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EFFECTS OF PACKAGING AND STORAGE CONDITION ON THE SHELF STABILITY OF ROASTED AND PULVERISED DATE (*Phoenix dactylifera L.*) SEED

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ABSTRACT

Food packaging helps maintain food safety, minimize environmental impacts and protect from spoilage/damages. This study evaluated the effects of packaging materials and storage conditions on the shelf-stability of roasted and pulverised date seeds. Date fruits were gathered and the seeds obtained and processed through the stages of sorting, soaking, washing, drying at 90°C for 6 hours, roasting at 220°C, cooling, blending and sieving. Tightly sealed transparent cellophane bags and Plastic containers were used for packaging. Storage conditions assessed were: Ambient (room) condition, free table and dark cupboard. Microbial analyses were carried out on the powdered date seed samples at 2, 7, 14, 28, 42 and 56 days of storage to determine the bacterial and fungi load count, using nutrient agar, plate count agar and potato dextrose agar. There were no visible microbial growths on the fresh sample and on samples at 2 days post-production for the two storage conditions considered in this study. As storage duration increased from day 7, bacteria such as *Pseudomonas spp, Bacillus subtilis* and *Staphylococcus aureus* were isolated and identified. Also, fungi such as *Aspergillus flavus and yeast* were isolated and identified. There were no differences between microbial loads of samples stored in a dark cupboard and on the table in open space as storage duration increased. The study showed that a transparent cellophane bag was a more suitable packaging material.

Keywords: Packaging Materials; Storage Conditions; Shelf Stability and Date Seed

INTRODUCTION

Date (*Phoenix dactylifera L.*) is a palm fruit native to the Arab nations. However, it is highly cultivated in some other nations of the world today. The date fruit has a fleshy mesocarp layer covering a hard oblong-shaped seed as the endocarp part. Both the fleshy layer and the seed of date fruit have been reported to have tremendous use in human nutrition and health. The mesocarp part of date fruit has been reported to be useful in the manufacturing of date sugar, syrup in the pharmaceutical and confectionery industry and fortification of baby foods. The date seed has also been reported to be of great use in the production of caffeine-free date-pit beverages, which is commonly consumed in the Middle Eastern part of the World. Earlier researchers (Ishrud *et al.*, 2001) reported that date seed beverage helps in reducing blood sugar and lowering the effect of body temperature. Date seed has also been reported by Ishrud *et al.* (2001) as a critical component of medicinal food used in treating weak memory, hyperactivity, renal stone, bronchial asthma and cough. El-Rayes, (2009), Basuni and Al-Marzooq, (2010), Ardekani *et al.* (2010) have reported that oil can be extracted from date seed, and it is also valuable for pharmaceuticals and cosmetics production. Date seed has also been reported to have nutritional benefits as it contains carbohydrate, protein, oil, dietary fibre, bioactive polyphenols and natural antioxidants (Ayuba 2019).

The emergence of various kinds of complicated diseases and ailments and the resistance of some diseases and ailments to existing drugs and therapy has increased the global attention towards the search for functional foods capable of promoting healthy living.

The packaging of substances has been reported to play a significant role in protecting the physical, chemical and biological constituents of food products (Oyelade, 2019). Food packaging has been reported to help in maintaining food safety, and it minimizes environmental impact post-production (Coles *et al.*, 2003).

Various kinds of packaging materials exist, as they are also manufactured from different kinds of raw materials. Therefore, when an inappropriate packaging material is used for a product or an appropriate packaging material used but stored in an inappropriate storage condition, the packaging material can also react with the product kept in it in different ways. Packaging materials also interact with environmental conditions in different ways. Likewise, the product kept in these packaging materials may not behave the same way in varying storage conditions.

The efforts of previous researchers on the various uses, processing and benefits of date seed and its constituents is acknowledged. However, little or no information has been reported on how the processed date seed can be packaged and stored to ascertain its longevity in storage with its potency and quality attributes retained before consumption. On this basis, this study is focused on assessing the effects of packaging materials and storage conditions on the shelf stability of date seed powder.

