International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(6): 2798-2804

Received: 15-09-2018
Accepted: 19-10-2018

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Bio-deterioration of kokum seeds as influenced by different packaging materials during storage

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Abstract
A lab experiment was carried out to study that seed physiological and biochemical parameters of kokum seed (*Garcinia indica*) as Influence by different packaging materials. Kokum is found all over the world, mostly in Asia and Africa. It is a fruit bearing tree. Seeds of kokum are used in extracting oil, which has many food and non food applications. Kokum seeds were stored in different packaging materials viz., cloth bag, polyethylene bag, jute bag, one in open pan under ambient conditions for a period of 270 days. The results of the study revealed that the least fluctuations in all seed physiological parameters such as moisture content and seed biochemical parameters viz., oil content, total mould count, free fatty acid and saponification values were recorded in polyethylene packed seeds than seeds in open pan, jute bag and cloth bag for kokum seeds throughout the storage period. Among the packaging materials, the seeds stored in polyethylene bags maintained the seed biochemical and seed physiological parameters with least deterioration compared to seeds stored in jute bag, cloth bag and open pan.

Keywords: Kokum seed, packaging material, mould count, FFA, oil content

Introduction

Kokum (*Garcinia indica*) is a plant in the Clusiaceae family. It is a fruit bearing tree. The Clusiaceae family includes about 200 species found all over the world, mostly in Asia and Africa (Parthasarathy *et al.*, 2014) [22]. Kokum is found in forest lands, riversides and wastelands. These plants prefer evergreen forests, but sometimes they also flourish in areas with relatively low rainfall. Kokum is native to the Western Ghats region of India. It is found mainly in the Konkan region of Maharashtra, Goa, North coastal Karnataka, Kerala, forests of Assam, West Bengal and part of Gujarat in India (Haldankar and Somavashi, 2015) [10].

Kokum is grown on about 1000 ha area in Konkan region with production of about 4500 MT fruits. According to survey conducted earlier by chief conservator of forest, out of total 46,600 kokum trees in the state of Maharashtra; 43,000 trees existed in Ratnagiri and Sindhudurg Districts only. The production of kokum is generally 70 % from Sindhudurgh and 30% from Ratnagiri (Anonymous, 2015) [2]. The production of kokum fruits in Maharashtra is around 50,000 tonnes with an average productivity 10tonnes/ha (Sengar *et al.*, 2012) [28]. It was also reported that in South Konkan 1674 MT of kokum fruits were used for production of dried kokum rind, 757 MT for preparation of kokum syrup and 40 MT for manufacture of kokum butter (Wadkar *et al.*, 2001) [33]. Thus Konkan region in Maharashtra is enjoying the monopoly status with respect to kokum fruit production and its processed products.

The seeds of kokum contain oil, which is in solid state at room temperature. Seeds are traditionally decorticated by wooden mallets that need be crushed to obtain kernels. The kernels are 60% of kokum seed by weight and contain about 44% fat. This fat is called Kokum Butter. The kokum butter is in light grey or yellowish in colour with greasy texture and a bland oily taste (George *et al.*, 2002) [8]. It has food and non-food applications (Krishnamurthy, 1982) [13]. It is used as vaseline for cracks of skin. It is useful in manufacture of ointments, cosmetic preparations and pharmaceutical products, for candle making and soap manufacture. It is also used as confectionery butter. The cake left aft er the extraction of oil is used as manure.

