International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 2, Issue 3, March 2015
Available at http://internationaljournalofresearch.org

Biochemical Changes of Storage Pulses in Different Conditions

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ABSTRACT
In this study three pulses were taken which are widely uses in India. These pulses are Urad (black gram), Arhar (red gram) and Masoor (massor). Estimation of concentration of Protein, Carbohydrate and Fatty acids were done with fresh pulses, then they were stored for three months in different storage conditions like in polythene, earthen pots, papers, plastic cans and in jute sacs. After Storage the concentration of Protein, Carbohydrate and Fatty acids were decreased. Before the storage of pulses analysis the concentration of protein, carbohydrate and fatty acids. Higher protein concentration (0.78 mg/ml) present in Red gram and lowest concentration in (0.33 mg/ml) in Massor. After storage of pulses in jute bag, polythene, and earthen pots concentration of protein, carbohydrate and fatty acids were decreased. Highest protein degradation found in Black gram (0.29mg/ml) and lowest in Massor (0.11mg/ml). In Pulses highest protein degradation are found in jute Bag (0.029mg/ml) and lowest in polythene (0.087mg/ml). Highest total Carbohydrate degradation found in Massor and lowest in Red gram. In Pulses highest total carbohydrate degradation are found in Jute Bag (0.05mg/ml) and lowest in Pollythene (0.09mg/ml). Highest Fatty Acid degradation is also found in Massor and lowest in Red gram. In Pulses highest Fatty Acid degradation are found in Clay Jar (0.06mg/ml) & Jute Bag (0.06mg/ml) and lowest in Pollythene (0.09mg/ml).

Key words- Pulses, Storage Methods, Food Spoilage

INTRODUCTION
Pulses production in any country varies from year to year and hence the grains should be stored strategically from years of overproduction for use in year of under production. Grain quality after harvest is influenced by a wide variety of biotic and abiotic factors and has been studied as a stored grain ecosystem. Spoilage of stored grain by fungi is determined by a range of factors which can be classified into four main groups including (a) intrinsic nutritional factors, (b) extrinsic factors (c) processing factors and (d) implicit microbial factors. The factors produce fungal colonization within the stored grains (Wallace and Sinha, 1981; Sinha, 1995). Bio-deterioration of various types of harvested and stored products due to ubiquitous presence of molds is a chronic problem in tropical and subtropical countries which gets aggravated due to hot and humid climate (Logrieco *et al.*, 2003). Food products are a rich nutrient source that will attract both bacterial and fungal colonizers. As such, the food product can be regarded as an ecological resource. After successful colonization of the product, its nutritional properties are altered. When the nutritional value, structure, and taste of the product are negatively influenced, this colonization is called food spoilage (Dijksterhuis *et al.* 2013). In present study Urad (black gram), Arhar (red gram) and Masoor (massor) pulses were used.

MATERIALS AND METHODS
1. **Bio-Chemical Test-** Before storing the meals we are investigate the concentration of protein, carbohydrate and fatty acid by following biochemical test.

1.1 Protein Estimation by Lowry's Method
Extraction of Protein from sample
Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500mg of the sample and grind with a pestle and mortar

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in 5-10 ml of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of Protein

Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes. Pipette out 0.1ml and 0.2ml and 0.2ml of the sample extract in two other test tubes. Make up the volume to 1ml in all the test tubes. A tube with 1ml of water serves as the blank. Add 5ml of reagent C to each tube including the blank. Mix well and allow standing for 10 min. Then add 0.5ml of reagent D, mix well and incubate at room temp in the dark for 30min.Blue colour is developed. Take the readings at 660nm. Draw a standard graph and calculate the amount of protein in the sample. This procedure can be done 3 times for accurate result.

1.2 Determination of total Carbohydrate by Anthrone Method;-

Estimation of Carbohydrate :-

Weigh 100mg of the sample into a boiling tube. Hydrolyse by keeping it in a boiling water bath for three hours with 5ml of 2.5ml N-HCL and cool to room temperature. Make up the volume to 100ml and centrifuge. Collect the supernatant and take 0.5 and 1ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6 and 0.8 and 1ml of the working standard.'0' serves as blank. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Then add 4ml of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green on dark green color at 630nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the axis. From the graph calculate the amount of carbohydrate present in the sample tube. This procedure can be done 3 times for accurate result.

1.3 Estimation of Free Fatty Acids

Dissolve 1-10g of oil or melted fat in 50ml of the neutral solvent in a 250ml conical flask. Add a few drops of phenolphthalein. Titrate the contents against 0.1N potassium hydroxide. Shake constantly until a pink color which persists for fifteen seconds is obtained. This procedure can be done 3 times for accurate result.

RESULTS

1. **Biochemical Analysis: -** Before storage of pulses in different condition we can analysis the concentration of Protein, Carbohydrate and Fatty Acid.

