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ASSESSMENT OF MICROBIOLOGICAL QUALITY OF DRIED VEGETABLES MARKETED IN VHEMBE DISTRICT, SOUTH AFRICA

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ABSTRACT

Fruits and vegetables are important sources of nutrients and are included as part of healthy and balanced diet globally. Contamination of these commodities with pathogens during growth, harvesting, storage, transportation, processing and handling might occur. However, some of the pathogens are originated from human, animal or environmental sources. This study was conducted in order investigate the microbiological quality of sixty (60) different types of dried vegetable (pumpkin flower, leaves and cowpea leaves) samples that were purchased from various street vendors around Vhembe district municipality, Limpopo Province, South Africa. Five (5) different locations were identified, and twenty (20) samples of each dried vegetables were randomly collected. Microbiological analyses were conducted for coliform bacteria, *Escherichia coli, Salmonella* species, *Bacillus cereus*, total plate count, yeasts and moulds. Data analyses were conducted using SPSS version 25. Coliform counts were present in all dried vegetables samples ranging from 0.00 - 3.70 log_{10}cfu/g. *Escherichia coli* counts ranged from 0.00 - 4.62 log_{10} cfu/g. *Salmonella spp.* varied from 0.00 - 3.75 log_{10} cfu/g. *Bacillus cereus* ranged from 0.00 - 3.72 log_{10} cfu/g. Total plate count ranged from 2.13 - 2.66 log_{10} cfu/g. Yeast counts ranged from 2.03 - 5.61 log_{10} cfu/g. However, mould counts did not grow after incubation for the dried vegetable samples. Most of the microbiological results were in line with the Food and Drug Administration standards of Philippines and Department of Health of South Africa. The study showed that most dried vegetable samples were safe for human consumption which means that good hygiene practices were properly implemented by street vendors However, there is a need for food scientist, policy makers and government officials to train street vendors on how to handle their food products as most of them are sold uncovered which lead to high microbial growth. The aim of the study was to assess the microbiological quality of dried vegetables such as pumpkin flowers (*Cucurbita moschata Duchesne*), pumpkin leaves (*Cucrbita pep* L.) and cowpea leaves (*Vigna sinensis*) sold in Vhembe district municipality.

Key words: microbial quality, dried vegetables, foodborne pathogens, microorganisms, food safety

INTRODUCTION

Vegetables are excellent sources of nutrients because they contain high amounts of dietary fibre, vitamins, minerals and phytochemicals. They are very essential in African food culture [1, 2, 3]. Fresh harvested vegetables contain 90% water which increases the microbiological growth because they are very perishable [4, 5]. o, In sub-Saharan Africa, vegetables are eaten raw [6] or dried, then cooked and included as part of daily diet and consumed with porridges and other starchy products to supplement the diet since they supply macro as well as micronutrients. They are usually abundant during the rainy season especially in poor rural communities. This is the reason why fresh vegetables need to be preserved to make them available throughout the year [5]. The most common traditional method of preserving vegetables is home sun drying [7, 8]. The final weight of moisture content of dried vegetables varies from 2 to 5% [9].

Drying is one of the oldest and most common methods of processing and preserving food in developing countries such as South Africa and Ghana [10, 11, 12]. Notably, vegetable drying is also practised in Asia and Europe. Advantages of drying vegetables include the inhibition of the growth of spoilage microorganisms and it is cost effective and easy to perform. However, vegetable drying permanently changes the plant tissue through shrinkage, sticking or browning and deformation of shape which affects the quality of the final product [13]. Other drawbacks are that sun drying takes long and result in low quality of the final products. Moreover, sun drying does not necessarily reduce the microbial load because it is usually not properly monitored since it relies on weather conditions [14]. The low water activity of dried vegetables extends their shelf life, and this promoted the perception that these products are microbiologically safe [15].

Clean storage facilities of dried vegetables are important in maintaining quality and reducing the growth of microorganisms. Alpa and Bulantekin [16] reported that most microorganisms isolated from dried vegetables are total plate count, coliform bacteria, Bacillus cereus, Salmonella spp., Escherichia coli, yeast and moulds. The recent outbreaks of foodborne illnesses associated with dried food products have corroborated that pathogenic microorganism can continue to grow in low water activity. [17]. Moreover, pathogenic microorganisms are likely to cause human illness because of low infectious dose or favourable temperature that allows them to grow. Since drying of vegetables inhibits the growth of spoilage and pathogenic microorganisms, very little attention has been given to their safety. Food-borne pathogens on dried vegetables and their involvement in food-borne outbreaks are not well documented [16]. Therefore, the objective of this study was to determine

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the microbiological quality of dried vegetables sold in Vhembe districts markets, Limpopo Province, South Africa.