MATERIALS AND METHODS

One hundred kilogrammes (100 kg) of dry date fruit was weighed and cleaned to ensure that dirty particles and rotten date fruits were excluded. The seeds were separated from the flesh, using knife to cut through the fruit to remove the seed. The seeds were further processed by washing, soaked for thirty minutes to remove any adhering flesh, dried at 90°C for 6hours in a cabinet oven drier. The dried seeds were roasted at 220°C for an hour and then allowed to cool before blending into powder, sieved with mesh size 250 micron to have a uniform texture. The powder was packed into the different packaging materials and stored at different conditions for fifty-six (56) days following the procedure of Oyelade (2019).

Packaging Materials and Storage Conditions

The packaging materials used in this study were; tightly sealed low density transparent cellophane bags and a tightly sealed transparent plastic container. Simultaneously, room condition on a

free table and dark cupboard out of reliving daylight were the storage conditions considered in the study. The treatments were coded according to storage condition as Plastic Container in Dark Cupboard (PCDC); Cellophane Bag in Dark Cupboard (CBDC); Plastic Container on Free Table (PCFT), and Cellophane Bag on Free Table (CBFT).

Determination of bacterial and fungal load count

Determination of bacterial and fungal load count were carried out for fifty-six days (56 days). Samples were collected and analyzed in the following order after production: the fresh sample (Control Sample) on the day of production (0-day), two (2) days, seven (7) days, fourteen (14) days, twenty-eight (28) days, forty-two (42) days, and fifty-six (56) days. Analyses were carried out for seven (7) durations of storage, starting from the day of production with one sample from the control and four samples from the various packaging materials and storage conditions for the rest of the six (6) analyses.

Identification of Bacterial Isolates

Bacteria isolates were identified morphologically and biochemically through Gram staining, Spore staining, capsule staining, motility, indole, citrate, urease, oxidase, catalase, coagulase, methyl-red, Voges-Proskuer (VP) and sugar fermentation test. The results were interpreted according to the Bergy Manual of Determinative Bacteriology (2000) as reported by Basuni and AL-Marzooq (2010).

Identification of Fungal Isolates

The inoculated plate was identified using a wet mount on a microscope, based on cultural and morphological characteristics such as colony diameter, colony colour on agar, reverse plate, colony and texture. Also, morphological features were studied under the microscope and the major microscopic features that were considered are conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles (Klich, 2002).

RESULTS

Bacterial count: There was no visible growth for bacteria and fungi on the fresh samples. After two days of storage time, there was still no visible growth of bacterial and fungi in the plate. However, from seven days upward, visible growth were observed across all the packaging materials and storage conditions. Bacterial count of date seed powder stored in the plastic container in dark cupboard (PCDC) and plastic container on free table (PCFT) kept increasing as storage time increased. However, date seed powder stored in cellophane bag in dark cupboard (CBDC) showed increase in bacterial count up to 28 days in storage and then declined after 42 days in storage ($5.0\pm0.0 \times 10^6$cfu/g – $3.0\pm1.4 \times 10^6$cfu/g). Similarly, date seed powder stored in cellophane bag on free table (CBFT) also show bacterial growth up to 14 days but the count declined from day-28 (Table 1).

