DEVELOPMENT AND FORMULATION OF CONCENTRATED NATURAL HERBAL DRINK USING IRAMUSU (*Hemidesmus indicus*)

By

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Declaration

The research work described in this thesis was carried out at the Kelani Valley Canneries Limited, and the Faculty of Applied Sciences, under the supervision of Mr. Lasantha Rathnayaka and Mrs.W.M.D.Priyadarshani.

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Dedicated
To
My Family Members
And To All My Teachers

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ABSTRACT

Iramusu (*Hemidesmus Indicus*) belongs to family Asclapadaceae and is a valuable medicinal plant in Sri Lanka. The roots of Iramusu have a medicinal value.2-hydroxy-4-methoxy benzaldehyde, is the key compound that is responsible for the characteristic fragrance, aromatic taste and medicinal properties of the root. Iramusu is successfully employed to recover diabetic mellitus, uninary diseases, gastrointestinal ulcers and cancers. Iramusu was used to make different types of Herbal drinks, which involves several unit operations. Therefore, it is difficult to prepare Herbal drinks for frequent consumption. In order to avoid those difficulties and hardships, several attempts were made to develop concentrated herbal drinks using dry Iramusu root.

Experiments were carried out to prepare concentrated Herbal drink using maha Iramusu root. Also, several experiments and trails were conducted to find out the optimum extraction conditions, optimum Iramusu amounts, optimum sugar levels and suitable flavor combination to produce concentrated Iramusu herbal drink with 25 Brix value. Sensory evaluation tests were conducted to find out optimum levels and conditions for the above factors using 9-point Hedonic scale subjectively. Shelf life evaluation studies were carried out in Chemical and Microbiological aspects. Titratable acidity, pH and TSS were tested for 1½ months of storage period at room temperature (27 ± 3 °C). After 1½ months Microbiology analysis were carried out.

From the findings of this study, it can be concluded that the optimum extraction condition for Iramusu roots is 100g of dry Iramusu roots with 500ml water and 30min boiling period. Optimum ingredient amounts that to be added for preparing 100ml of the herbal drink is, 40ml of extract with 20g of sugar level. Citric acid (0.25%) and salt (0.25%) combinated sample is the best sample, with good flavor enhancement capacity.

The results revealed that the product was acceptable for 1½ months and Coliform test was negative during this period. Total plate count was 30 per 1ml of concentrated herbal drink. A good quality Iramusu herbal drink can be achieved from the roots of Iramusu by using the above specifications.

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ABBREVIATIONS

App : Appendix
CMC : Carboxy methylcellulose
SMS : Sodium Meta bisulfite
TSS : Total Soluble Solids
TPC :Total plate Count
ml :mili liter
Min :minute
Et al :And others
L :Liter

CHAPTER 01

INTRODUCTION

1.1 Introduction

Now a days improving food security and providing healthy diets to rapidly expanding population is a serious challenge faced by the food scientists. Historical records indicate that a significant proportion of daily diets of people in Sri Lanka have been derived from forest and other natural vegetation. The new generation is unaware of the importance of what their parents ate, how they prepared their meals from locally available valuable foods (Costa and Gunasena, 2002). Proper education, which includes knowledge, attitude, practice are prerequisites in promoting traditional food plants in modern society. Therefore food technologists make efforts to increase the consumption of traditional food plants by introducing new food products using traditional and modern food recipes.

Ayurveda is a complete science of health and it aims to protect the health of the healthy and to alleviate disorders in the diseased. About 50-65% of human body consists of water and this shows the importance of water and other drinks in our diet. It can be consumed in plain water form or in the form of different drink preparation. The drinks, which have medicinal properties along with quenching thirst are called health drinks (Sethi, 2004). These different types of health drinks can fulfill nutritive or corrective (of disease) requirement of our body and can also add taste.

Many plant varieties are utilized in manufacturing of health drinks. Iramusu (*Hemidesmus indicus*) belongs to family Asclapanceae and it is a valuable medicinal plant in Sri Lanka.In Sanskrit it is called Sariva Anantamuli and in Tamil is known as Nannari and English it is called Indian Sarsaparilla (Fernando, 1993). There are two varieties, "Maha Iramusu "and "Heen Iramusu". The crop can be described as slender, laticiferous, twining, some times prostrate or semi erect shrub. The roots of the plant give the medicinal property. The 2 hydroxy-4-methoxybenzoic acid gives the characteristics fragrance, aromatic taste and medicinal properties, which is present in the root (Jayaweera, 1981).

Iramusu is used to make infusion or decoction as syrup, and also used to produce herbal soups, infusion bags, herbal tonic, sherbet and kola-kanda. Iramusu powder is also be used to produce confectionery products (Fernando, 1993). Tropical

Herbs (Pvt) Ltd, Balagolla produce infusion bags called Herb's Tea and Herb's Tea plus. These are produced using blend of five medicinal herbs including Iramusu, Beli, Neeramuliya, Polpala, Ranawara and Tea. The Agriculture Ministry is in collaboration with Prime Herb Lanka (Pvt) Ltd, and Herbarium Lanka Ltd has taken a major step to promote indigenous beverages in local and international market by launching seven herbal beverages with natural taste. The seven prime herbs and herbarium range include belifruit, Iramusu drink, Ashwaganda, Nelli drink, Soymilk and Aloevera health drink. Food and Nature (Pvt) Ltd, Maharagama produce infusion bags called Iramusu Ayurvedic Herbal Tea.

Dried root of Iramusu was chopped and made into a pillow and used for rheumatism and headaches. This root contains a property, which purifies the blood and cools the system (Fernando, 1993). And also gives good appetite and act as blood purifier, preserves the skins' natural vitality and cure Rheumatism and also controls the diabetic and protect kidney from urinary diseases. (Wealth of India, 1959) A decoction of the root bark with and sugar is good alterative tonic for cough and diarrhea in children (Jayaweera, 1981). Dried root of Iramusu cools human system so it can be taken as an herbal drink.

Owing to the global trend towards improved quality of life there is a considerable evidence of an increase in demand for medicinal herbs an their derivatives. Therefore this study is focused on developing concentrated herbal drink without added Flavor or color using hot extraction method. This Iramusu drink is 100% natural herbal preparation, which is well addressed for herbal conscious customer.

1.2 Objectives

Development of value added Herbal drink, using Iramusu (*Hemidesmus indicus).*

1.2.1 Specific Objectives

(1) Identification of best relationship for extraction time and amount of water.

(2) Determination of optimum amount of Iramusu extracts to be added in Herbal drink formulation.

(3) Determination optimum sugar level for the Herbal drink formulation.

(4) Evaluation of sensory appeal to determine the best product.

(5) Shelf life evaluation of the product.

CHAPTER 02

REVIEW OF LITERATURE

2.1 Classification

Family – Asclepiadaceae

Genus-*Hemidesmus*

Botanical Name – *Hemidesmus Indicus* (Jayaweera, 1981)

2.2 Varnacular Name

Sinhala – Iramusu

Sanskrit Name – Satavari, Sariva

English – Indian Sarasapailla

Hindi – Magrabu, Salsa

Bengal – Anantmool

Arabic – Zaiyana

Tamil – Arakkam, Aritinviyachi (Jayaweera, 1981)

2.3 Varities

Iramusu ***(Hemiesmus indicus)*** is one of the most import Herbs in the Sri Lanka.There are two verities of Iramusu called "Heen Iramusu" and "Maha Iramusu". "Heen Iramusu" is the most common and most abundant in the Sri Lanka. Large amount of this verity is utilized in the rural areas."Maha Iramusu" is imported from India. Demand for "Maha Iramusu" is much higher than the demand for "Heen Iramusu"(Compendium of medicinal plant, 2002).

2.4 Description and Morphology

2.4.1 About the Plant

Perennial, semi – shrubby twinner with a woody root stock and numerous, very long postrates or ascending, whip – like stems, slightly twinning cylindrical, thickened at nodes (Rajapaksha, 1998).

2.4.2 About Leaves

Simple, opposite, exstripulate, very varriable from oblong – oval to linear, 3.7 – 6.5 cm long 3.5 – 8 mm broad (Rajapaksha, 1998).

2.4.3 About Root

The Tuberous root is dark-brown ,coma silvery white,tuberous with teansversely cracked and longitudinally fissured bark. It has a atrong central vasculature and a pleasant smell and taste (Vaidysala, 1995).

