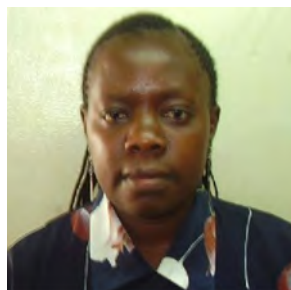


**REDUCTION OF NITRATES, OXALATES AND PHENOLS IN FERMENTED
SOLAR-DRIED STORED COWPEA (*Vigna unguiculata* L.) LEAF
VEGETABLES**

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ABSTRACT

This study was conducted to determine the effect of fermentation, solar drying and storage duration on the levels of anti-nutrients: nitrates, oxalates and phenols, in cowpea leaf vegetables. The rationale was reduction of the anti-nutrients. Reduction of nutritional stress factors in plant foods increases bioavailability of nutrients, hence improving their quality as foodstuffs. The cowpea leaves were purchased from the local markets, sorted to remove blemished leaves and foreign materials, washed in running tap water. Then, the vegetables were drained and divided into three batches of 16 kg each. One batch was heat-treated in hot water for 3 minutes and then cooled to ambient temperatures, drained and solar-dried. The second portion was acidified to a pH of 3.8, heat-treated, and solar-dried. The third portion was fermented for 21 days, heat-treated, and solar-dried. The three batches of vegetables were spread at different times on drying trays at the rate of 4 kg/m² and dried in a solar drier to an approximate moisture content of 10%. The dried vegetables were packaged in either polyethylene bags or Kraft paper bags and stored for three months at 18°C, 22°- 26°C or 32°C. Fermentation, heat-treatment and drying of vegetables led to significant ($P < 0.05$) reduction in nitrates compared to fresh cowpea leaves, but the reduction in oxalates and phenols was not significant. Storage for three months led to significant ($P < 0.05$) reduction in nitrates in the fermented sample compared to the other samples. The acidified sample had significantly ($P < 0.05$) higher levels of phenols after three months of storage than the other samples. Samples stored at 18°C had higher levels of oxalates and phenols but lower levels of nitrates, compared to those stored at higher temperatures. Packaging material had no significant effect on the level of nitrates, oxalates and phenols. Data obtained in this study reveal a novel technique for the reduction of anti-nutrients in cowpea leaf vegetables, namely; fermentation followed by solar drying. The increased acceptability of these fermented-dried vegetables would help rural communities in providing better foodstuff with fewer anti-nutrients, thus alleviating micronutrient malnutrition. This novel long-term storage technology can greatly help to deal with the issue of seasonality and will increase food security, especially during the dry season.

Key words: Fermentation, solar drying, vegetables, anti-nutrients

INTRODUCTION

Malnutrition due to nutritionally inadequate diets is a major concern in Kenya and many other developing countries [1]. The prevalence rates of micronutrient malnutrition remain high, with devastating consequences for health and productivity [2]. In Africa, people have always depended on traditional leafy vegetables to meet their nutritional needs. The vegetables represent cheap but quality nutrition for large segments of the populations in both urban and rural areas. The vegetables are rich in vitamins, especially A, B, and C, and minerals such as iron, zinc, calcium and phosphorus [3].

