat treatment at 95°C for 2 minutes

B3: Sample given heat treatment at 95°C for 3 minutes

B5: Sample given heat treatment at 95°C for 5 minutes

B7: Sample given heat treatment at 95°C for 7 minutes

B10: Sample given heat treatment at 95°C for 10 minutes

F1: Sample heaped at room temperature for 1 day

F2: Sample heaped at room temperature for 2 days

F3: Sample heaped at room temperature for 3 days

F4: Sample heaped at room temperature for 4 days

F5: Sample heaped at room temperature for 5 days

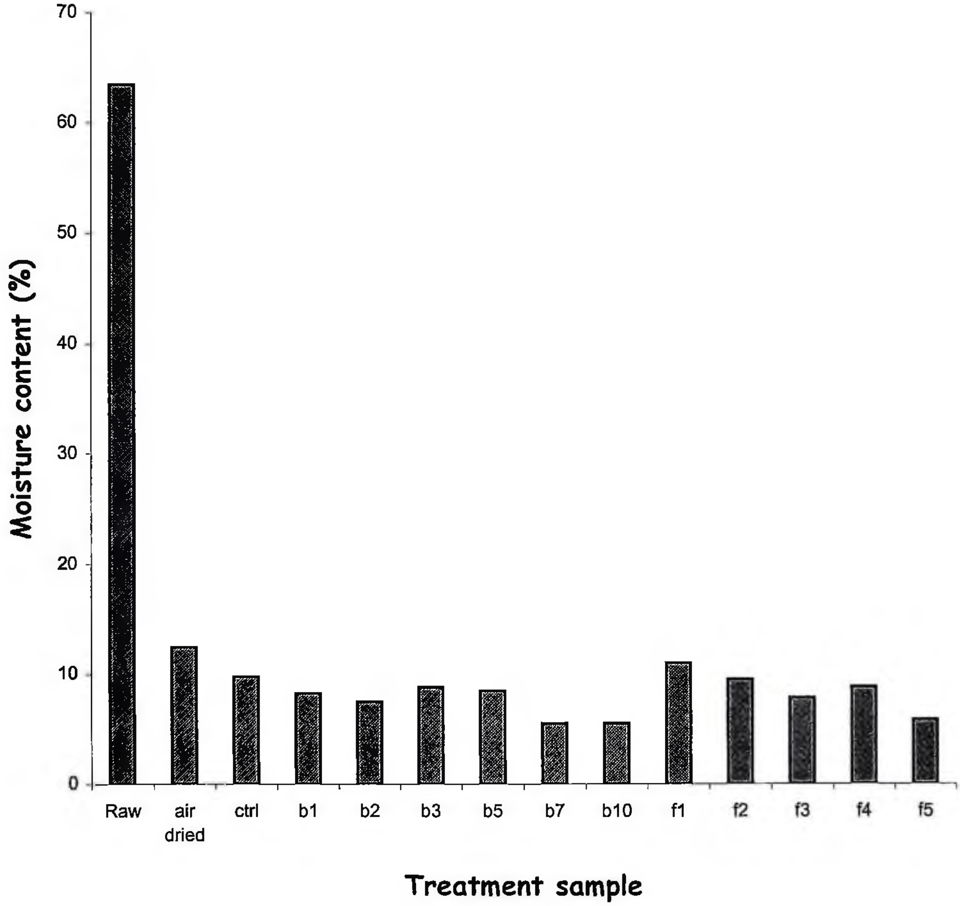

70
60
50
40
30
20
10
0
Moisture content (%)
Raw
air dried
ctrl
b1
b2
b3
b5
b7
b10
f1
f2
f3
f4
f5
Treatment sample

Figure 12: Variation In Unit weight of Black Peppercorn With Treatment

Ctrl.: Control

B1: Sample given heat treatment at 95°C for 1 minute

B2: Sample given heat treatment at 95°C for 2 minutes

B3: Sample given heat treatment at 95°C for 3 minutes

B5: Sample given heat treatment at 95°C for 5 minutes

B7: Sample given heat treatment at 95°C for 7 minutes

B10: Sample given heat treatment at 95°C for 10 minutes

F1: Sample heaped at room temperature for 1 day

F2: Sample heaped at room temperature for 2 days

F3: Sample heaped at room temperature for 3 days

F4: Sample heaped at room temperature for 4 days

F5: Sample heaped at room temperature for 5 days

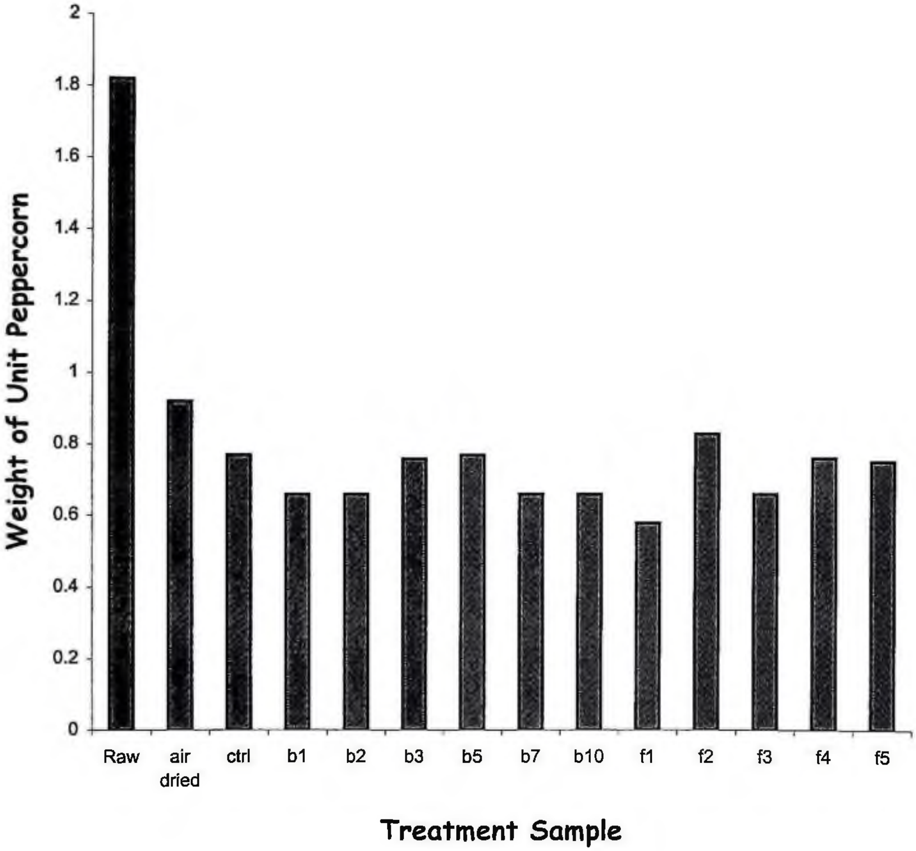

Weight of Unit Peppercorn
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Raw
air dried
ctrl
b1
b2
b3
b5
b7
b10
f1
f2
f3
f4
f5
Treatment Sample