Fungal Mold Count: No mold growth was found on the fresh samples, likewise on the samples examined two days after storage (Table 2). However, fungal molds were observed in samples examined from day-seven post storage up to the last day of storage (56 days) of the study. The mold count of date seed powder kept in plastic container in dark cupboard (PCDC) increased as storage time increased from day-7 ($0.3\pm1.4 \times 10^6$cfu/g) up to day-28 ($8.0\pm0.0 \times 10^6$cfu/g) and later stabilized between day 42 and day 56. Similar trend was also observed in the samples kept in plastic container on free table (PCFT); there was an increase in the mold count from day-7 ($5.0\pm1.4 \times 10^6$cfu/g) up to day 28 ($11.0\pm2.8 \times 10^6$cfu/g). The total mold count later stabilized between day-42 and day-56. Date seed powder stored in cellophane bag in dark cupboard (CBDC) and the sample stored in cellophane bag on free table (CBFT) also had fungal growth from the seventh day of storage ($2.0\pm0.0 \times 10^6$cfu/g) which observed to be stable up to day-42 in storage. A decline in the mold count was observed in day-56 ($1.0\pm1.4 \times 10^6$cfu/g) of storage for both packaging and storage media (Table 2).

There was no bacterial isolate in the fresh sample and the samples examined after two days of storage across all the packaging materials and storage conditions. However, as storage days increased from day-7 upward, there were bacterial isolates found in some of the storage media. *Pseudomonas* species, *Bacillus subtilis* and *Staphylococcus aureus* were found from day-7 till the last day (day-56) of storage in samples stored in plastic container in dark cupboard (PCDC) and on free table (PCFT). However, for samples stored in cellophane bag in dark cupboard (CBDC) and free table (CBFT), there was no *Pseudomonas* species found across all the days of storage. Also, there was no *Bacillus subtilis* found in the sample stored in cellophane bag on free table (CBFT) all through the period of storage; implying that, minimal bacterial isolates were found in samples stored in cellophane bag (Table 3).

There was no fungal isolate observed in the fresh sample and samples examined after 2-days of storage in all the storage media (Table 4). However, fungal isolates such as *Aspergillus flavus*, *Fusarium* species and yeast were found as storage time increased from day-7 to the last day (day-56) of storage. *Fusarium* species was not found in the samples stored in cellophane bag in the dark cupboard (CBDC) and on the free table (CBFT) all through the storage time. Fungal isolates and counts observed in the samples stored in cellophane bag were relatively low compared to samples stored in plastic container (Table 4). This implies that fungal growth is more inhibited in the cellophane bag.

DISCUSSION

Microbial Load Count of Pulverised Roasted Date Seed

Given the benefits of the domestic and industrial use of date seed to man, preservation against spoilage and prevention against contamination after the production of date seed powder becomes highly imperative. Globally, food safety is an increasing concern in the area of public health. Although microorganisms, usually the primary cause of spoilage, have specialized roles in the environment, they are extraordinarily diverse.

The microbial load of the date seed powder that showed no microbial growth in the fresh samples indicates that the microorganism in the powdered date seed had been destroyed during the roasting process because no bacterial or fungal growth was observed in the fresh samples. This serves as more or less a sterilization process for the product. Also, 2-days post-production, no visible microbial growth was observed. This may be attributed to low moisture, which could have limited microbial growth during the roasting process, thereby inhibiting spoilage from the microorganism's growth. However, after 7 days in storage, visible growth of bacteria and fungi were observed. Samples stored in a plastic container on free table (PCFT) had the most populated plate for bacteria and fungi. It was observed that the plastic container in dark cupboard (PCDC) had the most isolated organism in terms of bacteria. In contrast, the samples stored in plastic container on free table (PCFT) had the most isolated organism for fungi. An arithmetic reduction in bacterial and fungi mean count from samples packaged with the transparent cellophane bag was observed. In contrast, in the samples packaged in the transparent plastic container, there was a geometric increase. This implies an increase in moisture through an unsealed lid, which invariably increases the activity of micro-organism. The values of bacteria growth count reported by Shenasi *et al.* (2002) in pre-packed commercial dates were lower than the value reported in this study. Also, fungal growth values obtained in this study were higher than those reported in commercial date fruits (Aidoo *et al.*, 1996; Shenasi *et al.*, 2002). This could be due to the fact that this study focused on date seed only whereas, Shenasi *et al.* (2002) and Aidoo *et al.* (1996) utilized the whole date fruit for their study. However, yeasts and moulds were observed in all samples at various levels. The yeast and mould are considered as spoilage organisms of date (Shenasi *et al.*, 2002). A load of spore-forming bacteria (*Bacillus*) was detected in the samples analysed except for samples stored in cellophane bag on free table. Bacterial isolates belonging to