Unfortunately, most plant species contain nutritional stress factors (anti-nutrients) that increase the loss of essential nutrients from the body, interfering with the metabolism of absorbed essential nutrients, decreasing the digestion of food, or decreasing food intake. Reduction of nutritional stress factors in plant foods increases the bioavailability of nutrients in the plant and thus improves its quality as a foodstuff. The most commonly occurring antinutrients in plant foods include nitrates and nitrites, phenols, cyanogenic glycosides, glucosinolates, oxalates and saponins [4]. Toxicity to humans is due to nitrites that arise from microbial reduction of nitrates in the gastro-intestinal tract. This can cause methaemoglobinaemia or act as precursor in the endogenous formation of carcinogenic nitrosamines. This reduction is more likely in infants than in adults, due to low acidity in their digestive tract, which allows coliforms and clostridial bacteria to survive [5]. The leafy vegetables are the major vehicle for the entry of nitrates into the human system [6]. High concentrations of oxalate may be of great nutritional disadvantage to both humans and animals. Oxalic acid is a plant toxicant, which forms an insoluble salt with the essential nutrient calcium, thus inhibiting its absorption [7]. It also inhibits the absorption of iron and, to some extent, zinc [8, 9]. This manifests as calcium deficiency, even in diets with adequate levels of calcium. This is more significant in growing children, with developing bones and teeth than in adults [10]. In addition to potential toxicological concerns, phenolic compounds have been implicated in influencing the functional, nutritional and sensory properties of foods with which they are associated [11]. High levels of phenolic compounds are undesirable for women trying to become pregnant, since these compounds also decrease fertility, possibly by modulating hormone levels and even by interfering with the critical early stages of pregnancy [12].

The cowpea (*Vigna unguiculata* syn *Vigna sinensis*) is one of the most important legumes in Kenya. It is cultivated all over Kenya mainly for seeds, but the leaves are a popular local vegetable. The main problem with traditional vegetables is their lack of availability due to seasonality. However, in areas where seasonality is a critical factor that limits availability, promoting home gardening and appropriate local preservation technology can improve availability [13].

Fermentation of indigenous foods is considered an effective, inexpensive and nutritionally beneficial household technology, especially in the developing world. Likewise, sun drying has been a means of preserving food from earliest times [14].

The main problem with the conventional solar drying is huge nutritional losses. This study aimed at reducing these nutritional losses and reducing the stress factors by incorporating fermentation into solar drying. The study also considered the problem of food security, which is devastating during the dry season. The levels of nitrates, oxalates and phenols in fermented solar-dried cowpea leaf vegetables were assessed.

MATERIALS AND METHODS

Cowpea leaves

The fresh cowpea leaves were purchased from local markets in the morning and transported quickly to the University of Nairobi's Department of Food Technology and Nutrition. For the fermentation trials, the stalks, withered and dried leaves, weeds, stones and other foreign materials were sorted out from the rest of the vegetables. The vegetables were then thoroughly washed and well drained. They were cut manually with a kitchen knife into slices approximately 5mm thick.

Determination of optimal levels of salt and sugar for fermentation

To determine the optimal level for salt, the sorted cowpea leaves were divided into seven portions and fermented in lots of 500g each. Each lot was mixed thoroughly with 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 or 5.0% concentration, respectively, of table salt, followed by tight packing in 4-litre plastic beakers. Fermentation was carried out at ambient temperatures (22° – 26°C). To determine the optimal level for sugar, each sample was mixed with 3% salt (determined as the optimal level of salt for fermentation) and varying percentages of glucose and sugar, that is, 2.5%, 3.0% or 3.5%. The fermentation was carried out for 16 days with three replicates. Sensory analyses were performed on the fermented vegetables to determine the effect of added sugar on their acceptability.

Product manufacture

The fermented-dried vegetables were prepared in comparative trials with control and acidified samples as follows: Procurement and preparation of the raw materials were similar to those carried out during the determination of optimal levels of salt and sugar for fermentation. The amount of the cowpea leaves used was larger. The vegetables were sliced and then divided into three equal portions each of 16 kg. One portion was thoroughly mixed with 3% salt and allowed to stand for two hours, then heat-treated. This was treated as control sample. The second portion was thoroughly mixed with 3% salt and citric acid (EFF Chemicals Ltd, Kenya) to a final pH of 3.8 and allowed to stand overnight, then heat-treated. This was treated as an acidified sample. This was done to see whether acid alone could lead to the same results or different from fermentation. The third portion was thoroughly mixed with 3% salt and 3% sucrose, which were then tightly packed in a 60-litre plastic bucket. The salted and sugared vegetable sample was allowed to stand for 10 minutes before a polyethylene bag full of water was placed inside the bucket as a weight to ensure that the vegetables were immersed in the brine and fermented for 21 days. After fermentation, the sample was heat treated [15].