Figure 13: Variation In The Amount of Black Pepper Light Berries With Treatment

Ctrl.: Control

B1: Sample given heat treatment at 95°C for 1 minute

B2: Sample given heat treatment at 95°C for 2 minutes

B3: Sample given heat treatment at 95°C for 3 minutes

B5: Sample given heat treatment at 95°C for 5 minutes

B7: Sample given heat treatment at 95°C for 7 minutes

B10: Sample given heat treatment at 95°C for 10 minutes

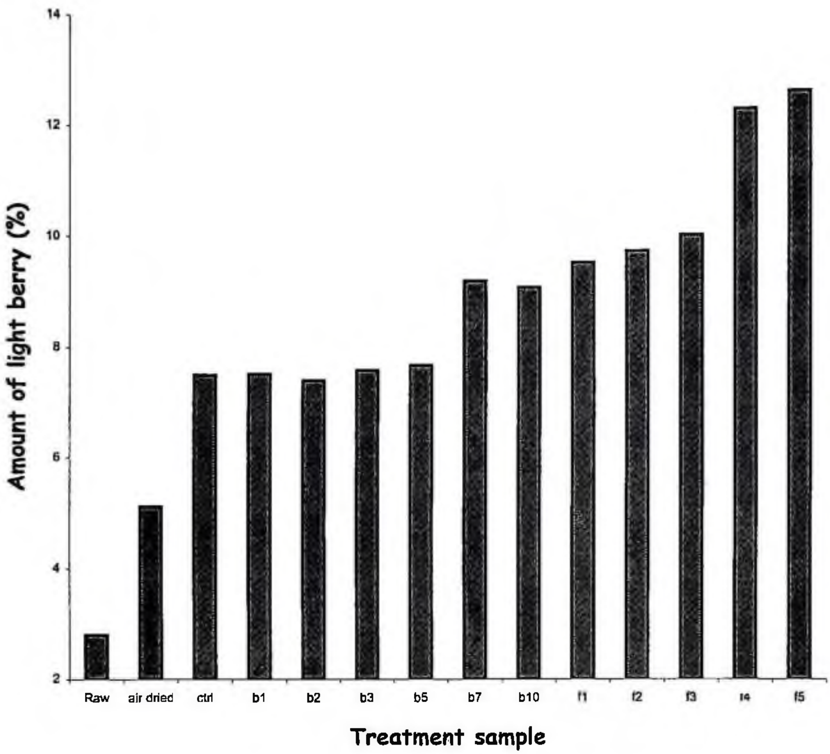
F1: Sample heaped at room temperature for 1 day

F2: Sample heaped at room temperature for 2 days

F3: Sample heaped at room temperature for 3 days

F4: Sample heaped at room temperature for 4 days

F5: Sample heaped at room temperature for 5 days


Amount of light berry (%)
14
12
10
8
6
4
2
Raw
air dried
ctrl
b1
b2
b3
b5
b7
b10
f1
f2
f3
f4
f5
Treatment sample

4.1.3 Dimensions of the treatment samples

The largest circumference, the length, generally had a higher value in all the samples compared to the corresponding width. Thus, by nature, the shape of the peppercorn ensures such dimensional difference. The raw sample had the largest set of dimensions. Its length and width were 5.12mm and 4.7mm respectively. Generally, drying had a significant impact on the dimensions, by reducing them to the region of 3mm (Figure 14)., indicating that moisture played a significant role in enhancing the dimensions of the peppercorns in their raw state. Heat treatment and fermentation seemed to have had a marginal effect by increasing the dimensions slightly, when compared to the control (no treatment). This, perhaps, was due to toughening of the seed coat prior to drying. The main deviation was in the F1 sample, which had much lower than expected dimensions compared to the control. This may be due to increased metabolic activity that may have influenced the size at drying.

Figure 14: Dimensional Variations in Black Pepper Treatment Samples

Ctrl.: Control

B1: Sample given heat treatment at 95oC for 1 minute

B2: Sample given heat treatment at 95°C for 2 minutes

B3: Sample given heat treatment at 95°C for 3 minutes

B5: Sample given heat treatment at 95°C for 5 minutes

B7: Sample given heat treatment at 95°C for 7 minutes

B10: Sample given heat treatment at 95°C for 10 minutes

F1: Sample heaped at room temperature for 1 day

F2: Sample heaped at room temperature for 2 days

F3: Sample heaped at room temperature for 3 days

F4: Sample heaped at room temperature for 4 days

F5: Sample heaped at room temperature for 5 days

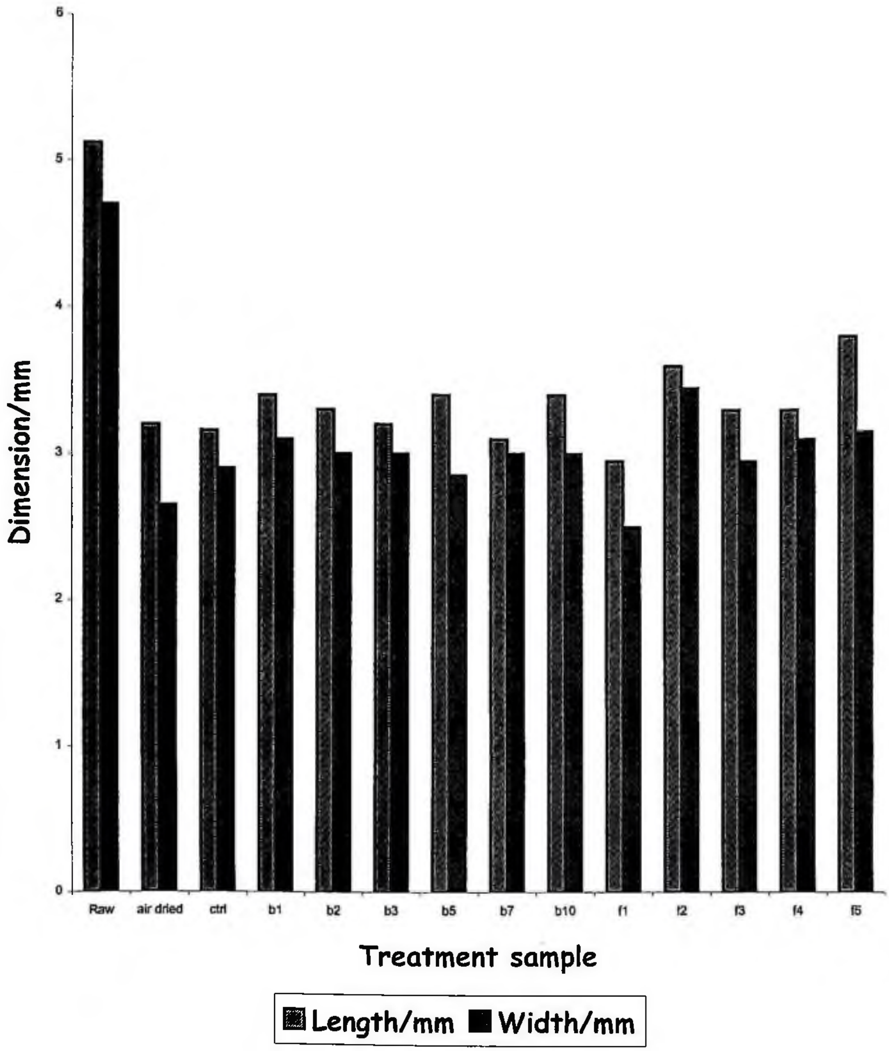

6
5
4
3
2
1
0
Dimension/mm
Raw
air dried
ctrl
b1
b2
b3
b5
b7
b10
f1
f2
f3
f4
f5
Treatment sample
Length/mm
Width/mm

