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CHANGES IN ANTINUTRIENT AND NUTRITIONAL VALUES OF FERMENTED SESAME (*Sesanum indicum*), MUSK MELON (*Cucumis melo*) AND WHITE MELON (*Cucumeropsis mannii*).

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ABSTRACT

The seeds of S. *indicum*, *C.melo* and *C. mannii* were subjected to traditional fermentation (a common way of producing local soup condiments in Nigeria) with the aim of improving the nutritient and reducing the antinutrient properties. The result of the study showed that the raw seeds have high nutrient level: *S. indicum*; protein, (24.5%), fat (44%), carbohydrate (10.8%), crude fibre (11.6%) and ash (5.9%). *C.melo*; protein, (27.8%), fat (45.2%), carbohydrate (20.6%), crude fibre (2.3%) and ash (1.8%). *C. mannii*; protein, (34%), fat (46%), carbohydrate (10.3%), crude fibre (2.4%) and ash (3.8%), while the vitamin C contents of the three raw seeds were 2.01, 0.76 and 1.0mg/g respectively, but with some antinutrients factors; phytate 38.4, 18.6 and 19.2 mg/g respectively; oxalate, 2.57, 2.1 and 1.35mg/g respectively; Saponin, 2.68, 5.1 and 3.48mg/g respectively; and tannin, 0.03, 0.022 and 0.0202mg/g respectively. As a result of fermentation there was an improvement in the nutritional status of the seeds of *S. indicum C.melo* and *C. mannii* (protein: 25.8, 30.4 and 38%; fat: 57.2, 58.
4, and 55.4%; carbohydrate: 3.4, 5.0, 2.5%; crude fibre: 7.3, 2.1 and 2.0%; ash: 4.1, 1.6 and 3.7% respectively), The antinutrient factors (phytate, saponin and oxalate) were remarkably reduced after day 7 of fermentation while only *S. indicum* showed a rapid decrease in the level of tannins in the extracts, only a slight decrease was observed for *C.melo* and *C. mannii*, as the fermentation progresses.
The total phenolic levels of the raw seeds of *S. indicum* (0.026) showed a remarkable decrease as the fermentation process continues to day 7 with a level of 0.010mg/g. *C.melo* and *C. mannii* (0.012 and 0.016mg/g respectively) had an increase in the phenolic levels to 0.023 and 0.020mg/g respectively at the day 7 of the fermentation. The raw seeds of S. *indicum* showed the highest flavonoid levels (0.095mg/g) than *C. melo* and *C. mannii* (0.05 and 0.04mg/g respectively), when fermented the level of flavonoid was remarkably reduced for S. *indicum* (0.04mg/g), while an increase was observed for *C. melo* and *C. mannii* at day 7 of fermentation with values 0.07 and 0.081mg/g respectively. A decrease in the vitamin C contents of their raw seeds were generally observed within the period of fermentation.

INTRODUCTION

The studies on most of the plants revealed that some of the underutilized oil crops are rich in health benefiting substances and could serve as functional foods [15]. Epidemiological research has shown a positive association between certain diseases and dietary intake of food rich in such essential components [33,29]. The investigation into the effect of processing on antioxidants, proteins, minerals, and vitamins constituent of these oil seeds are inexhaustible.

Every attempt is to ensure that the processing effect that may not destroy the functionality of ingredients in such food item is adopted. Functional ingredients are commonly defined as safe dietary substances that beneficially affect specific targets in the body beyond providing adequate nutrition [33,2]. Some authors in their articles refer to certain melon varieties with high lycopene content as so-called functional food. Food and nutrition scientists regard

phytonutrients as the third functional component of food, after nutrients and taste components [20,10]. Although the primary function of food is to provide nutrients, its secondary function concerns sensory attributes such as taste and flavor.

The tertiary function, said to be independent of the previous two, is to prevent disease at the molecular level [33,11,36]. However, it now appears that the secondary and tertiary functions are linked, if not at odds with each other. For the most part, plant-based phytochemicals are bitter and therefore aversive to the consumer. The discovery of a family of about 50 different bitter-taste receptors only confirms how important sensitivity to bitter taste was to evolution and survival. Research on plant-based functional foods presents several challenging opportunities for cancer biology and nutrition science [23]. The processing of plant originated foods improves their taste and flavour thereby increasing their acceptability [16], but little is known about the effect this could have on the chemistry of these foods. Most foods when subjected to heating processes lose their quality and the usefulness of such item to posses important component necessary to combat and prevent disease conditions are reduced. This study is aimed at examining the effects of local preparation and processing methods on the antinutrient and nutritional value of these seeds.

MATERIAL AND METHODS

Materials

The seeds of *Cucumeropsis mannii*. *Cucumis melo* and *Sesanum indicum* were obtained from Oja-oba in Akure, Ondo State, Nigeria in the month of October and identified at the Department of Crop Science and Pest Production, Federal University of Technology, Akure The chemicals used were of analytical grade, and glass distilled water was used.

