MALTING PROPERTIES OF PEARL MILLET, PENNISETUM AMERICANUM, AND ITS EFFECTS ON BREWERS' YEAST.

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (M .Sc) in MICROBIOLOGY

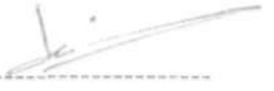
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DECLARATION

This is to certify that I did the work reported in this thesis at the laboratories of the Department of Microbiology, Ahmadu Bello University, Zaria under the supervisions of Dr. J. B. Ameh and Prof. L. J. Ogbadu.

This thesis is an original work carried out by myself and no part of it bas been presented elsewhere for the award of a degree or a diploma. The work of other investigators and researchers referred to in this dissertation are duly acknowledged.



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CERTIFICATION

This thesis entitled "The malting properties of pearl millet Pennisetum americanum (L.), and its effects on brewers' yeast" by Mr. Duro Damisa, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University Zaria and is approved for its contribution to knowledge and literary presentation.

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To the memory of

MALL. DAMISA EJIBADE OGAMAJIREBA

"OZI - ONIYA"

ACKNOWLEDGEMENT

I am pleased to acknowledge the persons and institutions whose assistance was crucial to the completion of this work. I thank the chairman of the supervisory committee, Dr. J. B. Ameh, who provided invaluable critical input from his view point as an experienced researcher. His intellectual insight into this work together with his pereceptive reading of the manuscript make the whole research a reality. Same goes to Prof. L. J. Ogbadu for her encouragement and scholarly contributions with regard to the experiemental design of this work. I also thank Dr (Mrs.) V. J. Umoh for reading through the manuscript and effecting the necessary corrections.

I appreciate my mother, Adisetu Damisa for her wise counsel. I am grateful to my special friend and beloved one, Hannah Funmilayo Momoh for all her encouragement and understanding. Kindest gratitude to my Cousin, Engr. Abdulkareem Jatto for his love and generous contributions. Sincere thanks to my Uncles, Mr. S. O. D. Ahmadu and Mr. D. O. Otaru for all their love. I also appreciate the efforts of Mr. P. W. E. Istead of the institute of brewing, London; Dr. Chris Marchbanks of Burton upon trent, staffs, England; Dr. T. C. S. Dolan of Highland distilleries, Banffshire, England; Dr. J. C. Harrison of Maidenhead, Berks, England and Dr. W. B. Ndahi of Institute of Agricultural research, ABU, Zaria for all the free literature and source materials they provided for this work. They are very wonderful people. A note of thanks to sister Florence Suleiman and to Prof. Edward I. Alcamo of State University of New York, USA, for their words of encouragement.

I acknowledge the many kindness extended by a number of good friends like Mr. Ayo Salami of AIICO Plc, Engr. J. J. Umar, Engr. Y. N. Mohammed, Miss Katumi Musa, Messrs George Nche Ndifor-Agwafor, Kola Oladunmoye (my brother), Abdulkarim Ibrahim, Abdulsalam Ihima Ali, Isah Sule, Abdulsalam Akande, U. K. Ikwechegh, Ejike Udobi, Kaharu Yusuf, Suleiman Abubakar, Salawu Saliu, Jaguda Enevene Nuru Olagunju and Ojo Damisa. Also to Husseina Sadiq, Christy Oniwon, Hassia O. Otaru, Awa Dzikwi, Asmau' Abdulkarim and host of other friends and colleagues whose names are not mentioned here but whose minds and other resources I often strained, I appreciate with gratitude, the elegant stenographic crafting of this work by Messrs. Pius Okogbenin and Odey N. Gabriel.

I must not forget to express my appreciation to Mr. J. U. Damisa and the rest of the other members of my family for their concern and message of good will.

Lastly, to the Almighty God without whom the programme would have been a far lesser work.

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ABSTRACT

Pearl millet, Pennisetum americanum (L). FV.19 Cultivar was used in this study involving two malting cycles, (2 - clay) and (3-day) and five kilning temperatures; (40°, 45°, 50°, 55° and 60 c). Malts were prepared from this cultivar by steeping for 24 hours, germinating for either 2 - days or 3 days and kilning at the live temperatures. Analysis were made for the malting loss, mall yield, diastatic power, cold - and hot- water extracts of the ground malts. Worts derived from each malt by the infusion method of mashing were fermented by batch method of fermentation for nine days using the brewers' yeast, Saccharomyces uvarum, Effect of the fermenting wort on the brewers' yeast were studied and the yeast flocculence determined Products of the unconverted starch in the fermented worts were analysed for all the worts. Results indicate that germination period has remarkable effect on the quality of malt produced as the 3-day regime malt gave better malt qualities than the 2-day regime malt although the cold-and hot-water extract, and diastatic power assessment of the malt were far inferior to those of commercial barley malt. Prolonged malting gave low malt yield. Malt yield was found to be high as only 10 to 18 percent of initial weight of grains were lost in both cycles. Steeping the grain at room temperature lor 24 hours is conducive before malting; malting for 3-days, and thereafter kilning at 50 c for 24 hours was found to be the best for producing good malt Fermented won prepared from the malt indicated that amylodextrins and erythrodextrins are largely the products of the unconverted starch in the wort for the 2-day and 3-day regime malts respectively Yeast floccculence was satisfactory (70 %) and a decline in the fermentable sugars promoted flocculation Yeast viability was maintained for the three pitching cycles although the generation time was high (.32 - 40 hours) with low number of generations (5- 8) and growth rate (4.2 x K) ^{4}-5.1 x 10^{-4}). Bud formation was observed nine hours into the initiation of fermentation and slowed down towards the termination of fermentation, with cells looking elongated with irregular buds The pH of the wort was satisfactory (3.8), but the alcohol percent by volume obtained was low (l.3%V/V). These results show that malting and kilning of millet have no adverse effects on brewers' yeast and as such could provide a source of raw materials in the brewing industry and also as substrate for single cell protein production The millet mall may be formulated to provide a good source of malt extract for microbiological media.

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INTRODUCTION

The beer brewing industry in Nigeria represents a vital industry in the generation of income for the country. There exists over thirty breweries in Nigeria with total installed capacity of 18.8 million hectolitres (RMRDC, 1989). Over half a million people derive their daily bread from this sub-sector either directly or indirectly. Majority of the breweries in the country now are operating below their total installed capacity due to the January 1988 ban by the Nigeria Government on the importation of malted barley. Beer consumption per person in 1986 was 10-12 litres (Ogazi et al 1984).

In compliance with the Federal Government policy on encouraging import substitution with respect to raw materials, it has become necessary to examine the suitability of locally available cereals for use as raw materials in the brewing industries. Using pearl millet, Pennisetum americanum (L) malt as a complete or partial replacement for barley or sorghum (lately) malt is likely to interest brewers in countries which have a suitable climate for growing it and therefore its use will act as an incentive to local farmers to cultivate more of this cereal. The recorded history of beer brewing with barley malt covers approximately 5000 years (Harrison et al, 1987). Barley has been the grain of choice because of its peculiar enzyme content which other grains have unrivalled (MacGregor et al,1988). Growing of barley in Nigeria and tropics is hindered by climatic factors. A hundred percent based beer produced from sorghum has been documented (Ogundiwin, 1989, Aisien and Ghosh, 1978). Millet too has been utilised extensively in the preparation of several starch - based foods and traditional low alcoholic beverages (Novellie, 1977, Kolawole et al 1987).

Pearl millet, Pennisetum americanum, formerly called Pennisetum typhoides is locally known as 'Gyero' and has not received much attention as a brewing material. Little is known about the history of the crop and is grown in dry regions especially the sahelian zone (Guillaumet, 1980) because it finds its main agricultural niche in this region where maize and sorghum cannot grow (Kochlar, 1981). It is the sixth most important cereal in the world (Panwal and Pawar, 1989) with total world output reaching 45 million tonnes in 1986 - representing 1.9% of total food production (Vietmeyer, 1986) while estimated output in 1987 in Nigeria was 39 million tonnes (RMRDC, 1990). Millet plays an import role in the farming system of savannah area (Nwasike, et al, 1982) and it also serves as a staple food in many parts of Africa, Asia, Central America and the middle East (Rachie 1975). Burukutu or Pito has been produced from malted millet (Ekundayo, 1969, Faparusi et al, 1973). In this tradition fermentation, the dregs of a previous brew are inoculated into the new wort to initiate the process and since the fermentation process is an uncontrolled inoculation, variations in the quality and stability of the products do exist (Sanni, 1993). Such brews normally contain an

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unusually large populations of fermentative organisms and very likely some pathogens too (Odeyemi, 1984). These brews differ from the European type beers because lactic acid is formed during fermentation, the finished beer contains a large amount of insoluble materials and is consumed while actively fermenting (Walls and Ross, 1970).

Industrial scale malting is a new practice in Nigeria, accounting for less than 0.1% in this field (Ikediobi, 1989) as only a few companies are currently engaged in this sector. The methodology of malting practised in the household for burukutu and pito fermentation differs from place to place

Sorghum has been researched into extensively in terms of its malting and brewing potentials but not much attention has been given to millet. Therefore, the objective of this work were:-

1. To produce an acceptable malt from the millet cultivar by varying the parameters for malting i.e germination regime and kilning temperature.

2. To produce wort from such malt .

3. To carry out fermentation studies in order to evaluate the effects of the wort on growth and fermentative ability of brewers' yeast Saccharomyces uvarum

CHAPTER 2.0

REVIEW OF LITERATURE

2.1 MALT

Malt is any cereal which has been germinated for a limited period of time and growth of embryo terminated by drying under a set of conditions (Adeyemi, 1989). Malting is aimed at promoting synthesis and accumulation by the cereal grain of hydrolytic enzyme such as amylases and proteases, to solubilise the walls of the grain endosperm and secure the enzymatic breakdown of these soluble components to low molecular weight compounds and to degrade the cereal protein to a desired mixture of amino acids and peptides (Uraih and Izuagbe, 1990). These free amino acids and small peptides are required for yeast nutrition in fermentation (Baxter, 1981) . Therefore , a malt of good quality is a malt which through a normal and problem free brewing process will produce a good beer (Jacobsen, 1986). The Amino acid composition of different malt extracts varies according to the grain used but proline always make up 50% of the total amino acids present (Crueger and Crueger, 1984). Dry malt extract consists of about 90 - 92% carbohydrates majorly hexoses (glucose, fructose), disaccharides (maltose, sucrose), trisaccharides (maltotriose) and dextrins. Purines, pyrimidine and vitamins are also present. Overheating malt can result in 'Maillard ' reaction due to low pH value and high proportion of reducing sugar. In this conversion, the amino groups of amines, amino acids (especially lysine) react with the carbonyl groups of reducing sugars, aldehydes or ketones to form a brown condensation product (Greenshield and Macgillviray, 1972). These reaction products are not suitable for yeast. Barley malt is the brewers grain of choice (Pomeranz, 1987) though use of millet has been suggested as substitute (Olaniyi, 1984), although it has also been used as adjuncts (Dhamija and singh, 1987). Sodium bisulphite treated malt extract can be stored over a period of six months than an untreated malt whose microbial contamination increases with increasing pH (Wagle, et al, 1975) .

Selection for malting quality in cereal depends primarilly on small scale malting tests (Morgan and Gothard, 1981). Parameters used for malt quality assessment include hot and cold water extracts, moisture content determination, dextrinising power, diastatic power and filtration time. These parameters help the brewer to determine the malt value with regard to the nutrient yield by the malt and also as a blueprint for malt evaluation and consequently a guide for commercial transaction (Palmer, 1989). Grain modification can be considered as the ultimate of a successful malting process. Malting loss is an indication of the percentage of extent of germination. It is the total loss in weight occuring when cereal is converted into malt. Steeping loss, root weight loss and respiratory loss all

contribute to malting loss. Malting loss is increased at higher moisture levels in germinating grain. Hot water extract (HWE) is a measure of the quantity of dissolved materials in a wort prepared mashing on a laboratory scale . Increased level of hydrolytic enzyme during mashing results often in increased yield of hot water extract (Palmer, 1983). Cold water extract (CWE) measures the performed soluble sugar level which is leached out into solution from ground malt under condition which excludes the participation of the enzymes. High extract yield is a direct consequence of the degree of grain modification (Okon and Etuk, 1992). Malting cereals produce amylases (diastase). The degree of amylolytic activity of the malt is therefore referred to as diastatic power. Malt with high diastatic power is best adopted for brewing and diastatic power is influenced by several factors such as temperature, moisture level, germination time, type of grain (Novellie, 1962). Diastatic power therefore assesses the total activity of enzyme that are involved in the hydrolysis of starch during mashing. Wort run - off rate and extract yield can be significantly improved by reducing the grist size of the husk fraction or optimising the malt grist profile for brewing operation (Stubits et al. 1986). The determination of moisture in malting cereal is important since the rate of germination and modification intensity of grains are controlled by regulating the moisture content and temperature. Grain storage stability is also a function of its moisture content (Briggs et al,1989).

2.2 THE MILLET.

Millet is one the major cereal grains consumed by many people in tropical Africa, parts of Asia and Latin America where the climate favours the cultivation of these crops. The pearl millet is one of the varieties of different millet strains cultivated. The other varieties are finger millet, foxtail millet, proso millet, kodo millet and barnyard millet.

Pearl millet, which is the grain of investigation, is similar to barley but it loses its husks during threshing whereas barley retains them (Skinner, 1987), and the lack of husks can make filtration difficult. Unlike barley grain which in spindle - shaped, pearl millet kernel varies in shape from sphere to sub-cylindrical. The grain has an irregular indentations of the pericarp into the endosperm (Kent, 1982). Pearl millet is a naked caryopses. The seed comprises of seed coat, germ and endoplasm. Husked types of millet have additionally, outside the fruit coat, the fused glumes (Palea and lemma) which constitute the husk. Histological examination of the embryo tissue reveals the presence of important structures such as the provascular tissue, the parenchyma cells of the scutellar tissue and the columnar epithelia cells. The aleurone tissue located beneath the thick pericarp, in the endosperm consists of a single layer of cells (Aisien et al , 1986). According to Macleod and Palmer as cited by

Aisien et al, (1986), the aleurone layer appears to encroach on the periphery of the scutellum, an observation which has also been made in barley. Unfermented whole grains contain about 84% carbohydrate and 9% protein (Oyenuga, 1968). Most of the millet have a crude fibre content of 2 to 5 times that of sorghum but the protein content is lower than that of all other cereals. Mineral content is higher in barley and millets than in maize and sorghum (Freeman and Bocan, 1973). Starch from millet is similar to that from sorghum and maize in most respects, but the granule size is slightly smaller and the starch has a slightly lower tendency to retrograde. Physicochemical attributes of starch isolated from pearl millet in an investigation showed that malted starches contained a majority of smaller granules, slightly more amylase and exhibited high gelatinisation temperature, lower swelling power, high solubility in water, lower intrinsic viscosity than native starches and more susceptible to amylosis (Malleshi et al, 1986 a). Starch granules are insoluble in cold water but when heated in the presence of water, it absorbs it, swell and burst (gelatinisation). Malleshi et al (1986 b) reported that malted samples of pearl millet contain significantly higher levels of free sugars (glucose, fructose and maltose) and water-soluble non starchy polysaccharides (WSNP) than native millets. The WSNP isolated were richer in hexoses and pentoses (Malleshi et al, 1986 b) . Since alcohol is derived from the carbohydrate of the grain, the total nitrogen of a grain which has a large effect on the protein content and in turn on the carbohydrate content has been found to have an effect on the possible alcohol yield by giving low yield when high in relation to the carbohydrate content (Dolan, 1976). Millets and sorghum are characterised by the relatively high leucine content. The germ of millet contains a lot of fat and so undegermed meals in hot climate become rancid. Lipid content is 4 to 6%, saturated fatty acids comprise 11 - 26% of total, while unsaturated fatty acids is 72 to 85% (Freeman and Bocan, 1973). Main phospholipid was found to be lipophosphatidyl choline (42%) and the main glycoside were esterified sterol glycolipids and cerebrosides i.e ceramide monohexosides (Osagie and Kates, 1984). The proportion of anatomical parts (endosperm, germ and bran) determination revealed that the germ forms a large part of the kernel and the amount of bran varies with seed size (Abdelrahman, 1984). About 95% of the mineral matter of these cereals with naked caryopsis (sorghum and some millet varieties) and the kernels of husked millet consist of phosphates and sulphates of magnesium, potassium and calcium (Kent, 1982). Malting has been found to decrease the phytate content and increase the lysine, tryptophan and ascorbic acid contents (Malleshi and Desikachar, 1986).

Investigations into the water absorption characteristics of selected cereal grains has revealed that millet takes about 24hrs to reach its water absorption capacities. Rupture stress increases just after soaking in water and generally decreases with increase in moisture content (Ituen, et al, 1986) Tests for sulphur compound and thiocynates were negative but the cyanide test were positive, although

low levels were detected. Animal assays of methanolic extracts indicted that natural giotrogenic activity increased on wetting and subsequent drying possibly due to an increase in the concentration of phloroglucinol and resorcinol which are potent goitrogens (Birzer, et al, 1987).