MATERIALS AND METHODS

Sampling and sampling preparation

A total of sixty samples (20 per category) of three different types of dried vegetables namely, pumpkin flowers, pumpkin leaves and cow pea leaves were randomly collected from five (5) different locations in Vhembe district. All samples were placed in cooler box at a temperature of 4°C and transported to the Food Microbiology laboratory for analysis. All analyses were conducted in triplicates immediately after sampling (n = 3) and were duplicated to validate results..

a- fresh pumpkin flowers, b- dry pumpkin flowers, c-fresh pumpkin leaves, d-dry pumpkin leaves, e-fresh cowpea leaves and f-dry cowpea leaves

Microbiological analyses

The dried vegetable samples were analysed for the presence of coliforms, Escherichia coli, Salmonella spp., Bacillus cereus, total plate count and yeast and

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moulds. The microbial analyses were performed according to the ISO International Standards methods, 2006. The dried vegetable samples were subjected to decimal dilutions and aliquots were inoculated in the culture media proposed by the ISO International Standards, 10^{-1} to 10^{-6}. *Coliforms* and *Escherichia coli* were assessed using *Chromocult coliform agar* (Neogen culture media, United Kingdom) and incubated at 37 °C for 24 h following ISO 4832 [18].

Salmonella spp. was detected using Xylose Lysine Deoxycholate agar (Neogen culture media, United Kingdom) according to ISO 6579 [19]. *Bacillus cereus* counts were obtained by spreading the diluents on solidified *Bacillus Cereus* agar (Neogen culture media, United Kingdom) at 37 °C for 48 h (ISO 21871) [20]. Total plate count was performed according to the ISO 4833 [21] and aliquots were pour-plated in Plate Count Agar (Neogen culture media, United Kingdom) and incubated at 30 °C for 48 h. The yeast and mould were plated on Potato Dextrose Agar and incubated at 25 °C for 5 days. All analyses were performed in triplicates.

Statistical analysis

Data capturing was done using Excel. Data was subjected to analysis of variance using SPSS version 25 and means were separated using Duncan's multiple range test at $p < 0.05$. The counts were calculated using log_{10}cfu/g.

RESULTS AND DISCUSSION

Coliform counts in dried vegetables

Coliform counts (log_{10}cfu/g) of pumpkin flowers ranged from 0.00 - 3.70, pumpkin leaves from 0.00 - 2.95 and cowpea leaves from 2.30 - 2.30 as shown in Figure 1. Coliforms are a group of mostly harmless bacteria that live in the soil, water and as well as the gut of animals. Martin *et al.* [22] stated that coliforms are indicators of the potential presence of disease-causing bacteria and indicate the general sanitary quality of food. The highest coliform counts were found on pumpkin flowers at Location 1 and the lowest count on pumpkin flowers at Location 2. Pumpkin flowers were significantly ($p < 0.05$) different compared to other samples at Location 1.