Kokum cultivation in Maharashtra is characterized by high rainfall and high relative humidity resulting in genetic integrity leading to rapid deterioration of seeds. Most of kokum seed is dried under the sun either on mat or on ground. During sun drying, the kokum seeds are exposed to open environment and are contaminated by dust and insects, resulting in poor quality seeds with less value addition. According to Shelar, (2008) [31] seed quality during storage depends upon the initial quality of seed and the manner in which it is dried and stored

stored. The kokum seeds are a valuable source of oil. Kokum seeds oil contain a fairly high amount of stearic acid suggesting ability to remain solid at room temperature. After harvest, some of the kokum seeds are sold to buyers while a considerable proportion is stored to obtain a higher price later in times of scarcity. Inadequate drying before storage contributes significantly to rapid deterioration of kokum seeds. Storage of kokum seeds in Konkan region of Maharashtra is done in jute sacks under ambient environmental conditions. This traditional method of storage inevitably provides suitable conditions for mould growth in stored seeds. The prevailing high temperature and relative humidity (80%) in Konkan region of Maharashtra enhance the colonization process by fungi during storage of agricultural products. Oil seeds should be of very high quality to meet the various purposes for which they are utilized. Deteriorated oil seeds cannot be satisfactorily used for consumption and other industrial purposes. The mould growth in stored oil seeds may cause various forms of deterioration leading to quality loss. This may manifest as decrease in the nutritive value of oil seeds or decrease in bleachability of extracted oil. Although the literature contains many references on mould deterioration of oil seeds such as palm kernels, melon seed and soybean however information on deterioration of kokum seeds is very scanty.

Keeping in view the enormous scope the kokum seed has in food, cosmetic and pharmaceutical industries, the present research was undertaken to study the influence of coastal conditions on microbial activities of stored kokum seed and assess the changes in moisture content, total oil content, free fatty acids and Soponification value in kokum seeds during the storage.

Materials and methods

Raw Material

Kokum fruits were collected from the orchards of Department of Horticulture, Dr. B.S.K.K.V. Dapoli. The raw kokum seeds used in this study were collected from fresh fruits. The cleaned fruits were cut into two halves. The seeds were separated manually and sun dried to initial moisture content less than 8% (d.b.).

The dried kokum seeds (after attaining the safe moisture content) were packed in bags made from three different packaging materials namely; jute bag, cloth bag, polyethylene bag and one stored in open pan and were placed on raised platforms inside a ventilated room. The packaging material types were replicated three times. Experiments were carried out during the period between July 2017 and March 2018. After every fortnight (15 days interval) of storage, three separate identical samples (from three bags) were withdrawn from each packaging material type and subjected to analysis.

These samples were used to determine indicators of quality during storage namely; moisture content, mould count and oil content of kokum seed, free fatty acid and saponification value of kokum seed oil.

Moisture Content

The moisture content of the kokum seed samples were determined using hot air oven by following method ASAE 1997 [5].

Total Mould Count

Serial dilution technique was adopted (Peterson, 1959) [23] for making colony counts. Kokum seeds with intact hull were put in sterilized water (10 g/90 ml), stirred and drained to obtain kokum seed wash sample. All the procedure of pouring plates and serial dilution was done in laminar flow to maintain aseptic condition and avoid contamination during the experiment.

The kokum seed wash (liquid) sample was used for serial dilution method. The pipettes were filled with nine ml dilled waster. One ml of well mixed sample draw into sterilize pipette. This sample was added to first tube. The volume of this tube became ten ml. This provides an initial dilution of 10^{-1}. The dilution was thoroughly mixed. This pipette was discarded into the pot of disinfectant. The used pipette was sterilized on the flame and used again. One ml sample from first tube of the 10^{-1} dilution was drawn and placed it in the second tube. It was mixed well as before. This gave 10^{-2} dilution. The pipette was also discarded in disinfectant. This was repeated for the remaining tubes, removing one ml from the previous dilution and adding it to the next 9 ml of diluents. A known volume of each dilution was used to make pour plates. The plates were incubated at room temperature. After incubation the plates were observed for the colonies. The colonies were carefully counted using a marker pen. The number of micro-organisms in the sample was calculated using following formula:

Number of microbes/ml = number of colonies × dilution of sample

Oil Content

The oil content of kokum seed was determined using Soxhlet apparatus by solvent extraction method. (AOAC, 2005, Official Method 996.06) [4].