Kernel size appears to make millet less susceptible to insect attack and so, in malting practice, the grain size causes inconveniences which would necessitate alteration in conventional malting equipment if the crop was to be malted on an industrial scale (Nout and Davies, 1982). Spike worm have recently been reported as a potential threat to pearl millet production in sub-sahelian zone of west Africa (Gahukar, 1987). Presence of mould in grains of pearl millet is evidence by grain discoloration. Ikediobi, (1989) observed that most of the mould species encountered during malting are either picked up from ware - houses during storage or are field derived. During malting, pearl millet may suffer mild fungal attack at later stages of germination. This may be avoided by washing the germinating seeds with 0.1% lime water once a day during germination and growth (Malleshi and Desikachar, 1986 a). Prolonged storage of millet will result in increase in Aspergillus species population with a concomitant decrease in field fungi, Drechslera oryzae, as observed by Emayavaramban and Ramanbadram, (1987).

2.3 THE YEAST.

The yeast organisms, specifically the Saccharomyces genus, are regarded as the "oldest pant cultivated by man" (Russell et al, 1986). They are unicellular fungi and have traditionally been used in brewing because of their ability to produce carbondioxide and ethanol. Yeasts as unicellular fungi are nucleated, nonmotile, chemosynthetic and either spherical, ovoid or elliptical in shape. Their lack of hyphae and existence as individual cells enveloped within thick cell walls distinguish them from other fungi. Cellular dimension vary in respect to the species, but in general, yeasts range in width from 2.5 to 10.5μ and 4.5 to 21μ in length (Reed and Peppler, 1973). The Saccharomyces species are ascosporogenous yeasts (true yeast) and generally have 14 pairs (haploid) of chromosome compared to bacteria with one (Lehniger, 1975). Yeasts are ubiquitous and reproduce asexually by budding. However, some strains can reproduce sexually under certain environmental conditions. In the budding process, a small localised area of the cell wall protrudes outwardly, cytoplasm from the parent cell streams into the area, nuclear material replicates, by mitosis and one of the daughter nuclei moves into the distended protuberance. The developing bud continues to grow and increase in size until it attains about a third of the size the mother cell. The cell wall then closes off the newly formed yeast cell, leaving a bud scar on the mother cell and a birth scar on the new yeast cell (Miller, 1982). Cells may reproduce as many as 9 to 43 buds per cell with an average of 24 buds in a medium

not limited by nutrients in Saccharomyces cerevisae species. Bud formation requires about 1.5 to 2hrs for completion under ideal conditions. Aged cells, however require longer time for duplication. Saccharomyces species are heterothallic, requiring compatible mating types for conjugation. Homothallic yeasts, have in contrast, the potential of converting from one mating type to another (Trivedi et al, 1986).

Yeasts are classified on their basis of activity. Active yeasts are those used for fermentation e g the brewers' yeast. Brewers yeast include select strains of Saccharomyces cerevisae for producing lager beer and ale. Lager is produced by the bottom fermenting yeast S. uvarum. As a bottom fermenter, the yeast settles to the bottom of the fermenter at the end of the alcohol generating process, leaving the lager beer as a clear liquid (Hardwick, 1983). The top fermenting strain is used to produce ale and often clumps together during growth and are buoyed to the surface of the fermenter from which it can be skimmed (Frazier, 1967). The two strains differ in their fermentation requirement. For example, top fermenters require higher temperatures and shorter time for ale production. Their principal point of difference is in their ability to ferment melibiose. Top fermenters cannot assimilate melibiose and, so, can handle only one - third of a raffinose molecule. Bottom - fermenters, on the other hand can ferment the entire raffinose molecules (Nagoda-withana, 1986). Recovered yeast after fermentation can be recycled up to 20 times before it needs to be replaced by a pure culture.

Young colonies of yeast when growing on solid media, have almost a very characteristic appearance, being moist and somewhat slimy (Joseph, 1974) with the colour whitish cream or pink. Majority of the numerous species grow as loose aggregates of single cells which may be globose, ovoid more or less pear shaped or elongated and almost cylindrical. (Joseph, 1974).

2.4 MALTING OPERATIONS

Malting is a well known process associated with beer production. The various stages are outlined below:-

2.4.1 STEEPING

Good quality seed grain is ideal for use and a container large enough to hold twice the required quantity will suffice for the steeping operation (Harrison et al, 1987). The composition of ware used in steeping can have an indirect effect on the colloidal stability of the beer made from the malt. It would seem advisable to use a water which in respect to its mineral composition is not very different from a potable water (Moll, 1987). At the initial stage of malting, sound cereal grain which has been cleaned with water to separate the dirt and other extraneous materials is steeped in water to a moisture level which will allow it to germinate, produce enzyme and develop an optimal level of brewers extract (Palmer, 1983). The grain absorbs moisture and increase rapidly at first but slows down progressively and ceases eventually when germination does not follow (Briggs et al, 1981). In order to counteract water sensitivity and to accelerate germination, the grain is not submerged continuously in water, but inter-spaced with 'air -rest ' period after 6 to 8 hours of steeping to ensure even germination (Harrison et al, 1987). The extent to which water absoption occurs during steeping in determined by seed composition, seed coat permeability, availability of water in seed environment, and presence of additives in steep liquor, (Mayer and Peljakoff, 1963). Steeping raises the water content of the grain to a level high enough to provide the aqueous environment required by various enzymes in the grain to act (Ikediobi, 1989). Deficiencies which may be induced by anaerobic conditions in steeping appear to be eliminated during germination (Cantrell, 1987). Conversely, malt quality may be affected by excessive steep aeration. In conventional malting practice, grain transferred to the germination phase of malting below 44% moisture will often require spraying with water to ensure good enzyme and extract development. A low moisture content in grain leads to a reduction in the diastatic power (Novellie, 1962), hence the choice of an appropriate steeping duration is critical during malting. The lower the water content, the slower the modification rate and hence, the poorer the malt (Olaniyi and Akinrele, 1987). This process reduces root growth and malting loss. Growth occurs during steeping with a resultant oxygen (B.O.D.) consumption by steeping grains,. This results in pH increase and bacterial growth, hence the need to change the steep liquor during steeping (Harrison et al, 1987). Beers from malt steep at higher optimum moisture level are in general more stable than those to lower optimum (Palmer, 1989). The final moisture content, which is a function of steeping regime, that a malting cereal must attain during steeping is important

in controlling the germination and modification intensity of the grain. In one particular study (Malleshi and Desikachar, 1986 b), the moisture uptake by seeds of finger - millet steeped at different temperatures was determined. Progressive increase in steeping temperature gave a corresponding increase in moisture uptake by the seeds until the seeds attained moisture saturation of 33 to 34%. Steeping barley grains to a moisture level of 43 to 46%, the optimum for this cereal, has been found to increase the amylase content of malt appreciably (Surminski et al, 1986).

Various additives can be added to the steep water to influence malt quality. Such additives include lime water (to reduce phenols), formaldehyde (to reduce phenol and improve germination), and chlorine or hypochlorite (to reduce growth of algae and fungi). Steeping plants vary in different malting industries. These extend from small old flat - bottomed concrete steeps with no aeration or emptying facilities to large conical steeps with aerating gravity discharge systems and to very large flat bottomed steeps with automatic aerating and discharging facilities (Palmer, 1983).

2.4.2 MALTING

Malting refers to germination of the grain under a set of conditions. Germination is the second stage of the malting process. Here, the chitted grain is allowed to grow and develop the endosperm enzyme which will modify the endosperm starch, proteins and cell wall materials to useful extract (Palmer, 1983). Grain modification can be considered as the ultimate of a successful malting process. Maltsters are however not unanimous in choosing a standard parameter to estimate the extent of modification of grain during malting (Okon and Etuk, 1992). Germination process in malting produces a spectrum of enzymes intended to cater for the physiological needs of the young seedling (Okechukwu et al, 1984). Malts are thus described as poorly modified, under-modified well modified or over modified (Palmer and Pathgate, 1976). The progress of malting is monitored by physical examination of, and assay of key enzyme systems in the germinating grain.

In malting, there are several measures through which the composition of malt can be varied so as to favour the colloidal stability of beer. Among these are; the duration and temperature of steeping, duration and temperature of germination; duration and temperature of kilning and, the effect of certain additives (Moll, 1987). Degree of modification of malt also affects its colloidal stability. Beers made from malt with longer days of germination was distinctly the more stable both for chill haze and in an agitated 110hours test (Moll, 1987). Before germination, grains from the steep are first drained of water for sometime and then made up into a heap or couch to build up heat (Briggs et al, 1981). Malting techniques vary and is an integral part of the type of malt produced. In floor malting, the

grain is spread out on a very clean floor at a predetermined thickness and left to sprout (Ikediobi, 1989). In traditional malting, the grain is turned regularly with shovels to prevent the rootlets from becoming entangled and the grains at the bottom becoming suffocated (Skinner, 1976). In barley grains, the sample size can affect the malting potential so that in thick beds there is non uniform sprout because of lack of air (Henry and Mclean, 1984). In pneumatic malting systems, the grains bed is turned by automatic turners and the temperature is controlled by cooled humidified air. On the other hand, in static (non rotating) germination, the malting grain is turned daily to avoid matting of the roots.

One of the most significant advances was the recognition of the role of the plant hormones, particularly the gibberellins, in germination and the exploitation of gibberellic acid (GA) in commercial malting that began in the late fifties (Hudson, 1986).The discovery that exogenous GA applied to barley greatly enhanced the synthesis of α -amylase and other hydrolytic enzymes and could significantly reduce malting time, came at a time of expansion in demand for malt and was undoubtedly a major factor in increasing production. It is noteworthy that neither gibberellic acid nor bromate does affect amylase and protease levels in sorghum (Aisien and Muts, 1987). GA is in itself produced by the growing embryo (Palmer and Pathgate, 1976). Commercial GA is produced from the fungus, Gibberella fujikuroi (VanDemark and Batzing, 1987). During the first day of germination, the maltsters may apply by spraying, low levels of GA onto the chitted grain to supplement that produced in the embryo of malting grain. The ungerminated embryo (non-chitted seed) does not absorb the hormone efficiently. In general, the hormone helps in enhancement of enzyme production like α -amylase, limit dextrinase, endo β --glucanases and endoproteases, acceleration of endosperm modification and reduction of malting time (Palmer, 1983). The activities of - β amylase and carboxy peptidase also increase during malting. Pearl millet has been found to exhibit higher α amylase activity with two to three days of germination while maize, sorghum, wheat and triticale showed high enzyme activity after 4 to 5 days of germination (Malleshi and Desikachar, 1986 a). It is also higher in B-amylase than sorghum malt (Hulse et al, 1980). Malting conditions such as germination temperature, time, kilning temperature, can strongly influence the quality of malt especially the availability of lysine, α -amylase activity, dry matter loss and the malt aroma (Malleshi and Desikachar, 1986 b).

During endosperm modification, the glucanases break down the β-glucan cell wall and expose the protein matrix to attack by proteases. In a well modified malt, about 75% of the B-glucan material is broken down and about 40% of the protein is solubilised. Curiously enough only about 5 to 10% of

the starch is broken down during malting. This is an ideal situation because while β-glucan and proteins are inefficiently broken down during infusion mashing at 65°C, starch is readily converted at this temperature. This is thus, one of the primary objectives of the maltster; to get efficient β-glucan and protein break down during malting while restricting starch break down and loss of its sugar products to the growing roots (Palmer and Pathgate, 1976). To curb root growth and malting loss, malting grains is sprayed with potassium bromate to reduce proteolysis in the endosperm.

Diastatic power of a malt may be defined as the total activity of enzymes that are involved in the hydrolysis of starch during mashing (Henry, 1984). According to Novellie, (1977) it is the degree of amyloytic activity of a malt and is a better index of malt quality than extract yield. The diastatic power of malt is influenced by several factors such as temperature, moisture level, germination time and type of grain ., (Novellie, 1962). It has been shown that sorghum malts generally posses inferior diastatic power and therefore give poor yield when compared with barley malts (Okon and Uwaifo, 1985). This has been attributed to various factors among which are quantitative differences in their amylase contents and probably the presence of endogenous amylolytic enzyme inhibitors. Malting often lowers the paste viscosity of cereal flours especially millet (Malleshi and Desikachar, 1986 a) Maltsters have always had to be on their guard against microbiological contamination damaging their product. Cereals from the field are heavily contaminated and malting conditions is conducive to growth of microorgainsims. Floor maltings are notorious for black and blue moulds especially in warm weathers (Hudson, 1986).

The extent of grain modification due to malting can be tested roughly by the sweet taste developed in the grain and to the length of the young plumule (Faparusi, 1970). The various enzymes formed breakdown some quantities of their respective substrates but the major breakdown takes place during mashing (Okafor, 1987). In any prolonged germination, malting losses can be very high due to the considerable length of the radicles compared to the grain dimension (Nout and Davies, 1982). Use of excessive quantities of unmalted cereals could have some negative effects on beer quality (Maiden, 1975).

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2.4.3 KILNING

Generally, further reactions in the grain are halted by kilning which consists of heating the 'green' malt in an oven first with a relatively mild temperature (drying phase) until the moisture content is thus reduced (Okafor, 1987). Subsequently, the temperature of heating (curing phase) depends on the type of beer to be produced. The purpose of kilning is to reduce the moisture level of the malt, to arrest the growth and enzymic activities of the green malt at the end of the germination process, and to develop malt flavour characteristic, beer quality factors and colour - forming potential (Palmer, 1983). In the manufacture of lager and pale ale malt, these enzymes and chemical reactions are kept to a minimum. In the manufacture of traditional ale malt, these enzymic and chemical reactions are kept to a minimum. In the manufacture of traditional ale malts the dried malt is subjected to a final curing phase, during which the temperature is raised to 100°C for about 2 hours. In this phase, thermolabile enzymes such as -β--amylase, endo-β-glucanase and endo-protease are denatured and lose much of their activities. In cured malts, the moisture level is about 2.3 to 3.0% (Palmer, 1983). During the first phase of drying when the malt temperature is about 25°C to 30°C, the green malt enzyme especially those degrading carbohydrates and proteins are very active. The sugar and the amino acid products react during kilning to from melanoidins and other compounds which give colour to the wort and beer (Palmer, 1983). Turning of the green malt during germination is essential to prevent matting of the roots but in traditional kilns, the grain is not turned (Palmer, 1983).

Curing reduces enzyme activity and so there is a trend towards using lightly kilned malts for both lager and ale production. These lightly kilned malts are used by some brewers to help convert unmalted adjuncts or less well modified malts (Palmer and Pathgate, 1976). Kilning not only removes water, denatures enzyme and increases colour forming potential and flavour, but it also coagulates protein which might otherwise reduce beer quality. Recent studies of the influence of kilning on beer flavour suggests that kilning at about 70°C to 80°C for extended periods can destroy the potential of the malt to produce dimethyl sulphide (DMS) (Palmer and Pathgate, 1976). Beers made from cured malts do not have high DMS levels, which are higher in beers from lightly kilned malts. 20 to 80 µg /L of DMS is regarded as an important flavour compound in lagers (Palmer and Pathgate, 1976). Kilning can be carried out in shallow trays, provided frequent turning is practised. Drum Kilning is far more superior, firstly because even temperature can be maintained and, secondly if the drum is constructed of fine mesh wire with approximately one sixteenth inch gap, then the drum will automatically remove the roots as it revolves (Harrison, 1987). Grain or malt roasting in small quantities can be readily effected using an ordinary domestic oven and some shallow trays made from stainless steel gauze or aluminium

sheet (Harrison, 1987). Sorghum can be kilned by drying in the open air sunshine (Aisien and Muts,

1987).

2.4.4 MILLING

The purpose of milling is to expose particles of the malt to the hydrolytic effects of malt enzymes during the mashing process (Okafor, 1987). Before the kilned malt is milled, it is cleaned of the germinated roots. Particles size must not be too small because wort run-off will be impaired, nor must it be too large as extract yield will be reduced (Palmer, 1983). The brewer has therefore to find a compromise particle size which will give him maximum extraction and yet permit reasonably rapid filtration rate. Investigation of milling showed that sorghum and millet bran coats are easily pulverised to a fine powder which makes it difficult to separate from the flour (Pertan, 1983). A particle size range of 300 - 100μ is considered ideal for larger beer production (Chukwurah, 1989). Therefore correct roller mill setting must be used to ensure that the particle size falls largely within this range during the milling operation. Alternatively, the malt may be milled coarsely or finely and two separate mashes carried out. The difference in the extract yield is an indication of the degree of modification. Modification is considered satisfactory for values less than 50^{0}L/kg for barley malt (Palmer, 1983). Unlike barely malting, millet and sorghum are percrushed by a hammer mill before feeding into a roller mill because of the relatively smaller sizes of their kernels, otherwise grist with poor extract yield would be obtained since small kernels pass through the feed and crushing rollers of the mill without being crushed (Chukwurah 1989).