Table 4.2: Effect of Fermentation on Black Pepper Characteristics

Heap period (Days)	1	2	3	4	5
1000 P.wt.(g)	78.0±1.0	73.0±2.0	68.4±0.9	71±1	65.0±2.0
Moist. cnt. (%)	11.0±0.3	9.5±0.2	7.8±0.3	8.8±0.1	5.8±0.4
Ash (%)	3.86±0.05	3.8±0.1	3.84±0.08	3.98±0.1	3.66±0.09
N-Veth. ext.(%)	4.71±0.3	4.8±0.4	5.0±0.8	5.1±0.5	7.0±1.0
V. eth. ext. (%)	1.0±0.5	1.0±0.6	1.2±1.0	1.8±0.7	1.6±0.5
Crude fibr. (%)	14.0±1.0	14.8±0.8	14.0±2.0	14.0±0.6	14.0±0.9
Starch (%)	30.0±0.8	29.0±1.0	28.0±1.0	28.0±0.6	28.0±0.9
Piperine (%)	5.9±0.6	6.0±1.0	6.1±0.8	6.5±0.4	6.5±0.8
Light berry (%)	10.0±1.0	9.8±0.8	10±2.0	12±1	12.6±0.5
Foreign mat.(%)	1.4±0.7	1.2±0.4	0.98±0.06	1.9±0.8	2.4±0.5
Length (mm)	3.0±0.2	3.6±0.1	3.3±0.2	3.3±0.2	3.8±0.3
Width (mm)	2.5±0.4	3.5±0.1	3.0±0.3	3.1±0.4	3.2±0.2

(Results are mean of 3 determinations ± standard deviation)

Fermentation, induced as a result of heaping the fresh peppercorns causes a change brought about by enzymes or living organisms such as bacteria or as microorganisms existing as unicellular plants including yeasts, molds and fungi (Bavappa and Ruettima, 1990). The chemical changes may include oxidation of nitrogenous organic compounds, and decomposition of sugars and starches to ethyl alcohol and carbon dioxide (Johnson and Peterson, 1974). One thousand peppercorn weight tended to generally decrease from day 1 heaping to day 5. That is, from 78.0 grams to 65.0 grams. The fourth day sample interrupted the downward trend, however, yielding a higher weight of 71.0 grams compared to day 3 (68.4 grams). The moisture content followed the same trend as one thousand-peppercorn weight. Beginning from a high of 11.0% moisture at day 1 and ending on 5.8% at day 5. Again, day 4 had higher moisture content than day 3, thus disrupting the downward trend. This higher moisture content at day 4 may account for its higher than expected 1000 peppercorn weight. Ash content did not show any remarkable changes as a result of heap duration, averaging 3.8% across the treatment period, except day 5, which recorded 3.66%. Period of heaping had very little effect on the crude fibre content. With the exception of day 2 which yielded 14.8% crude fibre, the remaining samples averaged 14.0%. These however fall within the commercially acceptable limit of not more than 15%. Heaping affected starch content as the value gradually reduced from 30% at day 1 to 28% at day 5 . This could be attributed to the depletion of nutrients as natural decomposition due to senescence sets in as the pepper ripens and tends to rot. The seed coat degenerated, thus giving microorganisms and natural enzymes access to the starch store, which was consequently degraded. Expected piperine content of balancotta is about 6.0%. Day 1 heaping through to day 5 caused an increase in piperine from 5.9% to 6.5%. Heaping/fermentation enhances pepper flavour (Bavappa and Ruettiman), hence the observed trend. At the same time, decomposition due to increased period of heaping causes more light berries to be generated, thus the observed increasing trend in the amount of generated light berries from a low of 10% at day 1 to a high of 12.6% at day 5. This value is higher than the acceptable commercial value of 10% maximum. Both volatile and non-volatile ether extract increased with increasing heap period. The volatile extract, however, dropped slightly at day 5. Variations in unit weight, length and width of the fermented dried pepper followed a similar pattern. The least weight was at F1 (0.58 grams). F1 produced the least length and width as well. That is, 3.0 mm and 2.5 mm respectively. These increased drastically at F2 in all three cases, but dropped at F3 and began to gradually increase until F5. Apart from the length whose increased value at F5 was above that of F2, the F5 values for weight and width increased but not above their corresponding F2 values.

Table 4.3: Effect of Heat Treatment on Black Pepper Characteristics

Heat (95°C) (Minutes)	1	2	3	5	7	10
1000 P.wt.(g)	75.2±0.8	66±2	76.4±0.9	76±1.0	63±1.0	58±1.0
Moisture (%)	8.3±0.8	7.5±1.0	8.8±0.5	8.5±0.2	5.5±0.4	5.5±0.8
Ash (%)	3.5±0.1	3.6±0.1	3.8±0.4	3.4±0.1	3.5±0.2	3.5±0.4
N-V eth. ext.(%)	6.5±0.5	6.4±0.2	5.0±1.0	4.2±0.8	4.0±0.4	3.1±1.0
V. eth. ext. (%)	1.2±0.5	0.8±0.2	1.0±0.3	0.7±0.3	0.5±0.2	0.5±0.3
Crude fibr. (%)	14.8±0.9	15.0±1	14.5±0.4	14.1±0.8	14.9±0.4	14±1
Starch (%)	28.9±0.5	28.1±1	29.6±0.9	29.0±2	29.0±1	30.1±0.8
Piperine (%)	5.2±0.7	5.4±0.9	5.3±1.0	5.0±0.4	5.0±0.8	4.9±0.4
Light berry (%)	7.5±0.4	7.4±1.0	8.0±1.0	7.7±0.9	9.2±0.7	9.0±1.0
Foreign mat.(%)	0.9±0.3	0.9±0.4	0.9±0.4	1.2±0.6	1.0±0.3	0.8±0.2
Length (mm)	3.4±0.2	3.3±0.1	3.2±0.3	3.4±0.1	3.1±0.2	3.4±0.3
Width (mm)	3.1±0.2	3.0±0.1	3.0±0.1	2.9±0.2	3.0±0.1	3.0±0.2

(Results are mean of 3 determinations ± standard deviation)

Generally, one thousand peppercorn weight reduced gradually with increasing time of heat treatment. For each deviation from this trend, there was appreciable level of increase in moisture content (B3 and B5). The highest and lowest values for both thousand peppercorn weight and moisture content were 76.4 grams (B3) and 8.8% (B3), and 58 grams (B10) and 5.5% (B7 and B10) respectively. Ash content, crude fibre, starch, foreign material, unit weight, length and width remained relatively stable with heat treatment. Non-volatile and volatile ether extract showed gradual reduction in content across the treatment range. Yielding a high of 6.5% and 1.2% respectively, as well as a low of 3.1% and 0.5% respectively. Piperine content showed a similar depleting trend with increasing time of heat treatment. Starting with

5.2% at B1 and a final value of 4.9% at B10. The increasing period of heat treatment, however, caused a corresponding gradual increase in light berry generation. B1 had 7.5% and B7, 9.2%.