2.4.4 About flowes

Greenish purple, Regular, bisexual on very short pedicels crowded in axillary cymes. Flowering period is februvary and March (Jayaweera, 1981).

2.4.5 About Fruits

2, distinct, divaricated, pollicies, linear, falcatetrete, smooth, 10 – 12.5 cm long, denisoing along the ventral suture (Rajapaksh, 1998).

2.4.6 About Seeds

Flattened ,black ,ovate- Oblong, coma silvery white (Vaidysala, 1995).

Fig. 2.1 *Hemidesmus indicus* external morphology

2.5 Centre of Origin and Distribution

2.5.1 Origin

Grows in nothern part of India.

2.5.2 Distribution

This plant spreads in the east wards as far as Bengal and Sikkim and South wards in to Sri Lanka. In Sri Lanka it is very common in the low country up to 800m or higher in grassy places (Jayaweera, 1981).

2.6 Propergation and Cultivation

2.6.1 Propergation

By seed and vegetative method. Can be propergate using seed root or pieces of stems which near the root and berry it under the soil (Compendium of medicinal plant, 2002).

2.6.2 Cultivation

Small plant of iramusu should be planted in the distance of 15*15 cm (Compendium of medicinal plant, 2002).

Loamy soils are well suited for the growth but soils intermixed with stones encourage production of long thick roots.The length and thickness of the root mostly depends on the soil profile rather than the environment (Vedavathy, 2004).

2.6.3 Harvesting

These plants can be planted along with rain ,and it can be harvestd after 12 months.Usually matured roots have the highest demand. But farmers used to sell whole plant (Compendium of medicinal plant, 2002).

2.6.4 Insect pest and disease

The plant is disease free and needs no special care (Vedavathy, 2004).

2.7 Food Uses & Parts Used

2.7.1 Parts Used

Roots, leaves and stem.

2.7.2 Food Use

The infusion of tender leaves in drunk as a beverage, and leaves extracted and added for prepairing porridge. It is used to prepare tonics and sherbets (Rajapaksh, 1998).

After boiling the roots and extract juice used as a produced ready to serve drink. The current trends in the market for Iramusu include herbal soups, infusion bags, ready to serve drinks (RTS) and herbal tonics. Iramusu powder can also be used to produce confectionery products. Iramusu root is used as a flavouring agent in the preparation of sherbet and medicinal mixture, for an example for the preparation of Nellie cordial Iramusu has used as a flavouring agent. A decoction of the root bark with milk and sugar is a good alternative tonic for cough and diarrhea.

Also Iramusu used to produce totally natural Caffeine Free Iramusu Ayurvedic Herbal Tea. It's offered many medicinal benefits. Iramusu Ayurvedic

Herbal Tea has its significant fragrance and a good and lasting taste and giving fresh breath.

2.7.3 Non food uses

Iramusu has special ability to enhance the growth of hair.

The milky latex of the plant is used ,in travancore,for relieving inflammation in the eye.

Ether extract of roots have some inhbitiry effect on the growth of Eschericha coli.

Found in many medical and cosmetic facial packs.

2.8 Nutrition, Therapeutic values and Physical composition

2.8.1 Nutritional value

Table 2.1 Proximate composition of the Iramusu root (100g edible portion)

Element	Amount
Moisture	92.1g
Energy	26cal
Protein	2g
Fat	.7g
Calcium	72mg
Phosphorus	21mg
	10.9mg
Carotene	5.586mg
Thiamine	30mg
Riboflavin	21mg
Niacin	.5mg
Vitamin C	28mg

(Source: Rajapaksha, 1998)

The root of this plant contains a volatile oil and hemidesmine. The root is a substitute for sarsaparilla.

2.8.2 Therapeutic Value

- Used in treating less of appetite.
- Used to treat skin diseases
- For inflammation of urinary passage.
- The plant is used in preparation of snakebite curses (Rajapaksha, 1995).
- Used to in the treatment of Fever
- For diabetic partitions.
- Have blood purification effect.
- For give to nursing mothers to increase milk.
- Act as anticancer agents.
- For general weakness
- A positive plant for investigation relating to an effective remedy for AIDS
- For Rheumatism(Jayaweera, 1981)

2.9 Physical Composition and Main chemical composition

2.9.1 Physical composition

Total ash	2.6 – 4.2 %
Acid in solvable Ash	15.5 – 18.8 %
Alcohol solvable extractive	1–1.5%
Water solvable extractive	16.6 –18.9%

(Sharma et al, 2000)

2.9.2 Main chemical composition

The aroma of the drug is attributed to this aldehyde.Other constituents present in the root are: ß-sito sterol, ∞-and ß-amyrins, lupeol,tetracyclic, triterpene alcohols, small amount of resin acid, fatty acids, tannins, saponins, a glycoside and ketone (Wealth of India, 1955). Acid like steropene distillation from root.50% crystallized material and 2-hydroxy-4-methoxy-benzaldehyde present in the

root.Coumarine influence the smell of the pharamaceuticals. In the root contains two sterols like Hemidesterol and Hemidesmol (Compendium of medicinal plant, 2002).

2.10 Chemicals and their Biological Activities

Table 2.2 Chemical and Biological activities of *Hemidesmus indicus*

Chemical	Position	Biological activities
Alpha-Amyrin	Root	Antitumor,Anti-inflammatory
Beta-Amyrin	Root	Antiedemic, Anti-inflammatory
Beta-Amyrin-Acetate	Root	Antioxiddant,Antinociceptive, Anti-inflammatory
Beta-Sitosterol	Root	Antibacterial, Antitumor (Breast), Antitumor (Lung) Anticancer (Breast),Hepatoprotective, Hypocholesterolemic,Hypoglycemic
Desinine	Plant	No activity reported
Eo	Root	No activity reported
Hemidesmol	Root	No activity reported
Hemidesterol	Root	No activity reported
Hexatriacontane	Root	No activity reported
Hyperoside	Flower	Antibacterial,Anticapillary,Antiviral Cancer-Preventive
Isoquercitin	Flower	No activity reported
Lupeol	Root	Antimalarial,Antirheumatic,Antioxidant
Lupeol-Acetate	Root	Antihyperglycemic,Antiulcer
Resin	Root	No activity reported
Rutin	Flower	Anticancer,Antidiabetic,Antimalarial Oviposition-Stimulant
Saponins	Root	Antitumor(Lung)Antitumor(Ovary)
Tannins	Leaf	No activity reported

(Source: Duke, 1992)

2.11 A description of *Hemidesmus indicus*

Parameter	Specification
Color	Brown
Odour	Characteristics
Taste	Characteristics
Solubility	
In water	60% w/w
In Alcohol	40% w/w
pH (1% W/V solution)	5-7
Loses on during at 100°C	5% w/w
Ash content	5% w/w
Volatile oil content	5% w/w
Microbiological analysis	
Pathogen (E.coli	Absent
Staphylococcus aureus, Salmonella)	
Total bacterial count	800cfu/gm
Total fungal count	500cfu/gm
Heavey metals	
As	8ppm
Pb	5ppm

(Source: Pioneer Enterprise, 2000)

2.12 Substitutes and Adulterants

Three species *Ictinocarpus frutescence, Cryptolepis buchanani* and Decalepis *hamiltonii* are used as substitutes due to non availability of *Hemidesmus indicus* in the market (Sharma *et al.*, 2000).

2.13 Herbal drink

2.13.1 Definition

The drinks, which have medicinal properties along with quenching thirst, are called as herbal drink

2.13.2 Basic ingredients of Iramusu Herbal drink

- **Sugar**

Sugar is the most common disaccarids. Sucrose is technical terms of the sugar. Sucrose is widely distributed in the plant kingdom although sugar cane or sugar beets are the commercial source of most sugar. Sucrose composed of one molecule of glucose and one molecule of fructose. Sugar is white or brown colored granules and is available commercially in many crystal sizes from extremely fine to very course. It has sweet taste. Turn into liquid when boiling and soluble in water (Pennington and baker, 1990).