Sample Preparation

The seeds of *C. mannii. C. melo* and *S. indicum* were sorted to remove grit, dirt and decomposing seeds, the whole seeds were divided into two portions; one part for fermentation and the other part were used as unfermented (raw) sample. The fermented and the unfermented portions were milled respectively using magic blender (SHB-515 model made by Sorex company limited, Seoul, Japan) to obtain very fine particles prior to analysis.

Production of fermented samples

A modified method of Omafuvbe et al., [32] was used. The seeds were rinsed with water and boiled for 6 hours to softness. The seeds thereafter were transferred into a pot, wrapped with banana leaves and allowed to ferment. The fermentation products were taken at the interval of 24 hours, 96 hours and 168 hours for analysis.

Sample Analysis

Determination of total phenol contents

The total phenol contents of the extracts were determined by mixing (0 - 1.0ml) of each extracts with equal volume of water; 2.5 ml Folin-Ciocalteau's reagent and 2ml of 7.5% sodium carbonate were subsequently added, the absorbance was measured at 765nm after incubating at 45^0C for 40 min. The amount of phenols in the extracts was expressed as tannic acid equivalent (TAE).

Determination of total flavonoid content

The content of flavonoids was determined using quercetin as a standard. 0-500µl of stock solution of the extracts was mixed with 50µl of aluminium trichloride and potassium acetate. The absorbance of the resultant solution read at 415nm after 30 min at room temperature. Standard quercetin solutions was prepared from 0.01g quercetin dissolved in 20ml of ethanol. All determinations were carried out in triplicate. The amount of flavonoids in the extracts was expressed as quercetin equivalent (QE).

Determination of Vitamin C

The vitamin C content of the samples was determined using Ascorbic acid as a standard compound prepared by dissolving 2mg/ml of ascorbic acid in water. 300µl of the sample preparation was mixed with 100µl of 13% TCA and 75µl of DNPH (dinitrophenylhydrazine), the resultant solution were incubated in a water bath

at 37^{o}C for 3 hours, then 500µl of 65% H_2SO_4 was added. The absorption was read at 520nm .

Determination of antinutrients

Phytate, Oxalate, Saponin and Tannin content were determined using the method of Association of Official Analytical Chemists [6].

Proximate analysis

Crude fat was extracted by the soxhlet method with petroleum ether (40-60 ^{o}C) for 8 h. Crude protein content was determined by the microkjeldahl method. This, as well as carbohydrate, crude fiber, protein, ash and moisture contents were estimated as described by the Association of Official Analytical [7].

RESULTS

Figure1. Shows the protein content of raw and fermented seeds of *S. indicum*, *C.melo* and *C. mannii*. The result indicated an increase in the protein contents of the three samples from 24.5, 27.8 and 34% to 25.8, 30.4 and 38.0% respectively at the end of day 7 fermentation period. From the result raw (unfermented) *C. mannii* has the highest level of protein with the least being *S. indicum.* Fermentation caused a remarkable increase in the protein levels.

Figure 2. Shows the crude fibre content of raw and fermented seeds of *S. indicum*, *C.melo* and *C. mannii.* From the result, a decrease in the crude fibre was obtained. The figure indicated a significant decrease from 11.6, 2.34 and 2.37% to 7.30, 2.10 and 2.00% respectively.

There was generally, an increase in the lipid content of the seeds after fermentation at day 7, from 44.4, 45.2 and 55.4% of the raw seeds to 57.2, 58.4 and 55.4 % of the fermented seeds respectively as shown in figure3.

The ash content of the raw and fermented seeds of *S. indicum*, *C.melo* and *C. mannii* are shown in figure 4. The result indicated that the raw seeds of these plant species have 5.90, 1.76 and 3.8% of ash, after the fermentation period of day 7, the levels of ash became 4.1, 1.6 and 3.7% respectively.

The carbohydrate content of raw *S. indicum*, *C. melo* and *C. mannii* were 10.8, 20.6, and 10.3% respectively after the inception of fermentation period, there was a total reduction to 3.4, 5.0 and 2.5% respectively as shown in figure 5

The antinutrient factors (phytate, oxalate, saponin and tannin) of the raw seeds of *S. indicum*, *C.melo* and *C. mannii* showed a decrease after fermentation; at the levels of phytate:- 38.4 to 17.3mg/g; 18.6 to 6.7 mg/g and 19.2 to 9.8mg/g respectively as shown in figure 6: Oxalate:- 2.57 to 0.36 mg/g; 2.1 to 0.27 mg/g and 1.35 to 0.14 mg/g respectively as indicated in figure 7; Saponin:- 2.68 to 1.01mg/g, 5.1 to 2.8mg/g and 3.5 to 1.9mg/g respectively as shown in figure 8; and Tannin: 0.019 to 0.008mg/g; 0.007 to 0.004mg/g and 0.008 to 0.005mg/g , respectively as shown in figure 9.

The total phenolic content of S. *indicum*, *C.melo* and *C. mannii* are shown in figure10. The total phenolic levels of the raw extract of *S. indicum* (0.026) showed a remarkable decrease as the fermentation process continues to day 7 with a value of 0.010mg/g. the *C.melo* and *C. mannii* (0.012 and 0.016mg/g respectively) had an increase in the phenolic levels to 0.023 and 0.020mg/g respectively at the day 7 of the fermentation.

