Original Research Article

EFFECT OF HARVESTING STAGE, DRYING METHOD AND PACKAGING OF COWPEA LEAVES ON MICROBIAL CONTAMINATION

ABSTRACT

Cowpea leaves are lost annually due to infestation and spoilage as a result of inadequate postharvest handling procedures. During harvesting, cowpea leaves have high moisture content which exposes them to greater respiration losses and predisposes them to microbial growth. Different packaging materials used by farmers also exposes the cowpea leaves to a range of microorganisms due to their different moisture retention capacity therefore reducing shelf-life and increasing their spoilage. Drying removes the moisture content; however, dried foods are susceptible to spoilage resulting from many microbial, biological, chemicals and physical reactions This research therefore aimed at prolonging the keeping quality of the dried cowpea leaves for use during off- season and coming up with information on the best and affordable packaging material that would ensure safety of dried cowpea leaves. Data was collected on fungal, bacteria and coliforms. The data was subjected to variance using Statistical Analysis System 9.2 edition and significantly different means separated using LSD at 5%. The combination of harvesting stage, drying method and packaging material significantly ($p<0.05$) influenced microbial load (bacterial and fungal), however no coliforms were observed. Open sun-dried cowpea leaves at 21 DAS, packaged in woven and aluminium foil reported a high number of bacterial and fungal counts compared to the kraft packaging. Oven dried cowpea leaves, harvested at 49 DAS, and in kraft paper resulted in the least bacterial and fungal contamination compared to those packaged in woven and aluminium foil. Sun drying and harvesting at 21, 35 and 49 DAS contained the highest bacterial and fungal contamination followed by solar drying and the least was recorded in oven drying method. This research shows that correct harvest stage, adoption of oven and solar drying methods and use of correct packaging material will prolong the shelf life of dried cowpea leaves therefore enhancing food security and food safety.

Key words: Cowpea Leaves; Harvesting Stage; Drying Method; Packaging; Microbial contamination

1.INTRODUCTION

Microbial post-harvest quality of leafy vegetables has been reported to be significantly influenced by pre-harvest handling practices, drying methods and even packaging. Poor harvesting practices and handling are also some of the predisposing factors to microbial contamination in cowpea vegetables i.e., mechanical injury like bruises and cuts [1]. Leafy vegetables contain higher moisture during the biological maturity period i.e., in the range of 25% to 85% [2]. The high moisture which is associated with the harvest stage reduces the possibility for long preservation since the value of moisture content is much higher than required one [3]. The decrease in moisture levels in the cowpea leaves can therefore slowdown the bacterial, enzymes and yeasts effects. The harvesting stage of cowpea leaves determines the moisture content of the plant. In the early stages of growth, the cowpea leaves are characterized by high moisture content which plays an essential role in microbial growth. Moisture presence increases the respiration rate of molds due to increased production of heat and water. This therefore contributes to spoilage of food quality and indirectly to reduction in quantity.

According to a study by Njoroge [4], spider plant, which had a higher moisture content than the other vegetables, recorded the highest total viable bacterial counts when compared to the other vegetables clearly demonstrating the importance of moisture content in microbial growth. Reducing moisture and drying cowpea leaves to suitable forms can therefore help to reduce this problem [5]. Drying hinders microbial growth survival in the dried food therefore reducing the water activity of food and has been used as the most popular means of food preservation. Drying methods have been reported to influence the amount of moisture within a plant sample [6]. Further research showed oven drying method to be one the most effective method in moisture reduction, since it's able to properly dry plant samples to required moisture content. Open -sun drying has also been effective in drying, but displaying the plant samples to the open atmosphere has been reported to contribute to increased microbial contamination. In comparison to oven drying method, the open sun drying retains higher moisture content which is key factor of microbial growth. The drying process creates a hard outer-layer which prevents micro-organisms from entering into the food product and therefore conserves the food for a long time. Solar dryer has been reported to be better preservers and produce items of higher quality than sun drying because they are free of microbial contamination [7].

Research by Njoroge [4] reported lower microbial load in solar dried vegetable samples. Application of solar drying brings about favorable product changes, deactivate enzymes and even kills microorganisms resulting in a product of good caliber, with increased storage life and safety to consumers [8]. Deterioration varies from extremely harmful toxic microorganisms to loss of quality aspects such as color loss or flavor [9]. To ensure product quality and safety is up to standard, inhibition of infectious pathogenic and toxicogenic microorganism should be adhered to [10]. Yeasts and molds have been reported to attack vegetables and fruits [11]. Most molds have been observed to develop in a pH range between 3 and 8 and also demonstrated to thrive at relatively low levels of water activity on dried foods (0.7-0.8). Their spores may also survive in harsh climatic circumstances, but the majority of them are sensitive to heat treatment. Post-harvest contamination of leafy vegetables is significant because all known food-grade post-harvest disinfectants are ineffective against the pathogenic organisms that are present on or in fresh vegetables [12].

Microbial growth is reduced in dried products and therefore when a significant number of pathogenic organisms remain after drying, it may cause a risk to the clients. Once the product is in the dried form, rate of microbial growth is prevented however, spores and vegetative cells can continue to live for several months [13]. Additionally, when the dried food products are rehydrated, it's possible to promote the proliferation of remaining organisms. This can lead to a faster deterioration and a high risk of end users' infection. Microbial contaminants are in many times introduced during first production stages, picking, storage and transportation [14]. The drying of the food products may be used as a key method for lowering the present microorganisms if no additional decontamination treatment is carried out post-harvest and during storage [15].