the *Enterobacteriaceae* family, which are potential public health concerns, were absent in the samples analysed in this study. This suggests that the product is safe for public consumption, if properly packaged and stored. As the storage period increased, the microbial load increased. The sample PCFT had the most bacterial and fungal count throughout the storage period, while those of sample CBFT had the least fungi and bacteria growth. Previous studies showed that yeast and moulds are the common contaminants of dates (Al-Jawally, 2010). These fungi included *Aspergillus spp., Stemphylium botryosum, Phomopsis diopspyri, Cladosporium spp., Citromyces ramosus, Macrosporium spp., Alternaria spp. and Penicillium spp.*

The bacterial species isolated from the pulverised roasted date seed include *Pseudomonas spp, Bacillus subtilis* and *Staphylococcus aureus.* The *Staphylococcus aureus* and *Bacillus subtilis,* which do not prove so harmful were isolated from the date seed powder. This could probably be due to environmental contamination, because the processing method involved using hands and the organisms are a body flora of the skin. Although the containers were sterilized before usage, the presence of *Pseudomonas species* could be related to an unhygienic production process. It could also be from other sources of contamination, such as utensils and equipment to prepare date seed powder produced with a roasting method. This indicates post-production contamination resulting from blending, sieving and packing of the powder.

The fungi species isolated from the powdered date seed sample in this study were *Aspergillus flavus and yeast.* The contamination may have occurred during the storage period due to not adequately covered containers and sealed nylon. Yeast *and Aspergillus flavus* were the most observed fungi associated with the spoilage of roasted date seed powder in this study. When left for a long time at room temperature, date seed powder would cake up due to air passage in the container and increasing humidity, thereby creating the moist condition for fungal action, especially yeast.

CONCLUSIONS

The study concludes as follows:

1. There was no microbial growth from the freshly produced and date seed powder stored for forty eight hours.
2. Yeast and *Aspergillus flavus* were isolated and identified as spoilage organisms in roasted date seed powder stored beyond forty eight hour.
3. The longer products stayed in storage, the lower microbial load stability.
4. The transparent cellophane bag used is the most suitable packaging material for date seed powder, under all of the storage conditions used in this study.
5. Unhygienic production processes, storage conditions and packaging materials have been implicated as a source of food contamination.

RECOMMENDATIONS

The study recommended that:

1. Good manufacturing practices (GMP) be put in place during and after roasting date seed powder, to reduce contamination of microbes to the bare minimum.
2. Food handlers should ensure good hygiene practices in the production and packaging of date seed powder.
3. An airtight container and properly sealed cellophane bag under any of the storage conditions described in this study would make roasted date seed powder less susceptible to microbial infestation that could invariably lead to deterioration.

Further investigations should be carried out on the chemical composition, nutritional value, active compounds present in date seed, and additives that could enhance its shelf life.

Table 1: Total bacterial count ($\times 10^6$ cfu/g) in date seed powder as affected by storage time and method

Storage Time (days)	Dark Cupboard		Free Table	
	Plastic	Cellophane	Plastic	Cellophane
0	0	0	0	0
2	0	0	0	0
7	10.0 ± 5.7^{a}	4.0 ± 0.0^{a}	13.0 ± 2.8^{a}	5.0 ± 1.4^{a}
14	16.0 ± 2.8^{ab}	6.0 ± 1.4^{ab}	17.0 ± 2.8^{b}	5.0 ± 7.1^{a}
28	24.0 ± 1.4^{b}	6.0 ± 1.4^{a}	29.0 ± 4.2^{b}	4.5 ± 5.0^{a}
42	30.0 ± 1.4^{b}	5.0 ± 0.0^{a}	32.0 ± 0.0^{b}	4.5 ± 0.7^{a}
56	31.0 ± 2.8^{b}	3.0 ± 1.4^{a}	35.0 ± 2.8^{b}	2.0 ± 0.0^{a}