Table 4.4: Characteristic Changes As A Result of Heaping

Heaping Period (Days)	0	1	2	3	4	5
pH	6.78	6.69	6.67	6.49	6.44	6.38
Temp(°C)	36	38.2	37.8	38.2	38.8	38.2
Ripeness(%)	0	0	0.1	6.8	42.5	78.4
Moldiness(%)	0	0	0.4	5.6	28.9	63.2
Ease of De-stalking(%)	0	0.3	28	41	56	89

Heaping/fermentation caused increasing acidity with extension of the treatment period. This may be attributed to microbial activity associated with ripening and decomposition of fresh agricultural produce such as the balancotta (Bavappa and Ruettiman, 1990). The trend started with a pH of 6.78 at F1 and closing at pH 6.38. Temperature changes over the period showed a leap by day 1 and then increased again from a low value at day 2 until it reduced slightly from that of day 4 at F5. This trend showed the activity or otherwise in the heaps due to microbial activity. This was supported by the drastic increase in the ripening and decomposition process and their concomitant increase in mold infestation as fermentation/heaping period was increased. Increasing the heap period also helped improve the de-stalking of the peppercorns. Improvement in de-stalking caused an increase in foreign material of the dried product, due to sticking of the peppercorn stalk to the pedicel on drying.

Table 4.5: Characteristic Changes During Two Minutes' Heat Treatment

Water Temperature (°C)	60	70	80	85	90	95	100
Onset of black colour(minutes)	45	37	8	5	3	2	1
Damaged peppercorn(%)	0.5	0.7	1.2	1.0	2.5	5.2	12.4

Black colour development and consistency is achieved, normally, by heat treatment of sample or by sterilization. This treatment, however, causes the loss of flavour compounds and oils (Tainter, 1992). Short-term heat treatment for the generation of appropriate colour was therefore necessary to this work. The time required for black colour formation decreased with increases in temperature. This led to the choice of 90°-95°C as heating temperature. The choice considered the rate of damage to the pepper, which increased with increases in temperature. At this temperature range, damage to pepper was relatively low considering the rate of black colour development and the need to preserve a lot of the oil and flavour of the pepper, which is attained with short time heat treatment.

Table 4.6: Comparison of Black Pepper from Different Sources

Source	Bunso	Tanoso	Kade
1000 P.wt.(g)	74.0±2.0	62±3	69±1
Moist. cnt. (%)	9.8±0.9	9.0±0.4	9.4±0.5
Ash (%)	3.72±0.04	4.0±0.1	3.5±0.1
N-V eth. ext.(%)	5.5±0.6	5.0±1.0	4.8±0.5
V. eth. ext. (%)	1.3±0.4	0.9±0.5	1.1±0.1
Crude fibr. (%)	14.4±2.0	14.0±0.9	13.7±1.0
Starch (%)	29.0±0.8	30.4±1.0	30±1
Piperine (%)	6.0±1.0	6.2±0.8	5.0±0.6
Light berry (%)	7.5±0.5	20.7±1.0	8.5±0.9
Length (mm)	3.2±0. 3	2.8±0.1	3.1±0.2
Width (mm)	2.9±0.1	2.5±0.2	3.0±0.1
Total coliform[1](cfu/g)	$1.3x10^2$	1.0x10	$1.8x10^3$
Total coliform[2](cfu/g)	1.0x10	1.0x10	1.0x10
Total coliform[3](cfu/g)	1.0x10	1.0x10	$1.4x10^2$
Total Count[1](cfu/g)	$2.1x10^3$	$1.0x10^3$	$2.6x10^3$
Total Count [2](cfu/g)	1.0x10	1.0x10	$1.0x10^2$
Total Count [3](cfu/g)	$2.5x10^3$	$1.8x10^3$	$9.0x10^3$
Faecal Coliform(cfu/g)	0.7x10	0.8x10	0.5x10
coliform (cfu/g)	0.2x10	1.0x10	$2.5x10^3$
E. coli (cfu/g)	Negative	Negative	Negative
Coli-count 2[a] (cfu/g)	None	None	None
Coli-count 3[a] (cfu/g)	2	5	3
Total count 2[a] (cfu/g)	8	12	6
Total count 3[a] (cfu/g)	Over 10^3	Over 10^3	Over 10^3

(Results are mean of 3 determinations ± standard deviation). 1= before cleaning/washing with 3ppm chlorinated water. 2= after cleaning/washing with 3ppm chlorinated water. a=Swab Test carried on drying tray. 3= after drying

Yield, cultivation conditions, and environment/soil type, affected composition of balancotta black pepper (Whatley, 1962). Thus the differently sourced balancotta samples would be expected to show some quality and/or composition variations. Samples from Tanoso were smallest in size, length, 2.8 mm and width, 2.5 mm. This culminated in a sample with the least moisture content, 9.0%, and the least thousand-peppercorn weight of 62.0 grams. Small size caused a more rapid evaporation of moisture from a unit of peppercorn. By this trend, the Bunso sample with the largest size yielded the highest one thousand-peppercorn weight of 74 grams and the highest moisture content of 9.8%. The Kade sample produced 9.4% moisture on drying, and one thousand-peppercorn weight of 69 grams. For all the samples, moisture content was below the maximum of 11% quoted for commercial black pepper. The relative peppercorn sizes imparted on the amount of light berry obtained. Whereas the quantity of light berry from Bunso and Kade were 7.5% and 8.5% respectively, that of the Tanoso sample was 20.7%, which was unacceptably higher than the maximum 10% expected for commercial balancotta black pepper. There were slight variations in all the parameters studied, even though their respective values conformed to or exceeded expected quality standards. Volatile oil of the Tanoso sample, 0.9%, was off the required range of 1-2.5%. The microbial quality of the samples before cleaning and after drying was poor, hence the high bacterial counts recorded. The cleaning process was effective as it resulted in no observed microbial activity in the samples immediately after cleaning. The absence of faecal coliform and *E. coli* is indicative of good sanitary conditions at the pepper sources at the time of harvesting and supply of the samples. The high microbial counts observed after drying may be attributable to post-cleaning/washing contamination from the external drying environment and/or the drier, and trays. Generally, the contamination was due, more, to total bacteria, with a maximum of 2.6×10^3cfu/g for the Kade sample before cleaning and after drying (9.0×10^3cfu/g), as against total coliform maximum (confirmed) of 1.4×10^2cfu/g also observed for the Kade sample after drying. Swab test results of the trays before cleaning and after the drying of the samples were very high. Thus the high microbial load of the dried samples may be confirmed to have been due to the surroundings.