Free Fatty Acid (FFA)

Free fatty acid is the relative measure of rancidity as FFA is normally formed during the decomposition of oil glycerides due to the action of moisture, temperature or enzyme lipase. The FFA is determined by directly titrating the oil in alcoholic medium against standard potassium hydroxide /sodium hydroxide solution (IS: 548-Part 1, 1964) [11].

Saponification Value

The saponification value is the number of milligram of potassium hydroxide required to saponify one gram of fat. The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution (AOAC, 2000, Official method 920.160) [3].

Results and Discussion

Figure 1 (a) and (b) shows the mean temperature and relative humidity profiles of the store throughout the whole period of the study. Biochemical changes in kokum seeds, moisture content, total mould count and oil content of kokum seed, free fatty acid and saponification value of kokum seed oil were estimated.

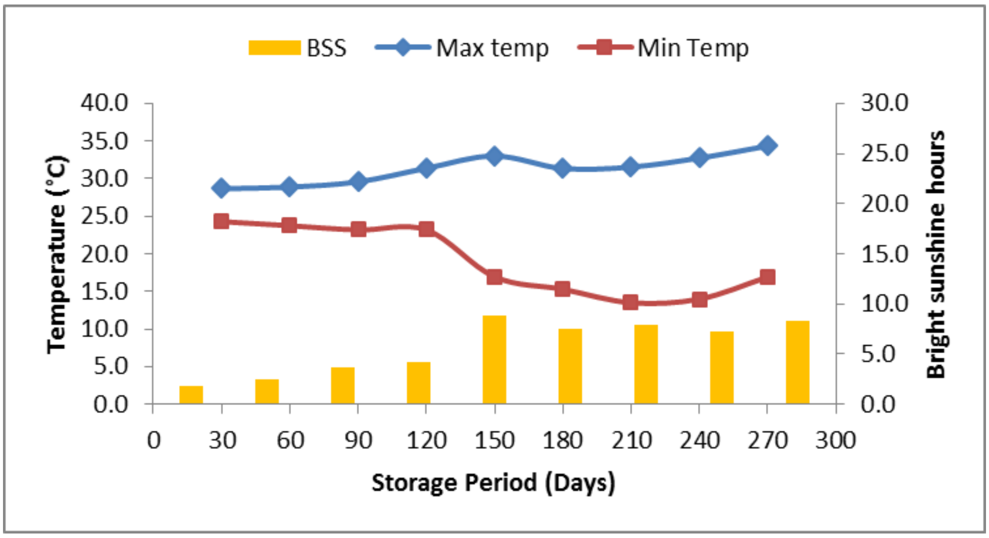

Fig 1(a): Mean monthly Maximum and Minimum temperature, Bright sunshine hours during the experiment