Wet - milling is generally preferred for barley malt or grain meant for beer production. This helps to preserved the husk, which facilitates wort separation when the lauter tun is used for mash filtration (Arri, 1989). However, sorghum and millet are huskless and cannot be successfully handled in a lauter tun.

2.5 MASHING

Mashing is an extension of malting and is aimed objectively at producing fermentable sugars and other soluble suitable for yeast nutrition during fermentation through the amyloytic and to a lesser extent, proteolytic degradation of gelatinised starch and protein respectively (Okon, 19880. An aqueous extract of malted cereal is an excellent substrate for many fungi and yeast (Crueger and Cruegar' 1984). Mashing is the central part of brewing as it determines the nature of the wort and hence the nature of the nutrients available to the yeasts. Successful mashing plays a major role in determining the final characteristics and quality of a finished beer, hence it importance cannot be over emphasised (Harrison et al, 1987). Mashing consists of mixing the ground malt at temperatures optimal for amylases and proteases derived from the malt. The aqueous solution resulting from mashing components is known as wort. In barely malt, two largest components in terms of dry weight are starch (55%) and protein (10 to 12%). The controlled breakdown of these two components has tremendous influence on beer character. (Okafor, 1987). Of the malt starch, 20 to 25% is made up of amylase. The key enzymes in the break down of malt starch are the α and β - amylases. Optimum temperature and pH for α - amylase action is 70^0C and 5.0 respectively but are destroyed at 75^0C. Malt protein (Albumin, Globulin etc.) breakdown starts during malting and continues during mashing by proteases. Free α-Amino nitrogen (FAN) is an essential nutrient for yeast growth (Taylor, 1983). During fermentation under normal conditions of sorghum beer mashing, 60^0C at pH 4.0 production of FAN by proteolysis account for approximately 30% of wort FAN the remaining 70% being performed in the malt (Taylor and Boyd, 1986). The quality of FAN in sorghum beer worts is good as it does not contain a high percentage of proline. Optimal conditions for FAN production during mashing are 51^0C and pH 4.6 (Taylor and Boyd, 1986). Protein breakdown has no pronounced optimum temperature but during mashing, it occurs evenly up to 60^0C. Proteolytic activity in wort is however dependent on pH and therefore wort pH must be maintained at 5.2 to 5.5 with lactic acid, mineral acids or calcium sulphate. Mash exposed to too high a temperature will be low in alcohol due to insufficient maltose production since β-amylase enzyme is inactivated (Okafor, 1987). Mash concentration is also important because thinner mash gives higher extract yield and maltose content, however, it is important to maintain a constant temperature over period of 1 to 2 hours throughout mashing (Harrison, 1987).

Three mashing methods are known: Decoction, infusion and the double mash.

Decoction involves mixing the mash at an initial temperature of 35^0C to 37^0C and the temperature is raised in steps to about 75^0 during this period. About one-third of the initial mash is withdrawn and

transferred to the mash kettle where it is heated slowly to boil and returned to the mash turn. the temperature of the mash becoming raised in the process. The enzymes in the heated portion become destroyed, but the starch grains are cooked, gelatinised and exposed. Another portion may be removed, boiled and returned. In this way. The process may be a one - two-, or three-mash process, the initial temperature of 35^{0}C to 40^{0}C favours proteolysis (Narzis, 1981); the three-mash method is the oldest and best known and it originated form Bavaria, in West Germany (Okafor, 1897). From technical port of view decoction method has been reported to give a better wort than the infusion process as far as mashing with sorghum malt is concerned (Skinner, 1976), Owuame and Okafor, 1987), although this poses filtration problems as a result of the viscous nature of the final product. Different cereals have different gelatinsation temperature and this temperature is important when raw grain is used with malt in either an upward infusion or decoction mash system in-order to achieve complete conversion (Marchbank, 1987). Inadequate starch gelatinsation and dextrinisation of sorghum starch may contribute significantly to the low extract yield of sorghum mails (Okafor and Aniche, 19887).

Infusion method is used in Britain and is carried out in a mash-tun which resembles a laughter tub of lager beer, but is deeper (Narizis, 1981). Mash is never boiled but the temperatures is gradually raised. The grist is mixed thoroughly with hot water (2.1 part by weight) to produce a thick porridge like mash and the temperature is carefully raised to about 65^{0}C. It is held at this temperature for a period varying from 30 minutes to several hours (Okafor, 1987). On the average, the holding time is one to two hours. The enzyme acts principally on the starch and it degradation products both the malted and unmalted cereal, and only a little protein breakdown occurs. Further hot water at 75^{0} C to 78^{0}C is sprayed on the mash to obtain as much extract as possible and to halt the enzyme action. No part of the mash is boiled from mashing-in to mashing-off.

Malts carry a large population of micro-organisms including potentially harmfully Gram-positive cocci and Lactobacilli, which can survive the mashing process where relatively low temperatures are used in order to protect the carbohydrates, which are required to continue to function in the fermenting vessel (Dolan, 1976).

2.6 WORT PRODUCTION:

At the end of mashing, insoluble materials are removed from the wort. The conventional method of separating the solids from the mash is to strain the mash in a lauter ('clarifying') tub which is a

vessel with a perforated false bottom on which the husks (for barley) themselves form a bed through which the filtration takes place. For huskless grains such as millet, mash filter is more suitable (Chukwurah, 1989). The spraying (washing with hot water) of the mash solids is done with water at about 80^0c and is continued till the extraction is deemed complete. Later the wort is boiled for one to one and half hours in a brew kettle of stainless steel. During the boiling, agitation and circulation of the wort help increase the amount of precipitation and 'floc' formation. Cooled wort is now ready for fermentation as it contains no enzyme but a rich medium for fermentation.

2.7 FERMENTATION.

Fermentation is the transformation of hexose sugars and other organic compounds, into the carbon skeleton needed for biosynthesis of proteins, nucleic acids and other cellular constituents. Two fermentation mechanisms used by yeasts are the Emden-Mayerhof - Parnas (EMP) pathway and the Hexose monophosphate shunt (pentose phosphate cycle) (Reed and Peppler, 1973). All yeasts can utilise glucose, mannose and fructose and some species can assimilate galactose. The efficiency of utilising carbon and nitrogen sources is dependent upon the enzyme systems with which the organism are equipped. For lager beer brewing, the bottom fermentation is employed. Wort is inoculated to the tune of $7x10^6$ to $15x10^6$ yeast cells per millilitre of wort at 6^0C to 10^0C (Okafor 1987, Uboaja 1991) All small scale brewers depend directly or indirectly on the brewing trade for supplies of good quality yeast. Once a successful brew has been made with yeast from any source, the clean yeast drop from the final racking can be stored under beer in a seated bottle kept at 3^0C to 5^0C in a refrigerator (Harrison et al 1987). Anaerobic conditions pre-dominate in beer and fermentation may last from 7 to 12 days (Aisien and Muts, 1987). The progress of fermentation is followed by wort specific gravity monitoring which tend to decrease as yeasts use up the extract. In a good fermentation, the specific gravity falls rapidly during the first 24 to 30 hours owing to the removal of sugars and the production of ethanol, and then tails off as less readily fermentable materials only are available (Dolan, 1976).

Wort spoilage by bacteria has been reported, for example Enterobacter species have been incriminated in beer wort spoilage resulting in objectionable flavours and odours, while contaminating wild yeast could also carry out competitively, reaction similar to those of species of Lactobacilli are known to be heterofermentative, producing lactic acid, acetic acid, ethanol and carbondioxide all of which could alter the taste and acidity of the final product (Ayres, et al 1980) Studies have shown that fermentation and growth activities of yeast at early stage of fermentation are elevated by wort aeration, reaching their maximal level after five hours of aeration (Ohno and Takahashi, 1986 a). In aerobic conditions supported with plentiful of nutrients, Saccharomyces will

grow and bud rapidly but it is also able to survive in low oxygen concentration and will undergo fermentation (Miller, 1986). Yeasts require more than 30mg of available oxygen per gram of dry yeast to synthesise the amounts of lipids necessary for desirable fermentation (Ohno and Takahashi, 1986 b). Experiments in a pilot plant as well as in the brewing industry have shown that a healthy yeast with a high viability is the guarantee for the production of a high quality beer (Runkel and Niemsch, 1987). It is therefore necessary to keep the yeast in optimal condition for its metabolism by offering sufficiently aerated wort and a convenient storage temperature. Yeast growth may be strongly influenced by environmental factors particularly temperature, pH and the development of staling substance as by products of yeast metabolism (Ingold, 1975). During alcohol fermentation, ethanol inhibition of Saccharomyces uvarum is not essentially the result of exocelluar product (Navarro and Finck, 1982) Indeed, alcohol accumulation takes place within the yeast cell, and it affects the hexokinase activity of the yeast (Navarro and Finck, 1982).

Factors which adversely affect beer fermentation include insufficient purity of the yeasts, lack of care in observing thermal conditions and faults in the composition of the wort i.e low levels of amino nitrogen, oxygen or trace elements in the wort (Vernerova and Cejka, 1987). Clover fermentation with high concentration of brewers yeast has resulted in ethanol content of 12% V/V in 3hours at 30°c with resultant death rate of yeast cells essentially logarithmic during this period (Nagoda - withinana, and steinkraus, 1976) whereas 6hours at 15°C was required to reach the same ethanol content, but the yeast cells retained their viability. Using a lower population , the yeast cells also retained their viability at 30°c. Ethanol added to the medium was much less lethal than the same or less quantities of ethanol produced by the cell in a ' rapid fermentation ' (high yeast population) It was considered possible that ethanol was produced so rapidly at 30°C that it could not diffuse out of the cell as rapidly as it was formed. The hypothesis was postulated that ethanol accumulating in the cell was contributing to the high death rate at 30°C (Nagoda - within and Steinkdraus, 1976) Low intra -cellular ethanol concentration will give high cell viability.

Fermentation performance i.e the time required in hour to attenuate an all malt wort from original gravity of 1040° to 1020° has been accurately predicted for a Saccharomyces cerevisiae strain using the acidification power test (Kara et al1988). The test measures the ability of a population of yeast cells to maintain different intracellular and extracellular H' (hydrogen ion) concentrations when placed in distilled water. It also measures the change in these concentration induced by the presence of glucose. In a separate study, product accumulation within the cells of Saccharomyces uvarum during alcoholic fermentation was found to be a consequence of the resistance to alcohol diffusion through the cell wall from inside to the outside of the cell (Navarro, 1980). Addition of peptone or

surfactants to the culture medium resulted in lower ethanol accumulation, hence the inhibitory effects are reduced. Fermentation environment such as pH , dissolved oxygen and carbondioxide concentration, ethanol and substrate inhibition effect together with other variables such as fermenter type, raw material type, can affect alcohol fermentation by yeast (Jones, et al, 1981).

The quantity of yeast in suspension depends on its nature, whether it is more or less flocculent, on the lagering time and the type of fermenter used for the lagering of the beer (Moll, 1987). Flocculation dispersion of brewers' yeast has been found to be influenced by environmental conditions (Kamada and Murata, 1984 a) Sugar consumption during fermentation is a factor in yeast flocculation. Dispersion of brewers' yeast early in fermentation was thought to be induced by synergistic effect of decreased yeast agglutination and environmental conditions. Fermentable and non-fermentable sugars are found to be effective in dispersing the yeast. Yeast has been dispersed more intensely after a lag time which decreased as temperature increased in the sugar solution. This suggests that dispersion depended on increasing carbohydrate metabolism (Kamada and Murata, 1984 b). Increased cell activity might act on the yeast cell surface to produce reducing conditions in the same way as reducing agents, hence causing dispersion. Lack of group B vitamins can hinder flocculation. Flocculating cells possess a stable cellular envelope structure with increased cell -wall contents of mannans and the lectin type components which are responsible for flocculation(Bonaly and Lematre, 1983). A number of economic and productive quality advantages exist in brewing when high -gravity worts of 16% to 18% dissolved solids are fermented (Casey et al 1984). Above this level , production problems such as slow or stock fermentation and poor yeast viability occur, due to ethanol toxicity to the brewers' yeast. Factors limiting the production of high levels of ethanol by brewers yeast in actually a nutritional deficiency. When a nitrogen source, ergosterol and oleic acid are added to worts, up to 31% dissolved solids, it is possible to produce beer's up to 16.2% V/V ethanol with high yeast viability (Casey et al, 1984) and can be repitched at least five times.

CHAPTER 3.
MATERIALS AND METHODS.

SOURCE OF MILLET

Millet grains, Pennisetum americanum, (L) IV 19 cultivar, were obtained from the National Seed Research centre, Institute of agriculture research (IAR), Ahmadu Bello University, Zaria for the study.

3.2 SOURCE OF YEAST

The brewers' yeast, Saccharomyces uvarum, was obtained from The Nigerian Breweries, Plc; Kaduna and was recognised as the third generation. The culture was maintained on malt extract agar slants

3.3 CLEANING OF THE GRAINS

The grains were cleaned according to the methods of Demuyakor and Ohta, (1992). They were picked by hands to remove the broken millet grains, foreign seeds, sand and other unwanted particles. Seventy gram sorted grains (duplicate samples) was then poured into 450millilitre capacity beaker containing water and the floating grains were strained off leaving the sinkers. The grains were immediately dried in a forced air oven at 40°C for 12 hours.

3.4 DETERMINATION OF MOISTURE CONTENT

The moisture content of the grain was determined using the method of Achoba et al (1991). Replicate samples, 10g each of the sorted samples were weighed into pre-weighed petri-dishes and transferred into a preheated oven and dried at 110°C until they attained constant weights. Moisture content was calculated from the results using the following relation:

$$M = \frac{W_1 - W_2}{W_s} \times 100$$

where M = Moisture content (Percent)

W1 = Initial weight of grain before drying

W2 = Final weight of grain after drying.

3.5 THOUSAND CORN WEIGHT DETERMINATION

The determination was done using the institute of Brewing (IOB, 1986) method. Twenty gram portions of the grains in duplicate samples were weighed and the number of grains in the sample were counted. The moisture content was determined as in 3.4 above for dry matter calculation. The thousand corn weight was then determined using the following relation:

$$TCW = \frac{W \times 1000 \times DM}{N \times 100}$$

where TCW = Thousand corn weight

W = Weight of sample taken

DM = Dry matter (%) of millet

N = Total number of corns counted

3.6 GERMINATIVE CAPACITY

The hydrogen peroxide (IOB, 1986) method was used. Two hundred - grains (duplicate samples) were counted into 200millilitre capacity glass beakers and steeped at 28^{0}C for 48hours in 7.5% hydrogen peroxide ($H_2 O_2$). The steep liquor was strained off and the grain incubated at ambient temperature for 24hours after the grains were transferred unto moist filter paper while ensuring that all the grains made good contact with the filter paper. Chitted grains were separated from unchitted ones and counted. Germinative capacity was calculated using the following relation:

$$G.C\%\ (H_2 O_2) = \frac{(200-N)}{2}$$

where N = Number of ungerminated grains

G.C = Germinative capacity.

3.7 GERMINATIVE ENERGY

The IOB (1986) method was used. Two filter papers (Whatman No.1) were placed at the bottom of a petri dish and 4millilitre of distilled water transferred onto it. One hundred grains duplicate samples were counted and placed on the filter paper taking care to ensure that all grains were in good contact with the moist filter paper. The petri dish was covered with the lid and incubated at 28^{0}C for 48hours, the percentage of chitted corn was calculated at the end of incubation period and expressed as the Germinative energy using the relation:

$$G.E\ (\%) = 100-N$$

where G.E = Germinative Energy

N = Number of ungerminated grains

3.8 MALTING CONDITIONS

In this method, some of the factors which determine the extent of grain modifications, such as steeping time, temperature and moisture content were kept constant at optimum level while the germination time was varied and the kilning temperature altered.

3.8.1 STEEPING

A 50g aliquot of the grains in duplicate samples were cleaned thoroughly and then steeped at room temperature according to the methods of Malleshi and Desikacha, (1986 a); and Panwal and Pawar, (1989) in a glass beaker containing tap water to completely cover the grains for 24hours with periodic stirring until the desired moisture content of 34% was obtained.

3.8.2 GERMINATION

The steeped grains were transferred unto a muslin cloth to drain off the water. The grains were allowed to germinate at ambient temperature in a dark cupboard (Aisien, et al, 1986) for 48hours or 72hours - corresponding to the malting regime of (2) or (3) days respectively, using the same glass beaker that was used in steeping, since the container permitted a depth of 6 inches (Harrison, et al, 1987.) Each beaker was covered with a black polythene paper to enable the sprouts' generate heat to hasten germination (Okafor, 1987) and also to prevent photosynthesis. Grain moisture was maintained by spraying a little quantity of water on the grains and the grains turned manually by hand at intervals of 12hours to prevent root matting.

