FEASIBILITY OF INCREASING RICE (*Oryza sativum*) USAGE AS AN ADJUNCT FOR BEER.

By

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DECLARATION

The research work described in this thesis was carried out by me at The Lion Brewery Ceylon Ltd and Faculty of Applied Sciences under the supervision of Mr. Janaka Bandara and Mrs. Shiromi Perera. A report on this has not been submitted to any other university for another degree.

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Affectionately Dedicated to

My Parents.

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ABSTRACT

Adjuncts are an additional source of carbohydrate extract used to partially replace or supplement the malt in beer. They are also used to reduce the overall material costs and to utilize indigenous sources. Rice (*Oryza sativum*) is widely used as an adjunct in the world for the production of alcoholic beverages such as beer. The ratio of malt to adjunct must fall within an acceptable range. Each brewer according to the quality specifications for the final beer and the cost estimation sets adjunct ratios. Thus the major objective of this project was to increase the rice usage in beer as an adjunct.

Alpha amylase and Beta amylase are the main two enzymes involved in brewing. Alpha amylase is more thermo stable than Beta amylase. Alpha amylase cleaves starch randomly except near ends of branch points while Beta amylase cuts off maltose from non-reducing ends of molecules. Alpha amylase mainly produces dextrin molecules and Beta amylase produces Beta maltose which is the major sugar in wort. It is considered that additional Beta amylase enzymes are in malt. This additional enzyme amount can be used for saccharification of other carbohydrate sources in mashing where the ground malt and the solid adjuncts (grist) are mixed with water at a set temperature and with a set volume of liquor (water) to produce wort.

The first approach was to identify the optimum ratio of malt and rice for saccharification. More than 50% rice amount can be saccharified by additional Beta amylase enzymes in malt, For Lion Larger it is used about 29.2 % rice and 70.2% malt amount as weight basis. That usage was increased up to 40% with keeping the quality of it. For that three different larger samples were prepared by changing the rice, malt and water amount of the original recipe. The amount of extract was calculated according to the IBD (Institute of Brewing & Distillation) method. The existing recipe was changed by gradually increasing the rice usage step by step. The rice usage starting from 30% and gradually increased by 35% and finally up to 40%.This was done in the commercial scale while the Lion Larger quality limits were maintained at all the stages of the beer production. Then beer samples were organoleptically analyzed by the well trained sensory panel. The Triangular test was done to identify the differences. Beer samples were scored for overall acceptability on 9-point hedonic scale using 12 well trained panels, where 4 samples were presented in 1 session in a well prepared sensory room. Data was statistically analyzed with Freidman test using MINITAB statistical analysis package (version 14.1).

According to that aroma and colour gave significant difference when comparing with other beer samples. About sixty thousand rupees cost saving could be achieved by this project for each batch of the beer annually that is about 7.2 millions rupees of cost saving.

CONTENTS

Title	Page No
ABTRACT	I
ACKNOWLEDGEMENT	II
CONTENTS	III
LIST OF ABREVIATIONS	VII
LIST OF FIGURES	VIII
LIST OF TABLES	IX
CHAPTER 01: INTRODUCTION	
1.1. Background	**01**
1.2. Overall Objective	**02**
1.3. Specific Objectives	**02**
CHAPTER 02: LITERATURE REVIEW	
2.1 Beer: An Overview	**03**
2.1.1 History of beer	03
2.1.2 Different types of beer	03
2.1.2.1 Lager	03
2.1.2.2 Ales	04
2.1.2.3 Wheat beers	04
2.1.2.4 Stouts	04
2.1.2.5 Low alcohol / alcohol-free beers	04
2.1.2.6 Low-carbohydrate beers	04
2.1.3 Beer styles	04
2.1.4 The health benefits of beer	04
2.1.5 Beer industry in Sri Lanka	05
2.1.6 The Lion Brewery Ceylon Ltd	06
2.1.7 Market overview of LBCL beer	07
2.2 Beer Processing	**08**
2.2.1 Raw materials for brewing and its characteristics	08
2.2.1.1 Malt	08
2.2.1.2 Barley	09
2.2.1.3 Adjuncts	11
2.2.1.4 Rice cereals	12
2.2.1.5 Hops	14

2.2.1.6 Brewing water 15
2.2.1.7 Principal ions and minor ions 16
2.2.1.8 Brewers yeast 17
2.2.1.9 Brewing salts 18
2.3 Brewing of beer 18
2.3.1 Preparation of sugar solution/wort 18
2.3.1.1 Malt milling 18
2.3.2 Mashing 19
2.3.2.1 Purpose of mashing 19
2.3.2.2 Properties of enzymes in mashing 19
2.3.2.3 Starch degradation 20
2.3.3 Wort separation (Lautering) 21
2.3.3.1 First wort and second wort 22
2.3.3.2 Sparging 22
2.3.3.3 Water quality 22
2.3.3.4 Water quantity 22
2.3.3.5 Water temperature 22
2.3.3.6 Wort boiling 22
2.3.3.7 Wort cooling 23
2.3.4 Beer fermentation 23
2.3.4.1 Yeast preparation & handling 24
2.3.5 Beer conditioning 24
2.3.5.1 Colloidal stabilization 24
2.3.6 Beer carbonation 24
2.3.7 Bottling and packaging 25
2.3.7.1 Bottle rinsing 25
2.3.7.2 Beer packaging 25
2.4 Technology 25

CHAPTER 03: MATERIALS AND METHODOLOGY
3.1 Materials and Equipments 31
3.1.1 Materials 31
3.1.2 Equipments 31
3.2 Methodology 32
3.2.1 Find out the optimum usage of rice for Lion Larger 33
3.2.2 Selection of the optimum rice usage for beer in keeping quality aspects 33

3.2.3 Calculation of rice and malt amount for Lion Larger 34
3.3 Beer production **36**
3.3.1 Handling of malt and rice 36
3.3.2 Preparation of sugar solution/wort (Lautering) 39
3.3.3 Water temperature 39
3.3.4 Wort boiling 39
3.3.5 Wort cooling 40
3.3.6 Basic brewery calculations 42
3.3.7 Fermentation 42
3.3.7.1 Yeast preparation & handling 44
3.3.8 Conditioning of beer 44
3.3.8.1 Treatment of beer 44
3.3.8.2 Beer filtration 44
3.3.6.3 Beer carbonation 45
3.4 Beer packaging **45**
3.4.1 Bottling 45
3.5 Technology **45**
3.6 Quality Analysis Testing of the Lion Larger **47**
3.6.1 Analysis of cooled wort 47
3.6.2 VDK measurement in each ferment vessels 47
3.6.3 Analysis of fermented beer 47
3.6.4 Analysis of treated beer 48
3.6.5 Analysis of bright beer tank 48
3.6.6 Analysis of unpasteurized beer 48
3.6.7 Analysis of bottled beer 49
3.6.8 The Original Gravity (OG) 49
3.6.9 Calculations for spectrophotometric methods 49
3.7 Sensory analysis **50**
3.7.1 Find out the significant difference between each beer sample 50
3.7.2 Preference test 50

CHAPTER 04: RESULTS AND DISCUSSION
4.1 Formulation of a newer Lion Larger recipe **51**
4.2 Comparison of results in the Brew House of trials **53**
4.2.1 Plato value of wort 53
4.2.2 Pre boil & post boil plato 55
4.2.3 pH variation of pre boil and post boil wort 56

4.2.4 Spent grain plato 56
4.3 Fermentation Records **57**
4.3.1 Comparison of fermentation records of 35% rice Lion Larger 57
4.4 Sensory evaluation results for 35% rice used LL beer **60**
4.5 Sensory Evaluation results for 40% rice used beer **60**
4.5.1 Summary of the triangular test 61
4.6 Cost calculation for Lion Larger with 40% rice 62
4.7 Benefits of rice as an adjunct for beer 64
4.8 Practical Issues and Recommendations **65**
4.8.1 Practical issues and recommendations for 35% rice beer 65
4.8.2 Sensory analysis of 35% rice beer 65
4.8.3 Practical Issues and recommendations for 35% rice beer 40% rice 65
4.8.4 Sensory analysis of 40% rice 65

CHAPTER 05: CONCLUSION AND RECOMMENDATION
5.1. Conclusion **66**
5.2. Further Studies **67**

REFERENCES 68
APPENDIX I 71
APPENDIX II 72
APPENDIX III 73
APPENDIX IV 74
APPENDIX V 75
APPENDIX VI 78
APPENDIX VII 80

LIST OF ABREVIATIONS

AOAC	Association of Official Analytical Chemists
BBT	Bright Beer Tanks
BU	Bitterness Unit
CB	Carlsberg
CBSB	Carlsberg Special Brew
Conc.	Concentrated
DO	Dissolved Oxygen
IBD	Institute of Brewing and Distillation.
EBC	European Beer Council
hrs	hours
kP	kilo Pascal
L	Litres
LBCL	Lion Brewery Ceylon Ltd
LL	Lion Larger
LS	Lion Stout
Ltd	Limited
MW	Molecular Weight
OE	Original Extract
pvt	Private
PVPP	Poly Vinyl Poly Pirolidine
RDF	Real Degree of Fermentation
SS	Strong Beer
SLSI	Sri Lanka Standard Institute
SLS	Sri Lankan Standard
TBT	Treated Beer Tanks
UV	Ultra Violet
VDK	Vicinyl Di Ketone
V/v	Volume to volume
W/w	Weight to weight

LIST OF FIGURES

Figures		Page no
Figure 2.1	Basic flow chart of beer production with the ingredients	03
Figure 2.2	Starch granules of barley grain prior to germination	09
Figure 2.3	Steeping process of barley in steeping tanks	10
Figure 2.4	Germination process of barley in malting box	10
Figure 2.5	Kilning process of malt in kilning chamber	11
Figure 2.6	Structure of hop plant cone and indication of alpha acid in cone	15
Figure 2.7	Structure of yeast cell under microscope	17
Figure 2.8	Spider diagram of a particular brand of beer	30
Figure 3.1	Basic flow chart of beer production with the ingredients	32
Figure 3.2	Outline of reformulation scheme of existing recipe of LL	32
Figure 3.3	Malt transport system at the Lion Brewery Ceylon Limited	37
Figure 3.4	Rice transport system at the Lion Brewery Ceylon Limited	38
Figure 3.5	Process flow of wort production	41
Figure 3.6	Yeast dosing of beer processing	43
Figure 3.7	Beer conditioning procedure of Lion Larger	44
Figure 3.8	Beer carbonation procedures	45
Figure 4.1	Graph no: 01-Plato variation of 35% rice used Lion Larger	54
Figure 4.2	Graph no: 02- Plato variation of 40% rice used Lion Larger	54
Figure 4.3	Graph no:03- pre boil and post boil plato analysis	55
Figure 4.4	Graph no: 04- pre boil and post boil plato analysis	55
Figure 4.5	Graph no :05- pH variation of pre boil and post boil wort	56
Figure 4.6	Graph no :06- spent grain plato variation	57
Figure 4.7	Graph no: 07- plato variation in the fermentation	57
Figure 4.8	Graph no: 08- VDK Level in fermentation	58
Figure 4.9	Graph no: 09- Temperature in the fermentation tank	58
Figure 4.10	Graph no: 10- pH in the fermentation tank	59

LIST OF TABLES

Tables		Page no
Table 2.1	Beer manufacture in liters (million)	05
Table 2.2	Commonly used adjuncts in brewery	12
Table 2.3	The composition of hops	15
Table 3.1	Existing Lion Larger recipe	33
Table 3.2	Quantity of rice usage and its percentage	34
Table 3.3	Reformulated recipes with selected ingredients	35
Table 4.1	Summary of the typical time at each stage of the brew house	52
Table 4.2	Plato variation with the wort volume at the brew process	53
Table 4.3	Sensory analysis of 35% rice used beer	60
Table 4.4	Sensory analysis of 40% rice used beer	60
Table 4.5	Triangular test results	61

CHAPTER 01

INTRODUCTION

1.1. Background

The Lion Brewery Ceylon Ltd (LBCL) is a company engaged in the process of manufacturing and marketing beer under five brands such as Lion Larger, Lion Stout, Strong Beer, Carlsberg Special Brew and Carlsberg. LBCL is the market leader who has about 85% share of beer market in Sri Lanka. It produces bottles (625ml, 325 ml) and kegs (30 L, 19L, and 15.5L) which it is distributing locally and internationally.

Brewing beer is almost as old as baking bread. The Sumerians produced an alcoholic beverage from fermented bread grain. The Assyrians are known to have brewed beer about 4000 B.C. (Uhlig, 1998). Beer is a fermented alcoholic beverage made from malted cereals, water, hops and yeast. Barley is the main raw material for beer production. Its use depends on the fact that barley has high starch content and the husk still adheres to the grain even after threshing and processing to malt. (Pollock, 1987) Adjuncts are an additional source of carbohydrate extract used to partially replace or supplement the malt. They are also used to reduce the overall material costs and to utilize indigenous sources. Raw barley, rice, raw wheat, maize grits, hydrolyzed starch syrup, semi-refined cane sugar, caramel, oats, sorghum and rye are commonly used adjuncts for brewing. (Journal of the institute of brewing)

Adjuncts are used to obtain fermentable sugars at a lower cost; they also play a very important and specific role in giving a characteristic quality to the beer to attract consumers. All malted beer is very rich in flavor has dark color. On the other hand the beer with adjuncts has a light color and taste, so that consumers tend to drink more because of its less satiating taste. The beer with adjuncts also has higher colloidal stability due to the lower nitrogen content. (Yoshizawa and Kishi, 1988) Rice is widely used as an adjunct in the world for the production of alcoholic beverages such as beer. (Yoshizawa and Kishi, 1988) The ratio of malt to adjunct must fall within a permissible range if a legal regulation on it is in effect. Each brewer according to the quality specifications for the final beer and the cost estimation sets adjunct ratios. (Haywood, 1996)

Rice is usually used for brewing in the form of rice grits or broken rice which is obtained as a by product of rice harvesting and the processing of edible rice. The rice used for beer brewing is an almost pure fragment of endosperm that contains starch exclusively. (Bhattacharya, 1995) Rice is considered as a mash tun adjunct. It is normally be solid based and may need pre-cooking if the temperature of gelatinization is greater than the malt conversion temperature in the mash tun. The gelatinization temperature of rice is 61°C-78°C. The central process of brewing is mashing where the ground malt and the solid adjuncts (grist) are mixed with water at a set temperature and with a set volume of liquor (water) to produce a hydrated mash. (Broderick, 1988)

The main objective of mashing is allowing the conversion of starch from malt and solid adjuncts into fermentable and unfermentable sugars to produce wort of the desired composition. In mashing a small quantity of crushed malt with hot water is sending to the cereal cooker. The quantity of malt used for this purpose is dependent upon the enzymatic power of the malt and the nature of the adjuncts. (Broderick, 1988)

Alpha amylase and Beta amylase are the main two enzymes involved in brewing. Alpha amylase is more thermo stable than Beta amylase. Alpha amylase cleaves starch randomly except near ends of branch points while Beta amylase cuts off maltose from non-reducing ends of molecules. Alpha amylase mainly produces dextrin molecules and Beta amylase produces Beta maltose which is the major sugar in wort. (Preece and Oliver, 1954)

1.2. Overall objective:

- To increase the rice usage in Lion Larger without changing the quality.

1.3. Specific objectives:

- Reformulate the existing formulas of Lion Larger in such a way to increase the rice usage.
- To study and record practical issues met at the brewing process when using high percentage of rice.
- To find out the optimum rice usage of beer with keeping the quality.
- To study the chemical composition of beer at each stage of the process (cold wort, beer fermentation, beer treatment, bright beer handling, unpasteurized beer, bottled beer) and compare it with different beer samples.
- To evaluate the organoleptic properties through sensory evaluation of the final product.

CHAPTER 02

LITERATURE REVIEW

2.1 Beer: An Overview

Manufacture of all kind of alcoholic beverages utilizes the ability of yeast to ferment sugars in to alcohol. Beer is a special kind of beverage where it is famous all over the world under the category of low alcoholic beverage. The recognized definition of beer would is an alcoholic beverage produced by the fermentation of sugars derived from malted barley and flavored with hops. (Broderick, 1988)

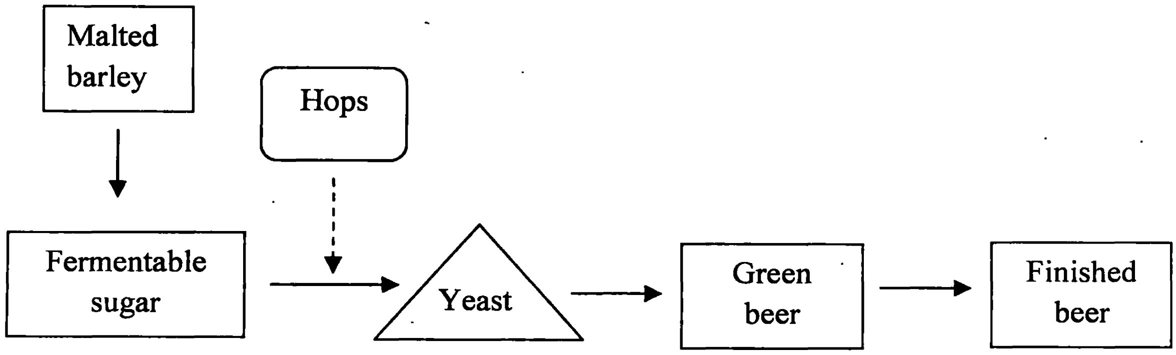


Fig 2.1 Basic flow chart of beer production with the ingredients. (Broderick, 1988)

2.1.1 History of beer

When concerning the history of brewing we have to move to 3000 BC where they have used wild yeast for the fermenting process in Egypt. In the period of 12 AD in Europe they have started the process in a commercial manner by introducing malt and hops. Lager was first produced in Germany and Ales /stouts produced in UK. From all those initiations, brewing of beer spread all over the world and now highly advanced fully automated breweries are under operation. (LBCL-Training Kit)

2.1.2 Different types of beer

All over the world, different areas developed their own types of beer. The variations among the types have come about through a combination of raw materials for its manufacture and the tastes of the consumers.

2.1.2.1 Lager

Lager is by far the biggest proportion of beer sold. Its delicate flavor comes from:-

- The use of a malt that is relatively under modified and lightly kilned.
- A relatively low bitterness.
- The use of a bottom fermenting yeast.
- Cold maturation.

2.1.2.2 Ales

Ales are produced mainly in the United Kingdom and the Republic of Ireland. Their flavors come from:-

- The use of well modified and biscuit flavored malt which is sometimes highly colored.
- The use of top fermenting yeast.

Ales come in various forms, bitter pale ales and mild beers.

2.1.2.3 Wheat beers

Wheat beers are lagers produced from the use of malted wheat instead of malted barley.

2.1.2.4 Stouts

Stouts are very dark in color and richly flavored from the use of highly colored malts or roasted barley.

2.1.2.5 Low alcohol / alcohol-free beers

Low alcohol / alcohol-free beers are produced by several different processes and their definition varies in different countries. Usually alcohol-free means less than 0.05% (vol/vol) alcohol and low alcohol means less than 0.5% (vol/vol) alcohol (less than 1.2% in UK).