The flavonoids content of the raw and fermented seeds of *S. indicum*, *C.melo* and *C. mannii* are shown in figure 11. S. *indicum* has the higher flavonoid levels (0.095) when compared to the raw seed of *C.melo* and *C. mannii* (0.05 and 0.04mg/g respectively), after the fermentation period, the level of flavonoid was remarkably reduced for S. *indicum* (0.04mg/g), while an increase was observed for *C. melo* and *C. mannii* with values 0.07 and 0.081mg/g respectively.

The vitamin C content of *S. indicum*, *C.melo* and *C. mannii* are shown in figure 12. The vitamin C contents of the seeds were reduced for *S. indicum*, *C.melo* and *C. mannii* from 2.01 to 0.93mg/g, 0.76 to 0.4mg/g and 1 to 0.67mg/g respectively as the fermentation progresses to day 7. The raw and fermented *S. indicum* has the highest of vitamin C. although; there was a general reduction in the level of vitamin C of the three fermented seeds.

DISCUSSION

The phytate content of raw *S. indicum, C.melo* and *C. mannii* were found to be 38.4, 18.6 and 19.2mg as presented in figure 6. The progressive increase in fermentation time significantly reduced phytate thereby improving the availability of mineral such as calcium and phosphorous. Phytate was reduced to a significant value of 6.3, 2.7 and 3.8mg/g respectively when compared to the value of the raw seeds. This is in line with what happened in the production of fermented products such as Tempeh in which phytate is being hydrolysed by the action of phytase produced by micro-organism during fermentation. High phytate content in the diet were undesirable; phytate binds essential and nutritionally important divalent cations, such as, iron, zinc, magnesium and calcium and forms insoluble complexes, making the minerals unavailable for absorption [13]. They also form complexes with proteins and starch, inhibiting enzymic digestion of the same [26].

The oxalate content of raw *S. indicum, C. melo* and *C. mannii* were found to be 2.6, 2.1 and 1.4mg/g respectively as shown in figure 7. After the fermentation period the levels of oxalate reduced to 0.6, 0.3 and 0.14mg/g respectively. Oxalate in the seeds of *S. indicum, C.melo* and *C. mannii* may limit the Ca availability, which binds with calcium to form calcium oxalate. A negative correlation between digestibility and lignin content in tropical browse has been observed [8]. They are therefore considered poisonous in large amount but harmless when present in small amounts, therefore the levels of oxalate in the fermented seeds is thus not harmful.

The seeds of *S. indicum, C.melo* and *C. mannii* were found to contain saponin as presented in figure 8, the raw seeds has the values of 8.57, 6.8 and 3.5mg/g respectively. The levels of tannin was low in the raw seeds of *S. indicum, C.melo* and *C. mannii* (0.019, 0.007 and 0.008mg/g respectively), after fermentation there was a slight decrease in the tannic levels of *the seeds. T*he tannin contents in the raw seeds could be considered low. According to Enujiugha and Agbede [17], tannin usually forms insoluble complexes with proteins, thereby interfering with their bioavailability and poor palatability is generally attributed to high tannin diets.

There was a significant decrease in the levels of saponin as the fermentation progresses from 8.5, 6.8 and 3.5mg/g in raw seeds to 2.4, 3.4 and 2.1mg/g respectively in day 7 of fermentation as presented in figure 8. Saponins have both beneficial and adverse effects on human health. Contrary to their hypocholesterolemic property [25], saponins also show hemolytic activity by reacting with the sterols of erythrocyte membrane [9]. The observed progressive reductions of antinutrient factors as the fermentation progresses is in line with the assertion that the antinutrients levels (phytate, oxalte, saponin and tannin) were generally reduced due to fermentation and the microflora activity [1].

The proximate analysis of the nutrients in *S. indicum, C.melo* and *C. mannii* seeds showed that their protein contents were significantly improved from 24.5 to 25.8, 27.8 to 30.4 and 34.0 to 38.2% respectively, with days of fermentation (figure1) with *C. mannii* having the highest and *S. indicum* having the least protein level. However, these oil seeds can be said to be considerably high in protein and can compete favourably with some other good sources of protein like *Colocynthis citrullus* and *Cucumeropsis edulis* which are other varieties used as soup condiments, that were reported to contain 28.4% and 31.85% protein respectively [4,5]. The increase in the protein level of the fermented *S. indicum, C.melo* and *C. mannii* agrees with the report of David and Aderibigbe, [12] where an increase in protein level of some fermented melon species were observed. This result was similar with the earlier report of Selma *et al*., [35]; where fermentation improved the protein level of the fermented pearl millet. The improvement in the level of protein due to fermentation was significant at ($p < 0.5$) and could be attributed to increased microbial

nitrogen during fermentation as a result of increased production of single cell proteins [27,28] and the secretion of some extracellular enzymes into the fermenting seeds by the microorganism during fermentation [3]. This high percentage of protein in these seeds are indicative of the fact that it could contribute to the daily protein need of 23.6 g of protein for adults as recommended by National Research Council, [24]. It should be noted that proteins functionally promote growth, tissue repair and maintenance. Dietary proteins are needed for the synthesis of new cells, enzymes, hormones, antibodies and other substances required for the healthy functioning and development of the body as well as its protection.

