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FERMENTATION OF C*ARICA PAPAYA* SEEDS AND ITS EFFECTS ON WEIGHT OF *RATTUS ALBUS*

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ABSTRACT

The fermentation of pawpaw seeds (C*arica papaya Linn.*) on weight of albino rats was investigated. Fresh pawpaw seeds were fermented for six days and the microbial populations for the fermentation were determined using standard microbiological techniques. Proximate analysis as well as temperature, pH and titratable acidity were carried out on the fermented seeds. Five rats were fed with dried and grinded pawpaw seeds after two weeks of acclimatization. The microbial population of bacteria and fungi was between 3.9 x 10^8cfu/ml to 4.6 x10^8cfu/ml and 2.0 x 10^3sfu/ml to 3.2 x 10^3sfu/ml respectively during fermentation. Four different bacteria and five fungi were identified, *Micrococcus roseus, Staphylococcus aureus, Bacillus subtilis,* and *Bacillus cereus. Aspergillus flavus, Aspergillus fumigatus, Neurospora crassa, Brachysporium nigrum, Articulospora inflaxa.* Moisture content of the fresh seeds was 67.37% while fermented was 11.34%. Ash, fat, fibre and crude protein contents for fermented pawpaw seeds were higher than the fresh seeds. The pH of fermented seeds of pawpaw during fermentation was 4.80 on the first day and 4.15 on the fourth day. On feeding the rats with dried grinded pawpaw seeds they all gained weight. Fermented dried pawpaw seeds can be used as food supplements for animal feeds.

INTRODUCTION

Pawpaw is the fruit of the plant *Carica papaya*, the sole species in the genus *Carica* of the plant family *Caricaceae.* It is native to the tropics of the Americas, and was first cultivated in Mexico several centuries before the emergence of the Mesoamerican classic cultures, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50-70 centimeters (20-28 in) diameter, deeply palmately lobed with 7 lobes. Pawpaw fruit is an excellent source of water, sugar, protein and ash, dietary fibre, folate, vitamin A, C and E (FAO, 19997). It is very rich in antioxidant nutrients, flavonoids and carotenes. It contain enzymes called papain and chymopapain which helps with the digestion, particularly it breaks down the protein from the food into amino acids. The papain enzymes are produced in the skinny peel of pawpaw. Eating pawpaw lower the inflammation in the body, alleviates the pain and edema caused by sport injuries, because of its high antioxidant content, pawpaw can prevent cholesterol oxidative and can be used in preventative treatments against atherosclerosis, strokes, heart attacks and diabetic heart disease (Emurewa, 1984).

Pawpaw seeds are a waste product of the fruit that is highly abundant in Nigeria. Seeds are often discarded after eaten the fruit due to its very limited uses at the moment. In Hawaii for example the seeds constitute 22% of the waste from papaya puree plants and oil extraction of the waste from papaya puree, plant and oil extraction has been examined as a possible method of utilization (FAO, 1992). Papaya seeds are recently gaining importance due to its medicinal value. The seed had recently been linked to curing sickle cell diseases (Imaga *et al.*, 2009), poisoning renal disorder (Olagunju *et al*., 2009) and as an anti-helminthes (Okeniyi *et al*., 2007). There is scarce information on this relatively underutilizes see despite its importance.

This study therefore is aimed at determining the microbial load, types of organisms responsible for the fermentation of pawpaw seeds, effect of fermentation on nutritional value of the seeds and on the weight of albino rats.

MATERIALS AND METHODS

Riped fresh pawpaw fruits were brought from City market of Masjed Soleiman and were brought to the laboratory for analyses. Albino rats were brought from Khuzestan animal House.

Sample Preparation

One hundred grams of each sample from wet and dried seeds was weighed into a transparent covered bowl which acts as the fermentor separately and fermented for five days. Each sample from the fermented seeds was taken for microbiological analyses.

Microbial isolation

One gram (1g) of each sample was macerated in 9ml of sterile physiological saline and diluted serially. Then 1ml and 0.1ml was pipette separately from each dilution factor unto sterile Petri-dishes. Thereafter, 20ml of nutrient agar and acidified potato dextrose agar was cooled to 45^0C and poured separately onto each of the plates in triplicate and the plates were gently swirled and allowed to solidify. The nutrient agar plates were incubated in an inverted position at $37^0 \pm 2^0$C for 24 hours (bacteria) while potato dextrose agar plates were incubated at $28^0 \pm 2^0$C for 72 hours (fungi). The viable colonies were sub cultured from mixed culture plate to obtain a pure culture. Bacteria cultures were characterized and identified using various morphological and biological tests such as Gram stain, spore stain, motility, catalase, coagulase, indole, urease, citrate, oxidase and sugar fermentation. Pure cultures of each isolate were obtained by streaking the specific colonies on suitable media and incubated appropriately; these were maintained in an agar slant in McCartney bottles. The identification of the microbial isolates was based on classification Scheme proposed by Harrigan and McCance (1976), Buchanan and Gibbson (1974), Holt *et al.*, (1994) and Collin and Lyne (1984). The identification was based essentially on morphological and biochemical reactions. The isolated fungi were then identified with reference to Barnet and Hunter (1972) and Frazier and Westholf (1998).

Proximate composition and pH were carried out according to the method of Association of Official Analytical Chemists (1990). These includes determination of, fat, fiber, protein and carbohydrate were determined by difference.