Dehydration and Storage

The fermented, acidified and control vegetable samples were heat treated by boiling in their own liquor at 90° – 95°C for 3 minutes. Each vegetable sample was cooled and drained immediately after heat treating and loaded onto a solar drier with shade provision [16]. The vegetables were spread evenly on trays (4kg/m²) and the trays inserted into the drier. They were then dried until the weight was constant, which took on average five days. The fermented dried vegetables were packaged in either Kraft or polyethylene paper. Each package contained 50g of the fermented dried vegetables. The packaged products were stored at: 32°C ambient temperatures (22° – 26°C) and 18°C in enclosed dry places for three (3) months. From each batch, one polyethylene and one Kraft paper bag were opened each month and the vegetables analyzed for ascorbic acid and beta-carotene. Two bags were used every month for sensory evaluation. The fermented dried vegetables were prepared in comparative trials with control and acidified samples as shown in Figure 1. All experiments were repeated twice.

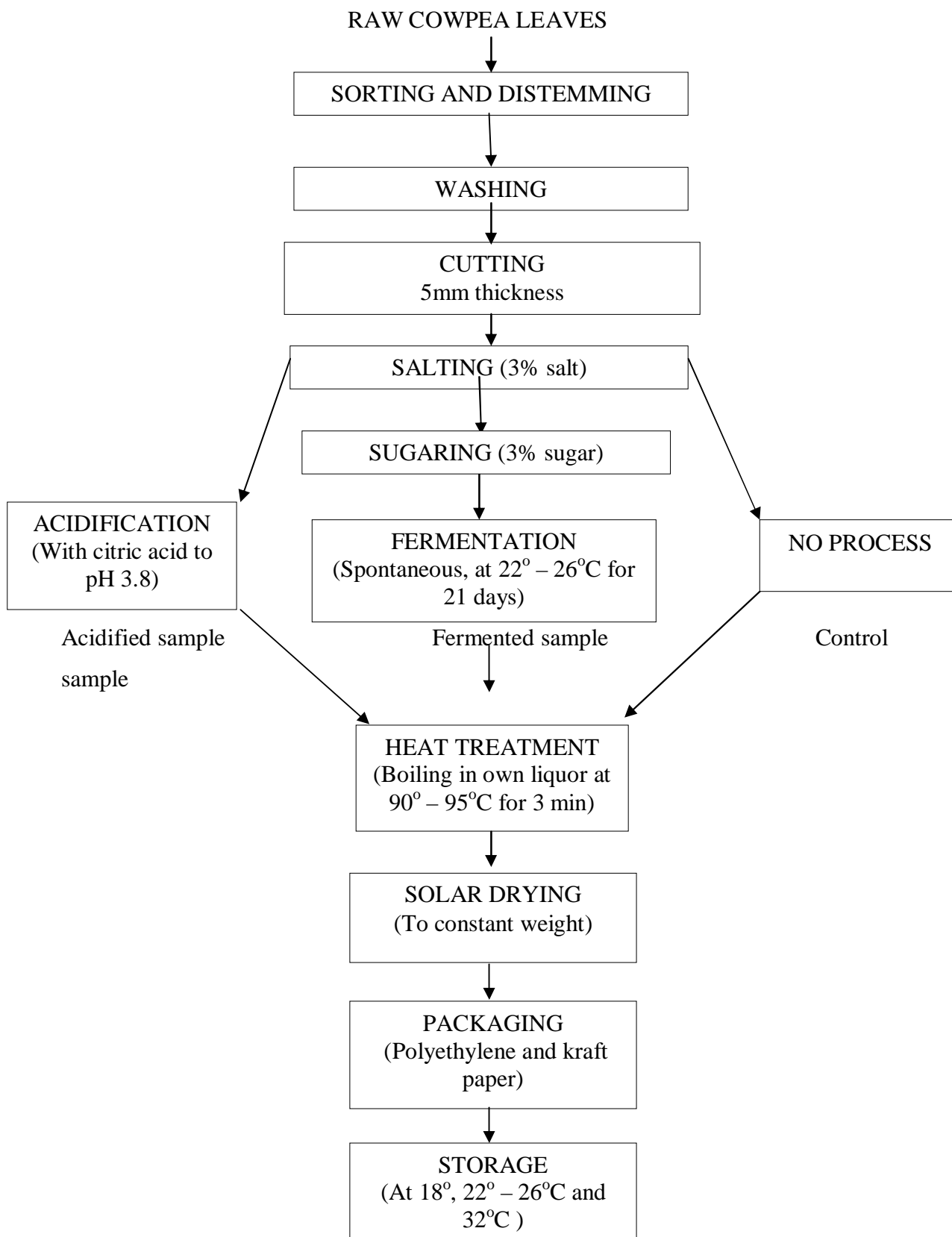


Figure 1: Product manufacture flow diagram

Anti-nutrient Analyses

Nitrates were determined using the following method: A standard curve was prepared using different concentrations of potassium nitrate, and nitrates were calculated as equivalent milligrams/100 g fresh weight. The sample was ground and re-dried overnight in a hot air oven at 70°C. A sample of 0.1 g was then suspended in 10 ml distilled water in 100 ml beaker and incubated at 45°C for 1 hr, to extract the nitrates, and then filtered through Whatman filter paper No. 1. An aliquot of 0.2 ml of the filtrate was pipetted into a 50 ml beaker and 0.8 ml of 5% (w/v) salicylic acid in sulphuric acid was added and mixed thoroughly. The mixture was allowed to stand for 20 min at ambient temperatures. Sodium hydroxide (19 ml) of 2 N concentration was added and the mixture allowed to cool for 30 min. The absorbance was measured at 410 nm against a common blank. The nitrate content was determined from a standard curve and the nitrates content calculated as mg/100 g [17].