Food packaging's primary purpose is to safeguard food from damage and contamination from the environment as well as to give consumers nutritional information in an economical manner that satisfies customer demands and upholds food safety [16]. The storage life of dried cowpea leaves can be increased by use of the right packaging. It has been reported that improper or no packing, particularly in underdeveloped nations, causes 25 to 50% of food loss and around 10% of exported fruits and vegetables [17]. The quality of the stored material must be maintained by carefully managing the initial water content and its disparity during storage [18]. According to Mauriello [19] the effect of microbial changes of any food material throughout storage duration is greatly dependent on storage temperature the ratio between gas and product volume, packaging material, type of the product, gas composition and hygiene during processing and packaging. A variety of bacteria that can shorten the shelf life of unpackaged foods are frequently present [20]. Appropriate packaging materials combined with drying methods for food processing can help lower post-harvest losses and increase the shelf life [21].

Due to their sensitivity to humidity and oxidation during storage, dried vegetables pose a special challenge for food safety; as a result, it is essential to choose the absolute best packing material to stop these undesired physicochemical processes [22]. Red pepper paste packaged on polyethylene plastic was reported to have an extended shelf life compared to other types of plastic [23]. Shelled walnuts packed on polyethylene, as opposed to polyethylene pouches, had a longer shelf life and less microbiological development [24]. The features of a packaging material are determined by its permeability for gases (CO_2, O_2, ethylene, water vapor) and the degradative agents penetrating from outside [25]. Mauriello [19] observed that hygienic practices used during processing and packaging as well as product type and packaging materials, storage temperature, appropriate gas composition, and ratio between gas and product volume all have a significant impact on how many microbes any food product will develop over the course of storage. Leafy vegetables are more susceptible to foodborne pathogen contamination due to the production, processing, and packing procedures.

Typically, microbial contaminants are added to vegetables during primary production or during harvest, transportation, and storage. Fresh food can become infected by pathogenic bacteria from sources like raw or badly composted manure or animal waste, including *Vibrio cholerae* Salmonella, *Campylobacter jejuni* and *Escherichia coli* O157:H7 [26]. Fresh vegetables and fruits have a high-water content (about 80%), making them very perishable [27]. Vegetables can be preserved in their natural form, structure, and nutritional value by being dried, which inhibits the growth of spoilage microbes, browning, and other moisture-driven deterioration responses [27]. Molds and yeast are the major microbes responsible for fruit and vegetable microbial deterioration [28]. Therefore, knowledge on integration of harvesting stage, drying method and packaging material on cowpea leaves will help enhance food security and food safety through adoption of correct processing and storage methods.

2.MATERIALS AND METHODS

2.1 Experimental Site

The cowpeas were planted in a farmers' field next to Chuka University horticultural demonstration farm in Tharaka Nithi County. The farm lies at 0^{o} 19` S, $37^{o}$ 38` E and 1535 m above sea level. The region receives roughly 1,200 mm of rainfall each year, which is distributed bimodally, with the long rains falling from March to June and the short rains from October to December. The predominant soil type is humic nitisol, which is deep, well-weathered, and has moderate to high natural fertility and the average annual temperature is about 20 ^{o}C [29].

2.2 Experimental Design

The field experiment was set up using a randomized complete block design. The treatments included three harvesting stages i.e., 21,35 and 49 days after sowing, (DAS) three drying methods, open sun, solar and the oven drying method, and three packaging materials, aluminium foil, kraft(khaki) and woven bag. The lab layout used was complete randomized

2.3 Data Collection

The collection of data collection was done over two cultivations, January-March and April-June 2022. Data collection was done sequentially following different stages of harvesting. The cowpea leaves harvested at 3 different harvest stages (21, 35 and 49 DAS) dried using solar, Open- sun and oven drying) and packaged in aluminium foil, a kraft (khaki) paper and a woven package was then stored at room temperatures and in a dry environment awaiting microbial analysis.

Bacteria counts, coliforms, mold and yeast analyses were determined through the microbiological analysis [30]. After being prepared, the samples were serially diluted and inoculated in various media. To count the total amount of bacteria, coliforms, and fungi, three different agars-Plate Count Agar (PCA), Violet Red Bile Agar (VRBA), and Rose Bengal (RB) were used. 1 g of dry sample was put in a test tube with 9 ml peptone water to give a 10^{-1} dilution. The samples were further diluted to 10^{-2},10^{-3} and 10^{-4} and spread in the petri-dishes. The PCA was incubated at 60 °C for one days, VRBA at 60 °C for 24 hours, and RB at for seven days with the help of a digital colony counter. For PCA, all the colonies were counted, for VRBA only the colonies showing growth as described for coli were counted. For RB all the colonies were counted, and registered if it was mold, yeast or both, growing on the agar. The numbers of microorganisms found in the samples were presented as the colony forming unit per g sample (cfu/g.

2.4 Data Analysis

The data on fungal and bacteria contamination was analyzed using the Statistical Analysis System version 9.3 at a 5% probability level. Significant means were separated using LSD at $\alpha = 0.05$ to determine the differences between the harvesting stages and drying method on fungi and bacteria, drying and packaging on bacteria and fungi and combination of harvest stage, drying method and packaging material on bacteria and fungi.

3.RESULTS AND DISCUSSION

The significance of this objective was to identify how the harvesting stage, drying method and packaging material contributed to microbial contamination of cowpea leaves. The investigation studied the microbial contamination by fungi, bacteria and coliforms. The study comprised investigating the effect of harvesting and drying methods on bacteria and fungi, the effect of drying method and packaging on bacteria, fungi and coliforms, and the effect of harvesting stage, drying method and packaging on fungi, bacteria and coliforms.

