STUDIES ON THE MOISTURE SORPTION ISOTHERMS OF SORGHUM, MILLET AND MAIZE GRAINS AND THEIR MALTS IN RELATION TO MICROBIAL SHELF STABILITY.

BY

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A Thesis submitted to the Postgraduate School, Ahmadu Bello University, Zaria in partial fulfilment of the requirements for the award of Master of Science degree in Microbiology.

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DECLARATION

I declare that this thesis is entirely a record of my own research work and has not been part of any presentation for any other qualification.

OLADUNMOYE, M.K

CERTIFICATION

This thesis entitled "Studies on the Moisture Sorption Isotherms of sorghum millet and maize grains and their malts"in relation to microbial shelf stability by Oladunmoye Muftau Kolawole meets the regulations governing the award of Masters of Science degree of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the memory of my late friend, Mr Bisiriyu Ajibike Wasiu (1967 - 1996).

ABSTRACT.

Moisture equilibrium behaviour for absorption of water by three cereal grains of industrial importance: Sorghum (Sorghum bicolor Sk 5912), millet (Pennisetum americanum EV) and maize (Zea mays Ex-Borno) and their malts were studied at 20, 30 and 37°c over a water activity range of 0.1 - 0.95. The malts were found to exhibit a higher equilibrium moisture content thean the corresponding raw grains at water activity > 0.65 at all temperatures.

Analysis of the Isotherms showed that the malts can tolerate more moisture than the grains. Futhermore, the results indicated that the storage stability of both the grains and their malts decreased with increase in temperature. The monolayer values of the grains and their malts were determined and which tended to decrease in relation to increase in storage temperatures. Isoteric heat of desorption was also determined and which was shown to be higher in the grains than the malts; but decreasing in relation to storage temperatures.

The microbial load on the raw grains was also estimated. The total count showed that millet had the higher value $5.76x10^5$ cells per gram, followed by sorghu.n $2.38x10^5$ cells/gram while maize had the least value of $1.8x10^5$ cells/gram. Viable counts showed that sorghum had the highest value of $1.81x10^5$ efu/g. followed by millet $1.03x10^5$ cfu/g. and maize had the least value of $3.8x10^4$ cfu/g. respectively. The identity of microbial isolates showed that fungi are the most abundant contaminating/spoilage organism of cereal grains. The mold genera isolated include: Penicillium, Fusarium, Aspergillus, Pythium, Phytophtora, Cladosporium, Colleotrichum, Al ternaria, Mucor, and Curvularia. The yeast isolates include the genera Saccharomyces, Candida and Torula.

The mold genera Pythium (AA), Curvularia (DD), Fusarium (GA) and Phytophtora (KA) were isolated from the interior of the grain.

The bacterial isolated included the genera Pseudomonas, Klebsiella, Staphylococcus, E Coli, Acinetobacter, Bacillus and Proteus.

All the isolates are external floral of the cereal grains expect the Pseudomonas SP.

The time of appearance of mould was also determined. Mold growth was detected earlier at 95% relative humidity than 85% at all the storage temperatures. But at any particular relative humidity, the time of visual detection of mold increased as temperature increased. It is therefore recommended that cereal grains be stored in the raw state than the malt form at low moisture content <. 6% for good storage stability.

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CHAPTER ONE

INTRODUCTION

Grain is a collective term applied to cereals and crop plant belonging to the grass family Graminae that are grown for their edible starchy seeds (Leonard, 1963). The term cereals applies to the entire plant as well as the grains and is sometimes loosely applied to the grain products. About half the ploughed land of the world is given to the growing of principal cereal (Wudiri and Fatoba, 1991). Cereal grains dominate world agricultural production because they directly or indirectly provide a large portion of human sustenance (Leonard, 1963). They are by far the most important source of concentrated carbohydrate for man and his animals (Nwashike and Abba, 1986). Cereal such as millet, sorghum, maize and rice are the chief items in the diet of many people particularly, in the Orient because they are relatively cheap source of calorie in terms of effort and cost of production (Leonard, 1967).

In Nigeria, grains are extensively eaten especially in the northern parts. The various forms of food prepared from food grains include: brabisco, tuwo, fura, kunu, waina, ogi, akamu and koko (Nwashike and Abba, 1986). Grains can also be fermented locally to give alcoholic drinks like burukutu and ogogoro (Okafor, 1987).

In the industries, grains such as maize, millet and sorghum serve as the essential raw materials. These grains can be processed in the industries to produce:

(i) Livestock feeds for ruminants and monogastric animals.

(ii) Amylose for plastics, cellophane, films and other products.

(iii) Carotenoid pigments and vitamin A.

(iv) Zein for special fabrics.

(v) Fats and oil

(vi) Pops and confections

(vii) Composite flour

(viii) Bakery products, snacks food and pasta products.

(ix) Instant pudding mixes, glues and branch chain starches

(x) Fermentable sugars used in brewing industries and

(xi) Malt drinks that are non-alcoholic and rich in B vitamins (Hulse et al, 1980).

In keeping with the Nigerian government policy on encouraging import substitution and self reliance with respect to raw materials for her manufacturing industries, it becomes necessary to examine the suitability of locally available raw materials (grains) for our food industries (Okechukwu and Okaka, 1991), particularly the brewing and baking industries where it is becoming increasingly difficult to import sufficient barley malt and wheat respectively. Recent researches had revealed that locally grown cereal grains such as millet, sorghum and maize could be used in beer production since the most prominent raw materials is any fermentable carbon source capable of microbial transformation into enthanol and these grains (sorghum, millet and maize) offer potential sources of these fermentable sugar and a good base for biomas growth (Kolawole et at, 1988).

Meaningful progress has been achieved in the attempt to boost grain production in Nigeria. However, the problem of grain shortage like that of any food either for domestic consumption or industrial use is not only that of inadequate production but of inadequate storage, processing and preservation (Awoyemi, 1984). Effort to combat grain shortage has centered on:

(a) Increasing grain production at accelerated rate through the use of high quality hybrid seeds, fertilizer and mechanisation.

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(b) Improving post harvest storage and handling facilities and

(c) Enhancing their availability by improving preservation techniques.

Report presented in this thesis addresses the latter pair of research foci. The determination of moisture sorption isotherm which is a very useful approach to the study of absortion of water by solid substances such as those occuring in cereal grains on three selected ones (sorghum, millet and maize) was carried out. An isotherm is simply a curve describing the equilibrium relationship, at a specific temperature, of the amount of water sorbed by the substance on one hand and the vapour pressure or relative humidity on the other hand and this influences the degree of moldiness in the substances concerned.

Although, moisture sorption isotherms have been determined for cereal products in order to study their shelf life (Iglesias and Chirifie, 1982; Pixton, 1982; Denloye and Ade-John, 1985 and Achoba et at 1991). However, there have been few reports on the sorption isotherms of the native raw grains obtained from the farm and their malts. So far the emphasis has been on chemical assaying for proximate nutrient, amino acid content, vitamin E, free fatty acid formation, hydroperoxides and aldehydes (Iglesias and Chififie, 1982; Banigo, 1984 and Chung and P fost, 1967).

In this study, sorption isotherms of these three currently tested grains for industrial use and their malts will be determined to ascertain the best set of conditions under which they can be stored to minimise or prevent the growth of spoilage micro-organism. Three temperature (20, 30 and 37°c) were used to simulate at least three different seasons of the year.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 CEREAL GRAINS

Cereals are flowering plant in the grass family Graminae cultivated for the good values of the grains. These include plants like sorghum, millet, maize, wheat, oats, barley, rice and acha. The most important cereal being grown in Nigeria are sorghum, maize, millet, rice and wheat (Wudiri and Fatoba, 1991).

2.1.1 *WORLD DISTRIBUTION OF CEREALS*

The principal regions of grain production are in the North America, China, Europe, India, Argentina and Australia. Northern Africa grows considerable barley and wheat. The most important areas of grain production exclusive of China are within Europe (Leonard and Martins, 1968). About 95% of world rice crop is produced and consumed in Monson Asia (Sumaro, 1993). Corn is produced from latitude 58°N in Canada and Soviet Union to about 45° S (Jungenheiner, 1976). About half the world acreage is in the U.S.A (Bland, 1971). Sorghum is fairly important throughout the world but particularly so in India, Africa and U.S.A (Dogett, 1988). Millet is used as food primarily in Eastern and Southern Asia, parts of Africa and Russia (Wudiri and Fatoba, 1991).
The total world production of cereal grain varies little from year to year. Shortages of certain crops in some areas are often balanced by above-average production elsewhere. The principal grain export nations are : Australia, Argentina, Canada and U.S.A. Europe has long been the world major grain importer (Bland, 1971).

2.1.2 ORIGIN AND EARLY HISTORY OF CEREALS

Cultivation of all cereal grains except oat and rye began before the dawn of history. Wheat, barley and millet were cultivated in many parts of the old world 10 - 15 thousand years before the Christian era (Haferkamp et al, 1953). Civilization were founded on the basis of cereal grains because when man learnt to cultivate cereals, he did not need to wander in search of food. The progenitors of all cultivated cereals were wild grasses that shed seeds at maturity (Hughes and Metacaife, 1972). Archaeological evidences suggest possible centre of origin of th cereals. Mutation, natural selection and mass selection by man gradual transform these wild grasses into plants worthy of domestication (Jurgenheimer, 1976).

2.1.3 CLIMATIC FACTORS INFLUENCING CEREAL PRODUCTION

Climatic factors determine those areas where each of the cereals can be grown economically. Within areas favourable to production, weather conditions have a marked influence on growth, yield and quality of harvested crop. Cereal grains grown in temperate climate of the world include: wheat, maize, rye, barley and oats, while sorghum, rice and millet grow in the hot climate (Leonard and Martins, 1968; Hulse et al, 1980).

2.1.4 CEREAL PRODUCTION IN NIGERIA

Nigeria has a total land area of 924,000 sqkm or 98.3million hectare (Olugbemi, 1988). 75% or 73.7ha of this is considered suitable for cultivation. 13 million ha is given to the growing of the principal cereals with a combined production of 15.303million tons in 1989 (Wudiri and Fatobi, 1991). The major cereals produced for food and raw materials in

Table 2.1: production figures of selected major food crops in Nigeria 1970-1989 ('000 tonnes)

Year	Rice	Maize	Sorghum	Millet	Wheat	Cassava	Yam	Potatoes
1970	344	1220	1600	2800	6	8600	12,000	204
1971	389	1042	1340	2688	7	8600	12,000	204
1972	447	1182	3561	3048	7	7900	12,000	205
1973	489	600	3000	2150	5	8100	12,500	204
1974	525	610	3500	2800	6	8800	13,000	216
1975	515	1260	3900	3000	18	10,500	12,950	199
1976	387	1295	3700	2900	20	10,800	12,880	217
1977	408	1395	3010	2600	21	10,600	12,995	219
1978	514	1450	3000	3100	21	10,500	12,339	240
1979	752	1500	3785	3130	21	11,000	13,090	251
1980	1090	1550	3800	3130	21	11,000	13,090	260
1981	1242	1580	3850	3230	21	11,000	12,895	260
1982	1250	1750	3275	3850	30	11,700	10,990	270
1983	1279	1600	2660	2300	35	9950	9089	240
1984	1300	1800	3551	3683	45	11,800	9730	260
1985	1428	1800	4822	4001	39	11,910	10,080	258
1986	800	1735	2636	2338	25	12,100	10,080	260
1987	1780	1357	3229.13	2286.54	110	12,100	10,990	260
1988	2000	3773.54	4920	5134	260	13,000	10,950	251
1989	2020	5628.02	3280	4090*	285	13,200	11,095	206

Sources: (1) FDARD Annual Report, 1989
(2) NCRI Survey, 1988
(3) Ogungbile and Abalu, 1982
(4) Olayide, *et al*, 1972
(5) Wudiri *et al*, 1989
(6) J. Gana, 1989

* Estimates

Table 2.2: Supply and demand for maize in Nigeria 1970-1989 ('000 Tonnes)

Year	Demand Estimate	Cultivated Area (000ha)	Domestic Production	Imports	Deficit/ Surplus
1970	1008	1016.7	1220	N.A	-212
1971	1008	694.7	1042	N.A	-34
1972	1139	788	1182	N.A	-43
1973	1189	400	600	—	589
1974	1231	406.7	610	—	621
1975	1299	804.0	1260	3.5	35.5
1976	1239	863.3	1295	1.3	-57.3
1977	1529	930.0	1395	36.8	97.2
1978	1779	966.7	1450	66.3	262.7
1979	2119	1000.0	1500	87.3	531.7
1980	2279	1033.3	1550	70.3	658.7
1981	2450	1053.3	1580	105.7	764.3
1982	2385	1166.7	1750	144.1	490.9
1983	2506	1066.7	1600	326.1	279.9
1984	2634	1200	1800	13.6	820.4
1985	2871	1200	1800	—	1070
1986	2881	1156.7	1735	—	1146
1987	3580	9047	1357	—	2223
1988	3840	2513.4	3773.54	—	66.46
1989	4000*	3752	5628.02*	—	1628.02

Sources (1) Olayide *et al*, 1978
(2) NCRI Survey, 1985

* Estimates

Table 2.3 Supply and demand of sorghum and millet in Nigeria 1970-1989 ('000 Tonnes)

Year	SORGHUM				MILLET			
	Demand estimate	Cultivated area (000ha)	Domestic production	Deficit/ surplus	Demand estimate	Cultivated area (000ha)	Domestic production	Deficit surplus
1970	3450	1334	1600	1850	2168	2800	2800	-632
1971	3360	1117	1340	2020	3005	2688	2688	317
1972	3561	2967.5	3561	0	3900	3048	3048	852
1973	3810	2500	3000	810	4015	2150	2150	1865
1974	3695	2916.6	3500	195	4225	2800	2800	1425
1975	3731	3250	3900	-169	4696	3000	3000	1696
1976	3771	3083.3	3700	71	4695	2900	2900	1795
1977	3009	2508	3010	899	4909	2600	2600	2309
1978	4191	2500	3000	1191	5395	3100	3100	2295
1979	4575	3154.2	3785	790	5759	3130	3130	2629
1980	4307.5	3166.6	3800	507.5	6059.82	3230	3230	2839.82
1981	4509.9	3208	3850	6599	5900	3850	3850	2050
1982	4721.9	2729	3275	1446.9	6558	2300	2300	4258
1983	4443.9	2216	2660	1783.9	7019	2683	3683	3336
1984	5176.2	2959	3551	1625.1	7811.68	4001	4001	3810.68
1985	5419.5	4018	4822	5975	7612	2338	2338	5274
1986	5674.2	2197	2636	3038.2	7916*	2286	2286	5630
1987	5940.9	2691	3229.13	2711.77	7916*	5134	5134	2782
1988	6220.1	4100	4920	1300.1	7616*	4090	4090	3826
1989	6512.5	2734	3280	3232.5	7916*	5109	5109	2507

Sources: (1) CBN Annual Report, 1987
(2) Ogungbile and Abulu, 1982
(3) J. Gana, 1987

* Estimates.

the industries are sorghum, millet, maize and rice (Olugbemi, 1988).

2.2 SORGHUM (Sorghum Vulgare)

Sorghum is one of the two cereal crops (the other, millet) that constitute the largest volume of cereal produced and consumed in Nigeria (Aluko, 1992). Sorghum is being grown in 20 States of the Federation (Olugbemi, 1988). Nwashike and Abba in 1986 reported that sorghum together with millet account for over 60% of total cereals grown in Nigeria. With the ban on importation of barley, wheat and maize coupled with the proliferation of breweries and search by flour millsfor alternative sources of local raw materials, the demand for sorghum has been staggering in recent years (Olugbemi, 1988).

Sorghum is a cereal of remarkable genetic diversity (Maria et al, 1995), and the most important character is the ability to tolerate and survive under condition of continuous or intermittent drought that results from low and uncertain rainfall. This ability is hormonally controlled (Hulse et al, 1980).

2.3 MILLET (Pennisetum Americanum)

Millet is the most drought resistant of all the cereals (Jiapeng, 1993) and they thrive well in drier parts of the world where maize and sorghum can not grow to maturity without irrigation. It is widely grown in the Guinea Savannah as a hunger crop because it is earliest crop to be harvested when the food reserve is at its lowest level. In terms of total area cultivated and production it is equal or higher to sorghum (Table 2.1).

2.4 MAIZE (Zea Mays)

Maize is being grown throughout the counrty but it thrives and gives highest yield per unit area in the Savannah zone. Current national production stood at 5,288,020 tons on an area of 3.5million ha. It has been estimated (Awoyemi, 1986) that only about 20% is being consumed directly by man, it forms 50 - 60% of livestock feeds and 20 - 50% of brewery raw materials, the balance being used in other industries such as textiles and starch. Maize contribute to the economy of Nigeria in terms of increasing Gross Domestic Product (GDP), employment, income to farming communities and raw materials to industries (Olugbemi, 1988).

2.5 FOOD PRODUCTION AND STORAGE

The problems of food shortage in Nigeria and developing world in general is of great concern. The situation is further compounded by inadequate technological know-how for production, processing and storage of foods. The current economic crunch, under development, inefficient management of resources and lack of concrete policy guidelines on food have further worsen the situation (Achoba, 1989).

Though accurate figures are not available, it has been reported (Adeyemo and Awoyemi, 1984) that 40% or more of agricultural produce in the third world is lost during storage/marketing (Table 2.4). Food processing, preservation and storage are important aspects of food production whereas the production of food with adequate technological backing may be in increase, the post harvest problems of processing, preservation and storage are not keeping pace with production (Eton, 1985). The consequences of such lapses is evident in massive losses and wastage (Gomwalk, 1985). There is

Table 2.4 : IMPORTANT CROPS IN NIGERIA AND ESTIMATED LOSSES

COMMODITY	PRODUCTION/'000TONS	ESTIMATED LOSS %
Cereals:		
Rice	2,500	0 - 15
Wheat	-	-
Sorghum	3,680	0 - 37
Maize	-	0 - 58
Oat	2,000	5.5 - 70
Millet	3,000	0.5 - 20
Non-Cereal Legumes:		
Cowpeas	11,000	6 - 0
Groundnut	400	1 - 0
Roots/Tubers:		
Yam	18,000	10 - 50
Cassava	30,000	10
Sweet Potato	1,800	15
Carrot	250	25
Potato	1,500	5 - 40
Vegetables:		
Onion	3,800	15 - 20
Tomato	6,000	20 - 60
Plantain	15,000	35 - 100
Cabbage	2,500	37
Okra	500	20
Fruits:		
Banana	35,000	20 - 80
Papaya	8,500	40 - 100
Citrus	35,000	20 - 95
Grape fruits	100,000	30
Pineapple	8,000	20 - 70

Adapted from Adeyemo R. and Adeyemi A.I (1984).

therefore the need to device an effective and efficient ways of storing our produce or processing them before storage to decrease wastage (Adelusi, 1984).