Oxalates were determined as follows: Standard sodium oxalate solution was prepared by dissolving 3 mg of sodium oxalate in 10 ml of 0.5 M sulphuric acid. This was followed by titration with 0.1 M potassium permanganate at 60°C, using a microburette to a faint violet colour that was stable for at least 15 seconds and a standard curve was plotted. A dried sample of 0.1 g was extracted with 30 ml of 1 M hydrochloric acid in a boiling waterbath for 30 min. The sample was cooled, then shaken and filtered through No. 1 Whatman filter paper. The filtrate was adjusted to a pH greater than 8 with 8 M ammonium hydroxide followed by re-adjusting it to pH 5.0 – 5.2 with 6 N acetic acid. An aliquot of 10 ml was precipitated with 0.4 ml of 5% calcium chloride, shaken thoroughly, allowed to settle at ambient temperatures for at least 16 hrs, and centrifuged at 3000 rpm for 15 min. The supernatant was discarded, rinsed twice with 2 ml of 0.35 M ammonium hydroxide and then the cake (pellet) drip-dried. The pellet was dissolved in 10 ml of 0.5 M sulphuric acid followed by titration with 0.1 M potassium permanganate at 60°C using a microburette to a faint violet colour that was stable for at least 15 seconds. Oxalates content in the sample was determined from the standard curve prepared earlier as mg/100 g [18].

Total phenols were determined as tannins by Folin-Denis method [19]. The Folin-Denis reagent was prepared by mixing 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml phosphoric acid with 750 ml water. The mixture was then refluxed for 2 hrs, cooled and diluted to 1 litre. Saturated sodium carbonate solution was prepared by dissolving 35 g anhydrous sodium carbonate in 100 ml water at 70° – 80°C, and allowed to cool overnight. The supersaturated solution was seeded with crystals of hydrated sodium carbonate and filtered through glass wool after crystallization. Tannic acid solution was prepared by dissolving 100 g tannic acid in 1 litre distilled water. Fresh solution was prepared for each determination. A standard curve was prepared by pipetting 1 – 10 ml aliquots of the standard tannic acid solution into 100 ml flasks containing 75 ml of distilled water. Five ml Folin-Denis reagent, together with 10 ml sodium carbonate solution were added. The solution was diluted to volume with distilled water and mixed thoroughly. Optical

densities were determined at 760 nm after 30 min and absorbance plotted against mg tannic acid/100 ml, to obtain a standard curve.