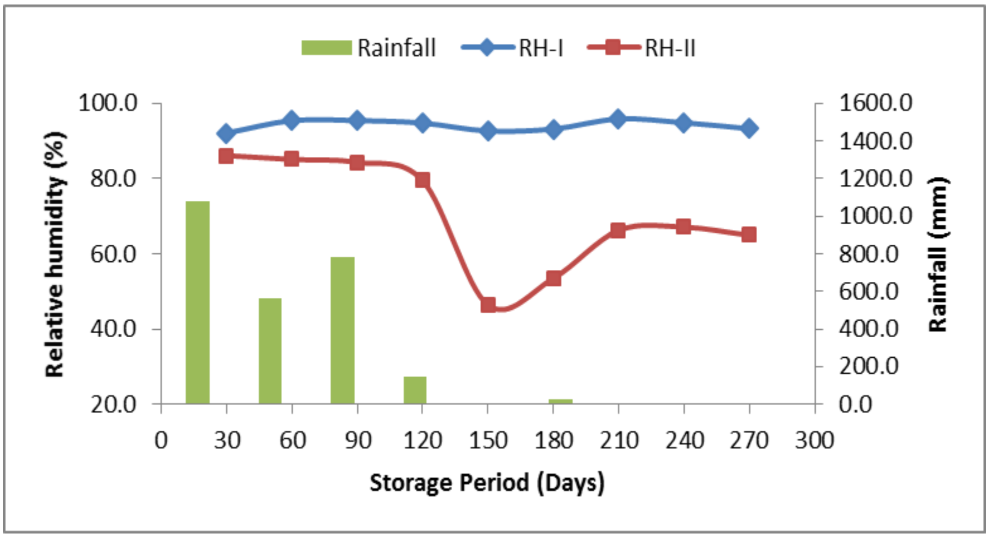

Fig 1(b): Mean monthly RH-I (morning), RH-II (afternoon) and rainfall during the experiment

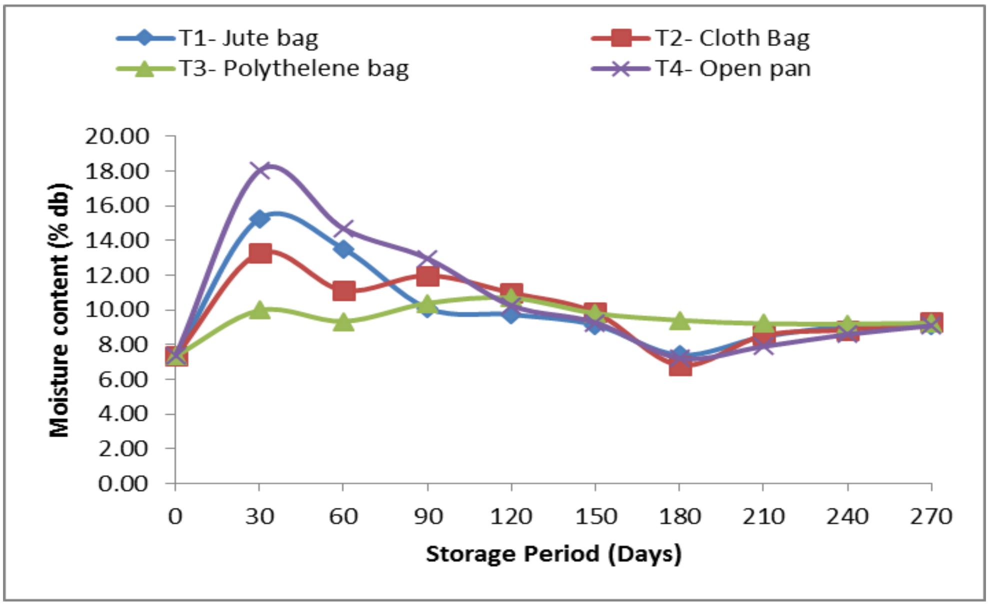

Fig 2: Effect of packaging and storage on moisture content of kokum seed during storage