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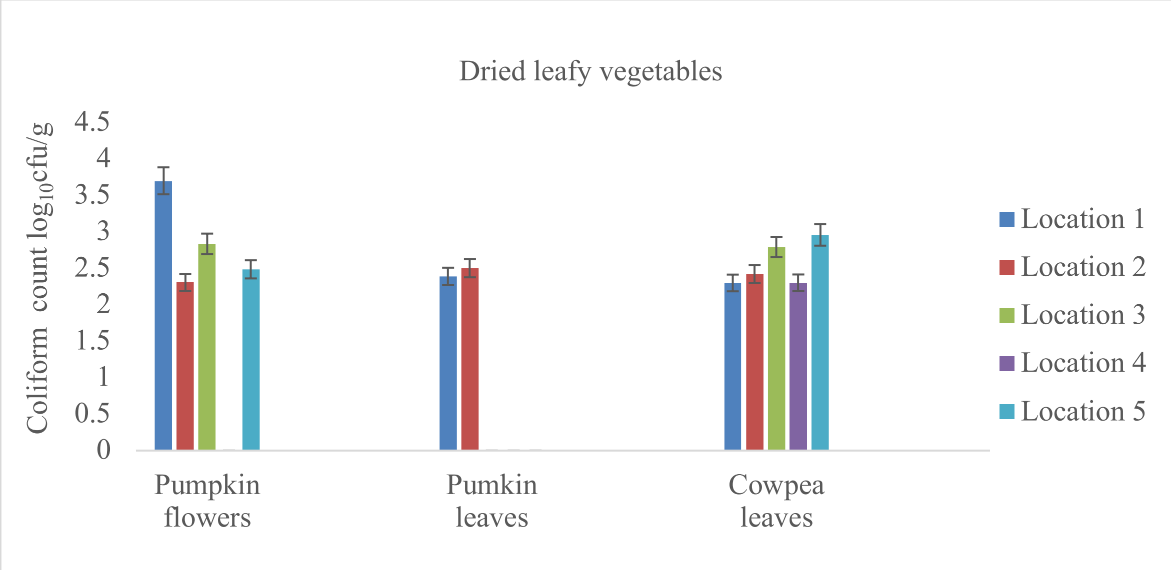

Figure 1: Coliforms count (log_{10}cfu/g) of different dried leafy vegetables. Values are mean ± standard deviation of the three replicates (n=3). Error bars are standard error of mean values (n= 3)

The current study results are different from those reported by Ntuli *et al.* [23] who obtained coliform count that ranged from 3.51 - 7.71 log_{10}cfu/g. The acceptance level for coliforms on dried vegetables is 10^3 cfu/g [24; 25]. Therefore, results obtained are within the specified coliforms level suggesting that hygiene practice were followed during cultivation, harvesting, post-harvest handling, processing, cooking and drying. However, blanching of vegetables before drying should be recommended for the street vendors to inactivate enzymes responsible for colour changes and to decrease microbial load. For example, drying of leafy vegetables is usually done in open space and with hands and this exposes the products to potential microbial contamination. Blanching of vegetables before drying is important since the temperature of home drying is generally not very high to kill all contaminating microorganism.

Escherichia coli counts in dried vegetables

Escherichia coli is a bacterium that lives in human and animal intestines and is responsible for most food-related infections. It is closely associated with faecal contamination. *Escherichia coli* is a common pathogenic bacterium that causes various diseases resulting in 2 million fatalities every year. This includes diseases such as diarrhoea, acute renal failure often leading to death, neonatal meningitis and sepsis. It is found in soil, food products, environmental factors, wastewater treatment and water [26, 27]. The counts of *Escherichia coli* varied in pumpkin flowers from 0.00 - 4.62 log_{10}cfu/g, in pumpkin leaves it ranged from 0.00 to 2.57 log_{10}cfu/g and in cowpea leaves from 0.00 - 3.51 log_{10}cfu/g, respectively (Figure 2).

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Pumpkin flowers showed a significant difference at Location 1 compared to other samples. Ntuli *et al.* [23] reported *E. coli* count of 3.21 cfu/g and Asime *et al.* [27] reported 2.04 x 10^2 cfu/g, however, these are similar to the results obtained in this study. However, the standard specification of *E. coli* on dried vegetables is 10^3 cfu/g.