Figure 2. Shows the crude fibre content of the raw and fermented seeds of *S. indicum, C.melo* and *C. mannii.* The result indicated a gradual reduction in the level of crude fibre content of these seeds from 11.6 to 7.3, 2.34 to 2.1 and 2.4 to 2.0% as fermentation progresses to day 7, with *S. indicum* having the highest level of crude fibre. There were remarkable differences in the dietary fibre contents of the raw and fermented seeds. This result is similar with the report of David and Aderibigbe, [12] where fermentation caused a significant reduction in the level of crude fibre content of the some melon species. Dietary fibres are constituents of plant foods that remain undigested by human intestinal enzymes [21], in effect they are essentially made up of cellulose, hemicelluloses, lignin and pectin. Consumption of significant quantities of dietary fibre has been shown to be beneficial to human nutrition, reducing the risk of certain types of cancer, coronary heart disease, diabetes and constipation [21]. High levels of dietary fibre in diets are advantageous for their active role in the gastrointestinal tract [19].

The fat content of the seeds of *S. indicum, C.melo* and *C. mannii* shown in figure 3 indicated a significant ($p < 0.5$) increase in the fat contents of the seeds from 44.4 to 57.2, 45.2 to 58.4 and 46 to 55.4% respectively as the fermentation progresses to day 7. These values were within the range of fat content of raw *C.melo* and *C. mannii* (42.67±3.43, 45.89±4.73) reported by Loukou *et al*., [22]. The increase in the fat contents of the fermented seeds was in agreement with the report of Omafuvbe et al., [32]; Enujiugha and Akanbi, [18] but disagrees with the report David and Aderibigbe, [12].Where it was reported that fermentation leads to reduction in the fat content of oil seeds. The increase in the fat content of the fermented seeds could be attributed to the transformation of the carbohydrate molecules to fat; Akindahunsi and Glatz (1998); reported that certain fungi could produce microbial oil during the course of fermentation.

The carbohydrate content of *S. indicum, C.melo* and *C. mannii* are presented in figure 5. The result showed a significant ($p < 0.5$) reduction of carbohydrate in the fermented seeds from 13.0 to 3.4, 20.6 to 5.1 and 10.3 to 2.5% respectively. The carbohydrate content of the three oil seeds are less than that reported by Enujiugha and Akanbi, [18] for *Pentaclethra macrophylla* (African oil bean) in both raw and fermented seeds. The reduction in the carbohydrate level was in agreement with the report of Udensi and Okoronkwo, [37] in the effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate. In this report fermentation was able to reduce the carbohydrate content of *Mucuna cochinchinesis* from 4.27% to 0.04%. Similar report was made in the work of Omafuvbe *et al,* [32], where fermentation of African locust bean and Melon species caused remarkable decrease in the carbohydrate content of the fermented seeds. Earlier reports by Popoola and Akueshi, [34] showed a significant reduction in the carbohydrate content of soybean seeds fermented into daddawa. As earlier stated the decrease in carbohydrate could be attributed to the possible utilization of carbohydrate as carbon source for the growth of microbial biomass and possible transformation of carbohydrate to other secondary metabolites [3]. The general reduction in all the various antinutrient factors as a result of fermentation suggest that health benefitting factors of these

seeds could be improved with improved fermentation process , except *S. indicum* seed whose tannin, flavonoid and phenolic contents showed a much higher reduction than *C.melo* and *C. mannii* which puts a question mark on its antioxidant status after fermentation. However, further investigation into the effect of fermentation on their antioxidant potential is suggested.

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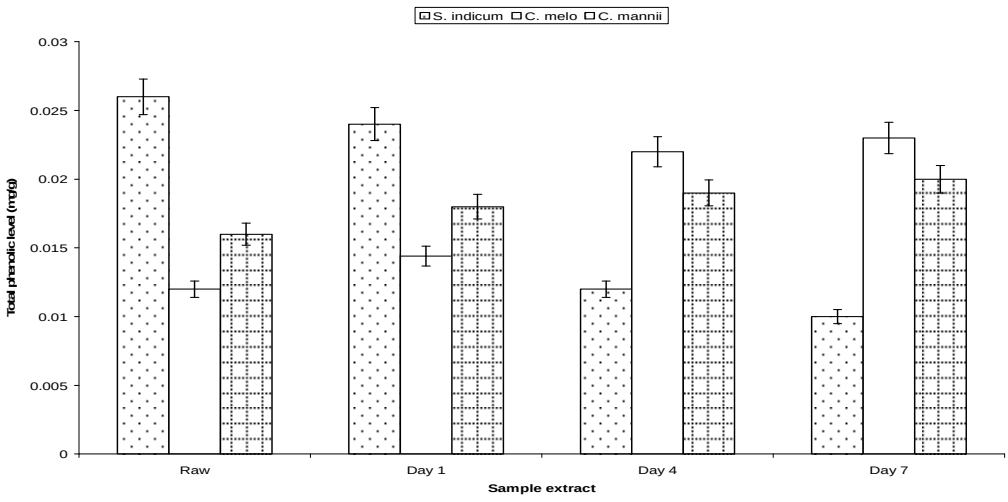