2.1.2.6 Low-carbohydrate beers

Low-carbohydrate beers are brewed by producing wort that is more fermentable than in "standard beers" by several techniques, but usually by adding additional enzymes to convert more of the non-fermentable sugars into fermentable sugars. (Goldammer, 2006)

2.1.3 Beer styles

Although beers are brewed from similar materials, beers throughout the world have distinctive styles. Their uniqueness comes from the mineral content of the water used, the types of ingredient employed, and the difference in brewing methods. In a strict sense, there are two classical beer styles, ales and lagers. However, in addition to ales and lagers, there are other classical beer styles such as wheat beers, porters, stouts, and lambics. (Broderick, 1988)

2.1.4 The health benefits of beer

Many of the health benefits of moderate beer drinking are unique to beer because of its ingredients such as hops and malted barley. Of all the alcoholic drinks on the market beer is also relatively low in alcoholic strength. Drinks such as beer have other nutrients and properties that can also be beneficial in terms of health. Another important aspect to the role of moderate drinking and health is that the benefits relate to many of the prime public health issues of today such as heart disease, osteoporosis, strokes, diabetes, cancer and Alzheimer's disease. (Pauls and White, 1988)

- Reduces the risk of heart disease.
- Helps keep blood pressure down and reduce the risk of stroke.
- Benefits the immune system meaning healthy adults are less prone to get infections.
- Has an anti-inflammatory effect which contributes to heart health.
- Could play a role in the battle against osteoporosis as it improves bone mineral density which contributes to healthy bones.
- Helps fight cancer because of compounds in hops called flavinoids.
- Decreases the risk of dementia due to its beneficial effect on preserving brain function in old age.
- Can protect against type II diabetes.

2.1.5 Beer Industry in Sri Lanka

The main argument, at least on paper, for lowering the taxes on beer and making it more accessible was the belief that the beer lobby has promoted the belief that it would resolve the increased production and consumption of unlicensed liquor. The phenomenal increase in beer production and consumption during the decade 1989 - 1999 is illustrated in the statistical table 2.1

Table 2.1 -Beer Manufacture in Liters (Millions)

	1989	1996	1997	1998	1999
Ceylon Brewery Ltd		19.1	22	19.2	10.5
McCallum Brewery Ltd		3.5	4.6	4.1	3.3
United Brewery Ltd				4.8	5.3
Lion Brewery Ltd				11.6	22.8
Total	7.8	22.6	26.6	39.7	41.9

(Source: BOI Annual Industrial Review, 2000)

This increase is an outcome of a series of policy changes by the government that were favorable to and in support of the beer industry on the one hand, and the intense marketing programme of the industry on the other. In 1995, the excise duty on all beers was reduced by 70 per cent creating a new situation of demand exceeding supply. Two BOI projects approved by the government came into production in 1998.

The Lion Brewery Ceylon Ltd., which is a BOI project, has become within two years of operation the market leader. The major shares of Lion Brewery are held by the Ceylon Brewery Ltd, which is the oldest brewery in the country and Carlsberg Ltd of Denmark. The layout of the new plant has been designed for a six-fold increase in production from 300,000 hectoliters (i.e. 30 million liters) to 1.8 million hectoliters (i.e. 180 million liters) to cater for an expanding market. (BOI Annual Industrial Review, 2000)

2.1.6 The Lion Brewery Ceylon Ltd.

This important oversight was however, rectified when Sir Samuel Backer set up Sri Lanka's first brewery, the Ceylon Brewery at the foothills of one of Nuwara Eliya's famous waterfalls, the Lovers Leap. At the time of establishing the brewery Backer would never have envisioned the extent to which his pioneering venture would expand. The company grew with time, its product appealing to both Scottish as well as native palates. Today Ceylon Brewery's most well known brand is Lion, along with Carlsberg. To cope with its ever expanding local market as well as its export markets a second brewery, Lion Brewery, was established in 1996. Carlsberg A/S joined hands with the new brewery took up a 25% in LBCL. The new brewery with its state-of-the-art production facility is poised to take this century old brewing company well into the future. (LBCL Annual report-2006)

Quality and excellence are two important adjectives used to describe brewing process in LBCL. The selection of equipment is also essential for the production of high quality beers and at Lion Brewery they use the latest in brewing and bottling equipment from Germany's leading suppliers, Steinecker and Krones. The Lion Brewery, rated as one South Asia's best, is a fully automated production facility where the entire brewing process takes place untouched by human hand. Further LBCL has occupied ISO 22000 Quality and Food Safety Management System.

The Lion Brewery currently has an annual capacity of 300,000 hl and the potential to expand to 1,800,000 hl. Currently they brew in the factory all their Lion brands namely Lager, Stout, Strong and international brand Carlsberg Larger & Special Brew. Today, the Lion Brewery Ceylon Ltd has occupied an unchallenged position as market leader in Sri Lanka penetrating markets even in Australia, UK, USA, France, Japan, Maldives, UAE, Sweden, Italy, Switzerland and Germany. (BOI Annual Industrial Review, 2000)

2.1.7 Market overview of LBCL Beer.

In Sri Lanka the alcohol industry can be categorized in to mainly two groups such as recommended alcoholic products and illicit. High alcoholic products (Spirits), low alcoholic products (Beer) are main two recommended beverage category while the other group is illicit products (Kasippu, Cider) which has 95% market share in Sri Lanka and recommended group has only 5% market share. In the alcoholic beverage market, beer has 5% market share and spirits has 95% market share. That situation is completely different in European countries. They have normally 95% beer market and 5% spirit market.

In Sri Lanka, there are three main beer producers such as Lion brewery Ceylon Ltd, Asia Pacific Brewery (APB), Macculum Brewery ltd (MBL). Out of them about 85% market shares has LBCL, while 10% to APB and 5% to MBL. Out of LBCL brands Lion Larger (LL) has 36% market share while Lion Stout (LS) has 24%, Strong Beer (SB) has 16%, Carlsberg (CB) has 17% and Carlsberg Special Brew (CBSB) has 7%.

Beer also can be categorized in to mainly two groups such as below 5% alcohol level and above 5% alcohol level. High taxation has to be paid for above 5% alcoholic beer such as CBSB, SB, and LS and low taxation has to be paid for below 5% alcoholic beer such as CB and LL. About Rs. 55/= should be paid for above 5% alcolic level beer and Rs. 35/= should be paid to below 5% alcolic levels to the government. When designing the market LBCL people not considering the elder group. Further they consider women as the secondary market for beer. Following are some attributes of target customers.

Lion Larger –Males, age 21-44, all socio groups, All Island,

Moderate Income level, Fun loving, friendly,

Personality-proud, confident and honest.

Strong Beer - Males of 21-34 years of age,

Moderate Income,

Low level socio groups,

Well strong, nice looking, hard working

Lion Stout- 25-45 of age, moderate income,
Urban & rural, hard working,
Physically good, mentally strong,
Conscious about health.

Carlsberg- 25-35 of age, decent, enjoying life, high income level,
Experience enables to make decisions to what is right for them.

CBSB- 25-35, well educated, independent,
Lot of experience in life, high income level.

LBCL exporting their products to 14 countries such as USA, European Union, Maldives, etc. They produce bottles of 625ml and 325ml while kegs of 30L, 19L and 15.5L.325ml pints are introduced those who need alcohol or soft drink while eating or instead of soft drink. Beer market is strongly price sensitive and people drink that to get maximum kick. CBSB and CB mostly taste by foreigners, so the quality of it should be maintained properly since it is an international brand. Climate is not a considerable factor for the beer market.

2.2 Beer Processing

2.2.1 Raw materials for brewing and its characteristics

Raw materials for brewing are as follows and the quality of beer depends on the quality and quantity of raw material as well as the process specifications.

- Malt
- Adjuncts
- Hops
- Brewing water
- Yeast
- Brewing salts

2.2.1.1 Malt

Malt is the major source for brewing; where, barley is the core source of malt in case of brewing. When the barley grain is subjected to the process of malting, it's called as Malt. Malting process also consists of several steps and it is not practiced in Sri Lanka currently, so we are importing the malt from several countries such as Denmark & China under great assurance of quality.

2.2.1.2 Barley

Malted barley is the main raw material used in the brewing of beer in every brewery. Malt provides the sugar that will be fermented into alcohol in the brewing process by yeast cells. Barley is a cereal traditionally grown in mild maritime climates and for centuries it has been used in the production of beer. Malt contains carbohydrates in the form of starch which is the source of food for the growing plant when the seed is germinating. Usually that starch is locked away until it is needed.

There are two major types of malting barley currently in use, six-row and two-row with respect to the row of kernels in the barley head. In general two-row barley is more plump with a tighter, thinner hull than six-row and it produces malt with higher extract, paler color and lower enzyme content than six-row. About 20-25% of the total malt used for the brewing purpose is two-row barley, but nowadays new barley varieties has been introduced with more disease resistant, more plump, higher extract and enzyme content and easier to malt, are widely used to facilitate the brewing. (Pollock, 1987)

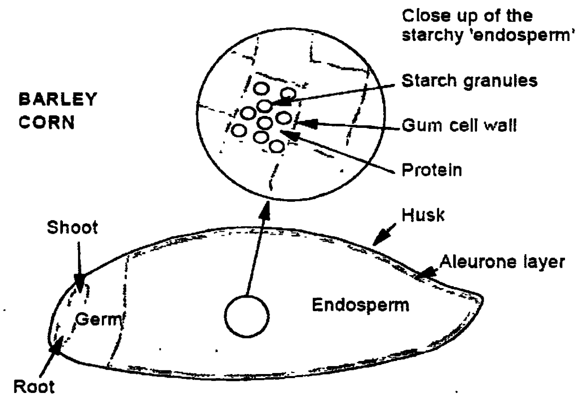


Fig 2.2 - Starch granules of Barley grain prior to germination

The diagram illustrates the key features of the barley corn. It shows the location of the starch granules which are the main carbohydrate food reserves. Starch is present as granules which are embedded in a protein matrix. This matrix is surrounded by cell walls containing a gum, β-glucan. The starch granules are therefore inaccessible and protected from attack by the amylase enzymes that are produced during germination. (Pollock, 1987) During the malting process, the cell walls and the protein will be dissolved by the enzymes produced naturally as the seed get germinated.

(a) Malting Process of Barley

There are three stages in the process of converting barley into malt:-

(i) Steeping

Barley is soaked in water to simulate the conditions that start germination or growth. This is done in a steep tank and usually the tank is aerated to encourage fast moisture uptake by the barley to increase the number of corns germinating and the rate of germination.

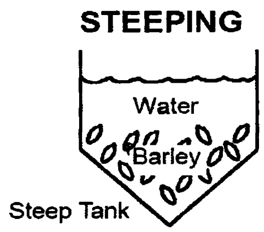


Fig 2.3 Steeping process of barley in steeping tanks

(ii) Germination

Soon after the completion of steeping, the barley seed is allowed to grow.

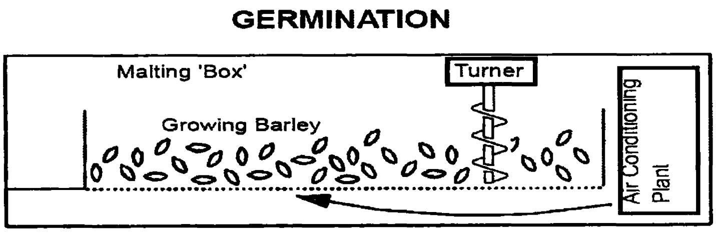


Fig 2.4 Germination process of barley in malting box

During germination two major changes occur, firstly, hormones stimulate the production of enzymes in the aleurone layer. Secondly, these enzymes start to act. So the enzymes will break down the gummy cell walls and break down the protein matrix. This breakdown releases the starch granules making them accessible for conversion into sugar by amylase enzymes. (Pollock, 1987)

(iii) Kilning

During this stage of the malting process, water is removed from the green malt by drying. The malt then becomes stable and can be stored without deterioration. Here malt is also slightly roasted to give it colour and flavour.

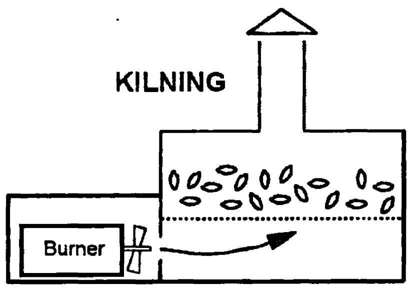


Fig 2.5 Kilning process of malt in kilning chamber

The combination of high grain moisture and high temperature would normally destroy the enzymes developed during germination. So that the malt is dried at a relatively low temperature using high flows of air. When the malt is dried with a moisture content of around 10%, then the kilning temperature is increased so that the malt can develop colour and flavour. At the completion of kilning, the malt's moisture content will be 4-5%.
(Pollock, 1987)

(b) Coloured malt

Coloured malts are used to increase beer colour, to modify flavour and because of their nature they produce a more stable beer. These types of malts are produced by small modifications in malting process.

- Carapils and Munich malt: Similar to crystal malt but they have a lower colour and a more delicate flavour from using under-modified malt followed by less kilning. These are used to colour and flavour lager.

- Black malt and Chocolate malts: Produced by roasting finished malt in a drum. Both malts have a very high colour and a dry bitter flavour. They are used in stouts to give a very dark and highly flavoured beer. (Pauls and White,1988)

2.2.1.3 Adjuncts

"Any carbohydrate source other than malted barley which contributes sugars to the wort,"
Adjuncts could be solid or liquid alternative source of brewers' extract.
Eg. rice, maize, raw barley, wheat, sugar syrups
Often a cheaper source of extract, contribute little to flavor and appearance and sometimes added for convenience, but contain no active enzymes

Table 2.2: Commonly used adjuncts in brewery, its quality benefits and quality hazards.

Adjuncts	Quality benefits	Quality hazards
Wheat	Head Retention	Poor wort filtration
Barley	Shelf life	Poor beer filtration
Maize / Rice	Shelf life, Flavour life	Rancid, oily flavours

(Source: Barros *et al.,* 2001)

Although adjuncts are used mainly because they provide extract at a lower cost (a cheaper form of carbohydrate) than is available from malted barley and because they are readily available, other definite advantage are also achieved. (Yoshizawa and Kishi, 1988)

Adjunct use results in beers with enhanced physical stability, superior chill-proof qualities, and greater brilliancy. The greater physical stability has to do with the fact that adjuncts contribute very little pertinacious in terms of colloidal stability. Rice and corn adjuncts contribute little or no soluble protein. Except for barley; adjuncts also contribute little or no polyphenolic substance. Adjuncts can be used to adjust fermentibility of wort. Many brewers add sugar and/or syrup directly to the kettle as an effective way of adjusting fermentability, rather than trying to alter mash rest times and temperatures.

Adjuncts are often used for their flavor contribution. For example, rice has a very neutral aroma and taste, while corn tends to impart a fuller flavor to beer. Wheat tends to impart dryness to beer. Semi refined sugars add flavor to ales that has been described as imparting a luscious character. Most of the brewer's adjuncts are based on a limited range of cereal grains. The non malt brewing materials used in greatest quantity today are those derived from corn and rice, although barley, wheat, and sorghum grain are sometimes used. (Yoshizawa and Kishi, 1988)

2.2.1.4. Rice Cereals

(a) History of rice

Rice is believed to have originated in Asia, as well as cultivation beginning as early as 4000 B.C. Ancient records show that the Greeks were introduced to the crop when Alexander the

Great invaded India around 326 B.C. From there, it spread across Europe for the next 2000 years. It was then brought to the new world by European settlers in the late 17th century. Rice became an important crop product in several countries with the development of irrigation methods. Now, rice production is practically worldwide. (Yoshizawa and Kishi 1988)

(b) World rice production

Rice is one of the world's most important food crops. People in the east use about a half to two-thirds of a pound of rice daily. Asia is the leader in rice production. It produces 90% of the world's rice supply, with the US only producing 1%. Asia produces over 485 million tons of rice, but only exports around 10 million. This is because 75% of the world supply is consumed by people in the Asian countries. The US produces about 9 million tons of rice each year and exports 2.6 million tons. This is roughly 30% of the US's production, partly because in the US, rice is served as a side dish. (Broderick, 1988)

(c) Rice in Brewing

Rice is currently the second most widely used adjunct in the world, in the production of light-colored larger beers. Rice has almost no taste of its own, which is regarded as a positive characteristic since the rice will not interfere with the basic malt character of the beer. It promotes dry, crisp, and snappy flavors and is employed in several premium brands. Some brewers prefer rice because it has lower oil content than corn grits. One disadvantage in using rice is the need to use an additional cooking vessel because its gelatinization temperature is too high for adequate starch breakdown during normal mashing. (Broderick, 1988)

Cereal adjuncts are classified according to the way they used in brewing, i.e., by whether they are added to the cooker, the mash tun or the brew kettle.

1) Cooker adjuncts
2) Mash tun adjuncts
3) Brew kettle adjuncts

- **Cooker Adjuncts**

The cooker mash adjuncts consist of nongelatinised cereal products (meal, grits, flour, or dry starch) whose starches are in their native forms. A non gelatinized adjunct needs to be heated in a separate cereal cooker to complete liquefaction since the starch gelatinization temperature of the adjunct needs to be heated in a separate cereal cooker to complete liquefaction since the starch gelatinization temperature of the adjunct is higher than that used for the malt saccharofication (starch hydrolysis) temperature. The cooked adjunct is then added directly to the mash in either the mash tun or mash conversion vessel, once it has completed its photolytic stand. The malt enzymes from the standard mash can be used to hydrolyze the starch from the adjunct, converting it to sugars ready for fermentation. (Broderick, 1988)

- **Mash tun adjuncts**

The adjunct can be mashed directly with the malt in either the mash tun or mash conversion vessel when the starch gelatinization temperature of the adjunct is lower than the malt saccharofication temperature required for mashing or the adjunct has been pregelatinised. Whole grain wheat and barley can be mashed this way after being milled. (Broderick, 1988)

- **Brew kettle adjuncts**

Kettle adjuncts consist of cereal grain syrups and sucrose sugar. The British and the Australians commonly use syrups and sugars, whereas the rest of the world uses cereal adjuncts such as corn, rice and wheat. (Broderick, 1988)

2.2.1.5 Hops

The Hop (*Humulus lupulus L.*) perennial, dioecious climbing plant of the hemp (Cannabis) family and belongs to the order (Urticales) which also includes the nettle family. In the brewery it is the inflorescences of the female plant which are used. These contain bitter resins and ethereal oils which supply bittering and aroma components to beer.

So far brewing is concerned; hops are the dried hop cones of the female hop plants and products made from them which contain only components from hops. Hops are grown in special growing regions where the necessary growth conditions exist. After the harvesting of the hops, they are dried and processed to avoid a reduction in their values. (Preece and Oliver, 1954)

(a) Composition and properties of the components

The composition of the hop is extremely important for the quality of the beer produced from them. His applies especially to pilsner type beers because the hop aroma makes an essential contribution to the beer character.

Table 2.3: The composition of hops

Compounds	%
Bitter substances	18.5
Hop oil	0.5
Polyphenols	3.5
Protein	20.0
Inorganic	8.0

(Source: Oliver, 2001)

The rest consists of cellulose and other materials without importance in beer production. Hops play a special role during beer production as they are responsible for the special bitter flavour and the floral aroma associated with hop plant of beer and also act as a beer preservative. The hop is a plant that develops bitter resins called α-acids and oils within glands around the base of the petals in the cone. These resins are dissolved when the hops are added to and boiled in the process. They remain as iso-humulone which is a strong flavour component of the finished beer. Hops are produced in large scale farms where it is more progressively grow under warm climates. Hops are of two types as aroma hops and bitter hops under two physical properties as pallets or extract. (Preece and Oliver, 1954)

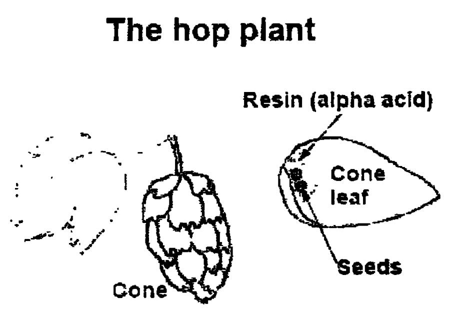


Fig 2.6 Structure of hop plant cone and indication of alpha acid in cone.