3.8.3 KILNING

Duplicate seed samples that were malted for 48hours or 72hours were kilned by the method of Taylor, (1983) using a forced draught air oven set at 40°C,45°C,50°C,55°C and 60°C - corresponding to the five kilning temperatures (used in this research) for 24hours to dry the green malt. The germinated roots and rootlets (which were kilned too) were devegetated by rubbing the roasted millet between the palms and winnowed. The malted grains were weighed to calculate the malt yields using the following relations:

$$\% \text{ malt yield} = \frac{W2 \times 100}{W1}$$

where, W2 = Weight of dry malt

W1 = Weight of dry raw grain taken

3.8.4 MILLING:

Devegetated roasted millet malt was dry- milled using Electrim hammer mill, - to particle size 1400 - 800μ

3.9 ANALYSIS OF MALT

3.9.1 PERCENTAGE MALTING LOSS

It was determined according to the standard procedure of the IOB (1986). The difference between the weight of malt (rootlets removed) and that of the original grains dry weight) expressed as a percentage of weight of grains malted, gives the percentage malting loss.

malting loss % $\frac{x}{y}$ x 100, where X is equal to difference between original weight of grains malted and weight of malt whose root-lets have been removed.Y = Original weight of grains malted.

3.9.2 COLD WATER EXTRACT (CWE)

The Cold water extract was determined by the method of Gothard et al (1980) and calculated by the method of Demuyakor and Ohta (1992). Three gram duplicate samples of malted millet (from 3.8.4) was extracted with 30millilitre solution (27.5millilitre distilled water mixed with 2.5millilitre of 0.1mol/Litre ammonium hydroxide, NH_4OH, solution) at 28°C for two hours in a shaker set at 100 r.p.m. The extract was filtered through whatman No.1 filter, paper and the amount of dissolved sugar in the clarified wort determined using the AOAC (1980) Plato table after measuring the specific gravity. Percent cold water extract was calculated using the following relation;

$$\% \text{ CWE} = \frac{P(M + 1000)}{(100 - P)}$$

This was corrected for dry weight using the (Demuyakor and Ohta, 1992) equation

$$\% \text{ CWE (dry basis)} = \frac{100x}{100 - M}$$

where CWE = Cold water weight using
P = Sugar (%) from Plato table
M = Malt moisture content (%)

X = Extract (%).

3.9.3 HOT WATER EXTRACT (H. W. E).

This was determined by the method of Gothard et al (1980), modified according to the recommendations of Palmer (1989) and calculated according to the method of Demuyakor and Ohta (1992). Three gram duplicate samples of malt grist was transferred into a 100millilitre capacity Erlenmeyer flask and extracted with 25millilitre of distilled water in a water bath pre-set at 45^{0}C and was allowed to remain for 30 minutes, with stirring after every 10 minutes. The wort was then decanted from the mash and kept separately, while the mash was brought to 100^{0}C in a boiling water bath. It was maintained there for two minutes and then cooled to below 65^{0}C. The mash and the wort were then recombined and further extraction carried out at 65^{0}C for one hour.

At the end of the extraction, the solution was cooled and the volume made up to 35millilitre with distilled water. It was well mixed and allowed to settle for twenty minutes after which it was shaken again and decanted into a fluted filter paper (Whatman No.1). The percentage sugar content of the clarified wort was determined by calculation from the Plato table of AOAC (1980) using the specific gravity values obtained from the work. The percentage hot water extract was calculated as for cold water extract in section 3.9.2.

3.9.4 DIASTATIC POWER

The diastatic power was determined according to the IOB (1986) method with some modifications. Malt infusion was prepared using C.W.E. except that the extraction was done for three hours instead of two hours. 3 millilitre duplicate samples of the unfiltered supernatant was transferred into 100millilitre of buffered starch solution a temperate at 28^{0}C and contained in 250millilitre capacity Erlenmeyer flask (as in appendix I). The flask content was mixed by swirling and allowed at this temperature for one hour in a shaker. At the lapse of time, 30millilitre of sodium hydroxide solution was added to stop the reaction and the contents made up to 200 millilitre at 28^{0}C with distilled water. The reducing substances in the solution were determined using 5millilitre portions of the digested starch solution which was transferred into 100 millilitre capacity Erlenmeyer flask and 10 millilitre of alkaline potassium ferricyanide solution was added. The solution was well mixed and transferred into boiling water bath for about twenty minutes. Care was taken to ensure that the boiling water level was above that of the flask content. The flask was cooled quickly under a running tap water, then 25millilitre of acetic acid salt solution was added, followed by 1millilitre of potassium iodide solution. The flask content was then mixed by swirling. Titration was carried out with 0.05mol/litre of sodium thiosulphate ($Na_2 S_3 O_4$) solution using a microburette, against the

solution until the end point was reached as indicated by the complete disappearance of the purple colour. The volume of this thiosulphate used was designated as "A" A blank was prepared by adding 30millilitre of 0.1mol/litre sodium hydroxide to 3 millilitre of the unfiltered malt infusion and 100millilitre of 2% buffered starchwas added. The solution was made up to 200millilitre with distilled water. The flask content was then treated the same way as the test sample, The titre of the thiosulphate used was designated as B.The diastatic power of the malt was calculated as follow:

$$DP\ (^0IOB) = \frac{B - A\,(23 \times 200)}{250 \times (1/C)}$$

where DP (^{0}IOB) = Diastatic Power by institute of Brewing method

B = Volume of $Na_2 S_3 O_4$ used for blank correction

A = Volume of $Na_2 S_3 O_4$ for direct titration

C = Volume of unfiltered malt extract used for digestion.

DP(^{0}IOB) were converted to DP(^{0}L) using the formula:

$$DP(^0L) = DP(^0IOB) \times 1.1.$$

DP(^{0}L) = diastatic power by Litner method

3.10 MASHING:

European Brewing Convention (EBC) infusion method with some modifications was adopted for the mashing procedure of standard wort (Analytica - EBC III, 1975). A 15g weight duplicate sample of each of the ground millet malt was mixed with 100millilitre of distilled water in a 250millilitre Pyrex Erlenmeyer conical flasks. The mixture was stirred thoroughly with a sterile glass rod to disallow the formation of lumps. The mash flasks were then placed in a thermostatically controlled water bath set at 45^{0}C and this temperature (45^{0}C) was maintained for thirty minutes and then subsequently increased to 5^{0}C for five minutes for a period of twenty minutes at which time, the temperature rose to 65^{0}C . At 65^{0}C, the mash was allowed to remain for thirty minutes in the bath before the temperature was subsequently raised to 70^{0}C and the mash held for one hour at this temperature. At the end of one hour, the temperature was finally raised to 75^{0}C (mashing off temperature) and then cooled promptly to room temperature.

After thorough stirring, the mash was emptied into a fluted Whatman No. 1 filter paper in a sterile funnel. The mash was completely filtered and 30millilitre quantity of boiling water was sprayed unto the residue until the washings coming out from it were clear. The resulting wort was boiled for thirty minutes and allowed to cool to room temperature.

3.11 ANALYSIS OF WORT

3.11.1 pH OF THE WORT.

The pH of the wort was determined by the use of PYE -UNICAM' pH meter, the meter was first standardised to pH 5 using acetate buffer solution (appendix II) according to Moffat, (1986)since the pH of the wort was slightly acidic. The electrode was washed and rinsed thoroughly with distilled water before dipping into the wort for pH determination.

3.11.2 EXTRACT YIELD

The amount of fermentable sugars and other soluble dissolved was determined by the use of the Analytical EBC III (1975) infusion method for the preparation of wort. Extract content was determined from the specific gravity by means of the official table (Plato table) for AOAC, (1980).

3.12 INOCULUM MEDIUM AND YEAST GROWTH ASSAY.

Inoculum medium peptone yeast extract glucose broth (PYG) made up according to Demain and Solomon (1986) with some modifications (appendix III) was used to grow and assay the yeast cell. Growth assay was carried out in nine millilitre of sterile distilled water to which has been dispensed one millilitre of the young yeast culture, and serially diluted and 0.1millilitre aliquot inoculated unto malt extract agar (MEA) by spread method. Inoculated triplicate plates were incubated for a predetermined period of time at ambient temperature.

3.13 WORT FERMENTATION

Batch method of fermentation was adopted. The hot wort which has now been cooled to room temperature was dispensed aseptically in one hundred and fifty millilitre portions duplicate samples into sterile two hundred and fifty millilitre capacity Erlenmeyer flasks. The flasks were aseptically plugged with cotton wool and allowed to remain at room temperature. The flasks were later each aseptically inoculated with ten millilitre (1.8×10^7 cfu / millilitre) of the yeast suspension obtained from 3.12 above. Inoculated flasks were agitated for eighteen hours in a stirred fermentation according to Okafor and Aniche (1986) before being allowed to ferment at ambient temperature (Dolan, 1987) for nine days.

3.14 ANALYSIS OF THE FERMENTED WORT WITH THE YEAST.

3.14.1 TURBIDITY MEASUREMENT OF FERMENTING WORT

Turbidity was measured by use of BECKMAN TYPE SPECTROPHOTOMETER to determine its absorbance. At time T=O, i.e when the yeast was introduced, the absorbance was measured and at time T=9 days i.e at the end of the fermentation, the absorbance was also measured. two millilitre aliquot was taken in each case, and put in one centimetre cuvette. All measurements were done at the wavelength of 660nm (Pringle and Mor, 1975).

3.14.2 YEAST MORPHOLOGY

The morphology of the pitching yeast was observed by wet mount stain using methylene blue dye at start of fermentation and at stop of fermentation. This was to provide a clue to changes in cell morphology of the pitching yeast in the course of the fermentation.

3.14.3 YEAST VIABLE COUNTS

From the fermented wort, the suspension was thoroughly mixed and one millilitre aliquot delivered into a nine millilitre sterile distilled water using sterile pipette. Further serial dilutions were carried out and the 10^{-6} dilution plated unto the (PYG) agar and incubated at ambient temperature for forty eight hours after which the colonies appearing on the agar were counted.

3.14.4 YEAST DEAD CELLS

The improved Neubauer counting chamber used for counting red blood and white blood cells was adopted from Baker and Silverton (1985). 1millilitre of the fermented wort was withdrawn and diluted twenty fold i.e 0.1millilitre of the fermented broth in two millilitre of methylene blue stain using the white blood cell (WBC) pipette. It was thoroughly mixed and the counting chamber of the haemacytometer was filled with the micropipette content. It was covered with a coverslip and placed under '40X' objective and counting was done. Cells dispersed in the four chambers were counted and cell in area of marginal error ignored.

Cell counted = N x DF x 10^6/A x D per litre.

Where N = Number of cells counted

DF = Dilution factor (20)

A = The area counted = 5x0.1

D = Depth of the counting chamber = 0.1mm

∴ % dead cells = A/A+B x 100

Where B = Living cells

A = Dead cells.

3.14.5 FILTRATION OF THE FERMENTED WORT.

The fermented wort was filtered to strain off the yeast cells using Whatman No. 1 filter paper. Because the Saccharomyces uvarum used was the bottom yeast and had already sedimented to the bottom of the flask, the supernatant that still contained some cells was the one from which the bulk of the fermented wort was obtained.

3.14.6 YEAST FLOCCULENCE.

The flocculence was determined according to the methods of Helm et al, (1953). one gram of the yeast dry weight was suspended in ten millilitre of acetate buffer (pH 5.0) in a 15millilitre centrifuge tube. They were kept in water bath at 20°C for twenty minutes. They were resuspended by shaking after ten minutes. The amount of sedimented yeast was read in the centrifuge tube and expressed in millilitre (millilitre).

3.14.7 IODINE REACTION FOR UNCONVERTED STARCH.

Performed according to AOAC, method (1980), by adding 0.02N iodine solution from dropper to form distinct layer on top of the fermented wort. Colour development was observed with transmitted light and reported as either blue, purple or reddish tinge indicating presence of starch, amylodextrin and erythrodextrin respectively.

3.14.8 TITRATION FOR TOTAL ACIDITY

The total acidity was determined according to the method of Pearson (1976). Total percentage acidity was measured as acetic acid by titrating ten millilitre with 0.1 molar sodium hydroxide (NaOH) using phenolphthalein as indicator. Titre values obtained were multiplied by 0.006005 in

order to obtain the total percentage acidity.

3.14.9 FINAL pH OF THE FERMENTED WORT.

The final pH of the filtered fermented wort was determined using pH meter (PYE UNICAM MODEL) Buffer solution prepared as in appendix II was used to calibrate the pH meter before the electrode was immersed into the wort to read off the values.

3.14.10 PERCENT (%) V/V. ALCOHOL

The alcohol content V/V. of the fermented wort was determined by direct but slow distillation of the broth at 78^0C using the 'quick - fit' apparatus. Amount of distillate was measured using the specific gravity (density) bottle at 20^0C and alcohol content estimated as percentage by volume from the appropriate custom and excise tables as recommended by AOAC (1980).

CHAPTER FOUR

RESULTS.

4.1 MOISTURE CONTENTS AND GERMINATION TESTS OF THE PEARL MILLET.

Germination tests were carried out to establish the germination potential of this grain and the result is as presented in Table 4.1. The native millet had a moisture content of 6% while the steeped millet attained a saturation moisture level of 34% after 24 hours. The moisture content of the steeped millet was 34%. This is because 50g was used for the analysis where the raw (native) millet was found to contain 0.6g of moisture. Thousand corn weight determination showed that it is 7.one gram, while the Germinative capacity and energy were found to be 93% and 97% respectively.

4.2 EFFECT OF MALTING REGIME AND KILNING TEMPERATURES ON MALT YIELD AND LOSS OF THE PEARL MILLET.

In figures 4.1 and 4.2 are presented the effects of malting regime and kilning temperatures on the malt yield and loss of the pearl millet respectively. Malt yield was observed to decline gradually for both malting regimes. The malt yield decreases with increase in the kilning temperature values (Table 4.2). Highest malt yield of 90% and 88% were observed for the lowest temperatures of kilning (40^0C) for the two malting regimes of 2 - and 3 - days respectively. Lowest malt yields of 84% and 82% were also observed for the highest temperatures of kilning (60^0C) for the two regimes of 2 - and 3 - days respectively. Grains germinated for 2 days had higher malt yield than those germinated for 3 days. Malting loss was generally found to increase gradually in proportion with increase in kilning temperature. Lowest malting loss was observed for malts kilned at 40^0C and the highest observed for those at 60^0C. Difference in malting loss between the two regimes (2 - day and 3- day) was not significant and higher malting loss was showed by samples malted for longer days. Malting loss is generally inversely correlated to other malting parameters except for the two day regime malt where it has slight direct correlation with HWE (0.69) and DP (0.33). differences between the temperature treatments (kilning) of the malts is highly significant (18.28) at 99% confidence level

TABLE 4.1 MEAN VALUES OF THE MOISTURE CONTENTS AND GERMINATION TESTS OF THE PEARL MILLET.

MOISTURE CONTENT (%)		THOUSAND CORN WEIGHT (TCW) (g)	GERMINATIVE CAPACITY (GC)(%)	GERMINATIVE ENERGY (GE) (%)
NATIVE	STEEPED			
6	34	7.1	93	97

TABLE 4.2 MALTING ATTRIBUTES OF THE PEARL MILLET
MEAN VALUES OF THE TWO (2) DAY AND THREE (3) DAY REGIME
MALTS KILNED AT DIFFERENT TEMPERATURES

	KILNING TEMPERATURE (^{0}C)									
Malting Properties	40		45		50		55		60	
	2D	3D	2D	3D	2D	3D	2D	3D	2D	3D
Malt Moisture content (%)	12.4	8.40	11.6	7.0	10.6	6.20	8.8	4.0	6.0	3.20
Malting Loss (%)	7.14	8.57	8.57	10.0	10.0	10.0	11.40	11.40	11.40	12.90
Cold Water Extract (%)	3.56	4.06	3.54	4.01	3.74	4.10	3.70	3.87	3.63	3.85
Hot Water Extract (%)	11.55	12.74	12.0	13.20	12.52	14.71	14.24	14.60	12.11	12.23
Malt Yield (%)	90	88	88	86	86	86	84	84	84	82
Diastatic Power (^{0}L)	2.02	4.05	3.04	6.07	5.06	7.08	4.05	5.06	2.02	4.05

- 2D is 2 day regime
- 3D is 3 day regime

4.3 EFFECT OF KILNING TEMPERATURE AND MALTING REGIME ON THE MOISTURE CONTENT OF MALTED MILLET.

Figure 4.3 shows the effects of kilning temperatures on the moisture content of the malted millet Moisture contents of the malts were observed to decrease with increasing temperature and period Kilning for 24 hours of a 2 - day regime malted millet at 40^{0}C resulted in moisture content of 12.4%. gradually leading to 8.4% after 3 days of malting at same kilning temperature. Kilning at high temperature of the prolonged malted millet reduced the moist content of the malts considerably to values between 8.4 - 3.2%.