RESULTS AND DISCUSSION

Table 1 represents the microbial population of fermented pawpaw seeds with 4.6 x 10^8cfu/ml for bacteria and 3.2 x 10^3sfu/ml for fungi on day zero during fermentation. While on the fifth day 3.9 x 10^8cfu/ml for bacteria and 2.0 x 10^3cfu/ml for fungi as shown in Table 1. Four bacteria and five fungi were isolated and identified as shown in Table 2. These are: *Micrococcus roseus, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger Articulospora inflate* and *Neurospora crassa.*

Table 1: Microbial population from fermented pawpaw seeds during fermentation

Day	Bacteria (10^8) cfu/ml	Fungi (10^3) sfu/ml
0	4.6	3.2
1	4.4	3.0
2	4.1	2.9
3	3.9	2.9
4	4.0	2.4
5	3.9	2.0

Table 2: **Bacterial and fungal isolated from pawpaw seeds during fermentation**

Bacteria	Fungi
Micrococcus roseus	*Aspergillus flavus*
Staphylococcus aureus	*Aspergillus fumigatus*
Bacillus subtilis	*Aspergillus niger*
Bacillus cereus	*Articulospora inflate*
	Neurospora crassa

Table 3, shows the daily pH of fermented seeds of pawpaw during fermentation, the highest pH was on the zero day 4.80 and the lowest was observed on the fourth day 4.15. There was gradual decrease of the pH of the fermented seeds.

Table 3: Daily pH of fermented pawpaw seeds during fermentation

Days	pH
0	4.80
1	4.60
2	4.55
3	4.50
4	4.15
5	4.45

Daily temperature of the fermented seeds of pawpaw during fermentation is shown in Table 4. The temperature was 32.50^0C in the zero day and this was the highest temperature of the fermented seeds. The lowest was 29.50^0C on the first day. The same value was observed for second and third day (31.50^0C).

Table 4: Daily temperature of pawpaw seeds during fermentation

Days	Temperature ^{0}C
0	32.50
1	29.50
2	31.50
3	31.50
4	32.00
5	31.00

The proximate analysis of fresh and dried grinded pawpaw seeds is shown in Table 5, moisture content was 67.37% for the fresh seeds and 11.34% for dried seeds. There was increase in the ash, fat, protein and crude fibre content of the dried grinded pawpaw seeds from 1.65 to 10.03%, 10.41 to 24.34%, 6.86 to 22.61% and 11.97 to 28.09% respectively.

Table 5: Proximate analysis of fresh and fermented dried seeds (%)

Nutritional composition	wet seeds	fermented dried seeds
Moisture	$67.37^{a} \pm 0.07$	$11.34^{b} \pm 0.06$
Ash	$1.65^{b} \pm 0.31$	$10.03^{a} \pm 0.14$
Fat	$10.41^{b} \pm 0.09$	$24.34^{a} \pm 0.16$
Protein	$6.86^{b} \pm 0.06$	$22.61^{a} \pm 0.25$
Crude fibre	$11.97^{b} \pm 0.18$	$28.09^{a} \pm 0.31$

Means with different superscript in the same roll are significantly different

The initial weight of albino rats, their weight as the feeding proceeds and the number of days they were fed is shown in Table 6. There was increase in the weight of all the rats fed with the dried pawpaw seeds. Rat A, increased from 172.0g to 173.0g, D and E shows the major appreciable increase from 179.0g to 180.5g and 177.0g to 178.2g respectively.

Table 6: weight of rat before and after feeding with fermented dried pawpaw seeds (g)

Rat	0 Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	172.0	172.0	172.4	172.5	172.5	172.8	173.0
B	175.0	175.0	175.5	175.6	175.9	176.0	176.0
C	168.0	168.0	168.3	168.4	168.7	168.9	169.0
D	179.0	179.0	179.0	179.5	180.0	180.0	180.5
E	177.0	177.0	177.3	177.5	177.8	178.0	178.2

The microbial population as shown in Table 1 during fermentation was high; this might have been due to the normal flora of the organic wastes. The isolated in Table 2 organisms include; *Micrococcus roseus, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Aspergillus niger, Aspergillus fumigatus, Neurospora crassa, Brachysporium nigrum* and *Articulospora inflate.* These isolates confirmed previous report of Gupta and Pathak, 1986 that isolated *Aspergillus flavus, Rhizopus* spp, *Botryodiplodia* and *Curvularia* spp. Table 3, shows the daily pH of fermented seeds of pawpaw during fermentation, the highest pH was on the zero day 4.80 and the lowest was observed on the fourth day 4.15. There was gradual decrease of the pH of the fermented seeds this could be due the activities of microorganisms present during fermentation.

The moisture content of fresh pawpaw seeds was the highest value of 67.37% (Table 5), while dried grinded seeds was11.34%. Moisture content is a critical factor in fermentation because these variables have influences on growth, biosynthesis and secretion of different metabolites (Krishna and Chandrasekaran, 1996). Lower moisture content causes reduction of solubility of nutrients of the substrate, low degree of swelling and high water tension. If the moisture content is higher than normal it causes a reduction in yield due to static hindrance of the growth of the producer strain by reduction in porosity of the solid matrix (Lonsane *et al.,* 1985). There was increase in the ash, fat, protein and fibre content this might be due to microbial activities Table 5.

There was increase in the weight of rats fed with dried grinded pawpaw seeds Table 6. The increase in the weight of the rats might be due to the high fat content, crude fibre and protein content of the dried pawpaw seeds. The antifertility activity of papaya seed has been demonstrated in female rat mice and man (Das, 1980).

In conclusion, pawpaw seeds are invaluable part of pawpaw fruit that is prevalent throughout tropical Africa. The seeds can be fermented, dried and grinded for animal feed formulation.

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