1.1 Protein Estimation: - Higher protein concentration (0.78 mg/ml) present in Red gram. And lowest concentration in (0.33 mg/ml) in Massor are shown in Table 1.

Table 1. Concentration of Proteins (mg/ml) in pulses

S.N.	Types of Pulses	Concentration of protein before storage (mg/ml)
1	Black gram	0.58
2	Red gram	0.78
3	Massor	0.33

1.2 Total Carbohydrate estimation: - Higher Total Carbohydrate present in (0.91mg/ml) in Black gram and lowest present in (0.7mg/ml) Massor are shown in Table 2.

Table 2. Concentration of Carbohydrate before storage (mg/ml) in pulses

S.N.	Pulses	Concentration of Carbohydrate before storage (mg/ml)
1	Black gram	0.91
2	Red gram	0.73
3	Massor	0.7

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1.3 Fatty Acid Estimation: - higher present Fatty Acid in (0.81mg/ml) in Black gram and lowest present in (0.73mg/ml) Red Gram are shown in Table 3.

Table 3. Concentration of Fatty Acid before storage (mg/ml) in pulses

S.N.	Pulses	Concentration of Fatty Acid before storage (mg/ml)
1	Black gram	0.81
2	Red gram	0.73
3	Massor	0.77

2. Biochemical Analysis: - After storage of Pulses in different condition presence of protein, carbohydrate and Fatty acid can be estimate.

2.1 Protein Estimation: - Highest protein degradation found in Black gram (0.29mg/ml) and lowest in Massor (0.11mg/ml). In Pulses highest protein degradation are found in Jute Bag(0.029mg/ml) and lowest in Polythene (0.087mg/ml). Shown in Table 4.

Table 4. Concentration of Proteins (mg/ml) in pulses

S.N.	Pulses	Concentration of protein after storage (mg/ml)		
		Jute Bag	Pollythene	Clay Jar
1	Black gram	0.029	0.073	0.031
2	Red gram	0.066	0.087	0.069
3	Massor	0.10	0.16	0.11

2.2 Total Carbohydrate Estimation: - Highest Total Carbohydrate degradation found in Massor and lowest in Red gram. In Pulses highest total carbohydrate degradation are found in Jute Bag (0.05mg/ml) & lowest in Polythene (0.09mg/ml). Shown in Table 5.

Table 5. Concentration of Total carbohydrate (mg/ml) in pulses

S.N.	Pulses	Concentration of Total carbohydrate after storage(mg/ml)		
		Jute Bag	Pollythene	Clay Jar
1	Black gram	0.050	0.089	0.054
2	Red gram	0.066	0.090	0.07
3	Massor	0.049	0.070	0.05

2.3 Fatty acid Estimation: - Highest Fatty Acid degradation is also found in Massor and lowest in Red gram. In Pulses highest Fatty Acid degradation are found in Clay Jar (0.06mg/ml) & Jute Bag (0.06mg/ml) and lowest in Polythene (0.09mg/ml) in Table 6.

Table 6. Concentration of Fatty acid (mg/ml) in pulses

S.N.	Pulses	Concentration of Fatty Acid after storage(mg/ml)		
		Jute Bag	Polythene	Clay Jar
1	Black gram	0.08	0.09	0.096
2	Red gram	0.07	0.09	0.075
3	Massor	0.06	0.078	0.06