Table 4.7: Effect of Storage on Sample Characteristics

Storage (Months)	2	6	8	10	12
1000 P.wt.(g)	74.6±0.9	73.9±1.0	74.0±1.0	72.8±0.7	73.5±1.0
Moist. cnt. (%)	9.5±0.6	10±1	9.6±1.0	9.8±0.5	10.2±0.8
Ash (%)	3.5±0.1	4.1±0.1	3.8±0.3	4.0±0.2	3.8±0.2
N-V eth. ext.(%)	4.9±0.7	5.2±0.5	5.5±0.9	5.0±0.4	5.2±0.4
V. eth. ext. (%)	0.9±0.2	1.0±0.1	1.2±0.5	1.0±0.2	0.8±0.2
Crude fibr. (%)	15.1±1.0	14.6±0.8	14.5±0.7	15±1	14.9±0.8
Starch (%)	29.8±1.0	30.2±0.5	28.7±0.8	29.6±0.4	29.0±0.8
Piperine (%)	5.2±0.1	4.8±0.2	5.0±0.1	4.6±0.5	4.2±0.2
Light berry (%)	6.8±1.0	6.1±0.4	7.3±0.8	7.0±0.5	6.9±0.4
Colour (L)	50.8±0.2	50.1±0.4	51.2±1.0	50.2±0.2	50±1
Length (mm)	3.0±0.1	3.1±0.2	3.0±0.1	3.2±0.1	3.0±0.2
Width (mm)	3.0±0.2	2.8±0.1	2.8±0.1	2.9±0.2	2.8±0.1
Total coliform°	0.2x10	0.1x10	0.5x10	0.2x10	0.3x10
Total Count°	$2.0x10^2$	$5.4x10^2$	$1.8x10^2$	$2.4x10^2$	$1.6x10^2$
Faecal Coliform°	None	None	None	None	None
coliform°(confirmed)	None	None	None	0.2x10	0.1x10
E. coli°	Neg.	Neg.	Neg.	Neg.	Neg.

(Results are mean of 3 determinations ± standard deviation) °=Microbial count (cfu/g) Neg.= Negative

The One thousand-peppercorn weight trend, suggests that storage time did not have much effect on sample quality. The slight variations may be due to the random nature of sampling for weighing. Generally, the characteristics of the sample were stable over the storage period. Although the initial sample stored had a total count of $2.0x10^2$cfu/g, storage conditions did not cause any further increase in total bacteria count. Apart from the sixth month storage sample, which showed some increase in microbial population with a total count of $5.4x10^2$cfu/g, the remaining samples all resulted in total count close the initial. If the reduction was real, then the package environment was not favourable to bacteria growth, or the warehouse conditions may have contributed to this, or both.

Table 4.8: Comparing Characteristics of Air-/Solar-dried Samples

Sample Type	Air Dried	Solar Dried
1000 Peppercorn wt.(g)	83±3	77±1
Moisture (%)	12.5±1.0	9.8±0.6
Ash (%)	3.72±0.6	3.8±1
Non-Volatile ether extract(%)	5±1	5.5±0.7
Volatile ether extract (%)	0.9±0.4	1.3±0.8
Crude fibre (%)	15.1±0.1	14.4±0.2
Starch (%)	28.9±0.5	29±1
Piperine (%)	5.6±1.0	5.6±0.8
Light berry (%)	5.1±1.0	7.5±0.8
Length (mm)	3.2±0.1	3.2±0.1
Width (mm)	2.7±0.1	2.9±0.1
Total coliform[1] (cfu/g)	6.2×10^2	5.8×10^2
Total coliform [2](cfu/g)	None	None
Total coliform [3](cfu/g)	4.1x10	0.8x10
Total Count [1](cfu/g)	1.2×10^3	1.0×10^2
Total Count [2] (cfu/g)	None	None
Total Count [3] (cfu/g)	2.5×10^3	2.0×10^2

(Result, are mean of 3 determinations ± standard deviation). 1= before cleaning/washing with 3ppm chlorinated water. 2= after cleaning/washing with 3ppm chlorinated water. 3= after drying

A moisture content of 12.5% for the air-dried black pepper failed to pass the commercially acceptable value of 12% maximum. The solar dried sample recorded 9.8% moisture content hence solar drying was a better drying system. Also, solar drying turned out a microbiologically better product

$2.0x10^2$cfu/g total count as opposed to $2.5x10^3$cfu/g obtained from the air-dried sample. Light berry amount of 5.12% for air dried pepper, as against 7.5% in solar drying, as well as thousand peppercorn weight of 83 grams, obtained for air dried pepper, and 77 grams for solar dried sample, may have been positive points for air drying but these may be explained to have been due to the high moisture content which disqualifies the drying method. Volatile oil for the air-dried sample also fell below the 1 - 2.5% acceptable range, yielding 0.9%. These not withstanding, both drying methods yielded products with similar composition values, though differing slightly in some cases.

Table 4.9 Variation in pepper colour due to Treatment

Peppercorn sample	Colour (L)
Control (No treatment) but solar dried	50.8±0.2
Air-dried	52.3±0.1
Solar dried chlorine washed	53.1±0.1
Blanched for 1minute (B1)	54±1
Blanched for 2minutes (B2)	52.2±0.4
Blanched for 3minutes (B3)	52.4±0.2
Blanched for 5minutes (B5)	52.8±0.2
Blanched for 7minutes (B7)	54.8±0.2
Blanched for 10minutes (B10)	52.5±0.1
Fermented for 1 day (F1)	51.7±0.3
Fermented for 2 days (F2)	50.8±0.1
Fermented for 3 days (F3)	50.2±0.1
Fermented for 4 days (F4)	50.22±0.04
Fermented for 5 days (F5)	50.1±0.3

Generally, heat treatment yielded samples with a more intense black colour than the heaped/fermented samples. Among the heat, treated pepper samples, B7 had the highest L value of 54.8 with B1 having 54.0. Samples B2, B3, B5 and B10 averaged 52 - 52.8. The highest value for the heaped samples was 51.7 at F1, with the remaining averaging 50.1 - 50.8. The air-dried sample also had better black colour intensity, 52.3, than the control, which recorded 50.8. The chlorine, washed sample exhibited a black colour level close to the control. An indication that, treatment had very little effect on the mechanism for black colour generation/intensity.

CHAPTER FIVE

Conclusion and Recommendations

5.1 Drying Characteristics of Black pepper

Generally, treatment did not have significant effect on the drying pattern of the pepper sample. An average daily temperature of 30°C was effective in drying each treatment sample by the fifth drying day, using the solar drying method whose environment had an average temperature of 38° - 44°C. This method, with its conditions, dried the samples to yield final products with moisture content, which were equal to or lower than the expected standard/commercial requirement of 12%. Air-drying was, however, not an effective method for drying the sample, since it resulted in moisture content higher than the commercially acceptable value, compared to solar drying which resulted in samples with much lower microbial counts than was obtained for air-drying.