2.2.1.6 Brewing water

The mineral content of brewing water has long been recognized as making an important contribution to the flavor of beer. This is especially important since water composes more than 90% of the beer. A wide range of brewing water is employed, giving rise to many classic styles of beers, that over the centauries have become world famous. For example, the famous brewing waters from the deep wells. (Broderick, 1988)

2.2.1.7 Principal ions and minor ions

(a) Major ions

The Calcium ion is by far the most influential mineral in the brewing process. Calcium reacts with phosphates, forming precipitates that involve the release of hydrogen ions and in turn lowering the pH of the mash. This lowering of the pH is critical in that it provides an environment for alpha-amylase, beta-amylase, and proteolytic enzymes. Magnesium is most important for its benefit to yeast metabolism during fermentation. Magnesium Carbonate reportedly gives a more astringent bitterness than does Calcium Carbonate. (Broderick, 1988)

Sodium has no chemical effect; it contributes to the perceived flavor of beer by enhancing its sweetness. Like Sodium, Potassium can create a "salty" flavor effect. It is required for yeast growth and inhibits certain mash enzymes at high concentrations. Sulfates positively affect protein and starch degradation, which favors mash filtration and trub sedimentation. Calcium and Magnesium Chlorides give body, palate fullness, and soft-sweet flavor to beer. The presence of Carbonate ions and their effect in rising pH can results less fermentatable worts (a higher dextrin/maltose ratio), unacceptable wort color values, difficulties in wort filtration, and less efficient separation of protein and protein-tannin elements during the hot and cold breaks. Nitrate, in and of itself, is not a problem; it has no effect on beer flavor or brewing reactions. (Broderick, 1988)

(b) Minor ions

Iron in large amounts can give a metallic taste to beer. Iron salts have a negative action at concentrations above 0.2 ppm during wort production, preventing complete saccharofication, resulting in hazy worts, and hampering yeast activity. Zinc plays an important for proper enzyme action and has a positive action on protein synthesis and yeast growth. Manganese is important for proper enzyme action and has a positive action on protein solubilization. It can inhibit yeast growth and negatively affects colloidal stability; and in appreciable amounts, it can impart an unpleasant taste. (Haywood, 1996)

Salts dissolved in the water affect the beer's flavour, they influence the pH (acidity/alkalinity) of the process and the final product and they provide essential trace elements for yeast growth such as Calcium, Zinc etc.Water sources of Lion Brewery are tube well and normal well located in vicinity of the factory. (Haywood, 1996)

2.2.1.8 Brewer's yeast

Yeast is single –celled microorganism that reproduces by budding. They are biologically classified as fungi and are responsible for converting fermentable sugars into alcohol and other byproducts. There are literally hundreds of varieties and strains of yeast. In the past, there were two types of beer yeast: ale yeast (the "top fermenting" type, *Saccharomyces cervisiae*) and larger yeast (the "bottom-fermenting" type, *Saccharomyces uvarum,* formerly known as *Saccharomyces carlsbergensis*). (Pollock, 1987)

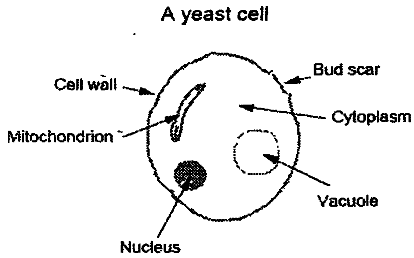


Fig 2.7 Structure of yeast cell under microscope

There are numerous strains of yeast used in brewing, many having characteristics that create unique flavors during fermentation. Yeast has nutritional requirements other than sugar which are listed below:

- Protein or nitrogenous compounds in the form of amino acids. These are created from the barley protein during malting and mashing.
- Lipids or fatty material. This is also supplied by the malt.
- Vitamins from the malt.
- Trace metals. Calcium is usually present in the brewing water, if not it must be added as brewing salts. Zinc may be present in hop products, if not it can be added to the wort.
- Oxygen is usually dosed into the wort. Oxygen is essential for healthy yeast growth and a large yeast population is required to ensure that the fermentation is healthy and fast.

(a) Top fermentation yeast

Saccharomyces cerevisiae - It floats to the top of the vessel at the end of fermentation because the Carbon dioxide bubbles stick to the yeast's cell walls. It thrives on relatively high fermentation temperatures, for example 20°C and consequently fermentations are fast, for example 3 days. They produce typical Ale flavour by fermentation. The way of cropping the yeast at the end of fermentation, that is skimming the yeast off the top of the beer, naturally selects the best yeast for re-pitching. (LBCL Training kit)

(b) Bottom fermentation yeast

Saccharomyces cerevisiae- Lager is the bottom fermenting yeast which is named as *S. carlsbergensis* or *S. uvarum*. It sinks to the base of the vessel at the end of fermentation because it has a different kind of cell wall. It likes low fermentation temperatures, for example 10°C and fermentations are slower, for example 7 days. They produce typical lager flavour by fermentation. The way of cropping the yeast at the end of fermentation, that is collecting from the base of the vessel is not selective and usually a pure culturing system is in use to maintain yeast purity.

LBCL uses bottom fermenting two different strains of *Saccharomyces cerevisiae* yeast cultures in two different forms for fermentation purpose. For Carlsberg brands, Carlsberg yeast culture in the form of pure culture and for Lion brands, lager yeast, it is in the form of freeze dried yeast. (LBCL Training kit)

2.2.1.9 Brewing salts

Calcium, Chloride, Sulphate are the main salts added during processing and which are added in different stages.

2.3 Brewing of beer

Basically four major steps are involving for beer manufacturing.

- Preparation of sugar solution/wort
- Fermentation
- Conditioning of beer
- Beer packaging

2.3.1 Preparation of sugar solution/wort

2.3.1.1 Malt Milling

The object of milling is to split the husk, preferably lengthwise, in order to expose the starchy endosperm for milling and allow for efficient extraction and subsequent filtration of the wort. However, it is necessary to compromise between the requirements for extraction and filtration because although a fine grind potentially yields more extract, it can lead to subsequent filtration problems and a loss of extract in the spent grains. In addition, excessive fine milling may lead to increased wort viscosity due to enhanced beta-glucan extraction. Furthermore, it may cause "balling" of the grist, which is the formation of clumps of malt that are wet on the exterior but dry inside. This will result in a loss of extract recovery since the grist is not converted during the mashing process. (Broderick, 1988)

Malt milling is usually done by either dry or wet milling. The selection of the type of milling and employment of a brewery-specific milling process is determined by the size distribution of malt kernels, their modification, moisture content, the mashing methods, and the wort separation method. For example, the degree of modification must be considered when milling. Poorly modified malts require fine grinding and well-modified malts can be more coarsely ground. Quality and uniformity of the crush is more important for an infusion mash than for a multi-temperature mashing program. Measurement of the particle size distribution of a grind is done by using a standard brewery sieve analysis.

2.3.2 Mashing

2.3.2.1 Purpose of mashing

Most of the substances in the malt grist are insoluble. Only soluble substances can pass into beer. It is therefore necessary to convert the insoluble materials in the grist into soluble materials during mashing. All the substances which go into solution are referred as extract. Examples of soluble substances are sugars, dextrin, inorganic substances and certain proteins. Insoluble substances include starch, cellulose, and part of the high molecular weight protein and other compound which remains as spent grains at the end of the lautering process.

The aim of mashing is to form as much extract and as good an extract as possible. Most of the extract is produced during mashing by the action of enzymes which are then allowed to act at their optimum temperatures. (Goldammer, 2006)

2.3.2.2 Properties of enzymes in mashing

The most important property of enzymes is their action in breaking chemical bonds in their substrates. This activity depends on various factors such as temperature, pH. The activity of enzymes increases with increasing temperature and each enzyme reaches its maximum value at its own specific optimal temperature. At higher temperature a rapidly increasing inactivation occurs as a result of unfolding of the three dimensional structure of the enzyme (denaturation).The inactivation and destruction of enzymatic activity is greater the more the optimum temperature is exceeded. (Preece & Oliver., 1954)

The enzymatic degradation processes of importance for the brewer are

Starch degradation

Beta-glucan (gum material) degradation

Protein degradation

A range of other degradation processes.

2.3.2.3 Starch degradation

The most important component of beer is the alcohol formed during fermentation from sugars. It is therefore important to degrade the starch to a large extent to maltose. In addition intermediate products, dextrins; are produced which are not fermented. Starch degradation occurs in three stages the sequence of which is unchangeable, but it merges into one another.

- Gelatinization
- Liquefaction
- Saccharofication.

(a) Gelatinization

In hot aqueous solution a large amount of water is incorporated into the starch molecules. This makes an increase in volume which causes the closely packed starch granules to swell and finally to burst. A viscous (sticky) solution is formed. The degree of viscosity depends on the extent of water uptake and is different for different types of cereal. For instance rice starch swells much more than malt starch. This process, during which no chemical degradation occurs, is called gelatinization. It plays an important role in everyday food preparation. (Pauls and White, 1988)

Because the gelatinized starch is no longer held together in the solid starch granules it can be directly attacked by the enzymes contained in the liquid mash. In contrast, degradation of ungelatinised starch takes several days. The gelatinization temperature is different for each type of cereals. Malt and barley starch gelatinize in the presence of amylases at 60°C , rice starch gelatinizes at 80 °C to 85 °C. (Yoshizawa and Kishi, 1988)

(b) Liquefaction

The long chains composed of glucose in starch (amylose and amylopectin) are very rapidly broken open to form smaller chains by Alpha amylase. This causes a very rapid reduction of the viscosity of the gelatinized mash. Beta amylase can only slowly degrade the long chains from the non-reducing end, and so degradation by this enzyme alone would take days. By liquefaction is meant the reduction of viscosity of the gelatinized starch by A-amylase. (Yoshizawa and Kishi, 1988)

(c) Saccharification

A-amylase progressively breaks open the chains of amylopectin to form dextrin containing 7 to 12 glucose residues. B-amylase splits off two residues (= maltose) from the new non-reducing ends produced on these smaller chains. This process inevitably takes longer than chains by A-amylase. (Yoshizawa & Kishi, 1988)

Because of the different lengths of the chains other sugars, such as glucose and maltotriose, are produced as well as maltose. In all cases the breakdown stops 2 to 3 glucose residues away from the 1,6-bonds in amylopectin because neither A-amylase nor B-amylase can break these 1,6 bonds. These limit dextrins are always present in normal wort.

A-amylase breaks down the long starch chains to smaller dextrins.It acts optimally at 72 °C to 75 °C (162 °F -167°F) and is rapidly destroyed at 80°C(176 °F). The optimum pH is 5.6 to 5.8. B-amylase splits maltose off from the non-reducing ends of chains, but it also produces glucose and maltotriose. It acts optimally at 60 °C to 65 °C (162 °F -167°F) and is very sensitive to higher temperatures. It is rapidly inactivated even at 70 °C (158°F) and is very sensitive to higher temperatures. It is rapidly inactivated even at 70°C (158°F).The optimum pH is 5.4 to 5.5. Starch breakdown must be monitored because residues of undergraded starch and larger dextrins cause starch hazes in beer. (Yoshizawa & Kishi, 1988)

2.3.3 Wort Separation (Lautering)

At the end of the mashing process the mash consists of a watery mixture of dissolved and undissolved substances. The aqueous solution of the extract is called wort; the insoluble part is refered to as the spent grains. The spent grains consist essentially of the husk, the seedling and other materials which do not go into solution in mashing. (Haywood, 1996)

Only the wort is used for beer production and for this purpose it must be separated possible from the spent grains. This separation process is called lautering.During lautering as much as possible of the extract should be recovered. Lautering is a filtration process in which the spent grains play the role of the filter material. It occurs in two stages which, strictly limited, succeed one another. (Haywood, 1996)

2.3.3.1 First wort and second wort

The wort drainoff from the spent grains is called the first wort.When this wort has been drained from the spentgrains the latter still contain the extract than the beer to be recovered. Consequently the spent grains are sparged after the first wort has run off.Sparging gradually dilutes the wort. In order to obtain the desired wort concentration at the end of lautering, the first wort must contain 4 to 6% more extract than the beer to be produced. E.g. for 12% beer it must be contain 16 to 20%. (Haywood, 1996)

2.3.3.2 Sparging

The extract retained by the spent grains is washed out by hot water. This process is called sparging. The thinner wort running off is called second wort.Its extract content at first decreases rapidly and then more and more slowly since the last extract is wash out of the spent grains only with difficulty. (Broderick, 1988)

2.3.3.3 Water quality

The quality of the beer may be affected if the sparging water is too alkaline. A pH greater than 7.0 is unacceptable; preferably, the pH should be around 6.0, which leads to better coagulation of proteins, better drainage of the grains, and a higher extract yield. (Broderick, 1988)

2.3.3.4 Water quantity

The total volume of sparge water will vary with beer styles and mashing processes. For both mashing and sparging, most designs assume a grist-to-water ratio of from 2.5 to 3.5:1, with 3:1 as the average. (Broderick, 1988)

2.3.3.5 Water temperature

The temperature of the sparge water (75 °C –78°C) must be higher than that of the mash to help maintain mash temperature and increase runoff. However, if the sparge water is too hot (above 80°C), it will extract unwanted materials such as tannins, proteins, and unconverted starch. (Broderick, 1988)

2.3.3.6 Wort boiling

Following extraction of the carbohydrates, proteins, and yeast nutrients from the mash, the clear wort must be conditioned by boiling the wort in the kettle. The purpose of wort boiling is to stabilize the wort and extract the desirable components from the hops. (Goldammer, 2006)

2.3.3.7. Wort cooling

After boiling and clarification, the wort is cooled in preparation for the addition of yeast and subsequent fermentation. The principal changes that occur during wort cooling are as follow:

- Cooling the wort to yeast pitching temperature
- The formation and separation of cold break
- Oxygenation of the wort to support yeast growth.

2.3.4 Beer fermentation

Fermentation is the process by which fermentable carbohydrates are converted by yeast into alcohol, carbon dioxide, and numerous byproducts. The byproducts have a considerable effect on the taste, aroma, and other characteristic properties of the beer. Fermentation is dependent upon the composition of the wort, the yeast, and fermentation conditions. Wort composition, as discussed in previous chapters, influences fermentation by the presence and concentration of various nutrients, pH, and degree of aeration and temperature. These factors can affect the rate of fermentation, the extent of fermentation, the amount of yeast produced, and the quality of beer produced. (Boulton and Bennett, 2001)

Traditionally, there are two types of beer yeast, based on their physical behavior: ale yeast (the "top-fermenting" type, *Saccharomyces cerevisiae*) and lager yeast (the "bottom-fermenting" type, *Saccharomyces uvarum*). Top-fermenting yeast flocculate and rise to the top of the fermenting wort producing stable yeast head, while bottom-fermenting yeast flocculate and settle on the bottom of the fermenter. However, some modern ale strains are selected for use in cylindronconical fermenters because they are poor head formers, the yeast crop being harvested from the base of the fermenter. Furthermore, some ale strains work equally well in both traditional and cylindroconical fermenters, forming a yeast head in a traditional vessel and settling to the cone at the end of fermentation in the conical vessel. The factors that affect fermentation conditions are time, wort temperature, volume, fermenter design, pressure, agitation, and currents in the wort. (Boulton and Bennett, 2001)

2.3.4.1 Yeast preparation & handling

After complete fermentation partially inactivated yeast due to temperature reduction is collect to yeast storage vassals and yeast is stored during the period between cropping and repitching as slurry under low temperature. Yeast is discharged completely after several generations because of the possibility of deduction in viability & efficiency of yeast as well as the mutant generation possibility. (Dueby and Maheshwari, 2004)

2.3.5 Beer conditioning

Following primary fermentation, many undesirable flavors and aromas are present in the "green" or immature beer. Conditioning reduces the levels of these undesirable compounds to produce a more finished product. The component processes of conditioning are: maturation, clarification and chill proofing. (Haywood, 1996)

2.3.5.1 Beer filtration

(a) Colloidal stabilization

To achieve beer stability it is necessary to remove the protein, the polyphenol, or both from the beer. These non biological haze precursors can be removed during the cold conditioning or filtration steps. The most commonly used stabilizers for removing proteins are amorphous silica gel (Lucite), Poly Vinyl Poly Pirolidine or PVPP (Polycar) is typically use for removing polyphenols.The stabilization procedures which directly interact with filtration or similar processes are:

- Addition of PVPP and silica gel powder to beer in conjunction with filter aids.
- Addition of PVPP in bright beer after Kieselghur filtration, followed by separation in horizontal leaf filters and subsequent regeneration for reuse.
- Combined treatment with sterilization using PVPP-impregnated sterilizing filter sheets.

2.3.6 Beer carbonation

The next major process which takes place after filtration and prior to packaging is carbonation. Carbon dioxide not only contributes to perceived "fullness" or "body" and enhances foaming potential; it also acts as a flavor enhancer and plays an important role in extending the shelf life of the product.

The level of dissolved Carbon Dioxide in beer following primary fermentation varies as a result of a number of parameters such as temperature, pressure, yeast, type of fermentation vessel, and initial wort clarity. Typically, Carbon dioxide levels range from 1.2 to 1.7 volumes of carbon dioxide per volume of beer (V/v) for non pressurized fermentations. The time required to reach a desired Carbon dioxide concentration depends on a number of physical factors. Temperature and pressure play an important role in determining the equilibrium concentration of Carbon Dioxide in solution. (Haywood, 1996)

2.3.7 Bottling and packaging

Once the final quality of the beer has been achieved, it is ready for bottling. Bottles must be rinsed before the bottling procedure.

2.3.7.1 Bottle rinsing

There are three types of bottle rinsers – twist, gripper, and rotary. Most craft breweries use twist rinsers, which are designed to invert the bottles before spraying. After being rinsed, the bottles are allowed to drain before being swung back up into the upright position and delivered to the bottle filler. Each bottle size and shape requires a different "twister," but the cost of twist rinsers is quite low. Gripper style rinsers are a more compact alternative to twist rinsers and can handle various bottle sizes and shapes without parts having to be changed. (Goldammer, 2006)

2.3.7.1 Beer packaging

Washed and inspected bottles are filled with beer at minus temperature and crowned. Then pasteurized beer is cooled to normal temperature. Finally corded, labled bottles of finished beer are arranged in containers.

2.4 Technology

Fully automated computerized operation system is used which controlled by PLC.

2.5 Quality Assurance System of Lion Brewery

Lion brewery considers quality as number one priority. The purpose of quality management is to establish a level where the characteristics of the product and process ensures conformity to quality levels set by the brewery. Lion Brewery Quality Assurance System is built upon a framework of the following elements:

- Sampling plans: raw materials, brewing outputs, packaging materials etc.
- Analytical systems: for the analysis of brewing outputs and raw materials.
- Recording & reporting systems: facilitate traceability and continuous improvements

2.5.1 Quality Assurance Laboratory and Chemical Analysis

Expectation of consistent high quality product by the consumer is highly satisfied by the quality assurance procedures followed by the laboratory. Fully equipped analytical laboratory with advanced technical background and highly skilled quality assurance team facilitate this concept in LBCL. All the procedures followed here are now based on the ISO 22000 Quality and Food Safety Management System and the Guidance of Carlsberg, Denmark. ISO 9001-2000 was the Quality Management System until year 2005 and in year 2006, they have updated their system to the ISO 22000 to ensure the Food Safety along with the Quality Management. Quality inspection is carried out fewer than three categories.

- Incoming material inspection: Includes raw material inspections and packaging material inspections.
- In process inspection: Covers the inspection of various factors from wort to finished beer.
- Product inspection: Final product inspection prior to market entry.
  (LBCL training kit)

2.5.1.1 Incoming material inspections

In case of incoming material inspections, it includes incoming raw materials used for brewing purpose and packaging materials used to pack the finished beer to market.

2.5.1.2 Raw material inspection

All the raw materials used for brewing purpose undergo inspection in terms of quality such as malt, rice, brewing liquor, hops, brewing salts and some miscellaneous includes black malt, caramel, sodium hypo chloride and filter aids.