3.1. Effect of Harvesting Stage and Drying Method on Microbial Contamination

3.1.1. Effect of Harvesting Stage and Drying Method on Bacteria

In both trials, harvesting at 35 DAS, and open sun, solar and oven drying of cowpea leaves in trial one, and open sun and solar drying in trial two were all significantly ($p<0.05$) different, in bacteria load with harvesting at 21 and 49 DAS (Table 1). In all harvesting stage open sun-dried recorded the highest bacteria load, followed by solar dried and the least in oven dried cowpea leaves in both trials one and two, respectively. Harvesting at 21 DAS and open sun-dried cowpeas leaves recorded the highest bacteria load followed harvesting at 49 DAS and solar drying and harvesting at 35 DAS and oven drying (Table 1). In trial two, harvesting at 49 DAS and oven drying of cowpea leaves differed significantly ($p<0.05$) with harvesting at 35 DAS and oven drying in bacteria load (Table 1). Harvesting at 49 DAS and open sun drying recorded the highest bacteria load, followed by solar drying at the same harvesting stage and the least bacteria load in cowpea leaves harvested at 35 DAS and oven dried. Trial, one recorded the highest bacteria load compared to trial two. The interaction of harvest stage and drying method on bacteria counts was significant in trial one.

The findings in both trials in all harvesting stages and open sun drying of cowpea leaves recorded the highest bacteria load followed by solar drying and the least load in the oven drying. This was attributed to high moisture content retained by the cowpea leaves coupled with contamination as a result of excessive drying time in the open environment where the cowpea leaves were dried. The low bacteria count in oven dried cowpea leaves could have been due to low moisture content and enclosed drying which ensured the cowpea leaves were not exposed to the open environment. Njoroge [4] reported that solar-dried indigenous leafy vegetables that were blanched for five minutes at 90 °C had the lowest total viable counts. Moreover, a 26-34% decrease in the total viable counts was caused by the effects of blanching and drying on native green vegetables

Table 1: Effect of harvesting stage and drying methods on bacteria load in counts

	Trial one			Trial two		
Harvesting Stage	Drying Methods			Drying Methods		
Days after Sowing	Open-Sun	Solar	Oven	Open-sun	Solar	Oven
21	119.42^{a*}	84.08^{b}	33.00^{C}	107.58^{a}	80.53^{b}	40.89^{cd}
35	110.14^{b}	54.83^{c}	23.89^{d}	103.75^{b}	54.25^{c}	23.81^{d}
49	118.92^{a}	86.86^{b}	47.69^{c}	111.33^{a}	84.94^{b}	45.75^{c}
LSD	9.85				12.75	
CV	18.16				17.88	

* Means with different letters along the column are significantly different at $p <0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance

The high rate of bacteria counts in trial one compared to trial two could have been due to increased contamination due to high temperatures during the trial period which could have favoured the growth of bacteria compared to low temperature in trial two. Research by Hamad [31] showed that treatments such as heating, drying, and even cooling had an impact on food's composition as well as the types and quantities of microbes that persisted in the food. Additional studies by Adu-Gyamfi [32] in an attempt to determine how drying method and irradiation affected the microbial quality of moringa leaves, reported that room-dried moringa leaves had a high total viable count of 6.45 cfu/g, whereas total viable cells were counted at 3.12 cfu/g for mechanically-dried moringa leaves, and at 2.61 cfu/g for solar-dried moringa leaves, further highlighting the importance of drying method.

All microorganisms have a specific temperature range in which they can flourish. In order to choose the best storage conditions for food products, it is crucial to comprehend how time and temperature interact. They have a minimum, maximum, and optimum temperature range. For several kinds of microorganisms, the relationship between the rate of growth and temperature varies [33]. Based on the temperature ranges in which they may thrive, microorganisms can be divided into four main categories: thermophiles, mesophiles, psychrophiles, and psychrotrophs [34]. Thermophiles are said to grow best at temperatures between 55 and 65 °C, while mesophiles, which include all human diseases, prefer temperatures between 30 and 45 °C. The optimum development range for psychophilic species is between 12 and 15 °C. In contrast, psychotrophs may thrive at low temperatures (a minimum of -0.4 °C and 3.3 °C to 5 °C). According to Lorenzo [35], psychotropic organisms, which include some foodborne pathogens as well as spoilage bacteria, yeast, and molds, are significantly more pertinent to food. The interaction of the harvest stage and drying technique on the number of bacteria was significant.

3.1.2 Effect of Harvesting Stage and Drying Method on Fungi

In both trial one and two, harvesting at 35 DAS and open sun, solar and oven drying of cowpea leaves differed significantly ($p<0.05$) with harvesting at 21 and 49 DAS on growth of fungi, However, harvesting at 21 and 49 DAS and open sun, solar and oven drying of cowpea leaves did not significantly ($p<0.05$) differ on fungi growth in both trials (Table 2). Open sun drying recorded the highest fungal growth, followed by solar and then oven drying having the least fungi load in all harvesting stages in both trials. Trial two recorded a higher contamination of fungi compared to trial one Interaction of harvest stage and drying method fungal growth was significant ($p<0.05$) in trial one only (Table 2).