Figure 1: Protein content of *S. indicum*, *C.melo* and *C. mannii*

Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

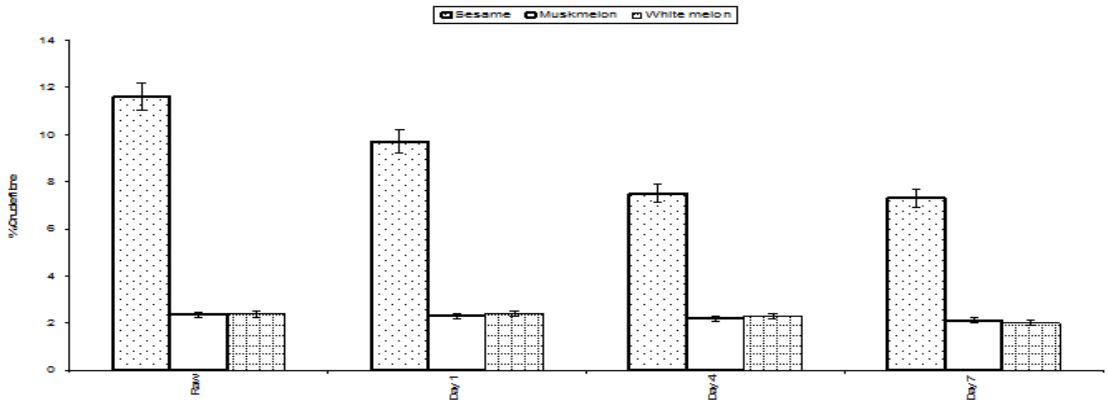

Figure 2: Crude fibre content of raw and fermented *S. indicum*, *C. meelo* and *C.mannii*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

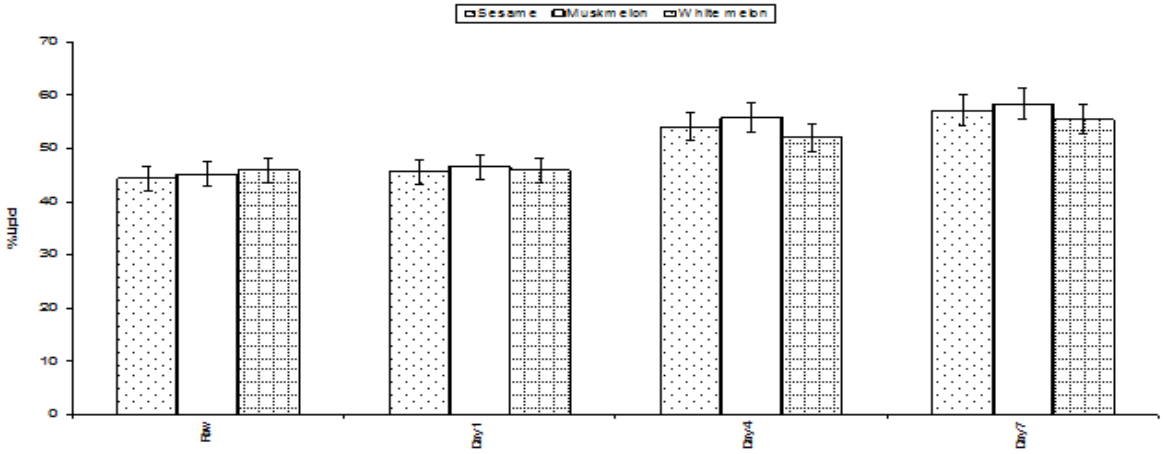

Figure 3: Lipid content of raw and fermented *S. indicum*, *C. melo* and *C. mannii*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