5.2 Effect of treatment on physical/chemical quality of black pepper

Heaping, done to induce browning fermentation, resulted in some physical and chemical changes to the black pepper. Starch content and one thousand-peppercorn weight reduced with increasing treatment period/duration.

Heaping gave samples with increased piperine levels as heap period was extended. There was a gradual reduction in the composition of volatile and non-volatile ether extract, as well as in piperine, as heating time increased. Light berry quantity was higher in the heaped samples compared to the heat-treated. Thus, heat-treatment was a better method in terms of light berry generation. Apart from the sample heaped for 1 day (24 hours), and which had its light berry value commensurate with the commercial requirement of 10% maximum, the remainder of the heaped samples failed to meet the standard. On the other hand, all the heat-treated samples had acceptable light berry levels, and they were below the maximum acceptable limit.

5.3 Effect of chlorine disinfection on microbial quality of sample

The Use of water with enhanced chlorine content (3 ppm) to wash/disinfect black pepper was very effective in drastically improving the microbial quality of the black pepper immediately after application. The same applied to its use on the drying trays. However, recontamination ensues on exposure to the environment during drying. Further work to ascertain how to avoid such recontamination of samples during the drying stage, is recommended. Artificial drying in a closed system may be considered.

5.4 Product stability at storage

All the samples showed marked stability, with regards to physical and chemical properties, during storage over a 12-month period. There was, however, no definitive pattern for the observed microbial load trend. This may require more studies into storage conditions and methods.

5.5 Optimum treatment levels

The most acceptable physical and chemical attributes were attained when heaping was made to last between 24 - 48 hours, and when the samples were steeped in hot water, at 95°C, for 2 to 3 minutes. Chlorine disinfection/washing optimum level would, however, depend on the initial condition of the sample, and this may vary with respect to geographical location, climatic conditions, as well as cultivation practices at a given time.

Figure 15: Flow Diagram Showing Primary Processing Of Black Pepper By The Traditional Fermentation Method

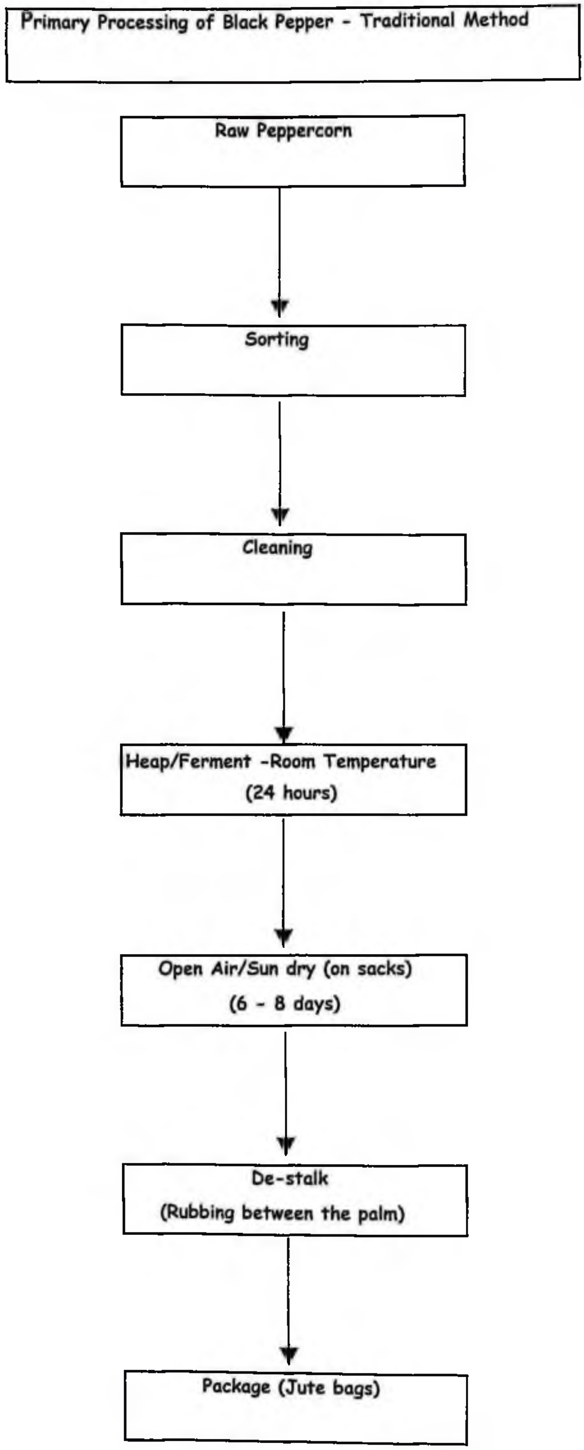

Primary Processing of Black Pepper - Traditional Method
Raw Peppercorn
Sorting
Cleaning
Heap/Ferment -Room Temperature (24 hours)
Open Air/Sun dry (on sacks) (6 - 8 days)
De-stalk (Rubbing between the palm)
Package (Jute bags)

Figure 16: A Flow Diagram Of the Laboratory Method For Primary Processing Black Pepper - Control