(a) Malt

Major brewing raw material malt is imported from Australia and China from the reputed suppliers of Carlsberg. These suppliers have been inspected by the Carlsberg team through out their cultivation period. Certified complete analysis report of malt will be provided each delivery. Pre shipment analysis also practiced here before the delivery. The parameters that are checked here to ensure the quality of malt delivered are

- Visual inspection (Hand evaluation)-Satisfactory
- Friability-85%
- Moisture content – 4.5%

If all these parameters satisfy the requirements based on the brewery's specifications, this malt will be used for the brewing purpose. (Carlsberg-Turbo manual)

(b) Rice

Major adjunct used here is rice in brands such as Lager, Strong and Carlsberg Special Brew. Supply is done by local reputed suppliers, who fulfill the requirements of brewery. The parameters checked during the delivery are

- Visual inspection: satisfactory
- Flavor: satisfactory
- Moisture: 13.6%(Carlsberg-Turbo manual)

(c) Brewing liquor (water)

Water sources are mainly 2 normal wells and 2 tube wells which located in-front of the factory premises. Complete raw water treatment is done prior to the usage for brewing. Seven samples of all water sources and treated water used for daily analysis of water quality. Following parameters are checked during the analysis

- Iron content: by spectroscopy method
- Chlorine content: by DPD No-3 tablets which indicate the presence of Cl_2
- pH

Treated water is directly used for brewing purpose, which should be free from Chlorine to avoid the bad odour in beer. Some other analysis in water like heavy metal analysis is conducted annual basis in external reputed institutions. All the other raw materials such as hops and other miscellaneous are received based on the supply quality certificate along with full analysis reports. (Carlsberg-Turbo manual)

(d) Packaging material inspection

Packaging materials used for the purpose of packaging of beer in LBCL also undergone the quality assurance test to ensure the best quality final product. Mainly glass bottles, labels, crown caps, crates and corrugated 6 pack boxes for export purpose. All the attributes with respect to the item should match with the standards available in laboratory for the acceptance of delivery. (Carlsberg-Turbo manual)

(e) In Process inspections

Major part of analysis which cover many aspects such as chemical, physical and microbiological. High attention is paid here because each and every step of analysis in any can affect the final quality of beer. This analysis starts from cooled wort to the finished beer and each points many factors are analyzed to optimize the quality.

2.5.2 Cooled wort

Taken from wort chiller and following parameters are checked

- Bitterness
- Colour
- Extract (% Plato)
- I_2 test for the confirmation of absence of starch.
- pH

2.5.3 VDK measurements

Sample is taken from fermentation tanks at close to the end of fermentation

2.5.4 Fermentation

Sample is taken when fermentation if completely finished

- Bitterness
- pH
- Alcohol (v/v)
- Colour
- Extract (% Plato)
- Yeast cell count
- Flavor

2.5.5 Treatment beer tank (TBT)

Sample is taken from treatment beer tank for analysis. All the parameters checked in fermentation vessels are checked here except bitterness. Other than that Dissolved Oxygen level is checked. (Carlsberg-Turbo manual)

2.5.6 Bright beer tank

Sample is taken from bright beer tank for analysis which is ready for bottling. All thee parameters checked in TBT are checked here, but CO_2 level and Haze will be checked additionally. (Carlsberg-Turbo manual)

2.5.7 Un-pasteurized beer

Sample will be taken from the beer filler while bottling. Few parameters are checked here such as alcohol, extract, CO_2 level and flavour. (Carlsberg-Turbo manual)

2.5.8 Product inspection

Sample is taken after pasteurization. Complete beer analysis is done to ensure the quality of finished product. Parameters checked here are

(i) Alcohol (v/v)
(ii) Bitterness
(iii) CO_2
(iv) Colour
(v) Haze- Using haze meter
(vi) Extract (% Plato)
(vii) pH
(viii) SO_2
(ix) VDK
(x) Head retention
(xi) Total and Dissolved O_2
(xii) Flavor score

Each and every parameter in each places have specifications. Once the samples satisfy the requirements only, the next step would be proceeding in the process. Non-Conforming Report (NCR) will be issued when the samples fail to meet its requirements in order to proceed next step. Action will be taken based on the quality management systems' manual by quality assurance members. Chemical analysis validation is done by Carlsberg Inter Laboratory Analysis Systems (CILAS) of Denmark.

2.6 Flavor Analysis and Tasting

In the sense used have "flavor" refers to the combine perception of taste (by the tongue) and of odour by the nose. Flavour testing may be conducted to establish which of two beer is preferred. (i.e.) "Performance testing", this is the information needed in producing a new beer of modifying the flavor of an existing one to suit the public and to record the verdict. (Roland *et al.*, 2000)

Flavor analysis is another important step of quality assurance which highly affects the image of the company. Well trained and validated tasting panel of brewery employees involved in this analysis. Tasting is done daily by the members of this panel in tasting laboratory. Own brands and competitor brands are tasted and analyzed for the improvement of the product consistency. (Roland *et al.*, 2000)

A common language has been used to describe beer flavours and brewers throughout the world. In this terminology, each of the recognizable flavours has been given a name and "the flavour wheel" is a pictorial summary of the main flavour characters. LBCL also following the same principle to identify the flavor. (LBCL training kit)

Spider diagram is a graphical form which used to check whether a beer is matches the standard flavour profile for that particular product or brand in LBCL. This diagram is drawn using that brand's typical flavours and intensities.

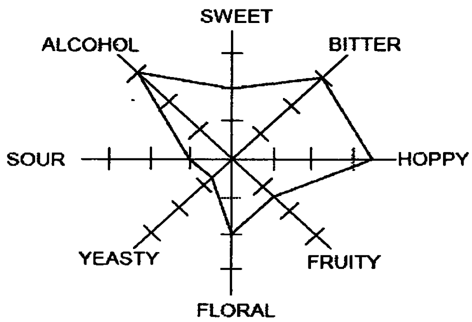


Fig 2.8 Spider diagram of a particular brand of beer

Tasting panel is trained with the extracted off flavor capsules of "Flavor active" institution and validated by the Carlsberg Inter Laboratory Analysis Systems (CILAS).

2.7 Microbial Analysis

Microbial analysis is also another important aspect which ensures the Food safety of LBCL products. Chance of contaminations of beer is very low because of its acidity and the in package pasteurization. Common contaminations that could occur in beer are

- Brewers' yeast contamination: cause haze problem
- Wild yeast contamination: cause haze and off flavour problems. Chance of this contamination is very low due to the fully closed plant for brewing.
- Lactic acid bacteria contamination: Such as ***Lactobacillus, Pediococcus,*** which can cause haze and off flavour problems.
- Wort bacteria: cause problems like off flavour and early fermentation.

All these microbial contaminations concerned while the analysis.

Yeast used for the pitching purpose from yeast holding tanks also checked for the contaminations. Haemo cytometer is used to obtain the cell counts and selective plating methods are used for the identification of contaminating micro organisms.

E.g.: For wort bacteria Universal Beer Agar (UBA) is used for plating purpose. (Dueby & Maheshwari., 2004)

CHAPTER 03

MATERIALS AND METHODOLOGY

3.1. Materials and Equipments

3.1.1. Materials

Double polished raw rice
Barley malt
Water
Calcium Sulphate
Calcium Chloride
Bitter hops
Aroma hops
Amylase enzyme
Phosphoric acid
Caramel
Sensory evaluation ballot papers

3.1.2. Equipments

Wet mill
Dry mill
Mash tun
Cereal cooker
Lauter tun
Wort kettle
Whirl pool
Fermentation cellers
Centrifugater
Buffer tanks
1L beakers

3.2 Methodology

The basic flowchart of Lion larger Processing is as follows.

Brew House Process

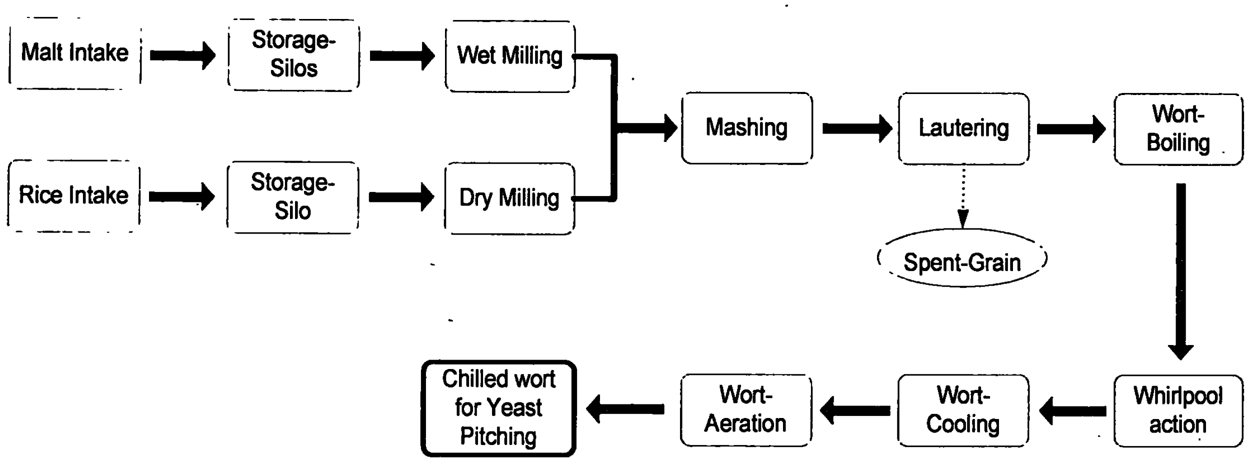


Fig 3.1 Basic flow chart of beer production with the ingredients.

In order to determine the effect of changing critical ingredients on the beer functionality, Lion Larger (LL) was manufactured in following approach at the LBCL Pvt. Ltd, Biyagama.

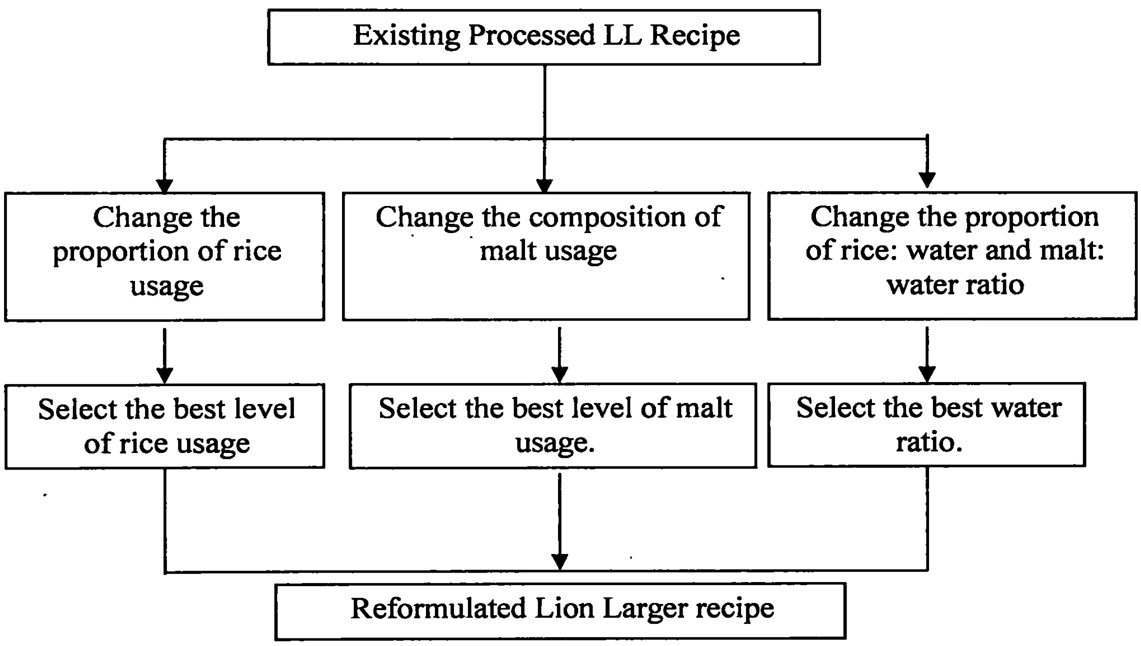


Fig 3.2 Outline of reformulation scheme of existing recipe of Lion Larger

3.2.1. Find out the optimum usage of rice for Lion Larger

Different amounts of double polished raw rice were taken out as an adjunct and precleaned before preparation of the mash. The mash was prepared by keeping the rice: water ratio at 1:3. The rice solution was heated up to 90°C and kept for 15 minutes for gelatinization. Then it was boiled up to 100°C while the rice was being heated small amount of thermally active A- amylase was added for the reaction of liquefaction. The pH was maintained at 5.6 to 5.8.

Malt was weighed and heated up to 45 °C -50°C for protein rest. After 20 minutes of rest rice was transferred to the malt solution. Then the mixture was kept at about 65°C for the saccharification. Iodine test was done to identify full starch conversion. Different amount of rice and malt were weighted and mixed in varying proportions to find out optimum rice usage for Saccharification. This is illustrated in Fig 3.2. Since the Iodine test was positive, the wort was processed to a beer as a commercial trial at the LBCL process flow which was fully automated. Following figures describe the facts of Brew House process in the LBCL beer factory flow.

Table 3.1 Existing Lion Larger recipe

Ingredients	Quantity
Barley malt	4200 kg
Rice	1800 kg
Brewing salt	6 kg
Hops	10 kg
Phosphoric acid	4 liters
Caramel	10 kg
Termamyl	0.5 kg
water added	180 hl

** Total grist to water ratio is 1:3

3.2.2. Selection of the optimum rice usage for beer in keeping quality aspects

Three different larger samples were prepared by changing the rice, malt and water amount of the original recipe. The amount of extract was calculated according to the IBD (Institute of Brewing & Distillation) method amount of each incorporated in the recipes are shown in table 3.1.

Table 3.2 Quantity of rice usage and its percentage

Sample Code	Rice quantity	Percentage of rice usage
001	1800	30%
002	2060	35%
002	2360	40%

3.2.3 Calculation of Rice and Malt amount for Lion Larger

The rice amount and the malt amount were calculated in the following way. The extraction of rice and malt was considered for the calculation of the recipe. The extraction percentage was taken according to the supplier quality record. Here the capacity of the mash tun was considered before the calculation. The capacity was 6000 kg, thus the recipe was formulated to tally with the total capacity of the mash tun at LBCL.

Extract % of Malt - 78%
Extract % of Rice - 80%

For Current Recipe

Current extract of malt
Quantity of malt used = 4200 kg
Extracted amount = (4200) x 78/100
= 3276 kg

Current extract of rice
Quantity of rice used = 1800 kg
Extracted amount = (1800) x 80/100
= 1440 kg

Total grist weight = (4200+1800) kg
= **6000 kg**

Total extraction = (Extract of Malt+ Extract of Rice)
= (3276+1440) kg
= **4710 kg**

Malt usage % = (3276/4710) x 100
= 69.55%

Rice usage % = (1440/4710) x 100
= 30.45%

When rice percentage increased up to 35 %
(malt: 65%,rice: 35%)

Malt extract = 4710 x 65/100
= 3061.5 kg

Rice extract = 4710 x 35/100
= 1648.5 kg

Quantity of rice and malt needed for 65%-malt & 35% - rice

Malt amount	=	(3061.5/ 78) x100
	=	3925 kg
Rice amount	=	(1648.5/ 80) x 100
	=	2060.62 kg
Malt→ 3925 kg (appro.)	=	3940 kg
Rice→ 2060 kg (appro.)	=	2060 kg

<u>When rice percentage increased up to 40 %</u>

(malt: 60%, rice: 40%)

Malt Extract	=	4710 x 60/100
	=	2826 kg
Rice Extract	=	4710 x 40/100
	=	1884 kg

Quantity of Rice and Malt needed for 60%-Malt & 40% - Rice

Malt amount	=	(2816/ 78) x100
	=	3623.07 kg
Rice amount	=	(1884/ 80) x 100
	=	2355 kg
Malt→ 3623 kg (appro.)	=	3620 kg
Rice→ 2355 kg (appro.)	=	2360 kg

Table 3.3 Reformulated Recipes with selected ingredients

Ingredients	**Formula 1 (35% rice) Trial 01**	**Formula 2 (40% rice) Trial 02**	**Formula 3 (40% rice) Trial 03**
Malt	3940 kg	3620 kg	3620 kg
Rice	2060 kg	2360 kg	2360 kg
Aroma hops	2 kg	2 kg	2 kg
Bitter hops	8 kg	8 kg	8 kg
Caramel	10 kg	12 kg	14 kg
Brewing salt	6 kg	7 kg	7 kg
Phosphoric acid	4 Ltrs	4 Ltrs	2 Ltrs
Termamyl	0.5 kg	0.75 kg	0.9 kg
Water	180 hl	180 hl	180 hl

3.3. Beer production

3.3.1. Handling of Malt and Rice

Preparations of malt and rice were the starting point. Here cleaning, weighing, dust removing, grinding, mixing with water during or after grinding were taken place. Both malt and rice were stored in silos and the needed quantity was taken by giving a command through the Programmable Logistic Controller (PLC) system. The malt and rice transfer is described in the fig: 3.3 and fig: 3.4.

The malt was kept for 20 minutes at 45 °C in the mash tun for protein rest. In this period normally proteins are breakdown by protease enzymes in the malt. That is very important to manufacture a quality beer since proteins are the major causual agent for the haze formation of beer. Not only protein breakdown but also starch are sequence proceed up to pre desired level under strictly control of pH, temperature and time in the protein rest.

Fig 3.3: Malt transport system at the Lion Brewery Ceylon Limited.

Fig 3.4: Rice transport system at the Lion Brewery Ceylon Limited.