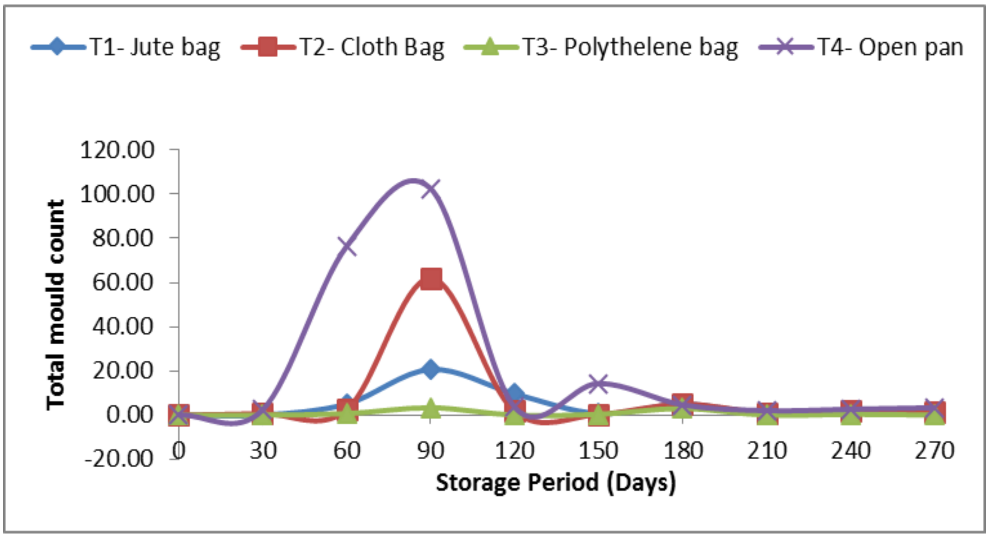

Fig 3: Effect of packaging and storage on total mould count of kokum seed during long storage

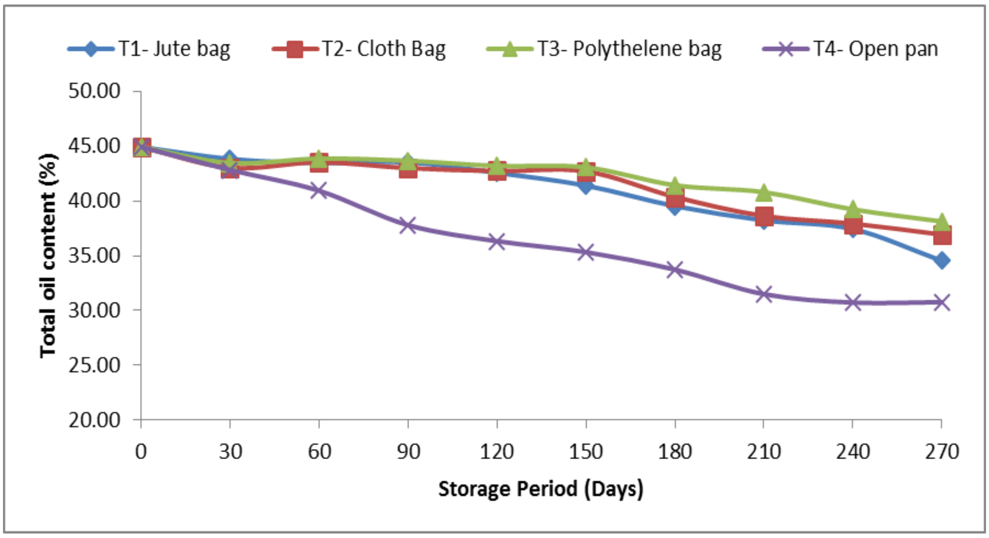

Fig 4: Effect of packaging and storage on total oil content of kokum seed during storage