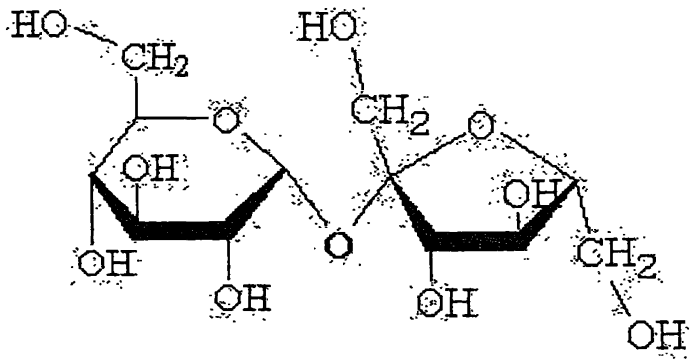

Fig.2.2 Chemical Structure of Sucrose molecules.

Sugar and syrup used in the preparation of drinks play in the flavor sensation. In addition to the sweet taste, sugar has three other principal flavor functions, namely

a) Sugar provides the sweetness necessary to balance the acid and other taste-producing components property and thus produce a balance flavored drink.

b) Sugar furnishes sufficient body to raise the beverage of the sweetened watery class.

Sugar serves to carry the flavor and thus deposit it uniformly when consumed (Jarcobs, 1959).

- **Preservatives**

Soft drink preserves some times require addition of chemical preservatives to improve their storage stability. These additives should be used judiciously and only when there is a clear need increase shelf life, prevent spoilage, or minimize the food-poisoning risk (Chapman, 2000). Sodium metabisulfite ($Na_2S_2O_5$) is a white to slightly yellowish, crystalline powder with an odor of Sulfur dioxide. Content of SO_2 at least 65.5%wt. Sulfur dioxide is able to stabilize the color of fresh and processed fruits and vegetables. Sulfur dioxide also inhibits the activity of common oxidizing enzymes and has antioxidant properties. Sodium metabisulphite (Na_2o, 2SO2 or $Na_2S_2O_5$)is commonly used stable source of sulphur dioxide.sulphur dioxide,SO_2, is colourless, with a pungent odour at normal temperature and pressure. Sodium metabisulphite added to drink to inhibit the growth of bacteria, yeasts, and/or molds (Potter and Hotchkiss, 1996).

- **Citric acid**

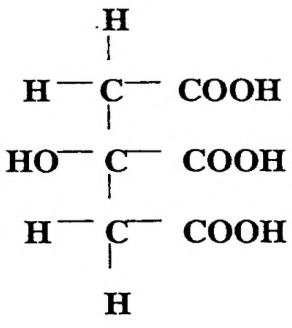

Fig.2.3 Chemical structure of the citric acid

Critic acid is a practically odorless, colorless solid, forming either translucent, crystals, or white granules or powder. The hydrated acid losses

water when it is heated at 70 to 75°C and has an apparent melting point at 100°C, but the anhydrous acid actually melts at 153°C (Kyzlink, 1990).

Citric acid is the fruit acid characteristic of the citrus fruits; citric acid has pleasant sour taste and a Flavour reminiscent of lemon. Citric acid is the principal acid used in the beverage industry. The major reason for its wide employment in the fact that combines well with fruity and light flavors.

Citric acid not only important as a flavor factor but has other function as well.

The more important of these are

a) Acid assists in preserving the syrup and beverage by killing microorganisms and making the environment that is beverage, unsuitable for their growth.

b) Acid catalyze the inversion of sucrose to invert sugar

c) Acid converts sodium benzoate to benzoic acid, which exerts its preservation action only on an acid environment. (Jarcobs, 1959).

- **Stabilizer**

Stabilizer, like xanthan gum, Carboxy methylcellulose, and guar gum significantly increased emulsion stability, and also stabilize and thicken foods by combining with water to increase viscosity and to form gels (Potter and Hotchkiss, 1996).

Carboxymethyl cellulose mainly used to increase the viscosity of foods. It dissolves in water to form a non-Newtonian solution, the viscosity of which decreases with increasing temperature. Solutions are stable at pH 5-10, with increasing temperature at pH 7-9.CMC helps solubilze common food protein. Due to its desirable rheologocal properties and lack of toxicity and digestibility, CMC has found broad use in foods. It acts as a binder and thickener in pudding; cheese spreads and its water binding capacity make it useful in ice cream and frozen desserts industry (Branen *et al*, 1989).

2.14. Product Requirements.

Appearance of the product: Product shall be uniform and characteristics colour, it shall be free from extraneous matter. Flavor and odor: Product shall have a pleasant characteristic Flavour. The flavor and odor shall be accordance with any claim made or implied by many factores. It should be free from scorching and caramalization (SLS 214, 1985).

Table 2.3 Requirements for reconstituted products

Sl. No (1)	Characteristic (2)	Requirement (3)
(i)	Sugar content (as Sucrose) per unit by mass, min	5
(ii)	Sulfur Dioxide content mg/kg, max	70
(iii)	Benzoic acid content, mg/kg max.	160
(iv)	Acidity (As anhydrous Citric acid) per cent by mass, min	10

(Source: SLS 214: 1985)

2.15. Packaging

2.15.1. Introduction

Packaging has been defined in several ways.

(1) A coordinated system of preparing goods for transport, distribution, storage, retailing and end-use.

(2) A means of ensuring safe delivery to the ultimate consumer in sound condition at minimum overall cost.

(3) A techno-economic function aimed at minimizing costs o delivery while maximizing sales.

Packaging materials used for food liquids should maintain good hygiene and have sufficient mechanical strength to prevent leakage and contamination from the outside. They should also be inert and provide barriers to light. Seals are important and low gas permeability is required (Paine and Paine, 1983).

2.15.2 Glass bottles

Glass bottles, the oldest industrial packaging, still have a high share of the packaged juice product market; they are used mainly for long life shelf. Glass bottles are seen as quality packs and have technical advantages where distribution places special demand on the package, as in long distance travel, and also provide many advantages in particular inertness, easy cleaning, durability and rigidity. Glass is not susceptible to mould growth and is impermeable to odors, vapors and liquids ((Paine and Paine, 1983).

2.16. Spoilage and Metal contamination

The characteristics indicating spoilage of concentrated syrup are extreme changes in taste and flavor, e.g.: - musty, sour, putrid, formation of sediment, cloud on turbidity, formation of slime, change in color and excessive forming or constant bubbling (Potter and Hotchkiss, 1996).

2.16.1 Microbiology Spoilage

Microbiological spoilage caused by yeasts, mold and bacteria. Yeast is the major cause of deterioration. Inflection with molds and bacteria are less common. Yeasts are mainly responsible for spoilage in drinks. Different kind may predominate in the juice, and their growth also depends upon the temperature, spoilage of raw juice at room temperature result in an alcoholic fermentation, followed by the oxidation of alcohol and acid by yeast and moulds, growing on the surface. To prevent spoilage every living yeast cell must be removed or suppressed by pasteurization, filtration or preservatives (Potter and Hotchkiss, 1996).

Table 2.4. Microbiological tolerance limits of Herbal drink

SI. No	Test	Limit	Method of test
(1)	Standard plate count, per, ml, max	50	SLS 516:Part 1
(2)	Coliform	Absent	SLS 516:Part 3
(3)	Yeast and moulds count, per ml	Absent	SLS 516:Part 2

(Source: SLS 729: 1985)

2.16.2 Contamination of extracted juice with metals.

The extracted juice can be contaminated with the heavy metals during processing. The cheap source that effect to contamination of extracted are, metal with in water, pane, pipes and other utensils used in the varies manufacture processes. The product shall not exceed the limits for heavy metals given in table.

Table 2.5 Limits for heavy metals

S1.No (1)	Characteristics (2)	Limit (3)
1	Arsenic (as As), mg/kg max	1
II	Copper (Cu)mg/kg max	20
III	Lead (as Pb), mg/Kg max	2.0
IV	Tin (as Sn), mg/kg max	250

(Source: SLS 214: 1985)

2.17 Shelf Life Evaluation

2.17.1 Introduction

Foods are perishable by nature. Numerous changes took place in food during food processing and food storage. It is well known that conditions used to process and store foods may adversely influence the quality attributes in food. Upon storage for a certain period, one or more quality attributes of a food may react an undesirable state. At that point food is considered unsuitable for consumption and it's said to reach of its self-life.

Self-life is an important feature of all foods. All those who involved in the handling of foods should aware of those facts. These may include growers, ingredients, suppliers, manufactures, whole sellers, retail sellers and the customers. Self-life of product may be defined as the time between the production and packaging of the product and the point at which it become unacceptable under defined environmental conditions (Man and Jones, 1994).