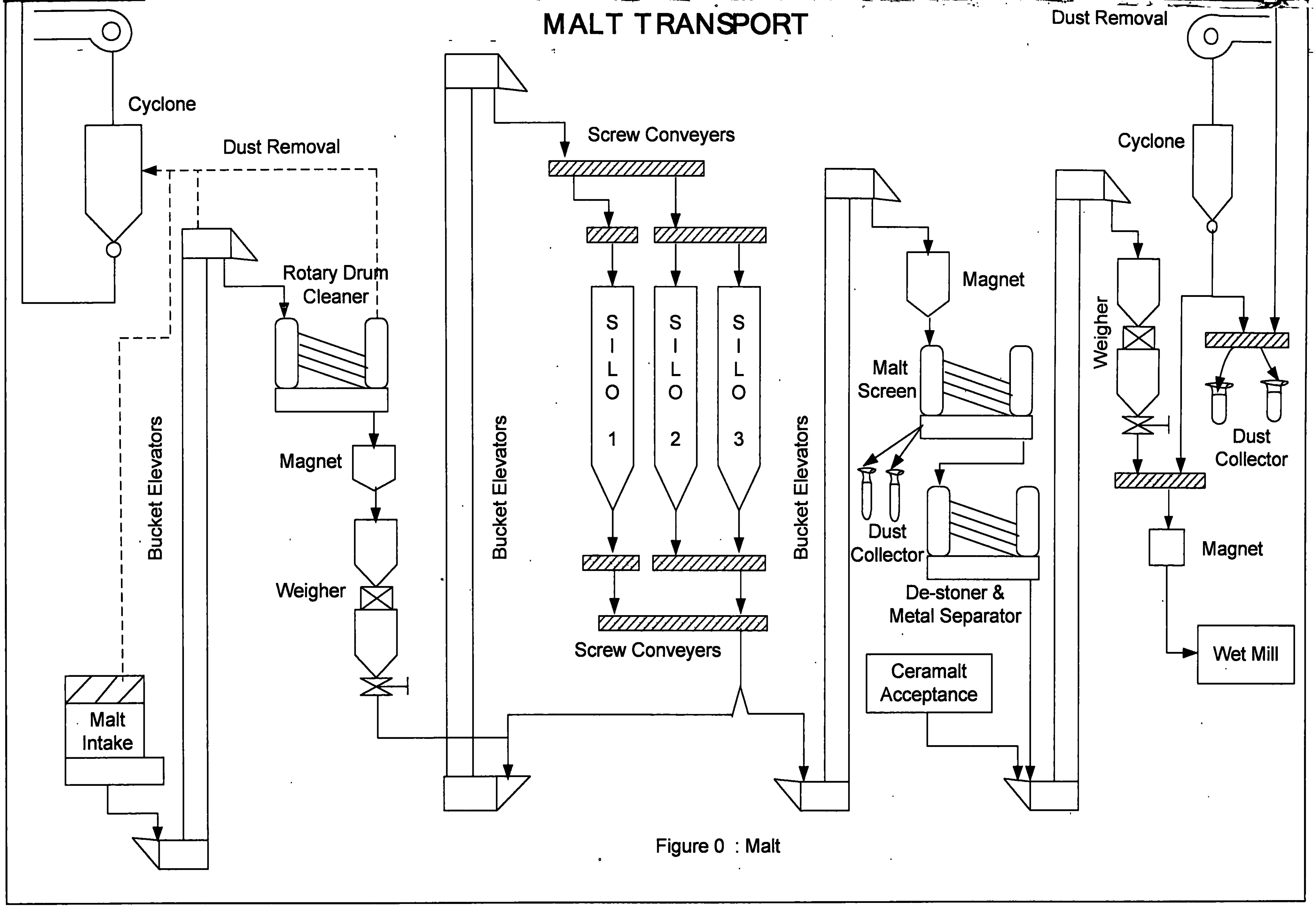


Fig 3.3. Malt transport

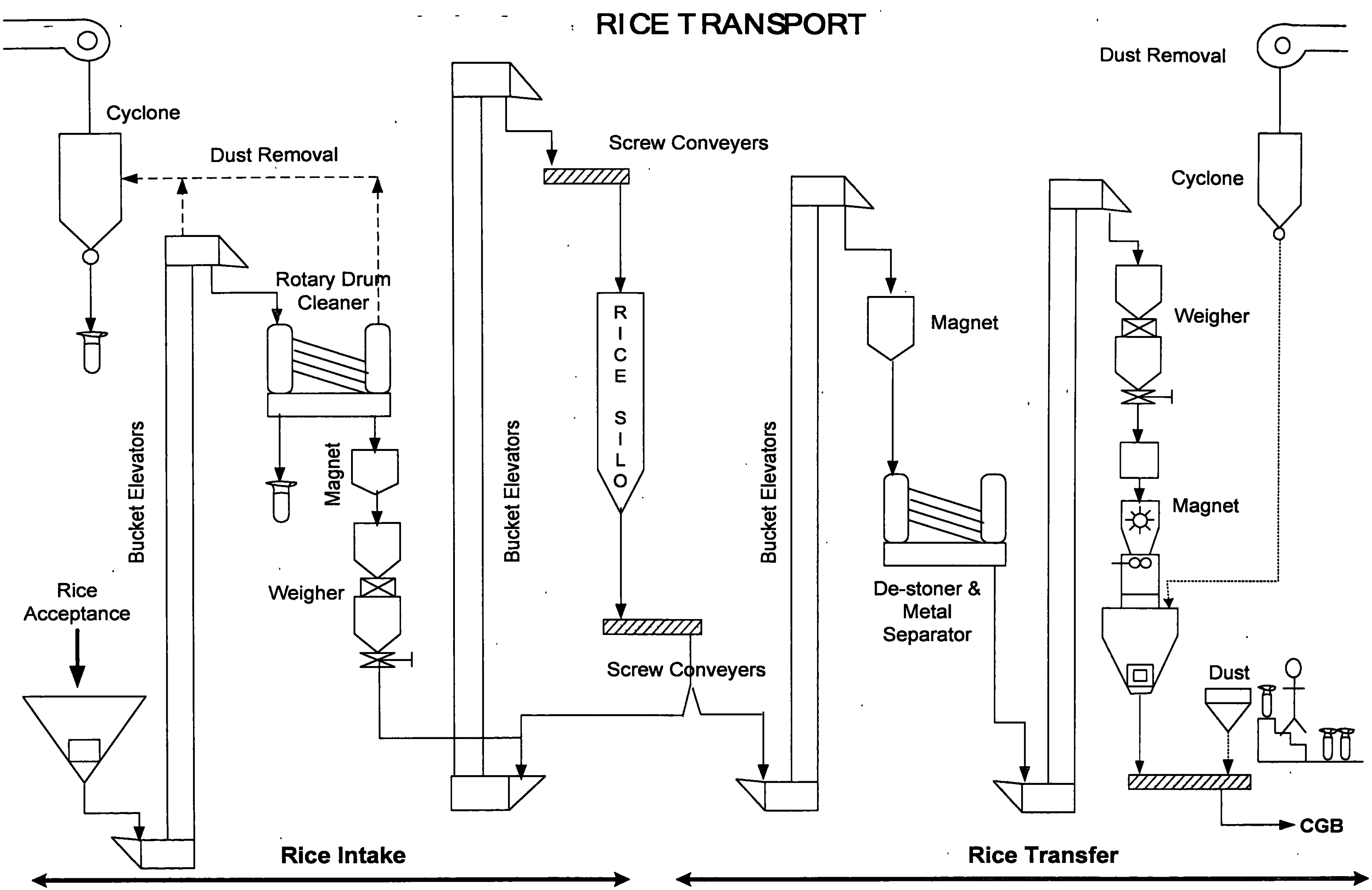


Fig 3.4 Rice Transport

3.3.2 Preparation of sugar solution/wort (Lautering)

This step proceed to prepare fermentable sugar solution using malt, adjunct, water and additives through pre planed and well control series of operations. Then filtration was help to get clear sugar solution. Addition of water during filtration was needed to increase the volume to reach desired extract level of wort by reducing viscosity and leaching soluble substances.

After filtration boiling was applied to extract the component from hops and sugar to filtered solution. Removing suspendable particles from boiled wort creates more clear sugar solution. Then clear hot wort was transferred to fermentation area. In the mean time wort was cooled up to the temperature, which was favorable to yeast, aerated in adequate level, and yeast pitched in correct quantity type.

3.3.3 Water Temperature

The temperature of the sparge water (75 °C –78°C) must be higher than that of the mash to help maintain mash temperature and increase runoff. However, if the sparge water was too hot (above 80°C), it would extract unwanted materials such as tannins, proteins, and unconverted starch.

3.3.4 Wort Boiling

Following extraction of the carbohydrates, proteins, and yeast nutrients from the mash, the clear wort must be conditioned by boiling the wort in the kettle. The purpose of wort boiling is to stabilize the wort and extract the desirable components from the hops. The principal biochemical changes that occur during wort boiling are as follow:

Sterilization
Destruction of enzymes
Protein precipitation
Color development
Isomerization
Dissipation of volatile constituents
Concentration
Oxidation

3.3.5 Wort Cooling

After boiling and clarification, the wort was send through a plate heat exchanger for cooling. The cooled wort has temperature of 4°C. This is needed prior to preparation for the addition of yeast and subsequent fermentation. As soon as the wort gets cooled it was send through an aeriater for oxygenation. Then the wort was send to the fermentation tanks. While the wort was transferred to the fermentation tanks yeast pitching was done.

The principal changes that occur during wort cooling are as follow:

- Cooling the wort to yeast pitching temperature
- The formation and separation of cold break
- Oxygenation of the wort to support yeast growth

Until the alcohol level obtained wort was fermented in the fermentation tanks.

Following figure shows the Brew House (BH) process of the Lion Larger processing.

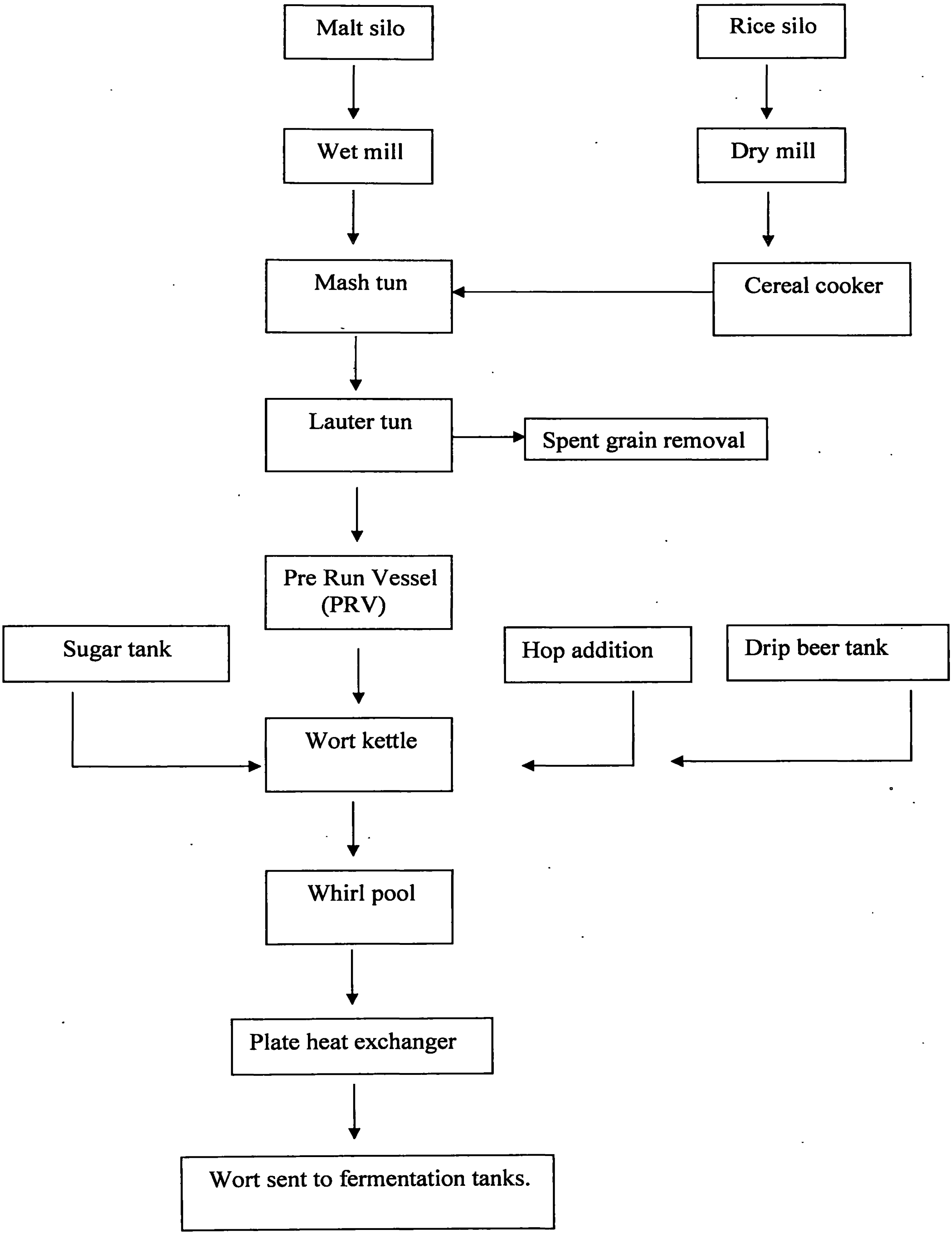


Fig 3.5 Process flow of wort Production

3.3.6 Basic Brewery Calculations

Following formulas were used to calculate the entire recipe. If the rice and malt quantity was calculated by considering the extraction of them, following formulas were used to decide the hop usage, yeast pitching rate and brewing yield.

(a) Calculations of Specific Gravity (SG) from %P of extract in wort at 20°C:

The extract content (Kg extract/hl wort at 20 °C) in the wort will be:

Kg extract/hl cold wort = OE%P*SG

SG=Specific Gravity.

(b) Calculation of Brew house yield

$$\text{Brew house yield} = \frac{\text{Amount of extract in finished wort}}{\text{Amount of extract from brewing materials}}$$

(c) Calculation of mash thickness

The proportion of brewing water to brewing materials is interesting as indicates the thickness of mash: The normal values for this figure for lautering process will be in the range 2.8-3.5 depending on the actual mashing recipe.

(d) Calculations of first wort extract

Assuming we want to calculate the theoretical first wort strength in % Plato we have

$$X = \frac{\text{Extract (mash)*100}}{\text{Extract (mash) +Added water+water (malt)}}$$

(e) Calculation of hop dosing

$$\text{Kg hops per brew:} = \frac{\text{Wanted B.U.* brew size (hl)}}{\text{\% Utilization*\%A-acid in hops used}}$$

3.3.7 Fermentation

With strike control of temperature fermentable sugars were converted to ethyl alcohol by yeast. The length of alcohol formation depends on the strength of fermentable sugars, pH, temperature, yeast healthy and degree of aeration etc. During the fermentation CO_2 and many other components were released as byproducts. The byproducts showed a considerable effect on the taste, aroma and other characteristic properties of the beer. Such byproducts (eg: VDK) were again absorbed by yeast when there was a rest period in the fermentation vessel after fermentation. VDK was critical byproduct produced during fermentation and cooling was applied up to minus centigrade after reducing VDK into threshold level of 0.15.By referring following calculation yeast pitched for the wort solution for facilitating the fermentation.

Pitching Volume calculation

$$\text{Amount of yeast pitched} = \frac{\text{Target count} * \text{FV volume after filling}}{\text{Yeast viability} * \text{yeast cell count}}$$

Actual pitching rate calculation

$$\text{Actual pitching rate} = \frac{\text{Yeast unit in FV after filling}}{\text{FV volume after filling}}$$

Yeast dosing for fermentation

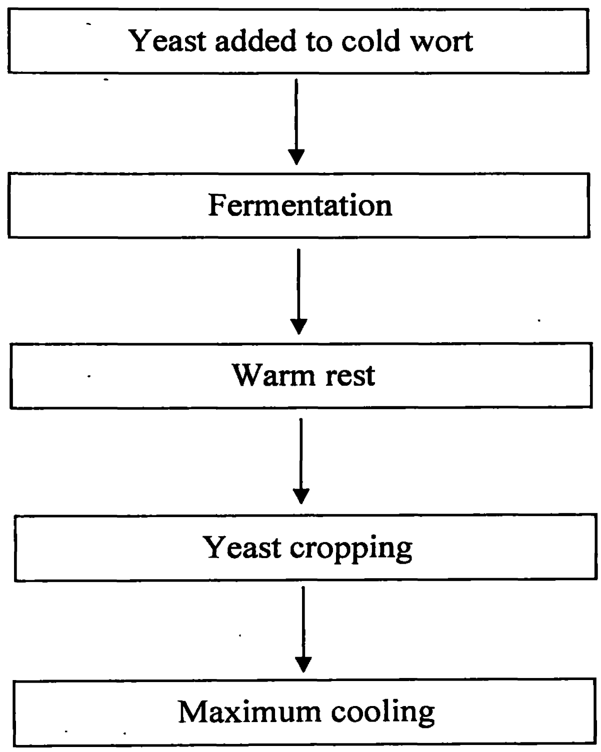


Fig 3.6 Yeast dosing of beer processing.

3.3.7.1 Yeast preparation & handling

After complete fermentation partially inactivated yeast was collected to yeast storage vessels and yeast was stored during the period between cropping and repitching as slurry under low temperature. Yeast was discharged completely after several generations because of the possibility of deduction in viability & efficiency of yeast as well as the mutant generation possibility.

3.3.8 Conditioning of beer

3.3.8.1 Treatment of Beer

When beer temperature was reached to minus, Flocculation aid (PVPP- Poly Vinyl Poly Pirolidine) was added to beer flow with specific rate after centrifugation to preliminary reduction of suspended particles and yeast from fermented beer. This flocculation substances aggregate with poly phenols and positively charge protein at minus temperature and settled down with time. Efficiency of flocculation substances depends on the temperature, ratio between beer volume and amount of flocculation aid and the resting period. This beer which consider as "Green beer" was sent to Treated Beer Tanks (TBT) as buffer storage.

3.3.8.2 Beer Filtration

Treated beer was send through a beer cooler and stored in a buffer tanks to ready for filtration. Then cooled beer was send through a filter bed, which was prepared by mixing Kieselghur powder and silica gel addition. This was called Kieselghur filter. The filtration was increased the brightness of beer. There was a significant quantity of suspended materials and removed in final polishing step and adequate carbonation was incorporated. (Fig 3.5)

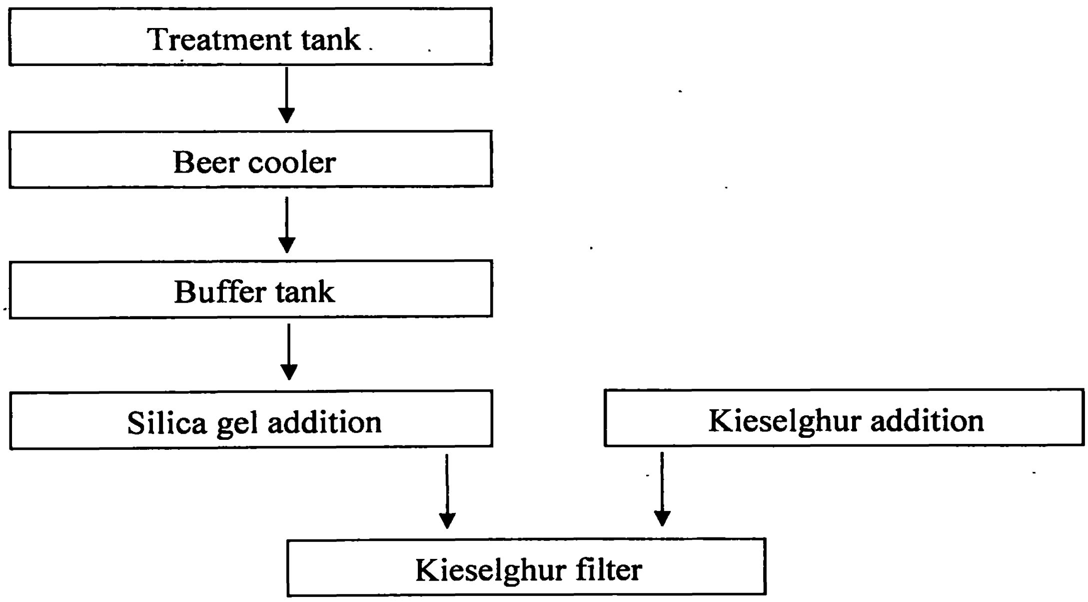


Fig 3.7 Beer conditioning procedure of Lion Larger.

3.3.8.3 Beer Carbonation

The next major processes which take place after filtration and prior to packaging were carbonation. Carbon dioxide not only contributes to perceived "fullness" or "body" and enhances foaming potential; it also acted as a flavor enhancer and played an important role in extending the shelf life of the product. Before carbonation beer was sent through a GAF filter and stored in a buffer tank to get a continuous flow via the carbonator. After the carbonation beer was send to storage vessels, which named as Bright Beer Tanks (BBT). That is ready for packaging.