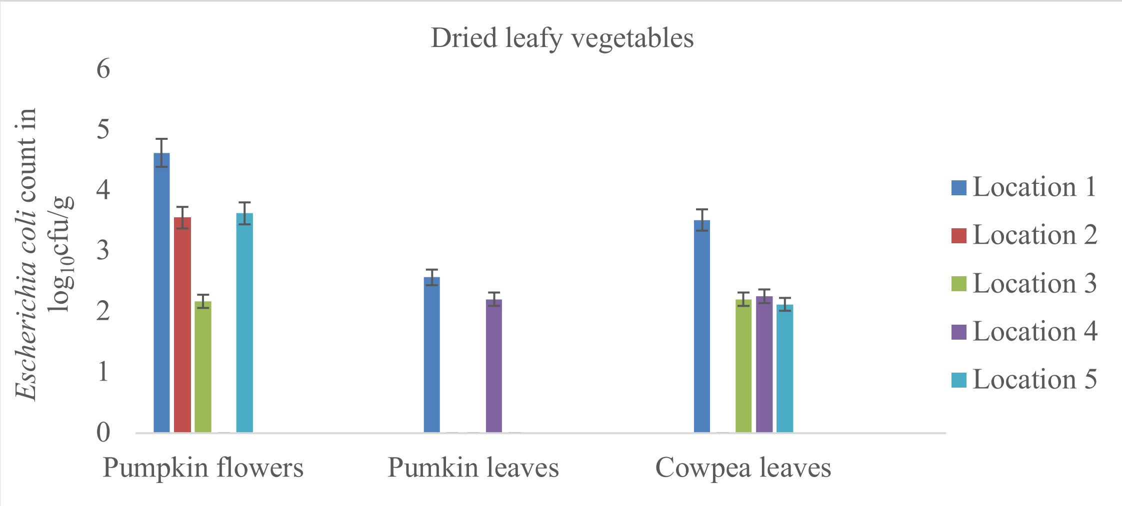

Figure 2: *Escherichia coli* count (log_{10}cfu/g) of different dried leafy vegetables. Values are mean ± standard deviation of the three replicates (n=3). Error bars are standard error of mean values (n= 3)

Thus, the amount of *E. coli* on pumpkin flowers from Location 1 is beyond specification. All other samples collected from different locations were within specified microbial levels. Nevertheless, pre and post-harvest sources of contamination such as soil, irrigation and rinse water, compost manure, unhygienic processing equipment and human handling should be minimised in order to prevent the growth of spoilage and pathogenic microorganisms in dried vegetable products [28]. *E. coli* and *Salmonella* can be found in animal faeces or from contaminated water used for both irrigation and additional processing steps. Transmission of *E. coli* from contaminated soil manure and irrigation water to processed vegetables has been reported [28].

Salmonella species counts in dried vegetables

Salmonella spp. is a well-known foodborne pathogen found in human and birds. *Salmonella spp.* is responsible for the infectious disease that causes gastrointestinal tract, chronic infections and Salmonellosis. *Salmonella spp.* results are recorded in Figure 3. Pumpkin flowers counts varied from 0.00 - 3.75 log_{10}cfu/g and the highest count was obtained from Location 1 and no count was detected in Location 4.

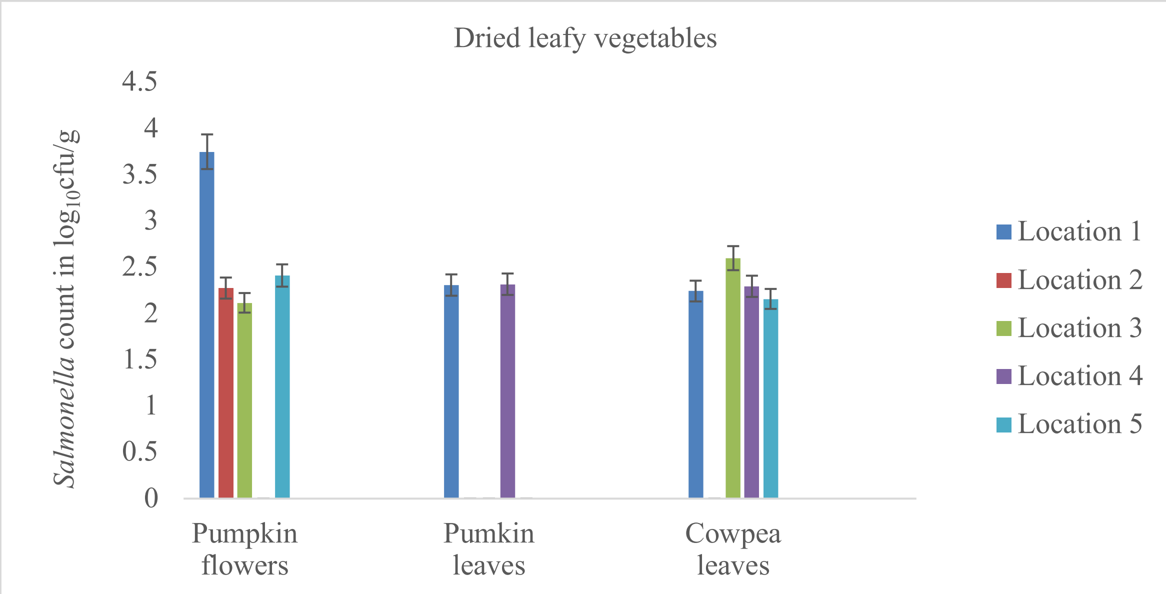

Figure 3: *Salmonella* species (log_{10}cfu/g) of different leafy dried vegetables. Values are mean ± standard deviation of the three replicates (n=3). Error bars are standard error of mean values (n= 3)