2.17.2 Major Modes of Food Deterioration.

During storage and distribution of food, they are exposed to a wide range of environmental conditions. Environmental factors such as temperature, humidity oxygen and light can trigger several reactions, mechanisms that may lead to food degradation. As a consequence of these mechanisms, foods may be altered to such extent so that the consumer either rejects them, or they may become harmful to the person. It is there for imperative that a good understanding of different reactions that cause food deterioration is gained prior to developing, specific procedure for the evaluation of the self-life of products. Chemicals, Physical and Microbiology changes are the leading causes of food deterioration (Man and Jones, 1994).

2.18 Sensory Evaluation

2.18.1 Introduction

A sensory evaluation system is made by the senses of taste, smell, touch and hearing when food is eaten. The complex sensation that results from the interaction of our sense is used to measure food quality in programs for quality control and new product development.

Sensory evaluation panels can be grouped in to three types, highly trained experts, laboratory panels and large consumer panels. Highly trained experts evaluate quality. Large consumer panels are used to determine consumer reaction to a product (Larmond, 1987). A sensory department may interact with many other departments in a food or consumer products company. Their primary interaction is in support of product research and development, such as marketing research supports to the company's marketing efforts. However they may also interact with quality control, marketing research, packaging and design groups, and even legal services over issues such as claim, substantiation and advertising challenges (Lawless and Heymann, 1999).

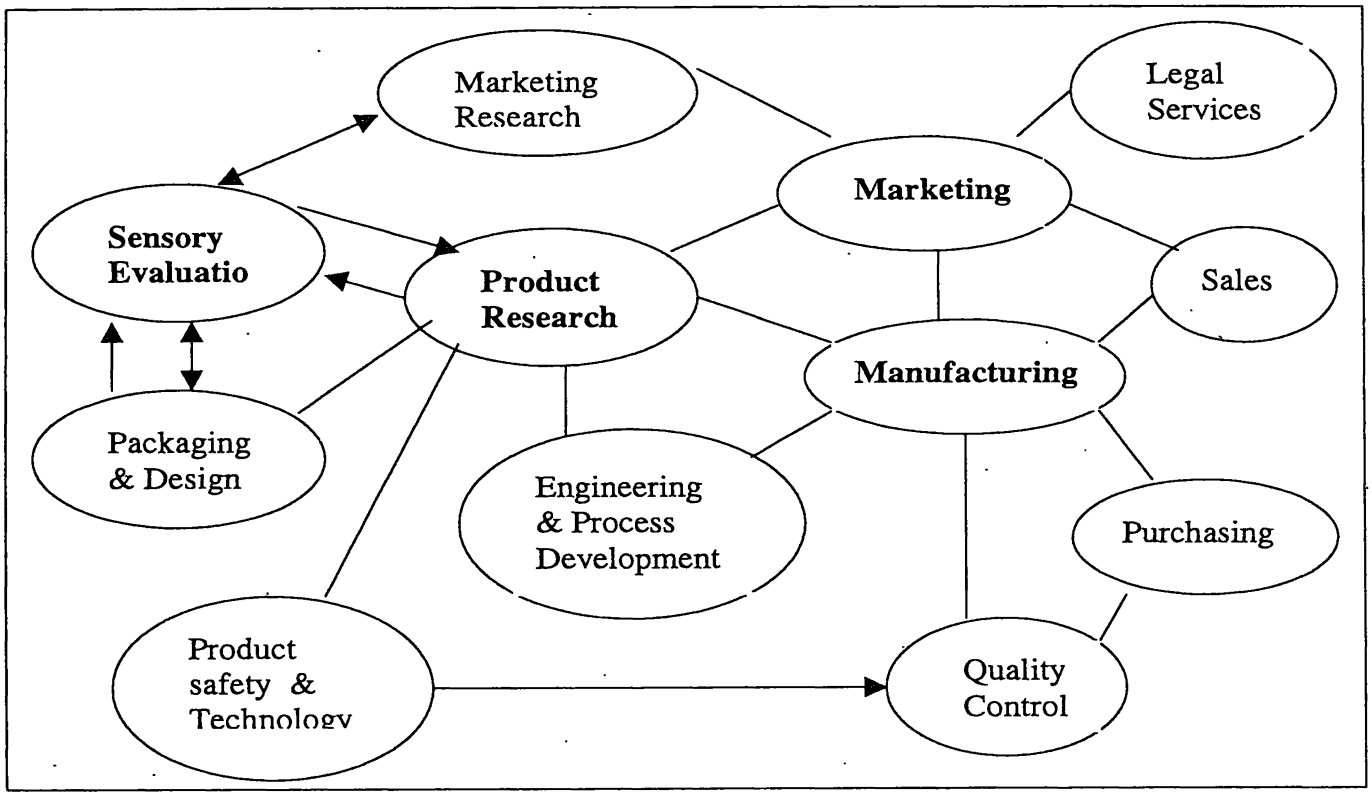

Fig.2.4. A sensory evaluation department may interact with many other department in a food or consumer products

2.18.2 Preparing for the test

- **Testing Area**

For sensory evaluation special testing area is used that distractions can be minimized and conditions can be controlled.

The preparation area should be separate from the testing area. The panelist should not enter the preparation area as they might gain information that would influence their judgment.

Foreign odors and odors from food preparation should be kept from the testing room. Smoking should not be permitted at any time and cosmetic odors should be avoided (Larmond, 1987).

- **Testing setup**

For most types of testing except profile methods, the panelists are required to make independent judgments. In order to eliminate distraction and prevent communication among the panelists, individual booths are

used. This arrangement is really preferable because the product to be tested can be passed through the preparation area to the panelists and the operators do not have to serve samples in the testing room (Larmond, 1987).

- **Lighting**

Lighting should be uniform and should not influence the appearance of the product to be tested. The type of light used should be carefully chosen. Color and appearance are important factors to be judged, since many fluorescent lights distort color. To eliminate difference in color between samples colored lights are sometimes used (Larmond, 1987).

- **Testing schedule**

The time of day that tests are run influences results, although this can not be controlled if the number of tests is large. Late morning and mid afternoon are generally the best times for testing (Larmond, 1987).

2.18.3 Methods for Sensory Testing

Several different sensory evaluation methods have been developed. The experiment should thoroughly familiar with the advantages and disadvantages of each method. The most practical and efficient method should be selected for each situation. No one method can be used universally. The experimenter must precisely define the purpose of the test and the information he wants to acquire.

There are 3 fundamental types of sensory tests. Preference/ Acceptance test, Discriminatory tests and Descriptive tests (Larmond, 1987).

- **Preference / Acceptance test**

These tests are effective tests based on a measure of preference or a measure from which relative preference can be determined. The personal feeling of a panelist toward the product directs his responses. Preference tests include the Paired comparison test, the Hedonic scale and Ranking (Larmond, 1987).

- **Discriminatory Test**

These tests are used to determine whether a difference exists between samples. The panelist does not allow his personal likes and dislikes influencing his response. Laboratory difference panels can be used to determine if there is a difference among samples (Larmond, 1987).

- **Descriptive test**

These tests are used to determine the nature and intensity of the differences (Larmond, 1987).

2.18.4 Hedonic Scale

The most commonly used scale for preference testing is the nine – point hedonic scale. The letter hedonic is defined as "having to do with pleasure". It should only be used in connection with scales in which the panelist expresses his degree of liking or disliking (Larmond, 1987).

2.18.5 Type of Panel

- **A Trained Panel**

Is the most usual type for conducting several type of testing? This is because a trained panel is more capable of describing the differences between samples. Small highly trained panel will give more reliable result than large untrained panel (Heymann and Lawless, 1999).

- **An Untrained panel**

Although untrained assessors tend to be less sensitive to small difference and are likely to be less consistent in their scoring of replicate products. The technique of free-choice profiling may be suitable for use with untrained panels, but may be more effective if the panel has received some basic training (Heymann and Lawless, 1999).

- **A Consumer panel**

Large number o respondents would be required in order to achieve a significant result, and if justified. It would seem more appropriate to have

them focus on preference question rather than difference question (Heymann and Lawless, 1999).

2.18.6 Sensory panel and panelists

2.18.7 Sensory parameters

- **Flavour**

The combination of taste and odour.It may be influenced by sensations of pain, heat, cold and tactile sensation (Heymann and Lawless, 1999).