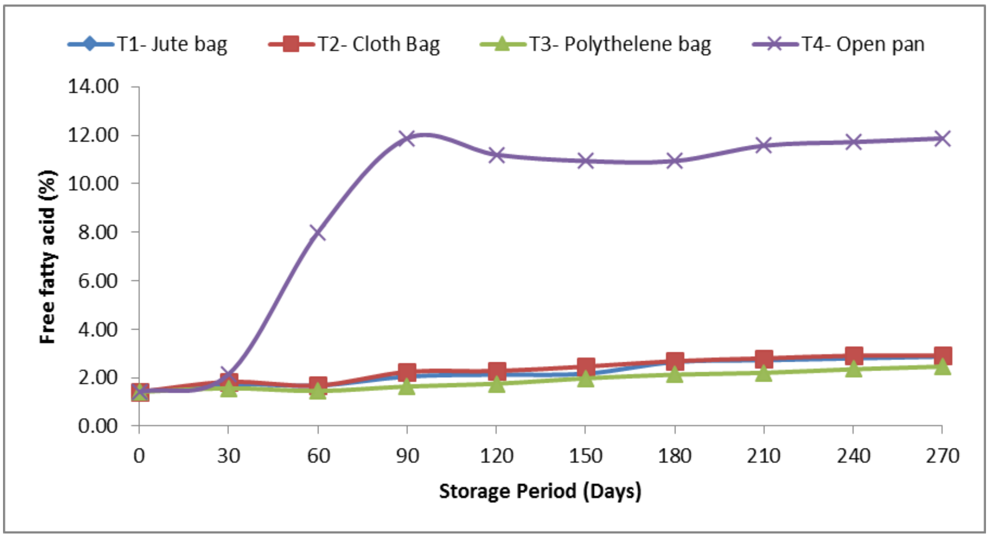

Fig 5: Effect of packaging and storage on free fatty acid of oil extracted from kokum seed during storage

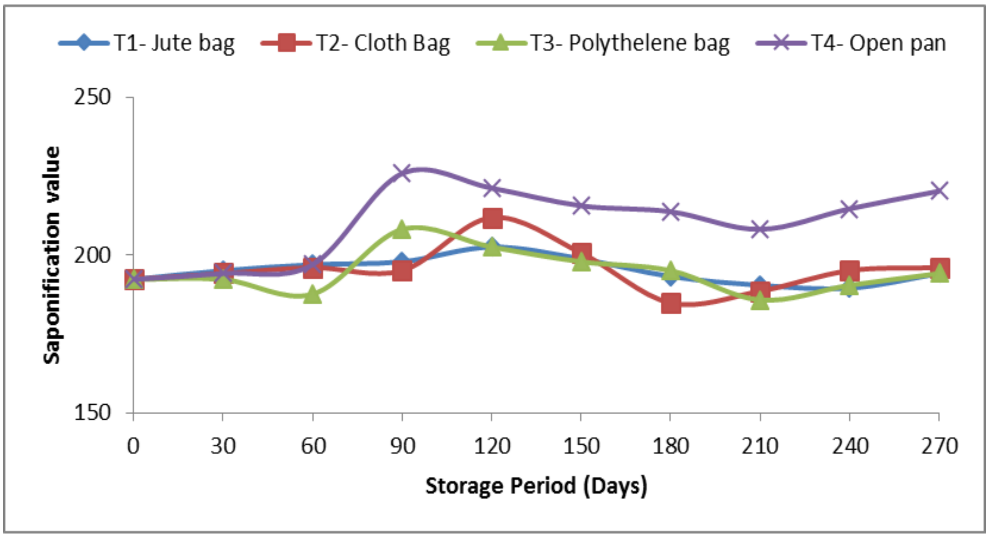

Fig 6: Effect of packaging and storage on saponification value of oil extracted from kokum seed during storage

Moisture Content

The moisture content of kokum seeds as influenced by different packaging materials such as Jute bag, Cloth bag, Polythene bag and Ambient condition is shown in Fig.2.

Moisture content of seed during storage is the most persuasive factor affecting the longevity of the seed. The period of longevity of seed is greatly determined by the amount of moisture present in the seed. In the present study freshly-harvest seed samples stored in different packaging material showed the maximum moisture content in initial months and the moisture content was observed decreased with the increase of storage period.

It was found to be differed significantly with storage period. The initial seed moisture content of kokum seeds was 7.34 % before seeds were packed into various packaging materials. At the initial period of storage (i.e., 2 months) the maximum moisture content (18.77 % d.b.) was observed in seeds stored in open pan at room temperature followed by Jute bags (15.58 % d.b.), cloth bag (14.22 % d.b.) and polythelene bag (9.97 % d.b.). With the change in atmospheric humidity (increased or decreased), significant variations have been observed in the rate of change in seed moisture content among all different treatments during storage.