Table 2: Effect of harvesting stage and drying method on fungi

	Trial One			Trial Two		
Harvesting Stage	Drying Methods			Drying Methods		
Days after Sowing	Open-Sun	Solar	Oven	Open-sun	Solar	Oven
21	5.53^{b*}	3.47^{cd}	2.72^{d}	6.55^{b}	4.45^{cd}	2.72^{d}
35	7.42^{a}	5.22^{b}	3.97^{c}	8.42^{a}	6.25^{b}	3.67^{c}
49	4.53^{b}	3.67^{cd}	1.74^{d}	5.53^{b}	4.47^{cd}	2.83^{d}
LSD	0.92			0.81		
CV	26.60			24.57		

*Means in the same column having different superscripts are significantly different ($p<0.05$); LSD is Least Significant Difference; CV is Coefficient of Variance.

The significant differences recorded in cowpea leaves harvested at 35 DAS and in all drying methods compared to harvesting at 21 and 49 DAS in both trials could be attributed to the differences in moisture content of cowpea leaves at this stage of growth and also the variation in the rains in both trials. Harvesting at 35 DAS in all drying methods recorded the highest fungal growth (Table 2). High moisture content at this stage of growth coupled with the contamination from the environment encouraged the growth of the fungal colonies. Fungi growth was highest in all harvesting stages and open sun drying compared to the solar and the oven drying of cowpea leaves. This could be as a result of contamination of the cowpea leaves due exposure to the open environment.

Studies by Amoah [36], also reported that open-sun-dried (*Zingiber officinale*) rhizomes samples to have the higher fungal load than solar-dried samples. This was attributed to increased rainfall in trial two compared to trial one which could have encouraged fungal growth. Guzman-Plazola [37] reported that high relative humidity levels favored spore germination of powdery mildew disease on tomatoes. A study by Alabi [38] revealed that the stage of harvest and drying technique had a substantial impact on the percentage incidence of Aspergillus spp. on maize. The highest proportion of Aspergillus spp. prevalence (68.5%) was found in maize seeds that had been collected 40 days after tasseling and sun dried. Research by Sahar [39] revealed that the samples under investigation showed positive aflatoxin contamination and moisture content. Additional studies reported that moisture content levels above 11% foster the growth of mold resulting in significantly increase in the generation of aflatoxin. The correlation between moisture content and aflatoxin contamination in these samples of chilies was also minor.

3.2. Effect of Drying Method and Packaging Material on Microbial Contamination

3.2.1 Effect of Drying Method and Packaging on Bacteria

In trial one, open sun-dried cowpea leaves packaged in Kraft paper, aluminium foil and woven bag were not significantly ($p<0.05$) different in bacteria counts observed. Solar dried cowpea leaves packaged in aluminium foil differed significantly ($p<0.05$) with kraft and woven bag packaged cowpea leaves in bacteria counts. However solar dried cowpea leaves packaged in kraft paper and woven bag did not differ significantly ($p<0.05$) in the bacteria counts. Oven drying and woven bag packed cowpea leaves differed significantly ($p<0.05$) with kraft and aluminium foil packaged ones. Oven drying and aluminium foil and kraft paper packaged cowpea leaves were not significantly ($p<0.05$) different in the bacteria counts (Table 3).

In trial two, open sun and solar dried cowpea leaves packaged in aluminium foil differed significantly ($p<0.05$) with kraft and woven bag packaged cowpea leaves. However, cowpea leaves packaged in kraft paper and woven bag and open sun and solar dried cowpea leaves were not significant ($p<0.05$) on the observed bacteria growth. Oven dried cowpea leaves packaged in aluminium foil, kraft and woven bags did not significantly ($p<0.05$) differ in bacteria counts. Trial, one recorded the highest bacteria load compared to trial two. The interaction of drying method and packaging was not significant ($p<0.05$) in both trials. Oven dried cowpea leaves packaged in aluminium foil recorded the least bacteria counts in trial one while oven dried cowpea leaves packaged in kraft paper recorded the least bacteria counts in trial two (Table 3).

Solar dried cowpea leaves packaged in aluminium foil differed significantly and recorded the least bacteria counts. This could have been due to reduced moisture content levels from the solar dried leaves, which lowered the growth rate of the bacteria. The key element controlling the development of microorganisms has been identified as the water activity of food [40]. Any food's ability to adjust its water activity has the power to affect how any food-related microorganisms develop. As moisture is transferred from the food to the environment, foods with a high-water activity that are packaged in materials with low relative humidity tend to lose moisture resulting to slowing down of microbial growth by the loss of available water [41].

Table 3: Effect of drying method and packaging method on bacteria

	Trial One			Trial Two		
	Packaging Material			Packaging Material		
Drying Method	Aluminium Foil	Kraft Paper	Woven Bag	Aluminium Foil	Kraft Paper	Woven Bag
Open-sun	117.67^{a*}	110.89^{a}	119.92^{a}	110.17^{a}	106.86^{b}	105.64^{b}
Solar	72.33^{b}	79.92^{a}	76.53^{a}	68.58^{c}	74.14^{b}	75.00^{b}
Oven	33.31^{b}	33.56^{b}	37.72^{a}	40.14^{bc}	33.28^{b}	37.03^{bc}
LSD	9.85			12.75		
CV	28.16			27.88		

* Means with different letters along the column are significantly different at $p <0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance

Aluminium foil has been reported to provide a complete barrier to light, oxygen, moisture and bacteria [42]. This therefore meant that it did not allow moisture re-entry from the atmosphere; thus, the reported low bacterial counts. Studies by Njoroge [43], reported the total viable counts to be low (in solar dried leafy vegetables. Further research by Sikorska [44] revealed that fewer bacterial counts were observed in crushed leaves samples and those that were packaged in Xtend packaging. Solar dried and aluminium foil packaged cowpea leaves in trial two showed the least bacteria counts. This could have been attributed to less contamination of the cowpea leaves during the drying process and also low moisture content. According to Njoroge [4], solar drying of indigenous vegetable samples resulted in a much-reduced microbial load than sun drying. The low bacteria count observed in the oven dried cowpea leaves packaged in kraft paper could have been due to less contamination of the cowpea leaves since they were not openly exposed to the atmosphere during drying. These, coupled with low moisture content of the solar dried leaves, lowered the rate of bacteria growth. According to research by Adejo [45], oven-dried tomato powders had a significantly lower bacterial population. A higher bacterial contamination was recorded in trial one compared to trial two. These could have been caused by the differences in weather conditions such as high temperatures in the first trial compared to the second trial.

3.2.2. Effect of Drying Method and Packaging on Fungal Growth

In trial one, open sun-dried cowpea leaves packaged in kraft paper differed significantly ($p<0.05$) with those packaged in aluminium foil and woven bags in fungal growth (Table 4). Solar and oven dried cowpea leaves packaged in woven bag differed significantly ($p<0.05$) with kraft paper and aluminium foil packaged cowpea leaves in fungal growth. Open sun dried and packaged in aluminium foil and woven bag recorded high fungal growth, whereas oven dried cowpea leaves packaged in aluminium foil recorded the least growth. Solar and oven dried cowpea leaves package in woven bag differed significantly ($p<0.05$) in fungal growth with those packaged in kraft paper and aluminium foil.

In trial two, open sun-dried cowpea leaves packaged in aluminium foil differed significantly ($p<0.05$) in fungal growth with those packaged in kraft paper and woven bags (Table 4), Solar dried cowpea leaves packaged in aluminium foil, kraft paper and woven bag were significantly ($p<0.05$) different in fungal growth. Oven dried cowpea leaves packaged in woven bag differed significantly ($p<0.05$) in fungal growth with those leaves packaged in aluminium foil. However, oven dried cowpea leaves packaged in kraft paper were not significantly ($p<0.05$) different with those packaged in woven bag in fungal growth. Open sun dried packaged in aluminium foil and woven bag recorded the highest fungal growth. Solar dried cowpea leaves packaged in kraft paper recorded the least growth in fungi. Trial two recorded higher fungal growth compared to trial one. The interaction effect of drying method and packaging significantly ($p<0.05$) influenced fungal growth.

Table 4: Effect of drying method and packaging method on fungi

	Trial One			Trial Two		
	Packaging Materials			Packaging Materials		
Drying Method	Aluminium Foil	Kraft Paper	Woven Bag	Aluminium Foil	Kraft Paper	Woven Bag
Open-Sun	6.94[a*]	4.92[b]	6.61[a]	8.94[a]	5.92[cd]	7.62[bc]
Solar	3.56[ef]	2.78[f]	5.83[d]	4.57[de]	1.79[f]	5.62[d]
Oven	2.11[f]	2.92[ef]	4.39[d]	3.12[f]	2.83[ef]	5.29[de]
LSD	0.92			0.94		
CV	14.60			16.69		

* Means with different letters along the column are significantly different at $p<0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance

In trial, one open sun-dried cowpea leaves packaged in kraft paper significantly recorded the lowest fungal colonies. This could have been due to the differences in moisture retention capacity of the kraft paper. The kraft paper absorbed the remaining moisture in the cowpea leaves onto the packaging material itself but did not permit moisture entry from the atmosphere. Solar dried and woven bag - packaged cowpea leaves recorded the highest significant fungal colonies compared to aluminium foil and kraft paper. These could have been caused by the porous characteristics of the woven bag which allowed moisture re-entry from the atmosphere and also allowed moisture escape from the dried cowpea leaves to the atmosphere. The reabsorption of moisture from the atmosphere could have increased moisture levels in the packaging material which favoured the growth of fungal colonies High moisture content shortens the shelf life of food goods that are stored and even encourages the growth of microbes [46]. The existence of oxygen is a crucial component that reduces a product's shelf life since it promotes the growth of aerobic bacteria, molds, and even insects [47].

Oven dried and woven bag packaged cowpea leaves recorded the highest fungal colonies compared to aluminium and kraft paper packaging. This was due to the high moisture content reabsorbed from the atmosphere which encouraged the fungal growth. The preservation of sensory qualities like texture, firmness, softness, and crispiness as well as the suppression of the growth of pathogenic microbes have been documented to depend heavily on the moisture barrier capabilities in packing of various foods, whether dry or wet [48]. The highest fungal growth colonies recorded in trial two in open sun dried and aluminium foil packaged cowpea leaves was attributed to increased contaminants from the atmosphere during open sun drying which could have promoted fungal growth. High moisture retention in open sun dried and aluminium foil packaged cowpea leaves due to lack of porosity in aluminium foil could have also contributed to an increase in fungal contamination.

The food's susceptibility to the effects of relative humidity is significantly influenced by the packaging. Moisture migration and changes in the ambient temperature may be factors in packaging materials. According to studies, temperature changes can cause moisture to condense on the surface of foods with low water activity, creating microenvironments that are ideal for the growth of microbes that cause spoiling. Open sun-dried indigenous green vegetables were reported to have higher counts of yeast and mold due to greater moisture contents (15.4–16.6%), which sped up their growth and contributed to their multiplication [4]. During the study it was observed that oven dried and woven bag packaged cowpea leaves resulted to the highest fungal contamination in trial two due to the high humidity as a result of moisture availability during the trial period and also from moisture reabsorption from the atmosphere by the woven bag which could have promoted growth of the fungal colonies.