Table 2: Total mould count ($\times 10^6$ cfu/g) in date seed powder as affected by storage time and method

Storage Time (days)	Dark Cupboard		Free Table	
	Plastic	Cellophane	Plastic	Cellophane
0	0	0	0	0
2	0	0	0	0
7	0.3 ± 1.4^{ab}	2.0 ± 0.0^{a}	5.0 ± 1.4^{b}	2.0 ± 0.0^{a}
14	6.0 ± 2.8^{a}	2.0 ± 0.0^{a}	7.0 ± 1.4^{a}	2.0 ± 2.8^{a}
28	8.0 ± 0.0^{ab}	2.0 ± 2.8^{a}	11.0 ± 2.8^{b}	2.0 ± 2.8^{a}
42	8.0 ± 2.8^{ab}	2.5 ± 3.5^{a}	11.0 ± 1.4^{b}	2.0 ± 2.8^{a}
56	8.0 ± 2.8^{b}	1.0 ± 1.4^{a}	11.0 ± 1.4^{b}	1.0 ± 1.4^{a}

Table 3: Bacterial isolates and counts ($\times 10^6$ cfu/g) in stored date seed powder as affected by storage method and time

Storage method	**Isolate**	**Storage Time (days)**						
		0	**2**	**7**	**14**	**28**	**42**	**56**
PCDC	*Pseudomonas spp*	0	0	3	1	1	3	3
	Bacillus subtilis	0	0	4	7	7	16	16
	Staphylococcus aureus	0	0	3	8	8	11	13
CBDC	*Pseudomonas spp*	0	0	0	0	0	0	0
	Bacillus subtilis	0	0	3	3	3	2	2
	Staphylococcus aureus	0	0	1	3	3	3	1
PCFT	*Pseudomonas spp*	0	0	0	8	0	0	0
	Bacillus subtilis	0	0	8	9	10	13	16
	Staphylococcus aureus	0	0	5	0	19	19	19
CBFT	*Pseudomonas spp*	0	0	0	0	0	0	0
	Bacillus subtilis	0	0	0	0	0	0	0
	Staphylococcus aureus	0	0	5	0	4	4	2

PCDC: Plastic Container in Dark Cupboard, CBDC: Cellophane Bag in Dark Cupboard
PCFT: Plastic Container on Free Table, CBFT: Cellophane Bag on Free Table

Table 4: Fungal isolates and counts ($\times 10^6$ cfu/g) in stored date seed powder as affected by storage method and time

Storage method	Isolate	Storage Time (days)						
		0	**2**	**7**	**14**	**28**	**42**	**56**
PCDC	*Aspergillus flavus*	0	0	1	2	2	2	2
	Fusarium spp.	0	0	1	1	1	0	0
	Yeast	0	0	1	3	3	6	8
CBDC	*Aspergillus flavus*	0	0	1	1	1	1	0
	Fusarium spp.	0	0	0	0	0	0	0
	Yeast	0	0	1	1	1	1	1
PCFT	*Aspergillus flavus*	0	0	2	2	4	3	3
	Fusarium spp.	0	0	0	0	0	2	2
	Yeast	0	0	3	5	7	8	8
CBFT	*Aspergillus flavus*	0	0	1	1	0	0	0
	Fusarium spp.	0	0	0	0	0	0	0
	Yeast	0	0	1	1	2	0	0

PCDC: Plastic Container in Dark Cupboard
CBDC: Cellophane Bag in Dark Cupboard
PCFT: Plastic Container on Free Table
CBFT: Cellophane Bag on Free Table

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