4.4 EFFECT OF MALTING REGIME AND KILNING TEMPERATURE ON THE COLD AND HOT WATER EXTRACTS OF MALTED MILLET.

The effect of malting regime and kilning temperature on the cold water extract (CWE) of the malts produced are presented in Table 4.2 and figure 4.4. With increase in temperature of kilning, the CWE was found to decline, rise up to reach its peak at 50^{0}C for both regimes and continue to decline again. There is a direct correlation between prolonged malting and CWE value, (0.88)

The 3 - day regime malt produced higher CWE than the 2 - day regime malt at same temperature of kilning. CWE of both regimes did not compare favourably with that of barley malt (28%), as the values obtained are smaller. The difference in values of the CWE with the variation in kilning temperature of both regime were not significant, 0.73 and 25.03 respectively The Hot water Extract (HWE) values of the millet malt produced are also shown in Table 4.2 and figure 4.4. HWE values are higher than CWE values for both malting regimes, although they are generally lower than that of commercial barley malt (92.6) at 50^{0}C. The highest HWE values was recorded for both regimes at 50^{0}C (12.5 and 14.7). HWE values are increased with increasing kilning temperature until they reach their peak and thereafter begin to decline. HWE values for the 3 -day regime malt are higher than HWE values obtained for the 2 - day regime malt.

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4.5 EFFECT ON KILNING TEMPERATURE AND MALTING REGIME ON DIASTATIC POWER OF MALTED MILLET

The diastatic power of malted millet as influenced by the kilning temperatures and malting regimes is as presented in Table 4.2 and figure 4.5 Diastatic power was observed to increase with increasing temperature of kilning until it gets to its peak at 50^0C for both regimes and thereafter continue to decline with further increase in kilning temperature. The 3 - day regime malt shows the highest diastatic power at 50^0C (7.0^0L) and the lowest at 40^0C and 60^0C (4.0^0L). The trend showed that the diastatic power decreases when kilning temperature is increased after having reached its peak. The 3 - day regime malt appears to show higher diastatic power values than the 2 - day regime. As kilning temperature is increased as well, the diastatic power decreases if the peak had been reached. The highest for the 2 - day regime was observed at 50^0C and the lowest at 40^0C and 60^0C (2.0^0L) as well. Diastatic power for both regime and the kilning temperature employed are much lower than that recorded for commercial barley malt (78^0L) (Palmer and Pathgate, 1976)

TABLE 4.3 MEAN VALUES OF pH OF WORT

SAMPLE	pH UNFERMENTED WORT	pH FERMENTED WORT
(2) . 40^{0}C	5.1	4.4
(2) . 45^{0}C	5.3	5.0
(2) . 50^{0}C	5.3	4.6
(2) . 55^{0}C	5.0	4.0
(2) . 60^{0}C	5.1	4.2
(3) . 40^{0}C	5.1	4.2
(3) . 45^{0}C	5.2	4.1
(3) . 50^{0}C	5.0	3.7
(3) . 55^{0}C	5.2	4.2
(3) . 60^{0}C	5.1	3.4

KEY: (2) 40^{0}C means 2 - day regime malt kilned at 40^{0}C for 24 hours

(3) 40^{0}C means 3 - day regime malt kilned at 40^{0}C for 24 hours

Values of the 2 day regime obtained (4.0 - 5.0) showed greater dispersion than the 3 day regime (3.4 - 4.2) and hence less reliable. The difference between the temprature treatments (kilning) is not significant (0.001162) to the pH value of the unfermented wort.

4.6 EFFECT OF KILNING TEMPERATURE ON THE WORT pH VALUE (UNFERMENTED AND FERMENTED)

The effect of kilned malts on the pH values of the worts derived from the two germination; 2 - day and 3 - day regimes, is as presented in Table 4.3. The pH of the unfermented wort falls within the range of 5.0 and 5.3. Kilning temperature does not seem to have any effect on the pH values of the unfermented wort, because the values obtained for the different temperatures employed is not significant. The fermented wort showed a wide variance in the pH range of all the worts: between 3.7 and 5.0, for (3).50^{0}C For each fermented wort, there is a corresponding decrease in the pH values as compared to the unfermented broth. The pH change of the fermented wort obtained seems to be influenced by malting regime. Worts obtained from malts kilned at higher temperatures showed higher percentage of change in pH values and the 3 - day regime malt showed even higher values than the 2 - day regime malt.

4.7 YEAST GROWTH ASSAY

Prior to inoculation of the wort, the yeast growth assay determined showed that 36 to 40 hours is the time required to grow the cell to the maximum level of 1.8×10^{7} cfu/millilitre and at this cell concentration, the culture is still in exponential phase.

4.8 YEAST MORPHOLOGY

At the point of inoculation, when time T = O, the yeast cells were ovoid in shape. Formation of bud was observed nine hours later. Bud formation in the yeast tremendously slowed down towards the end of the fermentation cycle, when cells now look elongated with buds becoming irregular. Kilning temperature adopted, and the malting regimes used do not seem to have any effect on the morphology of the yeast cells.

TABLE 4.4 MEAN VALUES OF WORT / YEAST CHARACTERS AND BROTH REACTION

SAMPLE	ABSORBANCE (%TRANSMISSION) OF WORT INOCULATED YEAST		(%) DEAD CELL NUMBER/L	(%) YEAST FLOCCULENCE	IODINE REACTION FOR UNCONVERTED STARCH	% ACIDITY AS ACETIC ACID
	T = 0hr	T = 216hrs				
(2).40^0	13.3	28	1.8	41	purple	0.0018015
(2).45^0	13.8	25	1.4	57	purple	0.0024020
(2).50^0	14	27	5.3	58	purple	0.0024020
(2).55^0	13.5	30	3.9	67	purple	0.0024020
(2).60^0	14	33	5.8	68	purple	0.0024020
(3).40^0	13.3	29	2.8	70	Reddish Tinge	0.0024020
(3).45^0	13.8	36	1.9	74	Reddish Tinge	0.0024020
(3).50^0	13.8	30	3.3	75	Reddish Tinge	0.0030025
(3).55^0	14	25	2.1	77	Reddish Tinge	0.0030025
(3).60^0	13.5	28	2.4	79	Reddish Tinge	0.0030025

4.9 TURBIDIOMETRIC VALUE DETERMINATION OF THE WORT (UNFERMENTED AND FERMENTED)

The absorbance values of wort derived from the kilned malts that has been inoculated with brewer yeast at time T= O (unfermented wort) and at time, T 216 hours (fermented wort) is as shown in Table 4.4. The amount of light transmitted for the unfermented wort samples is almost the same as the difference between them is not significant, being 0.06 and 1.62 for the malting regime and kilning temperature respectively. The amount of light transmitted in all the cases were high

At the completion of fermentation, the absorbance values of the broth were taken. In order to be able to determine the absorbance at this stage of growth, each sample was diluted four fold. The amount of light transmitted was very low because of the increase in the turbidity of the fermented wort, indicating that the yeast cells have multiplied appreciably in this broth (Table 4.4. The amount of light transmitted too is not significant to the malting regime (0.11) and kilning temperature (0.17).

4.10 YEAST DEAD CELL

The percentage of dead cell is as tabulated in Table 4.4 for each sample. Dead cells stained blue (and were counted), While the living cells remained colourless. Generally, the pattern of yeast dead cell in each of the worts derived from the variously treated malt has no correlation with either kilning temperature or malting regime. Percentage yeast dead cell for each wort sample was low per litre, the minimum and maximum being approximately 2 and 6 respectively .

4.11 YEAST FLOCCULENCE

The flocculation of brewers yeast in the fermented wort expressed as a percentage of the initial wet weight introduced into the buffer is as shown in Table 4.4. The degree of flocculence of the yeast seems to be influenced by wort constituents which is a factor of malting regime and kilning temperature. The coefficient of variation of yeast flocculence in the 2-day regime malt/wort are more dispersed than the 3-day regime. They all showed satisfactory flocculence, although the 3- day regime malt showed better flocculence than the 2 - day regime malt. There is no significant difference between malting regime and yeast flocculence and also with kilning temperature; the values being 24.7 and 3.52 respectively from the statistical analysis.

4.12 UNCONVERTED STARCH.

The unconverted starch reaction in the fermented wort with iodine is as presented in table 4.4. Reactions showing purple colour indicate the presence of amylodextrins in the wort while reactions showing reddish tinge characteristics indicate the presence of erythrodextrin. Generally the 2 - day regime showed the presence of amylodextrins while the 3 - day regime showed the presence of erythrodextrins.

4.13 PERCENT ACIDITY (AS ACETIC ACID)

The total acetic acid value in all the samples was low and almost same for all the worts excepts in the last kilning temperatures employed for the 3 - day regime malt, where figures obtained were same (0.0030025), and slightly higher than the 2 - day regime (0.002402), except for one sample which gave the least (0.0018015) - which was exhibited by the 2 - day regime kilned at 40^{0}C.

4.14 YEAST VIABLE COUNTS (MEAN VALUE)

Yeast viable counts obtained at the end of incubation after duplicate plating of 0.1millilitre of the broth on malt extract agar are presented in Table 4.5 and figure 4.6. Lowest viable count (7.5×10^{8}cfu/millilitre) was obtained in the 2 - day regime malt kilned at 40^{0}C representing slightly over a forty - one fold increase at the end of fermentation as compared to the amount (1.8×10^{7} cfu/millilitre) inoculated in the wort at start of fermentation. The highest viable count was obtained for the 3- day regime malt kilned at 55^{0}C which gave 1.9×10^{9} cfu/millilitre, representing over a hundred fold increase when compared with the amount (1.8×10^{7} cfu/millilitre) inoculated at initiation of the fermentation. The yeast generation time was high (32 to 40 hours) with low generation number (5 to 8) for the whole fermentation cycle and low growth rate (4.2×10^{-4} to 5.1×10^{-4})/minute.

TABLE 4.5 VIABLE COUNTS OF YEAST AFTER FERMENTATION

SAMPLE	INITIAL COUNTS (CFU/MILLILITRE)	FINAL COUNTS (CFU/MILLILITRE)	(%) INCREASE	n	g(hours)	k
(2).40^{0}C	1.8×10^7	7.5×10^8	40	5.4	40.1	4.2×10^{-4}
(2).45^{0}C	1.8×10^7	7.9×10^8	43	5.5	39.6	4.2×10^{-4}
(2).50^{0}C	1.8×10^7	7.7×10^8	42	5.4	39.9	4.2×10^{-4}
(2).55^{0}C	1.8×10^7	9.5×10^8	51	5.7	37.7	4.4×10^{-4}
(2).60^{0}C	1.8×10^7	1.6×10^9	87	6.5	33.4	5.0×10^{-4}
(3).40^{0}C	1.8×10^7	1.7×10^9	93	6.6	32.9	5.1×10^{-4}
(3).45^{0}C	1.8×10^7	1.8×10^9	98	6.6	32.5	5.1×10^{-4}
(3).50^{0}C	1.8×10^7	1.8×10^9	98	6.6	32.5	5.1×10^{-4}
(3).55^{0}C	1.8×10^7	1.9×10^9	104	6.7	32.1	5.2×10^{-4}
(3).60^{0}C	1.8×10^7	1.1×10^9	60	5.9	36.4	4.6×10^{-4}

KEY

cfu/millilitre = Colony forming unit per millilitre

n = Number of generation of yeast

g = Generations time of yeast

k = The growth rate of the yeast

TABLE 4.6 MEAN VALUES OF SPECIFIC GRAVITY AND PERCENTAGE ALCOHOL BY VOLUME OF FERMENTED WORT.

SAMPLE	SPECIFIC GRAVITY	% ALCOHOL V/V
(2) . 40^0C	0.9992	0.53
(2) . 45^0C	0.9989	0.73
(2) . 50^0C	0.9991	0.60
(2) . 55^{00}	0.9987	0.87
(2) . 60^0C	0.9984	1.07
(3) . 40^0C	0.9983	1.14
(3) . 45^0C	0.9984	1.07
(3) . 50^0C	0.9980	1.34
(3) . 55^0C	0.9983	1.14
(3) . 60^0C	0.9981	1.27

4.15 SPECIFIC GRAVITY AND ETHYL ALCOHOL % (V/V) OF THE FERMENTED WORT.

The percentages by volume of ethyl alcohol at 20^0C corresponding to the apparent specific gravity is shown in Table 4.6. Wort from malts with extended malting regime (3 - day) appear to have higher ethanol content than those of shorter malting regime (2 - day), although the alcohol content obtained from all the samples was very low. Least ethanol content (0.53% V/V) was derived from the 2 - day regime malt kilned at 40^0C while the highest (1.34% V/V) was obtained for the 3 - day regime malt kilned at 50^0C. There appears to be a stronger correlation in the 2-day regime malt/wort (r=0.89) with increasing temperature of kilning than in the 3-day regime malt/wort (r=0.47), although longer malting regime gave the highest amount of alcohol.