According to studies by Sikorska-Zimny [44], no fungi were seen on parsley leaves stored in extend foil, however more fungi were discovered in the complete leaf samples packaged in polyethylene. The increased fungal growth in trial two compared to trial one was attributed to the increased moisture availability since trial two was done during the rainy season. The availability of rainfall during the trial also made it difficult to thoroughly dry the cowpea leaves, which could have encouraged growth of the fungal colonies. Interaction of drying methods and packaging materials significantly influences fungal growth of dried cowpea leaves. According to studies by Gamuchirai [49] on the impact of drying methods and storage conditions on the quality and incidence of aflatoxins in dried chilies, packaging had no significant impact on the total levels of aflatoxin in the dried chilies. Additionally, there was no significant interaction between the drying method and the packaging material on the total aflatoxin levels of the chillies.

3.3. Effect of Harvesting Stage, Drying Method and Packaging Material on Microbial contamination.

3.3.1 Effect of Harvesting Stage, Drying Method and Packaging Material on Fungi Growth

In trial one and two, harvesting at 21 DAS, oven, solar and open sun-dried cowpea leaves packaged in aluminium foil, Kraft paper and woven bag differed significantly ($p<0.05$) in fungal growth. In trial one, harvesting at 21 DAS, oven drying and packaging in aluminium foil was significantly ($p<0.05$) different from kraft paper and woven bag packaging. Oven drying and packaging in kraft paper and woven bag packaging however did not differ significantly ($p<0.05$) in fungal growth. Where harvesting was done 21 DAS, solar drying and packaging in kraft, woven bag and aluminium foil packaging differed significantly ($p<0.05$) in fungal growth. Harvesting the cowpea leaves at 21 DAS, open sun drying and packaging in kraft paper, woven bag and aluminium foil did not significantly influence fungal growth. Harvesting at 35 drying and packaging the cowpea leaves in aluminium foil, Kraft paper and woven bag differed significantly ($p<0.05$) in fungal growth (Table 5).

Harvesting at 35 DAS, solar drying and packaging in kraft paper differed significantly ($p<0.05$) from woven bag and aluminium foil packaging. Solar drying and harvesting the cowpea leaves at 35 DAS, and packaging in woven bag and aluminium foil were however not significantly ($p<0.05$) different. Open sun drying the cowpea leaves, harvesting at 35 DAS, and packaging in kraft paper and woven bag differed significantly ($p<0.05$) in fungal growth observed. However, aluminium foil and woven bag packaged cowpea leaves harvested at 35 DAS, and open sun dried were not significantly different. Harvesting at 49 DAS, oven drying and packaging in aluminium foil packaging was significantly ($p<0.05$) different from kraft paper and woven bag packaging. The woven bag and kraft paper packaging however did not differ significantly in the fungal growth observed. Open sun-dried cowpea leaves harvested at 49 DAS, and packaged in aluminium foil, kraft paper and woven bag packaging did not differ significantly ($p<0.05$) in the fungal growth. Solar dried cowpea leaves harvested at 49 DAS, and packaged in kraft paper, woven bag and aluminium foil were significantly different ($p<0.05$) in the fungal growth recorded (Table 5)

In trial two, harvesting at 21 DAS, oven drying and packaging in kraft paper, aluminium foil and woven bag were not significantly ($p<0.05$) different in fungal growth recorded. Solar drying the cowpea leaves, harvested at 21 DAS, and packaging in kraft paper differed significantly ($p<0.05$) with woven bag and aluminium foil packaging. The aluminium foil and woven bag packaged cowpea leaves harvest at 21 DAS, were however not significantly ($p<0.05$) different (Table 5). Open sun drying, packaging in aluminium foil, kraft paper and woven bag and harvesting at 21 DAS, did not show significant differences in fungal growth ($p<0.05$). At harvest stage 35 DAS, oven dried cowpea leaves packaged in woven bag differed significantly ($p<0.05$) with those packaged in kraft paper and aluminium foil packaging (Table 5) in fungal growth recorded. Open sun-dried cowpea leaves, harvested at 35 DAS, and packaged in kraft paper and woven bag were significantly ($p<0.05$) different. However, the aluminium foil and woven bag packaging did not differ significantly ($p<0.05$) in fungal growth (Table 5).

Solar dried cowpea leaves harvested at 35 DAS, and packaged in kraft paper were significantly ($p<0.05$) different from those packaged in the woven bag (Table 5). Harvesting at 49 DAS, open sun drying and packaging in aluminium foil, woven bag, Kraft paper did not differ significantly ($p<0.05$) in fungal growth. Oven drying, packaging in aluminium foil and harvesting at 49 DAS, differed significantly with ($p<0.05$) the cowpea leaves packaged in kraft paper and woven bag. However, the kraft paper and woven bag packaging were not significantly different. Solar dried cowpea leaves harvested at 49 DAS, packaged in Kraft paper woven bag and aluminium foil differed significantly ($p<0.05$) in fungal growth (Table 5).

