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# Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022)

Organized by

**Department of Zoology and Fishery Science,**
**Rajarshi Shahu Mahavidyalaya (Autonomous),**
**Latur- 413531, Maharashtra**

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# Index



 

 

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# Altered quality aspects of deteriorated seeds of peanuts (*Arachis hypogaea* L.) With response to ageing

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**Abstract**

Initial seed quality and storage environment plays a key role to prolong the self life of the oilseeds. In groundnut seeds, the attack of fungal pathogen affects a lot in deteriorating the seed quality. Fungi growing on stored seeds can reduce the germination rate along with loss in the quantum of total oil content, free fatty acid content and enhancing other biochemical changes. The tropical climate with high temperature and high relative humidity result in to deterioration of seeds. So it is necessary to study the changes in fatty acid composition that occur during storage of seeds which become deteriorated as a result of the attack of different pathogens and altered environmental conditions. The present work will give an idea about relationship between oil extracted from normal seeds and deteriorated seeds With respect to their relative biochemical changes occur during storage. Declination in the oil content is recorded in deteriorated seeds (infected) of groundnut. Increase in age of the oil alters the fatty acid composition drastically in oil from deteriorated seeds than that of control.

***Key Words:*** Groundnut, Fatty acid composition, ageing, rancidity.

## Introduction:

Peanuts (*Arachishypogaea* L.) also called as groundnut is foods rich in fat. They contain nearly 50% fat, of which almost 70% are unsaturated. Among them the main are oleic (C 18:1) and linoleum (C18:2) (Omega 6) both are extremely important in controlling cholesterol. The ratio of these two fatty acids, the oleic/linoleum (O/L) ratio, naturally fluctuatesas a function of seed genetics, maturity, and growing envi-ronment.Shelf life of peanut based foods, including peanut butter, roasted snack nuts, confections, and peanut oil is primarily limited by the oils oxidative stability. In turn, this oxidative stability is largely a function of the peanut oil fatty acid profile

  

(FAP), especially the O/Lratio. As the O/L ratio increases, the total level of instauration within the oil decreases resulting in a more stable product. As such, there have been extensive research efforts to breed peanut seed with altered O/Lratios Worthington et.al.(1972).Different workers studied oil content and fatty acid composition from different oil seeds sunflower by Balesevic(2007), in sesame by Savant and Kothekar (2011).

Oils can be particularly susceptible to rancidity because their chemistry which makes them susceptible to oxygen damage. When food scientists talk about rancidity, they are often talking about a specific type of rancidity involving oxygen damage to foods, and this type of rancidity is called "oxidative rancidity. Oxidation of fats, generally known as rancidity, is caused by a biochemical reaction between fats and oxygen. It is also caused due to microbes called microbial rancidity. In this process the long-chain fatty acids are degraded and short-chain compounds are formed. In addition to that the amount free fatty acid also increases.

Acidification is the decomposition of fats, oils and other lipids by hydrolysis or oxidation, or both. Hydrolysis will split fatty acid chains away from the glycerol backbone in glycerines. These free fatty acids can then undergo further auto-oxidation. Oxidation primarily occurs fats by a free radical-mediated process. These chemical processes can generate highly reactive with unsaturated molecules in rancid foods and oils, which are responsible for producing unpleasant odour. Changes in chemical constituents of cell have been related to viability of seeds. Vertucci*et al*. (1994) studied the changes in lipids during storage of groundnut and other oil seeds and suggested that the changes in lipid components of seeds were associated with seed deterioration and could be measured using differential scanning colorimetry.

Braccini*et al*. (2000) observed reduction in protein, lipid and poly unsaturated fatty acids content and increased hexanal production in storage of soybean seeds. Murali*et al*. (2002) stated that germination and field emergence of the pulse seeds decreased while the electrical conductivity of seed leachate increased with increase in storage period. Peroxidation of unsaturated fatty acids led to leaching of electrolytes and other solutes in soybean (Singh and Dadlani, 2003). Narayanaswamy (2003) concluded that oil, protein and field emergence of groundnut seeds decreased but free fatty acid and EC increased with advancement of storage period. Simic*et al*. (2007) noticed a decrease in oil content of sun flower, soybean and maize seeds during storage. *Aspergillus* is a common mould in tropical and sub tropical countries and causes aflatoxin contamination as a result of moulding of badly stored commodities, such as groundnut, cereal , cotton, sesame, lentil, mustard, peanuts, castor, coconut, and other oil seeds.