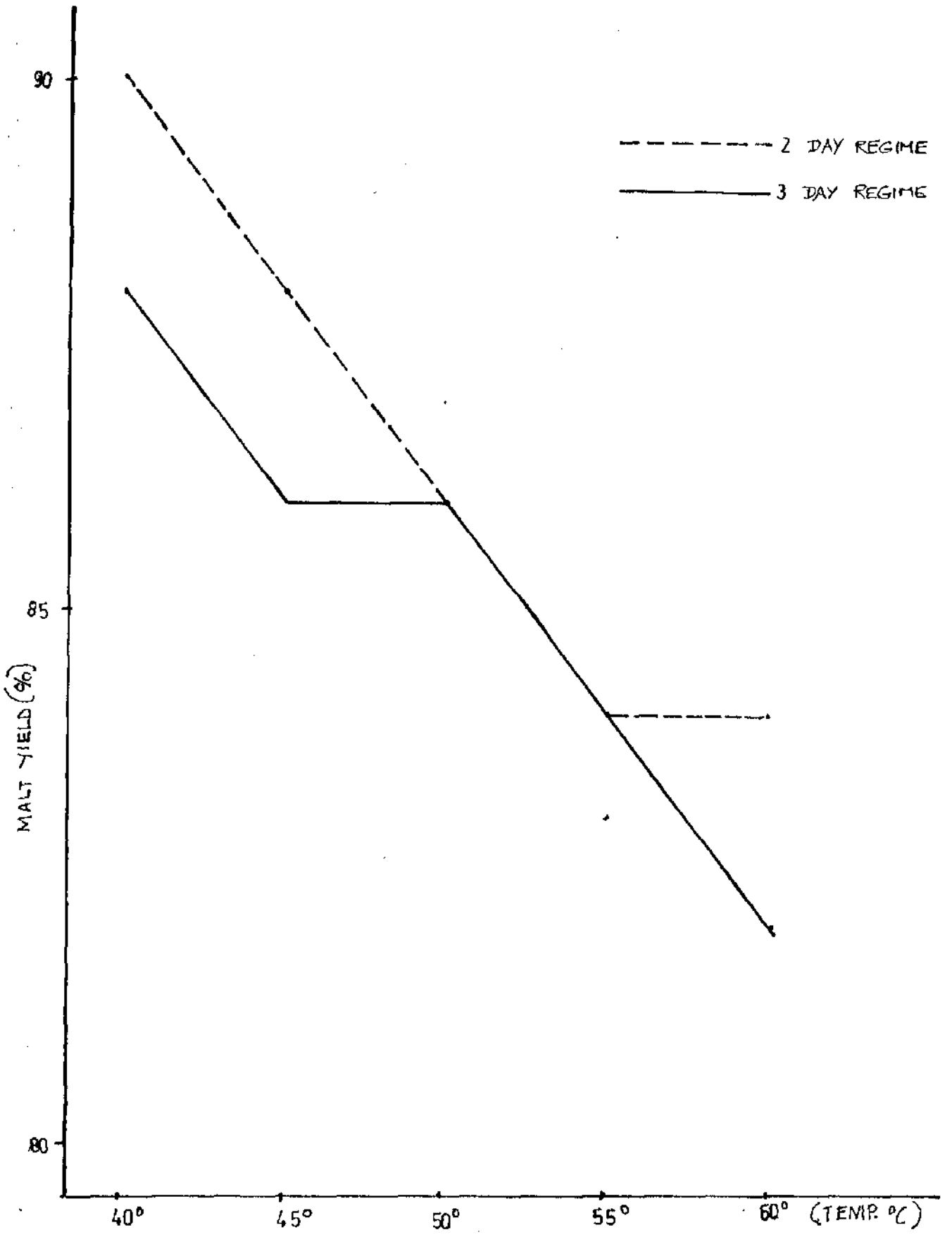


FIG 4.1 : EFFECT OF MALTING REGIME AND KILNING TEMPERATURE ON MALT YIELD

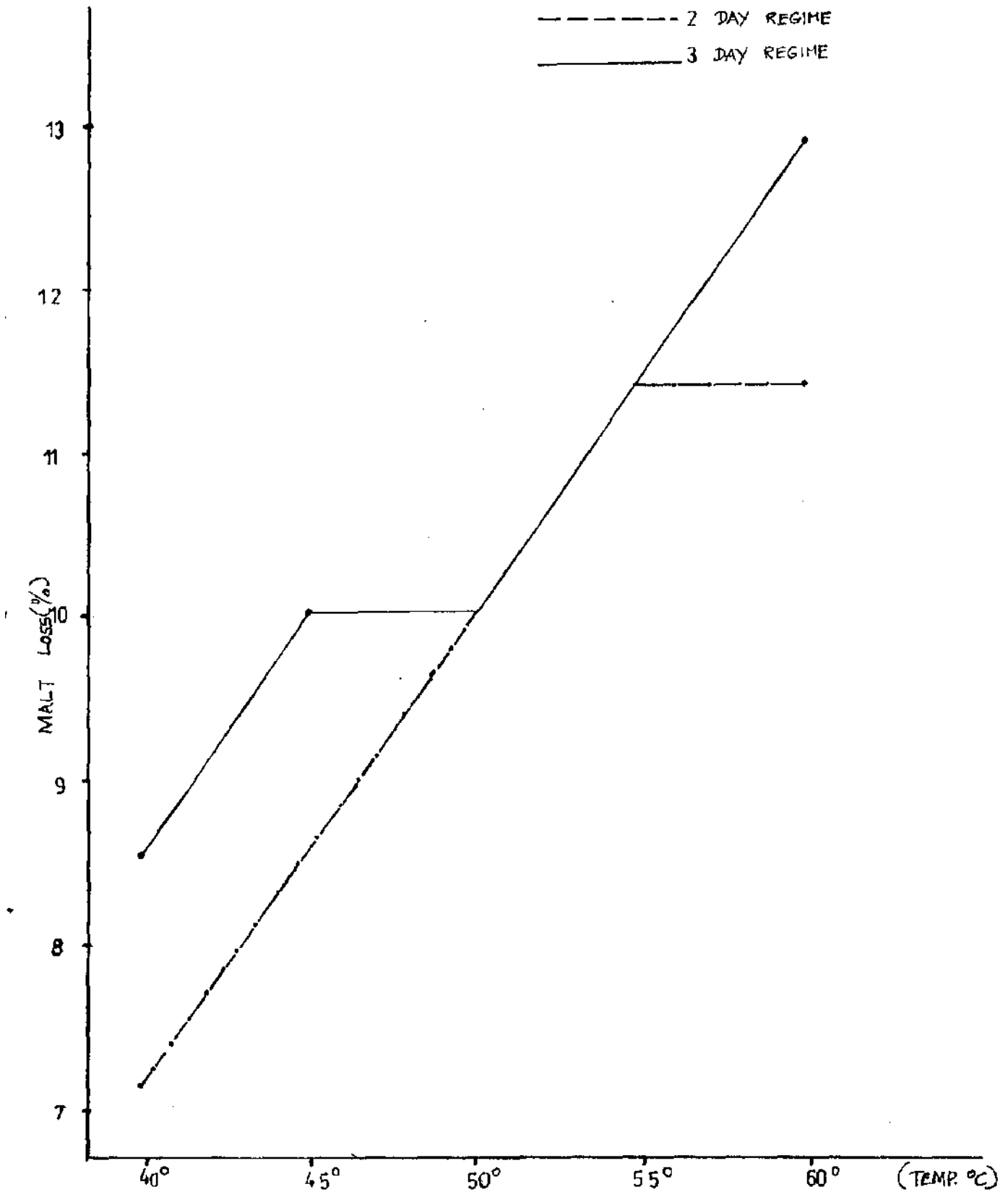


FIG 4.2 : EFFECT OF MALTING REGIME AND KILNING TEMPERATURE ON MALT LOSS

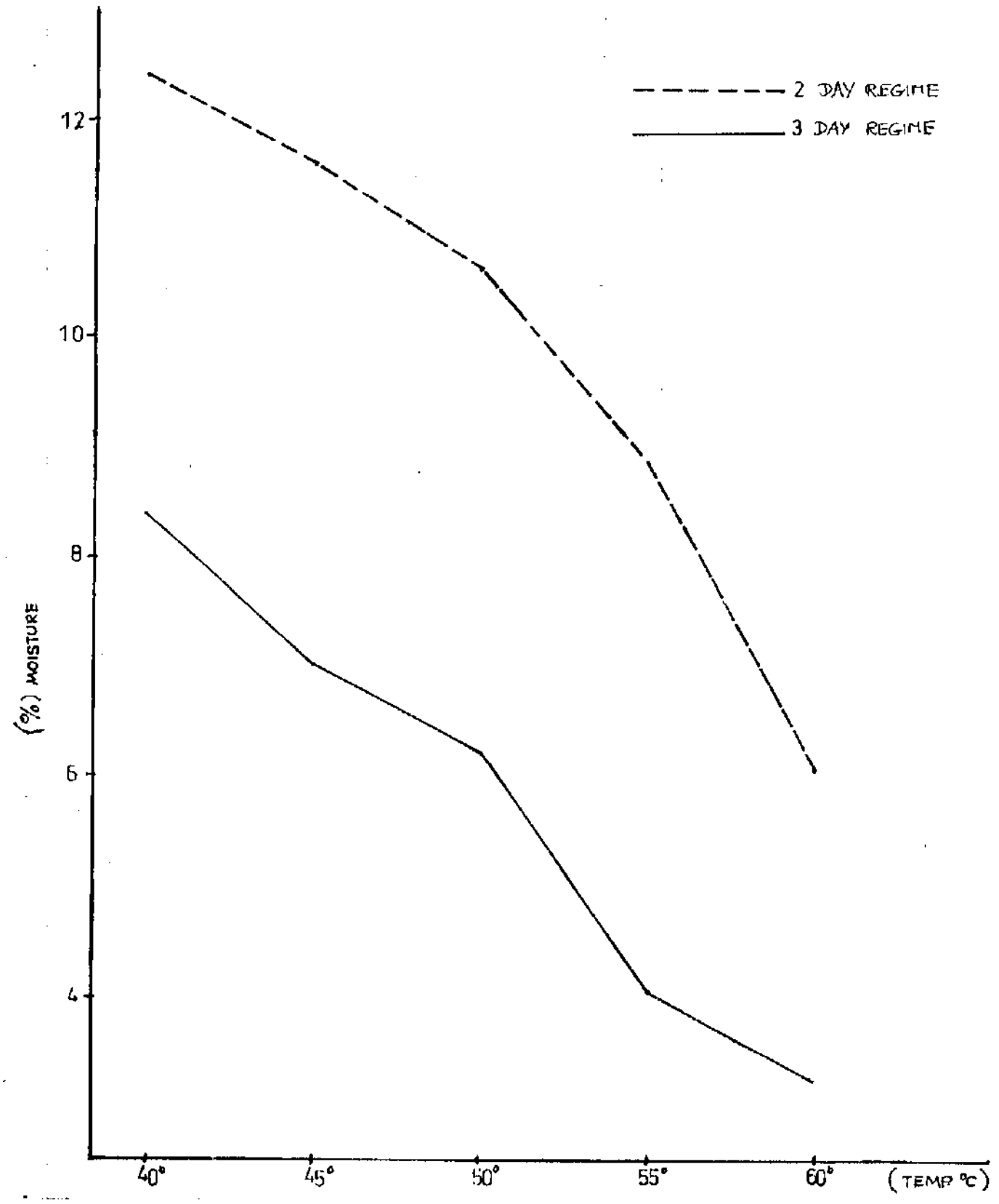


FIG 4.3 : EFFECT OF KILNING TEMPERATURE AND MALTING REGIME ON THE MOISTURE CONTENT OF THE MILLET GRAINS

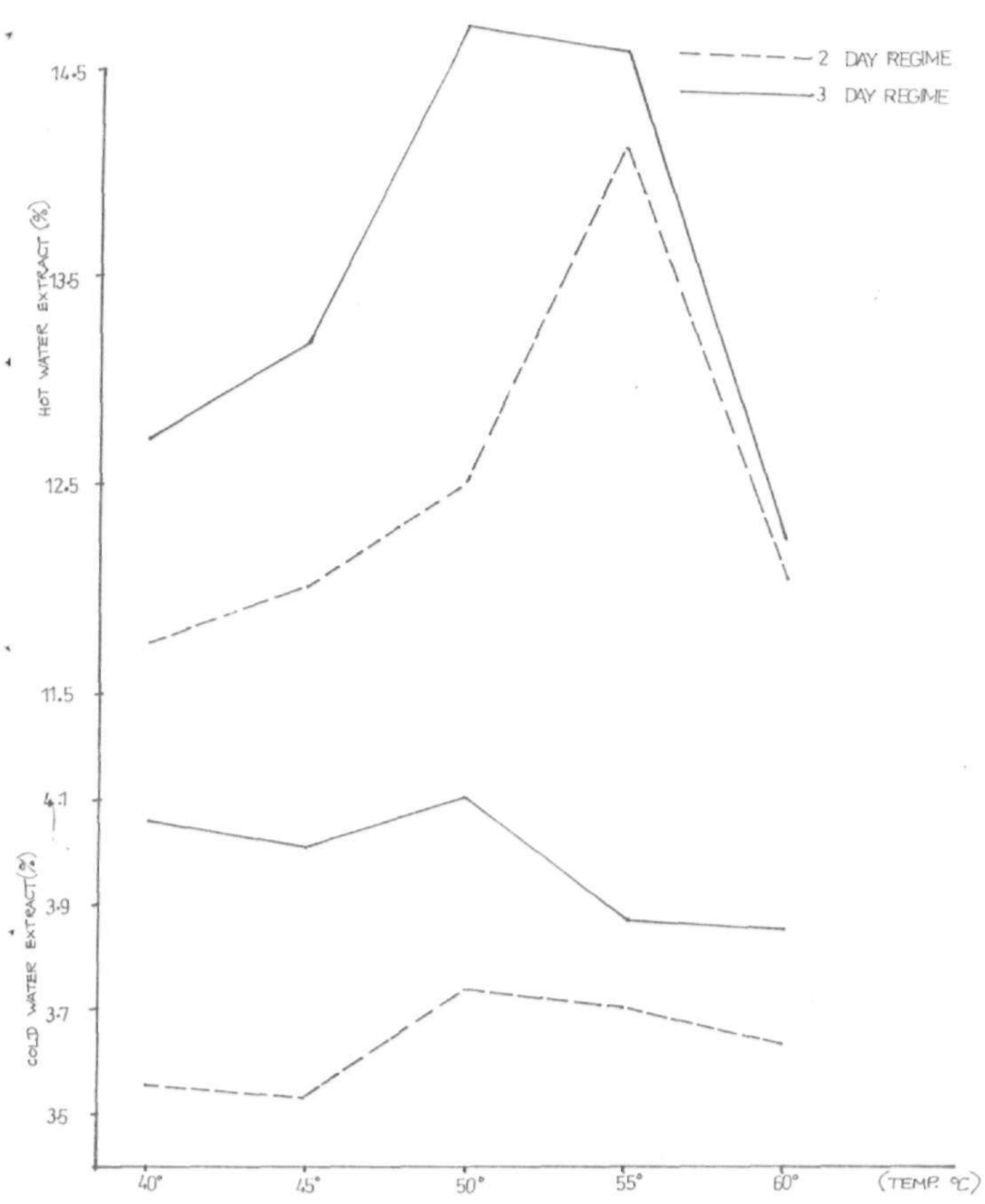


FIG 4.4 : EFFECT OF MALTING REGIME AND KILNING TEMPERATURE ON THE COLD WATER EXTRACT AND HOT WATER EXTRACT OF MALTED MILLET