Harvesting at 21 DAS, oven drying and packaging in aluminium foil significantly differed from kraft paper and woven bag packaging and even recorded the least fungal growth. This could have been caused by low moisture content from the oven drying. Studies by Martins [50] observed that a bread sample wrapped in aluminium foil and low-density polyethylene had a total fungal count that increased with storage temperature and days. Therefore, the storage condition, moisture and nature of packaging material when combined could contribute to deterioration of dried cowpea leaves during storage. Further studies reported the rate of fungal multiplication in all the packaging materials to be determined by the amount of inoculum existing in the sample and the amount of moisture retained.

Similar research by John [51] revealed that aspergillus spp. growth could be effectively and cheaply inhibited by storing peanut kernels in polyethylene-laminated aluminium in a dry environment at ambient temperature. Studies by Pessu [52] observed that chilies stored at 9% to 10% moisture content avoided fungal infestation; however, inadequate storage conditions can lead to the production of aflatoxins. Zand [53] noted that storage time increased the overall plate count of yeast and molds, and that pre- and post-contamination processes occur throughout the process. These results of Zand [53] could therefore be used to explain why the open sun drying method and the woven packaging material in this study recorded a higher microbial load compared to kraft and aluminium foil. The woven bag exposed the stored cowpea leaves to post drying contamination during storage. Research by Alabi [38] showed that DMRLSR-Y cultivar recorded the most prevalent *Penicillium spp*. percentage (30.5%) in maize seeds

Research by Poulsen [54], showed that pathogenic microorganisms could be found more or less everywhere and consequently could be transferred from processing surfaces to foods. Research by Krishnamurthy [55] reported that infrared radiations showed effectiveness in inactivating molds, bacteria, yeasts and spores in solid foods considering resultants moisture content and temperature. Therefore, combination of drying technology and application of correct packaging material could provide the required barrier between dried cowpea leaves and external environment that had been collected and sun dried 30 days after tasseling. Studies by Gamuchirai [49] on effect of drying techniques and storage conditions on quality and incidence of aflatoxins in dried chilies reported that the amount of total aflatoxin in the dried chilies was significantly impacted by the various drying techniques as the chilies dried using the solar cabinet drier had the highest aflatoxin levels, which were substantially higher than the levels found when the chilies were dried in the sun.

Vegetables should be protected from rehydration, microbiological contamination, and environmental contact through proper packing to ensure a long shelf life. A good packaging method necessitates certain microbe barriers, which are attained through regulated circumstances to measure moisture levels, microbial growth, levels of oxygen, hazardous fungal toxins and bacterial and indicators for temperature and time [56]. Further research by Zahra [56] showed that minimizing one or both of these elements or maintaining optimal conditions could help decrease microbial activity. The high fungal contamination observed in trial two compared to trial one could have been due to increased rainfall. Studies have reported fungal spores to be abundant in the air and as a result, when they land on moist or warm objects, they germinate and develop, encouraging the fungal growth as seen in trial two. The high rainfall in trial two which promoted fungal growth due to increased moisture in the dried cowpea samples.

3.3.2 Effect of Harvesting Stage, Drying Method and Packaging on Bacteria.

Cowpea leaves harvested at 35 and 49 DAS, solar, oven and open sun-dried and packaged in aluminium foil, Kraft paper and woven bag did not significantly ($p<0.05$) influence bacterial growth in trial one and two, respectively. In trial one, harvesting at 21 DAS, oven dried cowpea leaves packaged in aluminium foil, Kraft paper and woven bag were not significantly ($p<0.05$) different in bacterial growth.

Table 5: Effect of harvesting stage, drying method and Packaging material on fungal growth

		Trial One			Trial Two		
		Drying Methods			Drying Methods		
Harvesting Stage	Packaging Material	Oven	Solar	Open- sun	Oven	Solar	Open-sun
21	Aluminium Foil	1.50[i]*	2.67[ef]	5.92[cd]	4.30[ij]	6.88[fgh]	9.44[cd]
	Kraft Paper	3.25[efg]	2.08[gh]	4.67 [def]	4.45[ij]	4.69[i]	6.77[fgh]
	Woven Bag	3.42[ef]	5.67[cde]	6.00 [cd]	5.02[i]	6.87[efgh]	7.62[e]
35	Aluminium Foil	3.33[efg]	5.33[cde]	9.00 [a]	4.93[i]	7.52[ef]	11.81[a]
	Kraft Paper	2.25[hi]	4.17[efg]	5.42[cde]	4.85[i]	7.07[egf]	9.62[cd]
	Woven Bag	6.33[cd]	6.17[cd]	7.83 [ab]	6.92[efgh]	9.36[cd]	11.53[ab]
49	Aluminium Foil	2.50[i]	2.37[efg]	3.92[cd]	4.10[gh]	5.05[i]	8.01[egf]
	Kraft Paper	5.35[efg]	4.08[hi]	4.67[cde]	4.45[ij]	6.78 [ef]	6.80 [efgh]
	Woven Bag	5.42[efg]	5.34[cde]	5.52[cd]	3.04[j]	6.86[gh]	10.20[fg]
LSD	1.60				2.21		
CV	18.15				17.88		

*Means with different letters along the column are significantly different at $p<0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance

Solar dried cowpea leaves packaged in aluminium foil differed significantly (p<0.05) with those packaged in woven bag. However, the kraft paper packaged cowpea leaves were not significantly (p<0.05) different from woven bag and aluminium foil packaged cowpea leaves. Open sun-dried cowpea leaves packaged in the woven bag differed significantly (p<0.05) with those packaged in aluminium foil. However, the kraft paper and woven bag packaging were not significantly (p<0.05) different in bacteria counts (Table 6). In trial two, harvesting at 21 DAS, solar dried cowpea leaves packaged in aluminium foil, kraft paper, and woven bag were not significantly different in bacteria counts. However, oven dried cowpea leaves packaged in kraft paper, differed significantly (p<0.05) with those packaged in aluminium foil and woven bags in bacteria growth. Oven dried cowpea leaves packaged in aluminium foil and woven bag were not significantly (p<0.05) different in bacterial growth. Cowpea leaves packaged in aluminium foil and woven bag and open sun dried differed significantly (p<0.05) in bacteria counts, unlike kraft paper and woven bags that did not differ significantly (p<0.05) in bacteria counts. The interaction of harvest stage, drying method and package material did not significantly (p<0.05) bacteria counts (Table 6).