- **Smell**

Our sense of smell can detect many difference odours when shifted through the nose, but it also important for detecting volatiles given of by food item in the mouth as part of the Flavour perception. The sense of smell is key input to sensory analysis (Heymann and Lawless, 1999).

- **Taste**

The sensation of taste is a result of the effect of water-soluble molecules interacting with receptors on the tongue and in the oral cavity (Heymann and Lawless, 1999).

- **Texture**

The attribute of a substance resulting from a combination of physical properties and perceived by the senses of though, slight and hearing. Physical properties may include size, shape, number, nature and conformation of constituent structural elements (Heymann and Lawless, 1999).

- **Color**

Color is an important appearance factor.colour used in to assessing of the food. The colour of a food often affects our perception of and evaluation by other senses. Consumer preferences based on the colour of the product.colour characteristics of foods can result from both pigmented and originally nonpigmented compound (Heymann and Lawless, 1999).

2.19 Physicochemical properties assessment

2.19.1 Acidity

In evaluating the acidity of any beverage, two aspects must be considered. These are (1) the quantity aspect, that is, the total amount of available hydrogen ion present in a given volume of the beverage as estimated by a determination of the total titratable acidity and (2) the intensity aspect, that is, the apparent hydrogen-ion concentration usually expressed in terms of pH (Jacobs, 1959).

2.19.2 pH

The symbol.pH has been adopted for the logarithm of the reciprocal of the hydrogen-ion concentration. If the hydrogen-ion concentration (represent by $[H^+]$) of a solution is known, the corresponding pH of the solution may be calculated from the formula:

$$pH=\log 1/ [H^+]$$

It is not really necessary to consider the meaning of pH in terms of the theory of solution. The pH numbers need only be accepted as a practical scale of acidity and alkalinity with a pH of 7.00 being the neutral point, that is, the point where the concentrations of hydrogen ion and hydroxyl ion are equal. Solution in the hydroxyl-ion concentration, that is, acid solution, has lower pH values ranging down to 1.0 or lower. Solution in which the hydroxyl-ion concentration is greater than the hydrogen-ion concentration is alkaline, and the pH is expressed by higher value ranging up to 13 or 14 (Jacobs, 1959).

2.19.3 Total Soluble Solid (TSS)

- **Brix value**

Since the amount of sucrose dissolved in water is important industrially, a whole series of measuring indices were developed to indicate the relative proportion of the two materials. The most Important of these is the Brix scale, which relates the percentage by weight of sucrose in a water solution. Therefore, 65° Brix would represent a solution that is

65 percent sucrose and 35percent water. The Brix scale is also used to measure solution other than pure sucrose and water (Knecht, 1987).

2.20. New Food Product Development.

2.20.1. Introduction

A simple definition for a new product might be a 'product not previously marketed or manufactured by a company'. However, this breaks down if one includes new packaging (shape or size) or if one enters a product into a new market niche – the food service sector, for example. The definition of new product development and introduction of a product not previously manufactured by a company into the market place or the presentation of an old product in to a new product into a new market not previously explored by a company (Fuller, 1994).

2.20.2 Classification and Characterization of New Products

New food products fall into one of the following classifications:

- Line extensions
- Repositioned existing products
- New form of existing products
- Reformulation of existing products
- New packaging of existing products
- Innovative or added – value products
- Creative products (Fuller, 1994).

2.20.3 Why Go Into New Food Product Development?

If new food product development is fraught with so much difficulty, if it is so costly, and if it has a high rate of failure, why go in to it? Would it not be simpler to coast along with the existing products?

This certainly would be simpler but it would not be profitable for very long. Food companies must grow to make money and survive. New food products are the major avenues open to a food company to be profitable and to survive.

The need for new food product development can be seen to be driven by five dominant forces:

- All products have life cycles. That is, they enter the marketplace, flourish for an indeterminate time, then die, and must be replaced.
- A company's management may adopt a policy that requires an aggressive growth program to satisfy long – range business goals.
- The marketplace may change, requiring new products more suited to respond to the changes.
- New technology may take new food products available and new knowledge may tailor new food products more suited to the lifestyles of today's consumers.
- Change in government legislation, health programs, agriculture policy, or agriculture support programs may dictate that development of new food products be pursued (Fuller, 1994).

2.20.4 Phases in New Food Product Development

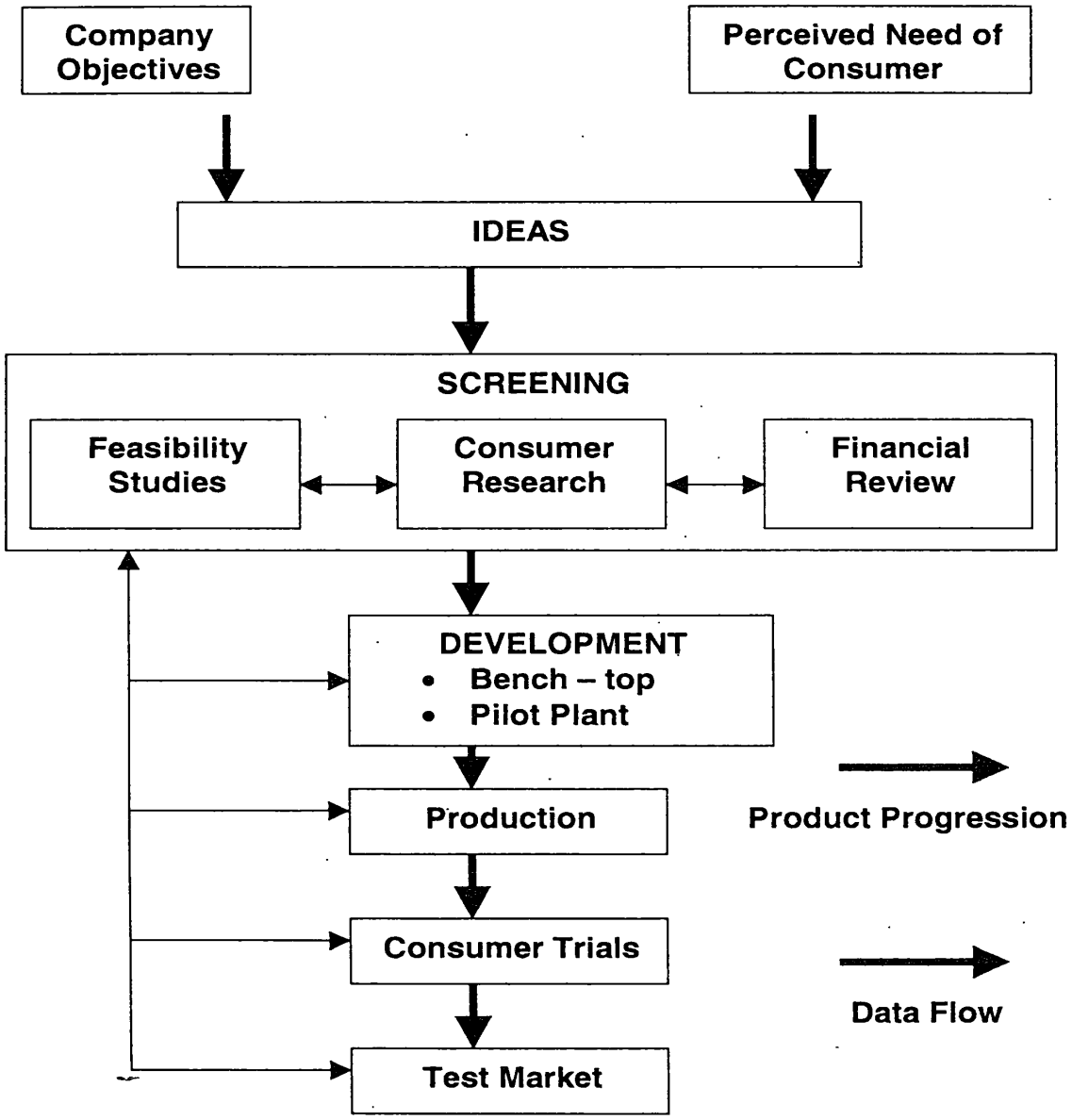

Fig 2.5 Phases in New Food Development
(Source: Fuller, 1994)

CHAPTER 03

MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Materials for clean and crushed the Iramusu root.