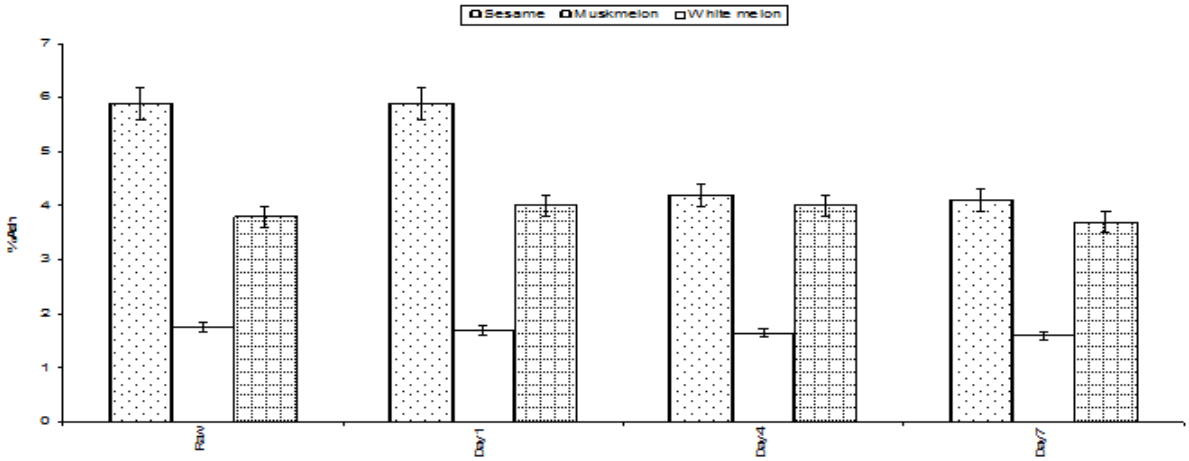

Figure 4: Ash content of raw and fermented *S. indicum*, *C. melo* and *C.mannii*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

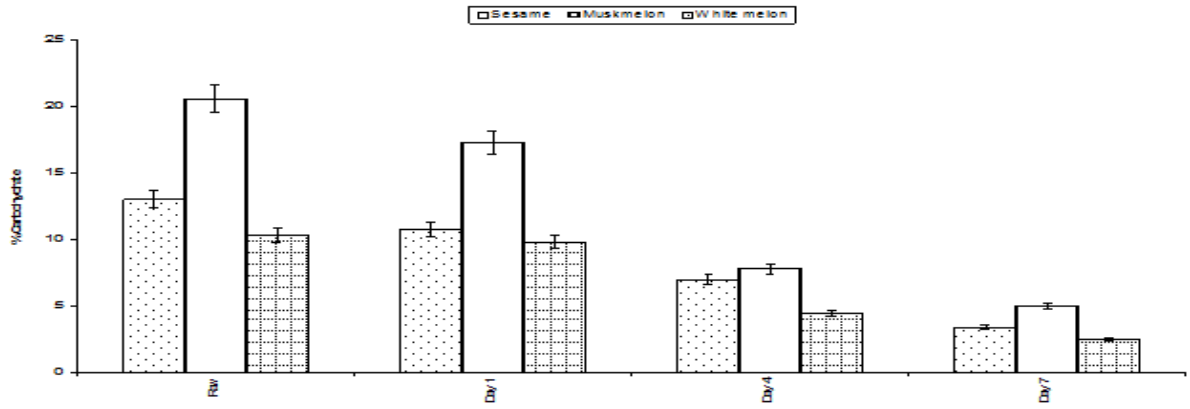

Figure 5: Carbohydrate content of raw and fermented *S. indicum*, *C. melo* and *C. mannii.*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

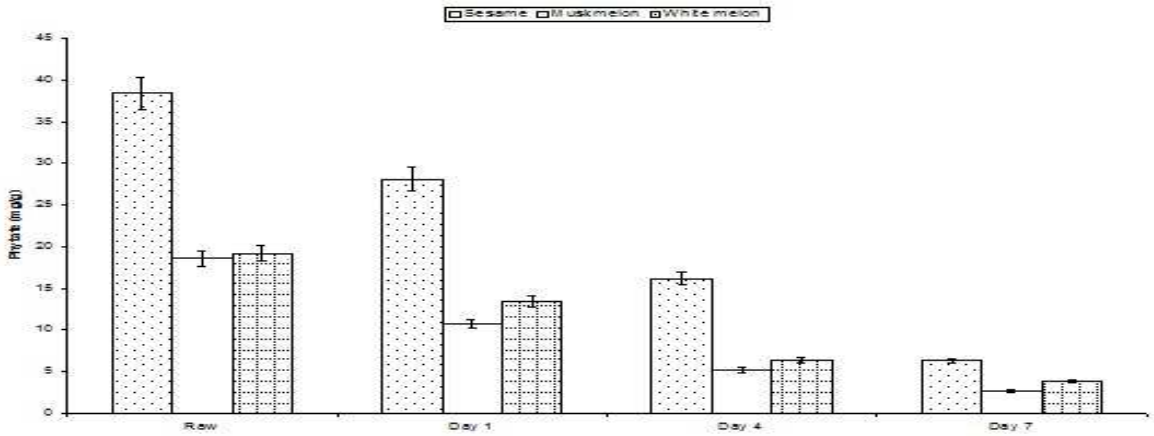

Figure 6: Phytate level of raw and fermented *S. indicum*, *C. melo* and *C. mannii*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