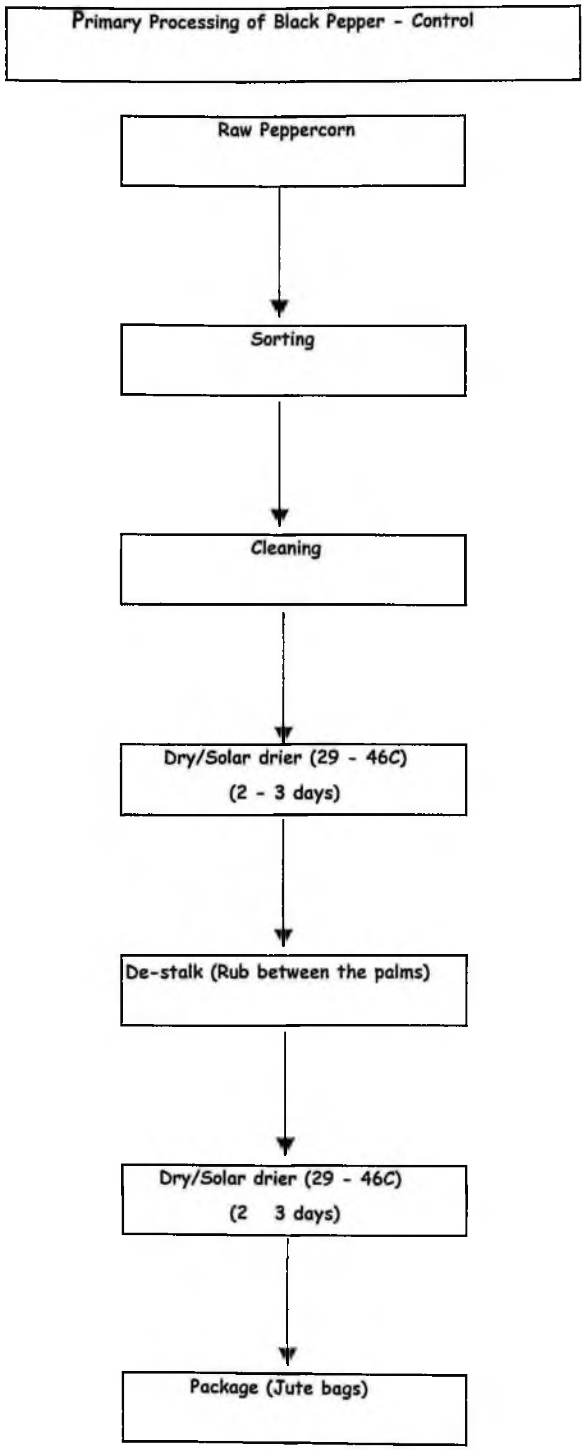

Primary Processing of Black Pepper - Control
Raw Peppercorn
Sorting
Cleaning
Dry/Solar drier (29 - 46C)
(2 - 3 days)
De-stalk (Rub between the palms)
Dry/Solar drier (29 - 46C)
(2   3 days)
Package (Jute bags)

Figure 17: A Flow Diagram Of The Laboratory Fermentation Method For Primary Processing Black Pepper.

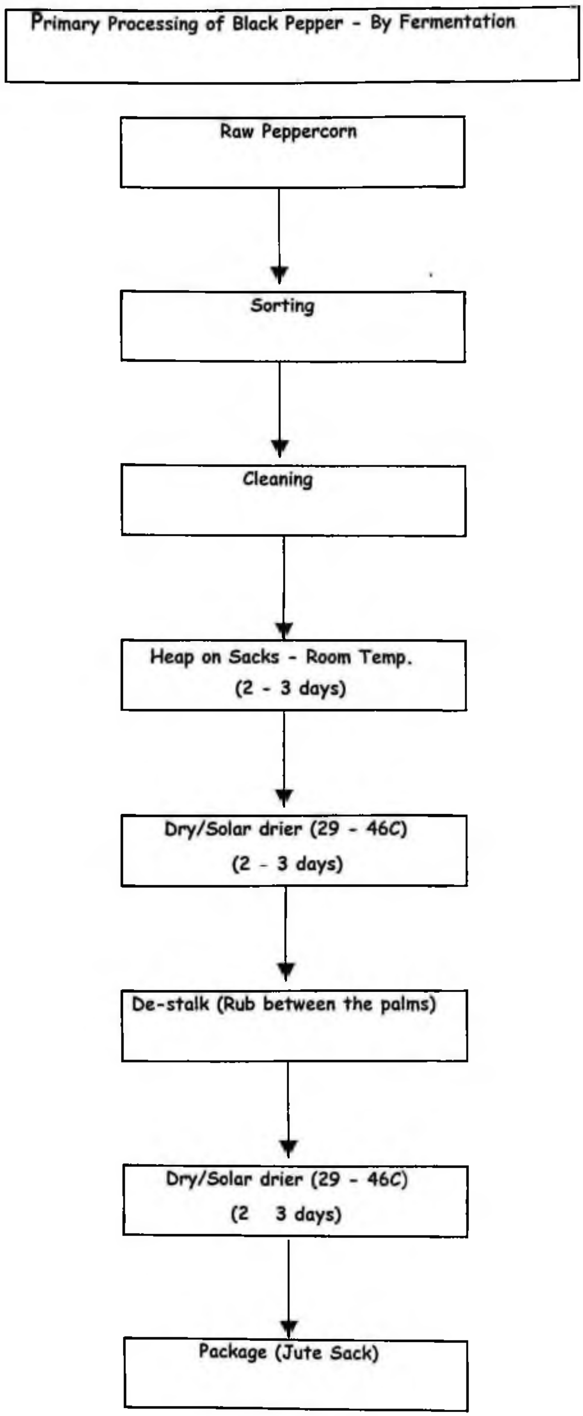
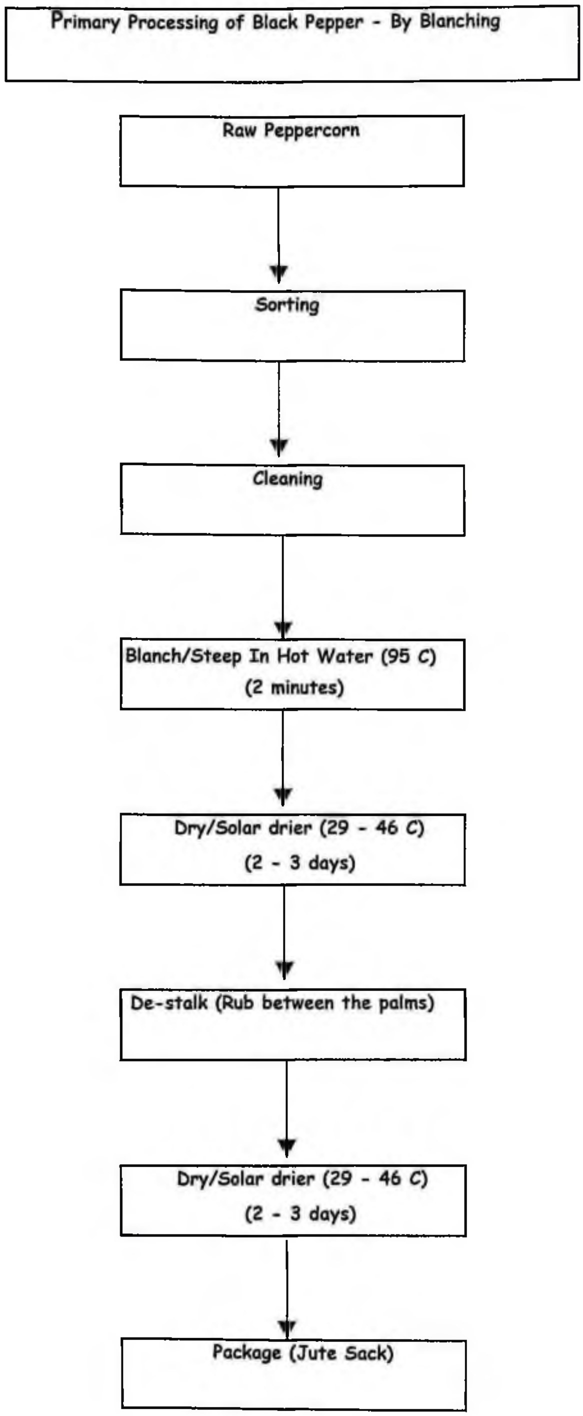

Primary Processing of Black Pepper - By Fermentation
Raw Peppercorn
Sorting
Cleaning
Heap on Sacks - Room Temp.
(2 - 3 days)
Dry/Solar drier (29 - 46C)
(2 - 3 days)
De-stalk (Rub between the palms)
Dry/Solar drier (29 - 46C)
(2 3 days)
Package (Jute Sack)

Figure 18: A Flow Diagram Of Primary Processing Of Black Pepper By The Blanching/Steeping In Hot Water Method.