  

*Aspergillusniger*,*Aspergillusflavus*,*Alternariadianthicola*,*Curvularialunata*,*Curvulariapellescens*,*Fusariumoxysporum*,*Fusariumequiseti*,and*Penicilliumchrysogenum* causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oil seeds.

Fungi growing on stored seeds, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces increased moisture content, free fatty acid content and enhancing other biochemical changes. The tropical climate with high temperature and high relative humidity along with unscientific storage conditions adversely affect the preservation of cereal grains, oilseeds, etc., which lead to the total loss of seed quality. The quality of oil also decreases with increasing age of oil . This study was designed to better understand the potential biochemical changes in oil of normal and deteriorated seeds which are altered with ageing.

**Material and Method:**

Deteriorated seedsof groundnut are collected from stored seeds and are classified in three groups as Type-I as semi deteriorated, Type-II as moderately deteriorated and Type-III as heavily deteriorated.These seeds are crushed into fine paste with the help of mortor and pistle to extracted Oil from the pest. Estimation of oil content in groundnut seed is undertaken by Soxhlet extraction apparatus (Sadasivam and Manickam, 2008). Oil from a known quantity of the seed is extracted with petroleum ether. It is then distilled off completely, and the percentage oil is calculated. A thimble (by using Whatman No 41 filter paper) was prepared by taking 5 g of fine macerated seed powder and was placed in Soxhlet apparatus. Dried pre-weighed solvent flasks containing solvent (petroleum ether, the b.p.40-60ºC) and were subjected to heating. The heating rate was adjusted at $70^0$ C to give 5-6 drops for 5 hours. Then solvent flasks extracted with oil in petroleum ether were evaporated .The weights of the solvent flasks were taken after evaporation of petroleum ether. The oil content was calculated by subtracting the weight of the solvent flask from the solvent flask with oil. The oil content was expressed in terms of percentage and it is calculated by using following formula.

Calculations:

$$\text{Oil in ground sample \%} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

The oil extracted from all samples is then analyzed for its odour and color. Small quantity (0.2 ml) oil from each type of deteriorated seeds is added in to2 mlfresh oil from normal seeds. These oil samples are incubated ineppendorf tubes for six months and analyzed for their fatty acid composition after three months interval.

**Fatty acid composition:**

  

The fatty acid analysis of the control seeds and selected infected seed carried out by using gas chromatographic analysis. Sesame oil was etherified according to the method of Marguard (1987) at Tinnaoil and chemicals Ltd.1, MIDC, Latur,Maharashtra. 1ml sample of oil was placed into a tube and 1 ml of Na-methylate was added to the mixture. The sample was left at room temperature overnight, and then 0.25 ml of isooctane was added to it. 0.5 µl sample of the mixture was injected into the gas chromatographer. The composition of fatty acid was determined by gas liquid chromatography (GC) performed on a Fison GC equipped with a flame ionisation detector (FID), and fitted with a fused capillary column FFAP-DF (25 m x 0.25 mm ID). The detector was operated at 260º C and the injector at 250ºC. The column was ballistically heated from 150 to 200º C at the rate of 5º C min-1. The carrier gas (helium) inlet pressure was 0.15 MPa and flow rate was 1 ml/min. The fatty acid identification was done by comparing the retention time of standards (Sigma Co.) and area under each peak by automatic integrator to give relative comparison using software.

**Result and discussion:**

The biochemical analysis of Normal and Deteriorated seeds of groundnuts was undertaken in present investigation. The different biochemical features studies comprised, oil content, and fatty acid profile.

The pertinent efforts have revealed that the considerable variations are found in oil content in Normal and infected seeds of groundnuts. It is noted that the amount of oil in the normal seeds is higher (46 %) than all types of seeds (Table- 1). The lowest amount of oil content is found in heavily deteriorated seeds. The oil content in seeds is gradually decreased with increasing deterioration in seeds.