Moisture content of seed plays an important role in influencing the fungal colonization during pre-harvest, post-harvest and in storage. Variations in the chemical composition of the seeds are known to be influence by the seed moisture content. There was a decrease in moisture content in seed samples during storage noted in the current study. According to Shelar *et al*., 2008 seeds absorb or lose moisture till the vapour pressure of seed moisture and atmospheric relative humidity reach equilibrium. Relatively greater change in moisture content was observed in moisture pervious packaging material *viz*. Jute bag, and cloth bag. On the other hand, kokum seeds stored in polyethylene bag maintained relatively stable moisture content.

These results are in agreement with the findings of Meena *et al*., (2017) [17] in soybean, Negedu *et al*., (2014) [20] in castor seed, Rajendra prasad *et al*., (1998) [24] in groundnut kernels. Saxena *et al*., (2015) [27] also observed a decrease in moisture content during storage in Soybean. Marginal variations in seed moisture content in moisture impervious packaging materials have been reported in rice (Padma and Muralimohan Reddy, 2000) [21] and groundnut (Meher, 2013) [18]. Although the impervious packaging material prevented the influx of moisture into the seed from outside environment, the increase in seed moisture content with advancement of storage period might be attributed to greater accumulation of metabolic water.

Total Mould Count

The total mould count of kokum seeds as influenced by different packaging materials such as Jute bag, Cloth bag, Polythene bag and Ambient condition is shown in Fig. 3.

Storage conditions in most parts of India are very conducive for mould attack, proliferation and elaboration of mycotoxins (Girish and Goyal, 1986) [9]. In the present study, variation was observed in total mould count of kokum seed from freshly harvested stage to at the end of the storage period in different packaging material. The change in the predominance of various molds found to change with seed moisture. Fungi were found to present in low percentage in freshly harvest seed samples and became leading as the storage period increased. (Saxena *et al*, 2015) [27].

The oil seeds crops are associated with a large number of storage mycoflora and it is one of the major factors for the low productivity of oil seeds in India. Seed borne Mycoflora (fungi) plays an important role in determining the quality and longevity of seeds. The dominance of a particular of the fungi seems to depend upon the moisture requirements of that species. The climatic conditions of tropical and sub-tropical countries favor the growth of *Aspergilli*.

Fungi colonies were found to be associated with kokum seeds stored in different packaging materials. Their prevalence varied with storage packaging materials. Sultana (2016)[32] and Jamandar *et al*. (2001) [12] reported similar fungi trend association with okra seeds. In the initial month of storage, seeds stored in open condition showed the highest prevalence of fungi followed by seeds stored in cloth bag as shown in Fig 3. Kokum seeds stored in plastic container yielded minimum prevalence of the fungus. Similar findings have been reported by Sultana *et al.* (2016) [32].

Oil Content

The oil content of kokum seeds as influenced by different packaging materials such as Jute bag, Cloth bag, Polythene bag and Ambient condition is shown in Fig. 4. In the present study the oil content (%) of kokum seeds differed significantly between treatments and there was a gradual decline seen with progress in storage period from the initial stage and until up end of storage, stored under room temperature.

It was found differed significantly with storage period. The initial oil content of kokum seeds was determined as 44.93% before seeds were packed into various packaging materials. At the end of storage i.e., 270 days polythelene packed seeds stored under room temperature recorded significantly higher oil content (38.13%) over all other treatments. The lower oil content (34.43%) was recorded in seeds stored in Jute bags samples, followed by cloth (34.53%).

Reduction in oil content is one of the important parameter which is influenced by ageing of the seeds. Martini and Anon, (2005) [16] reported that storage condition of oil seeds before industrial extraction may influence the quality of the crude oil. Different longevity of seed storage as well as storage conditions exerts significant influence on oil content. It is clear from the results that the kokum seed kept in polythelene bag could maintain higher oil content values compared to all other treatments. The results are in agreement with the findings of Bhattacharya and Raha (2004) [7] who reported decrease in the fat contents of soybeans, maize and rice as result of fungal growth on the seeds. The decrease in oil content might have resulted from the fat utilization by the storage fungi. Bankole *et al.* (2005) [6] observed a progressive decline in total oil content of melon seeds after three months of storage. Similar observations were reported by Saxena and Karan, (1991) [26] in sesamum seeds. There was a decrease in oil content from freshly seed samples to storage samples was observed. Sharma and Chauhan, (1976) [30] reported that the reduction in oil content, rancid smell and change in colour was due to *Aspergillus flavus* and *Cladosporium herberum* and stated that fungal enzyme lipase is responsible for the reduction in oil content.