A ground sample of 0.5 g was extracted in a mortar and pestle with 50 ml distilled water, and filtered. One millilitre of the filtrate was pipetted into a 100 ml flask containing 75 ml distilled water. Five milliliters of Folin-Denis reagent and 10 ml sodium carbonate solution were then added. The solution was made to volume, mixed thoroughly and then absorbance determined at 760 nm after 30 min incubation. Milligrams of tannic acid per 100 g of sample were calculated from the standard curve.

Data analysis

All experiments were designed as complete factorial with three main factors: storage temperature, processing treatment and type of packaging. Storage temperature had three levels: 18°C, 22° – 26°C and 32°C, which were fixed-effect treatments representing various agro-climatic zones in Africa. Processing treatments had three levels: fermentation, acidification (citric acid – positive control) and untreated control; each followed by blanching and solar drying. The type of packaging had two levels: polyethylene and Kraft paper, representing airtight and aerated packaging, respectively. The experiments were laid on a completely randomized design with three replicates. All experiments were repeated twice.

All data were then subjected to analysis of variance (ANOVA) and means were separated by Duncan Multiple Range Test using Genstat 6th Edition and Costat Statistical Software Programmes.

RESULTS

Levels of nitrates, oxalates and phenols in raw, fermented-, acidified- and control-dried cowpea leaves are given in Table 1. The levels of nitrates in raw cowpea leaves were significantly higher ($P < 0.05$) than those in the fermented-, acidified- and control-dried samples. There was no significant difference among the raw cowpea leaves and the fermented-, acidified- and control-dried samples ($P < 0.05$) in the levels of oxalates and phenols.

There were apparent losses in nitrates, oxalates and phenols during storage for three months compared with those before storage. The effect of fermentation and acidification on the retention of nitrates, oxalates and phenols during the three months of storage is given in Table 2. After three months of storage, the fermented-dried sample had the lowest levels of nitrates, oxalates and phenols, as compared with the other samples. This indicates that fermentation has a reducing effect on the levels of nitrates, oxalates and the phenols during storage. After drying, the three processed samples' levels of nitrates were not significantly different in nitrates (see Table 1), but after storage, the fermented dried sample had a significantly ($P < 0.05$) lower nitrate level compared to the acidified and control dried samples (Table 2). The acidified dried sample had a significantly higher level of phenols compared to the fermented and control dried samples after storage, whereas before storage there was no

significant difference. This suggests acidification has a significant retention effect on phenols during storage.

The effect of storage temperature on the retention of nitrates, oxalates and phenols is shown in Table 3. Samples stored at 18°C had a significantly higher level of oxalates than those stored at either 22° - 26°C or 32°C. Level of phenols was significantly lower for samples stored at 32°C compared to those stored at 18°C and 22° - 26°C. This indicates that the higher the storage temperature the lower the retention rate of oxalates and phenols.

There were apparent losses in the levels of nitrates, oxalates and phenols in samples packaged in Kraft paper as compared to those in polyethylene, but the differences were not significant.