Solar dried cowpea leaves harvested at 21 DAS and packaged in aluminium foil differed significantly with those packaged in the woven bag in trial one. This could have been due to differences in moisture content between the two packaging materials. A study by Bhila [57] reported that sun drying reduced microbial load in beetroot and the total bacterial counts were high in beetroots compared to cabbage. By affecting the degrees of moisture retention after drying and the degree of contamination during the drying process, drying methods have an impact on microbial development [58]. The findings of this study are in agreement with those of Njoroge [43], findings that different drying techniques had a significant impact on the moisture content of dried native leafy vegetables, potentially reducing the number of microorganisms that could be counted. Further studies by Njoroge [43] showed that total viable counts decreased by 26-34% when indigenous leafy vegetables were blanched and dried at a temperature of 90 °C for 5 minutes.

The total viable counts were lowest in solar-dried indigenous leafy vegetables. Oven dried cowpea leaves harvested at 21 DAS and packaged in kraft paper in trial two recorded the least bacteria counts. This could have been due to the low moisture content. Packaging material plays the role of reducing the rate of deterioration, protecting food products against biological and chemical contamination and enhancing food quality and safety [47]. Due to internal factors like humidity and temperature within the packaging material, the packaging materials may have contributed to the contamination of the stored cowpea leaves. According to Patrignani [59] study, peach fruits wrapped in plastic had a higher probability of contamination than those packed in cardboard. Therefore, the combination of the stage of harvest, drying method and packaging material either increased or slowed the bacteria growth on the dried cowpea leaves.

The stage of harvesting influenced the cowpea leaves in terms of the moisture and nutrient availability which ultimately favored growth of the bacteria. The method of drying significantly influenced growth of microorganisms [60]. The results revealed that *A. paniculata* extracts at harvesting ages of 90, 100, 115, and 127 DAS and drying at 65 ^{o}C and 80 ^{o}C yielded the minimal inhibitory concentration values between 3.12–25.0 mg/ml. The maximum antibacterial activity was seen in the 90 DAS/65 ^{o}C extracts, which had minimal inhibitory concentration values of 3.12 mg/ml against *S. aureus*, *B. cereus, and M. luteus* as well as 12.5 mg/ml against *S. epidermidis*.

Table 6: effect of harvesting stage, drying method and packaging material on Bacteria

		Trial one			Trial two		
		Drying methods			Drying methods		
Harvesting Stage	Packaging Material	Oven	Solar	Open-sun	Oven	Solar	Open-Sun
21	Aluminium Foil	29.83^{gh*}	84.5d	123.33^{c}	54.75^{ij}	82.83^{efg}	119.00^{a}
	Kraft Paper	34.17^{ghi}	88.00^{cd}	110.83^{ab}	33.33^{mn}	80.67^{fg}	107.50^{abc}
	Woven Bag	35.00^{ghi}	79.75^{c}	124.08^{a}	34.58^{ijkl}	78.08^{fg}	96.25^{bcdefg}
35	Aluminium Foil	18.00^{g}	49.75^{efg}	112.92^{ab}	17.58^{n}	49.42^{ijkl}	104.58^{abcde}
	Kraft Paper	24.92^{gh}	58.67^{e}	102.67 ^{bc}	25.08^{mn}	57.67^{hi}	101.41^{abcde}
	Woven Bag	28.75^{gh}	56.08^{ef}	114.83^{ab}	28.75^{mn}	55.67^{ij}	105.25^{abcd}
49	Aluminium Foil	52.08^{ef}	82.75^{c}	116.75^{ab}	48.08^{ijkl}	79.5^{gh}	106.92^{abc}
	Kraft Paper	41.58^{fg}	84.08c	119.17^{ab}	41.42^{ijklm}	84.08^{defg}	111.67^{abc}
	Woven Bag	49.42^{efg}	93.75^{cd}	120.83^{a}	47.75^{ijkl}	91.25^{cdefg}	115.42^{ab}
LSD		17.06			19.25		
CV		18.12			20.65		

*Means in the same column having different superscripts are significantly different ($p<0.05$) LSD is Least Significant Difference; CV is Coefficient of Variance.

4.CONCLUSION

Combining the harvesting stage, drying method and packaging influenced fungal and bacterial growth of dried cowpea leaves. Correct harvest stage coupled with the right drying method and proper packaging if adopted will result to a prolonged shelf life and maximum nutrient retention in cowpea leaves.

5.RECOMMENDATIONS

The integration of correct harvesting stage, drying method and correct packaging will result in a longer shelf life a dried cowpea leaves therefore ensuring availability even during off seasons. This therefore shows that proper drying in protected environment preferable solar drying and oven gave coupled with kraft packaging material will give effective results in terms of microbial safety and nutritional quality as earlier results showed.

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