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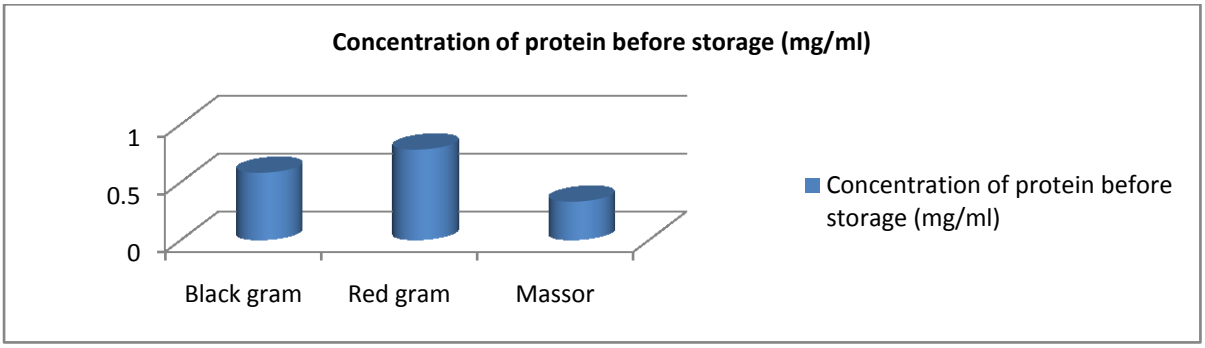
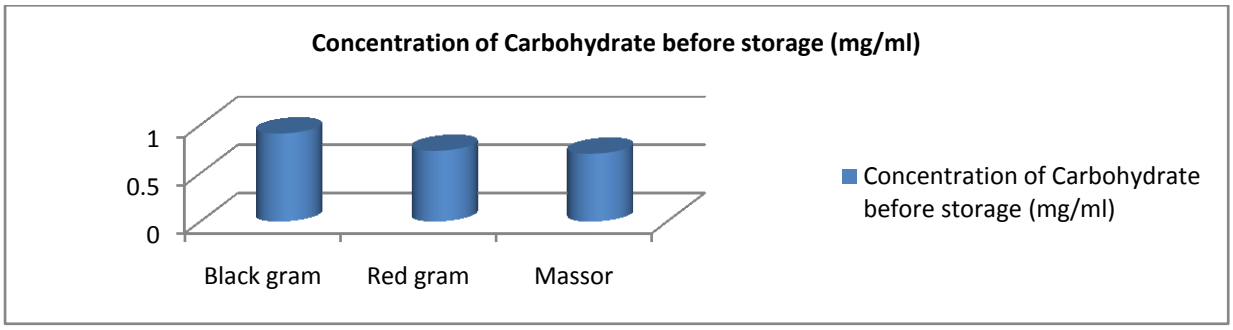
Before the storage of pulses analysis the concentration of protein, carbohydrate and fatty acids. Higher protein concentration (0.78 mg/ml) present in Red gram and lowest concentration in (0.33 mg/ml) in Massor. After the storage of pulses in Jute Bag, Pollythene, and Clay Jar concentration of protein, carbohydrate and fatty acids

Fig 1 : Different storage conditions.

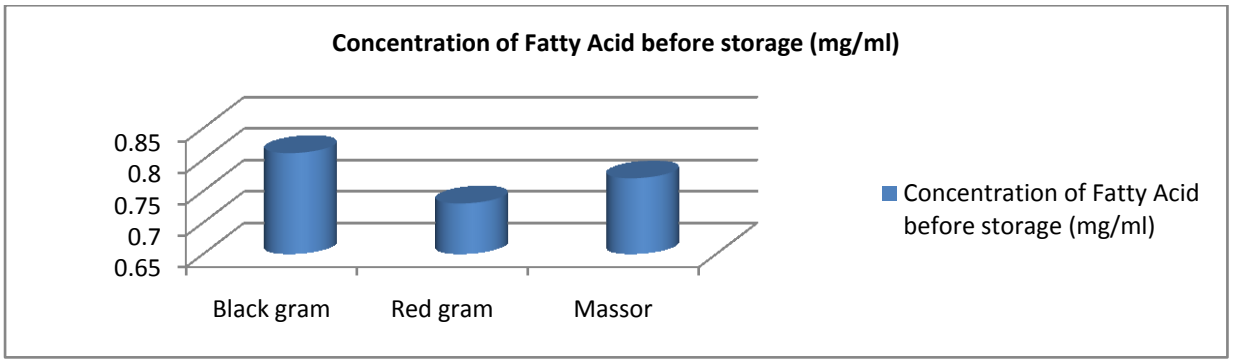
Graph 1. Shows Concentration of Protein before Storage (mg/ml)



Graph . 2 Shows Concentration of Total Carbohydrate before Storage (mg/ml)



Graph 3. Shows Concentration of Fatty Acid before Storage (mg/ml)