DISCUSSION

The lower levels of nitrates in fermented-, acidified- and control-dried cowpea leaves than in the raw leaves indicate that much of the nitrate leached into the blanching water. Leaching of nitrates has been reported [20, 21, 22]. Reduction in nitrate concentration represents added value for vegetable products rich in carotenoids, vitamin C and E, selenium, dietary fiber, plant sterols and so on [23]. Blanching, fermentation, acidification and dehydration resulted in minimal reduction of oxalates and phenols in the three samples. It has been reported that oxalates and phenols could change in form during food processing. However, the methods used for their determination in this study could not differentiate these forms; hence their levels did not change significantly with the treatments. Another researcher, when working with fermented *Uji* (a traditional porridge consumed in Kenya, made out of maize, millet and sorghum) reported that drum-drying directly, or in combination with fermentation with or without boiling, did not affect the content of phenols [24]. It has also been reported that fermentation, dehydration or storage of noni (*Morinda citrifolia* L.) fresh juice resulted in minimal reduction of total phenols [25]. However, domestic processing such as cooking in boiling water, seems to have a dramatic effect on phenolic content on edible vegetables [26]. High levels of oxalate can be reduced or eliminated by cooking, especially boiling [27, 28]. Unfortunately, in this study the contents of oxalates and phenols of the cooked vegetables were not determined.

Generally, oxalates and phenols are easily vaporized organic compounds. Possibly low storage temperature (18°C) hindered the vaporization of both oxalates and phenols compared to the higher temperatures of 22° - 26° C and 32°C [29]. The apparent lower levels in samples stored in Kraft paper could be due to vaporization also, as opposed to those in polyethylene, which is impermeable.

It is, therefore, concluded that blanching, fermentation, solar-dehydration and storage of cowpea leaf vegetables results in a more valuable food product due to the reduction of anti-nutrients. This reduction effect is significant in the long run, since such vegetables form the bulk of foods consumed by rural communities. The increased

acceptability of the fermented-dried vegetables, as demonstrated in this study would assist rural communities in providing a better foodstuff with lower levels of anti-nutrients, thus alleviating micronutrient malnutrition. This novel technology; fermentation followed by solar-drying, would ensure long-term storage and thus help deal with issues of seasonality and increase food security, especially during the dry season. It is, therefore, recommended:

1. Transferring this technology, which is cheap and effective, to local communities and women groups to preserve and improve seasonal vegetables like cowpeas.
2. Promoting increased acceptability and consumption of fermented and dehydrated vegetables among rural communities.

Table 1: Levels of nitrates, oxalates and phenols in raw, fermented-, acidified- and control-dried cowpea leaves expressed in mg/100 g edible portion on dry matter basis^a

Sample	Nitrates	Oxalates	Phenols
Raw	771 ± 36 ^a	1889 ± 98 ^a	2783 ± 88 ^a
Fermented- dried	217 ± 27 ^b	1679 ± 84 ^a	1992 ± 115 ^a
Acidified -dried	166 ± 13 ^b	1859 ± 67 ^a	2119 ± 89 ^a
Control - dried	352 ± 34 ^b	1830 ± 103 ^a	1959 ± 96 ^a
L. s. d.	376.1	536.2	871.1

^a Mean ± Standard Deviation (n = 4)
Means within columns superscripted by different letters are significantly different at (P < 0.05)

Table 2: Effect of fermentation and acidification on the nitrates, oxalates and phenols during storage for three months (mg/100 g solids)

Samples	Nitrates	Oxalates	Phenols
Fermented-dried	96.2 ^b	729.5 ^a	1438 ^b
Acidified-dried	205.3 ^a	847.0 ^a	1712 ^a
Control- dried	227.3 ^a	819.7 ^a	1485 ^b
L. s. d.	51.2	276.5	167.6

Means within columns superscripted by different letters are significantly different at (P < 0.05)

Table 3: Effect of storage temperature on the nitrates, oxalates and phenols during storage for 3 months (mg/100 g solids)

Storage Temperature	Nitrates	Oxalates	Phenols
18°C	161.4 ^a	1035 ^a	1616 ^a
22° – 26°C	174.2 ^a	702 ^b	1596 ^a
32°C	193.2 ^a	659 ^b	1424 ^b
L. s. d.	51.5	276.5	167.6

Means within columns superscripted by different letters are significantly different at (P < 0.05)

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