Cereal grains like any other food are subject to massive loss both before and after harvest. Millions of tons of cereals are wasted each year through spoilage of various sorts (Table 2.4). 50% of all food grains harvested are lost before consumption in developed countries while in Africa and some South American countries it is estimated that 30% of annual harvest is lost (Christensen and Kaufmann, 1968). Yet hunger is wide-spread and population increases ever more rapidly. Protection of food grains supplies through sound storage practices is thus a matter of the most vital importance.

2.6 FOOD MICRO-ORGANISMS AND THEIR SIGNIFICANCE

Food may contain only one nutrient as in glucose for energy production or may contain varieties of nutrient as in milk and perform many functions. Micro-organisms including bacteria, yeasts and moulds like other living organisms needs these nutrient for various functions (Baltzing, 1983). They gain access into foods through contamination or when they are deliberately introduced into them as in dairy or other fermentation industries (Carr, 1983) and also in palmwine where some microorganisms which are indigenous to the sap bring about fermentation (Okafor, 1972).

All raw food ranging from raw agricultural products, semi-processed and finished products are frequently contaminated by microorganisms and/or their products (Anderson, 1978). Although, shelf life of food depends on both the indigenous enzymes which bring about autolysis of food and the presence

of microorganisms which bring about spoilage. Different foods have different keeping qualities (Banwart, 1989). Some foods such as rice, maize, millet and sorghum will stay for a long time without showing obvious signs of deterioration while others such as fresh fruits, vegetables, meat and milk will only keep for a short time (Awoh and Egonnlety, 1986). The presence of microorganisms in food may be beneficial or harmful. When microorganisms are deliberately introduced for their useful metabolic activities, they produce useful by-products as in the production of yoghurt, lager beer, cheese and pharmaceuticals (Okafor, 1987). When microorganisms contaminates foods, they may be potentially harmful. This makes them unfit for human consumption and if consumed can lead to food borne diseases (Lindsey et at, 1983).

Certain moulds are capable of manufacturing chemical substances which are toxic to man and animals (William and Rao, 1989). These poisonous chemicals are called Mycotoxins (Marvins et at, 1995). One group aflatoxin produced by strains of Aspergillus flavus has been established as a great potential danger to livestock. A new mycotoxin called fumonisin B (FBI) recently been isolated from Fusarium Moniliforme which is implicated in hepatoxicity and equine leucoencephalomalacia, porcine pulmonary oedema, cancer promoting activity and also affect macrophage dependent immune system (Chartterjee and Mulchergee, 1994).

Certain undesirable conditions may also be produced by microorganisms (Bonwart, 1989). Some authorities consider food as being spoilt when it acquires changes in flavour, odour and constituency markedly different from its normal state. Banwart (1979) considered sight, smell and touch as parameters that can used to evaluate food qualities.

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2.7 FOOD SPOILAGE AND SHELF LIFE

The deterioration of food stuff is due to a number of interrelated factors which can be modified by man either by aggravating their effect through negligence or nullifying their impact by prudent and good storage techniques (Jarvis et al,1979).

Among the physical, chemical and biological factors that causes food spoilage include temperature, humidity, water content and microbial infestation. All these also determines the shelf life. The shelf life of a product (storage life or storability) is the period of time that a product is expected to maintain a pre-determined level of quality under specified storage condition (Shewfelt, 1986). To ensure a longer shelf life of a product, a combination of food preservation and packaging techniques would have to be employed. Boxal et al (1985) listed storage conditions, quality of raw materials, processing techniques and temperature as factors affecting the shelf life of a product. The ability of a food product to retain its nutritional content especially those that have protective roles also help in determining the shelf life of the product (Onigbinde and Akinyele, 1993).

2.8 GRAIN STORAGE

Just like any other stored product of the world, grains are attacked by a number of storage enemies. Moisture content, temperature of storage and some other factors must be taken into consideration when storing grains. The principal aim of grain storage is to fight all forms of grain loss and enable them to fulfil their most important task in national economy (Denloye and Ade-John, 1985).

Also, seasonal aspect of grain production and the need to have available throughout the year sufficient reserves of grains for the manufacturing of food products and for the provision of cattle and poultry feeds and seeds for sowing (Adeyemi and Awoyemi, 1984).

Broker et at (1974) listed drying, sealed storage, cooling or chilling and chemical treatment as the various methods available for cereal grain preservation. Microbial inhibition using chemical compounds like acetic acids, sorbic and benzoic acid has been demonstrated by some workers (Neves et al, 1994) on some food grains. Traditional methods of storing grains include aerial storage, storage in grand, platforms, cubs, dwelling, ventillated granaries, bags, baskets and gourds. Modern methods include plastics shelling, ventillated granaries reinforced concrete silos, corrugated iron silos, plastics sacks, metal drums, use of hermetically and non-hermetically seals systems, use of inert gases and pyramids (Apert, 1987). A large quantity of grain products are stored in undertakings in the food industries and in commercial and retail networks (Awoyemi, 1980).

2.81. WATER CONTENT AND TEMPERATURE OF STORED GRAIN

Labuza (1982) defined moisture content as the quantity of water held by a unit mass of a product expressed as percentage which be based on wet or dry weight of the product. In grains generally, moisture content exists in two forms (i) water of composition which is contained in the plant cell and (ii) free water on the surface of the cell (Apert, 1987), part of which is absorbed superficially by their cells. It is this free moisture content that is measured and this actually influences grain preservation. Cereals and legumes will not keep if

their moisture content exceeds 13% and 15% respectively (Adelusi, 1984). This therefore implies that there exists a safe storage at which the grain must be stored (Table 2.5). According to Anderson et at. (1976) grains that contain 15% moisture usually will keep during cold winter provided they have been cooled by aeration or by storing them in cold. Stored grains suffer damage when the moisture is sufficiently high to permit organisms (insects included) to thrive (Brooker et al, 1974). Grain infesting organisms are inactive when moisture content is below 9% and low temperature (Pixton, 1967). The high temperature which prevail throughout the year in tropics is a major problem connected with preservation of grains (Apert, 1987). The high temperature speeds up biological activities (Brooker et al, 1984). This in turn causes spoilage of cereal grains.

2.8.2 TYPES OF SPOILAGE IN CEREAL GRAINS

The type of spoilage of cereal grains is determined largely by its moisture content (Brooker et al, 1974; Adelusi, 1984). Grains that contains somewhat more than 20% moisture content usually sour due to fermentation of soluble carbohydrate (Pixton, 1982). Organic acids and heat are generated by the subsequent oxidation of carbon and hydrogen. Grains that contain 16 - 20% moisture usually heat and become mouldy as a result of fungal growth and respiration (Leonard, 1967). Grains when shelled without drying at 20% moisture content or more usually get soured and heated up in few days (Anderson, 1974).

Table 2.5: SAFE STORAGE MOISTURE LEVEL FOR SOME GRAINS

Cereal	Max. during harvest	Optimum at harvest	Usual when harvested	Required for 1yr.	Storage 5 years
Barley	30	18 - 20	10 - 18	13	11
Maize	35	28 - 32	14 - 30	13	10 - 11
Oats	32	15 - 20	10 - 18	14	11
Rice	30	25 - 27	16 - 25	12 - 14	10 - 12
Rye	25	16 - 20	12 - 18	13	11
Sorghum	35	30 - 35	10 - 20	12 - 13	10 - 11
Wheat	38	18 - 20	9 - 17	13 - 14	11 - 12

C.W. Hall (1957); D.W. Hall (1970); Matz (1969) and Sunha (1973).

Other forms of spoilage of cereal grains include:

(a) Damp grain heating. This causes caking and fermentation or rotten according to the percentage of oxygen in the air.

(b) A reduction of food value as a result of degradation of glutids protids.

(c) Misty smell in cereals

(d) Appearance of mycotoxin and fumonisin B1 and B2 due to fungal metabolism (SmithaKumeir, 1994).

(e) Discolouration of seeds

(f) Decrease in germinability and

(g) Loss in dry matter.

Grain is a living organism in which a variety of living processes occur (Agboola, 1985), the intensity of these depends on the ambient medium. If the latter favours the process of active metabolism in the grain cells which will inevitably leads to significant loss in grain weight and may be accompanied by deterioration on grain quality (Brooker et al, 1974) because it aids proliferation of cereal grain microorganisms.

2.8.3 CEREAL GRAIN MICRO-ORGANISMS

Right from harvesting, grain is populated by a large number of different types of bacteria, moulds and yeasts both ordinary and cosmopolitan (Gwant, 1991). The population continue to increase during the various handling operations which the grain undergo until such time it is consumed. The microflora of cereal grains and their products is made of a wide variety of bacteria and fungi (Leonard, 1967).

The kind and abundance depend on climatic factors under which the grains are produced, processed and stored (Agboola, 1985). Micro-organisms outside the grain are more abundant than those inside it. Parasites and saprophytes constitutes the internal

floral of grain which include Hehmithesperivin, Alternaria, Curvularia, Aspergillus Mucor, Xanthosoma, Pseudomonas, (Brooker, 1974). Saprophytes are the main components of the external microfloral of grains. These include mould genera of Aspergillus, Fusarium, Colleotrichum, Alternaria, Cephalothecium, Helmintosporium, etc, yeast and yeast like fungi such as Manilia, Torula, Candida, Saccharomyces, Hasenula and Cryptococcus are common. Pseudomonas, Micrococcus, Lactobacillus Enterobacteria, Bacillus and Achromobacteria are the commonly occuring bacterial genera on cereal grains (Harrold et al, 1972). It is the presence of these micro-organisms cn grains that usually brings about spoilage as a result of their metabolic activities. Most of these reactions that cause grain spoilage are related to moisture content and the environment which is usually assessed through the sorption isotherm studies of the grains (Denloye and Ade-John, 1985).

2.9 MOISTURE SORPTION ISOTHERMS

The concept of equilibrium moisture content is important in the study of food preservation (Achoba, 1991). The minimum moisture content under which food can be preserved is influenced by the ambient equilibium moisure content (Agbaji and Agbaji, 1989). The aim of drying food stuff is to lower the moisture content in order to lower the chemical and biochemical reactions (Labuza, 1970) to the bearest minimum and prevent the growth of spoilage microorganism (Agbaji et al, 1992).

A very useful approach to the study of the absorption of water by solid subtances such as those occuring in cereal grains is by means of its isotherms (Hylinka, 1974). When a product is exposed to an atmosphere of uniform relative humidity at constant temperature for a defined period of time, the intergranular

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relative humidity reaches a constant value known as the equilibrium relative humidity (erh) (Pixton, 1967). A plot of the moisture content of the product at equilibrium on a dry basis against the relative humidity is known as the moisture sorption isotherms of the product (Denloye, 1985). It is essential in enhancing the storability potential of the product (Labuza, 1982).

The relationship between equilibrium relative humidity (erh) and equilibrium moisture content (emc) has been known as basis to predict microbial growth and to determine the extent of enzymic reactions in food (Labuza et al, 1970; Fennema, 1985). This is because the 'emc' determines the minimum moisture content to which it is sensible to dry the food.

2.9.1 SORPTION ISOTHERM CURVE

The typical moisture sorption isotherm may be of adsorption (a moisturing process) or of desorption (a drying process). The isotherm varies with temperature (Agbaji and Agbaji, 1990) for different products. A close loop hysteresis has been observed by many workers (Houston, 1952; Benson and Richardson, 1955; Bushuk, 1956; Wrinkler and Ptost, 1967; Pixton and Wonbinton, 1971, 1976). **For most biological materials,** with the desorption isotherm showing longer equilibrium moisture content at a given relative humidity. According to Troller (1983) equilibrium will occur given sufficient time so that only one line is evident.

Three areas are apparent in most isotherms (Iglesia, 1982). In the lowest part of the curve, the water is in a mono-layer on the product or the so called bound water (Pixton, 1967) and is not available for biological activities. In the middle of the curve, additional layer of water is added to the mono-molecular layer film and there is rapid increase in moisture

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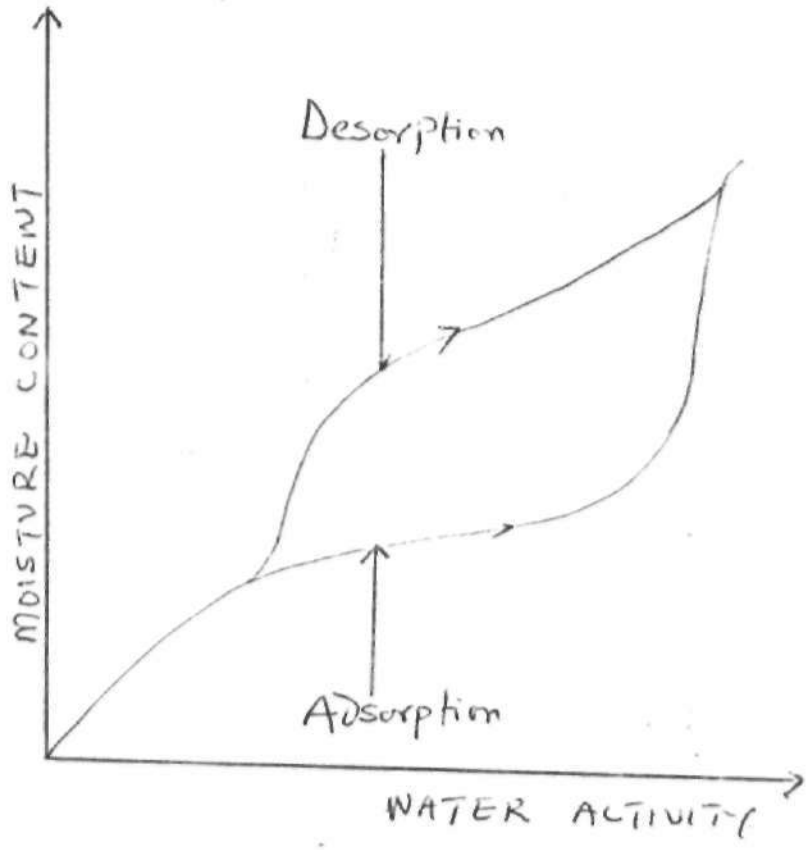


FIG 1: TYPICAL MOISTURE SORPTION ISOTHERM CURVE

(Labuza, 1983). In the upper region of the curve, some water becomes available to support the growth of some moulds and yeasts. In the upper part of the isotherm the moisture content increases rapidly with comparatively small incease in water activity and the product contains less water than is available for bacteria as well as fungal growth (Jarvis, et al, 1979).

This curve describing the water sorbed by a product as a function of relative humidity at a particular temperature is usually sigmoid showing no discontinutios. The shape of the curve is generally governor by the binding of the water within the product (Baki and Ohno, 1991).

As the binding energy decreases the relative humidity increases (Pixton, 1982) towards the end of the curve. This part of the line is almost linear curving gently and consistently (Ajibola, 1986).

Above 75% rh, the final part of the curve is concave to the moisture content axis. At this humidity, the capillary of the product is gradually filled with water and becomes saturated (Labuza, 1983). In this region, large moisture content increase results from small increase in relative humidity so that relatively large amount of water become available to be metabolically utilized by the product itself or by micro - organisms associated with it (Hung and Byerly, 1991). This encourages undesirable deterioration process in storage.

The relationship between moisture content and equilibrium relative humidity is not the same for absorbing and desorbing product (Kraener and Taylor, 1931). The desorption curve is closer to the equilibrium relative humidity axis (Agbaji et al, 1992). This phenomenon is termed hysterisis (Achoba, 1991). A number of theses have been advanced to explain hysterisis in grain. The ink bottle theory (Kraemer and Taylor, 1931) is probably the best known. For a given moisture content, therefore, a product has a range of equilibrium relative humidity values (Labuza, 1979).

.9.2 MOISTURE SORPTION ISOTHERM EQUATIONS

Equations for moisture sorption isotherms are of practical importance in drying (Boki and Ohno, 1992), in evaluating properties of dry mixes and in formulating stable dried products (Iglesias and Chriffe, 1976; Crapiste and Roststein, 1982; Ratti et al, 1989). Numerous mathematical equations have been used to describe sorption isotherms (Aguerre, Suanez

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and Viollaz, 1983); Ferro Funtan, et al, 1983). Seventy seven equations to represent sorption data were summarised by Van den Berg and Brum (1981). These equations were categorised into four models: Monolayer, Polymer sorption, Multilayer and Food sorption (Van den Berg and Brum, 1981). Langmnir (1918) and Freindlich (1926) equations represented monolayer model. The BET (Brunauer et al, 1938); Harkins and Jina, (1944) and Halsey (1948) equations represented multilayer models. Smith equation (Smith, 1947) was considered as a polymer sorption model, based on surface sorption and solution theory.

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Henderson (1952) and Chung and Pfost (1967) equations were emperical model proposed for food sorption. Baquet et al in 1976 concluded that the Hailwood and Horrobri (1946) equation seems to be the most versatile.

However, one of the best known relationship for predicting the equilibrium moisture content of cereal grains is the same emperical model proposed by Henderson (1952). Using Gibbs absorption equation, Henderson derived the following equation for the moisture equilibrium curve of biological products including grains:

$$1 - a = \text{Exp}\ (-Km^n)$$

Re-expressed as:

$$\text{Log}\ (-\ln\ [1-a]) = n\log m + \log K$$

where a = water activity $(\frac{RH}{100})$, n and k are constant depending on the product, m is moisture content as percentage of dry basis.

2.9.2 SORPTION ISOTHERM AND THE EFFECTS OF CHANGE IN TEMPERATURE

The relationship between moisture content of a product and the equilibrium relative humidity is different at different temperatures (Manuel Sa and Sereno, 1993).

Over the erh range of 20-80% for a given moisture content, the erh rises about 3% when the temperature is increased by 10°c (Achoba et al, 1991). Although, this is a small difference, it is important if the product is on the border line for safe storage or if large changes of temperature need to be allowed for it (Cardoso and Labuza, 1983).

A temperature gradient in a bulk of uniform moisture content always causes vapour pressure gradient with translocation of water from the warmer to the cooler region (Pixton, 1982).