Apparatus

- Stainless steal knife
- Cutting board
- Plastic tray
- Portable water
- Plastic basket

3.1.2 Materials for extraction of Iramusu juice

Raw Materials

- Dried Maha Iramusu Root

Apparatus

- Electronic balance (MS – 100, Capacity 2g – 100g, 100 mg)
- Measuring cylinder (Volac, Capacity 1000ml ±10ml)
- Plastic jug
- Strainer
- Stainless steal spoon
- Stainless steal sauce pan
- Gas cooker
- Thermometer (Quartz, - 50 ^{0}C ~ +260 ^{0}C)
- Glass bottles

3.1.3 Materials for concentrated herbal drink preparation

Raw Materials

- Extracted Iramusu Juice
- Portable water
- Sugar
- Citric acid
- Salt
- Carboxy Methyl Cellulose (CMC)
- Sodium Meta Bisulphite(SMS)

Apparatus

- Electronic balance (MS – 100, Capacity 2g – 100g, 100 mg)
- Small Measuring cylinder (MC, 50ml ±0.5ml)
- Plastic jug
- Stainless steal spoon
- Stainless steal source pan
- Gas cooker
- Thermometer (Quartz, - 500 ~ +200 C)
- Glass bottles
- Sterilize bottles and lids
- Plastic wash bottle
- Hand Refractometer(Atago,ATC-1E,Brix 0-32)
- pH meter(pH Scan WP2,+0.1pH)

3.1.4 Materials for Sensory Evaluation

- Sensory evaluation ballet paper
- Coded samples
- White glasses
- Serviette
- Glasses of portable water

3.1.5 Materials for chemical analysis

Apparatus

- Hand Refractometer (Atago, ATC-1E, Brix 0-32)
- pH meter (pH Scan WP2,+0.1pH)
- PH buffer capsules (pH = 4.0 ±0.05, pH = 7.0 ± 0.05)
- Small measuring cylinder (MC 50 ml ± 0.5 ml)
- Measuring cylinder (GT Britain, 1000 ml ± 10 ml)
- Burette
- Volumetric flask
- Metal stand
- 0.1 M NaOH solution
- Phenolphthalein
- Dropper
- Plastic wash bottle

3.1.6 Materials for Microbiology analysis

Raw Materials

- MacConky broth
- Standard methods agar
- Peptone water

Apparatus

- Incubator
- Pipette (ISO lab, Germany, 10ml ± 0.05ml)
- Small Conical flask (ISO lab, Germany, 250ml)
- Conical flask (Borosil, 1000ml)
- Measuring cylinder (Borosil, 50ml)
- Magnetic stirrer (Biocraft)
- Petridish (Borosil)

3.2 Methodology

3.2.1 Disinfection of Iramusu

Maha Iramusu roots purchased from Ayurvedic shop and it was stored on better condition under room temperature (27±3°C). Then Iramusu root was weighted and washed. Finally root was cut in to small pieces.

3.2.2 Determination of best Relationship for time and amount of water added.

100g of Maha Iramusu root was taken and it was chopped in to small pieces. 1L of water was added for 2 samples and for another two samples 2L and for the next 2 samples 1.5 L and 0.5 L water was added. One of 1L, 2L, 1.5L, and 0.5L water contained sample was boiled for 20 min and for the other samples were boiled for 30 min. Then those 8 extracts were cooled and after that TSS of the extracts were measured using Refractometer.

3.2.3 Determination of amount of Iramusu extract to be added in drink formulation.

Different amounts of water extracted Iramusu were mixed with different amounts of water to maintan final volume of 100ml while maintaining constant amount of SMS, Citric acid and CMC as given in the Table 2.1.

Table 3.1 Ingredients for the formulation of Herbal drink with different Iramusu amounts.

Sample Code	Extracted Iramusu (ml)	Water (ml)	Sugar (g)	CMS (mg)	SMS (mg)	Citric Acid (mg)	Final Volume (ml)
359	30	60	10	500	15	500	100
514	40	50	10	500	15	500	100
188	50	40	10	500	15	500	100

3.2.3.1 Evaluation of sensory appeal to determine the optimum amount of Iramusu extract added in drink

Sensory evaluation was done by 30 untrained panelists of Kelani Valley Canneries Ltd., and Faculty of Applied Science with use of ballot paper. Acceptability of 3 samples was evaluated using 9 – point hedonics scale subjectively. Three samples were coded as three digits number (See App. 01). Three glasses of diluted herbal drink were prepared by mixing one part of concentrated drink with three parts of water. Coded samples, ballot papers and water glasses were given for each and every panelists and suitable environment was provided for them to do their evaluaton unbiasely.

Results were analyzed using computer aided MINITAB Statistical Analysis package according to Kruscal wallis test at 95 % level of significant level (See App. 05).

3.2.4. Determination of optimum sugar level for the herbal drink formulation.

Constant amount of extracted Iramusu (40 ml) juice was mixed with different amounts of sugar (10g, 15g, 20g, 25g) and water along with constant amount of 5 mg citric acid, CMC separately as given in the Table 3.2.

Table 3.2 Ingredients required for formulation of Iramusu herbal drink with different sugar levels.

Sample	Iramusu Amount (ml)	Water (ml)	Sugar (g)	Citric Acid (mg)	CMC (mg)	SMS (mg)	Final Volume (ml)
897	40	50	10	500	500	15	100
195	40	45	15	500	500	15	100
174	40	40	20	500	500	15	100
192	40	35	25	500	500	15	100

3.2.4.1 Evaluation of sensory appeal to determine the Optimum Sugar level

Sensory evaluation was done by a untrained 30 panelists of Kelani Valley Canneries (KVC) Ltd, and faculty of Applied science with use of herbal ballot paper (See App 02).

Results were analyzed using computer aided MINITAB Statistical Analysis package according to Kruscal wallis test at 95 % level of significant level (See App. 06).

3.2.5 Enhancement of suitable flavor combinations

Constant amount of Iramusu extracts were taken and for one sample citric acid and salt was added as given in the Table 3.3. Citric acid and lime juice combination was added to another sample and for final sample only citric acid was added. These final samples were prepared while keeping the Brix value constant (25^0).

Table 3.3 preparation of Iramusu herbal drink with different Flavour combination

Sample Code	Iramusu (ml)	Sugar (g)	Water (ml)	Citric Acid (mg)	Salt (mg)	Lime Juice (mg)	CMC (mg)	SMS (mg)
853	40	20	40	500			500	15
921	40	20	40	250		250	500	15
799	40	20	40	250	250		500	15

3.2.5.1 Evaluation of sensory appeal to determine the best flavour enhancement combination.

The three prepared samples were sensed by trained sensory panelists of Kelani Valley Canneries (KVC) Ltd. Acceptability of three samples were evaluated using 9 point hedonic scale subjectively. Three samples were coded as three digits number (See App 03).

Results were analyzed using computer aided MINITAB Statistical Analysis package according to Kruscal wallis test at 95 % level of significant level (See App. 07).

3.2.6 Preparation of concentrated Iramusu herbal drink.

Water was put in to the vessel and heated. Part of the sugar was added during heating. It was heated until sugar gets completely dissolved. The measured Iramusu amount was added to it. Then the sugar and stabilizer were added and stirred well while heating the mixture up to about 85°C.The measured amounts of citric acid and salt was added and mixed thoroughly. The measured amount of preservative was added to the mixture. Hot filling to the sterilized bottles was carried out immediately.