The highest count of *Salmonella spp.* in pumpkin leaves was obtained from Location 3. *Salmonella spp.* was not detected in Location 2, 3 and 5. In cowpea leaves, *Salmonella spp.* ranged from 2.16 - 2.60 log_{10}cfu/g. Location 3 was significantly different when compared to other locations. The results are in line with the findings of Ntuli *et al.* [23] who reported *Salmonella* counts of 3.01 log_{10}cfu/g on dried vegetables. The microbial limit of dried vegetables is $>10^3$ cfu/g. All samples collected from different locations were within specification. *Salmonella spp.* is active and can survive on dried food products for at least eight months [23]. Nevertheless, traditional processing methods such as drying are effective when utilised in high moisture food but they usually fail to decrease microbiological contamination of low moisture food to non-detectable levels [16].

Total plate counts in dried vegetables

The results of total plate counts (TPC) are shown in Figure 4. Total plate count is an acceptable measure of the general degree of bacterial contamination in hygienic conditions of processing plants [29]. Total plate count in pumpkin flowers ranged from 2.31 - 2.68 log_{10} cfu/g. In pumpkin leaves, TPC ranged from 2.13 - 2.66 log_{10}cfu/g and from 2.34 - 2.64 log_{10}cfu/g in cowpea leaves.

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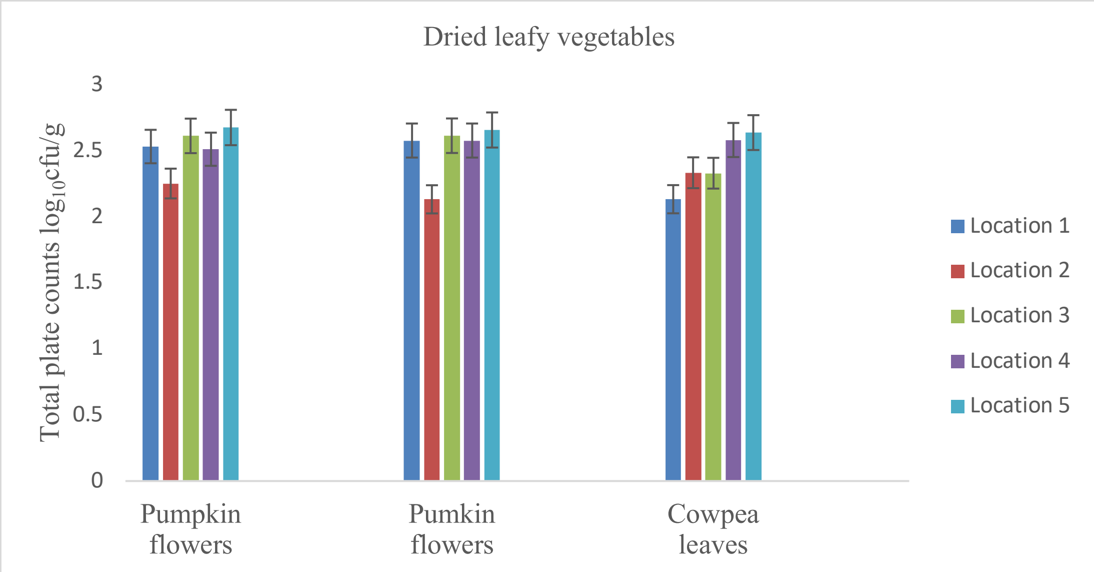

Figure 4: Total plate count ($\log_{10}$cfu/g) of different dried leafy vegetables. Values are mean ± standard deviation of the three replicates (n=3). Error bars are standard error of mean values (n= 3)