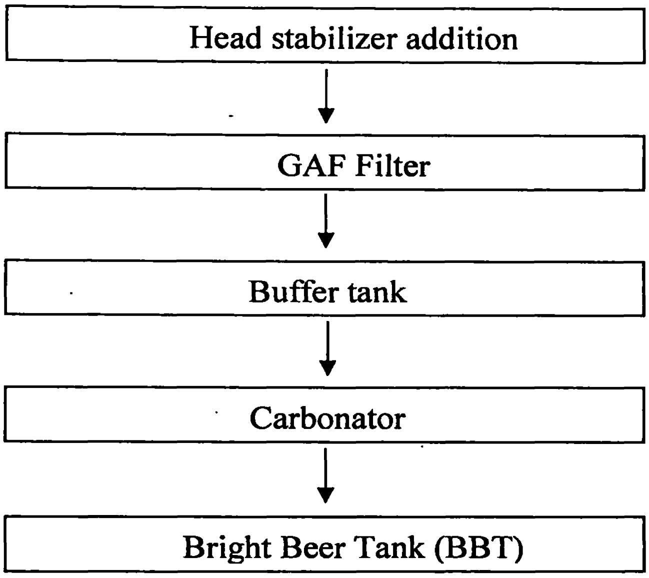


Fig 3.8 Beer Carbonation procedure

3.4 Beer packaging

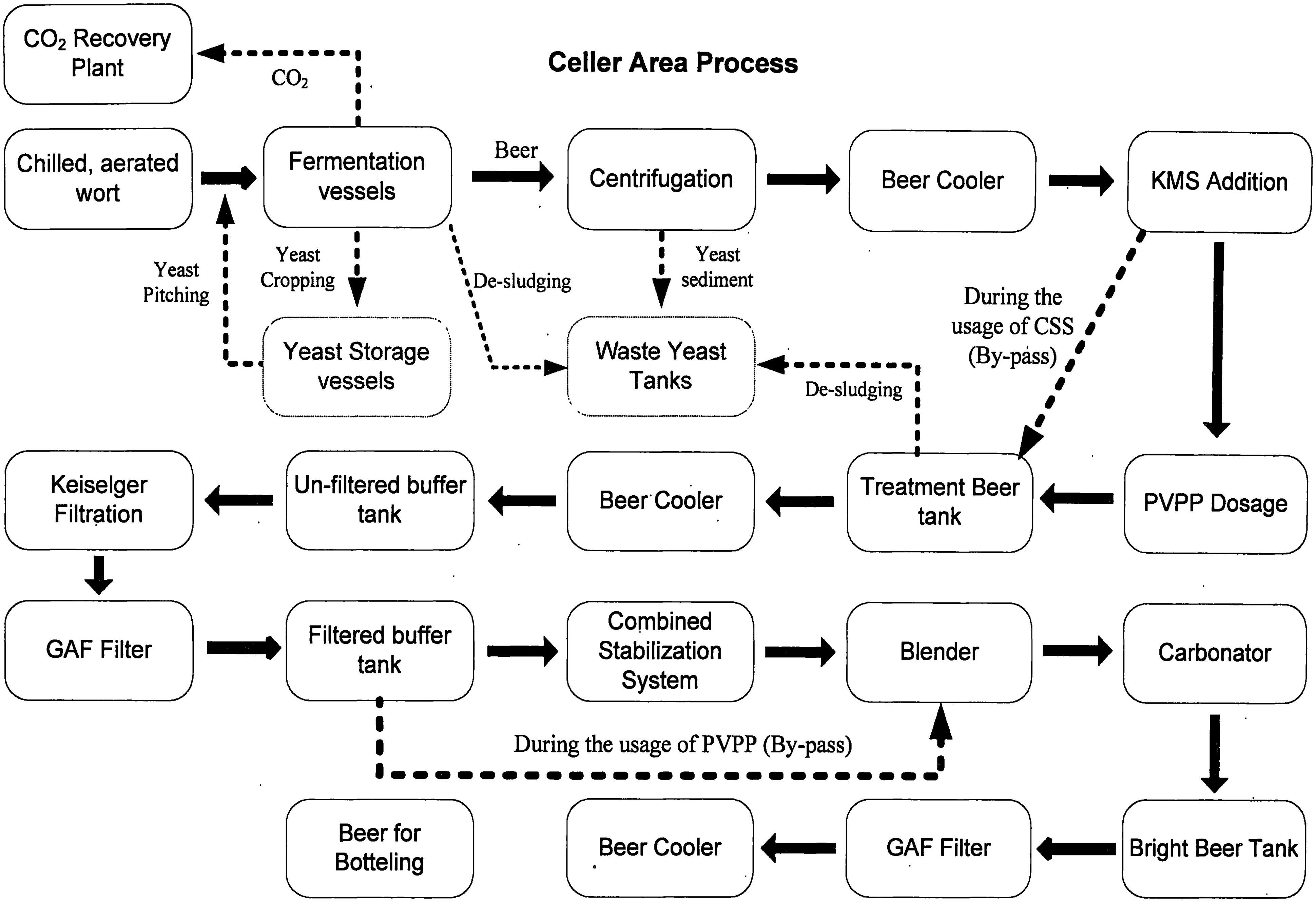
3.4.1 Bottling

Washed and inspected bottles were filled with beer at minus temperature and crowned. Then pasteurized beer was cooled to normal temperature. Finally corded, labeled bottles of finished beer were arranged in containers.

3.5 Technology

Fully automatic computerized operation system is used

Celler Area Process



3.6 Quality Analysis Testing of The Lion Larger

Following quality tastings were done to maintain the quality of beer from the stage of Cooled wort to bottled beer. All the tastings in LBCL were done according to the Carlsberg International Methods.

3.6.1 Analysis of cooled wort

Test	Unit
Bitterness	BU
I_2 Test	
Extract	% Plato
pH	

The bitterness was analyzed using the spectrophotometer at the reference wave length (λ max) of 275 nm. Then the given value as multiplied by a factor value of 50 to get the BU value.
The Extract or the sugar amount was analyzed by using the digital Plato meter. The Plato meter directly gave the reading. The pH was measured using the digital pH meter.
Iodine test was done to determine whether saccharification completed. Wort was put on to a marble and drops of iodine solution was put in to the wort solution, the brown color indicate the negative result which indicates the not of starch in the solution.

3.6.2 VDK measurement in each ferment vessels

VDK (Vicinyl Di Ketone) is a critical byproduct produced during fermentation of beer. With the time that was reabsorbed by yeast. The beer was kept in fermentation tanks until the VDK level became 0.15 which was the threshold level of it. The beer samples were filtered and distilled first. The distilled amount was analyzed using the UV visible spectrophotometer under the reference wave length of 330 nm. Following are the some analysis done at each stage of brewing process.

3.6.3 Analysis of fermented beer

Test	Unit
Bitterness	BU
pH	
RDF	%
Alcohol	%v/v
Color	EBC
OE	% Plato
Real Ex	%
Yeast C.C	$*10^6$/ml
Flavor Score	sat/nqs/ns

The pH was measured using the pH meter. The RDF (Real Degree of Fermentation), Alcohol level, OE (Original Extract) and Real Extract were analyzed by the Beer Analyzer. The bitterness and the color were analyzed by using the UV visible Spectrophotometer. The absorbance of the wort is measured at a wave length of 430nm for colour and 275 nm for bitterness. The colour in EBC units and the bitterness in BU units are obtained by multiplying the absorbance by a factor. Spectrophotometric method can be applied to all worts and that is used to analyze colour, bitterness, Iron, VDK and SO_2. The flavor score was identified by tasting of members of the trained panel. Plate count was done to get the cell count of yeast.

3.6.4 Analysis of treated beer

Test	Unit
Alcohol	%v/v
Color	EBC
DO	ppm
OE	% Plato
pH	
RDF	%
Real Ex.	%
Yeast C.C	$*10^6$/ml
Flavor Score	sat/nqs/ns

The Dissolved Oxygen (DO level) was measured by using the DO meter. Other testings were done according to previous method.

3.6.5 Analysis of bright beer tank

Test	Unit
Alcohol	%v/v
CO_2	g/l
Color	EBC
DO	ppm
Haze	EBC
OE	% Plato
pH	
RDF	%
Real Ex.	%
Yeast C.C	$x10^6$/ml
Flavor Score	sat/nqs/ns

The Haze amount was measured using the Haze meter. Other testings were done according to previous methods.

3.6.6 Analysis of unpasteurized beer

Test	Unit
O.E	% Plato
Alcohol	%v/v
Real Ex.	%
CO_2	g/l
Flavor	sat/nqs/ns

The CO_2 was measured using a titration. Other tastings were done according to previous methods.

3.6.7 Analysis of bottled beer

Test	**Unit**
Alcohol	%v/v
Bitterness	BU
CO_2	g/l
Color	EBC
pH	
RDF	%
OE	% Plato
Haze 60 D (20 °C)	EBC
Haze 5 D (60 °C)	EBC
VDK	ppm
Head retention	Seconds
Real Ex.	%

The head retention was measured by keeping the beer sample to retain the foam level. The time that has taken for retaining the foam level was measured by seconds.

Every beer sample was kept at 60 °C for five days and at 20 °C for 60 days and measured the haze formation using the haze meter.

3.6.8 The Original Gravity (OG)

The alcohol produced during fermentation was separated by distillation. The values of both fractions were restored with water to those of the original sample and the specific gravity of each fraction determined.

3.6.9 Calculations for spectrophotometric methods

The color, bitterness, iron, VDK and SO_2 were calculated by referring following formulas.

Color (EBC) = A*f*25

Where A= absorbance at 430 nm in 10 mm cell.

f = dilution factor

Results were expressed in 2 figures.

Bitterness (BU) =A*f*50

Where A= absorbance at 275 nm in 10 mm cell.

f = dilution factor

Iron = A*f*4.9

Where A= absorbance at 505 nm in 10 mm cell.

f = dilution factor

VDK = A*f*2.7

Where A= absorbance at 335 nm in 10 mm cell.

f = dilution factor

SO_2 = A*f*2.7

Where A= absorbance at 570 nm in 10 mm cell.

f = dilution factor

3.7 Sensory analysis

3.7.1 Find out the significant difference between each beer samples

Bottled beer samples were taken and stored in a refrigerator where even temperature was maintained. Then the well-trained sensory panel organoleptically analyzed beer samples. The triangular test was done to identify the differences. The results were obtained by referring the Carlsberg method.

3.7.2 Preference Test

Then the samples were prepared for sensory evaluation and sensed for taste flavour, colour, appearance and overall acceptability. Sensory data refers to newly developed formulae were analyzed along with sensory rating scores.

Beer samples were scored for overall acceptability on 9-point hedonic scale using 12 well-trained panels, where 4 samples were presented in 1 session in a well-prepared sensory room. Data was statistically analyzed with Freidman test using MINITAB statistical analysis package (Version 14.1).

CHAPTER 04

RESULTS AND DISCUSSION

4.1 Formulation of a Newer Lion Larger Recipe

Adjuncts are used to obtain fermentable sugars at a lower cost; they also play a very important and specific role in giving a characteristic quality to the beer to attract consumers. All malted beer is very rich in flavor and it has dark color. On the other hand the beer with adjuncts has a light color and taste, so that consumers tend to drink more because of its less satiating taste. The beer with adjuncts also has higher colloidal stability due to the lower nitrogen content. Rice is widely used as an adjunct in the world for the production of alcoholic beverages such as beer. Each Brewer uses rice for brewing as a cost effective raw material and to improve some quality aspects of it. The cost of malt is rather high when comparing with the cost for rice. Further Rice is indigenous to Sri Lanka and malt is importing by local brewers from European countries. Locally we have much of rice thus it is cost effective to use rice as a raw material instead of malt. Further that gives benefits to local farmers. More over rice gives higher extraction than malt.

LBCL imports malt from Australia and China. The cost of malt is rapidly increasing day by day, In order to maintain the price of beer at a constant level, the cost of production should be reduced. It can be done by increasing the rice quantity instead of malt. Therefore major part of this research was aimed at formulation of a newer Lion Larger recipe considering above facts at optimum conditions.

The Lion Brewery Ceylon Ltd occupies an unchallenged position as market leader of beer industry in Sri Lanka. They consider quality as number one priority. Lion larger is one of the major brands of them. Both rice and malt are used for brewing of Lion Larger. Therefore this study was aimed to develop the Lion Larger recipe by increasing the rice usage while keeping the quality aspects of it.

Beer is an alcoholic beverage produced by the fermentation of sugars derived from malted barley and flavoured with hops. The characteristic of beer is depends on type of raw material, additives, yeast type, manufacturing technology and processing factors etc.

During the malting process starch is converted in to fermented sugar.

The reaction was catalysed by α amylase and β amylase. When α amylase attacks to side chain of starch molecules, β amylase attacks to main chain. The results of breakdown were fermentable sugars (glucose, maltose & maltotriose) and unfermentable sugars (limit dextran). Optimum temperature and pH for α amylase were 70-75 °C & 5.6-5.8 and those parameters for β amylase were 60-65 °C & 5.4-5.6. Calcium salts were added for maintaining correct pH during the mashing period. Gelatinisation and liquefaction of starch were help to change 3D structure of starch molecules making easy access to breaking points. Following are the basic brewery calculations, which considered formulating the recipe and making the brew process.

Normally it is needed about 10 hours to the entire brew house process. Figures of table 4.1 reveals the typical time at the each stage of the brew house process.

Table 4.1 Summary of the typical time at each stage of the brew house.

(Source: LBCL training kit)

Activity	Typical Times	Total Time
1. Milling	½ hr	½ hr
2. Mashing	3½hr	4 hrs
3. Lauter tun	3½ hrs	7½ hrs
4. Wort Kettle	1½ hr	9 hrs
5. Wort Chilling & Aeration	1 hr	10 hrs

According to the data in the brew house process about ten hours processing is needed to prepare the wort solution for fermentation. For the mashing and lautering more time is needed since enzymatic reactions happening at that mashing stage more time is needed to pass that stage. Because a natural filter bed is forming at the lautering process that is also needed more processing period.

4.2 Comparison of results in the Brew House trials

4.2.1 Plato value of wort

The plato value was decreased with the wort volume. Normally the first wort gives a plato between 18%-20% in Lion larger. Normally water is sparging to the Lauter tun to get maximum wort yield. With the water sparging, the plato level regularly decreased. In the Lion Brewery water sparging is done until the plato value reduce to 4% .The table 4.2 and graph no 1 and graph no 2 reveals the plato variation at the Lauter tun when yielding the wort.

Table 4.2 Plato variation with the wort volume in the brewing process.

Wort volume (hl)	35% rice -LL	40 % rice-LL
25	18.41	19.01
40	18.83	19.51
55	18.73	19.35
70	18.80	19.50
85	18.63	19.38
100	18.20	19.13
120	18.00	18.55
140	16.30	16.31
160	13.36	12.23
180	10.60	10.75
200	8.65	7.80
220	6.73	5.17
240	5.18	3.98
260	4.26	3.43

Graph no 1 reveals the graphical overview of the plato variation of 35% rice used Lion Larger. According to that Initial plato values were between 15%-18%, with the wort volume the plato value was reduced gradually. The reason for this was gradual sparging of water to the Lauter tun to get maximum wort volume. Graph 2 reveals the plato variation of 40% rice incorporated beer.

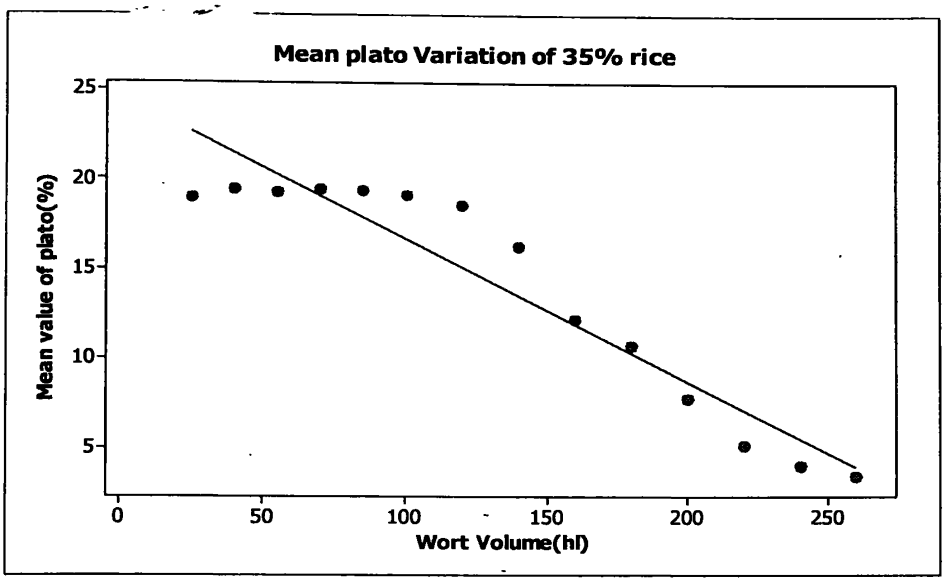


Fig 4.1 (Graph no: 01-Plato variation of 35% rice used Lion Larger)

Graph no 2 reveals the plato variation of 40% rice used beer. When increasing the rice usage the plato was also increased since the rice gave more extraction than malt.

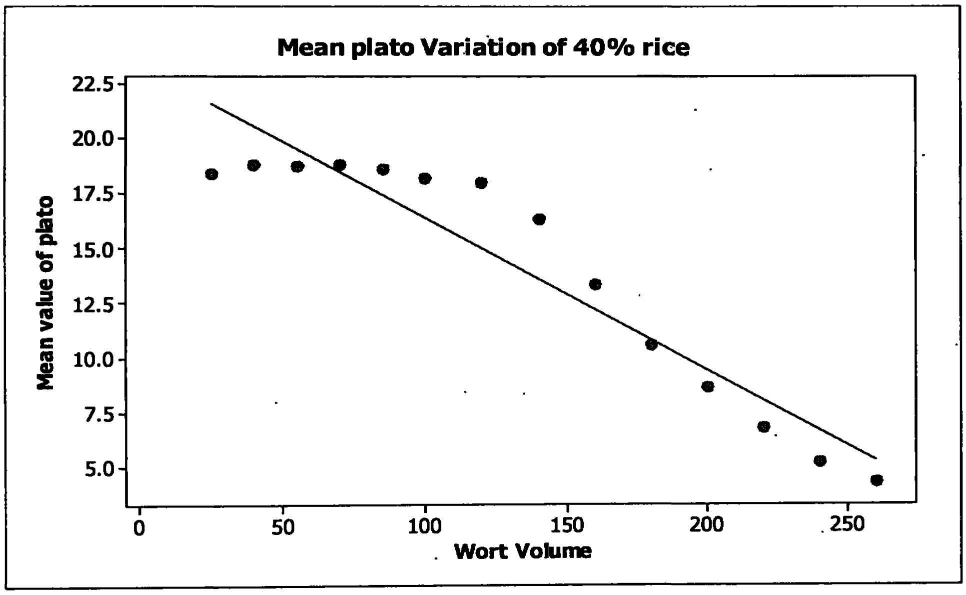


Fig 4.2 (Graph no: 02- Plato variation of 40% rice used Lion Larger)

4.2.2 Pre boil & post boil Plato

The wort which gained from the lauter tun is transferred to the wort kettle for boiling. The wort pato before sending the wort kettle is considered as the pre boil Plato and the other is considered as the post boil plato. Because water evaporation at the wort kettle the post boil plato is higher than the pre boil plato.Graph no 3 and graph no 4 reveal the pre boil plato and post boil plato variation of wort.

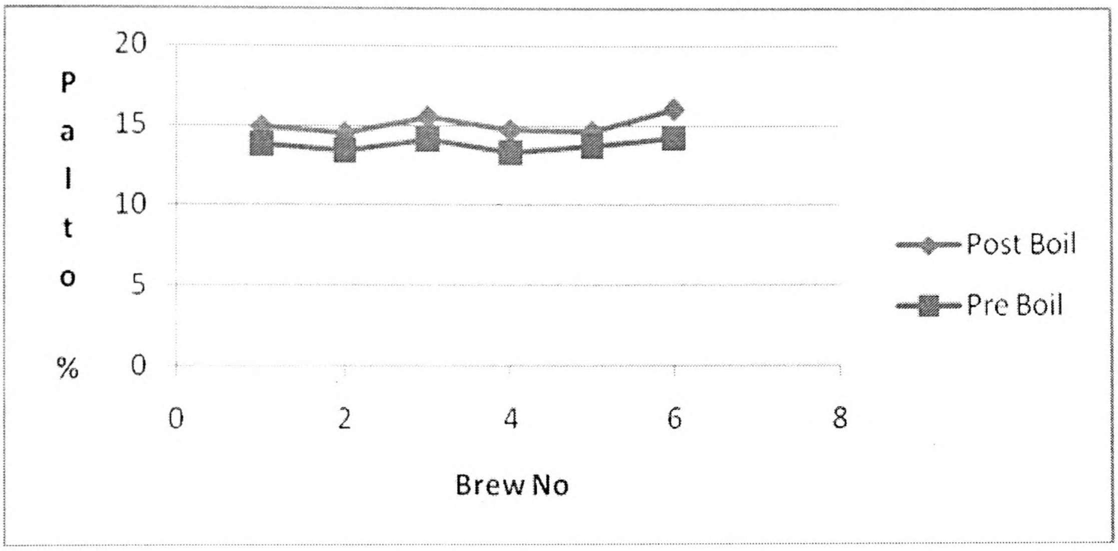


Fig 4.3 (Graph no:03- pre boil and post boil plato analysis of 35% rice used Lion Larger)

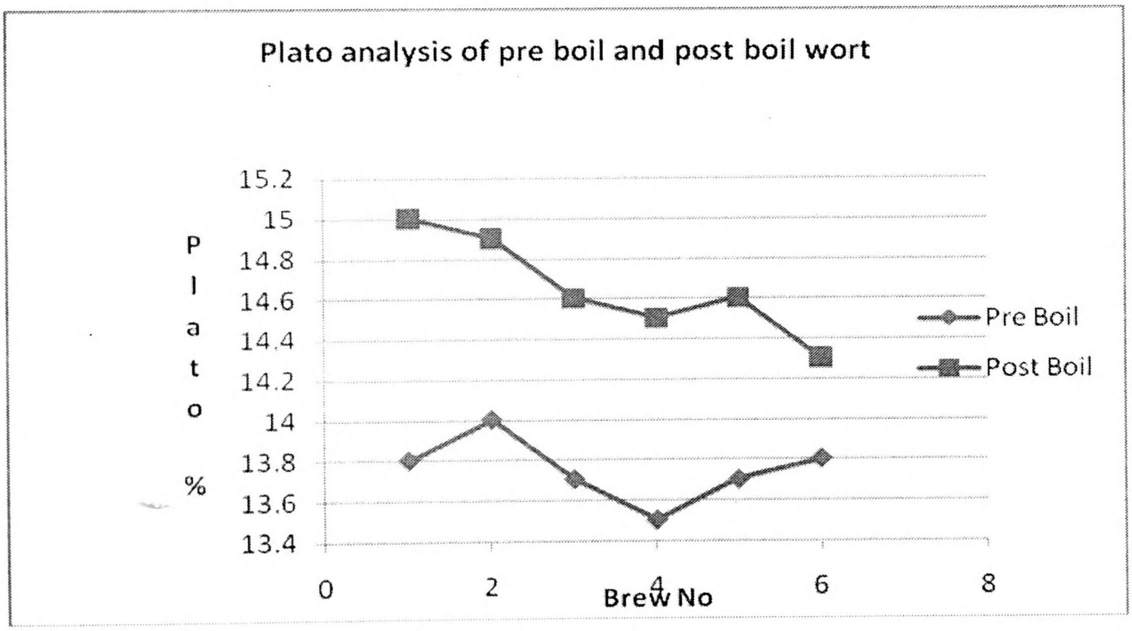


Fig 4.4 (Graph no: 04- pre boil and post boil plato analysis of 40% rice used Lion Larger)

4.2.3 pH variation of Pre boil and Post boil wort.

The post boil pH and the pre boil pH are major factors which consider in the brewing industry for a processing of a quality beer.Normally the post boil pH is higher than the pre boil pH , Because the evoparation of water in the wort kettle the concentartion is higher of the post boil wort. Normally the pre boil pH is maintained between 5.4 -5.6 while the post boil pH 5.2-5.4 . The graph no 05 indicates the pH variation of pre boil wort and post boil wort in the brewing process.

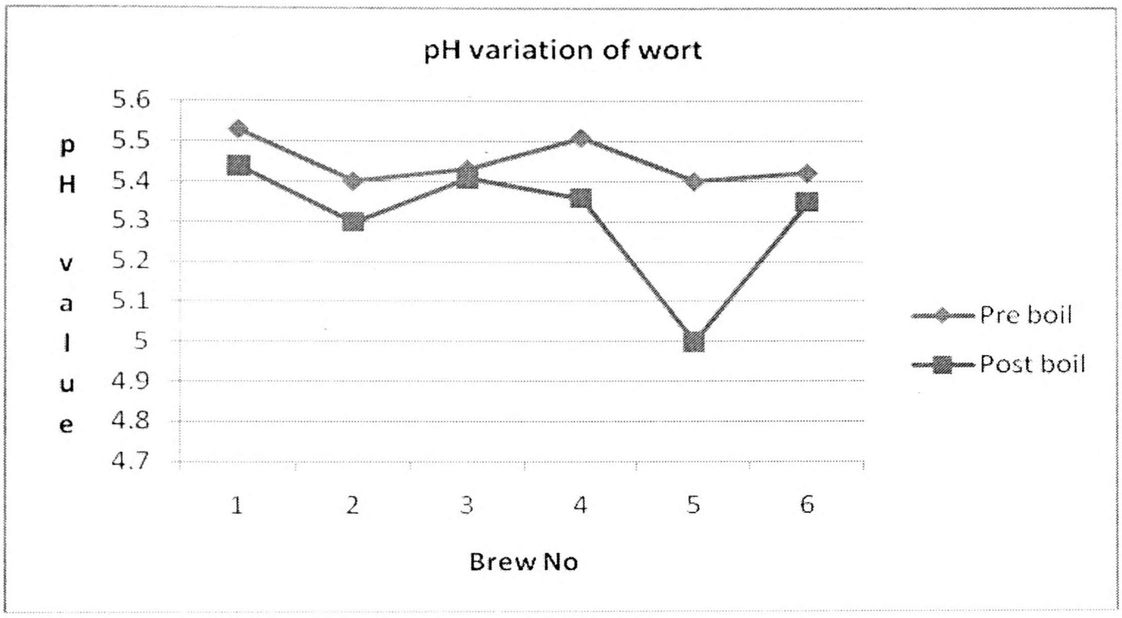


Fig 4.5 (Graph no :05- pH variation of pre boil and post boil wort)

4.2.4 Spent Grain Plato

Spent grains are the waste of the lautering process. In lautering the wort is filtered through a naturally form filter bed which form by the accumulation of malt husk. Some quantity of sugar may in the spent grain. The spent grain is not taken to further processing that is selling as cattle feed. In LBCL the plato value of spent grain is maintained below 3%. If that is more than 3% that is a lost to the company. The graph no 06 indicates the plato variation of spent grains which gained from the lauter tun as waste.

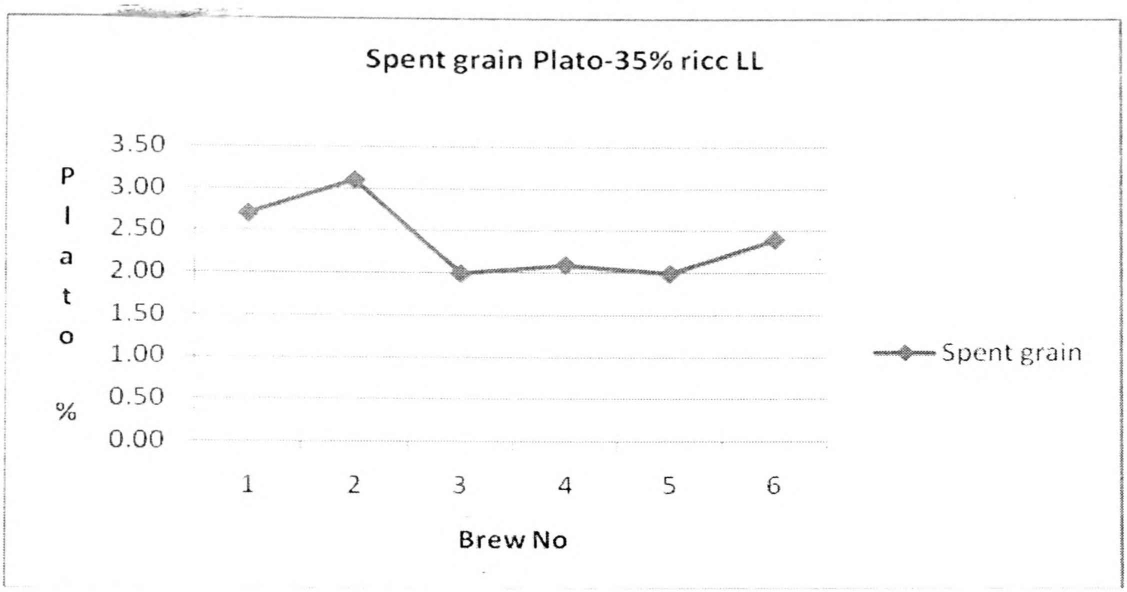


Fig 4.6 (Graph no :06- spent grain plato variation of 35% rice used Lion Larger)

4.3 Fermentation Records

4.3.1 Comparison of fermentation records of 35% rice Lion Larger.

With strick control of temperature fermentable sugars were converted to ethyl alcohol by yeast. The length of alcohol formation depends on the strength of fermentable sugars, pH, temperature, yeast healthy and degree of aeration etc. During the fermentation CO_2 and many other components were released as by products. They gave a considerable effect on the taste, aroma, and other characteristic properties of the beer. Vicinal Di Ketone (VDK) is a by product of yeast which was reabsorbed by them after the fermentation. In order to re absorb the VDK low temperature level should be maintained in fermentation tanks.

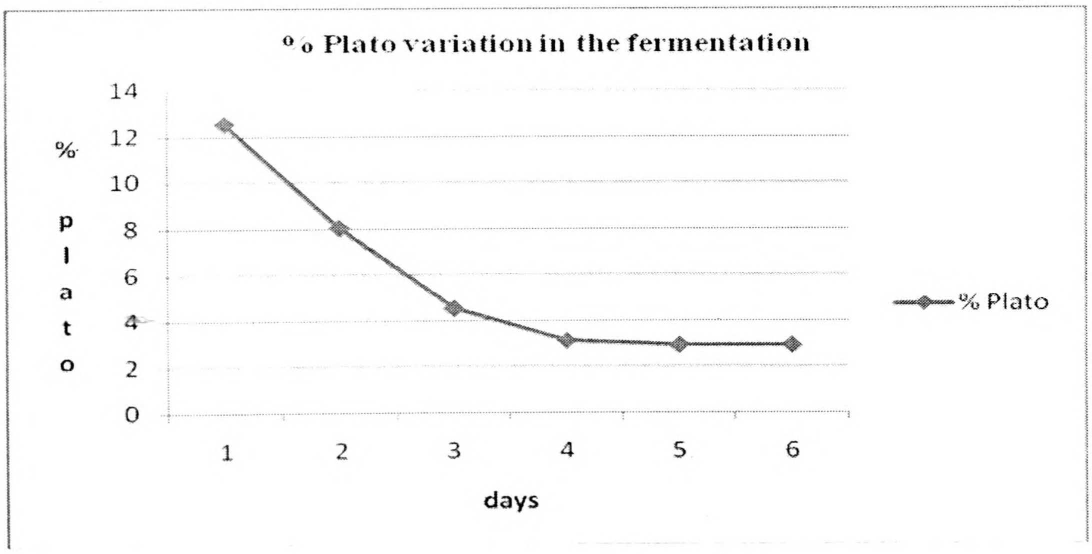


Fig 4.7 (Graph no: 07- plato variation in the fermentation.)

Since the sugar convert in to alcohol by yeast the plato level is decreased gradually. Generally the plato level is lying below 3.0 when the needed alchol level is reached to a satisfactory level. The length of alcohol formation depends on the strength of fermentable sugars, pH, temperature, yeast healthy and degree of aeration.

(a) VDK level in the fermentation

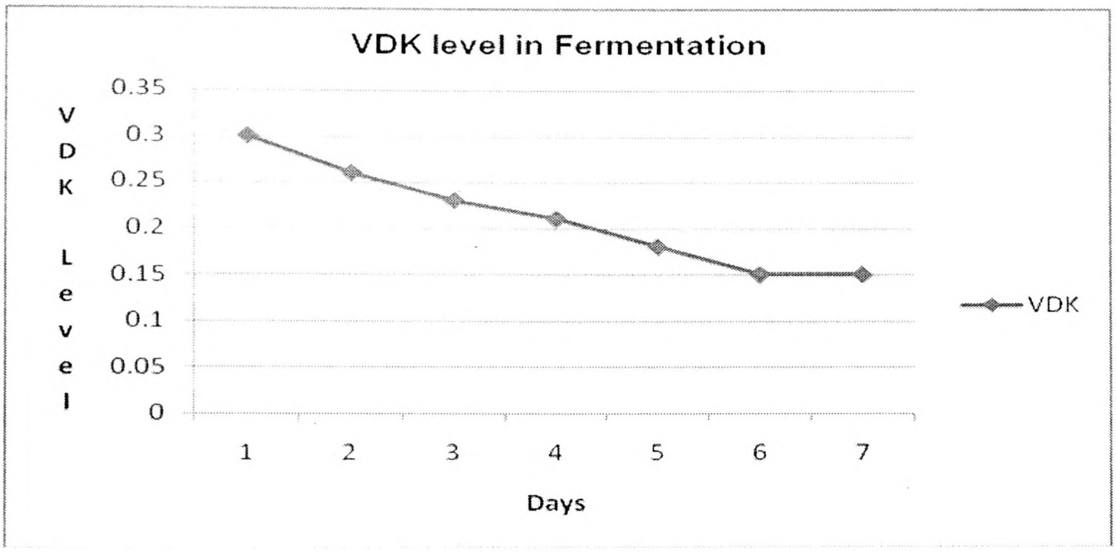


Fig 4.8 (Graph no: 08- VDK Level in fermentation)

In LBCL beer is keeping in fermentation vessels until the VDK level becomes to the threshold level (0.15) for humans. Graph no 08 describes the VDK level in the fermentation.

(b) Temperature in the fermentation tank

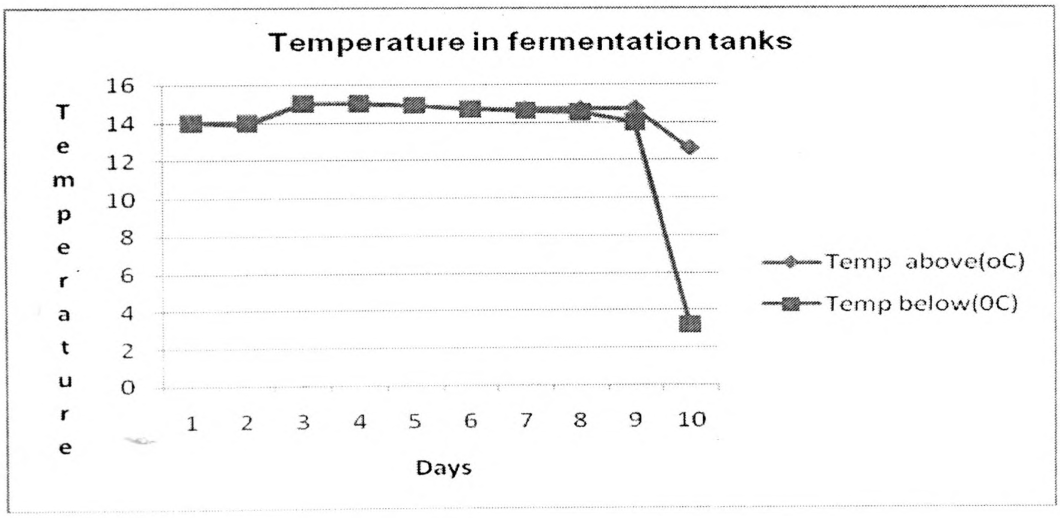


Fig 4.9 (Graph no: 09- Temperature in the fermentation tank)

The temperature of fermentation tanks should be maintained below 14 °C to supply the optimum temperature for the propogation of brewing yeast.

(c) pH in the fermentation tank

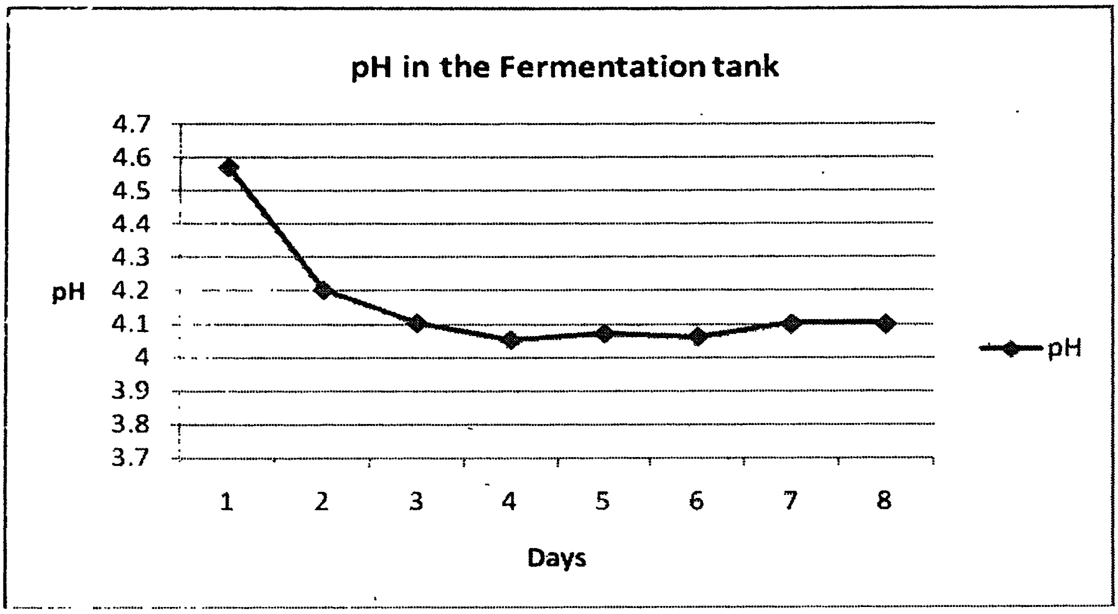


Fig 4.10 (Graph no: 10- pH in the fermentation tank)

Initially the pH of wort reduced to a certain level in the fermentation period. In the fermentation tank normally the pH is maintained at 4.0-4.2.

4.4. Sensory evaluation results for 35% rice used LL beer

Beer samples were scored for sensory attributes on 9-point hedonic scale using 12 well trained panels, where 4 samples were presented in 1 session in a well prepared sensory room. The results were analysed by performing Friedman test by using Minitab 14 Software. Sensory evaluation reveals that there is a statistically significant difference in the bitterness only (p value <0.05). The P value of color (0.092), aroma (0.095), mouth feel (0.123), appearance (0.259) and Overall acceptability (0.172) was larger than the 5% significant level (alpha=0.050).Therefore according to statistical interpretation there was not significant difference of those sensory attributes in between 35% rice used Lion Larger beer samples and other Lion Lager beer samples.

Table 4.3 Sensory analysis of 35% rice used beer.

Organoleptic attribute	Color	Aroma	Mouth feel	Appearance	Bitterness	Overall Acceptability
Grand Median	6.763	5.138	5.150	6.700	5.900	5.550
P value	0.136	0.280	0.027	0.022	0.008	0.051

4.5 Sensory Evaluation results for 40% rice used beer

The p-values for mouth feel (0.027), appearance (0.022), bitterness (0.008) indicates that there was sufficient evidence for a significant difference in those attributes between considered beer samples since their p values were less than 0.05 when alpha was set at 0.05. However The Color, Aroma and Overall Acceptability were not showed significant difference according to statistical analysis.

Table 4.4 Sensory analysis of 40% rice used beer

Organoleptic attribute	Color	Aroma	Mouth feel	Appearance	Bitterness	Overall Acceptability
Grand Median	6.542	6.692	5.154	5.000	6.308	5.846
P value	0.092	0.095	0.123	0.259	0.028	0.172

4.5.1. Summary of the triangular test

Table 4.5 Triangular test results

Sample Type	No of Assessors	Identified the odd sample.	Not identified the odd sample.
35% rice-LL	12	5	7
40% rice-LL	12	8	4

In order detecting differences between samples the triangular comparison was done to well trained sensory panel in the Lion Brewery Ceylon Ltd. Set of three samples were presented to assessors simultaneously, two of which were identical for identification of the odd sample. Then the results were analyzed by referring the ISO 4120:1983(E) significance table. According to that at least 8 correct replies should be needed from 12 assessors to reveal a significant difference at 5% significant level. According to the test result only 5 correct replies was got out of 12 assessors for 35% rice. Thus it can be concluded that no difference in between the 35% rice used Lion lager and other Lion lager beer samples.

Eight correct replies were got out of total 12 assessors for 40% rice used Lion Larger. It reveals a significant difference between 40% rice incorporated Lion Larger and normal Lion Larger at 5% significant level.

4.6. Cost calculation for Lion Larger with 40% Rice (with effect to the 15th July 2007)

Ingredient	Cost of 1 Kg (LKR)
Malt	52.00
Rice	31.00

<u>For Current Recipe</u>

Quantity of malt used	= 4200 kg
Cost of 1 kg	= 52.00
Cost for malt	= 52 x 4200
	= 218,400.00
Quantity of rice used	= 1800 Kg
Cost of 1 kg	= 31.00
Cost for rice	= 31 x1800
	= 55,800.00
Total cost of malt & rice for 1 brew	= (218,400+55,800)
	= 274,200.00
Total cost of malt & rice for 1 batch	= (274,200.00) x 6
	= 1,645,200.00

<u>When rice increased up to 35%</u>

Quantity of malt expected to use	= 3940 kg
Cost of 1 kg	= 52.00
Cost for malt	= 52 x 3940
	= 204,880.00
Quantity of rice expected to use	= 2060 Kg
Cost of 1 kg	= 31.00
Cost for rice	= 31 x 2060
	= 63,860.00
Total cost of malt, & rice for 1 brew	= (204,880+63,860)
	= 268,740.00
Total Cost of Malt, Rice & Enzymes for 1 Batch	= (268,740.00) x 6
	= 1,612,440.00
Expected saving for 1 batch	= (1,645,200-1,612,440)
	= **32760.00**
Expected saving percentage	= (32760/1,645,200) x100
(Relevant to malt and rice)	= **1.99%**

When rice increased up to 40%

Quantity of malt expected to use	= 3600 kg
Cost of 1 kg	= 52.00
Cost for malt	= 52 x 3600
	= 187,200.00
Quantity of rice expected to use	= 2400 Kg
Cost of 1 kg	= 31.00
Cost for rice	= 31 x 2400
	= 74,400.00
Total cost of malt & rice for 1 brew	= (187,200+74,400)
	= 261,600.00
Total cost of malt & rice for 1 batch	= (261,600) x6
	= 1,569,600.00
Expected saving for 1 batch	= (1,645,200-1,569,600)
	= **75600.00**
Expected saving percentage	= (75600/1,645,200) x100
	= **4.59%**

4.7. Benefits of rice as an adjunct for beer

1) Adjuncts provide extract at a lower cost than that available from malt. (Brewer's handbook)
2) High extraction can be obtained in cases of limited capacity than malt. (Brewer's handbook)
3) Rice contained high starch content than malt-rice extraction=80%- 85%, malt=75-78 %(Brewing Technology)
4) Help to improve the shelf life and flavor life of beer.(LBCL Training kit)
5) Low nitrogenous materials contain in rice than malt, So it causes for low haze formation (Biotechnology of Brewing)
6) High availability- rice is indigenous to Sri Lanka
7) Easy to handle –Low dust, less space needed to store than other cereals(The Practical Brewer)
8) Rice has neither a characteristic flavour nor a profound effect on the flavour of beer(Rice: Chemistry and Technology)
9) Rice contains less lipids than other solids adjuncts, (Rice: Chemistry and Technology)
10) Rice has an effect to reduce Coronary Heart Diseases (CHD); Lot of researches show that the moderate consumption of alcohol provides significant health benefits, primarily through reducing the risk of cardiovascular disease, also with respect to reduction in risks associated with other ailments such as stomach ulcers, gallstones, arthritis etc.
11) Beneficial for local farmers.

4.8. Practical Issues and Recommendations

4.8.1. Practical issues and recommendations for 35% rice beer.

Run without any practical issues.

Saccharofication occurred at 67 °C well with in 30 minutes.

4.8.2 Sensory analysis of 35% rice beer

(1) Out of 13 well-trained assessors 6 identified the odd sample while 7 could not identify the odd sample. Here 35% rice LL took as the odd sample and recently bottled normal LL was taken as duplicates.

(2) According to there preference 6 assessors preferred 35% rice LL, 5 preferred normal LL and 2 preferred other kind of larger type beer such as Carlsberg.

4.8.3 Practical Issues and Recommendations for 35% Rice beer 40% Rice

10 hl was added for each brew to maintain the saccharification temperature. Some adjustments of temperature and water were done.

Saccharification happens with in 30 mins in that trial too.

Iso hopes and caramel added.

4.8.4 Sensory analysis of 40% rice

According to the triangular test 9 out of 12 preferred the 40% rice beer while 1 preferred none.

According to the rating test 5 out of 12 expert panelists preferred 40 % LL, 4 out of 12preferred normal LL 2 out of 12 preferred 35% rice LL and 1 out of 12 preferred Carlsberg. Smooth presence flavour given by 40 % rice. A grainy, grassy and harsh flavour of normal LL is vanished with the high usage of rice for producing beer than the usage of grainy malt.

CHAPTER 05

CONCLUSION AND FURTHER STUDIES

5.1. Conclusion

Rice has a higher gelatinization temperature than malt and it must be gelatinized before addition to the mash. Adjunct can be used to adjust the fermentibility of wort which depends on the decrease of hydrolysis in processing or during mashing. Adjuncts are an efficient means of extending brew length and increasing the gravity of the wort.

It can be concluded that adjuncts effect for following attributes in beer manufacturing.

- Diluting the flavor to give a lighter, smoother beer in the case of bland adjuncts, such as cereals.
- Contributing their own distinctive character to the beer in the case of flavorsome adjuncts.
- Altering the Carbohydrate and Nitrogen ratios of the wort, thus affecting fermentation products in the case of adjuncts low in Nitrogen.
- Liquid adjuncts can be added after primary fermentation or primings to increase the sweetness or body/mouth feel or provide source of extract for a secondary fermentation.

Further the effects of adjuncts on beer stability are as follows:

- Enhance foam stability by providing additional glycoproteins and polypeptides in the case of wheat and barley.
- Reducing foam stability through diluting malt proteins (and possibly contributes foam negative lipids). E.g. maize, rice and liquid sugars.
- Improve chill haze and permanent haze stability by diluting malt Nitrogen and Polyphenols.
- Improve beer stability and shelf life, especially when maize and rice adjuncts are used.

Further more the rice usage can be improved more than 40%, How ever the quality condition will be changed. When the usage of rice increased some of the sensory attributes such as color, mouth feel and flavor become smooth. The basic challenge is to keep the saccharofication temperature between 65°C-67°C.when rice usage is increased the water ratio should be maintained well otherwise the temperature is increasing highly beyond the sacharofication temperature. The optimum rice proportion which was saccharafied by additional Beta amylase enzymes in malt was 50%. How ever that amount was not tried for manufacture a beer because that may deviate highly from the quality limits of Lion Larger.

5.2. Further Studies

Out of five beer brands which are manufacturing in the The Lion Beer Ceylon Limited , Rice is used as an adjunct for Lion Larger, Strong Beer and Carlsberg Special Brew. Other two brands are producing all malts only. Thus the rice usage can be improved in two of other brands of LBCL which are Strong beer and Carlsberg Special Brew. Now the LBCL Research and Development (R&D) division is trying to increase the rice usage in other two brands which they use rice.

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APPENDIX I

Triangular Test

Date:
....

Series
No:...

Name:...
......

---

1). Are two samples identical & one different. Yes ☐ No ☐

<u>If Yes:</u>
Which glass contains the single sample? Glass No. ☐

The identical samples are:..

Describe the
difference..
....
....
....
....

Comments:..
....
....
....
....

APPENDIX II

Triangular Test

(Used for 40% rice used beer analysis)

Name:...

Date:...

Product:...

Two of these products are Identical, One is different.

1. Taste the samples in the order indicated and identify the odd sample. Circle the number of the test sample which you decide is different.

314 347 381

2. Indicate the degree of difference between the duplicate samples and odd sample.

Slight	....
Moderate	....
Much	....
Extreme	....

3. Acceptability

Odd sample more acceptable	....
Duplicates more acceptable	....

4. Comments

....

....

....

....

....

APPENDIX III

Significance Table

Number of assessors	Paired Comparison test (Two -Sided)	Triangular test		
		5%	1%	0.1%
5	5	4	5	-
6	6	5	6	-
7	7	5	6	7
8	8	6	7	8
9	8	6	7	8
10	9	7	8	9
11	10	7	8	10
12	10	8	9	10
13	11	8	9	11
14	12	9	10	11
15	12	9	10	12
16	13	9	11	12
17	13	10	11	13
18	14	10	12	13
19	15	11	12	14
20	15	11	13	14
21	16	12	13	15
22	17	12	14	15
23	17	12	14	16
24	18	13	15	16
25	18	13	15	17
26	19	14	15	17
27	20	14	16	18
28	20	14	16	18
29	21	15	17	19
30	21	15	17	19
31	22	16	18	20
32	22	16	18	20

Source ISO 6658:1985(E)

APPENDIX IV

Sensory Evaluation Form (Rating Test)

Name:..
....

Date:..

Product Description: Larger type bottled beer.

Please assess each beer sample considering the Hedonic scale given below.

9-Like Extremely
8-Like very much
7-Like Moderately
6-Like slightly
5-Neither Like nor Dislike
4-Dislike Slightly
3-Dislike Moderately
2-Dislike Very Much
1-Dislike Extremely

Sample Code	Colour	Aroma	Mouth feel	Appearence	Bitterness	Overall Acceptability
267						
495						
653						
874						

Comments:...
....
....
....
....

APPENDIX V

Sensory Evaluation of 35% rice used beer.
Statistical Analysis (Friedman Test)

(1) Color

Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the color attribute.
H1: There is significant difference with the color attribute.

S = 18.75 DF = 12 P = 0.095
S = 20.55 DF = 12 P = 0.057 (adjusted for ties)

Grand median = 6.692

Decision rule:
Not reject Ho, Because P value >α value.
α=0.05
Interpretation
There is no sufficient evidence for a significant difference with the color attribute of each sample.

(2)Aroma

Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the aroma attribute.
H1: There is significant difference with the aroma attribute.

S = 17.76 DF = 12 P = 0.123
S = 18.75 DF = 12 P = 0.095 (adjusted for ties)

Grand median = 5.154

Decision rule:
Not reject Ho, Because P value >α value.
α=0.5

Interpretation
There is no sufficient evidence for a significant difference with the aroma attribute of each sample.

(3) Mouth feel

Friedman Test: Response versus Treatment blocked by Sample
S = 14.69 DF = 12 P = 0.259
S = 15.71 DF = 12 P = 0.205 (adjusted for ties)
Grand median = 5.000

Decision rule:
Not reject Ho, Because P value >α value.
α=0.05
Interpretation
There is no sufficient evidence for a significant difference with the mouth feel attribute of each sample.

Test Hypothesis:
Ho: There is no significant difference with the aroma attribute.
H1: There is significant difference with the aroma attribute.

S = 17.76 DF = 12 P = 0.123
S = 18.75 DF = 12 P = 0.095 (adjusted for ties)

Grand median = 5.154

Decision rule:
Not reject Ho, Because P value >α value.
α=0.5

Interpretation
There is no sufficient evidence for a significant difference with the aroma attribute of each sample.

(4) Appearance
Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the appearance attribute.
H1: There is significant difference with the appearance attribute.

S = 22.96 DF = 12 P = 0.028
S = 24.89 DF = 12 P = 0.015 (adjusted for ties)

Grand median = 6.308

Decision rule:
Reject Ho, Because P value <α value.
α=0.5

Interpretation
There is sufficient evidence for a significant difference with the appearance attribute of each sample.

(5) Bitterness
Friedman Test: Response versus Treatment blocked by Sample

S = 24.25 DF = 12 P = 0.019
S = 25.66 DF = 12 P = 0.012 (adjusted for ties)

Grand median = 5.846

Test Hypothesis:
Ho: There is no significant difference with the Bitterness attribute.
H1: There is significant difference with the Bitterness attribute.

S = 22.96 DF = 12 P = 0.028
S = 24.89 DF = 12 P = 0.015 (adjusted for ties)

Grand median = 6.308

Decision rule:
Reject Ho, Because P value <α value.
α=0.5

Interpretation

There is sufficient evidence for a significant difference with the bitterness attribute of each sample.

(6) Overall Acceptability

Friedman Test: Response versus Treatment blocked by Sample

S = 16.37 DF = 12 P = 0.175
S = 17.50 DF = 12 P = 0.132 (adjusted for ties)

Grand median = 5.846

Test Hypothesis:

Ho: There is no significant difference with the Overall acceptability.
H1: There is significant difference with the Overall acceptability.

S = 22.96 DF = 12 P = 0.028
S = 24.89 DF = 12 P = 0.015 (adjusted for ties)

Grand median = 6.308

Decision rule:

Do not Reject Ho, Because P value>α value.
α=0.5

Interpretation

There is no sufficient evidence for a significant difference with the overall acceptability.

APPENDIX VI

Sensory Evaluation of 40% rice used beer

Statistical Analysis (Friedman Test).

(1) Color

Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the color attribute.
H1: There is significant difference with the color attribute

S = 13.62 DF = 9 P = 0.136
S = 14.32 DF = 9 P = **0.111** (adjusted for ties)

Grand median = 6.763

Decision rule:
Not reject Ho, Because P value >α value.
α=0.05

Interpretation
There is no sufficient evidence for a significant difference with the color attribute of each sample.

(2) Aroma

Friedman Test: Response versus Treatment blocked by Sample
Test Hypothesis:
Ho: There is no significant difference with the aroma attribute.
H1: There is significant difference with the aroma attribute.

S = 10.94 DF = 9 P = 0.280
S = 11.57 DF = 9 P = 0.239 (adjusted for ties)

Grand median = 5.138

Decision rule:
Not reject Ho, Because P value >α value.
α=0.5

Interpretation
There is no sufficient evidence for a significant difference with the aroma attribute of each sample.

(3) Mouth feel

Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the mouth feel attribute.
H1: There is significant difference with the mouth feel attribute.

S = 18.82 DF = 9 P = 0.027
S = 20.80 DF = 9 P = 0.014 (adjusted for ties)
Grand median = 5.150
Decision rule:
Reject Ho, Because P value < α value.
α=0.5

Interpretation
There is sufficient evidence for a significant difference with the mouth feel attribute of each sample.

(5) Appearance
Friedman Test: Response versus Treatment blocked by Sample
Test Hypothesis:
Ho: There is no significant difference with the appearance attribute.
H1: There is significant difference with the appearance attribute.

S = 19.38 DF = 9 P = 0.022
S = 20.80 DF = 9 P = 0.014 (adjusted for ties)
Grand median = 6.700

Decision rule:
Reject Ho, Because P value < α value. (α=0.5)

Interpretation
There is sufficient evidence for a significant difference with the appearance attribute of each sample.

(6) Bitterness
Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the bitterness attribute.
H1: There is significant difference with the bitterness attribute.

S = 22.17 DF = 9 P = 0.008
S = 23.53 DF = 9 P = 0.005 (adjusted for ties)

Grand median = 5.900

Decision rule:
Reject Ho, Because P value < α value. (α=0.5)

Interpretation
There is sufficient evidence for a significant difference with the bitterness attribute of each sample.

(7) Overall acceptability
Friedman Test: Response versus Treatment blocked by Sample

Test Hypothesis:
Ho: There is no significant difference with the overall acceptability attribute.
H1: There is significant difference with the overall acceptability attribute.

S = 16.84 DF = 9 P = 0.051
S = 18.97 DF = 9 P = 0.025 (adjusted for ties)

Grand median = 5.550

Decision rule:
Reject Ho, Because P value < α value.(α=0.5)

Interpretation
There is sufficient evidence for a significant difference with the bitterness attribute of each sample.

APPENDIX VII

Analysis of Plato, pH and Iodine test of 35% rice incorporated trial of Lion Larger

Parameters \ Brew No		576	577	578	579	580	581
Iodine Test (+/-)	30 mins.	(-)	(-)	(-)	(-)	(-)	(-)
	35 mins	(-)	(-)	(-)	(-)	(-)	(-)
	40 mins	(-)	(-)	(-)	(-)	(-)	(-)
Lautering (plato)	25 hl	19.4	19.0	19.1	18.7	19.1	18.8
	40 hl	19.4	19.4	19.4	19.3	19.5	20.1
	55 hl	19.5	19.3	19.4	19.2	19.4	19.3
	70 hl	19.2	19.4	19.4	19.5	19.4	20.1
	85 hl	19.2	19.3	19.3	19.3	19.2	20.0
	100 hl	19.1	19.2	19.2	19.1	19.1	19.1
	120 hl	18.6	18.8	18.7	18.2	18.6	18.4
	140 hl	16.0	15.8	16.7	16.0	16.7	16.7
	160 hl	13.3	13.7	13.2	13.0	13.0	13.2
	180 hl	10.7	11.3	10.6	10.7	10.5	10.7
	200 hl	7.6	8.4	7.2	7.5	7.2	8.9
	220 hl	5.8	6.2	4.9	4.7	4.7	4.7
	240 hl	4.4	4.7	3.6	3.9	3.4	3.9
	260 hl	3.4	4.0	3.6	3.4	3.0	3.2
Spent Grain(Plato)		2.7	3.1	2.0	2.1	2.0	2.4
Pre Boil	Plato	13.8	14.0	13.7	13.5	13.7	13.8
	pH	5.53	5.40	5.43	5.51	5.40	5.42
Post Boil	Plato	15.0	14.9	14.6	14.5	14.6	14.3
	pH	5.44	5.30	5.41	5.36	5.0	5.35
Boiling Time(mins)		45 mins	45 mins	45 mins	45 mins	45 mins	45 mins
Cold Wort	Plato	14.00	14.30	14.50	14.50	14.50	14.40
	Iodine Test(+/-)	(-)	(-)	(-)	(-)	(-)	(-)
	Wort Volume(hl)	245	244	242	245	241	250

APPENDIX VIII
Brew House Analysis of 40% rice incorporated trial.

Parameters \ Brew No		606	607	608	609	610	611
Lautering (Plato)	25 hl	19.0	17.2	18.7	17.6	18.5	19.5
	40 hl	19.2	18.6	19.1	18.4	18.3	19.4
	55 hl	19.2	18.5	19.0	18.4	18.2	19.4
	70 hl	19.2	18.6	19.0	18.4	18.2	19.4
	85 hl	19.1	18.4	19.1	17.5	18.5	19.2
	100 hl	19.0	18.3	18.9	15.5	18.5	19.0
	120 hl	18.7	18.2	18.8	15.3	18.3	18.7
	140 hl	15.8	16.2	17.5	14.7	16.8	16.8
	160 hl	13.1	13.6	13.7	13.3	12.9	13.6
	180 hl	10.5	10.4	11.5	11.7	9.9	9.6
	200 hl	9.2	7.5	9.4	9.3	8.7	7.8
	220 hl	7.1	6.1	6.9	8.0	6.1	6.2
	240 hl	6.2	4.6	5.3	5.2	*	4.6
	260 hl	4.9	3.7	4.3	4.9	*	3.5
Spent Grain(Plato) (Digital meter reading)		4.8	3.6	3.0	2.7	3.0	2.9
Pre Boil	Plato	13.8	13.4	14.1	13.3	13.7	14.2
	pH	5.62	5.59	5.72	5.68	5.69	5.7
Post Boil	Plato	14.7	14.5	15.5	14.7	14.6	16
	pH	5.27	5.24	5.32	5.34	5.24	5.23
Cold wort	Plato	14.6	14.4	14.6	14.4	14.5	14.5
	pH (5.3+-0.2)	5.18	5.20	5.22	5.20	5.28	5.25
Colour	15+-1.5	15.3	13.5	13.7	13.5	13.9	14.1
BU	38+-4.0	36	34	34	34	36	35
Wort volume	(hl)	214	239	248	243	213	249
Cold wort Plato	(%)	14.5	14.4	14.7	14.4	14.5	14.5

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