**Table- 1: Percentage of oil in different seed samples.**

| Sr. No. | Seed sample | % of oil | Color | Odour |
|---|---|---|---|---|
| 01 | Normal Seed | 46 ± 0.39 | Light yellow | Agreeable |
| 02 | Type-I Seeds | 38 ± 0.54 | Dark yellow | Unpleasant |
| 03 | Type-II Seeds | 34.27 ± 0.87 | Yellowish red | Unpleasant |
| 04 | Type-III Seeds | 32.74 ± 0.44 | Yellowish red | Unpleasant |

The normal Peanut oil was odourless or have agreeable nutty odour, its flavour is nutty and mild. It is light yellow in color whereas oil from different deteriorated (infected) seed samples of peanut have rancid unpleasant odour, its flavour is unpleasant and it is bitter in taste. Degree of the deterioration of seeds negatively affects ocular and odour of oil. Color of the oil from Type-III is

  

darker than any other type with highly unpleasant odour.Kakde and Chavan (2011) concluded that *Aspergillusflavus*was responsible for maximum depletion of oil content and reducing sugar in safflower, soybean, sesamum and groundnut due to *Fusariumequiseti*and*Rhizopus*.The results of biochemical analysis of different oil samples are given in **Table -2.**The results showed that there is a great variation in fatty acid profile of oil from infected and normal seeds. The maximum variation is found in case of oleic acid and linoleum acid. The oleic acid content found in normal seed oil is 46.7% where as it is reduced upto44.1% in oil from Type -I seeds and the lowest value for oleic acid content is 41.1%, recorded in oil from heavily deteriorated Type- III seeds. The amount of linoleum acid recorded in control sample is 29.4% whereas the lowest amount of linoleum acid is again recorded in Type –III seeds where its value is decreased upto 22.8 %. Bhattacharya and Raha (2002) observed a decrease in carbohydrate, oil content and some unsaturated fatty acid content with a gradual loss followed by a small increase in protein content of maize, groundnut and soybean seeds during storage due to storage fungi. It is found that the levels of MUFA and PUFA values are gradually declined from normal to Type-III seeds. Among deteriorated seeds the levels of unsaturated fatty acids are decreased with increasing degree of deterioration. Little variation is also recorded in saturated fatty acid content. The palmitic acid content in oil from normal seeds is 5.8 %which isslightly increased in Type-II ( 5.9%) and Type –III (6.2%).

**Table -2: Fatty acid composition of Fresh Oil Extracted from different seed samples of groundnut**

| Fatty acid Type | Oil from Normal Seeds | Oil from Type-I Seeds | Oil from Type-II Seeds | Oil from Type-III Seeds |
|---|---|---|---|---|
| Capric | 1.5 | 1.9 | 2.2 | 2.4 |
| Lauric | 3.8 | 3.5 | 3.7 | 4.2 |
| Palmitic | 5.9 | 5.5 | 5.9 | 6.2 |
| Myristic | 0.07 | 0.1 | 0.1 | 0.1 |
| Arachidic | 1.6 | 1.9 | 1.8 | 2.0 |
| Palmitolic | 1.0 | 0.9 | 0.8 | 1.1 |
| Stearic | 0.7 | 0.8 | 0.9 | 0.9 |
| Oleic (18:1) | 46.7 | 44.1 | 42.7 | 41.1 |
| Linoleic(18:2) | 29.4 | 26.4 | 25.1 | 22.8 |
| Behenic | 1.4 | 1.4 | 1.1 | 1.2 |

Table: 3 includes the variations in fatty acid composition caused due to their response to three months ageing of oil. It is found that values for unsaturated fatty acids are fluctuated a lot than that of values for saturated fatty acids in response to ageing of oils. The unsaturated fatty acid contents

  

are decreased in all types of oils. The lowest percentages of oleic acid and linoleic acid were recorded in Type –III sample with 40.1% and 22.8%, as compare to normal 46.7% and 28.2%, respectively. Reduction in fatty acid content due to seed borne fungi during storage was recorded by Ushamalini*et al.* (1998).Palmitic acid percentage in different oil samples is also altered with ageing.The variation of fatty acid composition with climatic conditions and ageing was indicated by Gecgel et al.,(2007) and FadulOnemli (2012). Among deteriorated seeds the lowestpercentage of palmitic acid is found in Type –I sample whereas its highest percentage is found in Type –III sample. The valuesfor Palmitic acid percentage in inoculated samples are more than that of deteriorated seed samples.In case of inoculated oil samples the levels of unsaturated fatty acids are less than that of control but more than deterioratedsamples .In generala little increase in saturated fatty acid contents are found in all of the samples, in contrast unsaturated fatty acid contents are decreased in most of the oil samples with ageing of oil.