Free Fatty Acid (FFA)

The free fatty acid of oil extracted from kokum seeds as influenced by different packaging materials such as Jute bag, Cloth bag, Polythene bag and Ambient condition is shown in Fig. 5.

Increase in free fatty acid of oil is considered as a measure of deterioration. Fungal attack on stored seeds results in an increase in free fatty acid. The present study shows increase in free fatty acid content of kokum seed oil with an increase in the storage period of kokum seed sample.

It was found to be differed significantly with storage period. Free fatty acid value was least in the freshly harvested seed samples. The initial free fatty acid content of kokum seeds oil was 1.42 % before seeds were packed into various packaging materials. The maximum free fatty acid content (11.87 %) was observed in seed oil stored in open pan at room temperature followed by kokum seed packed in cloth bag (2.91%), Jute bags (2.87%) and polythelene bag (2.46%).

Saxena *et al.*, (2015) [27] reported similar trend of increase in free fatty acids in soybean stored for six months in cloth bag. In the same vein, increase in the level of free fatty acid had been reported by Bhattacharya and Raha, (2002) [7] on stored maize, groundnut and soybean. This increase in the level of free fatty acid could be as a result of the conversion of the oil into fatty acids (Angelo and Ory, 1983) [1]. It may be due to production of the free fatty acids at a rate faster than was utilized by the associated fungi growing on the seeds resulting in an increased free fatty acid level. Free fatty acids are produced by the actions of lipases on fats which break down into free fatty acids and glycerol during the storage. The accumulation of free fatty acids indicates a decline in the cellular pH and harmful to seed health (Malik and Jyoti, 2013, Saxena, 2015 *et al.*) [14, 27]. The deteriorative changes in oilseeds may be either oxidative resulting in typical rancid flavor or hydrolytic resulting in the production of free fatty acids. The results conclusively show that there are certain fungi which are responsible for the reduction of total yield of oilseeds; whereas others spoil the quality of oil by increasing the free fatty acids. The chemical changes indicate increase in saturated fatty acids, which is undesirable as it causes obesity and cardiovascular disorders. Hence, such seeds are unfit for extracting oil for human consumption and the oil cake as animal feed.

Saponification Value

The saponification value extracted from kokum seeds as influenced by different packaging materials such as Jute bag, Cloth bag, Polythene bag and Open condition is shown in Fig. 6. In the present investigation, increase in soponification value was observed in all the seed samples during the storage. The initial saponification value of kokum seeds oil was 192.27 before seeds were packed into various packaging materials. The initial saponification value 192.27 of the kokum seed stored in open pan increased to higher value 228.67 and subsequently decreased to lower value 220.27 at the end of storage period. According to Shankaram, (1966) [30] soponification value changes due to the formation of number of short chain fatty acid glycerides during the lypolysis of oil by the enzyme lipase. The similar trend of increase in saponification value was found for the soybean seed stored for six months by Saxena *et al.* (2015) [27].

Conclusion

In conclusion, the study has revealed that the fungal infection to kokum seeds induced bio-deteriorative changes in the seed constituents such as moisture content, total fat as well as free fatty acid and saponification values. All these together reduce the economic value of the seeds and therefore, kokum seed value chain operators (producers, processors, marketers etc.) are advised to prevent the growth and development of moulds in seeds along the entire kokum seed value chain.

Acknowledgement

We would like to thank our Head, Department of Agricultural Process Engineering, Dr. BSKKV, Dapoli for providing necessary facilities and encouragement.

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