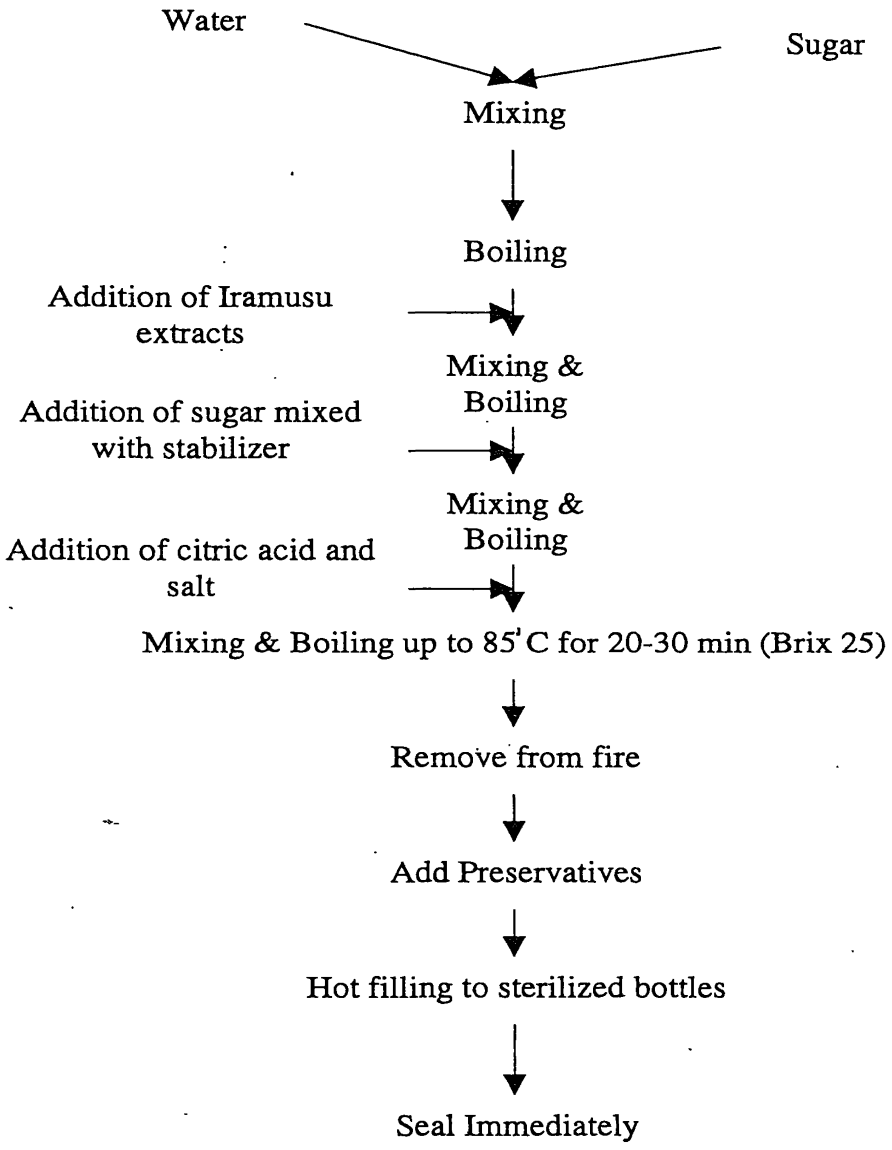

Fig 3.1 Processing flow of Concentrated Herbal drink

3.2.7 Studies on Physico- chemical Changes during storage and shelf life evaluation of Natural concentrated herbal drink.

Stored samples under normal condition were evaluated over 1 ½ month for pH, Acidity and TSS with one-week interval.

3.2.7.1 Physicochemical Assessments

Determination of pH

(1) Calibration of pH meter

- The protective cap was removed and tip of the electrode was dipped into stylized water heater and waited until stable value was displayed.
- Two buffer solutions were (PH 7.0 and PH 4.0) prepared by dissolving buffer capsules in distilled water.
- After that the pH meter was cleaned and dried and the tip of the electrode was immersed into the pH 7.0 buffer solution.
- The pH meter was calibrated to pH 7.0 by using screw type equipment.
- After that pH meter was immersed into pH 4.0 buffer solutions and calibrated to pH 4.0.

(2) Measuring the pH

- Prepared Iramusu sample was put into a beaker and pH meter was inserted into it.
- pH value was read directly.

Determination of Total Soluble Solid (TSS) in herbal drink

- 2-3 drops of Iramusu herbal drink was placed on the glass platform of the Refractometer.
- Then the reading was taken
- After that glass platform of Refractometer was cleaned by washing with distilled water and dried it using blotting paper.

Determination of Acidity

- 10g of diluted sample was weighted.
- 100 ml – 150 ml distilled water was added to it.
- Then 1ml of phenolphthalein indicator was added.
- Finally it was titrated with standard Sodium Hydroxide (NaOH) solution (01. M) (See App. 04).

3.2.7.2 Microbiological Evaluation

Microbial counts (Total Plate Count, Coliform) were taken after 1 ½ months from the stored sample.

Preparation of media:

Standard Method Agar

23.5g of medium was suspended in 1L of distilled water and boiled to dissolve completely. Then it was sterilized by autoclaving at 121°C for 15 minutes.

Peptone water

15g of sample was added in to 1L of distilled water and mixed well and transferred in to final containers. Then it was sterilized by autoclaving at 121°C for 15 minutes.

MacConkey broth

35g of sample was suspended in 1L of distilled water and boiled to dissolve completely. Then it was sterilized by autoclaving at 121°C for 15 minutes.

Table 3.4 Composition of MacConkey Broth.

Peptone	20 g
Lactose	10 g
Bile Salt	05 g
Neutral red or Brom cresol purple	0.075g 0.01g

Preparation of the serial dilution:

First dilution

1ml of original sample was measured and mixed with 9 ml of peptone water and shaked well. It was labeled as 10^{-1} solution.

Second dilution

From the first dilution, 1ml was transferred into second dilution tube containing 9ml of peptone water. It was labeled as 10^{-2} solution. This was repeated in preparation of 10^{-3},10^{-4}, 10^{-5}, 10^{-6}.

(A) Coliform test

This test is called "9 tubes test". MacConkey broth was used as the culture media. pH was Adjusted to 6.6 and 10 ml of Macconkey broth were put in to each 9 test tubes and Durham tubes were introduced to all the tubes and they were sterilized after filling of culture media without air bubbles. Test tubes were covered with cotton plugs and Aluminum foil. All tubes were sterilized in an autoclave at 121° C, 15 Psi pressure for 15 min.

After that 1ml of original sample was taken into a pipette. It was put into 3 separate tubes, which contained 10 ml quantities of Macconkey broth with Durham tubes. Tubes were labeled as10^{0}. 1 ml was pipetted out from serial decimal dilution solution (10^{-1}) and introduced in to 3 test tubes of 10 ml of Macconkey broth with Durham tubes. Tubes were labeled as 10^{-1}.After that 1ml of 10^{-2} solution was pipetted into the remaining 3 test tubes with 10 ml of Macconkey broth and Durham tubes. Tubes were labeled as 10^{-2}.

After that, each tube was incubated at 37 ^{0}C and was examined for the gas formation in the Durham tubes after 24 hrs and 48 hrs. Number of tubes out of each set of 3 were recorded, which gave positive results for the particular organism and was calculated using MPN tables (SLS 516: Part 3, 1991) (See.App.08).

(B) Pour plate method (Aerobic plate count)

The distribution serial of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} solution were prepared as above. Then 15 ml of the prepared standard method Agar medium was poured into petri dishes at 45 ± 0.5 °C. Then 1 ml from each sample of serial dilutions was pipptted out and introduced aseptically in to sterilized plates. Its were labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. 1 ml was pipetted out form the original sample and it was introduced into petri dishes with 15 ml of the prepared standard method Agar medium. It was labeled as 10^{0}. Then lid was closed immediately and shaken gently for even distribution of media on plate. After that plates were kept inverted on a clean horizontal surface for few minutes to solidify the nutrient agar. Then the dishes were surface and incubated at 36 ± 1 °C for 72 ± 3 hrs. After the specified period of incubation, colonies were counted in each petri dish using the colony counter (SLS 516: Part1, 1991).

CHAPTER 04

RESULTS AND DISCUSSION

4.1 Results

Results of the TSS (Total Soluble Solids) for find best relationship for time and amount of water, sensory evaluation test of selecting best iramusu amount, best level of sugar, best flavor enhancement combination and Result of the shelf life evaluation are discussed below.

4.1.1 Best Relationship for time and amount of water

According to the result obtained in the Table 4.1 Brix value of the samples increasing with increasing time and reducing water amount. It is due to the increase of the total soluble solides. Shastry (2005) reported that out of all compound present in the root, not less than 13% can be extract in to the water soluble extraction. Rai, Shrivastara and Pawarsun (2005) were reported coumarins, tannic acid, triterpenoid, saponins and,sterols like Hemidesterol, Hemidesmol and resin acid are affect the TSS of extracted Iramusu Juice. A stearopten smilasperic acid was also obtained by hot water extraction. Except sample No.7 all of other samples were low in Brix value. Sample No.7 has highest Brix value. Therefore sample No.7 has the suitable time and water combination to extract Iramusu Juice form raw Iramusu.The best relationship for time and amount of water was 30 min and 500 ml for 100g of maha Iramusu root sample. According to the Sethi (2004) Herbal Juice can be extracted out from dry herbal plant by soaking the coarse powder of the drug overnight and then next morning mashing it by hand. And also warm infusion was obtained by putting one part of powdered herb in eight parts of hot water and then boiling. The bitter taste was less in sample No.1 and 4 this may be due to less extraction time and more water.Sample No.8 and 7 have slightly bitter taste, because of less amount of water used for extraction.Table 4.1 presence the result of extraction.