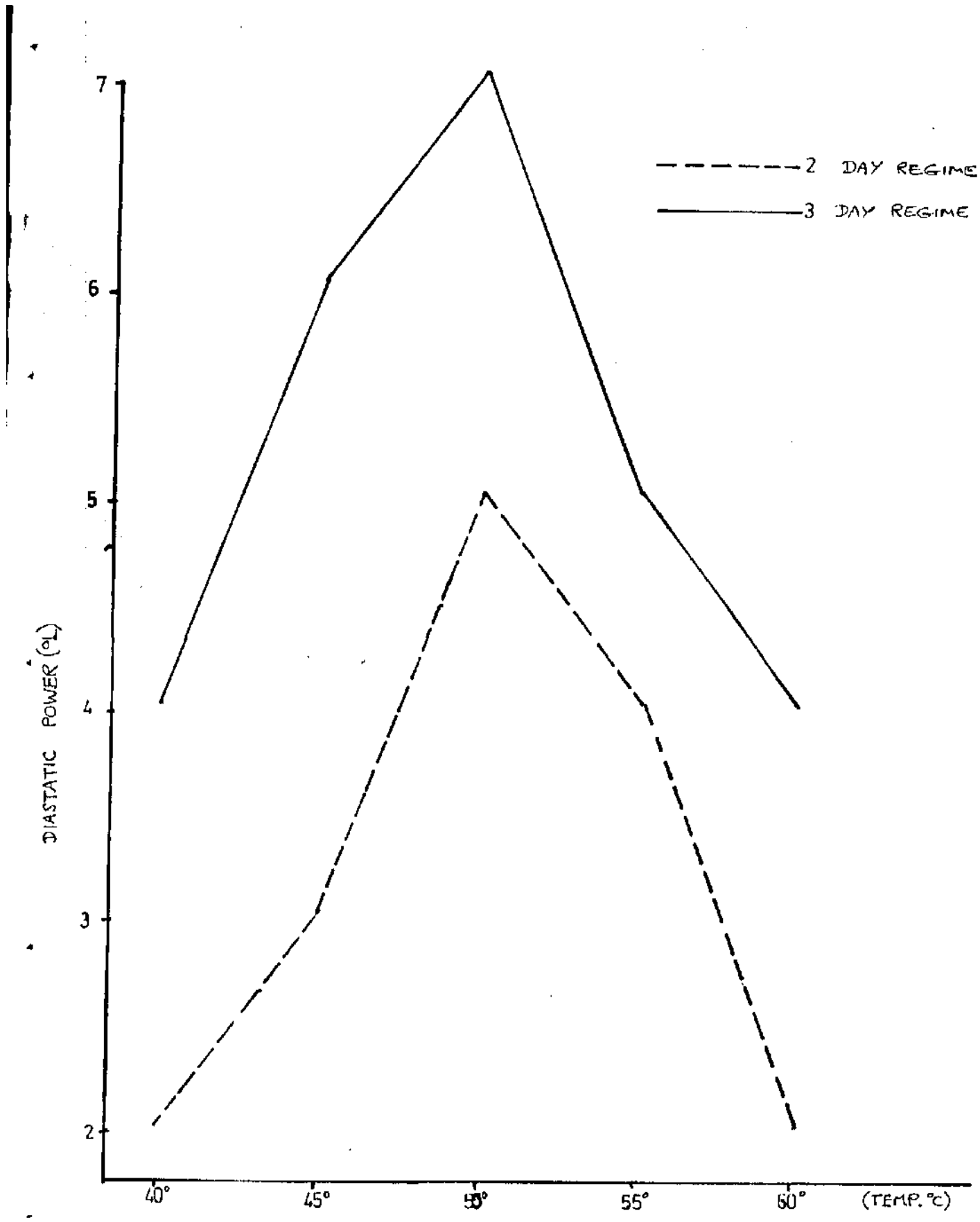


FIG 4.5 : EFFECT OF KILNING TEMPERATURE AND MALTING REGIME ON DIASTATIC POWER OF MALTED MILLET

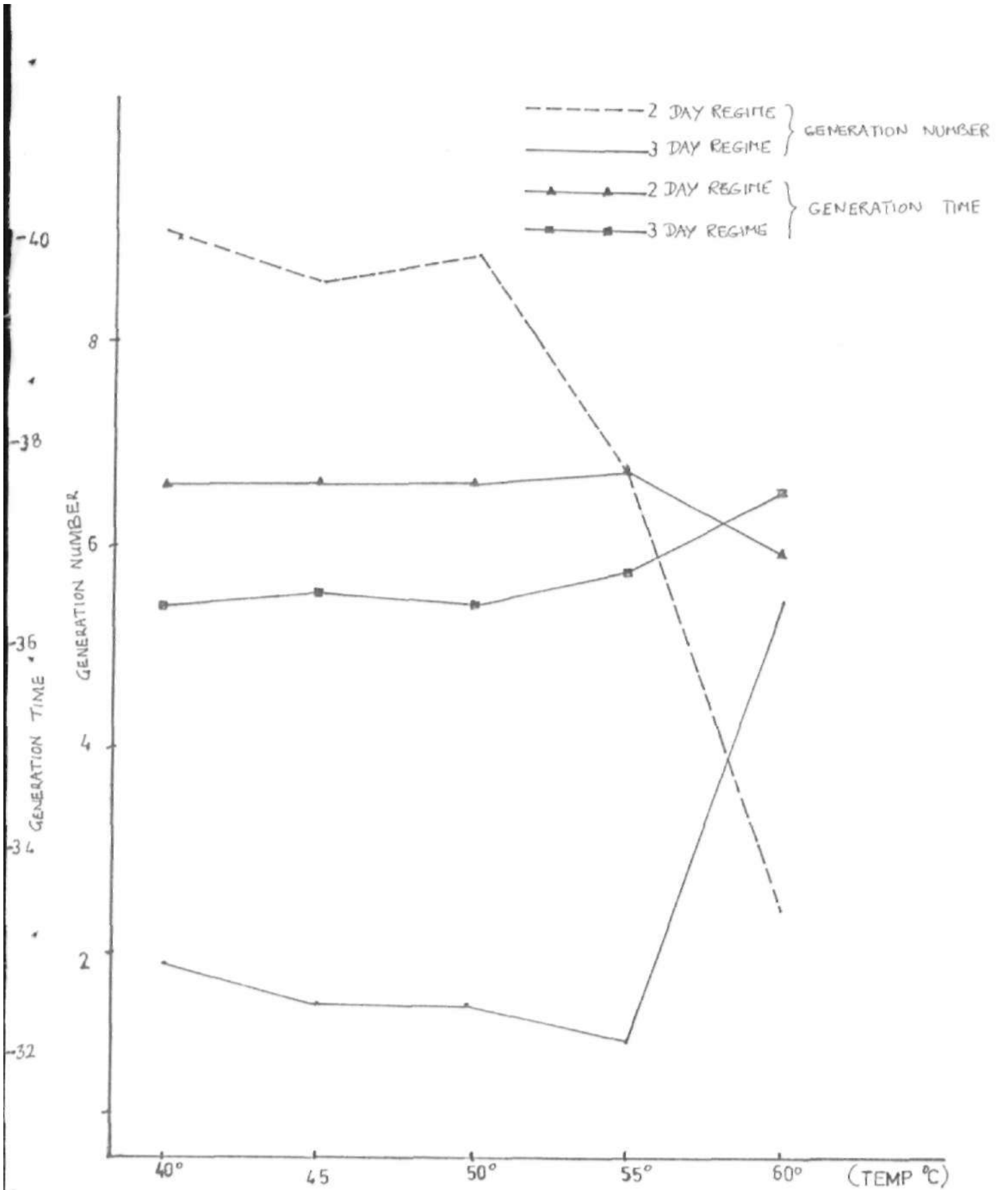


FIG 4.6 : EFFECT OF KILNING TEMPERATURE AND MALTING REGIME OF MALT -DERIVED WORT ON YEAST GENERATION NUMBER AND TIME

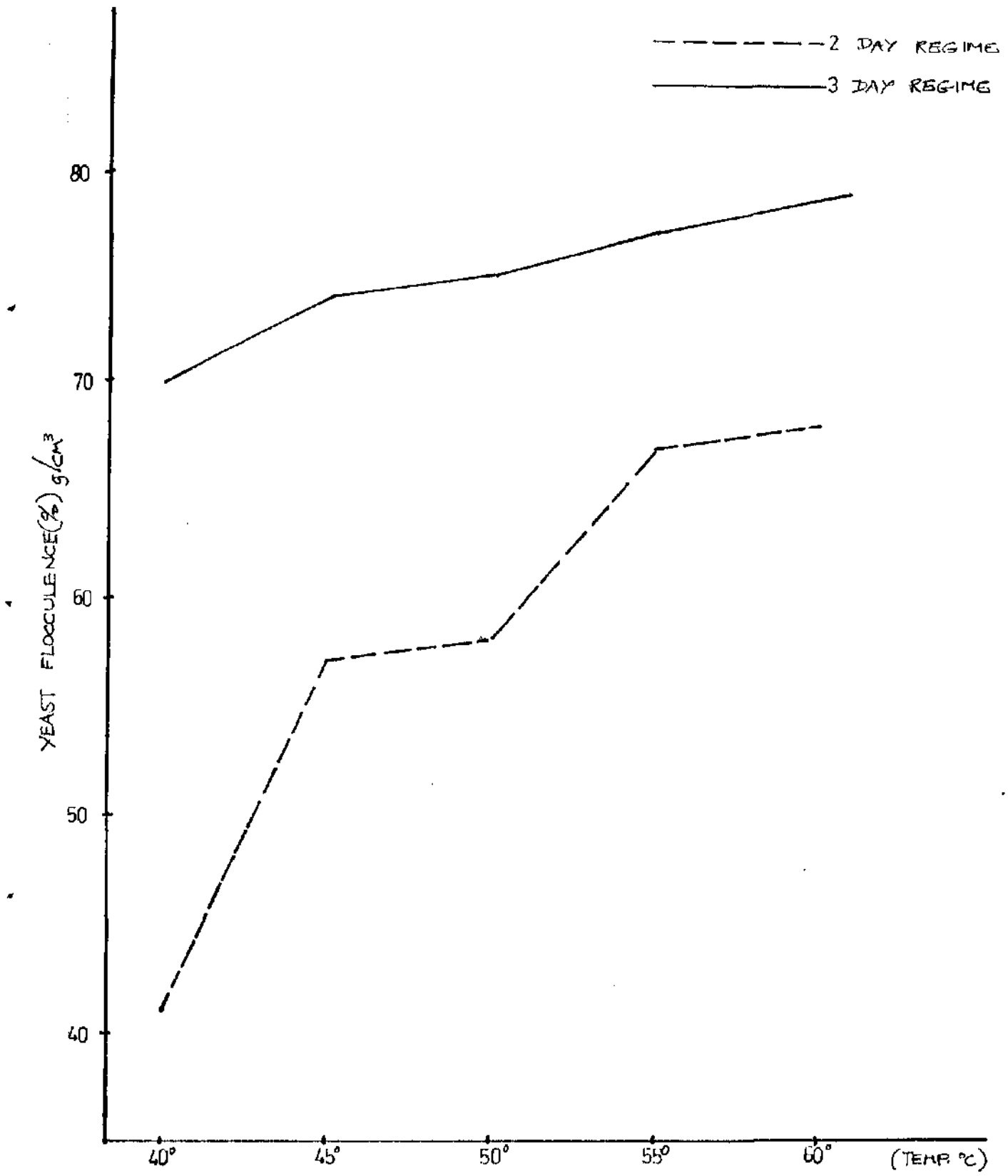


FIG 4.7 : EFFECT OF KILNING TEMPERATURE AND MALTING REGIME OF MALT -DERIVED WORT ON YEAST FLOCCULENCE

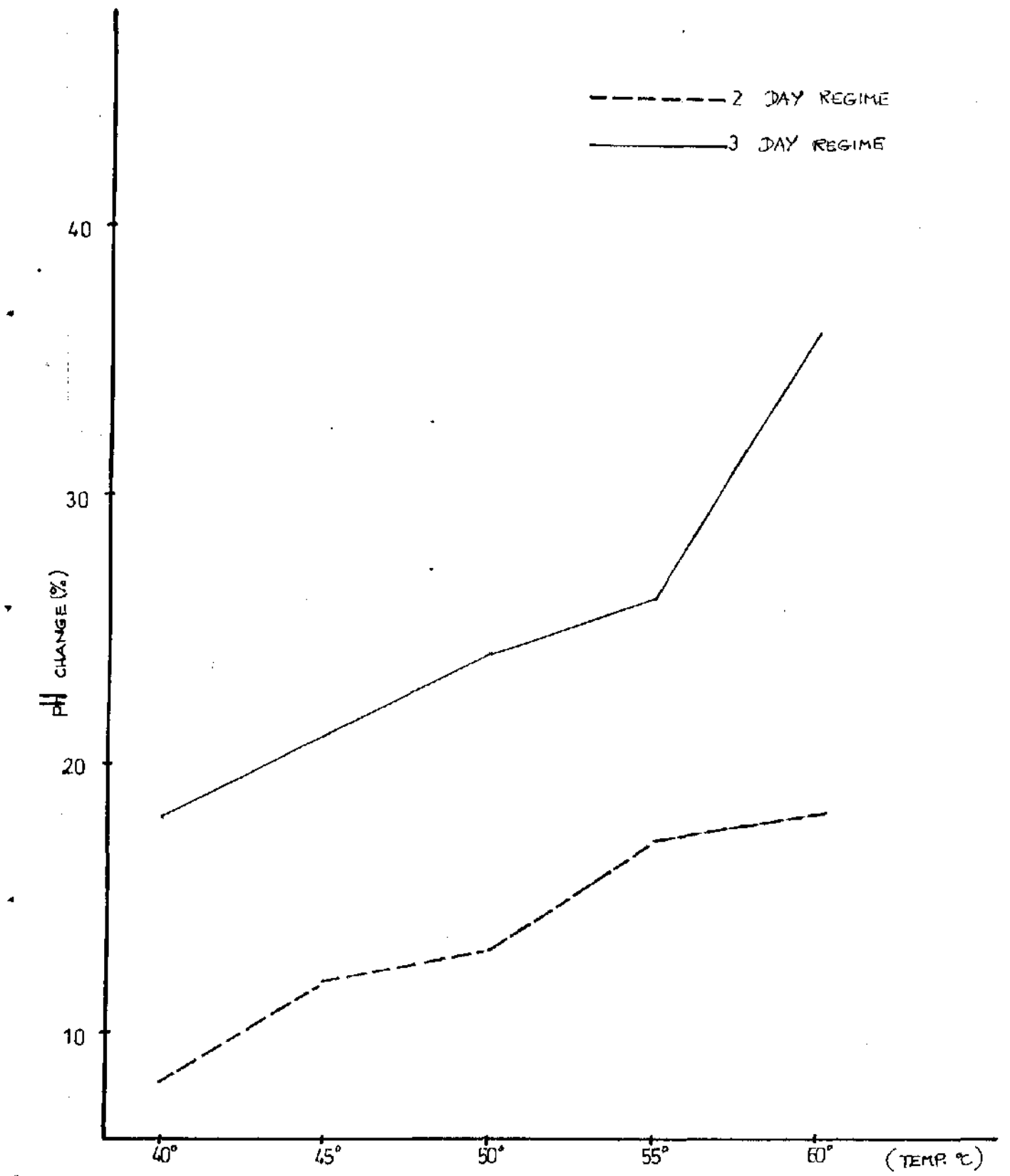


FIG 4.8 : EFFECT OF KILNING TEMPERATURE AND MALTING REGIME ON THE PERCENTAGE CHANGE IN pH OF FERMENTED WORT

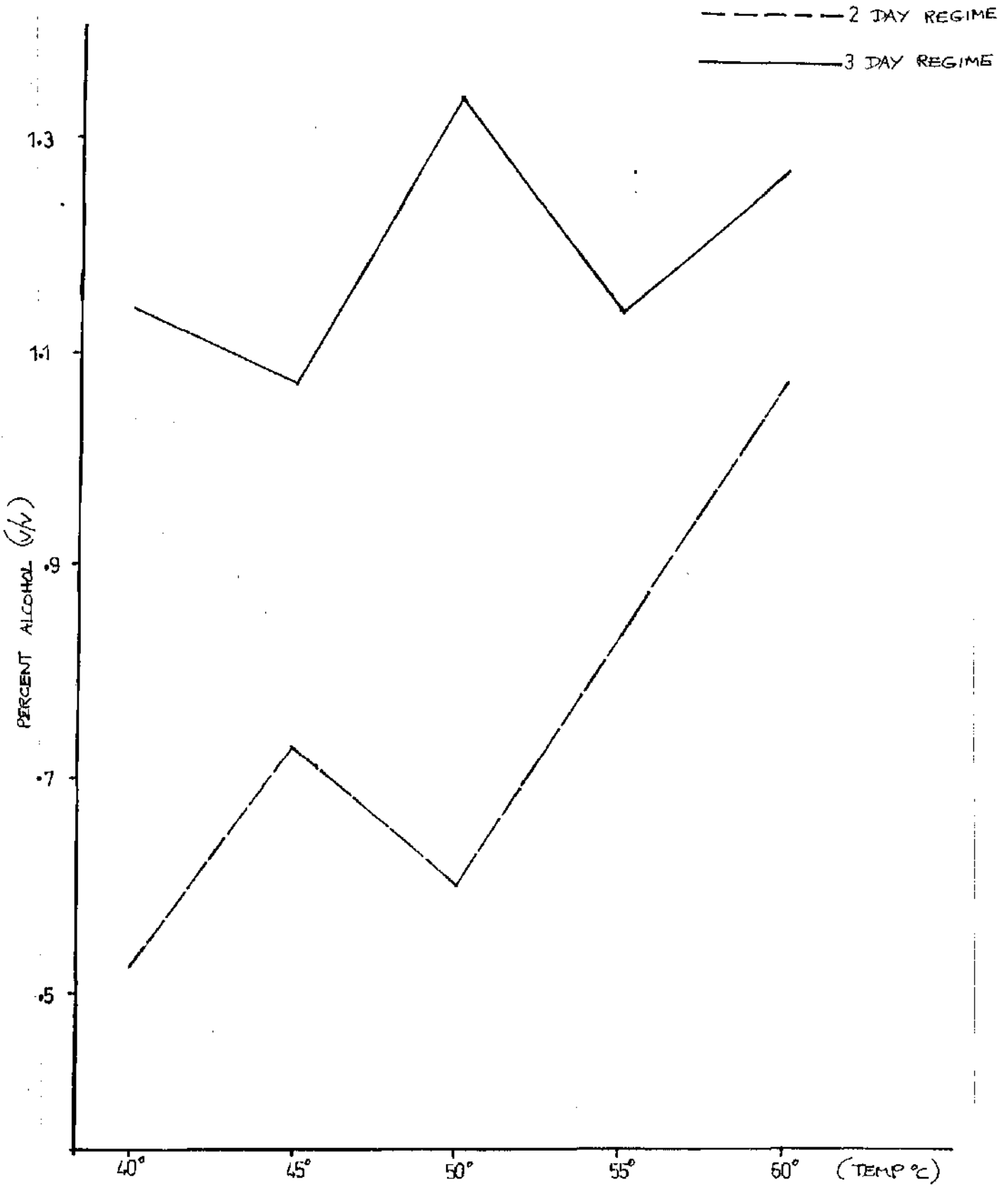


FIG 4.9 : EFFECT OF KILNING TEMPERATURE AND MALTING REGIME ON THE PERCENTAGE ETHANOL (V/V) OF FERMENTED WORT

CHAPTER FIVE
DISCUSSION

The amount of moisture contained in the millet grain may be regarded as an index of grain storage conditions and has direct correlation with weather. After steeping for 24 hours at room temperature, the moisture content was found to be lower than that for barley grains (40% - 50%). It is necessary to point out that the amount of moisture contained in the grain did not appear to have any effect on the malting characteristics of the millet under study. This has also been reported in similar observation (Malleshi and Desikachar, 1986 a). The grains attained their saturation moisture content (34%) within 24hrs of steeping (Table 4.1). Under this condition of steeping, pearl millet suffered no fungal attack. Under - steeping could result in slow germination, where as over-steeping would result into mould growth during germination (Okafor et al, 1988). It has also been reported for barley grains (Matz, 1970). A low moisture content in grain could also lead to a reduction in diastatic power value (Novellie, 1962).

The germination potentials of the millet grains are indicated from the results of the germination tests (Table 4.2). The water absorption capacity of this grain is smaller than that of barley probably because of the grain size or its starch content.

The Thousand corn weight assesses the quantity of fermentable material contained in a thousand grain of the sample. The malting quality of grains is therefore reflected by the thousand corn (TCW). Value obtained was 7.1 gram and this appears to be consistent with the findings of others (Hulse, 1980). The TCW is however very low. The germinative energy indicates the percentage of the grains that will be expected to germinate under standard conditions of normal germination, while germinative capacity indicates the percentage of living grains in a test sample. Germinative capacity of 93% would mean that the grain will give impressive results when germinated for malting. The grain also had a germinative energy of 97% which is also an acceptable germination potential because germinative energies and capacities below 90% would lead to such grains yielding malt of poor quality (Palmer. 1983). The germinative characteristics shown appears to be satisfactory for grains intended for malting as it exhibited values that are over 90%. It therefore follows that on this basis of good germination potentials, they could be used for further studies on the malting characteristics. It should be known that high germination energy is associated with high malting loss. Germination as a major unit process in malting produces a spectrum of enzymes intended to cater for the physiological needs of the young seedlings.

Malt yield is an expression of the percentage of weight of grains remaining in malt as compared to the original weight of malted sample. There is progressive decrease in malt yield in relation to the regime and more so with increasing temperature of kilning (Pathirana et, al 1983). The decrease observed in the malt yield for the 3 - day regime than the 2 - day regime is therefore to be expected (Table 4.2). There is weight loss through the roots and shoots and they are often winnowed after malting. This clearly shows that the lowest malt yield had vigorous germination (Figure 4.1). Although higher malt yields were obtained in both regimes, the malt yield alone is not enough to judge the maltability of the grain because other good malting properties such as diastatic power, cold and hot water extracts must accompany it.