Pumpkin flowers, pumpkin leaves and cowpea leaves had the highest levels of TPC in Location 5 and showed significant ($p < 0.05$) difference compared to other locations. The microbial limit for TPC on dried vegetables is 10^5 to 10^6 cfu/g [24]. All samples purchased from different locations were within the specified levels. Results of this study differ from that by Ntuli *et al.* [23] who reported TPC of 6.71 $\log_{10}$cfu/g. Since TPC is a real indicator of the presence of microorganisms and the levels of hygiene and the sanitary conditions of a given place, the low count in dried vegetables in this study implies high levels of hygiene and good sanitary conditions in the handling and processing of these products [28]. Nevertheless, vegetables are part of the food groups that have been implicated to cause enteric diseases via the use of treated and composited animal manure which was not the case in this study [25]. The use of drip irrigation in vegetable production is recommended since it reduces this effect [30].

Yeasts and moulds count in dried vegetables

Although most yeasts and moulds are aerobes requiring free oxygen for growth, their acidic or alkaline requirement for growth is quite broad. The presence of yeast and moulds may cause change in the appearance, texture, flavour of products which can affect the sensory attributes of the final products [16]. Yeast counts ($\log_{10}$cfu/g) varied from 2.35 - 4.38 in pumpkin flowers, from 2.05 - 4.51 in pumpkin leaves and from 2.35 - 5.61 in cowpeas leaves (Figure 5). Location 3 for pumpkin flowers, Location 4 for pumpkin leaves and Location 4 for cowpea leaves showed significant ($p < 0.05$) differences compared to other locations.

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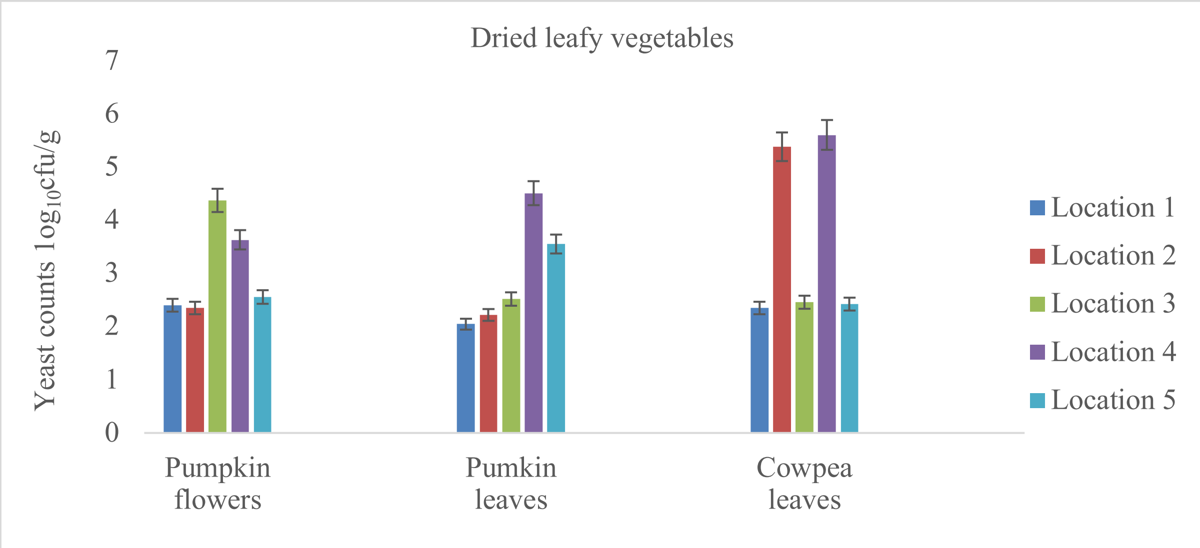

Figure 5: Yeast counts (log_{10}cfu/g) of different dried leafy vegetables. Values are mean ± standard deviation of the three replicates (n=3). Error bars are standard error of mean values (n= 3)

Mould did not grow after incubation in all samples tested. Ntuli *et al.* [23] reported that dried leafy vegetables (pumpkin leaves) produced at home were highly contaminated by fungi which varied from 6.5 x 10^5 to 9.4 x 10^6 cfu/g. The results of this study show that all samples are within the microbial level of 10^3 to 10^5 cfu/g [25]. Nevertheless, yeast and mould have been identified as the main spoilage microorganisms in dried food products. They are more tolerant to low water activity than bacteria and can survive below water activity of 0.80. [31]. In general, dried food products with higher water activity consist of significant amount of unbound water molecules that favour the growth of microorganisms [16].