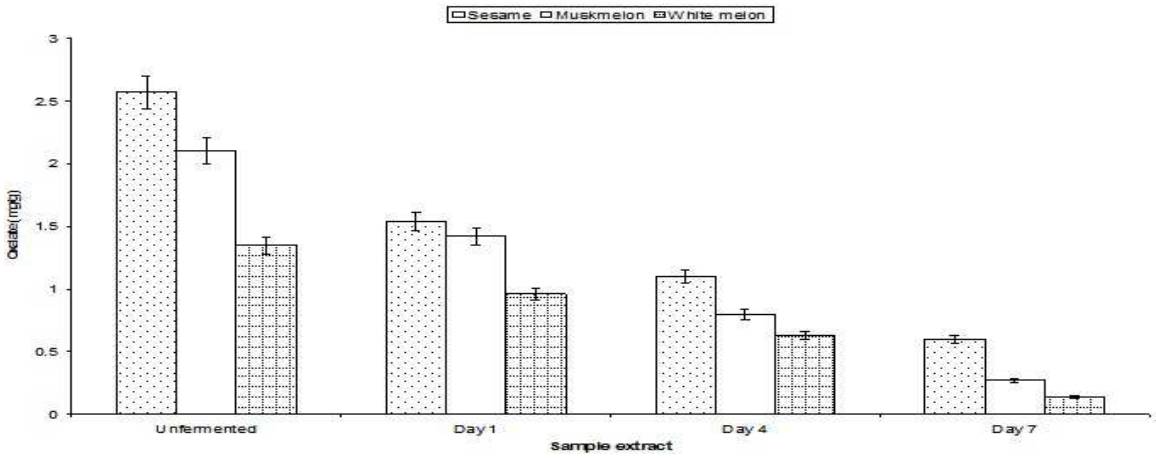

Figure 7: Oxalate level of raw and fermented *S. indicum*, *C. melo* and *C. mannii.*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

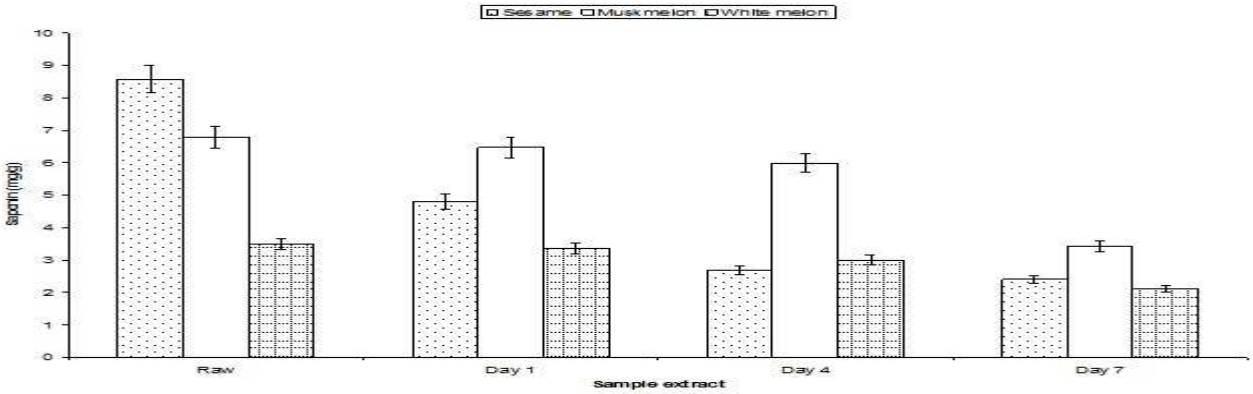

Figure 8: Saponin level of raw and fermented *S. indicum*, *C. melo* and *C. mannii*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

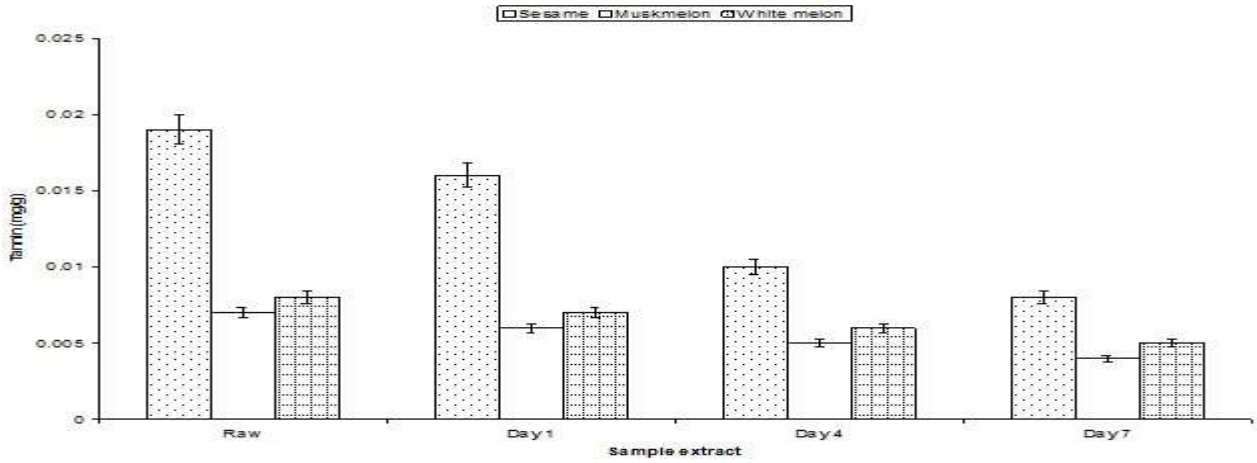

Figure 9: Tannins level of raw and fermented *S. indicum*, *C. melo* and *C. mannii.*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