At any given relative humidity, air contains lower water vapour at higher temperatures than at lower temperatures (Manuel Sa and Sereno, 1993.

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At any given moisture content, the water activity increases with increase in temperature in agreement with Clausius and Claperyran equation (Fennema, 1985).

$$\frac{d\ln aw}{dt} = \frac{DH}{RT^2}$$

where T is the absolute temperature, R is the gass constant and DH is the isoteric net heat of sorption at the water content of the sample. Thus, a temperature change, through its effects on water activity can influence the stability of stored product (Agbaji and Agbaji, 1990).

2.9.3 SORPTION ISOTHERM AND RELATIVE HUMIDITY

If food (grains) are not stored in a moisture proof containers, it will equilibriate with the relative humidity of the surrounding air according to its desorption or adsorption isotherm (Pixton, 1982). The duration will be determined by the moisture of the product and the relative humidity of the air (Labuza, 1983).

Dry products may pick up moisture to the extent that mould will be able to grow while those with higher water activity stored in low humidity may loose moisture causing shrinkages and other organoleptic changes (Labuza and Cantreras-Medellin, 1981).

The rate of equilibrium depends upon the relative humidity of the air (Achoba et al, 1991), the initial water activity of the product (Agbaji and Agbaji, 1992) and temperature (Manueal Sa and Sereno, 1993).

In mixtures of food such as cereal grains and raisins the moisture from one food will be transfered to the drier one. For raisins in cereal, the result is the cereal being less crisp and the raisins becoming hard (Labuza, 1983).

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2.10 WATER ACTIVITY AND RELATIVE HUMIDITY

Water activity is an index of the availability of water for chemical reactions and microbial growth (Bran, 1976). Water in food, its location and availability is one of the most important factors influencing microbial growth there in (Scott, 1957).

Water that is generally associated with food is of three types: Bound water, adsorbed water and absorbed water. Bound water has no influence on the vapour pressure and it is not removed by drying at ordinary temperatures, (Dawes, 1976) though it is removed at high temperatures which also decompose some organic matter. In quantity, it remains almost constant (Troller et al, 1978).

The absorbed and adsorbed water together are often termed free water but they exert a lower vapour pressure than that of normal water exposed to the atmosphere. This free water can be removed by drying using normal heat of vapourisation (Kraener and Taylor, 1931) and it is this variable quantity of the free water that is assessed by a determination of the moisture content of the product (Cardoso and Labuza, 1983). Water activity is the ratio of the vapour pressure above a material or solution and that of the pure water at the same temperature (Troller et al, 1978). The value ranges from 0 - 1. The vapour pressure of a pure liquid depends upon the rate of escape of molecules from the surface. The escape of water to the air is measured by the equilibrium relative humidity (ERH). This vapour pressure and ERH are related. The water activity of a solution is defined in terms of VP and ERH by the formula:

$$aw = \frac{P}{Pv} = \frac{ERH}{100}$$

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where P is the vapour pressure of the solution and Pv is the vapour pressure cf pure water (Duckworth, 1975; Davies et al, 1976).

2.10.1 WATER ACTIVITY DETERMINATION

Many systems have been discussed for calculating, predicting or determining the water activity of various substances including food grains (Farelto et al, 1983; Ferrofouten, 1982; Chrife, 1981; Labuza et al, 1976; Northolt and Heuvelman, 1982; Troller, 1983).

Volatile compounds in food may result in erroneous measurement of water activity when electric hygrometer is used (Faretto et al, 1984). The water activity of food generally decreases as the temperature is raised from 23°c to 27°c (Scott and Bernard, 1983).

2.10.2 WATER ACTIVITY AND MICROBIAL GROWTH

Not all water in a medium is free and available to micro-organisms. Many molecules are associated with solute molecules, the association resulting in lower vapour pressure for the solution than for pure water.

The growth of micro-organisms is progressively inhibited as the water activity is reduced to growth threshold characteristics for each organism and depend on interracting environmental factors (Brock et al, 1984).

Generally, with some notable exceptions bacteria are inhibited at aw values below 0.90. Yeast can grow at aw 0.84 and above and some moulds are capable of growth at aw down to 0.60 (Carry, 1978).

Growth inhibition is commonly manifest as extended lag, diminished rate of cell division and reduced maximum cell yield

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(Brock et al, 1984). Physiological responses in cells exposed to sub optimal water activity include compositional changes e.g, accumulation of compatible solutes (proline and γ - amino butyric acid for bacteria and polyhydroxyl alcohol for fungi) (Stainer et al, 1987), changes in respiration rate, permeability changes and altered tolerance to selective agent (Davies, 1976). The prevention of microbial growth is of paramount importance to microbiologists. Hence, the minimum aw at which growth can occur has been of interest. The limiting aw for growth is influenced by other environmental factors which should be at optimum values for the organism being tested if the absolute minimum water activity is to be determined. Organisms tend to grow better in substrate in which aw is altered by desorption rather than adsorption (Labuza, Cassil and Sunkey, 1972). Other aspects of water activity besides the minimum activity for growth are important. These aspects include the germination of spine, toxin production, resistance to heat and survival of micro-ogranisms (Brock et al, 1984). Sperber in (1983) reported that the aw values requirement for bacterial sporulation is the same for growth but generation can occur at lower values. Production of entenotoxin by staphylococcus aureus is at higher water activity values for the production of mycotocin than for growth by several species of Aspergillus and Penicillium (Willis et al, 1993).

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TABLE 2 .7: APPROXIMATE MINIMUM WATER ACTIVITY VALUES FOR GROWTH OF MICROORGANISMS

ORGANISM	MINIMUM WATER ACTIVITY	ORGANISM	MINIMUM WATER ACTIVITY
Most spoilage bacteria	0.90-0.91	Staph albus	0.88-0.92
Acinetobacter	0.95-0.98	S. aureus	0.83-0.92
Acromonas	0.95-0.98	Streptococcus	0.92-0.98
Alcaligens	0.95-0.98	V. parahaemolyticus	0.94-0.98
Athrobacter	0.95-0.98	Most yeast	0.75
Bacillus	0.90-0.99	Most Moulds	0.87-0.94
B . cereus	0.92-0.95	Xerophilic yeast	0.60-0.78
Citrobacter	0.95-0.98	Aspergillus	0.70-0.80
Cl botulinum	0.90-0.98	A . glaucus	0.68-0.88
Type A	0.93-0.95	A . flavus	0.70-0.75
Type B	0.93-0.96	A . halophilicus	0.70-0.90
Type E	0.93-0.97	A . niger	0.68
C. perfiringes	0.95-0.98	Botrytis cinera	0.80-0.84
Corynebacterium	0.95-0.98	Debrayomycis	0.93
Enterobacter	0.91-0.97	Fusarium	0.87-0.91
E. coli	0.95-0.98	Hansenula	0.80-0.92
Flavobacterium	0.95-0.98	Mucor	0.89-0.90
Klebsiella	0.96-0.98	Penicillium	0.80-0.93
Lactobacillus	0.90-0.98	Rhodotorula	0.78-0.90
Leuconostoc	0.96-0.98	S . Cerevisiae	0.89-0.92
Micrococcus	0.90-0.95	S . rouxii	0.62-0.81
M. roseus	0.96-0.98	Xeromyces blosporus	0.60-0.61
P. aeruginosa	0.94-0.97		
P. flurescence	0.96-0.93		
Salmonella	0.93-0.96		

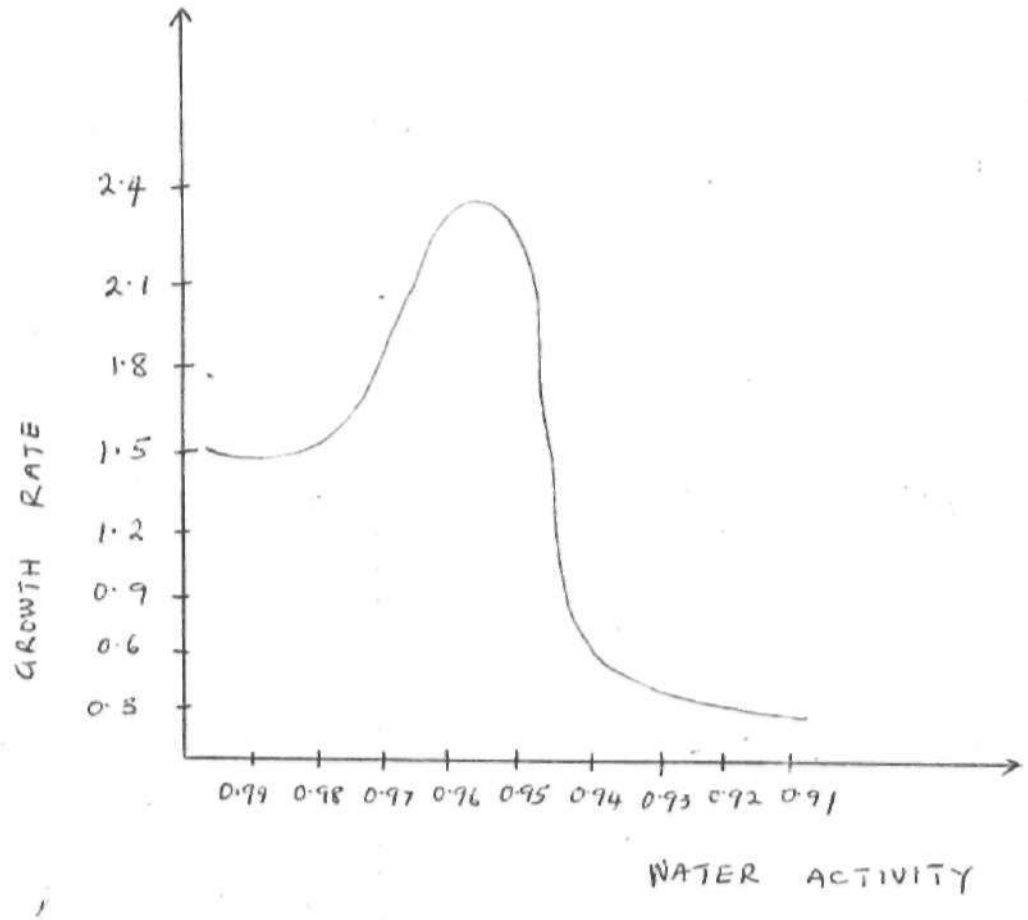


FIG 2: EFFECT OF WATER ACTIVITY ON BACTERIAL GROWTH

2.11 MICROBIOLOGY OF DEHYDRATED FOODS

Sorption Isotherms vary for different food materials and thus the alarm moisture content corresponding to water activity 0.7 also varies (Tsami et al, 1990). Usually, dry foods have lower moisture content with aw less than 0.4. Fennema in 1985 demonstrated how desorption and adsorption isotherms exhibited by foods reflect the state of water binding within the matrix. This also influenced the binding characteristics of food where constant and falling rate stages can be identified. The surface of drying foods remains close to wet bulb temperature in the late stages (Tsami E, 1991), when water activity is reduced. The most drying processes have microbicidal effect (Duckworth, 1975). This is reflected in the typical microbial count of various dry foods where number (and types) can vary. Count rages from 10^1 - 10^5 per grain. Food that can be eaten without rehydration but are microbiologically stable without refregration are called intermediate moisture food (IMF) (Davies, 1976).

2.12 INTERMEDIATE MOISTURE FOOD (IMF)

An intermediate moisture food is one that is preserved either by lowering the water activity by drying or adding solutes, often in combination with chemical preservative anaerobic packaging or both (Davies, 1976). IMF have been defined as having moisture level ranging from 15-50% and aw value of 0.6 - 0.9. Regardless of the exact aw or moisture, IMF can be eaten without rehydration but are microbiologically stable without refrigeration or thermal processing (Davies, 1976). Traditional IMF have been available for centuries but new interest in IMF technology reflect recent advances in understanding physical, chemical and biological principles governing the mechanism of aw reduction (Duckworth, 1975).

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Some types of IMF include high moisture dry fruits (oats, figs) raisins, jams, apple, molosses, horney and syrup. With the creation of semi moist product foods, there have been efforts to develop more human foods that have IMF characteristics. Substances that have been added to lower aw of food to obtain IMF include glycerol, sodium chloride, lactic acid, sorbitol as well as lactose (Plahar et al, 1982).

2.13 METHODS OF MEASURING MOISTURE CONTENT

Several methods exist for measuring moisture content among which are:

i. Oven method: This method involves standard combination of temperature and time of drying in an oven e.g, 130°c for 1 hour, 120°c for 1½ hours and 105°c for 16 hours (AOAC, 1984). For cereal grains except maize, the international standard organisation recommended drying a grand sample for two hours at a temperature of 130°c.

ii. Electrical Method: This depends on the degree of electrical conductivity by water in products (Labuza, 1983). But this method gives results that are not consistently related to those given by oven method. This is because there is no demarcation between bound water and free water. They are generally unreliable for materials that have been relatively wet or dried. They do however give result quickly and facilitate the testing of samples in circumstances in which slower method would be of no use.

iii. Chemical Method: This assumes that all the free water is extracted from the material. The reagent used which is the Karl Fischer method is methanol. Such method should be calibrated against a reference method, usually, one in which a grand sample is dried to standard constant weight under reduced pressure.

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Since all methods of moisture content determination are empirical, it is important that they are carefully, standardized and the test procedure used is fully described when quoting results. Organisation that deals with grains used to know the moisture content and all ought to use same (standard) method of determination.

2.14 INFLUENCE OF MOISTURE CONTENT ON HANDLING CHARACTERISTICS AND PROCESSING.

If a product is too wet, it will not keep in store, neither will it flow as easily or packed as drier products. However, for processing purposes, a relatively high moisture content may be preferred. The bulk density of the product also varies with moisture content.

The storage potentials of product are also affected by moisture content. At low moisture content, grains and other agricultural produce will keep for a relatively long period without deteriorating (Awoyemi, 1984). A knowledge of the relationship between moisture content and relative humidity is essential in appraising the importance of environment and other changes observed in storage and assessing the storage potential of the product (Duckworth, 1975; Davies, 1976).

2.15 MALTING

The purpose of malting is to develop in the grains amylases and proteases. These enzymes break down the carbohydrate and protein in the grain to nourish it before photosythetic systems are developed enough to support the plant (Gibson, 1989). Malt has been described (Palmer, 1991) as cereals that have been germinated and kilned. The full process of malting involves three carefully controlled steps:

i. Steeping
ii. Germination
iii. Kilning

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2.15.1 STEEPING

Prior to steeping, the grains are cleaned. Sand particles, bits of metal and other unwanted materials are removed. The cleaned grains are then steeped in water to effect absorption of water. Once enough water has been imbibed, the seedling undergo metabolic changes. Preformed enzymes become activated and new ones formed. Moisture uptake leading to increase in weight to around 35 - 40% of original weight is optimal for malting (Denmyaker and Ohita, 1992). The temperature at which grains are steeped and germinated is of vital importance to the quality of the malt. Barley steeping is usually done at relatively cool temperatures 12 - 14°c, sorghum and maize 30°c for 18 hours. In steeping grains for malting it is pertinent to consider the factors such as (i) The physiological requirement of grain. These include water sensitivity, dormancy and grain hardness; (ii) Variety and size of grain; (iii) Temperature of steep and (iv) Duration of steep.

According to Gibson (1986) steeping was initially carried out in a rectangular ditches which were later replaced by self emptying conical bottom tanks. Two methods of steeping can be practised:

(a) Spray Steeping:

This type of steeping is used in plants where it is desirable to maintain water economy along side water uptake. Water jets are used to spray water on the seeds. This method has the problem of creating uneven moisture uptake and modification. This problem is more important with seed that has mealy steely endosperm. However, such a problem can be eliminated by immersion steeping.

(b) Immersion Steeping:

The grains are completely submerged in water in this type

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of steeping. Running water or tap water may be used. Several types of steeping plant have been developed for this kind of steeping: Conical steeps, Flat bottom, Norden Girachen steeps, Sav[illegible] Germination, Kilning vessels (SGKVs). Of the two types of steeping, immersion has been recording high above spray steeping because the latter causes uneven hydration even though it accelerates germination (Palmer, 1983).

2.15.2 GERMINATION

Well steeped grains are germinated under carefully controlled conditions of temperature and moisture. Gibson (1989) reported that the traditional method of germination was to use concrete floor as the surface on which the grains are spread to a depth of 3 - 4 inches. Because this method was labour intensive, pneumatic maltings were later developed. Attemperated humidified air can be passed through the bed to generate the required germination conditions.

Several temperatures have been utilised for malting. Pathirana et al (1983); Ezeogu and Okolo (1994) conducted theirs at 30°c. Palmer (1991) recommended a slightly lower temperature of 25°c. All workers carried out their studies in the dark (to eliminate photosynthesis), under humidity level approaching saturation point. A moisture content of 40% is maintained. The findings of the various studies indicte that a temperature range of 25 - 30°c will be favourable for germination.

To prevent rot malting and to encourage sufficient aeration of all the grains, the germinating grains are turned at regular intervals. Several physical and biochemical changes occur in the endosperm during germination. Important hydiolytic enzymes such as α - amylase, β - amylase and proteases (McGregor et al, 1994) are synthesised and released in the endosperm when water is sprayed to maintain seed culture (Ezeogu and Okolo,1994)

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They extensively hydorlyse starch and protein (Okafor, 1982). Varied optimum duration of germination have been reported by different workers - Pathirana et al, (1983) - 4 days; Morrall et al, (1986) - 5 days; Palmer (1991) - 6-8 days and Ezeogu and Okolo (1994) used 4 days. According to Morrall et al, (1986) the diastatic power of malt increases rapidly upto 4 days then increase slowly with germination time and reaches a peak and then decline slightly. This report also indicated the effects of temperature, moisture and germination time on malt are closely related.