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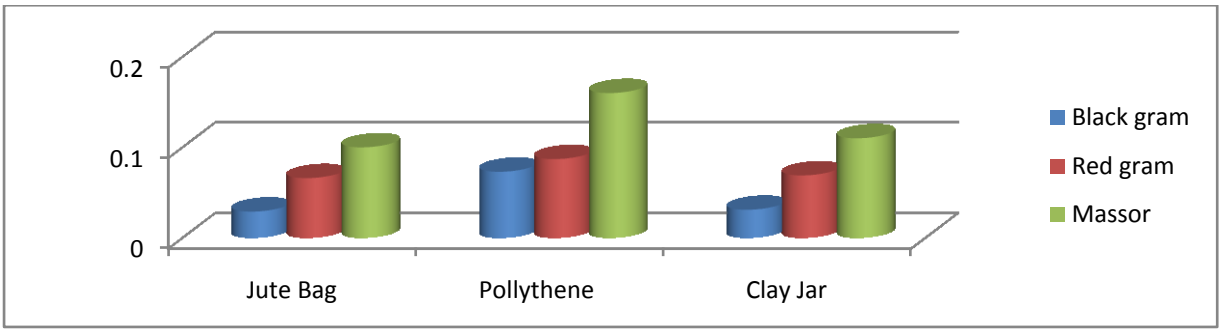
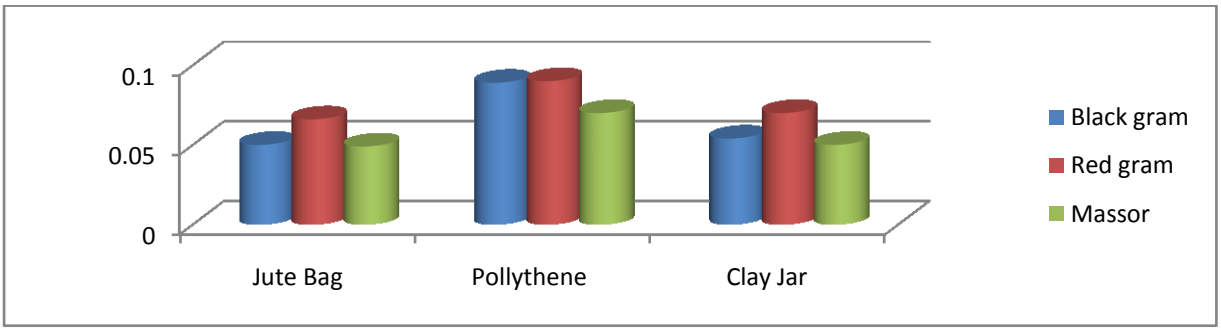
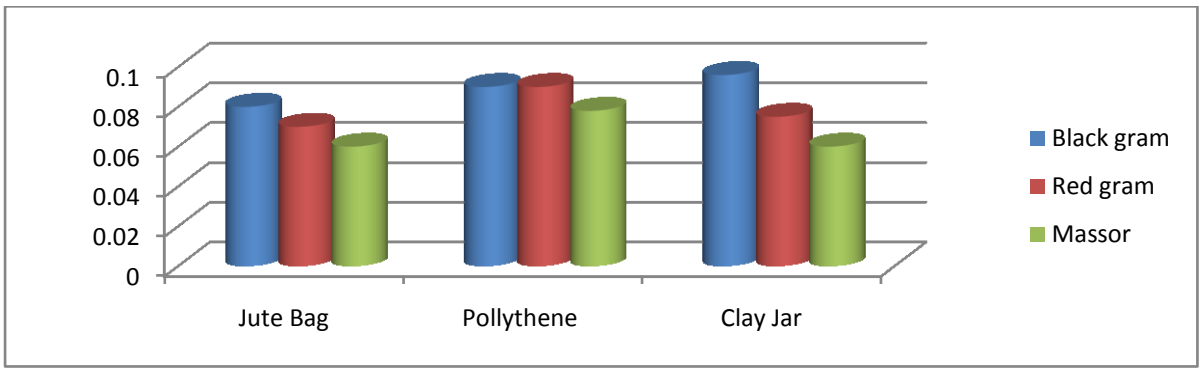
Graph 4. Shows Concentration of Protein after storage in Different Condition (mg/ml)



Graph 5. Shows Concentration of Total Carbohydrate after storage in (mg/ml)



Graph No. 6 shows Concentration of Fatty acid after storage (mg/ml)



are decreased. Highest protein degradation found in Black gram (0.29mg/ml) and lowest in Massor (0.11mg/ml). In Pulses highest protein degradation are found in Jute Bag (0.029mg/ml) and lowest in Pollythene (0.087mg/ml). Highest Total Carbohydrate degradation found in Massor and lowest in Red gram. In Pulses highest total carbohydrate degradation are found in Jute Bag (0.05mg/ml) and lowest in Pollythene (0.09mg/ml). Highest Fatty Acid degradation is also found in Massor and lowest in Red gram. In Pulses highest Fatty Acid degradation are found in Clay Jar (0.06mg/ml) & Jute Bag (0.06mg/ml) and lowest in Polythene (0.09mg/ml).

DISSCUSSION

Most of the households surveyed used bags to store pulses often with no use of chemical insecticides **(**Diop *et. al* 1996). In the present study three pulses Black gram, Red gram and Massor are tested. Before storage test the concentration of Protein, Total Carbohydrate and fatty acid. Then the pulses are stored in Different Condition (Jute Bag, Polythene, and Clay Jar). Storage of pulses in Jute bag and Clay jar are impact on the quality pulses. And decreased the

concentration of Protein, Total Carbohydrate and fatty acid. Contamination occurs through small quantities of spores contaminating the grain as it is going into storage from the harvest in handling and storage equipment or from spores already present in storage structures (IRRI, 2006). In India pulses are eat as a main dietary food so that this study show that storage of pulses in Jute bag and Clay Jar are impact on decreasing the concentration of protein, total carbohydrate and fatty acid due fungi abundant.

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