**Table -3: Fatty acid composition of different oil samples after Three Months of Extraction and inoculation**

| Fatty acid Type | Normal | Type-I | Type-II | Type-III | Type-I Inoculated Oil | Type-II Inoculated Oil | Type-III Inoculated Oil |
|---|---|---|---|---|---|---|---|
| Capric (*C.10*:0) | 1.5 | 1.9 | 2.2 | 2.4 | 1.9 | 2.2 | 2.4 |
| Lauric | 3.5 | 3.8 | 4.1 | 4.5 | 3.8 | 4.0 | 4.1 |
| Palmitic | 6.0 | 6.1 | 6.0 | 6.8 | 6.3 | 6.3 | 6.7 |
| Myristic | 0.06 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Arachidic | 1.6 | 1.8 | 2.1 | 2.1 | 1.6 | 1.8 | 1.8 |
| Palmitolic | 1.0 | 1.1 | 1.1 | 1.3 | 0.9 | 1.1 | 1.1 |
| Stearic | 0.8 | 0.8 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Oleic (18:1) | 46.7 | 42.2 | 42.1 | 40.1 | 45.2 | 45.0 | 43.1 |
| Linoleic(18:2) | 28.2 | 26.4 | 25.1 | 22.8 | 26.1 | 25.1 | 25.8 |
| Behenic | 1.4 | 1.4 | 1. | 1. | 1.4 | 1. | 1. |

  

**Table -4: Fatty acid composition of differentoil samples after Six Months of Extraction and inoculation**

| Fatty acid Type | Normal | Type-I | Type-II | Type-III | Type-I Inoculated Oil | Type-II Inoculated Oil | Type-III Inoculated Oil |
|---|---|---|---|---|---|---|---|
| Capric (*C.10*:0) | 1.5 | 1.9 | 2.2 | 2.4 | 1.9 | 2.2 | 2.4 |
| Lauric | 4.0 | 4.1 | 4.9 | 5.2 | 4.3 | 4.7 | 4.9 |
| Palmitic | 6.5 | 6.3 | 7.2 | 7.5 | 6.6 | 7.0 | 6.9 |
| Myristic | 0.06 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Arachidic | 1.6 | 1.8 | 2.1 | 2.1 | 1.6 | 1.8 | 1.8 |
| Palmitolic | 1.0 | 1.1 | 1.1 | 1.3 | 0.9 | 1.1 | 1.1 |
| Stearic | 0.8 | 0.8 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Oleic (18:1) | 45.9 | 42.0 | 41.4 | 39.5 | 44.0 | 43.7 | 43.0 |
| Linoleic(18:2) | 26.6 | 25.1 | 25.0 | 20.9 | 25.1 | 24.8 | 23.5 |
| Behenic | 1.8 | 1.4 | 1.3 | 1.2 | 1.0 | 0.8 | 1.9 |

The results of fatty acid composition of different oil samples after six months of extraction and inoculation are given in **Table- 4.** The continuity is found in case of gradual reduction in the amount of unsaturated fatty acids content in different oil samples from three months to six months of ageing. Little increase in the levels of saturated fatty acid contents is also recorded in different oil samples in response to increase in the age (three months to six months). The increase in fatty acid content in a little amount in all of the samples could be due to significant decrease in concentration unsaturated fatty acids because of that naturally, saturated fatty acid values seems to be increased. In fact there is no change in saturated fatty acid content due to the absence of any double bond in carbon chain.

Great variations in unsaturated fatty acid contents are obtained due to the effect of fungal pathogens which deteriorated the seeds. These fungi also secrets enzymes leading to degradation of the unsaturated fatty acids to form free fatty acids in the oil which spoils the oil and changes color as well as odour of the oil. Jain (2008) reported a rapid increase in concentration of free fatty acids in damaged seeds by fungal invasion. Embayed *al.* (2006) observed a reduction in carbohydrate,

  

reducing sugar and crude fat due to *Fusariumoxysporum* in legume seeds. Change in the composition of fatty acid with ageing of oils is also studied by Anna*et.al*. (2006)

**Conclusion**:

The change in lipid content and fatty acid content in deteriorated (infected) seeds is due to the eattack of pathogens and ageing. Both oxidative and microbial rancidity increases with ageingdecompose the oil and reduces the amount of different unsaturated fatty acids in deteriorated (Infected) seeds than normal seeds. The microbial rancidity occurs in large amount in infected seeds it leads to produce unpleasant odour in oil. It also leads to change in color and forms free fatty acids in oil.

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