2.15.3 KILNING

Further reactions in the grain are halted by Kilning which consist of gradual dehydration of specified temperature (Seaton, 1987); Palmer, 1989). According to Gibson (1987) the process generates alot of interest because of the amount of energy required. There may be many designs of kiln but the basic principle is the same with the green malt being transfered into a perforated floor and spread to a suitable depth. Hot air is then passed over the malt at the same time as it is being turned. Kilning of malt at elevated temperature is necessary to remove the raw flavour of green malt and to promote chemical reactions responsible for the formation of flavour components which impart the characteristics flavour to the malt. However, the activities of heat labile enzymes such as α-amylase are reduced during the process (Pathirana et al, 1983; Palmer, 1989) and may be responsible for the mashing problems often encountered in sorghum brewing. Therefore temperature around 50°c in forced air drought ovens have been favoured by most workers (Agu and Okaka, 1991; Ezeogu and Okoli, 1994). To improve colour and flavour, special colour malt treated at high temperature are added to the highly kilned malts.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLES

Samples of the grains were obtained from the departments of Crop Production and Plant Science both of the Institute of Agricultural Research and Training, Ahmadu Bello University, Samaru, Zaria. The Millet grains EV and Maize, Ex-Borno were obtained from the former while the sorghum grains SK 5912 was obtained from the latter. All the grains samples are those that have good germinative capacity and as such can be a good source of malt. The grains were packaged in a polythene bag to prevent further contamination and to disallow water loss. All the samples were kept at room temperature (28-30°c).

3.2 MOISTURE CONTENT DETERMINATION

The method described in Association of Official Analytical Chemists (AOAC, 1980) was used. 10g of grain sample whose moisture content is to be determined is accurately weighed into a clean, dry, weighed petridish (previously dried in an oven at 80°c for 30 minutes). The sample (grain + petridish) was dried in a vacuum oven(25mmHg) at 90°c until constant weight was obtained. Three replicate determinations were made for each sample. The moisture content is the difference between the initial and final weight.

If M_1 = Initial Weight

M_2 = Final Weight

Moisture content (mc) = (m1 - m2)g

Expressing the moisture content as percentage:

$$mc = \frac{\text{loss in weight after drying}}{\text{initial weight before drying}} \times 100$$

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3.3 EQUILIBRIUM MOISTURE CONTENT DETERMINATION

The equilibrium moisture content (emc) of oven dried sample were determined using the method of Tsami (1991). Pre-weighe samples were stored in triplicates, placed on metal gauze in a medium size dessicators maintained at different relative humidities. The dessicators were placed in thermostated ovens at 27°, 30° and 37°c. Constant relative humidity was maintained in each dessicator by the sulphuric acid (specific gravity = 1.84): water mixtures (Solomon, 1951) as shown on table 3.1 (Ragianna, 1977). The samples were weighed (metler balance, model P12 metler instrument A.G Switzerland) at intervals of 24 hours until constant weight is attained usually within seven days. The relative humidity was varied between 10-95%. The average of the three samples were taken as the emc values.

3.4 WATER ACTIVITY DETERMINATION

The water activity was determined by using the formula:

$$\frac{\text{Equilibrium Relative Humidity}}{100}$$

The method for measuring equilibrium relative humidity have been described in section 3.3 above.

3.5 SORTION ISOTHERMS DETERMINATION

The determination of the sorption isotherms of the grain samples and their malts were carried out by using the method of Screedharan (1985). Equilibrium mositure content of oven dried samples was plotted against the equilibrium relative humidity (Denloye and Ade-John, 1985). The resulting curve is known as the sorption isotherms of the samples which is essential in evaluating the stability of any product.

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Table 3.1: CONCENTRATED SULPHURIC ACID WATER MIXTURES FOR THE ATTAINMENT OF VARIOUS RELATIVE HUMIDITIES.

Dessicator No	H_2SO_4 Conc (mol/dm^3)	H_2SO_4 (ml)	Water Volume (ml)	Molarity	Relativ Humidity
1	22.54	12.25	187.75	2.3	10
2	50.92	26.67	172.23	5.2	20
3	71.52	39.41	160.59	7.4	35
4	90.16	49.00	151.00	9.2	40
5	120.54	65.51	134.49	12.3	45
6	128.38	69.77	130.23	13.1	65
7	136.22	74.03	125.97	13.9	75
8	164.64	89.48	110.52	15.8	85
9	191.10	103.86	96.14	19.5	95

Adapted from: Ragianna, S. 1977.

3.6 ESTIMATION OF MICROBIAL LOAD

3.6.1 TOTAL COUNT

The method described by Collins (1979) was used. Ten grains of the grains whose microbial load is to be determined is washed in 10ml of sterile distilled water. 0.1% methylene blue was added as stain. 0.05ml was then mounted in a counting chamber. Counting was made under the x100 objective of the microscope.

3.6.2 VIABLE COUNT

For viable count, the method described by Norris (1970) was used. Ten grains of grain sample was put into 10ml of sterile distilled water. One millimetre of water was pipetted into 9ml of diluent to give a 1.10 dilution. More serial dilutions were made up to 10^{-5}. 10ml of plate count agar cooled down to 45°c was poured into petridishes. Three plates were made for each dilution. 1ml of each dilution was pipetted onto the centre of the agar. Fresh pipette was used for each dilution. The petridish was moved gently and with the aid of sterile glass spreader, the water was spread uniformly on the agar. The medium was allowed to set, inverted and incubated for between 24 - 28 hours. To count:
Plates showing 30 - 300 colonies were selected. Total colony count was the average of the colonies obtained on countable plates. The reciprocal of the dilution multiply by the average colony count was reported as colony count per grain.

3.7 MICROBIAL ISOLATION AND IDENTIFICATION

3.7.1 ISOLATION OF FUNGI

Isolation of fungi was carried out for those that grow on the surface and those that grow within the grains. Isolation of fungi that grow on the surface of the grains and their malts

was carried out by using the method described by Anderson in 1976. The samples were washed in sterile water and the washed water was inoculated on the appropriate agar. Potato dextrose agar and malt extract agar (oxoids) was used for isolation of mold and yeast while yeast extract agar (oxoids) was specifically used for yeast isolation.

The potato dextrose agar was prepared by boiling 400g of peeled Irish potato (Solanum tuberosum) in 500ml of water for 15minutes. Agar-Agar (Oxoid) was then added (15%) to make it gel and 2% glucose. The mixture was autoclaved at 121°c for 15 minutes. Streptomycin sulphate and benzyl penicillin were added as bacteriostats to sterile molten PDA to give a final concentration of 30ug/ml. In case of the malt extract and yeast extract agar, mineral acid (sulphuric acid) was used as bacteriostat. All incubation for fungal isolation was done at room temperature (28 - 30°c).

Isolates representative on each of the media were picked and purified by subculturing several times on the same medium and the purified isolates stored at 5-10°c on agar slants on the same medium of isolation.

For the isolation of fungi within the grains, a method similar to that described by Anderson, (1976) was used. The grains were surface sterilised in milton solution which is sodium hypochlorite (16.5% NaCl). The grains were later rinsed in sterile distilled water and with the aid of sterile forcept, the grains were placed on fixed points on the various agar media incubated at room temperature. Isolates growing from within the grain on the media were picked and purified as discussed for those that grow on the surface. Isolates were also stored at 5-10°c on agar slants of the same medium of isolation.

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3.7.2 FUNGAL IDENTIFICATION

Each of the distinct colonies obtained above were inoculated on the appropriate agar media using point inoculation, and were incubated at room temperature (28-30°c). Observations were made of the appearance and pigmentation of the colonies of each isolate, the number of days at which growth is first noted, rapidity and luxuriance of growth, texture of growth, gross topographic characteristics. Ascospore formation, Pseudomycelium or true mycelium, pellicle formation were also observed on corn meal agar. The distinct colonies were also stained on a slide with lactophenol cotton blue.

For cultural morphology to identify fungi, the method described by Thompson and Lim (1965) was used. A piece of aerial mycelium is removed with two teasing needles and touched momentarily to a small drop of 95% alcohol previously placed on a slide, then removed quickly to a drop of 10% KOH also previously placed on the slide. The mycelium is carefully teased apart with the needle and covered with a cover slip. A finger was used to rub across the slide to give a thin film. Lactophenol blue was used as the mounting fluid (Skinner and Lovelock, 1979).

3.7.3 IDENTIFICATION OF SPORE FORMER

For those fungi that produce in sufficient quantities, two methods are available but the one described by Funder (1968) was used. Concentrated suspension of spores in sterile water was made. Inoculating needle was used to streak the surface of the agar medium. The needle bears a loop full of spore suspended in sterile water. The streaking was made in a zig-zag manner over the surface of the agar plate. Incubation was carried out for three days at room temperature (28-30°c).

The germinated spores were then transfered into a fresh medium. Several dilutions were made (1ml of aliquots for two selected dilutions - 10^{-2} and 10^{-3}) and transfered to sterile petridishes. Melted agar at approximately 45°c was added to the plates which were rotated to secure uniform mixing of still liquid agar and the suspension. The plates were incubated at room temperature (28-30°c) and isolates were removed from plates which show limited number of colonies with uniform dispersion. This method is known as the streak method. General characteristics of the aerial mycelium and spore producing structures after removing the cover and examination of the growth with magnifying glass, inspection of the under surface and formation of sclerotia and comparison with known culture were also employed to accomplish identification.

3.8 IDENTIFICATION OF YEAST

The appearance of culture on agar, shape and size of vegetative cells, sugar utilization, methods of propagation are among the features considered for the yeast identification. For the vegetative cell examination, a wet preparation of the yeast culture was made by mounting some of the growth from a young actively growing culture in a drop of water. The cells are then mounted in 5% solution of lactophenol cotton blue. The fungi identities was confirmed in the Crop Protection department, Institute of Agricultural Research, Ahmadu Bello University, Zaria.

3.9 BACTERIAL ISOLATION

The method described by Anderson (1977) was used for the isolation of bacteria on the grains. The grains were washed in sterile water. The wash water was then inoculated onto the appropriate enrichment media. The enrichment media used

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were selenite F and peptone water. The enrichment was done overnight and then the enriched culture was inoculated onto the various agar media.

Eosin methylene blue (EMB) agar was used for the enterobacteriaceae while salmonella-shigella agar was used in attempt to isolate the indicated organism. Other agar media used are: Trichocholate Bile Salt (TCBS) - (for vibrio), MacConkey, Blood, Manitol salt agar and Nutrient agar. Mixed colonies were obtained and to get the pure colony, the growing edges of the mixed colonies were subcultured. Several dilutions were made and several plates with small volume of each were poured. Each pure colony was stored on agar slant of the same media of isolation at 5°c - 10°c. To isolate bacteria that grow within the grain, the procedure similar to that described for fungal isolation in the interior of the grain was used.

.9.1 IDENTIFICATION OF BACTERIA

For the identification of bacteria, the method described by Chessbrough (1985) was adopted. The purified bacterial colonies were first Gram stained and examined for their gram reaction. The isolates were also cultured on the agar plate to examine their colonial morphology, colony forms, elevation and margin. Each colony was also stained for spore observation and motility tests were performed on each of them.
Further characterisation and identification were carried out by testing each organism biochemically. The following biochemical tests were carried out (where applicable) on each distinct colony:

(i) Oxidase Test

A piece of filter paper was placed in a clean petridish

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and 3 drops of freshly prepared oxidase reagent added. A sterile glass rod was used to remove a colony of the test organism and smeared on the filter paper. Development of blue purple colour on the filter paper within 10 seconds indicates positive oxidase test.

(ii) Catalase Test

3ml of Hydrogen peroxide (3% H_2O_2) was poured into a test-tube. A sterile glass rod was used to remove a good growth of the test organism and immersed onto the hydrogen peroxide solution. Bubble formation indicates a positive catalase test.

(iii) Coagulase Test

A drop of physiological saline was placed on a slide. The drop was emulsified with a colony of test organism to make a thick suspension. A drop of plasma was added to the thick suspension and mix gently and clumping signifies a positive coagulase test.

(iv) H_2S Production

A tube of nutrient broth was inoculated with the test organism. Lead acetate paper strips were prepared by soaking strips of filter paper on a 50g/l solution of lead acetate and allowed to dry. The paper is then inserted in the neck of the tube above and medium are stoppered well. the inoculated medium incubated at 37°c and was examined daily for a black thickening of the lower part of the strip.

(v) Motility IndolUrea (MIU) Test

A straight sterile wire was used to inoculate 5ml of sterile MIU medium. For indole test, an indole paper strip was placed on the neck of the MIU tube above the medium and the tube stoppered. The tube incubated at 37°c overnight. Indole production was examined by looking for the reddening of the lower part of the strip.

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(vi) Motility Test

For motility test there is spreading turbidity for the stab line or turbidity throughout the medium.

(vii) Urease Activity

For Urease activity the tube was observed for red-pink colour in the medium that indicate positive urease production.

(viii) Citrate Utilisation

A straight sterile wire was used to inoculate 4ml of sterile Koser's citrate medium with a broth culture of the test organism. The tube incubated at 37°c for 4 days checking each day for growth (turbidity) and change in colour from light green to blue. (Cowan and Steel, 1966)

3.10 MALTING

The procedure described by Palmer (1991) was used for malting. During malting grains were cleaned, sands and other unwanted materials are removed. The grains were then steeped in water at room temperature (28-30°c) until the moisture content was about 40%. The water was changed after about 12 hours so that micro organism that have grown will not deteriorate the grains. The steeping took about 3 days. The grains were drained of the water. The grains were then allowed to germinate. Water was sprinkled on the grains at intervals. The growth was completed in 5 days except for the maize that was completed within 4 days. The germinated grains were disallowed from further growth by kilning. The germinated grains were heated until the moisture content was reduced to about 6%. The malt was then winnowed to remove the rootlets that were dried by now.

CHAPTER FOUR

4.0 RESULTS

4.1 MOISTURE CONTENT

The values of the moisture content of the grains used are given in table 4.1. The result shows that Sorghum grain has the highest value (0.88g) or 8.8% when expressed as percenta The maize grain has the least value of 0.520g (5.2%) while the value for millet (0.75g) is in the intermediate range. Each value is a mean of three determinations.

4.2 EQUILIBRIUM MOISTURE CONTENT (EMC)

The equilibrium moisture content expressed on percentage dry weight basis, at different temperatures and water activi (aw = erh/100) are shown on tables 4.2 - 4.4 for the cereal grains and tables 4.5 - 4.7 for the malts. The result indicates that as the relative humidity increases, the equilibrium moisture content also increases. The increase in equilibrium moisture content per unit rise in equilibrium relative humidity is less at rh values between 10 - 40%. The emc however, increased rapidly at higher relative humidi values $>$40%. At extremely high relative humidity of 95%, the increment in emc is more pronounced than at 85%. The pattern of emc value variation is similar for the grains and their malts.

However, the emc values were shown to be higher for the rains at some low relative humidity than the malt, especial at higher temperatures of 30 and 37°c (Tables 4.3 and 4.6; 4.2 and 4.7). The emc values for the malts are lower than those of the grains at relative humidity values between 10 - 65% for the millet at all the three temperatures

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Table 4.1: MOISTURE CONTENT OF MAIZE, MILLET AND SORGHUM GRAINS

GRAIN	MC	PMC(%)
Maize	0.520	5.2
Millet	0.750	7.5
Sorghum	0.880	8.8

MC = Moisture Content

PMC = Moisture content expressed as percentage

investigated but between relative humidity (rh) values of ,5-95% the emc values for the millet malt become higher. The increase in emc value of the millet malt when the relative humidity value was raised from 65% to 75% was found to be extremely rapid.

For the sorghum grains and its malt, the latter emc values were shown to be less than the former at rh values from 10% up to 75% at 37°c (Tables 4.3 and 4.6). At rh of 85% and 95%, the malt was shown to have a high water sorbing capacity

At 20° and 30°c, the sorghum malt showed a higher water adsorption capacity than the malt at rh values of 75, 85 and 95% but at 30°c, the emc values of the malt were higher between 10 - 40% rh and dropped to a lower value at 65%. At 20°c, the emc values of the malt was higher at 10, 40 and 65% rh. The maize malt shows less water adsorption potential at relative humidity values between 10 - 65% at all the temperatures tested. (Tables 4.2 and 4.7). But at the relative humidity values between 75 - 95%, this tendency increased markedly and hence surge its emc value more than that of the raw grains.

The emc values for the grains and their malts were found to decrease as temperature increases (Tables 4.2 - 4.7). The emc values also varied among the three grains. Although it does not follow any general pattern but the results show that at 95% rh, the maize grain has the highest emc values (19.48, 15.97 and 14.09) at 20, 30 and 37°c respectively. At the same treatment sorhum had 14.5, 13.28 and 12.50 while millet had 17.62, 15.50 and 14.84 as its emc values (Tables 4.2 - 4.4).

The emc values of the malts too did not follow a specific pattern among the grains. At 95% rh, sorghum malt has the

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Table 4.2 :EQUILIBRIUM MOISTURE CONTENT VALUES OF MAIZE GRAIN AT VARYING EQUILIBRIUM RELATIVE HUMIDITIES & TEMPERATURES

	E M C AT		
ERH (%)	20 c	30 c	37 c
10	1.61±0.005	1.32±0.006	1.30±0.004
20	2.52±0.005	1.98±0.006	1.32±0.004
40	3.22±0.005	2.36±0.006	1.35±0.004
65	8.68±0.005	7.45±0.006	4.39±0.004
75	10.50±0.005	9.55±0.006	6.33±0.004
85	11.37±0.005	11.36±0.006	9.61±0.004
95	19.48±0.005	15.97±0.006	14.09±0.004

KEY

ERH = Equilibrium Relative Humidity (%)

EMC = Equilibrium Moisture Content (% dry basis)

Table 4.3 :EQUILIBRIUM MOISTURE CONTENT VALUES OF SORGHUM GRAIN AT VARYING EQUILIBRIUM RELATIVE HUMIDITIES & TEMPERATURES

	E M C AT		
ERH (%)	20 c	30 c	37 c
10	.98±0.005	.81±0.005	.81±0.004
20	3.45±0.005	1.36±0.005	1.28±0.004
40	4.52±0.005	3.39±0.005	2.60±0.004
65	9.11±0.005	5.91±0.005	4.46±0.004
75	13.37±0.005	9.00±0.005	5.45±0.004
85	13.47±0.005	12.09±0.005	9.77±0.004
95	14.48±0.005	13.28±0.005	12.50±0.004

KEY

ERH = Equilibrium Relative Humidity (%)

EMC = Equilibrium Moisture Content (% dry basis)

Table 4.4 :EQUILIBRIUM MOISTURE CONTENT VALUES OF MILLET GRAIN AT VARYING EQUILIBRIUM RELATIVE HUMIDITIES & TEMPERATURES

	E M C AT		
ERH (%)	20 c	30 c	37 c
10	2.90±0.002	1.95±0.005	.87±0.003
20	3.76±0.002	2.23±0.005	2.15±0.003
40	4.80±0.002	4.52±0.005	4.00±0.003
65	11.53±0.002	10.14±0.005	4.46±0.003
75	12.26±0.002	12.11±0.005	10.40±0.003
85	14.30±0.002	12.76±0.005	10.75±0.003
95	17.65±0.002	15.50±0.005	14.85±0.003

KEY

ERH = Equilibrium Relative Humidity (%)

EMC = Equilibrium Moisture Content (% dry basis)

Table 4.5 :EQUILIBRIUM MOISTURE CONTENT VALUES OF SHOGUM MALT AT VARYING EQUILIBRIUM RELATIVE HUMIDITIES & TEMPERATURES

	E M C AT		
ERH (%)	20 c	30 c	37 c
10	1.83±0.005	.96±0.003	.13±0.003
20	2.33±0.005	1.98±0.003	0.23±0.003
40	5.50±0.005	3.66±0.003	0.65±0.003
65	8.50±0.005	5.69±0.003	1.91±0.003
75	10.00±0.005	10.04±0.003	5.53±0.003
85	18.21±0.005	17.00±0.003	11.38±0.003
95	29.53±0.005	22.00±0.003	19.96±0.003

KEY

ERH = Equilibrium Relative Humidity (%)

EMC = Equilibrium Moisture Content (% dry basis)

Table 4.7 :EQUILIBRIUM MOISTURE CONTENT VALUES OF MAIZE MALT AT VARYING EQUILIBRIUM RELATIVE HUMIDITIES & TEMPERATURES

	E M C AT		
ERH (%)	20 c	30 c	37 c
10	1.40±0.006	.98±0.005	0.85±0.005
20	1.55±0.006	1.25±0.005	1.00±0.005
40	5.00±0.006	3.11±0.005	1.06±0.005
65	8.21±0.006	6.00±0.005	4.39±0.005
75	13.21±0.006	11.54±0.005	8.00±0.005
85	16.64±0.006	14.50±0.005	12.30±0.005
95	26.78±0.006	21.00±0.005	16.19±0.005

KEY

ERH = Equilibrium Relative Humidity (%)

EMC = Equilibrium Moisture Content (% dry basis)

Table 4.5 EQUILIBRIUM MOISTURE CONTENT VALUES OF MILLET MALT AT VARYING EQUILIBRIUM RELATIVE HUMIDITIES & TEMPERATURES

	E M C AT		
ERH (%)	20 c	30 c	37 c
10	1.98±0.006	.99±0.004	.99±0.006
20	2.97±0.006	1.17±0.004	1.00±0.006
40	3.89±0.006	2.92±0.004	2.00±0.006
65	8.31±0.006	4.21±0.004	3.19±0.006
75	15.62±0.006	13.34±0.004	12.58±0.006
85	16.67±0.006	14.81±0.004	14.59±0.006
95	25.00±0.006	21.32±0.004	20.10±0.006

KEY

ERH = Equilibrium Relative Humidity (%)

EMC = Equilibrium Moisture Content (% dry basis)

highest emc values at temperatures of 20 and 30°c (Table 4.6) while the millet malt had the highest value (20.10) at 37°c (Table 4.5). At lower relative humidity values of 10 - 20%, millet malt had the highest value at all the temperatures tested (Table 4.5 - 4.7) while at intermediate rh between 40 - 65%, sorghum malt had the highest values among the three grains (Table 4.6).

4.3 WATER ACTIVITY (a_w)

The values of the water activity $\frac{ERH}{100}$ obtained for the grain samples and their malts are shown on Tables 4.2 - 4.7. The values varied from 0.10 - 0.95. The aw values affect the emc values of the samples and made them to increase as its own value increases.

4.4 SORPTION ISOTHERMS

The results of the sorption isotherms are shown in figures 3 - 5 for the grains and 6 - 8 for their malts. The isotherms are closer to the ERH axis at lower rh values of 10 - 40%. But deviate markedly from it as the ERH values increase from 65 - 95%. There is consistent shift of the isotherms to the right as the temperature increases. All the isotherm curves are sigmoidal.

The sorption isotherms of the grains and their respective malts are also represented in figures 9 - 11. The isotherms shows that the sorption curves of the malt are closer to the ERH axis while those of the grains are closer to the EMC axis. At ERH above 40%, slopes of the isotherms are much steeper for the malt than those for the grains. This produces a large increase in EMC per unit rise in relative humidity.

4.5 MICROBIAL COUNT ON THE CEREAL GRAINS

4.5.1 TOTAL COUNT

The total microbial count is shown on Table 4.8. Millet has

the highest value of 5.76 x 10^6 cells/g while maize has the least value of 8.45 x 10^5 cells/g. The value for sorghum (2.38 x 10^6 cell/g) is in the intermediate range. Each val is a mean of two determinations.

4.5.2 VIABLE COUNT

The values of the viable count obtained for the cereal grai are presented on table 4.8. The values shows that there is slight variation from the total count for each samples. Sorghum has the highest value of 1.81 x 10^5cfu/g as opposed to millet that has the highest value for the total count. Maize still has the least value of 3.84 x 10^4 cfu/g as with the total count while millet has its value (1.03x10^5cfu/g) i the intermediate range. All the values are means of two determinations.

4.6 ISOLATION AND IDENTITY OF MICRO-ORGANISMS

4.5.1 FUNGI

The genera of the molds isolated from the cereal grains and tentatively identified to the genus level are shown on table 4.9. These include Penicillum, Fusarium, Aspergill Cladosporium, Pythium, Phytophtora, Colleotrichum, Alternari Helminthosporium, Mucor and Curvularia, Sp. The yeast isola include the genera Saccharomyces, Candida and Torula Sp. Only Pythium (AA), Curvularia (DD), Phythophtora (KA) and Fusarium (GA) were isolated from the interior of the grains. The other genera were isolated from the surface of the grains From the malt only two mold genera (Aspergillus and Mucor Sp) were isolated and identified. No yeast was isolate from the malts. The malt was shown to be inhabited by less number of fungi than the raw grains.

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Table 4.8: MICROBIAL COUNT ON THE CEREAL GRAINS

	TC (Cell/g)	VC (cfu/g)
SORGHUM	2.38×10^6	1.81×10^5
MILLET	5.76×10^6	1.03×10^5
MAIZE	8.45×10^5	3.8×10^4

TC = Total Count

VC = Viable Count

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4.6.2 BACTERIA

Table 4.10 shows the identity of bacteria isolated from the cereal grains and their malts. All the bacteria were isolated from the surface of the grains except Pseudomonas specie that was isolated from the inside of the cereals. From the malt, only proteus specie was isolated. Majority of the isolates belong to the family Enterobacteriaceae.

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Table 4.9: MOLD ISOLATES AND THEIR APPEARANCE ON POTATO DEXTROSE AGAR (PDA)

Isolate	Appearance on PDA	Identity
RA	Greenish Colour	Penicillum
GA	White fluffy	Fusarium
BA	Dark brown	Cladosporium
TA1	Yellowish	Aspergillus
SA1	Black	A.niger
KA	Whitish felted	A. fumigatus
AA	Fluffy grey/Silky	Pythium
KA	Silky	Phytophtora
TA2	Dark	Colleotricum
SA2	Dark conidiophore	Alternaria
AO	Dark with extensive conidiophore	Helmintosporium
SA3	Whitish with extensive mycelium	Mucor

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TABLE 4.10 : BIOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATES FROM THE CEREAL GRAINS

CODE	Gram Reaction	Colonial Morphology: Elevation	Margin	Colour on BA	Colour on Mac	Biochemical Reactions: Oxidase	Catalase	Motility	Glucose	Lactose	H_2S Production	Gas	Citrate	Urea	Indole	Coagulase	Identity
MC	gnb	Flat	round	grey/white	NLF	+	NA	+	-	-	-	-	+	-	-	NA	Pseudomonas
EC	gnb	raised	entire	grey/white	LF	-	NA	-	+	+	-	+	+	+	NA	NA	Klebsiella
MM1	gnb	Flat	round	grey/white	NLF	+	NA	+	-	-	NA	-	+	-	NA	NA	Pseudomonas
MM2	gpc	Flat	round	grey/white	NA	NA	-	-	NA	NA	-	NA	NA	NA	-	NA	Staphylococcus
EM	gnb	raised	round	grey/white	LF	-	NA	-	+	+	-	+	+	-	-	NA	Klebsiella
ES	gnb	raised	entire	grey/white	LF	-	NA	-	+	+	-	+	+	+	-	NA	Klebsiella
MB	gnb	Flat	round	grey/white	NLF	+	NA	+	-	-	-	+	+	-	-	NA	Pseudomonas
MM2	gnb	raised	round	grey/white	LF	-	NA	-	+	+	-	+	+	-	-	NA	Klebsiella
NS	gnb	Flat	round	grey/white	LF	-	NA	+	+	+	-	+	-	-	-	NA	E. coli
NC1	gnb	Flat	round	grey/white	NLF	-	NA	-	-	-	-	-	-	-	-	NA	Acinetobacter
NC2	gnb	Flat	entire	grey/white	NLF	+	NA	+	-	-	-	-	+	NA	NA	NA	Pseudomonas
MS	gpc	Flat	round	yellow	NLF	-	+	-	NA	NA	-	NA	NA	-	NA	-	Staphylococcus
MK	gnb	raised	round	grey/white	NLF	-	+	-	NA	NA	NA	NA	-	-	-	-	Bacillus
K	gnc	raised	entire	colourless	NLF	-	+	+	+	+	NA	+	+	+	-	NA	Proteus

KEY:

gnb	gram negative bacilli
gpc	gram positive coccus
gpb	gram positive bacilli
BA	Blood Agar
Mac	MacConkey agar
NLF	Non Lactose fermentor
LF	Lactose fermentor
NA	Not Applicable
-	Negative Reaction
+	Positive Reaction

CHAPTER FIVE

5.0 DISCUSSION

The result shows that out of the three grains (Maize, Sorghum and Millet), Sorghum has the highest moisture content of 8.8% followed by Millet, 7.5% while Maize has the least value 5.2%. The variation in moisture content may be due to unequal distribution of the chemical compounds that made up each grain. Maize is made up of 71% carbohydrate, 4.5% crude fat, 10% protein and 2.1% ash. Millet's carbohydrate, crude fat, protein and ash are 75%, 1.5%, 9% and 1.9% respectively, while Sorghum has 71% carbohydrate, 2.0% crude fat, 1.5% protein and 2.2% ash (FAO,1989). Moisture content has been shown to be intimately related to chemical structure of food (Hylinka and Robison, 1954) and also on the interaction of these constituent polymers with themselves and water (Trinette et al, 1985).

Intermolecular forces to some extent play a significant role in the amount of water in cereal grains (Trinette et al, 1985). Therefore, Sorghum that has the highest moisture content can be due to high percentage of starch and low crude fat and oil content. Starch is known to be a natural high polymer formed from glucose having OH groups and O_2 rings which are points of polarity in the molecule and therefore suitable foci for interaction with water molecule (Hylinka and Robinson, 1954). Maize that has the least value can be due to the presence of high oil content which has been shown to lower the amount of water in grains (Trinette et al, 1985) and relative high value of protein which offers an ample opportunity for water to interact with a variety of polar groups (Constantinan, 1995).

Other factors that may be responsible for the variation can be due to the nature and arrangement of the various structural components of the grain seed (Trinette, et al, 1985). A seed

is made up of four distinct components (Hulse et al, 1980). The pericarp or the testa/seed coat, the aleurone layer, starchy endosperm and the embryo. The pericarp and testa have variable thickness and variable content of polyphenols in different grains (Palmer, 1991), hence the amount of moisture expected to be held by each grain is expected to vary. The flow of water from various parts of the grain kernel depends on the internal characteristics of the seeds, the permeabilityof the aleurone layer and the outer pericarp and the extent of cracking of these layers (Brooker et al, 1974, Chung and Lee, 1985).

Other factors that have been shown to influence moisture content include bulk density, degree of filling, amount of foreign materials maturity and condition of storage (Brooker, 1974). Thermal properties parameters have also been investigated by other workers (Anderson, 1974, Hylinka and Robison, 1974) in relation to moisture content of cereal grains.

These include specific heat capacity, thermal conductivity, isotherms and heat of vapourisation (Bukker-Arkena and Saleh, 1985). The moisture content is very important in safe storage of foods like cereal grains and their malt (John and Shellwood, 1994). The equilibrium moisture content (emc) of the grains and their malts was found to increase only gradually with relative humidity at low value (10 - 45%) at all the temperatures suggesting that fluctuations in temperature and in low environmental relative humidity will only have minimal effects on the storage stability of cereal grains and their malts. Conversely, at relative humidities above 45% there is large increase in emc per unit increase in relative humidity (rh) suggesting that fluctuations in temperature and environmental relative humidity will greatly affect the storage stability of the cereal grains and their malts confirming the report of Sa and Sereno (1993) on marmalada jam.

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The equilibrium moisture content also varies among the cereal grains and their malts. Maize was found to have the least value at relative humidity between 10 - 85% (except millet at 10% rh); at all temperatures millet had the highest value and sorghum value is in the intermediate range. This is due to high oil content of maize (4.5%) since water adsorption is intimately related to chemical structure (Hylinka and Robinson, 1974) and it has also been reported that grains with high oil content absorbs less moisture from the surrounding air than seeds high in starch (Brooker et al, 1974). Millet that has the highest emc value may be due to high starch content (FAO, 1989). At high relative humidity greater than 85%, the emc value does not follow any specific pattern confirming the report of Fennema in 1985 that at extremely high rh, the water holding capacity of foods become highly unpredictable.

Variation in the emc values of the grains can also be due to grain variety, inactivity, history of the grain, rh measuring device and emc determination methods (Mill et al, 1994).

The malts were found to have high equilibrium moisture content values than the corresponding grains at all the temperatures at high relative humidity > 45%. This might be due to the fact that after germination during malting the permeability of the seed coat/testa and the aleurone layer to water has increased. During steeping and subsequent kilning, crevices have been created within the germinated grains (malt) which made them to hold on to higher volume of water than the raw grains (McGregor et al, 1994). Another factor may be as a result of increase in particle size of the malt than the corresponding grains. It has been reported by Lima and Cal-vidal (1985) that one of the factors affecting isothermal equilibrium of a dried food would be the particle size. During the process of malting, the components of the grains might have become polarised thus creating opposite charges that allow

more water to be dissolved in them since "like dissolves like" (Murray, 1985). The same process might have also enhanced the thermal properties of the malts that allow a higher water holding capacity.

In the adsorption isotherms of the grain samples and their malts, the influence of temperature on water uptake is shown, confirming the findings of Cal-vidal and Lima (1985) that with increasing temperature, the water uptake reduces. Though the isotherms have the usual sigmoidal shape, a characteristic shift to the right (Gustavo and Labuza, 1983) is observed, as the storage temperature increases (Figure 2-7). The shift is as a result of the temperature dependency of the water activity of any sample (Lima and Cal-vidal, 1983; Fennema, 1984; Saravacos et al, 1986). The adsorption isotherms of the grain samples are shown in figures 2-4. Similar isotherms for the malts are represented in figure 5-7. Each data point is a mean of three determinations. At any moisture content, the water activity increases with increase in temperature in agreement with Clasius-Claperyon equation (Huang and Byerly, 1992):

$$\frac{d\ln aw}{dT} = \frac{DH}{RT^2}$$

where T is absolute temperature, R is gas constant and DH is the isoteric net heat of sorption or the water content of the sample. Thus a temperature change, through its effect on water activity can influence the stability of stored product (Agbaji and Agbaji, 1990).

The adsorption isotherms of each grain and their malt are shown in Figure 8-10 for comparison. At relative humidity value below 40%, only slight differences are observed between the curves. Marked differences however occur at higher relative humidity values with the malts showing a higher water adsorption capacity than the grains.