Malting loss is an indication of the percentage of extent of germination. Malting losses included ungerminated grains, root-lets, shoots and losses due to respiration. Result indicates that malting loss tends to increase with increasing kilning temperature because the elevated heat tends to dry off the water and enabled the radicles and cotyledons to be removed. Prolonged germination (3 - day regime) tends to show higher malting loss than the 2 - day regime due to the considerable length of the radicles compared to the grain dimension. For both regimes higher malting loss was observed for the malts kilned at 60^0C and lower malting loss for the malts kilned at 40^0C (Table 4.2). It is noteworthy to point that either the display of low malting loss or high malting loss is a criterion to indicate the malt quality produced, rather, is an expression of what has beer lost vegetatively - during germination and kilning. Wainwright et al, (1986) has suggested the application of ammonium persulphate to malt beginning to grow to reduce root growth and malting loss, which can also lead to greater yield of malt and extract too.

In Table 3.4, the moisture content of the malts from both regimes appears to be inversely correlated to the kilning temperatures because the coefficient of correlation range from - 0.97 to - 0.99 (appendix iv). Higher malt moisture contents were observed for both regimes as compared to barley malt (5.5%) even though the kilning temperatures was increased (Nout and Davies, 1982) (Palmer,1983). Since kilning was done for all the malts for 24 hours, it therefore means that to bring the moisture content of the malt in the 2 - day regime to the moisture level of that of barley malt would require extended period of kilning. Higher moisture levels have been reported (Pathirana et al. 1983), to accelerate inactivation of enzymes at high temperatures. However, the malt moisture content from the 3 - day regime kilned at 50^0C and the 2 - day regime kilned at 60^0C showed some degree of nearness (6.2 and 6.0) respectively with the 5.5% for that of commercial barley malt. The malt moisture content is indirectly proportional to the malting regimes as well, because the 3 - day regime showed lower malt moisture content than the 2 - day regime due to the water that has been

obsorbed so much and utilised in prolonged germination by the radicle and plumule. Malting for 3 days and kilning at 50°C was found to be suitable for making good millet malt.

As can be observed in Table 4.2 the Cold Water Extract (CWE) of the malts obtained from the millet tend to decrease as kilning temperature is increased, although at lower temperatures, it tends to increase rising up sharply at 50°C (peak) and thereafter begins to decline (See figure 4.3). The CWE of millet is inferior to that of the barley malt (28%) for both regimes. Prolonged malting would yield a higher CWE value. Low cold water extract observed indicated low enzyme activity

With increasing kilning temperature, the HWE values appears to increase too, until they reached their peak (55°C for the 2 - day regime and 50°C for the 3 day regime) (Table 4.2) and thereafter continues to decline with further rise in kilning temperature. Generally, the result of the study sugests that HWE is far inferior to barley malt (92.6%). Pathirana et al (1983) had discussed that the decline in HWE of malt as kilning temperature is increased is related to the loss in enzyme activity of the malt due to heat susceptibility.

Kilning presents a problem in as much as temperature and time are fairly critical to preserve high diastatic and protease levels. Too high a temperature while grain is still wet will certainly destroy the enzymes very quickly (Harrison et al, 1987). The treatment of millet malt at the five different temperatures; (40°, 45°, 55° and 60°C) in the current study was to establish the optimum temperature for kilning the millet malt. Diastatic power is defined as the total activity of enzymes that are involved in the hydrolysis of starch during mashing. Nout and Davies, (1983) observed that the application of heat adversely affects enzymes of the malt and may result in wort deficiency of diastatic enzyme. It should be understood that kilning of malt is necessary to remove the raw flavour of the green malt and to promote the development of characteristic malt flavour. The diastatic power is low for malts under both regimes as compared to barley (78°L). Millet malts possess inferior diastatic power and therefore appears to give poor extract yield when compared with barley malts because of the quantitative difference in their amylase content and probably the presence of endogenous amyloytic enzyme inhibitors (Kneen and Sandstedt, 1946). From lower values, as the kilning temperature in increased, there is a corresponding increase in diastatic power (see figure 4.5) until both regimes reached their peak at 50°C and thereafter continues to decline steadily with further increase in kilning temperature. Since the flavour of the malt is to be maintained, malting for 3 days and kilning at 50°C exhibited moderate losses in diastiatic power with corresponding low malt moisture level comparable to barley malt. There is a direct relationship between diastiatic power and malting regime, because higher malting regime yielded a rise in the diastatic power value although

the difference was small. (see appendix iv)

The wort pH of kilned malt was neither affected by temperature of kilning, nor the malting regime. The difference in the wort pH of all the millet malts appears not to be significant because the values lie between 0.001 and 5.47 and are all in the acidic region (Table 4.3). This is to be expected because the wort contains some enzymes (acidic in nature) elaborated by the germinating millet (Malleshi and Desikachar, 1986 a) and more so at pH 5.3, there's more effective contact between enzyme and substrate molecules (Okon, 1988). Lower pH values has a deleterious effect on sugar formation particularly for the low diastiatic power malts (Novellie, 1966). Degree of pH change in the worts is a function of the malting regime. Percentage change in the pH values is low for all the wort samples, none up to 40% (fig. 4.8). The 3 day regime showed higher pH change because this is to be expected since the α - amylase enzyme which is acidic in nature, has its highest activity at 96 hours (Malleshi and Desikachar, 1986 a), hence it brings down the pH of the wort. Barley wort has a pH of 3.8 and only the 3 day regime malt kilned at 50^{0}C has some what value (3.7) close to it

The inoculum media used for the growth and assay of the brewers' yeast was satisfactory because high cell yield was obtained (appendix III). Inoculum media usually differ in composition from production media, and are often compounded to quickly yield large number of microbial cells in their proper physiological and morphological states but without sacrificing the genetic stability of the cell (Casida, 1993).

The absorbance measurement of the wort/yeast suspension read at 660nm at the start of the fermentation is shown in Table 4.4. Percentage amount of light transmitted is high (above average) for all the samples and the differences between them is not significant irrespective of the malting regimes and kilning temperatures for example for the unfermented wort at T=0hrs, the malting regime and kilning temperature gave 0.06 and 1.62 from the analysis of variance while that of the fermented wort i.e at T=216 hrs gave 0.11 and 0.16 respectively. The absorbance of a suspension of light scattering material depends on the concentration of suspended material (Pringle and Mor, 1975).Turbidiometric values obtained expressed as percentage transmission at end of fermentation indicate that yeast cells have multiplied, hence increase in the light scattering properties of the mixture. Suffice to mention that due to the turbid nature of the fermented broth, each sample was diluted four times to enable the determination of the absorbance.

The formation of buds by the yeast cells significantly decreased with prolonged fermentation. The cells now look elongated with irregular buds and more than a bud for each cell that exhibited it. This

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may be due to the presence of certain organic acids e.g lactic acid, that is produced into the medium in the course of fermentation as demonstrated by Mairoella et al (1983). Kilning temperature and malting regimes adopted have no effect on the yeast morphology because of their consistent and stable morphological characteristics.

Malting regime has a direct correlation with yeast count that has been grown in the wort derived from the malt. Prolonged germination (3 - day regime) would mean increase in α-amylase activity (Malleshi and Desikachar, 1986 a), hence resulting in corresponding increase in the free sugar content of the malt sample which supports yeast growth. This is why the 3 - day regime malt - wort showed high values of yeast count than the 2 - say regime (Table 4.5). Temperature too, has a direct relationship with the yeast count obtained from the wort. Fast maltose production from starch is principally the action of β - amylase and is optimum at 50^0C (Nout and Davies, 1982). In this study, wort obtained from the 3 - day regime malt kilned at between 50^0C and 55^0C gave the maximum yeast yield in terms of viable count (see Table 4.5). They also demonstrated increased recovery on yeast extract agar. Diluting the cells in sterile distilled water rather than in broth medium or in buffer results in no loss in viability. Difficulty in obtaining reasonable counts of the yeast viable cells are the clumping problem due to flocculation. Yeast number of generation was low with high generations time (Table 4.5 and fig. 4.6) probably due to the enzymatic composition or nutrient composition of the wort which seem not to support fast growth of the yeast cells.

The percentage of yeast dead cell as calculated using the improved Neubauer counting chamber indicated that the wort composition supports yeast growth although not fast (figure 4.6 - due to low generation number) since the values obtained are very low (in the range of 2% to 6% per litre). This suggests that the composition of the wort derived from the malt is assured for yeast nutrition.

The degree of yeast flocculence was affected by wort composition; because the yeast cells recovered from the 3 - day regime malt wort flocculated better (figure 4.6).Statistically the coefficient of variation displayed by the flocculating yeast in the 2 day regime wort (4.0) are more dispersed than the 3-day regime wort (16.7) This may be due to the prolonged germination which gave a better enzyme yield for the degradation of the starch and so, made them available yeast which is uses in probably building up mannan and lectin components of the cell wall, hence the capacity to flocculate (Bonaly and Lematre, 1983). kilning temperature too has a direct relationship with the flocculence. Better yeast flocculation in the 3 - day regime malt might be due to the a decrease in fementable sugars since this should repress cell metabolism (Kamada and Murata, 1984). Flocculating cells possess a stable cellular envelope structure with increased cell-wall contents of mannans and the

lectin type components are responsible for flocculation (Bonaly and Lematre, 1983).

Amylodextrins seem to be more in the 2 - day regime malt then the 3 - day regime malt which contains mainly erythrodextrins (Table 4.4). Both of them appear to be the unconverted starch in the wort; Presence of dextrins here in the millet wort might be due to the enzyme composition of the malt. Millet malt contains more α - amylase than β - amylase (Malleshi and Desikachar, 1986 a), when compared to barley malt which bas higher β-amylase than α- amylase, hence it suggests the reason why the millet malt contains so much of these unconverted starch.

Total acetic acid recorded for each wort is lower than that for barley wort (0.08 to 0.17) which has been fermented. The trend showed that there is a direct relationship between the total acetic acid and malting regime (Table 4.4). The 3 - day regime showed slightly higher values than the 2 - day regime It is unlikely that kilning temperature has any effect on it.

The specific gravity of the distillate (Table 4.6) was used to calculate the percentage alcohol by volume. β- amylase is the principal enzyme that degrade starch to produce maltose. This is low in millet (Malleshi and Desikachar,1986 a) and thus, will account for the low fermentability of the millet worts together with the low thousand corn weight to give a value of 1.34% alcohol V/V Hence the highest value obtained was 1.34 V/V which is very low. There is a direct relationship between the malting regime and alcohol values obtained (figure 4.9). There is also a direct relationship between the kilning temperature and alcohol values although the value begins to decline after reaching the peak at 50^0C for the 3 - day regime malt.

CHAPTER SIX

CONCLUSION

From the results of this study, the quality of millet malt is inferior to barley malt. The low malt yield indicate high capacity for malting loss. Steeping the millet grains at room temperature (25^0C to 28^0C) for 24 hours is conducive before malting. Malting for 3 days (3 - day regime) and kilning at 50^0C for 24 hours was found to be conducive for preparing good malt from millet although the diastatic power, cold and hot water extracts are far too low than those of barley malt. Germination period was found to have remarkable effect on the quality of malt, wort and alcohol produced from the millet. The use of 100% pearl millet alone for the production of lager beer will not appear adequate mainly because of their low extract content and diastatic power which would mean very light beer. Breeding studies is suggested to increase the starch and β-amylase content of the millet; although the millet malt could be used as adjuncts with sorghum or barley grains for beer production.

The yeast cell biomas and count was found to be high for all the samples. Since the viable count achieved was high together with low number of dead cells, it indicates that the wort composition was satisfactory, and as such the malt could be compounded for use in isolation media and may be used in the production of single cell protein. The low generation number, high generation time and low growth rate suggests that even though the wort composition was satisfactory, it does not enhance the vigorous multiplication of cell within a short time, which is a disadvantage to the brewer since fermentation are operated for maximum efficiency and minimum production cost. The brewers' yeast did not lose its flocculating ability for the three pitching cycles for which it was used, which suggests that the wort composition has not sacrificed the yeast cell genome.

In conclusion, if pearl millet malt is to be considered as potential malt for the production of conventional, lager beers, other factors such as filtrability, total soluble nitrogen, colour, clarity and flavour are of utmost importance to be considered. The millet malt may be formulated as weaning food for infants; as isolation media and for single cell protein production.

APPENDIX I

DIASTATIC POWER REAGENTS [SOURCE: AOAC, 1980]

Acetate buffer solution (pH 4.6): Dissolve 68g sodium acetate in 500millilitre 1moL/L. acetic acid (28.65millilitre glacial acetic acid at 20^0C diluted to 500millilitre) make up solution to one litre

Sodium hydroxide solution, (0.1mol/L): Dissolve 4g of sodium hydroxide in distilled water and make up to one litre.

Fresh starch solution (2%): Buffered to pH 4.6. Dissolve 10g of starch into a cream with distilled water. Pour cream into 400millilitre of boiling water with constant stirring and boil for two minutes. Cool to 28^0C and add ten millilitre of acetate buffer. Make the volume up to 500millilitre with distilled water.

4) Acetic acid solution: 70g of potassium chloride and 20g Zinc sulphate pentahydrate should be dissolved in distilled water and 200millilitre glacial acetic acid added. Make up to one litre with distilled water.

Potassium iodide solution: Dissolve 50g in 50millilitre distilled water and add three drops of concentrated sodium hydroxide. Make up to 100millilitre with distilled water.

6)Sodium thiosulphate solution (0.05mol/L): Take 12.4one gram of sodium thiosulphate and three gram duplicate samples of sodium tetraborate decahydrate. Dissolve in distilled water and make up to one litre.

7) Alkaline ferricyanide solution (0.05mol/L): Weigh 16.5g of potassium ferricyamide and 2g of anhydrous sodium carbonate and dissolve in distilled water to make one litre.

APPENDIX II

BUFFER [SOURCE: moffat, A.C; 1986]

1) Acetate Buffer (pH5): Dissolve 13.6g of sodium acetate and 6millilitre of Acetic acid in sufficient water to produce 1000millilitre.

2) Ammonia Buffer (pH 9.5): Dissolve 10.7g of Ammonium chloride in 40millilitre of 5M ammonia and dilute with water to 1000millilitre.

3) Phosphate Buffer (pH 5.6): Dissolve 62.4g of sodium hydrogen phosphate in sufficient water to produce 100millilitre.

4) Phosphate Buffer (pH 7.4): (a)Dissolve 9.465g of anhydrous disodium hydrogen phosphate in water and dilute to 1000millilitre (b) Dissolve 9.07three gram duplicate samples of potassium hydrogen phosphate in water and dilute to 1000millilitre Now mix 80millilitre of (a) with 20millilitre of (b).

APPENDIX III

GLUCOSE YEAST PEPTONE EXTRACT BROTH (GYP)[SOURCE: DEMAIN AND SOLOMON, 1986]

.) 10g Glucose
·) 10g yeast extract
·) one gram Dipotassium phosphate
·) ten millilitre Salt solution
·) 990millilitre distilled water
·) pH 7.0

APPENDIX IV

COEFFICIENT OF CORRELATION

1. KILNING TEMPERATURE AND MALTING REGIME RELATION TO THE MALTING PROPERTIES

A. TWO DAY REGIME

Malting Properties	Kilning Temperatures (°C)
MMC	-0.97
ML	0.97
CWE	0.54
HWE	0.51
MY	-0.97
DP	0.12

B. THREE DAY REGIME

Malting Properties	Kilning Temperatures (°C)
MMC	-0.99
ML	0.97
CWE	-0.81
HWE	0.05
MY	-0.97
DP	-0.12

2. CORRELATION OF MALTING PROPERTIES WITH EACH OTHER

C. TWO DAY REGIME

	MMC	ML	CWE	HWE	MY	DP
MMC	0	-0.88	-0.37	-0.34	0.88	0.13
ML	-0.88	0	0.67	0.69	-0.99	0.33
CWE	-0.37	0.67	0	0.65	-0.67	0.78
HWE	-0.34	0.69	0.65	0	-0.69	0.58
MY	0.88	-0.99	-0.67	-0.69	0	-0.32
DP	0.13	0.33	0.78	0.58	-0.32	0

D. THREE DAY REGIME

	MMC	ML	CWE	HWE	MY	DP
MMC	0	-0.97	0.88	-0.06	0.97	0.18
ML	-0.97	0	-0.89	-0.14	-0.99	-0.24
CWE	0.88	-0.89	0	0.24	0.89	0.56
HWE	-0.06	-0.14	0.24	0	0.13	0.72
MY	0.97	-0.99	0.89	0.13	0	0.24
DP	0.18	-0.24	0.56	0.72	0.24	0

MMC = Malt moisture Content

ML = Malt Loss

CWE = Cold Water Extract

HWE = Hot Water Extract

MY = Malt Yield

DP = Diastatic Power

Note: Correlation lies between +1 and -1 and is positive for direct correlation and negative for inverse correlation

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