B. cereus counts in dried vegetables

Figure 6 shows the results of *B. cereus* of different dried leafy vegetables from different locations. The counts of *B. cereus* (log_{10} cfu/g) on pumpkin flowers varied from 0.00 - 3.56. High counts for pumpkin flowers were obtained from Location 4 while there were no counts from Location 5. Location 4 was significantly different compared to other locations. The counts (log_{10} cfu/g) for pumpkin leaves ranged from 2.17 - 3.72 and the highest count was obtained from Location 5 while Location 3 had the lowest count. Cowpea leaves ranged from 0.00 - 3.70 log_{10}cfu/g and Location 4 showed a significant difference. Ntuli *et al.* [23] reported Bacillus species counts on dried vegetables which included pumpkin leaves and results varied from 5.6 x 10^1 to 6.1 x10^1 cfu/g.

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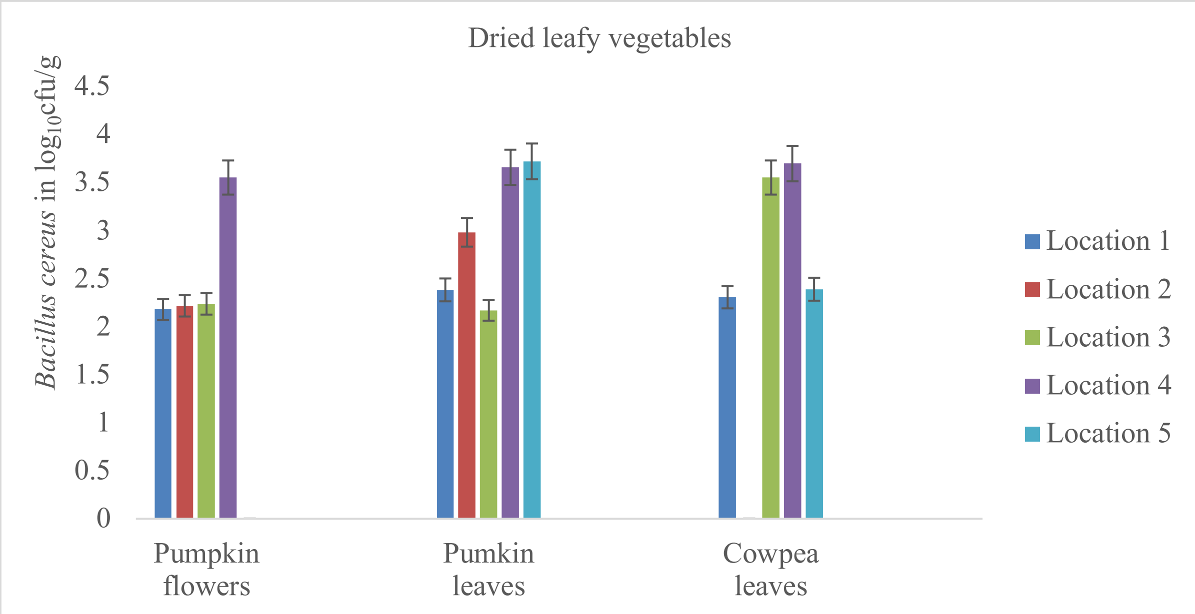

Figure 6: *Bacillus cereus* count (log_{10}cfu/g) of different dried leafy vegetables. Values are mean ± standard deviation of the three replicates (n=3). Error bars are standard error of mean values (n= 3)

The acceptable microbiological limits of *B. cereus* in dried vegetables must not exceed 10^3 cfu/g counts [31]. Despite the fact that samples were taken from home sun-dried vegetables that have a high potential risk of microbial contamination, the results of this study show that the counts were within specified microbial levels.

CONCLUSION

The findings demonstrate that dried vegetables sold in Vhembe district, South Africa are safe for human consumption since microorganisms isolated are within the level of South African Department of Health regulation. However, the presence of coliforms in dried vegetables shows that good hygiene is not practised by the street vendors. This shows that vegetable could easily become contaminated during the drying process. The need of training street vendors on hygiene and good manufacturing practices are the most vital points to report in this study.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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