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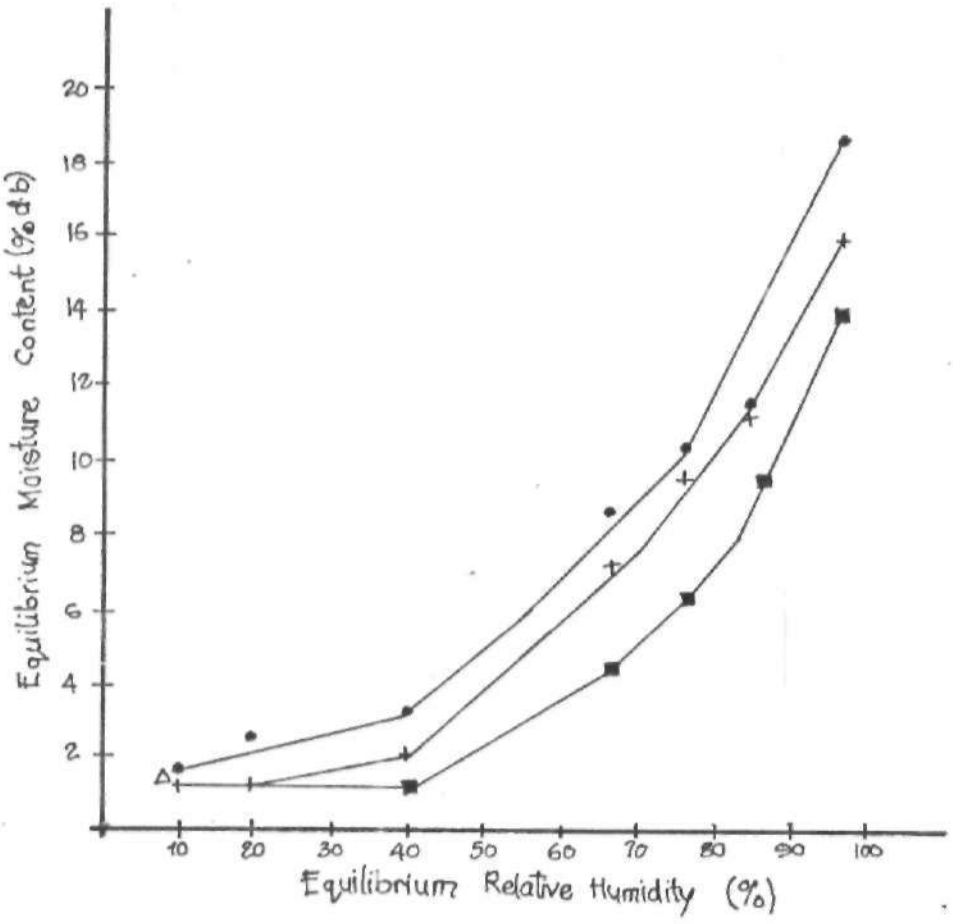