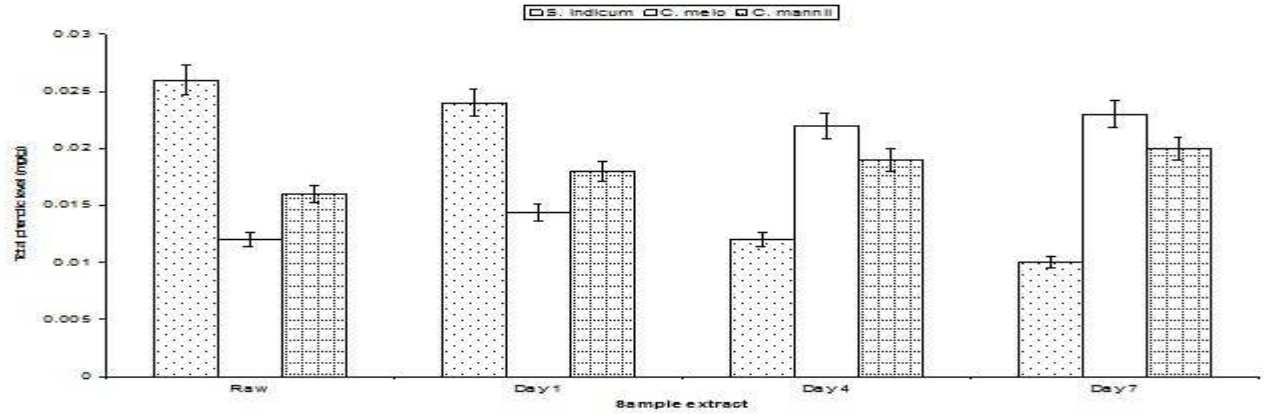

Figure 10: Total phenols level of raw and fermented *S. indicum*, *C. melo* and *C. mannii.*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

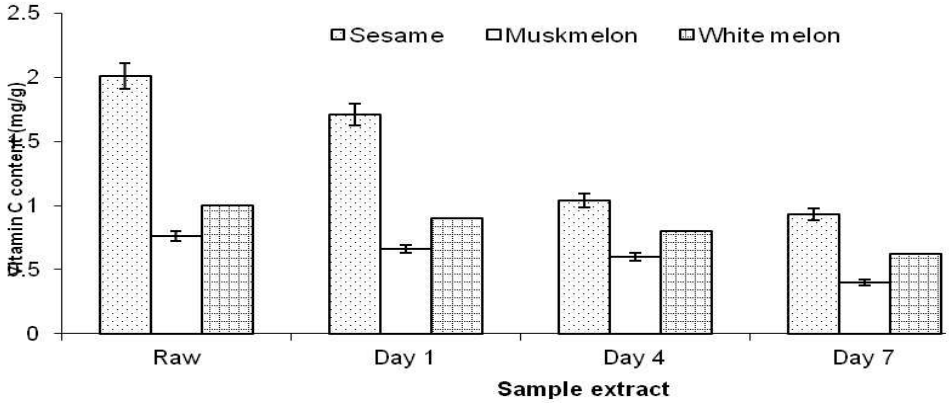

Figure 11: Flavonoids level of raw and fermented *S. indicum*, *C. melo* and *C. mannii.*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate

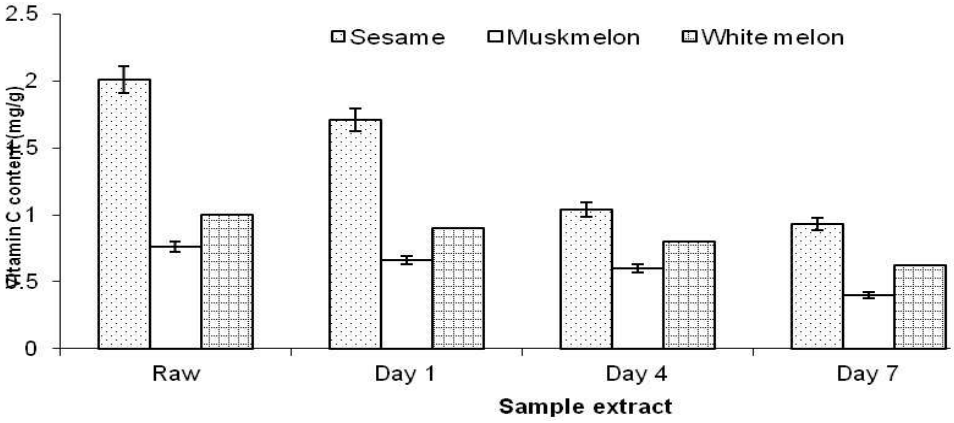

Figure 12: Vitamin C content of raw and fermented *S. indicum*, *C. melo* and *C. mannii.*
Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate