USE OF SWEET TODDY (COCONUT SAP) AS A BEER ADJUNCT.

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Declaration

The research work described in this thesis was carried out exclusively by me at Lion Brewery Ceylon Limited under the supervision of Mr. Janaka Bandara (B.Sc.Eng, Dip.Brew) and Mr. Saman Perera (MBA, MSc, and Dip.Brew). A report on this thesis has not been submitted to any other university, for another degree.

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Dedication

Affectionately Dedicated to

My Parents

&

My Teachers.

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Abstract

Sweet toddy is the phloem sap of coconut tree (*Cocos nusifera*), with the composition of 16.5g/100ml of Sucrose, 17.5g/100ml of Total Solids, 0.4 g/100ml Ash, & 0.6g/100ml of Protein, etc. The physical characters of freshly gathered sweet toddy are, whitish in colour with sweet taste and pleasant odour. The responsible flavour components of toddy are, alcohols, glycerols, aldehydes, ketones, amino acids, proteins, carboxylic acids, dissolved gasses etc.

There are few toddy manufacturers in Sri Lanka, but their manufacturing process is not streamlined in scientific manner. Therefore the product efficiency and the quality are inconsistent. This is the effort of looking at toddy manufacturing process scientifically and, to check whether the sweet toddy could be able to ferment by brewing yeast.

Malt is the major source of starch, part of which can be supplemented by another carbohydrate source known as adjunct. Considering the cost aspects as well as the availability and composition of sweet toddy, this study was aimed to explore the feasibility of using toddy as brewing adjunct.

The key quality parameters of sweet toddy were tested, before, during and after fermentation, using beer analyzer and spectrophotometer mainly. The sensory evaluations of the final products were done by internationally validated tasters.

There was high H_2S production during fermentation, but the flavour was smoother with a pleasant odour than marketed toddy. High sweetness was resulted in toddy, fermented by brewing yeast than, that was done by wild yeast.

Brewing toddy in controlled conditions, result more smooth flavour and pleasant odour. Even sweet toddy can be fermented by brewing yeast but a complete fermentation was not performed. Moreover, Sweet toddy is an ideal adjunct for Lion Stout.

It is also expected to conduct a shelf life evaluation further.

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Abbreviations

LS	Lion Stout
LL	Lion Larger
CBSB	Carlsberg Special Brew
VDK	Vicinal diketones
BU	The unit of Bittersness

Chapter 01

Introduction

1.1 Introduction

There are few Toddy manufacturers available in Sri Lanka, but their products are not used efficiently & effectively as they haven't standardized their manufacturing process in scientific manner or, they have no proper manufacturing profile. Therefore the quality of product is not in satisfactory level. Also the product quality is inconsistence due to variation in processing technology. Still these problems haven't been eliminated by the manufacturers as the individuals & organizations are not actively involved in research in this area.

Bottled Toddy Manufacturers:

The names of bottled Toddy Manufactures, and the quantities they manufactured and the duties recovered from each of them are as follews:

Manufacturing Institution	Qty (Ltrs)	Duty (Rs. Cts)
Eagle Bottle Toddy Manufactory	487,055.626	116,893.33
Singha Bottle Toddy Manufactory	780,605.000	187,345.20
Commander Bottle Toddy Manufactory	255,378.750	61,691.05
New Commando Bottled Toddy Manufactory	182,682.500	43,819.80
Manori Lanka Bottled Toddy Manufactory	687,206.250	164,929.50
Three Lions Bottle Toddy Manufactory	137,626.250	33,030.30
Chankanai Palmyrah Development Co-operative	106,368.000	21,278.60
Horse Power Bottled Toddy Manufactory	239,661.000	57,518.70
Waymba Super Bottled Toddy Manufactory	264,641.250	63,513.90
Godlden Eagle Bottled Toddy Manufactory	389,673.750	93,521.70
Rio Marketing Bottled Toddy M factory	147,913.125	35,499.15
Empire Bottled Toddy Manufacto	126,117.500	30,268.20
Golden Eagle Bottled Toddy Manufactory	29,223.750	7,013.70
Total	**3,834,152.751**	**916,318.13**

Fig. 1.1. Bottled Toddy Manufacturers.

Source: Administration report for the excise commissioner general, (2004)

In addition, in beer manufacturing process, malt is the major source of starch, which is extracted by barley. The importation & raw material cost of malt is too high. Hence, adjuncts are used as alternative sources of starch. Rice, Maize, Sorghum & raw barley are normally used as beer adjuncts, but they have to be imported too. Therefore the cost of production is being increased. However, sweet toddy, which can be found easily in Sri Lanka, contains a large amount of sucrose. Since the sap contains sucrose, it can be used as an adjunct & may act as the wort solution too. So that, the final out come is expected to be different in taste & odour.

Fermentation is one of the oldest processing techniques in the world in obtaining alcohol. The food is processed by using natural microflora, & has been prepared and consumed for thousands of years and are strongly linked to culture and tradition of the community.
Fermentation is a process of converting one substance to another by means of microbiological agents, alcoholic fermentation of sugary palm saps obtained by tapping the inflorescences.

Fermentation is a relatively, efficiently low energy processing method, which increases the shelf life & decreases the refrigeration or other forms of food preservation technology.

There is a tremendous scope & potential for the use of micro-organisms towards meeting the growing world demand for food & beverages, through efficient utilization of available natural foods, (Stanton 1985). Most traditional fermented products are made by natural fermentation carried out in a non-sterile environment conditions cause a gradual selection of micro organisms responsible for the desired final product. If the processes are to be refined, with a view to production on a larger scale, it is essential to have a scientific understanding of the fermentation processes. It is essential with any fermentation to ensure that only the desired bacteria, yeasts or moulds start to multiply and grow on the substrate. This has the effect of suppressing other micro-organisms which may be either pathogenic and cause food poisoning or will generally spoil the fermentation process, resulting in an end-product which is neither expected nor desired, (Sugihara 1985).

Beer is a potable alcoholic beverage produced by cereals & malt which is flavored with hops. Even it is a refreshing drink with 3-8% of alcohol content. Four major ingredients of beer are Barley, Water, Hops, & Yeast. Malted barley is the magic ingredient for beer because it contains the enzymes necessary to convert starch in to fermentable sugars. Beer is about 90% of water, which is an important element in brewing. Hops provide the characteristic flavor & aroma, and act as a natural preservative to protect the beer from spoilage micro-organisms. In

brewing process, the fermentation is carried out by brewing Yeast varieties that are reacting on sucrose and convert it to Ethyl alcohol & $C0_2$, even contribute the flavor development too. In other words, "Malt is the soul of beer and yeast gives it life but the kiss of the hop is the consummation of that life."

Yeast fermentation

$C_6H_{12}O_6$	⟶	$2C_2H_5OH$	+	$2CO_2$
Glucose	yeast	ethyl alcohol	+	carbon dioxide

If the yeasts and bacteria exist together in a form is known as commensalisms. The acetobacter are dependent upon the yeasts to produce an easily oxidisable substance (ethyl alcohol). It is not possible to produce vinegar by the action of one type of micro-organism alone.

Bacterial oxidation

$C_2H_5OH + O_2$	⟶	$CH_3COOH +$	H_2O
Alcohol		acetic acid	Water

Sweet toddy is the phloem sap of coconut tree (*Cocos nusifera*), with the composition of 16.5g/100ml of Sucrose, 17.5g/100ml of Total Solids, 0.4 g/100ml Ash, & 0.6g/100ml of Protein, etc. In brewing process, vitamin B content will be gradually developed during fermentation, due to the yeast reaction (Fandialan et al., 1983).

Sweet toddy is fermented to toddy by wild yeast, and have a by product complexity too. Those by products may be affected to the off flavor & odor development. Therefore the uniqueness of product or the product consistency is changed and it may not be pleasant. Hence it is needed to preserve the sweet toddy, to delay the process when the fermentation is unnecessary. As a precaution, the process is to be conducted in controlled environment or, controlled yeast type has to be used. Heat sterilization & chemical preservation can be applied to preserve the sweet toddy. The major problem of toddy industry is the formation of Hydrogen Sulphide (H_2S) which is responsible for off flavor & odor development, in the final product (Kauyananda 1961).

1.2 Objectives

1. Brewing of toddy in more efficient basis, aiming of a smooth flavor & aroma.
2. Use the Sweet toddy as a beer adjunct.
3. To check whether the sweet toddy could be able to ferment by brewing yeast.
4. Comparative evaluation of sweet toddy & toddy composition

1.2.1 Specific objectives:

1. Establishment of a recipe for new products.
2. Establishment of critical parameters for toddy manufacturing.
3. Evaluation of the quality of product using quality control tests including sensory evaluation

Chapter 02

Literature Review

2.1 Introduction

Sweet toddy is a refreshing drink with pleasant sweet taste. This sap is tapped from the flowering spadix of coconut tree is mildly acidic, rich in sugar & vitamins. Apart from 10 -15 percent sugars, it contains necessary proteins vitamins & minerals which make it nutritious drink & also an excellent fermenting medium. It is used for variety of disorders & the hydropaths recommend its regular use for the preservation of normal health. The chemical composition of sweet toddy is such that it may be considered as a wholesome beverage from the nutritional point of view. In 1917 – Government analysts found more than 35 kinds of wild yeast & bacteria from distillery toddy (Kauyananda 1961).

2.2 Collection of sweet toddy

The sap as it is collected by the traditional method is not liked & consumed by the urban elites because of several contaminations. In fact, toddy is considered as low class drink, since several insects fall in to it during the collection period. This is an unfortunate situation and has to be changed radically. Even processing and bottling of toddy cannot be undertaken unless hygienic collection procedures are followed.

2.2.1 Collecting Methods

1. Traditional Methods
2. Improved Methods

2.2.1.1 Traditional Methods

When the phloem sap is collected, the pots are hanging directly on to the spathe. Mouth of the pot is not covered, keep as it is. The pot brings down twice a day.

In the traditional methods of collecting, it was found that the whole process is unhygienic, using unclean pots, contaminated lime, unclean hands, poor personal hygiene, permitting the entry of ants, honey bees, butterflies, etc., in to the pots during collection. To prevent the spoilage of

sweet toddy by micro organisms, lime (CaO) is used in high concentration (10 g). The sap with its lime odour and taste makes it less acceptable.

2.2.1.2 Improved Methods

a) In other countries, plastic pots are used which can be easily cleaned.

b) To prevent the entry of insects and to collect the sweet toddy hygienically, the pots are cleaned well and hung directly on to the spathe. Mouth is tied with polythene ducts of 250 gauge of thickness. A cylindrical duct is prepared by the heat sealing of a plastic sheet. One or two pinholes are made using pin to facilitate displacement of air during the collection of sap. One end of the duct is tied to the mouth o the pot and the other end is tied to the spathe, as shown in following figures (Banumathi et al. 1987).

Fig. 2.1. The pot tied with plastic duct

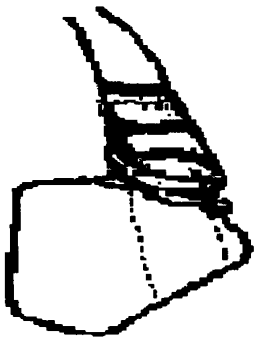

Fig. 2.2. The pot hanging on the spathe with the plastic duct.

The plastic duct prevents the entry of flies, honey bees cockroaches, lizards etc., and their falling in to the pots.

c) To prevent the entry of ants, castor oil is smeared with the index finger at the base of the spathe.

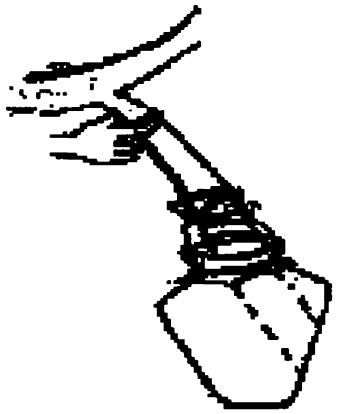

Fig. 2.3. Smearing castor oil at the base of the spathe, to prevent the crawling insects.

d) 7g of lime per approximately 1000ml of sap, Tannin containing materials like "Hal", Bark, and *Rhizophora muronata* is added to the pot before the collection of sweet toddy, to prevent the fermentation. But the addition of a large amount of lime is responsible for the strong odour, burning hot taste and for the turbidity.

2.3 Composition

2.3.1 Sweet Toddy

Table 2.1. Chemical composition of sweet toddy

Characteristics	Value	Units
Specific Gravity	1.058-1.077	g /ml (at 29^0C)
Total Solids	17.5	g /100ml
Acidity (as acetic acid)	0.2	g /100ml
Alcohol	Nil	
Ash	0.4	g /100ml
Sucrose	16.5	g /100ml
Invert Sugar	Trace	g /100ml
Proteins	0.6	g /100ml
pH	4.5-5.0	

(Source: Kauyananda, 1961)

2.3.2 Toddy

Table 2.2. Chemical composition of Toddy

Characteristic	Value	Units
Specific Gravity	1.01	g /ml (at 29^{0}C)
Total Solids	3.72	g /100ml
Acidity (as acetic acid)	0.68	g /100ml
Alcohol	6	% v/v
Ash	0.41	g /100ml
Sucrose	0.29	g /100ml
Invert Sugar	1.95	g /100ml
P^H	4.0	

(Source: Samanigo and Symons, 1970)

In addition to the constituents given in table 2 and 3, Toddy also contains vitamins, mineral salts and Flavour components in minor quantities.

Flavour Components of Toddy.

a) Higher alcohols
b) Glycerols
c) Aldehydes
d) Ketones
e) Amino acids
f) Proteins
g) Carboxylic acids
h) Dissolved gasses

Table 2.3. Composition of micro nutrients in Sweet Toddy and Fermented Toddy

Fresh Sap (g/100ml)		Fermented Sap (g/100ml)	
Nitrogen	0.033	Nitrogen	0.033-0.038
Phosphorus	0.026	Phosphoric acid (P_2O_5)	0.015-0.023
Calcium	0.002	Calcium Oxide (CaO)	0.006-0.0085
Magnesium	0.004	Potassium (K_2O)	0.144-0.203
Vitamin C	0.3	Manganese	44-66 µg/100ml

(Source: Kauyananda, 1961)

It should be noted that the Composition of marketed toddy does not always conform to the values given in table 2.3. It varies over a wide range.

It has been found that the Potassium & Vitamin C content remains unchanged through out the alcoholic fermentation, but Vitamin B content is gradually been increased during the fermentation process (Kauyananda 1961).

Table 2.4. Proximate Principles, Minerals, and Vitamins in Sweet Toddy & Fermented Toddy
(Values are per 100g. of edible portion)

Nutrient	Sweet Toddy	Fermented Toddy
Moisture (g)	84.7	97.6
Energy (kcal)	59	38
Proteins (g)	0.1	0.1
Fats (g)	0.3	0.3
Carbohydrates (g)	14.3	1.8
Calcium (mg)	150	-
Phosphorus (mg)	10	-
Iron (mg)	0.3	-
Vitamin A (mcg)	-	-
Carotene (mcg)	-	0
Thiamine (mcg)	-	10

Riboflavin (mcg)	40	10
Niacin (mg)	-	0.2
Vitamin C (mg)	-	-

(Source: Kauyananda, 1961)

2.4 Physical characterics of sweet toddy

Table 2.5. Sap after 15 hours of collection

Characteristics	Observation
Colour	Whitish
Taste	Sweet
Odour	Pleasant

(Source: Jayasundara et al. 2004)

2.5 Preservation

In sweet toddy preservation, there are 2 major types call

1. Primary Preservation
2. Secondary Preservation

2.5.1 Primary Preservation

a) Sweet toddy, if carefully collected in sterile glass vessels, will remain unfermented for a considerable time.

b) In addition to the sterilization of pots, a small quantity of slaked lime or Tannin containing materials like 'Hal' bark (*Verteria acuminta*) or *Rhizophora muronata* is added to inhibit the fermentation process.

- Tannin might help to clear toddy from albuminous impurities.
- 5g of powdered bark of *Vateria* per 100ml of sap will slightly retard the fermentation.

- The dose of lime which is to be used in the pot is generally 0.2% - 0.3% of lime of the weight of juice. It will create a medium of pH 9.5 – 10 which the micro organisms cannot be survived. In addition, Enzyme Invertase (Sucrase) is inactivated at this pH. Therefore sucrase in the sap will be remains unhydrolyzed

c) Pot is sterilized or smoked before liming.

d) Collection of sweet toddy to polythene lined pot is also an effective method.

e) Minimize the collection time period. It should not exceed 20-24 hours.

f) Since sun shine tends to promote fermentation, juice should be brought down from the tree in the early hours of morning, and processing should commence without delay.

g) 0.2% of Benzoic acid will necessarily, completely inhibit both acetic & alcoholic fermentation of sweet toddy.

h) 10-60 ppm sulphanil amide or Methyl, Propyl, Putryl esters of p-hydroxy benzoic acid are also promising preservatives for sweet toddy.

2.5.2 Secondary preservation

If the sap is to be used after some time (eg: as a drink), secondary preservation is needed.
Major preservation types are

- Chemical preservation
- Thermal preservation

2.5.2.1 Chemical preservation

Chemical food preservatives permissible for use under the food and Drugs Act were found ineffective at there maximum concentrations in preserving the product.

- Sodium metabisulphite (SMS)
- Diethyl pyrocaronate
- Sorbic acid is used as chemical preservatives.

- Tannins are recommended to add to inhibit the growth of undesirable yeast and bacteria.

The low yield of ethanol and off flavours of coconut toddy could be solved by the addition of Sodium metabisulphite up to 200mg/l a it suppresses the non ethanol producing microorganisms and permit ethanol production by pure yeast cultures to different extends under controlled conditions (Samarajeewa et al. 1985).

The chemical which had been used are no longer allowed by pure food regulations, while the use of 70 ppm of sulphur dioxide which is allowed by the regulations has been found inadequate to arrest the fermentation at any particular stage.

Sodium metabisulphite (SMS) is not an effective preservative for direct consumption of sweet toddy, which is at P^H 4.5. Concentration of sulphite is to be needed to suppress the micro floral activities (up to 2000ppm); would be excessive for human consumption.

Disadvantage of pyrocaronate is that it imparts a residual pungent odour.

2.5.2.2Thermal preservation

The method of Heat sterilization was found to be most satisfactory in destroying the micro-organisms and enzymes (Mohandas 1974).

- Heat sterilization at 80 ^{0}C
- Pasteurization at 70 ^{0}C for 30min
- Flash heating at 75 ^{0}C for 15 min are effective preservation techniques.
- The critical temperature for batch pasteurization is 60 ^{0}C. It ca be vary within 65 – 85 ^{0}C with the heating time of 15 – 45 min.

2.6 Sap Fermentation

2.6.1 Microbiology

- Unfermented fresh coconut sap normally contains 10^5 - 5×10^6 yeast cells/ml.
- The required initial cell concentration for sweet toddy fermentation is 10^6 cells/ml.
- When after the fermentation occur, the fermented toddy contains nearly 10^7 - 10^8 cells/ml.

The cell count changes of two major collecting methods are summarized in the following table.

Table 2.6. Yeast cell count in the traditional and improved methods of collecting sweet toddy.

Trial No.	Time	Traditional Method(Cells/ml)	Improved Method (Cells/ml)
1	0hr	25×10^7	3×10^7
	4hr	121×10^7	6×10^7
2	0hr	47×10^7	5×10^7
	4hr	114×10^7	14×10^7

(source: Banumathi et al.1987)

The types of micro organisms present in a fermenting pot could vary as they are contributed from the environment. The micro organisms have the potential of producing many chemical compounds in addition to ethanol. Some of them produced considerable quantities, cause marked changes in the flavour of sap. Many are produced in traces. As a result, the qualities of the fermented sap vary widely depending on the combination of micro organisms active in the pot. (Theivendirajah et al. 1979).

The survival of micro-organisms in fermented coconut toddy with temperature is given in following graph.

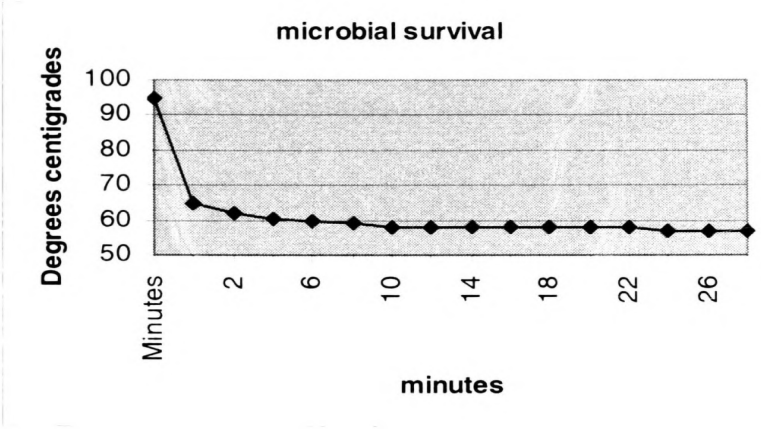

Fig. 2.4. The effect of Temperature on yeast Survival
(Source: Jeyaraj 1961)

2.6.2 Factors Affecting the Alcohol Production

The alcohol production by different yeast strains resulting different values. Some yeast strains take lower time to reach the maximum levels of alcohol, but some are taken more time to complete the fermentation. The longer duration taken for complete the fermentation may be attributed by the factors such as

1. Number of yeast cells at the start of the fermentation
2. The pH of the sap
3. Sugar concentration
4. Mineral salts
5. Sodium metabisulphite (SMS)

Experiments were carried out to test the effect of the above mentioned factors on sweet toddy with few isolated strains.

The effect of initial cell number for the alcohol production has been checked by using different cell concentrations of the same yeast strain. The results are shown in following figures.

2.6.2.1 Initial cell count

The results are shown that higher the cell number, lower the fermentation time.

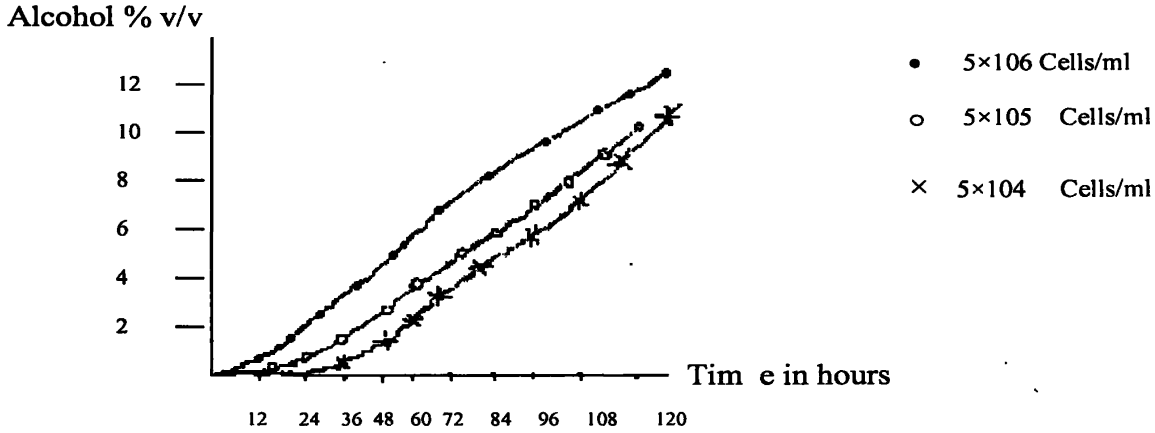

Fig. 2.5. The effect of initial yeast cell number on alcohol production, using different concentrations of an isolated yeast strain.

Table 2.7. The effect of different initial cell concentrations of an isolated yeast strain on alcohol production in coconut sweet toddy.

Time of fermentation in hours	Alcohol produced (percent by volume) under different initial yeast concentrations in cells/ml				
	1.2×10^4	1.2×10^5	1.2×10^6	1.2×10^7	1.2×10^8
12	0.00	0.10	0.70	1.70	4.80
24	0.50	1.40	2.65	3.95	5.80
36	1.60	2.75	4.00	5.50	7.85
48	3.35	4.25	5.75	7.60	9.70
60	4.80	5.90	6.80	9.30	9.80
72	6.50	7.45	8.10	9.65	9.75
96	8.60	9.40	9.70	9.70	9.75

(Source: Theivendirajah et al. 1979)

2.6.2.2 Hydrogen ion concentration or pH

The literature shows that the three isolated yeasts strains could be able to tolerate a wide range of pH values, but the optimum pH was found to be between 4.0 -5.5.

Table 2.8. The effect of Ph on alcohol production in coconut sweet toddy.

Yeast strain	Alcohol after 48 hrs. of fermentation under different pHs.								
	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
P4	4.90	5.90	6.20	6.20	6.45	6.60	5.30	4.00	3.70
K4	4.60	5.395	6.40	6.30	6.50	6.30	53.40	4.20	3.60
C3	4.85	6.00	6.35	6.10	5.95	6.40	5.90	5.00	3.80

(Source: Theivendirajah et al. 1979)

2.6.2.3 Concentration of sugar

The studies have shown that sweet toddy samples with higher sugar concentrations take relatively more time to complete the fermentation by yeast than the ones with low sugar concentrations. The experimental results are shown below.

Table 2.9. The effect of concentrations of sugar on alcohol production in coconut sweet toddy.

Time of fermentation in hours	Alcohol production under different initial sugar concentrations				
	13%	15%	17%	19%	21%
24	6.0	7.40	7.85	7.90	8.00
36	7.50	8.30	9.30	9.80	10.00
48	7.60	8.80	9.95	10.40	10.90
60	7.60	8.80	9.90	10.90	11.90

(Source: Theivendirajah et al. 1979)

2.6.2.4 Mineral salts

The addition of ammonium salts in the form of ammonium chloride and ammonium sulphate has been found to increase the rate and efficiency of fermentation by enhanced utilization of sugars available in toddy. Hence these salts were added to sweet toddy and the rate of fermentation has been compared with that of a control, using the same yeast strain. The average increase in the total yield of ethanol is 13% after addition of Ammonium salts.

Table 2.10. The effect of inorganic salts on the fermentation of coconut sap

Treatment	Percentage of Alcohol in			
	24 hrs	48 hrs	72 hrs	96 hrs
Sweet toddy *	3.10	5.10	7.05	6.95
Sweet toddy + 0.005% Ammonium chloride	4.70	7.85	8.20	8.10
Sweet toddy + 0.1% Ammonium chloride	4.90	8.00	8.00	8.05
Sweet toddy + 0.05% Ammonium sulphate	5.00	7.85	8.10	8.10
Sweet toddy + 0.1% Ammonium sulphate	4.95	7.95	8.00	8.00
Sweet toddy + 0.025% Magnesium sulphate	3.30	5.90	7.10	7.20
Sweet toddy + 0.05% Ammonium chloride + 0.025% Magnesium sulphate	5.20	7.80	8.05	8.10

*Sweet toddy sample contains 14% sugar by weight.

(Source: Theivendirajah et al. 1979)

2.6.2.5 Sodium metabisulphite (SMS)

The addition up to 200mgl^{-1} of Sodium metabisulphite to coconut inflorescence sap has been found to suppress the non-ethanol producing micro flora and permit to ethanol production by pure yeast cultures to different extends. At matabisulphite concentration is higher than 150 mgl^{-1} the ethanol yields from sap, fermented with a natural inoculums, increased under laboratory conditions. But if the SMS concentration is higher than 200 mgl^{-1}, it will result the off flavour or objectionable odour in the sap, and may be human hazardous too (Samarajeewa et al.1985).

2.7 Reactions and Changes during Fermentation

Under conditions are normal for the collected sweet toddy, very rapid changes take place in the sugary solution. The overall changes brought about may be summarized thus:

2.7.1 Chemical Changes

Sweet toddy as it obtained at the foot of the tree has pH around 7 and the sugar content is about 15%.

a) The pH of coconut sap commenced to drop by the fourth hour, showed a rapid drop between 6-10 hours, and reached a constant value of 3.8 – 4.0 in a day.

b) The drop in sugar content occurs in two stages. The total sugar drop by about 2% and remain constant for about 15 hours before a second drop, which commenced around the 30th hour.

The second drop in the sugar content is due to the conversion of sugar in to alcohol. The invert sugar reached the maximum in about 30 hours. No alcohol is produced in first 20 hours. Alcohol is produced rapidly after 30 hours, reaching the maximum in about 5 days. The alcohol is dropped after the 5th day due to acetification (Fig. 2.5). (Vidanapathirana et al. 1983).

2.7.1.1 The responsible chemical reactions.

1. $C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{Invertase}} 2C_6H_{12}O_6$

   Sucrose (16.5%) — Yeast — Invert Sugar (18.3%)

2. $C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2C_2H_5OH + CO_2$

   Alcohol (8.9%) — Carbon dioxide

3. $C_2H_5OH + O_2 \xrightarrow{\text{Acetic bacteria}} CH_3COOH + H_2O$

   Acetic acid

4. $CH_3COOH + 2O_2 \xrightarrow{\text{Acetic bacteria}} 2CO_2 + H_2O$

The first two processes occur during the fermentation of the sugar to alcohol, while the later occur during further fermentation of alcohol.

The equations given are the sum totals of a number of enzyme catalyzed reactions, while a number of side reactions bringing about the formation of other compounds in smaller quantities are not shown. Also, some strains of micro-organisms are poisoned by the products formed by them.

In addition part off the energy in the sugar is used up for the growth of yeast and other micro-organisms, so that the theoretical yields are never obtained in practice. It is usual to expect an efficiency of 90% in controlled fermentations using selected cultures of micro-organisms.

Alcoholization is normally complete in 12- 24 hours after collection of partially fermented toddy from the tree. During this period, both alcohol content and the acidity are increased. This is followed by acetification of the toddy to vinegar and then by oxidation of acetic acid.

Regulations prohibit the sale of toddy containing over 0.6% acetic acid.

2.7.2 Physical Changes

At the start, as the sap began to ooze out, it is a yellowish brown or whitish brown in colour and appears as a clear liquid. And also a pleasant flavourd, Semi sweet drink with an alcohol content of 4-6%. But when it is fermented totally, it may consist with high alcohol content and somewhat sour taste.

During the alcoholic fermentation, yeasts grow and multiply. The yeast cells are rich in high-grade proteins, amino acids and vitamins in B- complex. A portion of these compounds is dissolved in toddy but most of it is found within the yeast cells. Presence of these cells which cause the characteristic cloudiness of fermenting toddy and for the most of sediments as well (Jeyaraj, 1961).

The first phase of the fermentation is dominated by bacteria about 15-20 hours and a rapid drop of pH occurs, while the specific gravity is sharply dropped simultaneously.

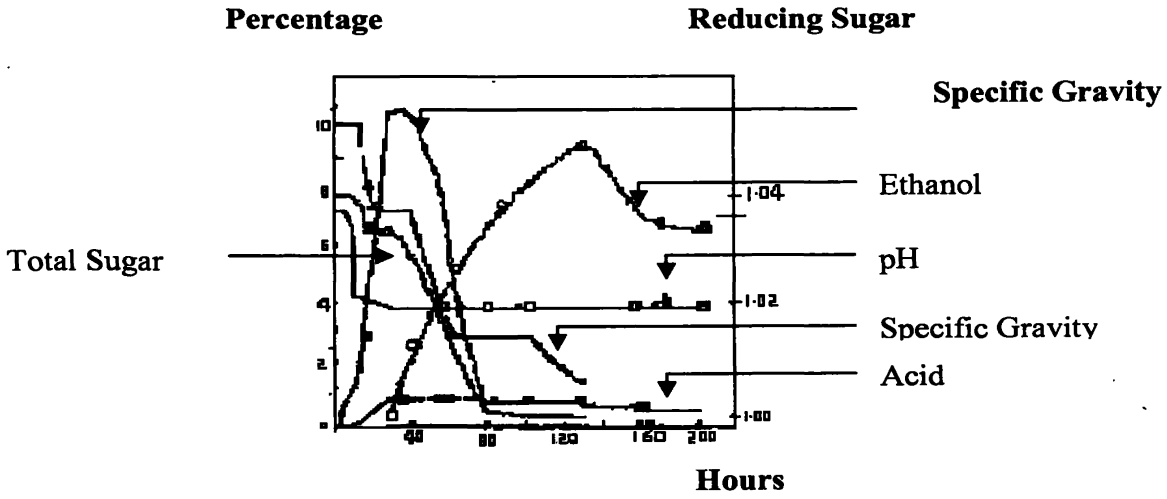

Fig. 2.6. Changes in Physical and Chemical parameters in coconut sap during fermentation

2.8 Bottling and keeping quality of sweet toddy.

a) The sweet toddy, which was subjected to a primary preservation in polythene lined pots and a secondary preservation after bottling (sterilized at 80^0C for 20 min) will show a good keeping quality even after 6 months; from the date of bottling.

b) Presence of trace of S^{-2} (sulphides) 50 parts par bottle and small quantities of acids in bottled sweet toddy, some inhibitory action could be observed by Citrate pyridoxal phosphate on the fermentation of Hydrogen sulphide (H_2S) in sweet toddy.

c) Keeping a sufficient head space of the bottle is also a critical point of retardation the off flavour development.

d) Some times the effervescence may occur even after pasteurization. This phenomenon can be imparted to preserved toddy, by bottling it under carbon dioxide under pressure.

e) Spontaneous chemical reactions after bottling, which can be given flavours, can be controlled by giving an adequate heat treatment after bottling.

f) Fresh toddy was found to have a common "bitter" after- flavour, pasteurization does not remove it. Chilling of pasteurized toddy was found to improve the flavour positively.

2.9 Techniques used for identification of key parameters.

2.9.1 Spectrophotometry

Spectrophotometers are a standard research tool used worldwide. A spectrophotometer is employed to measure the amount of light that a sample absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector.

If a beam of light is monitored to shine through a sample containing a substance that can absorb one of the beam's wavelengths, a plot of the amount of light absorbed versus the wavelength can be obtained. This plot is known as an absorption spectrum, and shows which particular wavelengths of light a chemical species can absorb.

When a light beam of a certain wavelength (l) and initial intensity (I_0) is shined through an absorbing sample contained in a spectrophotometer cell, the intensity of the light beam *transmitted* through the sample (I_t) is dependent on three factors. The first factor is whether the sample will absorb light at that wavelength. The second is the amount of sample which the light must pass through or, the *cell width* (b). The third factor is the *concentration of the absorbing species* in the sample solution (C). The fraction of light transmitted, or transmittance (T), is defined as the following:

$$T = \frac{I_t}{I_0}$$

This equation when written in terms of how much light is absorbed by the sample, a new term, *absorbance* (A) is defined as follows:

$$A = \log (1/T) = -\log T$$

(Shrewsbury, 1996).

2.9.2 Beer analyzer

2.9.2.1 Principle calculations of key parameters.

a) Density

The density is calculated from the oscillation period of the density measuring cell of the analyzer, by its own.

$$\text{Density} = A * P^2 - B$$

A, B : Calibration constants at 20 ^{0}C

P : Oscillation period of the density measuring cell

b) %A v/v = %Alcohol volume/volume

$$\%A\ v/v = \frac{\%A\ w/w * \text{density}_{beer}}{\text{density}_{Alcohol}}$$

$\text{density}_{Alcohol} = 0.78924\ g/cm^3$

c) % E real = % al extract weight/weight

The calculation of % E real is done, using a function of density and sound number.

$$\text{Sound number} = \frac{\text{sound velocity} - \text{sound velocity}_{water}}{\text{sound velocity}_{water}}$$

$\text{Sound velocity}_{water}$ at 20 ^{0}C = 1482.657 M/S

d) kcal/kg = "calories"

kcal/kg = % E orig * 36.4

The calculation is done according to the STEINER formula. (Steiner, 1963).

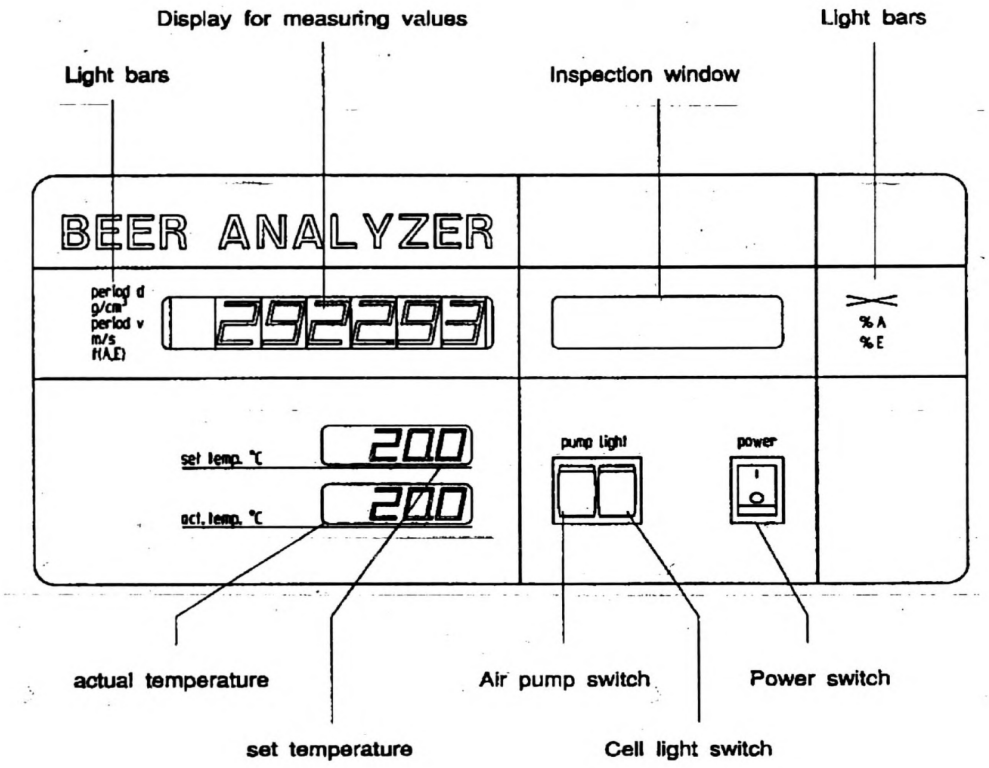

Fig. 2.7. The front side of the analyzer

2.9.3 Plato meter

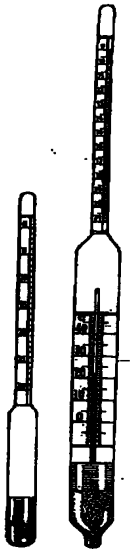

Fig. 2.8. The front side of the Plato meter

2.10 Sensory Evaluation

Aroma

Aroma is the best assessed when the beer is freshly poured from the bottle into the tasting glass. So, the impressions should be noted down immediately. The glass should be closed with tasters hand; a swirl is to be given and, then one good strong sniff should be given. Finally the sample has to be inhaled deeply.

Appearance

The glass has to be held up against a strong light and, the appearance (clarity) and colour at leisure should be evaluated.

Tasting

Tasting comes last because it is the best and most important part of the evaluation. One small sample has to be taken in to mouth and rolled around slowly. Then after, it is to be swallowed. Base your impressions on this. To refresh the memory on the beer, let time pass and the taste buds can be refreshed with water.

Mouth aroma

Beer aroma shows itself twice,

- once when you take a sniff (nose aroma) and
- Secondly when the beer is entrapped in the mouth and the aromatics pass to the Olfactive zone via the anterior nasal passage.

Mouth aroma indicates that specific flavors that are responsible for beer.

Overall Balance

Balance is probably the most fundamental characteristic of any beer. The expected balance differs from style to style and it is crucial that a reputable taster correctly interprets the required balance for each style of beer. Beer should be always tasted bitter and sweet together in different proportions according to the brewer's interpretation of style or unique design. When the formal evaluation is over and when after the beers are scored, forget the entire technical ramble and just enjoy what's on offer for what it is.

2.11 VDK (Vicinal diketones)

VDK is for Vicinal diketones, a substance which is secreted by yeast cells, during the fermentation process. An off flavour development can be occur due to this chemical compound, when it exceeds the threshold level. Threshold level for human tongue is 0.15. Distillation method can be used to measure the amount of VDK in the fermented sample.

Chapter 03

Materials and Methodology

3.1 Preparation of sweet toddy before processing

Sap was first examined for taste, smell, pH and Plato. Then it was filtered using a strainer to remove the coarser impurities before it was used for the experiment.

3.1.1 Materials and equipments

- Sterilized container
- Muslin cloth
- Sterilized bottles and crowns
- A basket
- A funnel
- Pasteurizer

3.1.2 Method

a) The sap was brought to the lab using a container which was sterilized by per acetic acid. Then the pure sap was filtered in to a cleaned basket using a muslin cloth. "A" part of the filtrate was separated for initial tests and the rest was filled in to sterilized bottles up to 2/3 volume of it. The bottles were crowned as soon as possible after filling.

b) Separated "B" part of the fresh sap was kept as un-pasteurized for further steps and the remaining quantity was pasteurized using pasteurization unit.

c) Finally, both pasteurized and un-pasteurized samples were labeled, and store in a cold room.

3.2 Trial 1.
Fermenting sap as it is

3.2.1 Common Materials and equipments for all the Trials

- Unfermented Sweet Toddy
- Flasks (2l)
- Cotton wool
- Plato meter
- PH meter (electronic)
- Beer Analyzer
- GAF filters
- Kieselguhr (diatomaceous earth)
- Haemocytometer
- Electronic microscope

3.2.2 Method (common in entire process)

Sap – before fermentation

Steps

1.1 The Separated sap, part "A" was used to check the following parameters of fresh sweet toddy.

1. Plato
2. pH
3. Density
4. Alcohol
5. Odor &
6. Flavor

1.2 A cell count was done to calculate the initial number of yeast cells in the sample.

1.3 A selected volume of sap was allowed to ferment as it is, in 2L well cleaned flasks. The flasks were sealed by cotton wool and the two samples were stored at ambient temperature.

Sap- During fermentation

1.4 Following chemical and physical parameters were measured daily, by using Beer analyzer.

- Alcohol % v/v
- % E real (Plato)
- % Fermentation
- Density
- Calories

Final pH of the sample was measured by electronic pH meter.

Sap- After fermentation

1.5 a) Step 1.1 was proceeded again.

b) The resultant solution was filtered using Kieselguhr (diatomaceous earth) and the GAF filters.

c) Finally, the filtrate was filled in to clean bottles. Then they were sealed by crowns, and labeled immediately. After this step, the samples were pasteurized using pasteurizing unit and stored in the refrigerator until the samples are subjected to sensory evaluation.

3.3 Cell count method

3.3.1 Materials and equipments

- Electric microscope
- Haemocytometer
- Beakers (25 ml)
- Dropper 1 ml
- Tissue papers
- Calculator

3.3.2 Method

a) First the haemocytometer and the cover slip were wiped using paper serviettes.
b) Then a small volume of the sample was taken out using 1ml Dropper, and an adequate amount of that was placed on the haemocytometer through the edges of cover slip.
c) Number of cells was counted and multiplied by the factor of 0.0625

3.4 Preparation of the sample for beer analyzer

3.4.1 Materials and equipments

- Flask (1L)
- Holders
- Funnels
- Filter papers
- Round bottom flasks
- Beer analyzer
- Caps and cells (sample tubes)

3.4.2 Method

a) After assembling the holder, funnel, filter papers and the round bottom flask, the toddy sample was degassed using 1l flask and was poured on to the filter papers.
b) Secondly, two cells of beer analyzer were filled with the filtrate and covered by caps.
c) The machine was started and the following parameters were measured.

- % Alcohol v/v
- % E real (Plato)
- % Fermentation
- Density
- Calories

3.5 Trial 2.
Fermentation of Pasteurized sap in controlled environment

3.5.1 Materials and equipments

- Unfermented Sweet Toddy
- Flasks (2l)
- Cotton wool
- Plato meter
- pH meter (electronic)
- Beer Analyzer
- GAF filters
- Kieselguhr (diatomaceous earth)
- Haemocytometer
- Electronic microscope
- Dropper 1ml

3.5.2 Method

2.1 Before fermentation

Steps 1.1 & 1.2 were conducted for unfermented sap and the samples were stored in cold room where the controlled conditions are maintained.

2.2 During the fermentation

All the parameters in step 1.1 and 1.2 were measured daily.

2.3 After fermentation

Same procedure in step 1.5 was followed.

3.6 Trial 3.

Pasteurized sap with Lion Larger Yeast

3.6.1 Additional Materials and equipments

- Autoclave
- Aluminium foil
- Lion Larger yeast
- Cold room

3.6.2 Method

3.1 Before fermentation

a) Step 1.1 was carried out again.

b) A calculated amount of Lion Yeast was inoculated to the sap and the flask was covered by a cotton cap.

c) Then the top part of the flask was covered using aluminium foil, in a way that aluminium foil covers the cotton cap also. Samples were stored in a cold room at controlled temperature (12 ^{0}C) and other conditions.

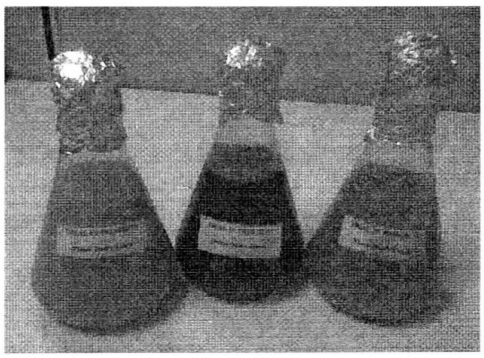

Fig. 3.1. The way of fermentation vessels are assembled

3.2 During the fermentation

The parameters in step 1.1 and 1.2 were measured daily.

3.3 After fermentation

Same procedure in step 1.5 was followed.

3.7 Yeast Pitching Rate

$$\left[\frac{\text{FV vol.} \times \text{Rate}}{\text{Cell count} \times \text{Viability}} \right] \times 100$$

FV volume of the fermented vessel

Yeast Pitching Rate

Cell count of the selected yeast samp le

Viability of he yeast sample

3.8 Trial 4.

Fermentation of Pasteurized sap with Lion Stout wort solution

3.8.1 Additional Materials and equipments

- Autoclave
- Aluminium foil
- Lion Larger yeast
- Lion Stout wort solution
- Cool room
- Spectrophotometer
- Distillation Apparatus

3.8.2 Method

4.1 Before fermentation

a) After proceeding step 1.1 and 1.2, pre determined quantity of Lion Stout wort was blended with pasteurized sweet toddy and, a calculated amount of Lion Larger yeast was added to the samples.

b) Steps in trial 3.1.c) was conducted for these samples also and, they were allowed to ferment.

4.2 During the fermentation

The step 1.4 was carried out and the key parameters were measured.

4.3 After fermentation

Same procedural steps in 1.5, were followed.

3.9 Trial 5

Fermentation of Pasteurized sap with Carlsberg Special Brew wort solution

3.9.1 Additional Materials and equipments

- Autoclave
- Aluminium foil
- Carlsberg yeast
- Carlsberg special brew wort solution
- Cold room
- Spectrophotometer
- Distillation Apparatus

3.9.2 Method

Same procedural steps as Trial 4, were conducted with

- Carlsberg Yeast, as a substitution for Lion yeast and
- Carlsberg Special wort, as a substitution for Lion wort solution.

3.10 Trial 6

Fermentation of Pasteurized sap with Lion Larger wort solution

3.10.1 Additional Materials and equipments

- Autoclave
- Aluminium foil
- Lion Larger yeast
- Lion Larger wort solution
- Cold room
- Spectrophotometer
- Distillation Apparatus

3.10.2 Method

Same procedural steps as Trial 4, were carried out with

- Lion yeast and
- Lion Larger wort solution.

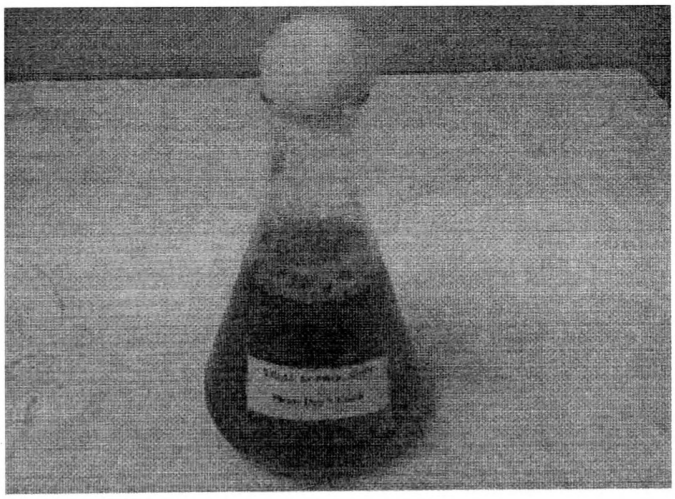

Fig. 3.2. Appearance of the blended sample

3.11 Trial 7.

Pasteurized Sweet toddy processing as a wort solution

3.11.1 Additional Materials and equipments

- Autoclave
- Aluminium foil
- Lion Larger yeast
- Hop extract
- Caramel
- Cold room
- Spectrophotometer
- Distillation Apparatus

3.11.2 Method

7.1 Before fermentation

After proceeding step 1.1 and 1.2, all the ingredients namely,

- Sweet toddy
- Hop extract
- Caramel

were blended together according to Lion larger proportions and the samples were fermented according to the steps followed in Trial 3.1.c .

Two separate sets of samples were prepared using above ingredients.

1. Sweet toddy + Stout wort + Caramel
2. Sweet toddy + Stout wort + Caramel + Hop extract

7.2 During the fermentation

The parameters in step 1.1 and 1.2 were measured daily.

7.3 After fermentation

Same procedure in step 1.5 was carried out.

When the fermentation is completed, the samples were filtered using both GAF filters and Kieselguhr (diatomaceous earth) and filled in to bottles leaving a head space. Then the filled bottles were carbonated using a carbonation unit.

The bottles were crowned immediately after filling, and were pasteurized by using pasteurization unit.

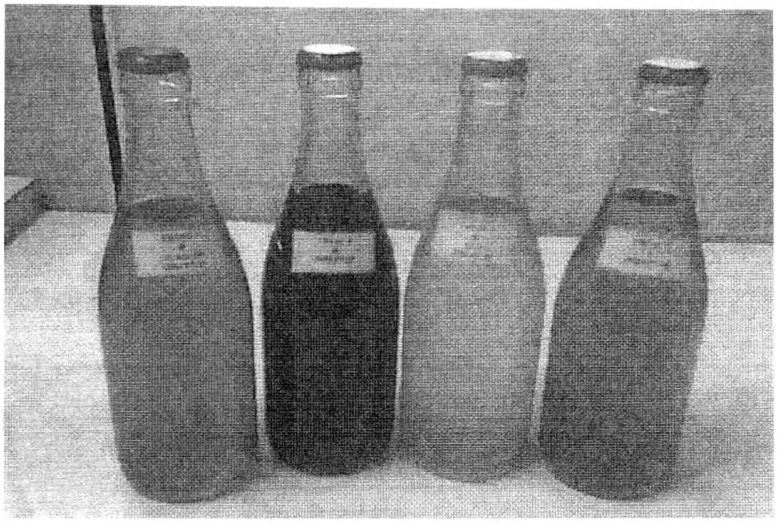

Fig. 3.3. The Final appearance of blended samples

Then after, the bottles were stored in a refrigerator.

Finally, the products were tasted by tasting experts.

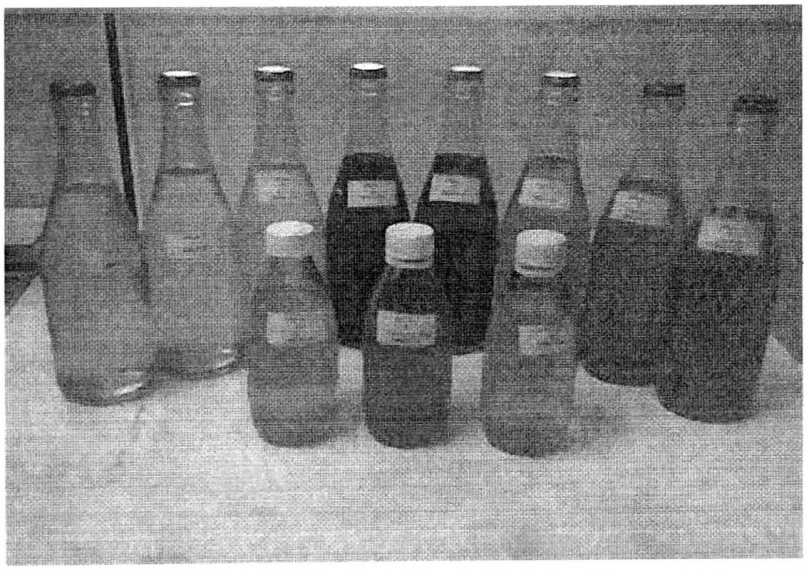

Fig. 3.4. The appearance of Final products

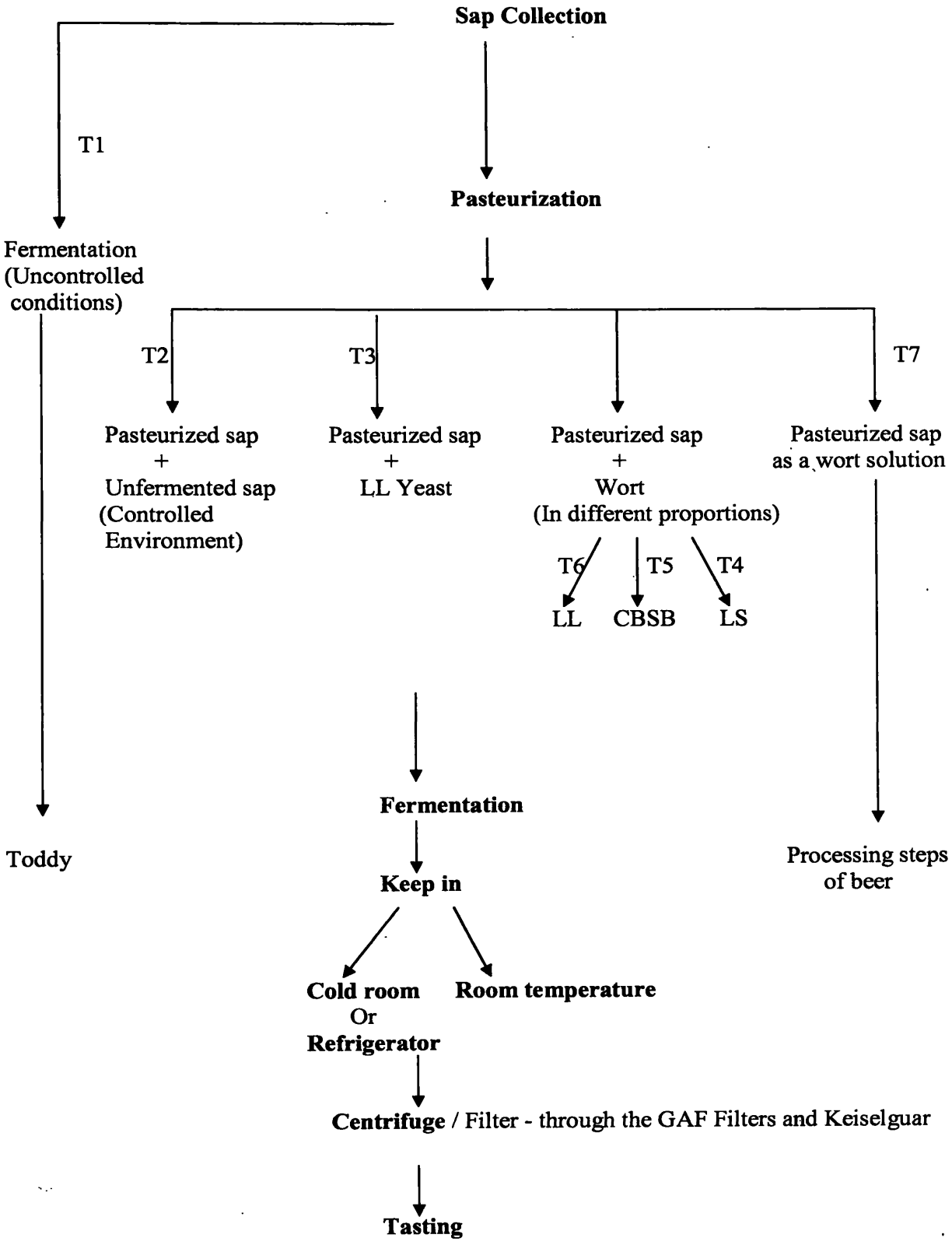

Fig. 3.1. Flow Diagram for the procedure followed in the methodology

3.12 Sensory Evaluation

3.12.1 Materials and equipments

- Beer glasses
- Light beam
- Cream crackers

3.12.2 Method

Aroma

Aroma is the best assessed when the beer is freshly poured from the bottle into the tasting glass. So, the impressions were noted down immediately. The glass was closed with tasters hand; a swirl was given to it, then one good strong sniff was given. Finally the sample was inhaled deeply.

Appearance

the glass was he ld up against a strong light and, the appearance (clarity) and colour at leisure were evaluated.

Tasting

The best and most important part of the evaluation is this. Hence it was done at the last. One small sample was taken to mouth and rolled around slowly. Then after, it was swallowed. Base your impressions on this. To refresh the memory on the beer, let time pass and the taste buds were refreshed with water.

Mouth aroma

Mouth aroma indicates that specific flavors that are responsible for beer.

Overall Balance

The bitterness and sweetness together was tasted in different proportions according to the brewer's interpretation of style or unique design.

Chapter 4

Results and Discussion

TRIAL	Sample		Date	Time	Description	Conditions	Observations	Plato (°P)	PH	Alcohol(v/v)%	Density (g/cm^3)	Plate count	Ferment r (%)	Calories (kcal/kg)	Odour	Flavour	Coments
			2006.06.14		pure sap		Sedimentation,Off white in colour, Bees & Flies were in the sap,	13.2	3.7	2.2	1.05	7.5×10^6	2.3	Did not read	A pleasent smell with a little alcoholic odour	Sweety, Sour	
1			6.15		Unpasteurized Sap												
Fermenting As It Is	A																
		1.1		4.30pm	Sap Before Fermentation			13.2	3.7	2.8	1.05	7.5×10^5	2.6	Did not read			
		1.4	6.16		Sap During Fermentation	Room											
				10.30am		Temperature (25^0C)	No air bubles,No foams,When it is shaking a little amount of foams were given, Sedimetation was appeared on the bottem of the flask.	12.7	3.7	3.2	1.05	10×10^6	4.5	528			
			6.17	10.30am			No change	12.2	3.7	4.6	1.05	12×10^6	6.5	537			
			6.18	10.30am			No change	11.3	3.7	4.9	1.05	18×10^6	8.3	557			
			6.19	10.30am			Air boubles were given slowly.A certain amount of foams were observed,Sedimentation was appeared on the bottem of the flask.	10.9	3.6	5.4	1.04	20×10^6	20.6	568	A strong toddy smell	Low Sweetness little soury	Too early to comment
			6.20	10.30am			more forms were appeared than yesterday Air boubles were expelled rapidly,	8.2	3.6	6.2	1.02	31.9×10^6	47.7	578	A strong odour with ester smell (Isomyle Acetate)		
			6.21	10.30am			The foam amount was retarded, No colour change, A strong toddy smell was given.	6.2	3.6	7.2	1.01	55×10^5	67.2	580	A strong odour with ester smell		
			6.22	10.30am			The foams were dissapeared. The boubling rate was lowered than 20th, but on the same day it was higher than trial 5. Sedimentation was appeared on the bottem of the flask. When it was shaking high amount of foams were given. A strong toddy odour & H_2S was felt.	4.3	3.6	8	1	19.4×10^6	79.8	586	A strong odour with ester smell		
		1.5	6.23	10.30am	Sap After Fermentation		No foams were seen.When it is shaking, little was given.No air boubles. No colour change.	2.3	3.6	8.5	1		82.6	587.8	A strong toddy odour With H_2S smell		
			6.24	10.30am			No change	2.2	3.6	8.5	1		84.4	587.7	same		
			6.25	10.30am			No change	2.2	3.6	8.5	1		85.9	587.9	same		
			6.26	10.30am			No change	2.2	3.6	8.5	1		85	591.7	same		
			6.27	10.30am			No change	2.2	3.6	8.5	1		85.6	592.57	same		
			6.28	10.30am	End Of The Trial		No change	2.12	3.6	8.5	1		85.6	592.6	same	Alcohlic(visky) taste	Watery, Low body

TRIAL	Sample		Date	Time	Description	Conditions	Observations	Plato (^{0}P)	PH	Alcohol(v/v)%	Density (g/cm^3)	Ferment r (%)	Calories (kcal/kg)	Odour	Flavour	Coments
2			2006.06.15		Unpasteurized sap					2.1						
Fermenting	A															
As It Is																
			6.16		Sap											
					During	Cold										
				10.30am	Fermentation	room	More lighter solution,No foams,	13.2	3.7	2.4	1.06	2.1				
						(12^{0}C)	when shaking little amount of									
							foams were given.Sedimetation was									
							appeared on the bottem of the flask.									
			6.17				No change	13.1	3.7	2.8	1.06	5.06				
			6.18				No change	12.9	3.7	3.2	1.06	7.21				
			6.19	10.30am			No change	12.1	3.7	4.4	1.06	10.2			sweetness is high,	
															Soury	
			6.2O	10.30am			No change	11.9	3.7	4.7	1.05	13.3		A strong odour		
			6.21	10.30am			No change	11.3	3.6	4.9	1.05	18		A strong odour		
			6.22	10.30am			No change	11	3.6	5.4	1.05	29.8		Ester smell, not a		
														strong odour		
			6.23	10.30am			No foams were seen.When it is shaking, a little	10.6	3.6	5.9	1.05	35.9				
							was given.No air boubles. No colour change.									
			6.24	10.30am			No change	9.3	3.6	6.6	1.02	41.7				
			6.25	10.30am			No change	8.6	3.6	7.2	1.02	49.9				
			6.26	10.30am			No change	8.2	3.6	7.7	1.02	54				
			6.27	10.30am			No change	6.6	3.6	8.1	1.02	59.2				
			6.28	10.30am			A little foamy appearance was seen.	5.3	3.6	8.2	1.02	62.5	449			
			6.29	10.30am			Higher foamy appearance was seen than	4.4	3.6	82	1.02	68.9	498			
							yesterday.									
			6.3O	10.30am			No change	3.6	3.6	8.3	1.02	73.7	550			
			7.01	10.30am			No change	2.3	3.6	8.3	1.02	79.8	587			
			7.02	10.30am			No change	2.1	3.6	8.3	1.02	84.5	603			
					Sap											
			7.03	10.30am	After Fermentation		Foams were dissapeared.	2.1	3.6	8.3	1.02	84.9	605			
			7.04	10.30am	End Of The Trial		No change	2.1	3.6	8.3	1.02	85.1	605	Alcohlic taste	Watery,	
															Low body	

TRIAL	Sample		Date	Time	Description	Conditions	Observations	Plato (°P)	PH	Alcohol(v/v)%	Density (g/cm³)	Plate count (cells/ml)	Ferment r (%)	Calories (kcal/kg)	Odour	Flavour	Coments
3			2006.06.15														
Pasteurized sap	A																
with					Sap												
CB Yeast		3.1		4.30pm	Before		light solution, Sedimentation on the	13.2	3.7		1.05	7.5×10^6					
					Fermentation		bottom of the bottle										
		3.2	6.16		Sap												
				10.30am	During	Cold Room	light solution,No foams,	13.1	3.7		1.05						
					Fermentation	(12^0C)	when shaking little amount of foams										
							were given.Sedimetation was										
						Asceptic	appeared on the bottem of the flask.										
						Conditions											
			6.17					12.9	3.7		1.05						
			6.18					12.1	3.7		1.05						
			6.19	10.30am			light solution,No foams,	11.9	3.7		1.05				Off smell	high sweetness	
							when shaking little amount of									Soury	
							foams were given.Sedimetation was									Worty	
							appeared on the bottem of the flask.										
			6.2O	10.30am			No change	11	3.7	1.5	1.05	14.38×10^6	15.02		Off smell		
							when shaking little amount of foams										
							were given.										
			6.21	10.30am			No change	10.6	3.7	1.9	1.05	11.25×10^6	17.81		A Strong odour		
			6.22	10.30am			Very little amount of foams were	9.3	3.7	2.2	1.05	19.4×10^6	19.43		A Strong odour		
							sean.No change in other parameters.								with ester smell		
			6.23	10.30am		Cold room	Very little amount of foams were	8.6	3.7	2.9	1.05		25.22				
						(14^0C)	sean.Other parameters were same.										
			6.24	10.30am		Ascepti	Little amount of foams were seen.	8.1	3.7	3.4	1.02		30.34				
						Conditions	When it is shaking, a little was given.										
							No air boubles. No colour change.										
			6.25	10.30am			No change	7.3	3.7	3.8	1.02		35.31				
			6.26	10.30am			No change	6.5	3.7	4.2	1.02		39.91				
			6.27	10.30am			No change		3.7	4.9	1.02		43.24				
			6.28	10.30am			high amount of foams and air bubbles	5.3	3.7	5.3	1.02		48.62	589			
			6.29	10.30am			No change	4.4	3.7	6	1.02		54.25	591			
			6.3O	10.30am			No change	3.6	3.7	6.7	1.02		60.52	592			
			7.01	10.30am	Sap		No foams. No air bubbles	3.1	3.7	7.2	1.02		65.72	607			
					After												
			7.02	10.30am	Fermentation		No change	2.3	3.7	7.6	1.02		70.34	611			
			7.03	10.30am	End Of The		No change	2.1	3.7	7.6	1.02		70.34	611	Iso amyl acetate	Sweet, Sour,	
					Trial										smell with above	Fruity taste	
															characters		

TRIAL	Sample		Date	Time	Description	Conditions	Observations	Plato	PH	Alcohol(v/v	Density	%Ferment	Calories	Bitterness	Colour	VDK	Odour	Flavour	Comments
														at the end of trial					
5	A		6.15																
Pasteurized					Sap														
sap with		5.1		4.30pm	Before			13.2	3.7	2.1	1.05								
CB Yeast +					Fermentation														
30% CBSB																			
Wort		5.2	6.16		Sap														
				10.30am	During	Cold Room	Solution was little darker, foams on		3.7	2.5	1.05								
					Fermentation	(12°C)	the top, when shaking little amount of												
							foams were given.Sedimetation was												
							appeared on the bottem of the flask.												
			6.17			Ascepti	No change		3.7	3.3	1.05	20.8							
						Conditions													
			6.18				No change		3.7	4.2	1.05	29.9							
			6.19	10.30am			Solution was little darker, More foams		3.7	4.9	1.05	38.4					Worty smell	sweetness is high	
							on the top, when shaking high amount of											Soury	
							foams were given.Sedimetation was											Worty	
							appeared on the bottem of the flask.Air												
							boubles were released rapidly.												
			6.2O	10.30am			A large quantity of foams were appeared		3.7	5.3	1.05	45.7	592				Off smell		
							on the top. When it is shaking, very large												
							amount was given. Air boubles waer												
							releasing rapidly,but the speed was lower												
							than trial 1.												
			6.21	10.30am			No change.		3.7	5.9	1.03	49.9	593				Little Toddy smell was given		
			6.22	10.30am			The quantity of foams were reduced, Little		3.7	6.5	1.02	58.2	595				Toddy smell+worty		
							amount was seen on the top. Air bubbles										smell + H_2S smell		
							were releasing,the speed was lower than												
							20th.Even the rate was lower than trial 1.												
			6.23	10.30am		Cold room	The quantity of foams were reduced, Little		3.7	6.9	1.02	63	587						
						(14°C)	amount was seen on the top. Air bubbles												
							were released very slowly.												
			6.24	10.30am		Ascepti	Little foamy appearance is seen.When it is		3.7	7.6	1	65.9	585						
						Conditions	shaking, a little was given.No air boubles.												
							No colour change.												
			6.25	10.30am			No change.		3.7	8	1	72.8	571						
			6.26	10.30am	Sap		No change.		3.7	8.3	1	76	578				Toddy smell+worty		
					After												smell +H_2S smell+		
					Fermentation												A strong smell		
			6.27	10.30am			No change.		3.7	8.3	1	77	576				same		
			6.28	10.30am			No change.		3.7	8.3	1	77.5	578				same		
			6.29	10.30am			No change.		3.7	8.3	1	78	578				same		
			6.3O	10.30am	End Of The		No change.		3.7	8.3	1	78	578	19.25	6.225	0.1134	same	Beer like feeling	Bitterness & colour is differ from
					Trial									29±3.0	12±1.0	≤0.15		Bitter	CBSB levels due to sweet toddy.
																			But VDK is in CBSB level.

Trial 1 Fermenting the sap As It Is

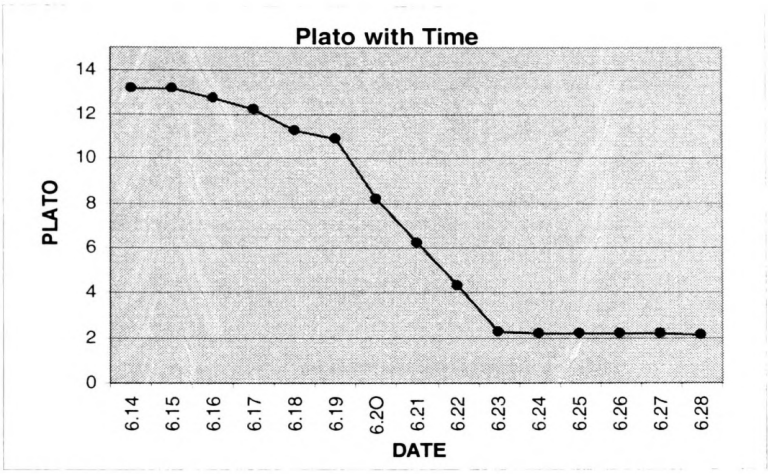

Fig. 4.1.1. Percentage of real extract (Plato) change with time

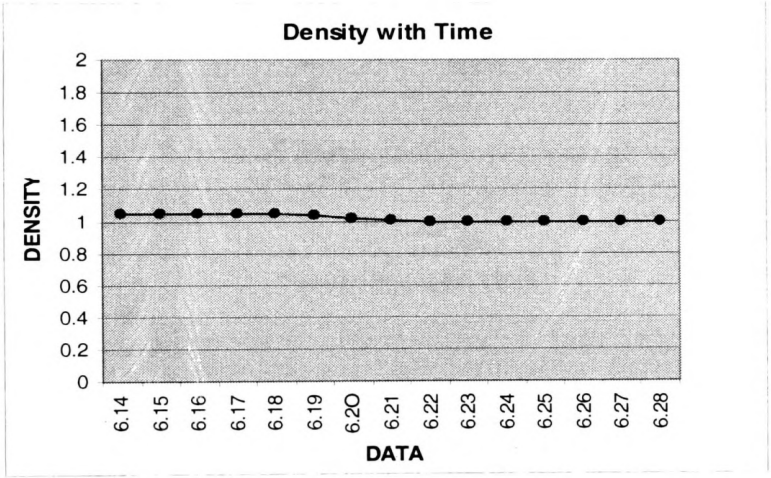

Fig. 4.1.2. The Density change with time

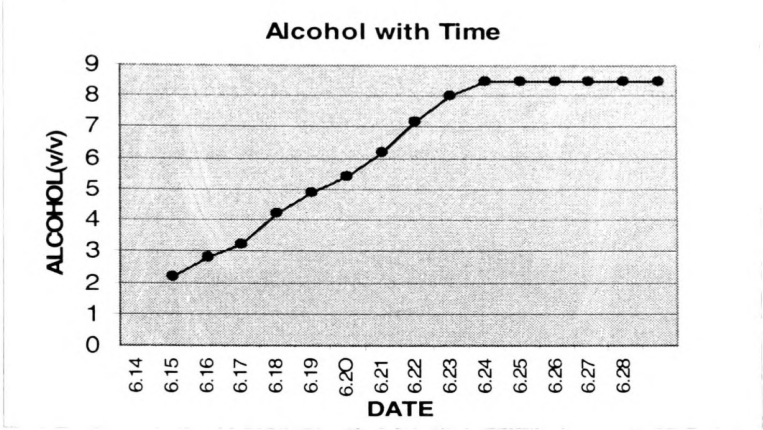

Fig. 4.1.3. The Percent Alcohol change, with time

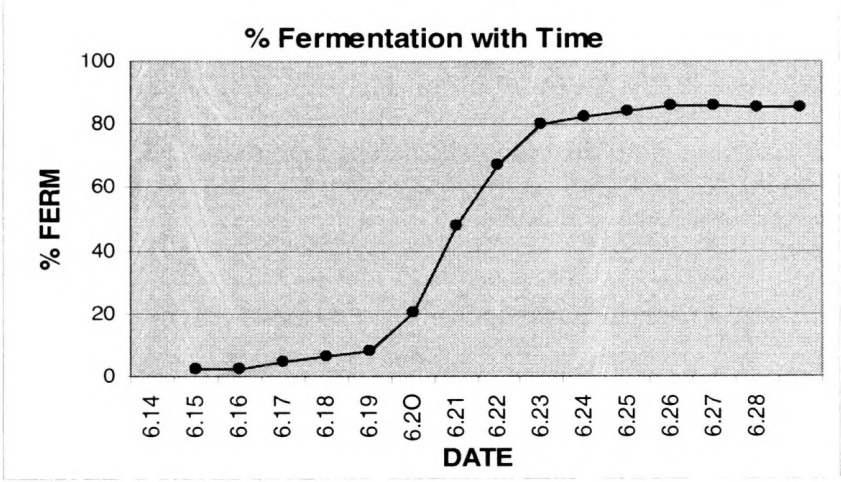

Fig.4.1.4. Change of Fermentation percentage with time

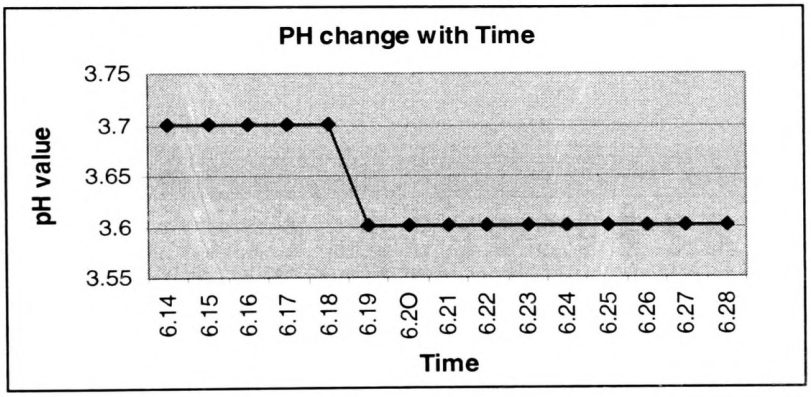

Fig.4.1.5. Change of pH with time

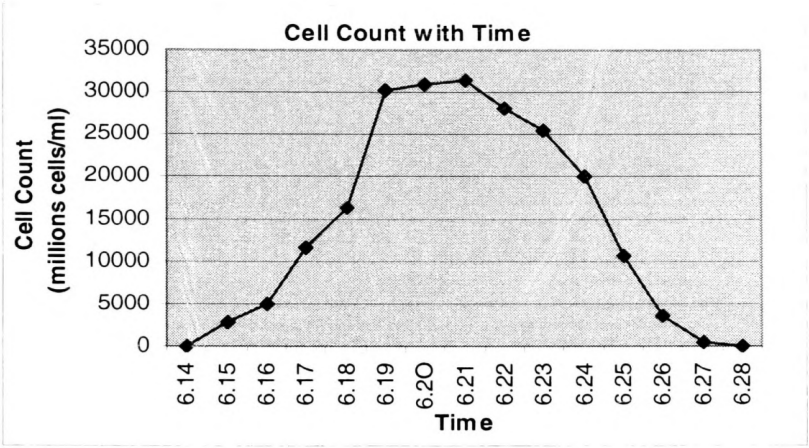

Fig.4.1.6. The variation of yeast cell count with time

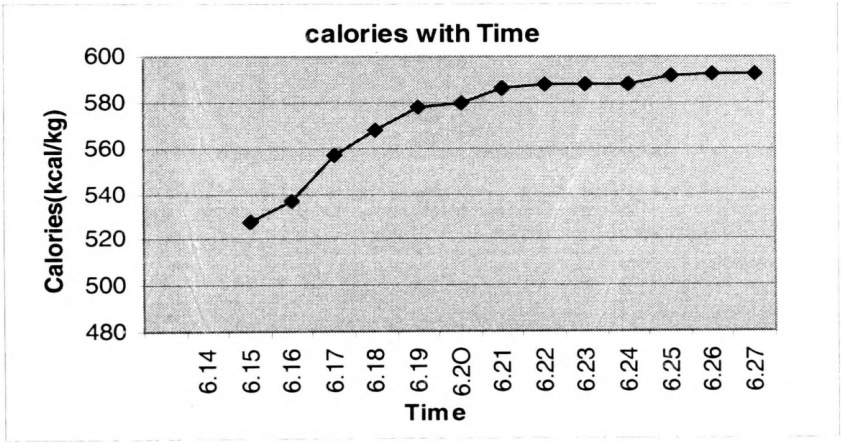

Fig. 4.1.7. The variation of caloric value with time

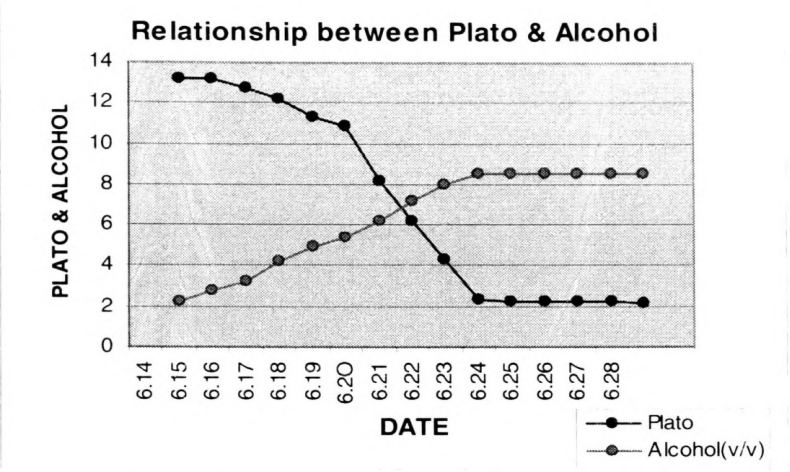

Fig. 4.1.8. The Relationship between Alcohol formation and the Real extract with time

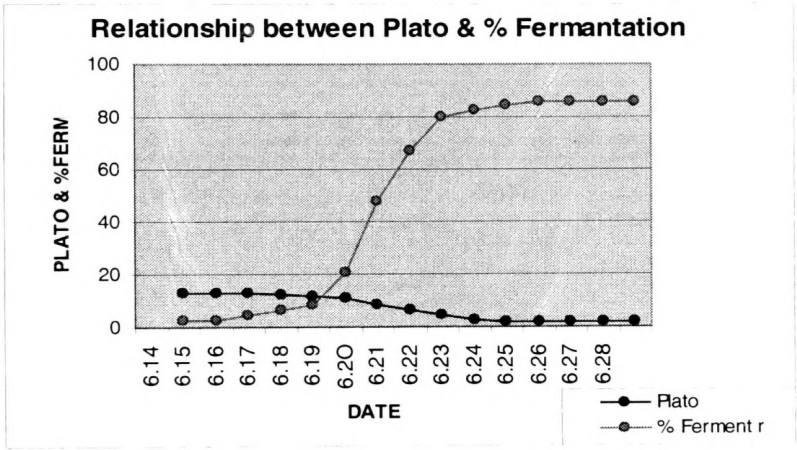

Fig. 4.1.9. The Relationship between Real extract and fermentation percentage with time

Trial 2. Fermentation of Pasteurized sap in controlled environment.

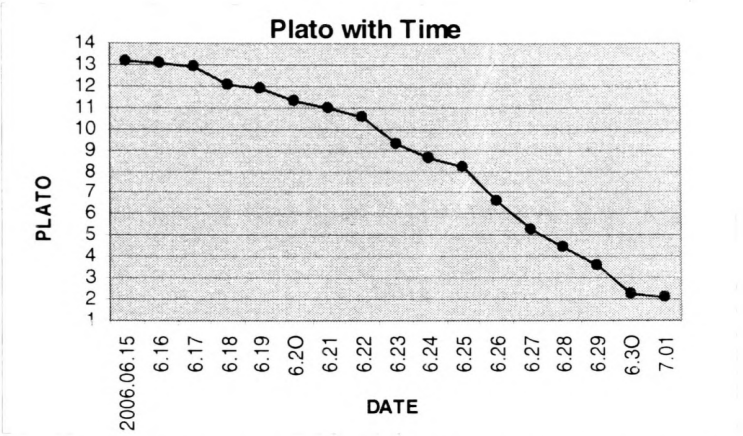

Fig. 4.2.1. Percentage of real extract (Plato) change with time

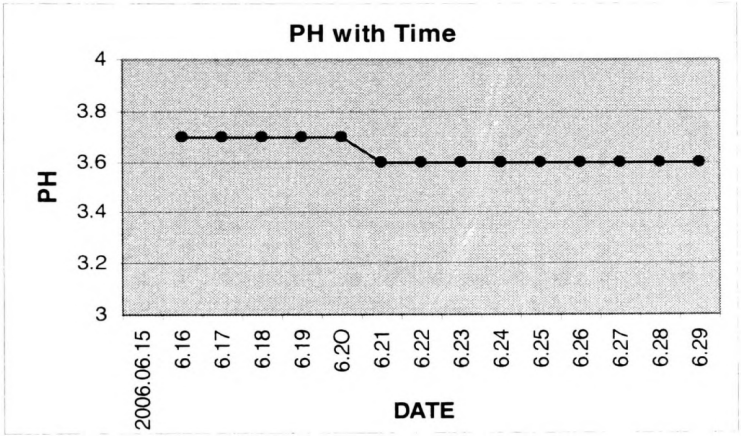

Fig. 4.2.2. The change of pH with time

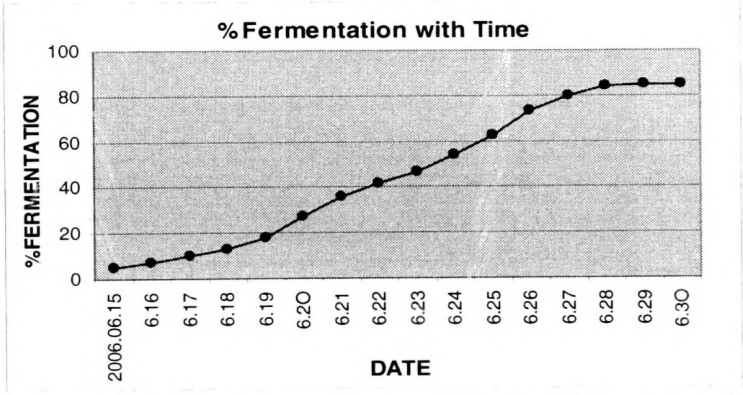

Fig. 4.2.3. Change the percent Fermentation with time

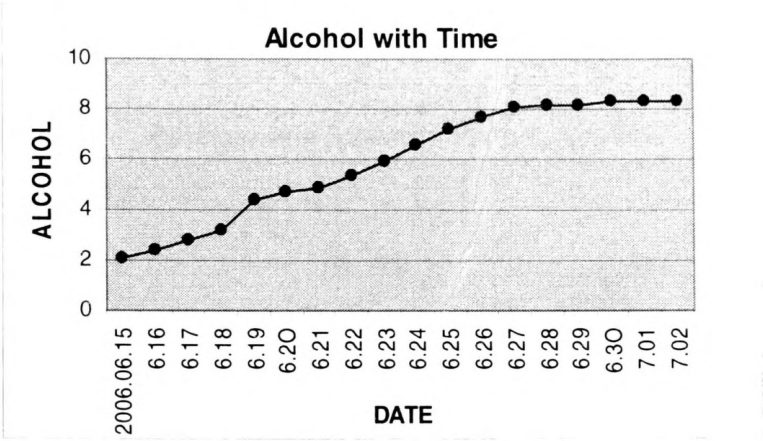

Fig. 4.2.4. Alcohol formation with time

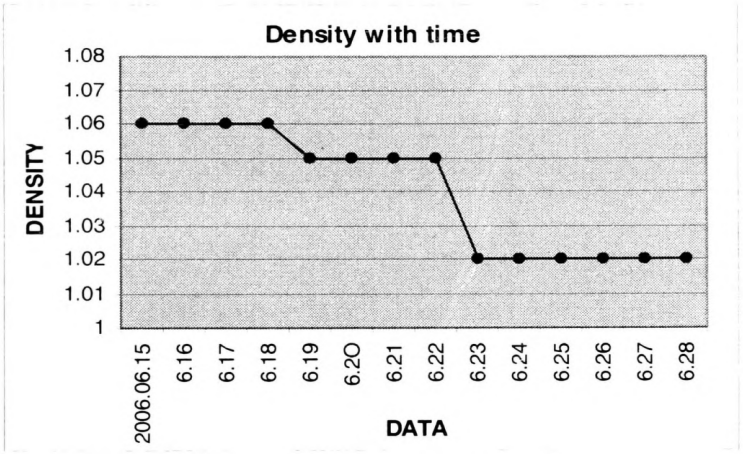

Fig. 4.2.5. Change of Density with time

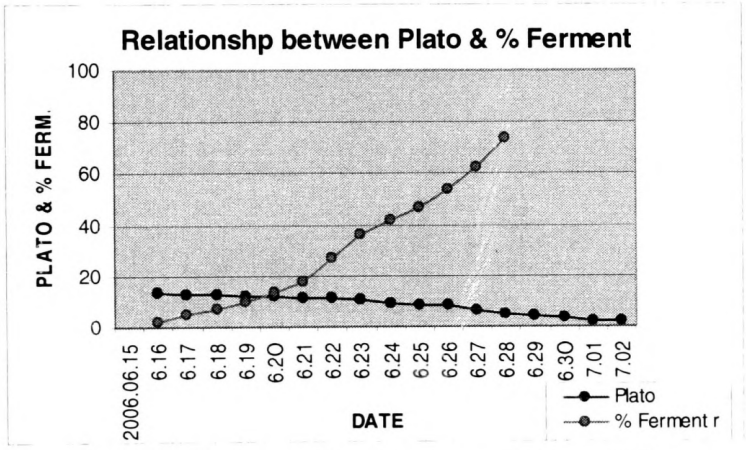

Fig. 4.2.6. The Relationship between and percent fermentation.

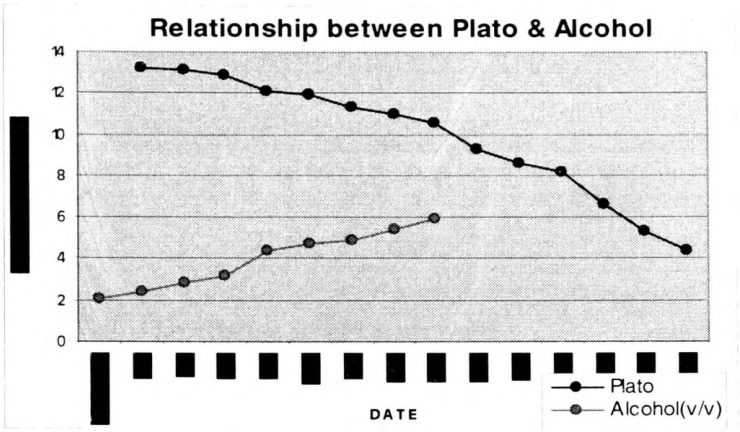

Fig. 4.2.7. The Relationship between Alcohol formation and the Real extract

Trial 3. Pasteurized sap with Lion Larger Yeast

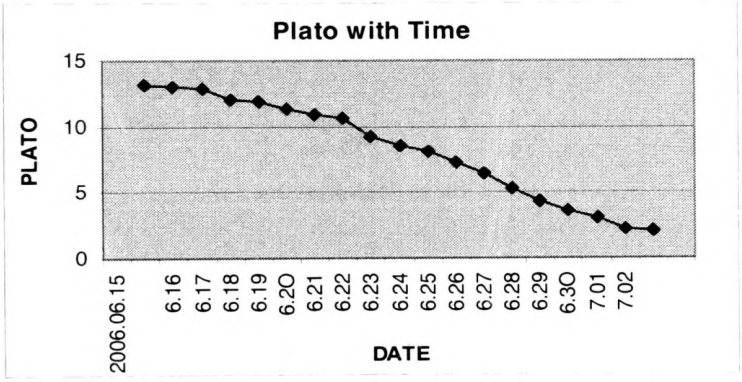

Fig. 4.3.1. Percentage of real extract (Plato) change with time

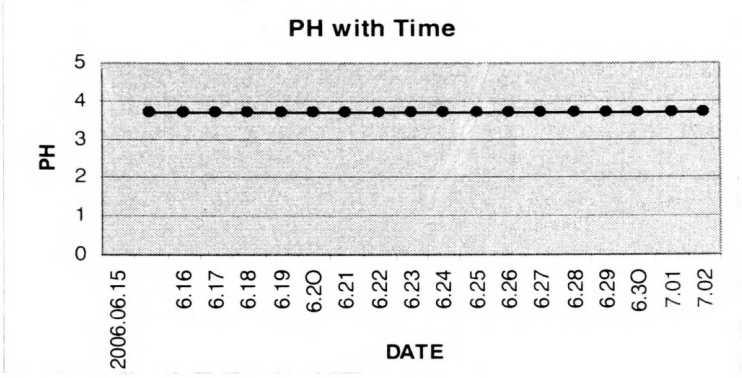

Fig. 4.3.2. The change of pH with time

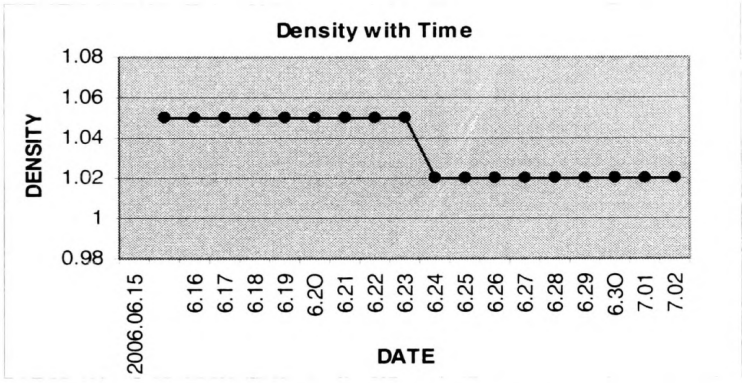

Fig. 4.3.3. Change of Density with time

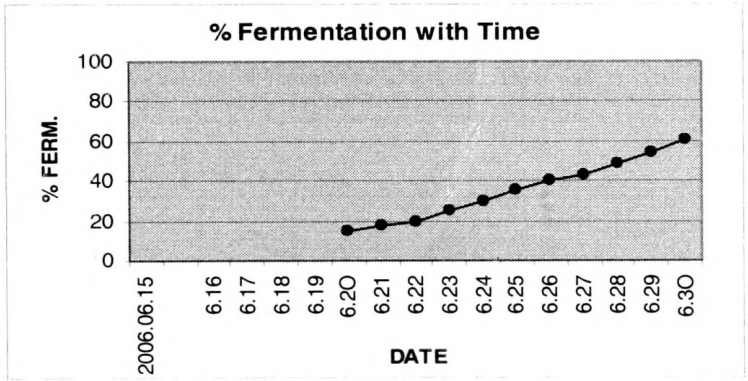

Fig. 4.3.4. Change of percent Fermentation with time

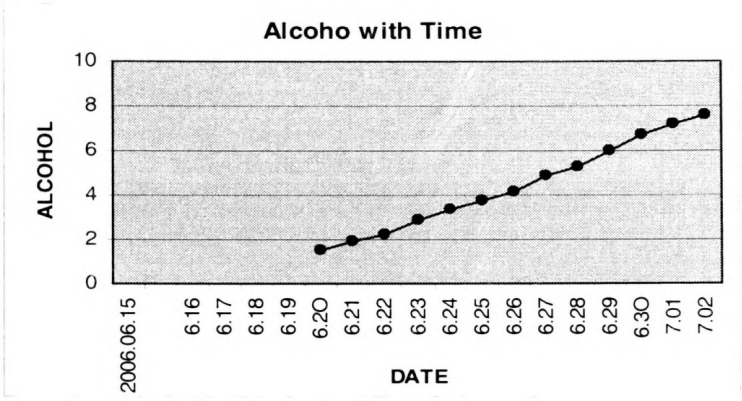

Fig. 4.3.5. Alcohol formation with time

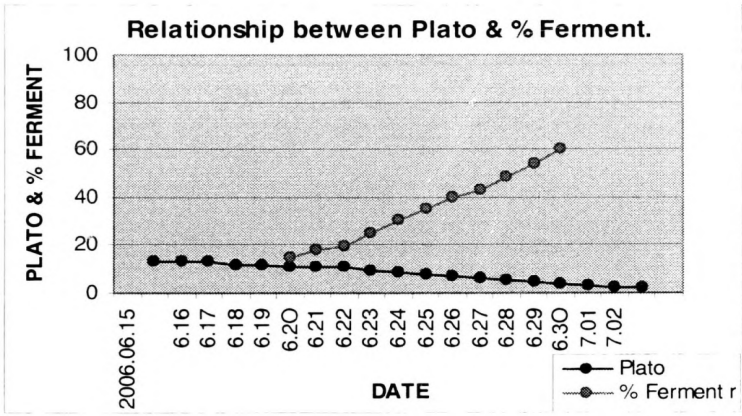

Fig. 4.3.6. The Relationship between Plato and percent fermentation

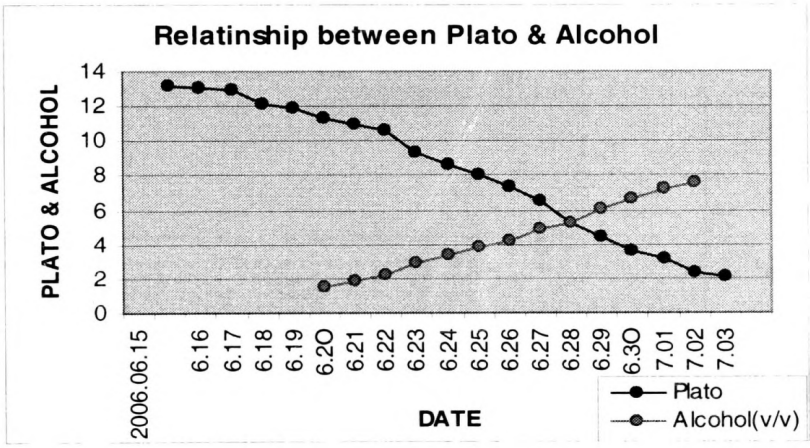

Fig. 4.3.7. The Relationship between Alcohol formation and the Real extract

Trial 4. Fermentation of Pasteurized sap with Lion Stout wort solution

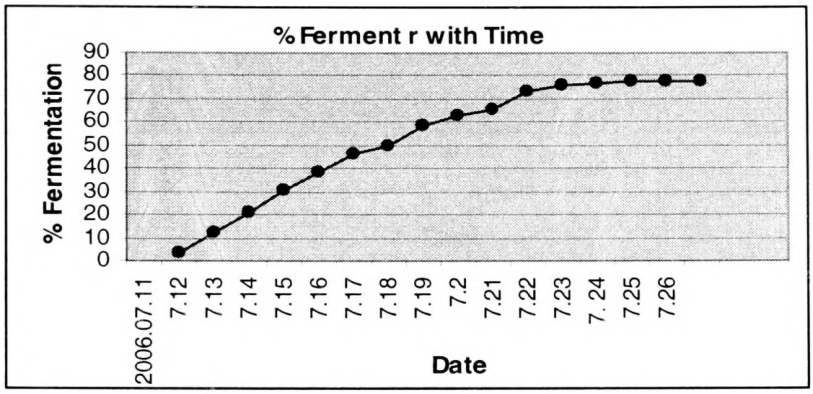

Fig. 4.4.1. Change of percent Fermentation with time

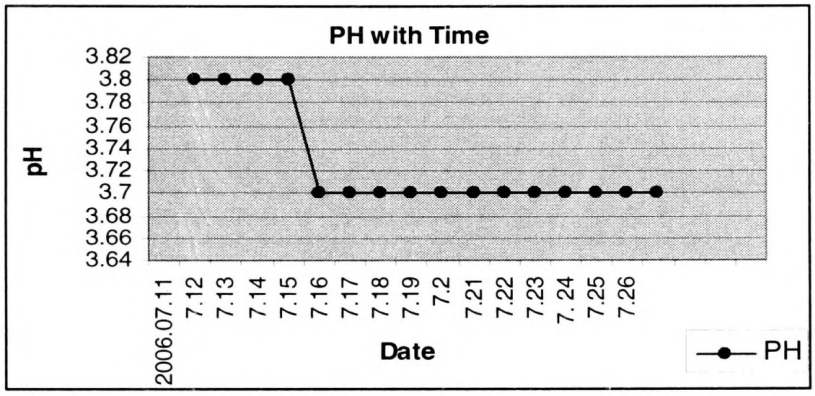

Fig. 4.4.2. Change of pH with time

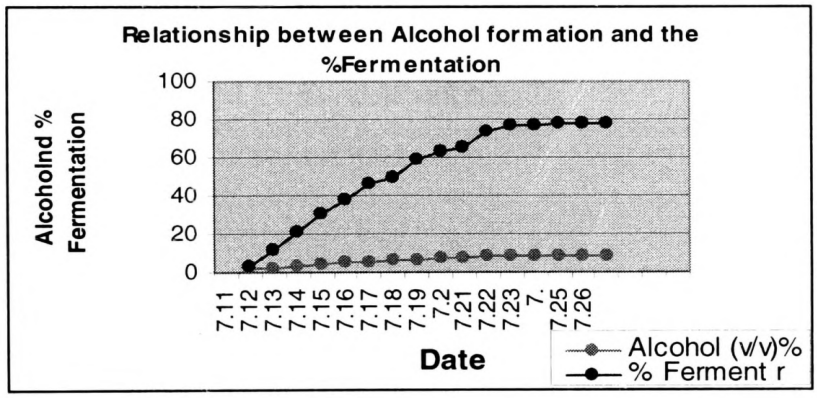

Fig. 4.4.3. Relationship between Alcohol formation and the fermentation percentage

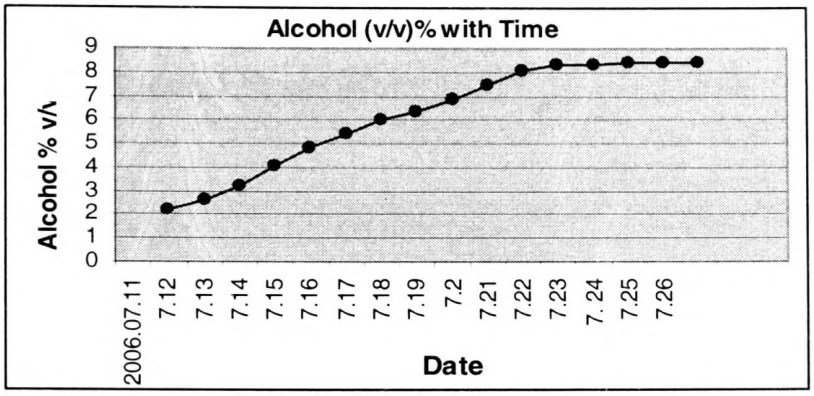

Fig. 4.4.4. Alcohol formation with time

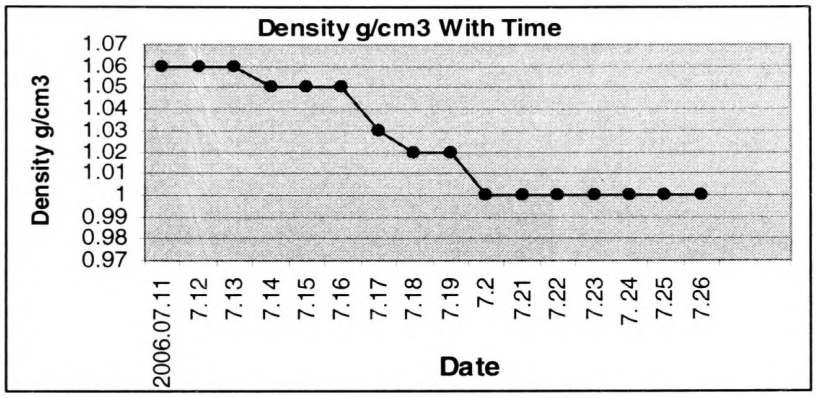

Fig. 4.4.5. Variation of Density with time

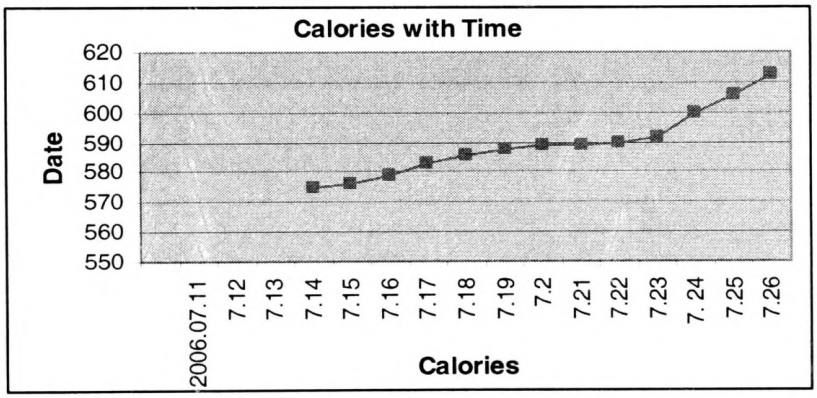

Fig. 4.4.6. The variation of calories with time

Trial 5. Fermentation of Pasteurized sap with Carlsberg Special Brew wort

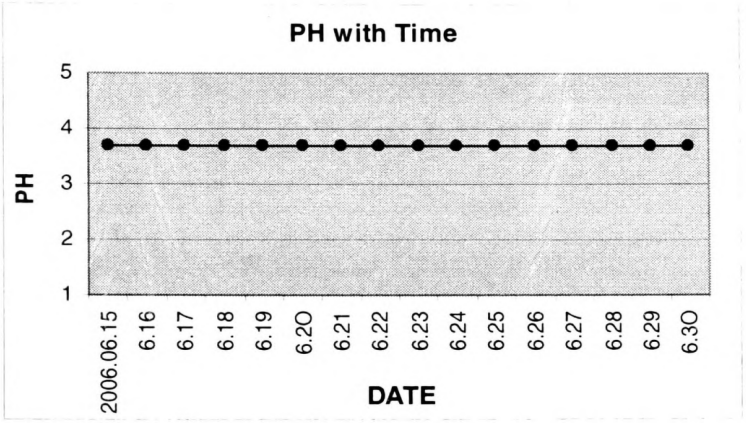

Fig. 4.5.1. The change of pH with time

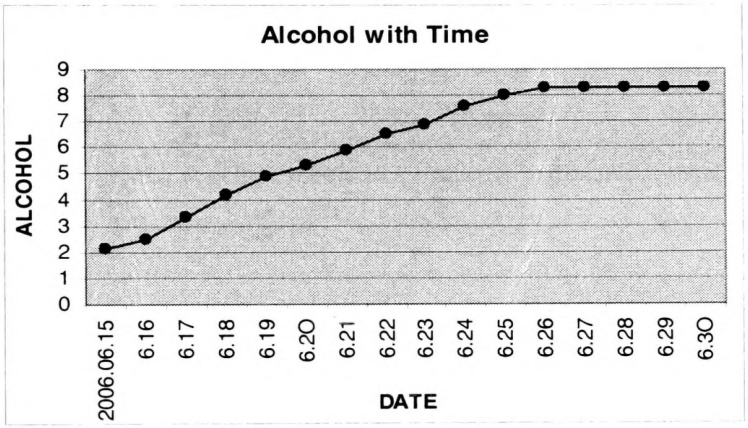

Fig. 4.5.2. Alcohol formation with time

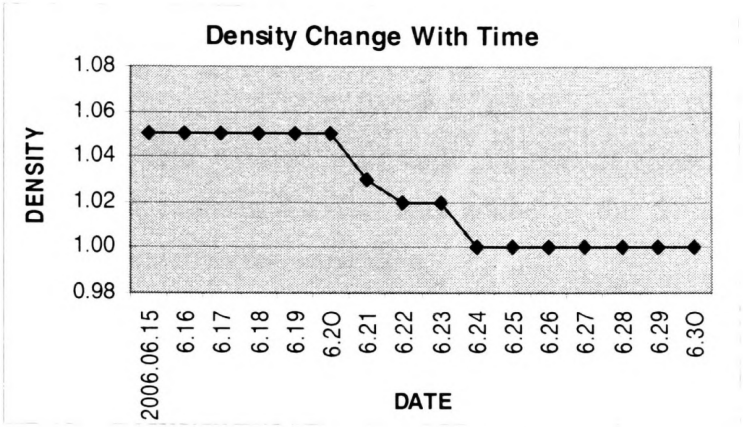

Fig. 4.5.3. Variation of Density with time

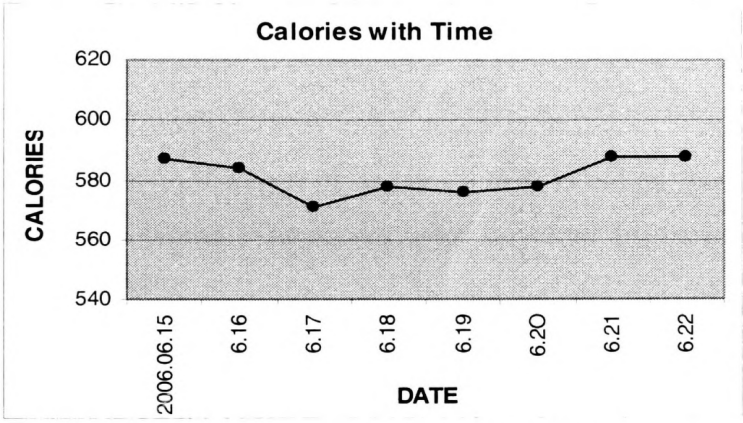

Fig. 4.5.4. The variation of caloric value with time

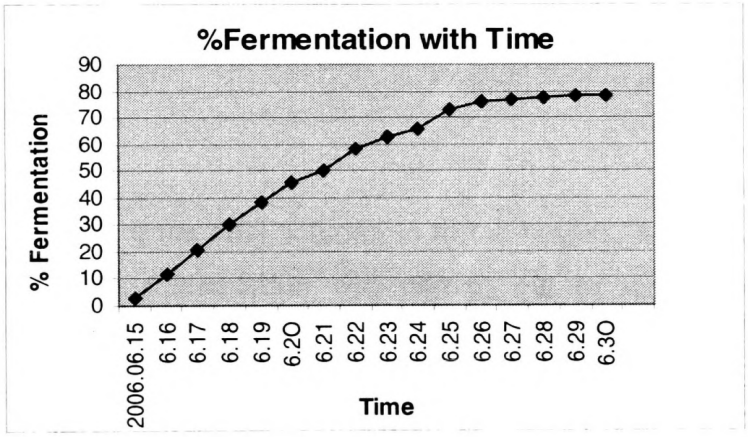

Fig. 4.5.5. Change of percent Fermentation with time

Discussion

Practical issues and suggestions

Sweet toddy is a highly fermentable solution with large amount of sugar (16.5 g/100ml). 'Hal' bark, a preservative which contains Tannin, was added to the fresh sap, during its transportation, to delay the fermentation for some time.

Even though 'Hal' barks were added, Sweet toddy was fermented up to a certain extent, as indicated by a rise in its pH from (4.5-5.0) to 3.8.

Samples were not fermented rapidly within first few days, Even though 'Hal' barks were removed before the trial. The remaining tannin compounds may have been affected to cause this problem.

Alcohol converts to Acetic acid with the help of oxygen which is called Acetification. This may cause an off flavour development. The flasks were cowered by cotton caps and an aluminium foil, as an oxygen barrier.

The pasteurized sap was directly used for fermentation without filtering. So, the dead yeast cells were retained in the fermenting vessels. The chemical reactions of dead cells can create objectionable flavours, which may be able to retard the characteristic toddy flavour. Therefore filtration should be carried out before filling, in further experiments.

Fermented solution was filtered using both GAF filters and, diatoms called Kieselguhr. But the clarity was not sufficient. So, more efficient filtering method should be followed in further trials.

Carbonation was done after filling the final solution in to the bottles. Available oxygen can make oxidization in the bottle. Carbonation was carried out to minimize the oxygen availability.

Chapter 5

Conclusions

Conclusions

1. There are enough evidences to conclude that, Sweet toddy functions almost same as beer, during the fermentation. Hence, it can be recommended as a beer adjunct.
2. Brewing toddy according to Trial 1 is more effective, because the product contains more favorable odour, pleasant flavour, and body as well as better appearance than marketed toddy products.
3. Sweet toddy can be fermented by Brewing Yeast, but a complete fermentation is not performed.
4. Sweet Toddy is an ideal beer adjunct for Lion Stout, as the substituted raw material (Sweet toddy) did not make much change of its own beer characters. But some proportional modifications have to be done.
5. The clarity of product is insufficient. More efficient filtering method is needed.
6. Bitterness and VDK amount of the products are within the acceptable range.
7. A water adulteration has been done by collectors.

Recommendations for Further Work

A detailed self life evaluation must be carried out to find out the exact shelf life of the product under ambient conditions.

Optimum conditions for toddy manufacturing process should be finalized by conducting further trials, in different conditions.

Critical process parameters should be established.

A suitable method should be found to minimize the strong H_2S odour.

More efficient filtration method should be applied.

More trials should be carried out for Trial 7 by changing the quantities, to optimize the product quality.

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Appendix 1

Questionnaire for Triangular Test

Date:

Sample:

You are given two identical samples and one different sample in each set. Taste these samples and identify the odd sample.

Is one sample differing from other two? **Yes / No**

If Yes: Identify the sample .....

What are the differences?

Odour .....

Taste .....

Colour .....

Appearance .....

Criticize the odd sample with respect to the other two.

.....

.....

.....

.....

.....

Name:

Date:

Appendix 2

TRIAL	Sample		Date	Time	%Real (Plato)	PH	Alcohol %(v/v)	Density g/cm^3	Plate count $\times 10^6$	% Ferment r	Calories kcal/kg
			6.14	4.30pm	13.2	3.7	2.2	1.05	7.5	2.3	
1		1.1	6.15	4.30pm	13.2	3.7	2.8	1.05	2800	2.6	
Fermenting	A	1.4	6.16	10.30am	12.7	3.7	3.2	1.05	5000	4.5	528
As It Is			6.17	10.30am	12.2	3.7	4.2	1.05	11500	6.5	537
			6.18	10.30am	11.3	3.7	4.9	1.05	16300	8.3	557
			6.19	10.30am	10.9	3.6	5.4	1.04	30200	20.6	568
			6.2O	10.30am	8.2	3.6	6.2	1.02	31000	47.69	578
			6.21	10.30am	6.2	3.6	7.2	1.01	31500	67.2	580
			6.22	10.30am	4.3	3.6	8	1	28200	79.83	586
			6.23	10.30am	2.3	3.6	8.5	1	25500	82.58	587.8
		1.5	6.24	10.30am	2.2	3.6	8.5	1	20000	84.35	587.7
			6.25	10.30am	2.2	3.6	8.5	1	10700	85.88	587.9
			6.26	10.30am	2.2	3.6	8.5	1	3600	85.7	591.7
			6.27	10.30am	2.2	3.6	8.5	1	400	85.62	592.57
			6.28	10.30am	2.12	3.6	8.5	1	12	85.62	592.6

Appendix 3

TRIAL	Sample		Date	Time	Plato	PH	Alcohol(v/v)	Density	% Ferment r	Calories
			2006.06.15				2.1			
2			6.16	10.30am	13.2	3.7	2.4	1.06	2.1	
Fermenting	A		6.17	10.30am	13.1	3.7	2.8	1.06	5.06	
As It Is			6.18	10.30am	12.9	3.7	3.2	1.06	7.21	
			6.19	10.30am	12.1	3.7	4.4	1.06	10.2	
			6.2O	10.30am	11.9	3.7	4.7	1.05	13.3	
			6.21	10.30am	11.3	3.6	4.9	1.05	18	
			6.22	10.30am	11	3.6	5.4	1.05	27	
			6.23	10.30am	10.6	3.6	5.9	1.05	35.9	
			6.24	10.30am	9.3	3.6	6.6	1.02	41.7	
			6.25	10.30am	8.6	3.6	7.2	1.02	47	
			6.26	10.30am	8.2	3.6	7.7	1.02	54	449
			6.27	10.30am	6.6	3.6	8.1	1.02	62.5	498
			6.28	10.30am	5.3	3.6	8.2	1.02	73.7	550
			6.29	10.30am	4.4	3.6	8.2	1.02	79.8	587
			6.3O	10.30am	3.6	3.6	8.3	1.02	84.5	603
			7.01	10.30am	2.3	3.6	8.3	1.02	84.9	605
			7.02	10.30am	2.1	3.6	8.3	1.02	85.1	605

Appendix 4

TRIAL	Sample	Date	Time	Plato	PH	Alcohol(v/v)	Density	% Ferment r	Calories
		2006.06.15							
3			4.30pm	13.2	3.7		1.05		
Pasteurized	A	6.16	10.30am	13.1	3.7		1.05		
sap with		6.17	10.30am	12.9	3.7		1.05		
CB Yeast		6.18	10.30am	12.1	3.7		1.05		
		6.19	10.30am	11.9	3.7		1.05		
		6.20	10.30am	11.3	3.7	1.5	1.05	15.02	
		6.21	10.30am	11	3.7	1.9	1.05	17.81	
		6.22	10.30am	10.6	3.7	2.2	1.05	19.43	
		6.23	10.30am	9.3	3.7	2.9	1.05	25.22	
		6.24	10.30am	8.6	3.7	3.4	1.02	30.34	
		6.25	10.30am	8.1	3.7	3.8	1.02	35.31	
		6.26	10.30am	7.3	3.7	4.2	1.02	39.91	
		6.27	10.30am	6.5	3.7	4.9	1.02	43.24	
		6.28	10.30am	5.3	3.7	5.3	1.02	48.62	588.5
		6.29	10.30am	4.4	3.7	6.01	1.02	54.25	591.1
		6.30	10.30am	3.6	3.7	6.7	1.02	60.52	592.45
		7.01	10.30am	3.1	3.7	7.2	1.02	65.72	606.57
		7.02	10.30am	2.3	3.7	7.6	1.02	70.34	610.55
		7.03	10.30am	2.1	3.7	7.6	1.02	70.34	610.57

Appendix 5

TRIAL	Sample	Date	Time	Plato	PH	Alcohol (v/v)%	Density g/cm^3	% Ferment r	Calories	Bitterness Bu	Colour	VDK
4		2006.07.11	4.30pm	13.2	3.8	2.2	1.06	3.3				
Pasteurized	B	7.12	10.30am		3.8	2.6	1.06	12.1				
sap with		7.13	10.30am		3.8	3.2	1.06	20.82				
LL Yeast +		7.14	10.30am		3.8	4.1	1.05	30.81	575			
LS Wort		7.15	10.30am		3.7	4.8	1.05	38.42	576			
		7.16	10.30am		3.7	5.4	1.05	46.71	579			
		7.17	10.30am		3.7	6.0	1.03	49.89	583			
		7.18	10.30am		3.7	6.4	1.02	58.88	586			
		7.19	10.30am		3.7	6.9	1.02	63.12	588			
		7.20	10.30am		3.7	7.5	1	65.56	588.8			
		7.21	10.30am		3.7	8.1	1	73.79	589			
		7.22	10.30am		3.7	8.3	1	76.43	589.9			
		7.23	10.30am		3.7	8.3	1	77.1	591.7			
		7. 24	10.30am		3.7	8.4	1	77.5	599.9 7			
		7.25	10.30am		3.7	8.4	1	78	605.9			
		7.26	10.30am		3.7	8.4	1	78	612.5	19.25	6.225	0.1134
										29±3.0	12±1.0	≤0.15

Appendix 6

TRIAL	Sample	Date	Time	Plato	PH	Alcohol (v/v)%	Density g/cm^3	% Ferment r	Calories	Bitterness Bu	Colour	VDK
5		2006.06.15	4.30pm	13.2	3.7	2.1	1.05	3.3				
Pasteurized	A	6.16	10.30am		3.7	2.5	1.05	12.1				
sap with		6.17	10.30am		3.7	3.3	1.05	20.82				
CB Yeast +		6.18	10.30am		3.7	4.2	1.05	29.89				
CBSB Wort		6.19	10.30am		3.7	4.9	1.05	38.42	578			
		6.2O	10.30am		3.7	5.3	1.05	45.74	578			
		6.21	10.30am		3.7	5.9	1.03	49.89	587			
		6.22	10.30am		3.7	6.5	1.02	58.22	584			
		6.23	10.30am		3.7	6.9	1.02	62.99	571			
		6.24	10.30am		3.7	7.6	1	65.87	578			
		6.25	10.30am		3.7	8	1	72.79	576			
		6.26	10.30am		3.7	8.3	1	76	577.8			
		6.27	10.30am		3.7	8.3	1	77	587.7			
		6.28	10.30am		3.7	8.3	1	77.5	587.9			
		6.29	10.30am		3.7	8.3	1	78	591.7			
		6.3O	10.30am		3.7	8.3	1	78	592.57			
									592.6			
										19.25	6.225	0.1134
										29±3.0	12±1.0	≤0.15

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