FIG 2:- ADSORPTION ISOTHERMS OF MAIZE GRAIN AT •20, +30 AND ▪37°C

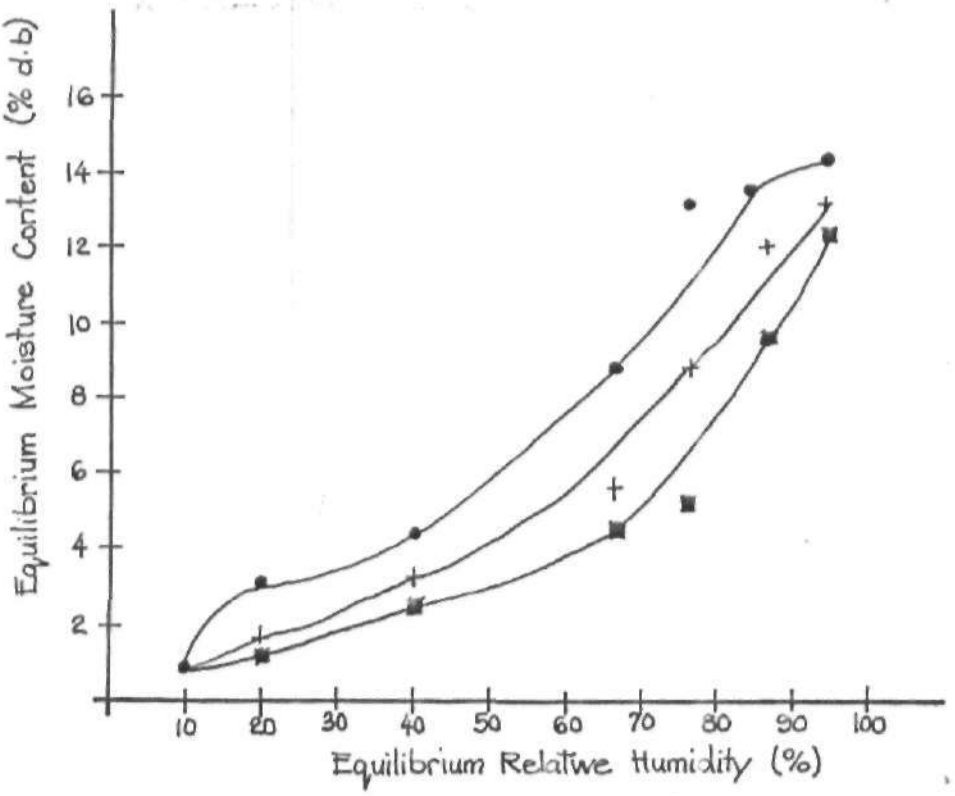


FIG 3:- ADSORPTION ISOTHERMS OF SORGHUM GRAINS AT • 20, + 30 AND ▪ 37°C

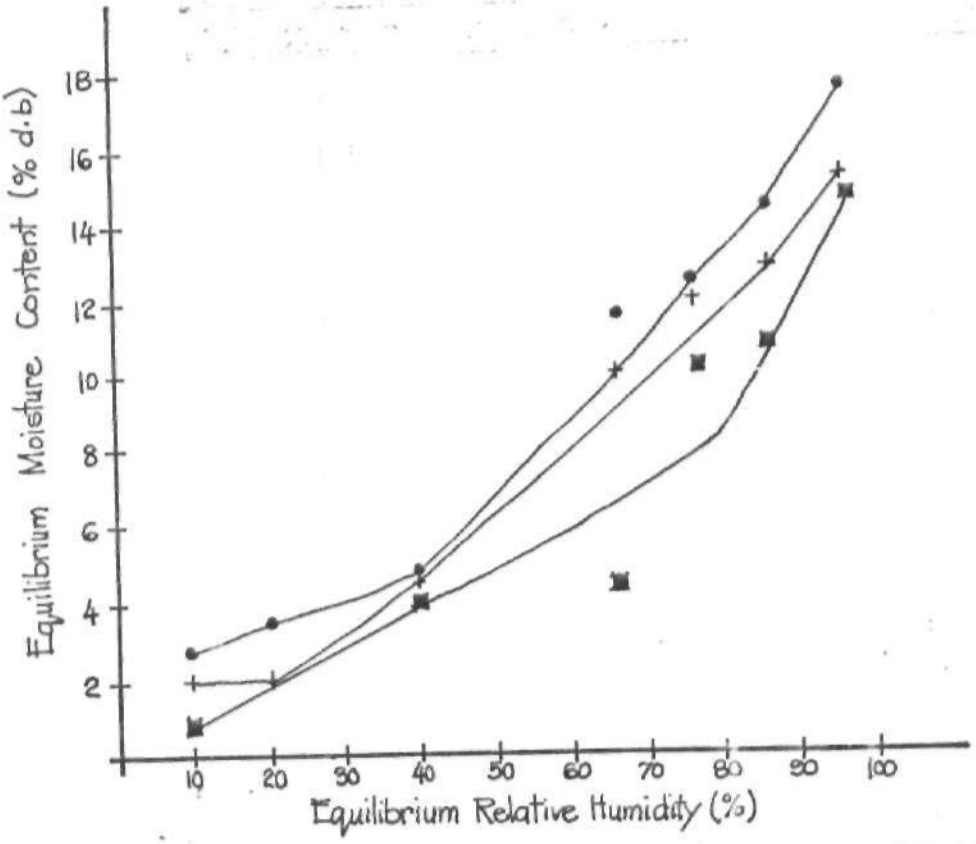


FIG 4:- ADSOPTION ISOTHERMS OF MILLET GRAINS AT •20, +30 AND ▪37°C

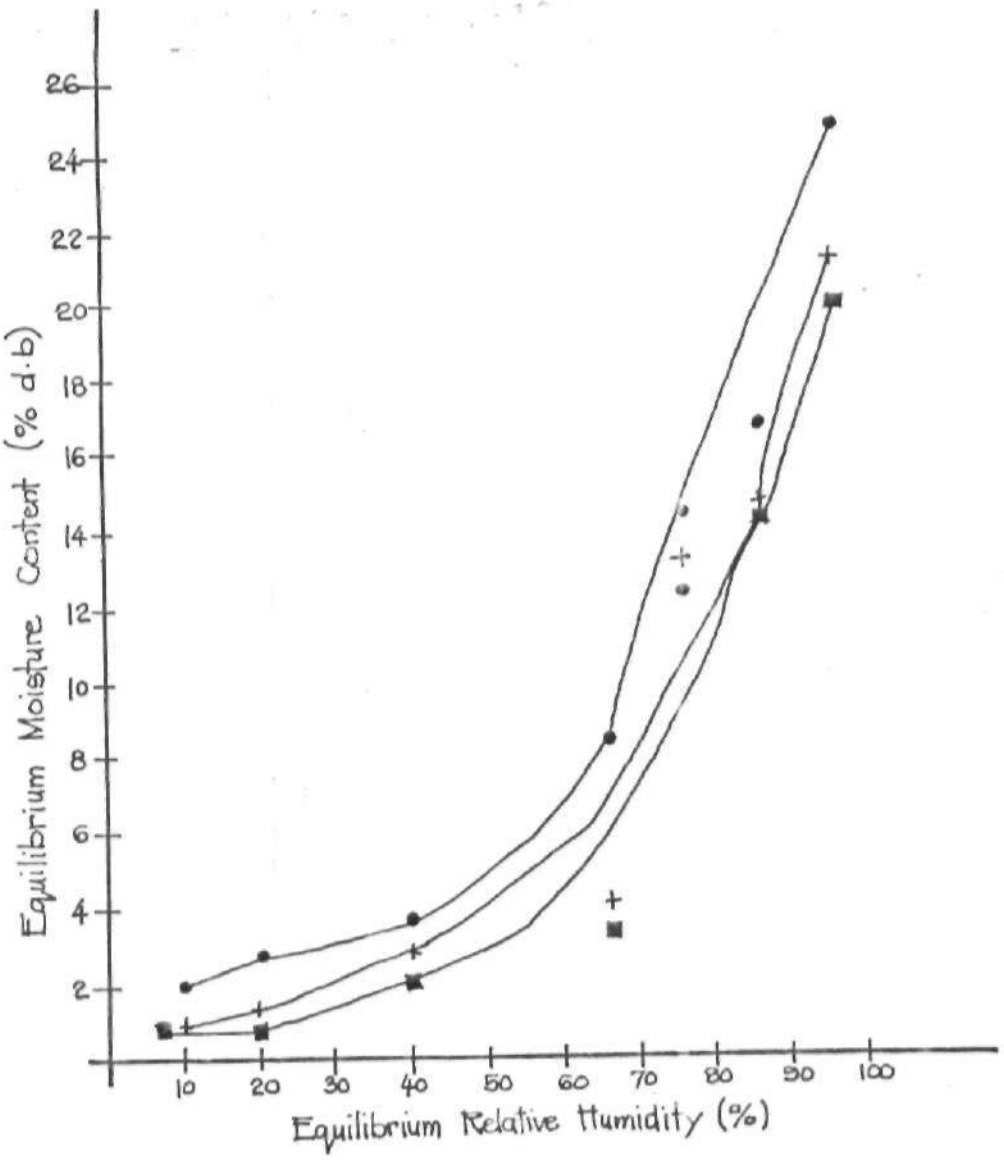


FIG 5:- ADSORPTION ISOTHERMS OF MILLET MALT AT •20, +30 AND ▪37°C

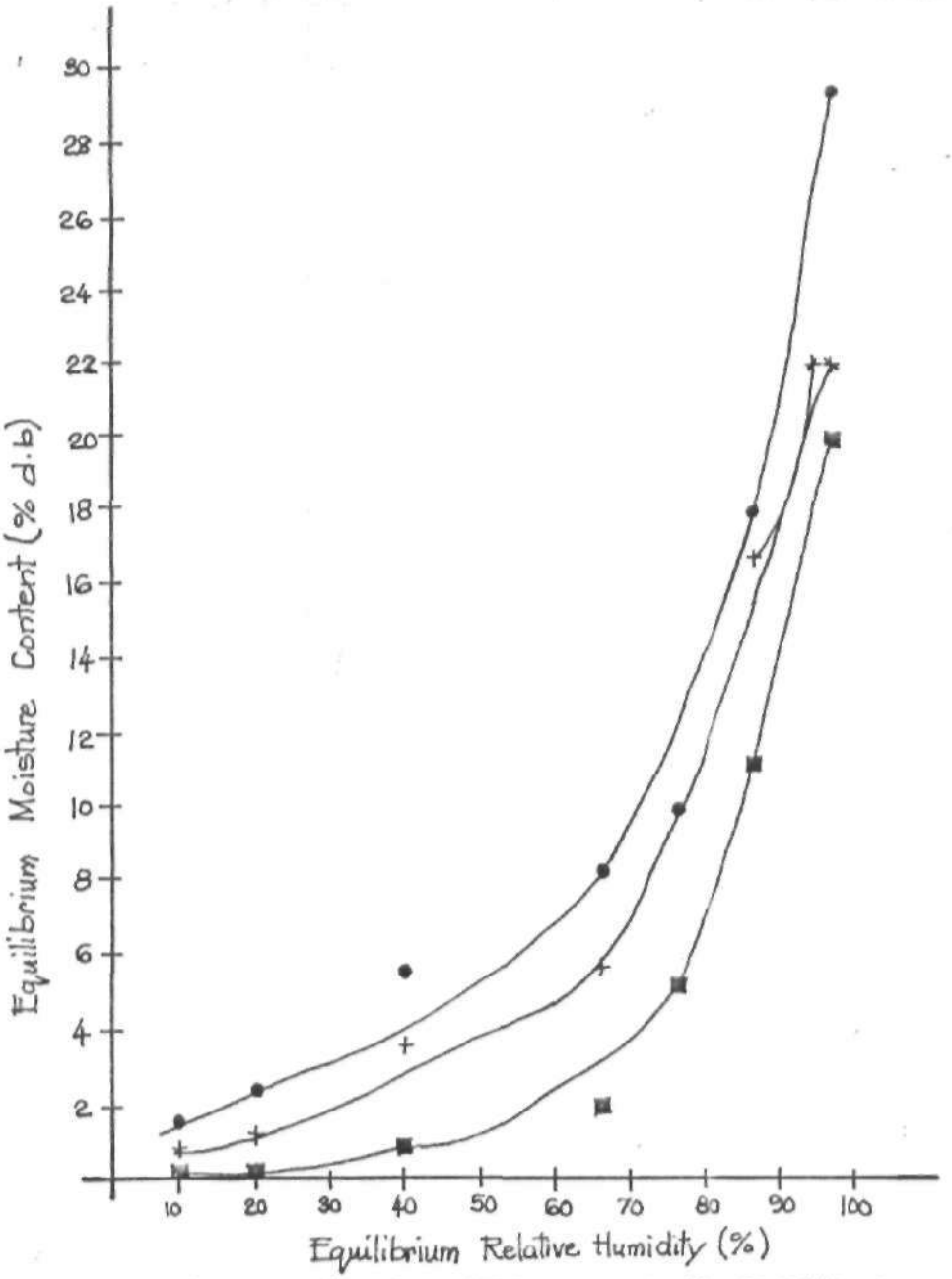


FIG5:-ADSORPTION ISOTHERMS OF SORGHUM MILLET AT •20, +30 AND ▪37°C

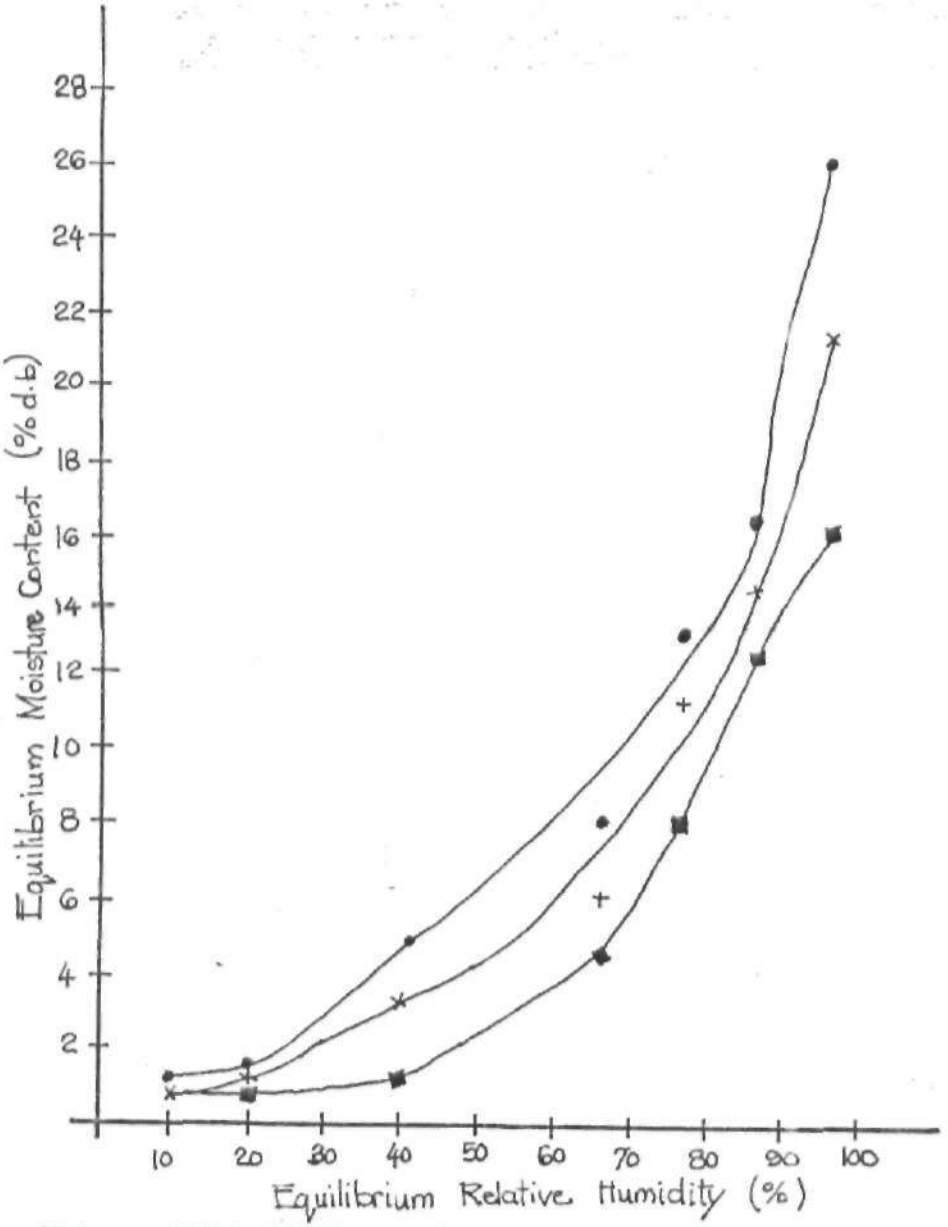


FIG 7:- ADSORPTION ISOTHERMS OF MAIZE MALT AT •20, +30 AND ▪37°C

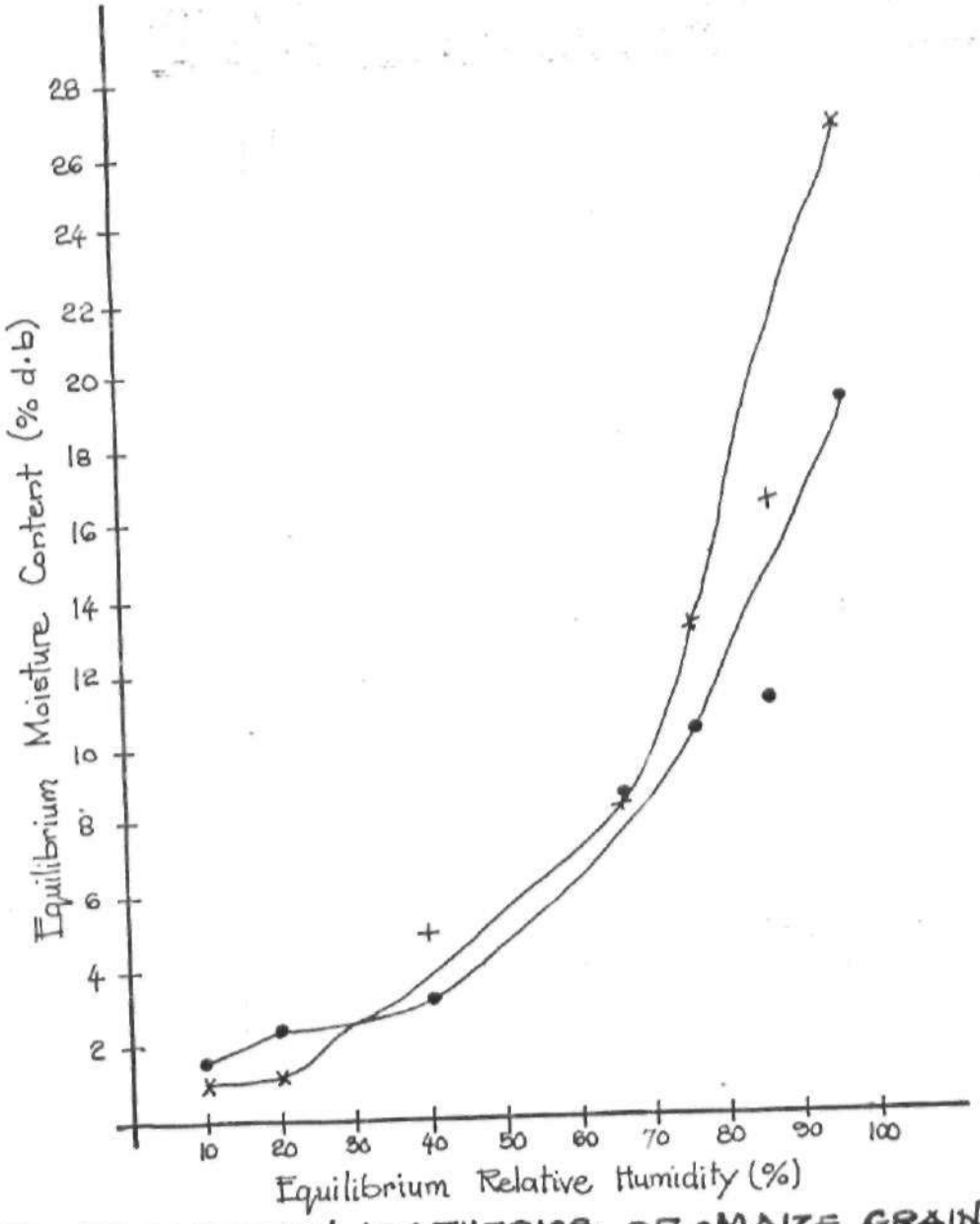


FIG 8:- ADSORPTION ISOTHERMS OF •MAIZE GRAINS AND ITS +MALT AT 20°C

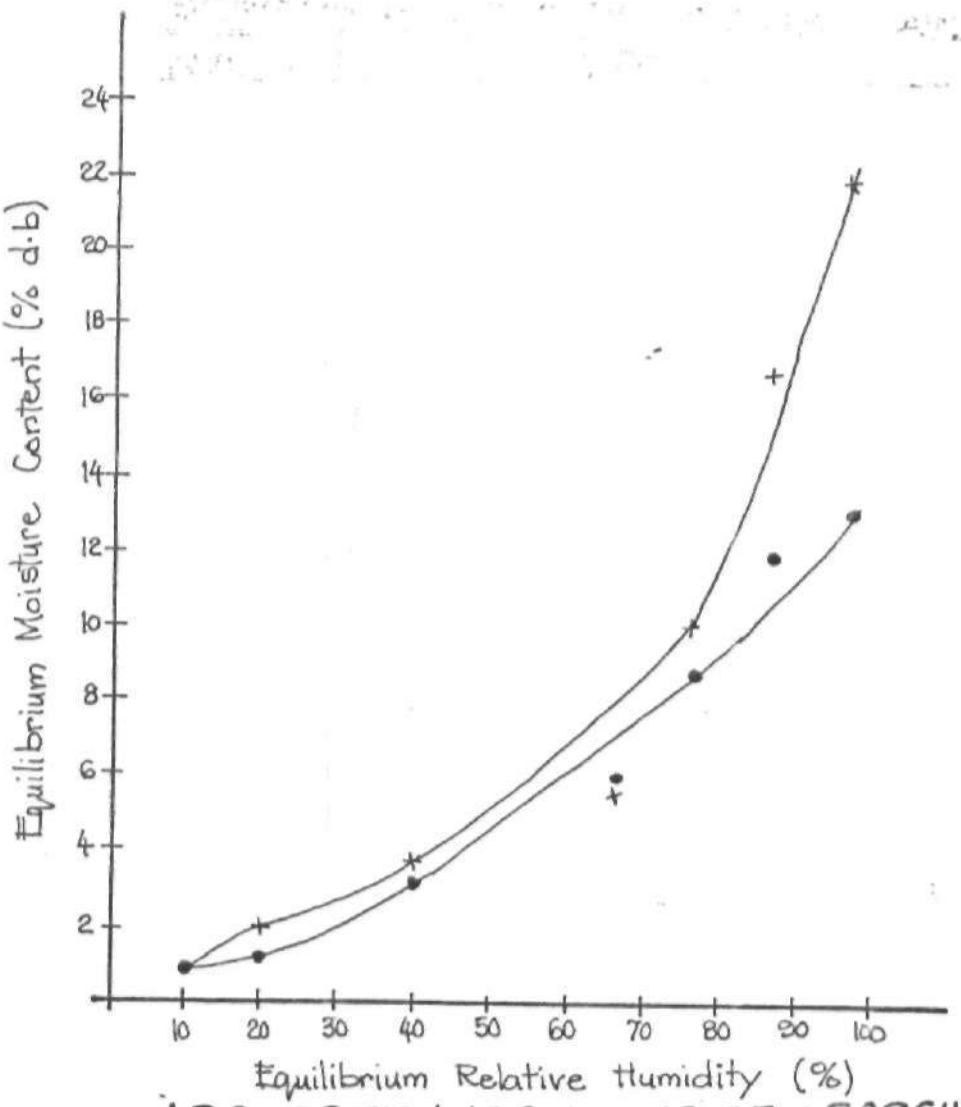


FIG 9:- ADSORPTION ISOTHERMS OF • SORGHUM GRAIN AND ITS + MALT AT 30°C

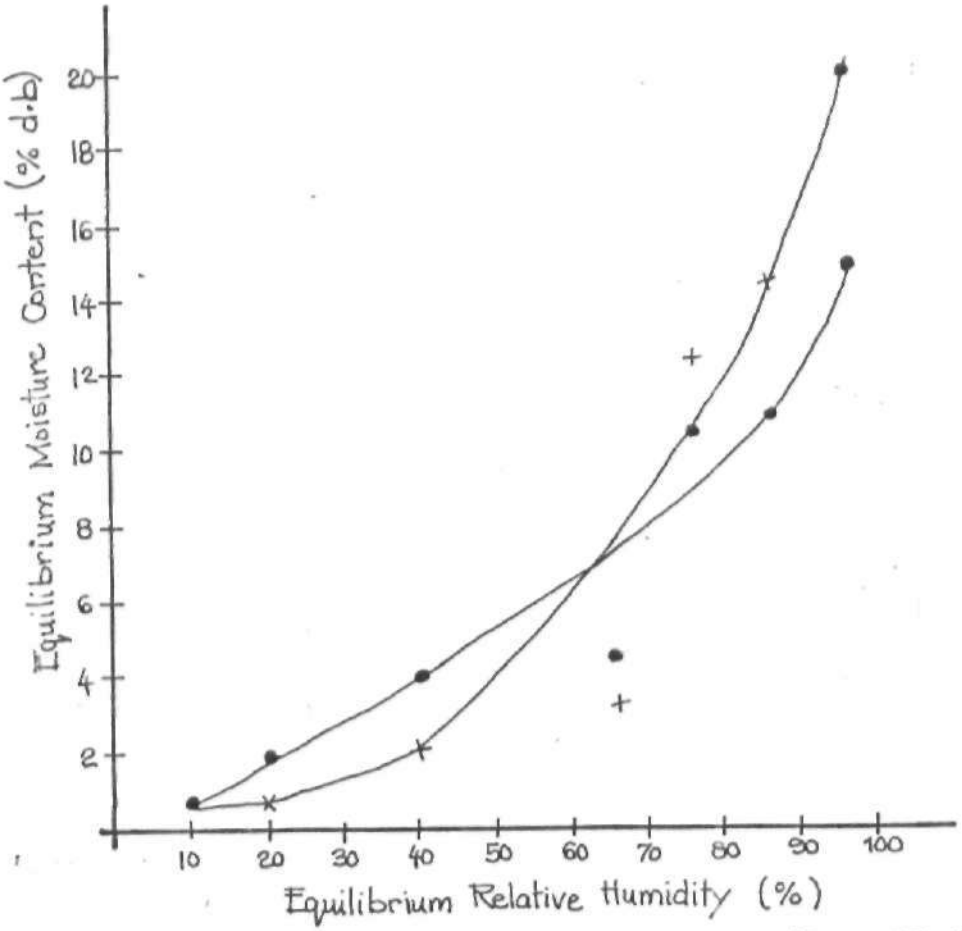


FIG10:- ADSORPTION ISOTHERMS OF • MILLET GRAIN AND ITS + MALT AT 30°C

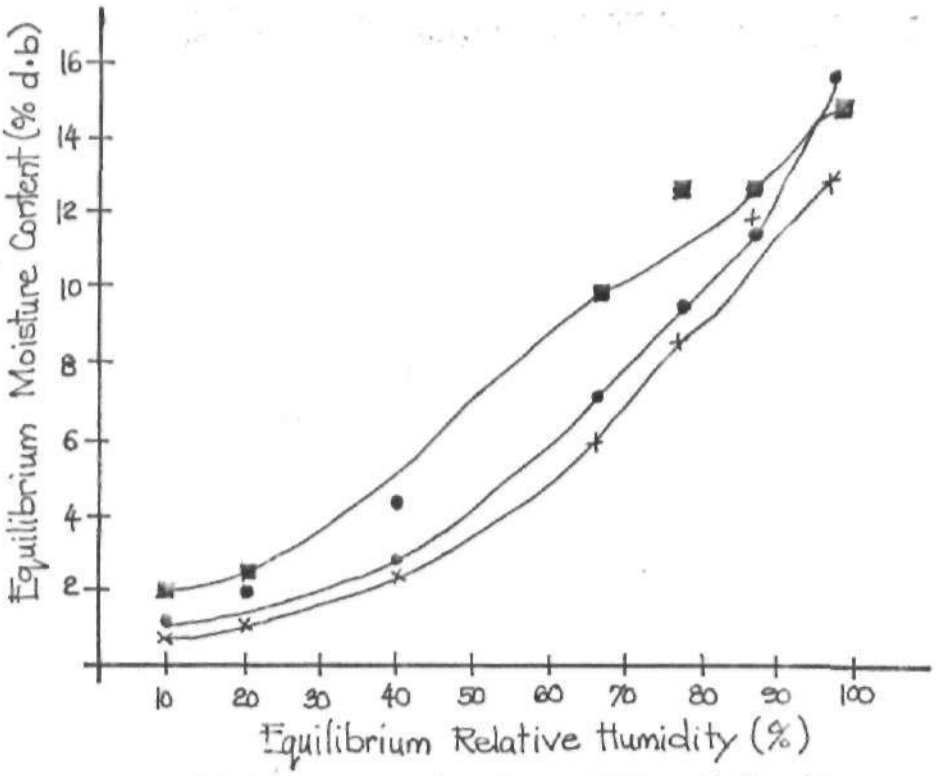


FIG 12:- ADSORPTION ISOTHERM OF • MAIZE +SORGHUM AND ■ MILLET GRAINS AT 30°C

This can be due to the fact that malt has a higher surface area (Lima and Cal-vidal, 1983) and at higher relative humidity > 40%, the adsorption of water had been shown to be a function of surface area (Agbaji and Agbaji, 1990). Other factors are likely to influence the absorption above this relative humidity.

The adsorption isotherms of the maize sorghum and millet are also represented in Figure 12. Maize was shown to have the highest value at extremely high rh > 85% but least at lower rh < 85%. At the same rh range sorghum has the least while millet has its value in the intermediate range. The reason for this variation might be due to differences in surface area and other intrinsic properties of the grains (Willis and Teixeira, 1993).

Analysis of the isotherms on figures 2-7 were carried out using the Henderson (1952) equation in order to establish a general equation relating moisture content and equilibrium relative humidity (erh):

$$1 - a = \text{Exp}\ (-Km^n)$$

where a = water activity $(\frac{erh}{100})$, K is a constant depending on the proudct, n is also a constant and m is moisture content (% of dry basis). The equation can be re-expressed as:

$$\text{Log}[-\ln(1-a)] = n \log M + \log K.$$

Plots of log[-ln(1-a)] against logM at each of the temperature were made for each sample. Typical plots for maize, sorghum and millet and their malts are shown in Figures 12-17. Clearly, more than one straight line can be fitted in each of the cases. In each case, two were fitted. The break in the lines indicates a change in the type of water binding. The moisture content at which the break occurs decreases with increase in temperature, indicating that moisture binding is a function of temperature (Tsami et al, 1990). The lines also show a constant shift to the left as the temperature increases. The values of the constant n and K with the moisture content are shown in Tables 5.1 and 5.2.