Primary Processing of Black Pepper - By Blanching
Raw Peppercorn
Sorting
Cleaning
Blanch/Steep In Hot Water (95 C)
(2 minutes)
Dry/Solar drier (29 - 46 C)
(2 - 3 days)
De-stalk (Rub between the palms)
Dry/Solar drier (29 - 46 C)
(2 - 3 days)
Package (Jute Sack)

CHAPTER SIX

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Abbreviations

P.wt.: Peppercorn weight

Moist. Cnt.: Moisture content

V. eth. Ext.: Volatile ether extract

Crude fibr.: Crude fibre

Foreign Mt.: Foreign material

Ctrl.: Control

B1: Sample given heat treatment at 95°C for 1 minute

B2: Sample given heat treatment at 95°C for 2 minutes

B3: Sample given heat treatment at 95°C for 3 minutes

B5: Sample given heat treatment at 95°C for 5 minutes

B7: Sample given heat treatment at 95°C for 7 minutes

B10: Sample given heat treatment at 95°C for 10 minutes

F1: Sample heaped at room temperature for 1 day

F2: Sample heaped at room temperature for 2 days

F3: Sample heaped at room temperature for 3 days

F4: Sample heaped at room temperature for 4 days

F5: Sample heaped at room temperature for 5 days

Appendix 6

Table 4.10 Cleaning/Sanitation Results - Free Residual Chlorine

Sample	Raw (Ctrl) Untreated	Heat Treated (2 Minutes)	Fermented (2 Days)
pH (Municipal Water)	7.0	7.1	7.0
No. Of Times Washed	6	4	9
Free Residual Chlorine at End (ppm)	0.7	1.0	0.8
Average pH	7.0	7.0	7.1
Coli-Count [1](cfu/g)	2.4x10	0.2x10	5.4×10^2
Coli-Count [2] (cfu/g)	None	None	0.3x10
Coli-Count [3] (cfu/g)	0.4x10	None	0.8x10
Total Count [1] (cfu/g)	8.2×10^2	3.6×10^2	4.9×10^2
Total Count [2] (cfu/g)	None	None	None
Total Count [3] (cfu/g)	3.6×10^2	2.4×10^2	4.6×10^2
Faecal Coliform(cfu/g)	None	None	None
Confirmed coliform (cfu/g)	None	None	None
Confirmed E. coli (cfu/g)	Negative	Negative	Negative

1= before cleaning/washing with 3ppm chlorinated water. 2= after cleaning/washing with 3ppm chlorinated water. 3= after drying

Appendix 7

Unit Weight And Dimensions of Treatment Samples

Sample	Length	Range	Width	Range
Raw	5.12	5-5.2	4.7	4-5.1
Air dried	3.2	2.6-3.8	2.65	2.2-3.1
Control	3.16	3-3.8	2.9	2.8-3.1
B1	3.4	3.2-3.6	3.1	3-3.2
B2	3.3	3.2-3.4	3.0	2.9-3.1
B3	3.2	3-3.4	3	2.9-3.1
B5	3.4	3-3.2	2.85	2.6-3.1
B7	3.1	3-3.2	3	3-3
B10	3.4	3-3.8	3	2.8-3
F1	2.95	2.9-3	2.5	2.2-2.8
F2	3.6	3-4.2	3.45	3-3.9
F3	3.3	3.2-3.8	2.95	2.9-3
F4	3.3	3.2-3.4	3.1	3-3.2
F5	3.8	3.6-4	3.15	3.1-3.2

Weight per grain/g
1.82
0.92
0.77
0.66
0.76
0.76
0.77
0.66
0.66
0.58
0.83
0.66
0.76
0.75

Appendix 8

Composition of Black Pepper (Treatment)

Sample	Moisture%	Ash%	N-V %	Volatile eth. Extract%	Crude Fibre%	Starch	Piperine%	Light Berry%	Foreign Mat.%	Black Colour(L)
Raw	63.5	3.38	-	-	-	-	-	2.8	5.62	
Air dried	12.5	3.72	5.04	0.9	15.09	28.9	25.62	5.12	3.27	52.28
Ctrl	9.8	3.72	5.49	1.3	14.36	29.01	5.57	7.5	2.1	5309
B1	8.3	3.46	6.48	1.2	14.84	28.85	5.2	7.52	0.9	54
B2	7.5	3.55	6.39	0.8	14.52	28.11	5.44	7.4	0.94	52.2
B3	8.8	3.82	4.99	1	14.5	29.57	5.3	7.59	0.88	52.4
B5	8.5	3.41	4.17	0.7	14.08	29.03	4.97	7.68	1.2	52.8
B7	5.5	3.53	4.02	0.5	14.91	28.55	5.0	9.21	1.0	54.8
B10	5.5	3.54	3.06	0.5	14.24	30.06	4.86	9.1	0.84	52.5
F1	11	3.86	4.74	1.0	14.38	29.96	5.88	9.55	1.4	51.7
F2	9.5	3.78	4.79	1.0	14.75	28.58	5.74	9.76	1.23	50.8
F3	7.8	3.84	4.96	1.2	13.98	28.43	6.51	12.32	1.91	50.21
F4	8.8	3.98	5.14	1.8	14.02	28.04	6.51	12.32	1.91	50.22
F5	5.8	3.66	6.52	1.6	14.0	27.98	6.5	12.64	2.41	50.1

Appendix 9

Observations: Heaped/Fermentation Sample

Time/days	pH	Temp/°C	Amount of Ripe Pepper%	Moldy Pepper%	Ease of De-stalking%
0	6.8	36	0	0	0
1	6.7	38.4	0	0	0.3
2	6.7	37.8	0.1	0.4	28
3	6.5	38.4	6.8	5.6	41
4	6.4	38.8	42.5	28.9	56
5	6.4	38.4	78.4	63.2	89

Appendix 10

Choosing Appropriate Sterilization Temperature

Temperature (Celsius)	Onset of Black Colour (Minutes)	Amount of Damaged Pepper (%)
60	45	0.5
70	37	0.7
80	8	1.2
85	5	1.0
90	3	2.5
95	2	5.2
100	1	12.4

Appendix 11

Weight Differential of Samples During Drying

Time/days	Ctrl	b1	b2	b3	b5	b7	b10
1	13	23	21	23	27	24	34
2	22	25	27	31	36	37	35
3	19	22	20	17	19	18	8.9
4	17	11	8	7	2	6	4.3
5	9.6	4	4	1	5	1	1.6
6	8.4	1	2	6	6	3	4.2
7	1.6	1	1	1	3	0	0.8
8	1	0	1	1	1	0	0.5

f1	f2	f3	f4	f5
16	22	39	41	50
22	25	26	30	24
12	21	20	13	14
5	11	0	2	4
1	0	0	0	2
0	0	0	0	1
0	0	0	0	0
0	0	0	0	0