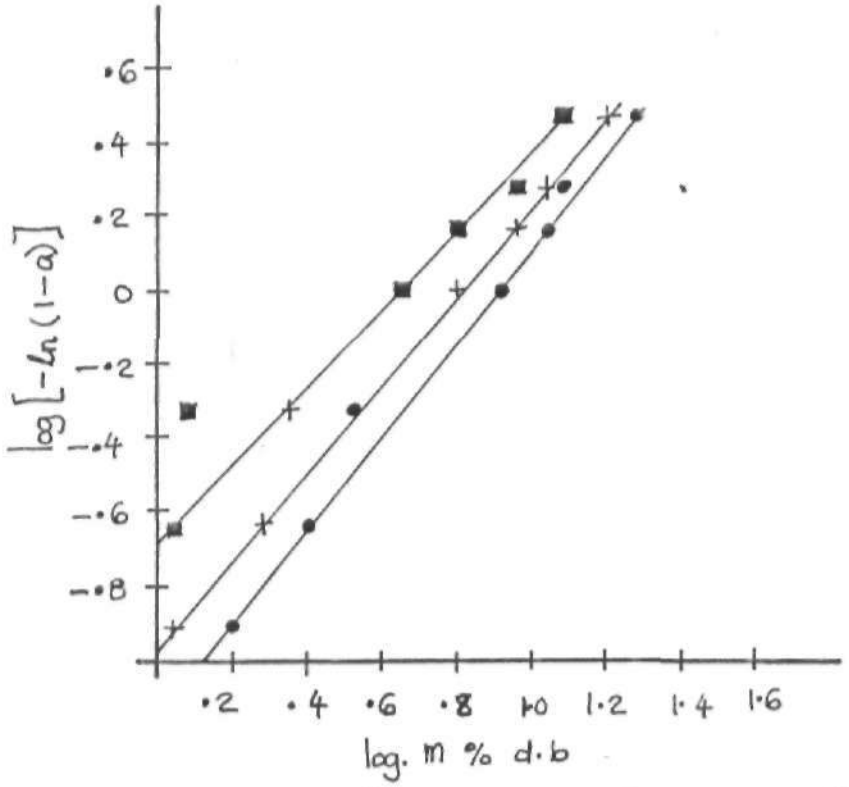


FIG12- HENDERSON PLOTS FOR MAIZE GRAIN AT •20, +30 AND ▪37°C

12 14

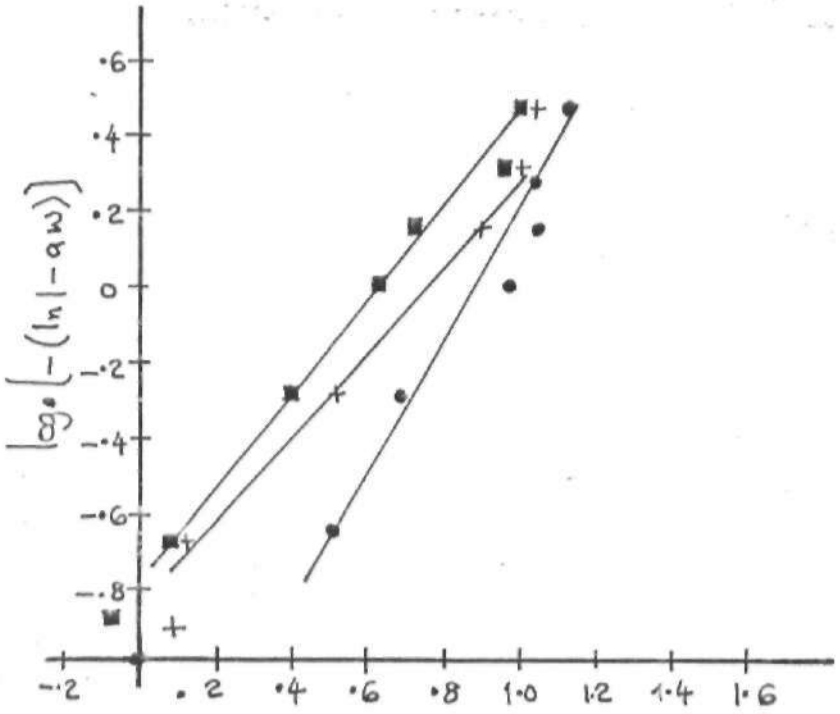


FIG 13:- HENDERSON PLOTS FOR SORGHUM GRAINS AT •20, +30 AND ▪37°C

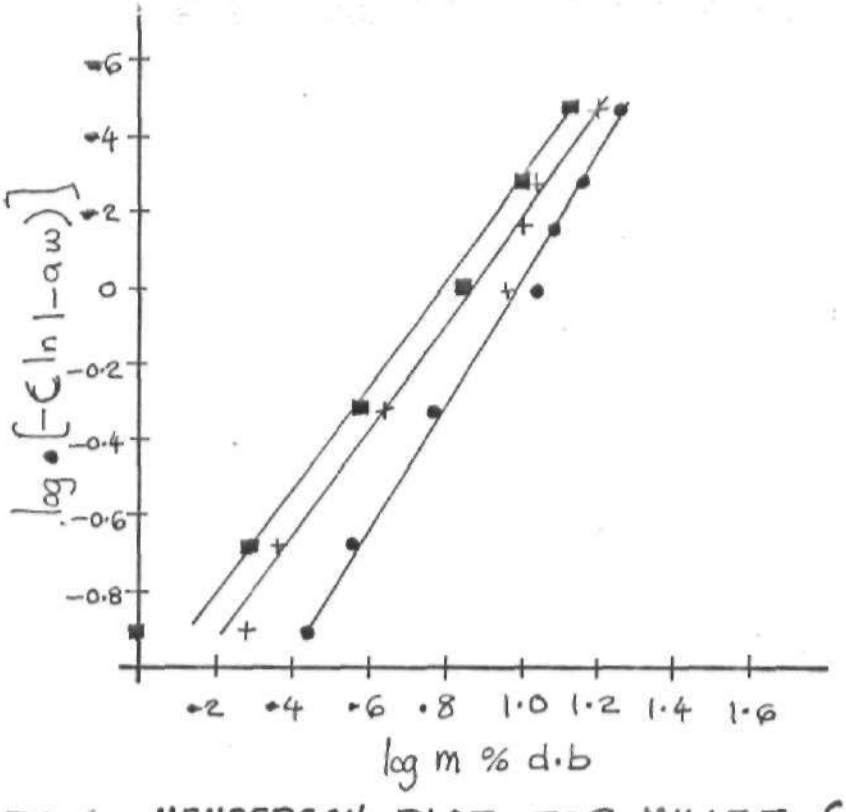


FIG 14:- HENDERSON PLOT FOR MILLET GRAINS AT •20, +30 AND ▪37°C

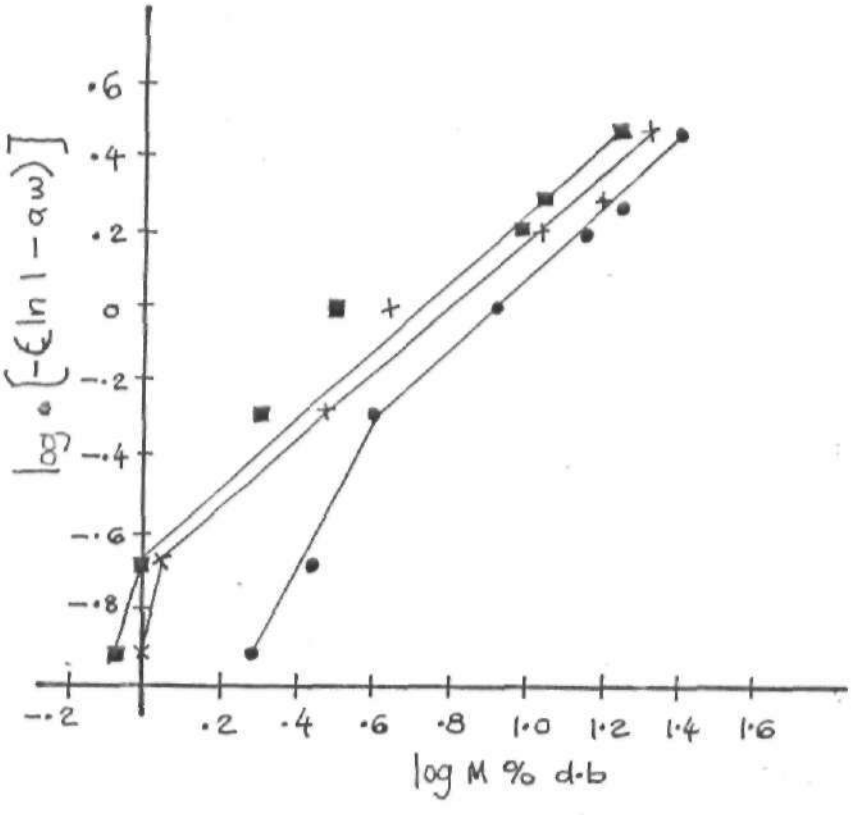


FIG 15:- HENDERSON PLOT FOR MILLT MALT AT • 20, + 30 AND ▪ 37 °C

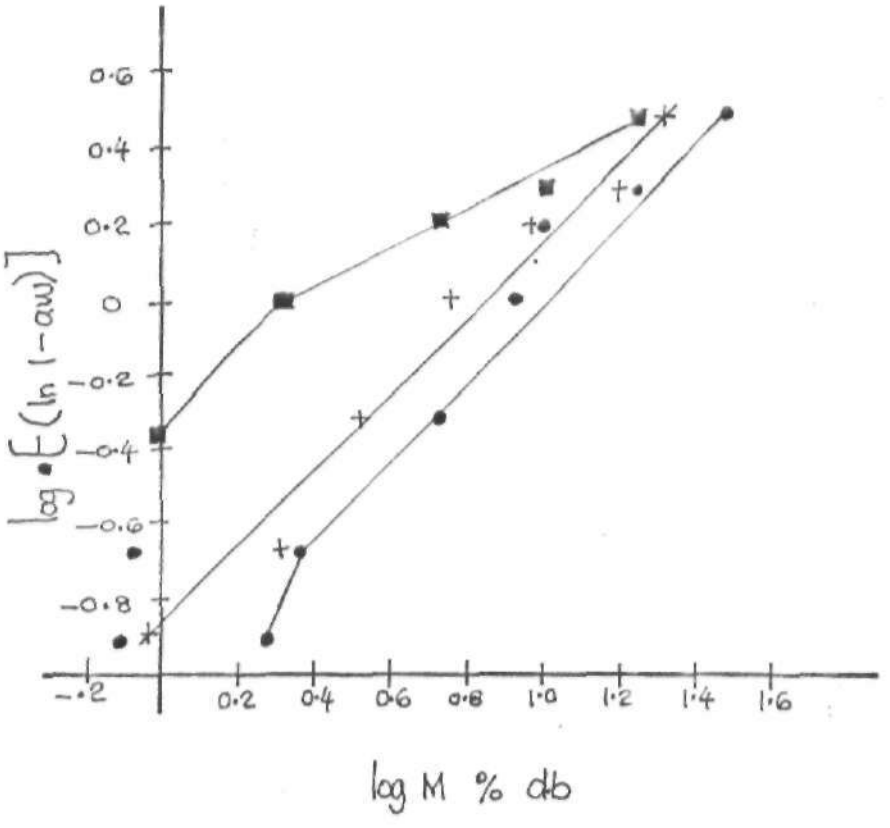


FIG 15:- HENDERSON PLOTS FOR SORGHUM MALT AT •20, +30 AND ▪37°C

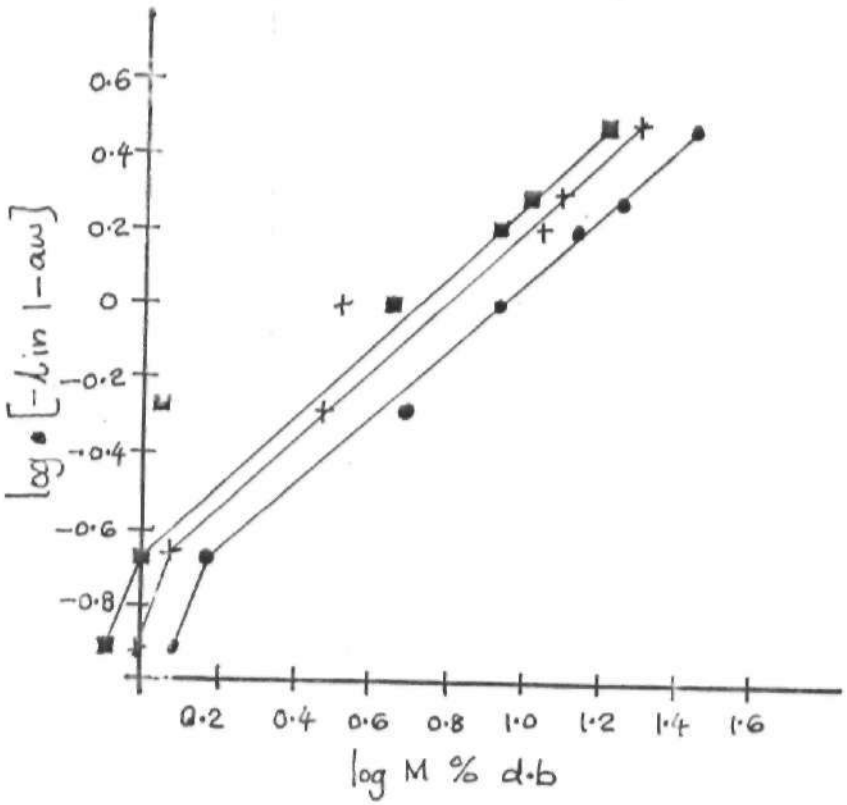


FIG 14:- HENDERSON PLOT FOR MAIZE MALT AT •20, +30 AND ▪37°C

Table 5.1: Constants n and K in Henderson plots for the cereal grains.

Temp °C	Maize		Sorghum		Millet	
	n	K	n	K	n	K
20	1.98	0.60	2.06	0.30	1.03	0.40
30	2.07	0.90	5.73	0.82	0.85	0.20
37	1.3	0.66	3.04	0.78	1.32	0.10

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Table 5.2: Constants n and K in Henderson plot for the cereal ma

Temperature°C	Maize		Sorghum		Millet	
	n	K	n	K	n	K
20	0.84	0.98	1.05	0.28	0.94	0.24
30	1.33	0.92	0.90	0.90	1.24	0.88
37	0.79	0.46	1.65	0.38	1.65	0.64

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Further analysis of the isotherms was carried out using Brunauer et al (1938) equation using water activity value less than 0.45 to obtain the monolayer values (Table 5.3). The monolayer value of a product provides the most desirable moisture content to which the product should be dried at the particular temperatur since the rate of all chemical reactions that could occur in the product are minimal at this moisture content (Salvin, 1959). The monolayer value of grain was significantly higher than that of the corresponding malts. The presence of more soluble sugar that had been formed during malting by the amylase enzyme may be responsible for the low monolayer value in agreement with Agbaji et al, (1992) that the presence of soluble sugars lowers the monolayer value of any product. Fennema (1985) has also reported that the addition of soluble sugars lowers the monolayer value of potato.

Monolayer values for all the samples decrease with increase in temperature in accordance with the report of Saravacos et al, (1986); Weisser, (1985); Denloye and Ade-John, 1985). This can be interpreted to mean that with increase in temperature, the stability of the product decreases, that is, the product can tolerate less and less water content with increase in temperature (Weisser, 1985, Sa and Sereno, 1993).

The heat of desorption, Qs, was determined for each sample using Clausius - Clayperon equation:

$$\frac{d\ln aw}{dT} = \frac{DH}{RT^2}$$

The equation re-expressed in the differential form as :

$$\frac{d\log aw}{d\ 1/T} = -\frac{Qs}{2.303R}$$

where aw = water activity, R is a gas constant and T is the absolute temperature.

Table 5.3: MONOLAYER VALUES OF THE GRAINS AND THEIR MALTS

	MAIZE		SORGHUM		MILLET	
TEMP °C	GRAIN	MALT	GRAIN	MALT	GRAIN	MALT
20	5.69	2.57	8.42	3.44	7.39	2.55
30	4.38	1.68	5.55	1.34	3.43	1.66
37	2.15	0.75	1.98	1.02	1.64	0.72

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A plot of log aw Vs $^{1}/_{T}$ at different moisture levels produced a linear relationship. Qs was calculated from the slope of each line which was determined by linear regression. The heat of desorption decrease with increase in moisture content and approaches a constant value (Weisser, 1985) - (Table 5.4).

Tables 4.9 and 4.10 show the genera of fungi and bacteria that were isolated from the grains and their malts. For the fungi, the genera Helmithosporium, Aspergillus and Fusarium were found to be the predominant organisms as they were isolated from virtually all the grains at the various conditions (temperature and relative humidity) of investigation. This implies that spoilage of cereal grains might be due to these contaminating organisms. For the malts, only two fungal genera of Aspergillus and Mucor were detected. This is obvious because after kilning, most contaminating fungi must have been destroyed. The isolated organisms must have come in contact with the malt from the air during weighing (Ajibola, 1986).

Some fungal genera were also isolated from the interior of the grains. These include Pythium, Helmithosporium and Aspergillus. These fungi must have gained entrance into the grain by the digestion of the seed coat of the grain using the extra cellular enzymes which they secrete (Pelzar et al, 1984) at the surface. The oxygen required for the survival will also be diffusing into the seed through the opening(s) already created by the digestion of the walls.

Verticillium, Curvularia, Alternaria, Phytophtora are other genera of fungi isolated but some of them are not known to be spoilage organisms of cereal grains. Verticilium is a soil organism and must have found its way into the grain during harvesting (Neves et al, 1994).

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Table 5.4: HEAT OF DESORPTION FOR THE CEREAL GRAINS AND THEIR MALTS.

SAMPLE	HEAT OF DESORPTION (KJ) AT 20	30	37°c
MAIZE GRAIN	63	51	40
MAIZE MALT	45	37	30
SORGHUM GRAIN	72	65	46
SORGHUM MALT	58	49	44
MILLET GRAIN	69	51	47
MILLET MALT	46	40	36

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The bacterial genera that were isolated mainly from the surface except Pseudomonas that was found in the interior of the cereal grains. Bacteria are generally not spoilage organisms of cereal grains but care must be taken so that they do not find their ways into the final product where they can be of health hazard to the consumers (man and animals) as most of these isolates are potential pathogen and sometimes opportunistic pathogen e.g E. coli. Some of the bacterial isolates, however, are normal floral of the grains and are important in fermentation during their processing into foods e,g, ogi and burukutu.

From this study, it was found that cereal grains and their malts contain varieties of bacteria and fungi. Their kind and abundance however, depend on climatic factors under which the grains were produced and stored. Microorganisms are more abundant on the surface than inside the grains. The micro-organism found on cereal grains can cause one type of spoilage or the other depending on the moisture content and temperature as reported by Jarvis, (1979). These include souring due to fermentation (especially species of Saccheromyces) which may be followed by rotten, mouldiness due to fungal growth that changes the physical appearance and consistency markedly, caking, reduction in food values, misty smell and damage to grain jeopardizing germination. Appearance of mycotoxins such as Aflatoxin may also result.

A new mycotoxin called Fumonisin B_1 (FB_1) have also been recently isolated from cereal grains. It is from Fusarium, Moniliforme which is implicated in hepatoxicity and equine lenco encephalomalacia, porcine pulmonary oedma, cancer promoting activity and also affect macrophage dependent immune system (Chatterjee and Mukherjee, 1994). They have been found in wide range of maize based food in some part of the World (Marrie et al, 1995).

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Discolouration, loss of texture, offensive odour are other spoilages that fungi can cause in cereal grains their products (Agboola, 1989).

Bacteria such as E.coli, Bacillus, Clostridium, and Proteus are agents of food borne illness (Santo et al, 1994). But generally fungi cause more grain damage than bacteria while the crop is growing in the field, before and after harvest, during transportation and more significantly during storage (William and Rao, 1981). Fungi such as Fusarium and Aspergillus apart from the spoilage they cause are often implicated in cases of otomycosis and mycotic keratitis (Glevin and Robert, 1983). Penicillium has been reported equally as an etiologic agent of human and animal penicillosis. Curvularia and Alternaria are equally implicated in phaeolyomycosis. Candidiasis is known to be caused by Candida alblicans (Chessbrough, 1985) while Aspergillus also causes Aspergillosis (Stainer, et al, 1984).

The time of the appearance of mould was also examined. Mould growth was detected earlier at relative humidity of 95% than at 85% at all the temperatures tested (Table 5.5). This is because at high relative humidity greater than 85%, fungal growth is greatly enhanced (Sa and Sereno, 1993). At relative humidity > 75% the grain appeared better protected against mould than their malts, this is because the malt has a higher emc value due to large size (Agbaji and Agbaji, 1990). On the other hand, the samples appeared better protected against mould growth as the storaged temperature increases because of the effect of temperature on sorption equilibria (Sarravacos et al, 1986; Weisser, 1985; Tsami, 1991).

The micorbial count (viable and total) was determined in order to ascertain the level of contamination which invariably will

Table 5.5: VISUAL DETECTION OF MOULD GROWTH ON THE GRAINS AND THEIR MALTS.

(a) GRAINS

Temperature	Days of detection of mould at RH of: 75%	85%	95%
20	5	4	2
30	7	7	5
37	15	12	9

(b) MALTS

Temperature	Days of detection of mould at RH of: 75%	85%	95%
20	3	3	1
30	5	4	3
37	10	8	7

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give an insight as to the ease of spoilage of these grains. If the count is high it means such grains will get spoilt earlier than those with low count provided other parameters are the same (Adelusi, 1984).

The total count was found to be higher on the millet (5.76×10^6 c/g) while it was least in maize (8.45×10^5 cells/g). Total count for sorghum was found to be 2.38×10^6 cells/g. The variation in the value may be due to unequal moisture content of the samples, their condition of storage and maturity of grain as well as extent of cracking (Rajue et al, 1995) which made the food nutrient of the cereal grains available for microbial growth. The total count for sorghum and millet exceeds the recommended value of 1.00×10^6 cell/g by WHO but that of the maize is still within the range of acceptable limits.

The result of the viable count is shown in Table 4.8. Sorghum had the highest value of 1.81×10^5 cfu/g while millet and maize had 1.03×10^5 cfu/g and 3.8×10^4 cfu/g respectively. The factors that cause these variations might be similar to those identified for the total count above.

5.1 RECOMMENDATIONS AND CONCLUSION

From the research work, it was observed that it is better to keep grains and their malts at high temperature and lower relative humidity. This is because at high relative humidity of > 75% microbial growth is enhanced (Sa and Sereno, 1993). At low temperature of 25°c, growth of microorganisms is known to be retarded (Pelzar et al, 1984) although more water will be sorbed (Tsami et al, 1990) but it has been observed that microbial growth is not a function of temperature alone but other optimum conditions such as water activity and pH must be present (Stainer et al, 1988).

Malt was shown to have a higher emc value than the other grains. Therefore, it is recommended that the grain should not be stored in this form. However, the malt was shown to have fewer number of contaminating microorganism. This is because after kilning, most of the flora had been destroyed. It then follows that grain may also be stored in the malt form provided the environmental relative humidity can be reduced considerably to the level that can not support microbial growth. Exception here is when the industry concerned is not interested in grain in the malt form. In Conclusion, grains should be kept in the raw form than processing them into malt because malt has a higher emc value and both should be kept at high temperatures and low moisture content for good storage stability.

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