Table 4.1 Extraction condition

Sample Number	Weight of Iramusu	Amount of water	Time	Brix
1	100g	2.0L	30min	0.9
2	100g	2.0L	20min	0.7
3	100g	1.5L	30min	1.0
4	100g	1.5L	20min	0.9
5	100g	1.0L	30min	1.2
6	100g	1.0L	20min	1.0
7	100g	0.5L	30min	4.0
8	100g	0.5L	20min	2.0

4.1.2 Result of sensory evaluation for Determination of Optimum amount of Iramusu extract to be added in drink formulation

The result of sensory evaluation conducted to determine the most appropriate Iramusu amount were given below. According to the analyzed data of the sensory evaluation the level of preference for each sensory attribute in all 3 samples were identified (See.App.05).

4.1.2.1 The effect of Iramusu extract on the colour of herbal drink

The results of effect on the colour of the herbal drink show the highest rank for the product, which contain 40 ml of Iramusu extract in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05. According to the data sample with 40 ml Iramusu juice gained the highest sum of rank value with the highest estimated median for colour. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.05).

Table 4.2 Effect of Iramusu Extract on Colour

(Sample 359:30 ml, 514:40ml, 188:50ml)

Sample code	N	Estimated Median	Sum of Rank
359	30	5.000	32.4
514	30	7.000	64.9
188	30	6.000	39.2

4.1.2.2 The effect of Iramusu extract on the Taste of the herbal drink

The result shows the highest rank for the product, which contained 40 ml of Iramusu extract in the formula. According to analyzed data there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data, sample with 40 ml of Iramusu juice gained the highest sum of rank value with the highest estimated median for Taste. Therefore this samples comes under the category of "Like very much" according to the 9-point hedonic scale (See.App.05).

Table 4.3 Effect of Iramusu Extract on Taste

(Sample 359:30 ml, 514:40ml, 188:50ml)

Sample code	N	Estimated Median	Sum of Rank
359	30	5.000	32.1
514	30	7.500	65.5
188	30	6.000	39.0

4.1.2.3 The effect of Iramusu extract on the Smell of the herbal drink

The highest rank for the smell of the product, gained by the sample which contained 40 ml of Iramusu extract in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data sample with 40 ml Iramusu juice gained the highest sum of rank value with the highest estimated median for Smell. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.05).

Table 4.4. Effect of Iramusu Extract on Smell

(Sample 359:30 ml, 514:40ml, 188:50ml)

Sample code	N	Estimated Median	Sum of Rank
359	30	5.500	33.6
514	30	7.000	62.9
188	30	6.000	40.0

4.1.2.4 The effect of Iramusu extract on the Appearance of the herbal drink

The product, which contained 40 ml of Iramusu extract in the formula achieved the highest rank for the appearance of the herbal drink.. According to analyzed data there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data sample with 40ml Iramusu juice gained the highest sum of rank value with the highest estimated median for Appearance. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.05).

Table 4.5. Effect of Iramusu Extract on Appearances

(Sample 359:30 ml, 514:40ml, 188:50ml)

Sample code	N	Estimated Median	Sum of Rank
359	30	5.000	34.0
514	30	7.000	64.4
188	30	6.000	38.2

4.1.2.5 The effect of Iramusu extract on the overall acceptability of the herbal drink

The results obtained for overall acceptability of the herbal drink shows the highest rank for the product, which contained 40 ml of Iramusu extract in the formula. According to analyzed data it the significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value

p=0.05.The sample with 40 ml Iramusu juice gained the highest sum of rank value with the highest estimated median for overall acceptability. Therefore these samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.05).

Table 4.6 Effect of Iramusu Extract on Overall Acceptability

(Sample 359:30 ml, 514:40ml, 188:50ml)

Sample code	N	Estimated Median	Sum of Rank
359	30	5.000	31.2
514	30	7.000	64.3
188	30	6.000	40.9

4.1.3 Result of sensory evaluation for Determination of Optimum sugar level for Herbal drink

The results of sensory evaluation conducted to determine Optimum sugar level for Iramusu Herbal drink given below. According to the analyzed data of the sensory evaluation the level of preference for each sensory attribute in all 4 samples were identified (See.App.06).

4.1.3.1 The effect of sugar level on the Colour of the herbal drink

The results of effect on the colour of the herbal drink show the highest rank for the product, which contains 20g of sugar level in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data sample with 20g of sugar level gained the highest sum of rank value with the highest estimated median for colour. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.06).

Table 4.7 Effect of Sugar level on Colour

(Sample 897:10g, 195:15g, 174:20g, 192:35g)

Sample code	N	Estimated Median	Sum of Rank
897	30	6.000	35.9
195	30	7.000	52.4
174	30	7.000	81.6
192	30	7.000	72.1

4.1.3.2 The effect of Sugar level on the Taste of the herbal drink

The results of effect on the taste of the herbal drink show the highest rank for the product, which contain 20g of sugar level in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data sample with 20g of sugar level gained the highest sum of rank with the highest estimated median for Taste. Therefore this samples comes under the category of "Like very much" according to the 9-point hedonic scale (See.App.06).

Table 4.8 Effect of Sugar level on Taste

(Sample 897:10g, 195:15g, 174:20g, 192:35g)

Sample code	N	Estimated Median	Sum of Rank
897	30	4.500	33.6
195	30	5.000	41.0
174	30	7.500	87.2
192	30	7.500	80.3

4.1.3.3 The effect of Sugar level on the Smell of the herbal drink

The results of effect on the Smell of the herbal drink show the highest rank for the product, which contain 20g of sugar level in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.003 of the test is less than the minimum probability value

p=0.05.According to the data sample with 20g of sugar level gained the highest sum of rank value with the highest estimated median for Smell. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.06).

Table 4.9 Effect of Sugar level on Smell

(Sample 897:10g, 195:15g, 174:20g, 192:35g)

Sample code	N	Estimated Median	Sum of Rank
897	30	6.500	49.6
195	30	6.500	50.1
174	30	7.500	77.1
192	30	7.000	65.3

4.1.3.4 The effect of Sugar level on the Appearance of the herbal drink

The results of effect on the Appearance of the herbal drink show the highest rank for the product, which contain 20g of sugar level extract in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data sample with 20g of sugar level gained the highest sum of rank value with the highest estimated median for Appearance. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.06).

Table 4.10 Effect of Sugar level on Appearances

(Sample 897:10g, 195:15g, 174:20g, 192:35g)

Sample code	N	Estimated Median	Sum of Rank
897	30	6.000	39.7
195	30	7.000	54.5
174	30	7.000	75.1
192	30	7.000	72.7

4.1.3.5 The effect of Sugar level on the Overall Acceptability of the herbal drink

The results of effect on the Overall Acceptability of the herbal drink show the highest rank for the product, which contain 20g of sugar level in the formula. According to data analysis there is a significant difference between the samples, since probability value p=0.000 of the test is less than the minimum probability value p=0.05.According to the data sample with 20g of sugar level gained the highest sum of rank value with the highest estimated median for Overall Acceptability. Therefore this samples comes under the category of "Like moderately" according to the 9-point hedonic scale (See.App.06).

Table 4.11 Effect of Sugar level on Overall Acceptability

(Sample 897:10g, 195:15g, 174:20g, 192:35g)

Sample code	N	Estimated Median	Sum of Rank
897	30	5.000	34.3
195	30	6.000	43.4
174	30	8.000	85.8
192	30	8.000	78.6

4.1.4 Result of sensory evaluation for Determination of best flavour enhancement combination for Herbal drink

The results of sensory evaluation conducted to determine best flavour enhancement combination were given below. According to the analyzed data of the sensory evaluation the level of preference for each sensory attribute in all 3 samples were identified (See.App.07).

4.1.4.1 The effect of flavour enhancement combination on the Colour of the herbal drink

The probability value (P=0.127) of the test was higher than the minimum probability value (P=0.05) reveled that there was no significant difference in all three samples (See.App.07).

Table 4.12 Effect of flavour enhancement combination on Colour

(Sample 853:citric acid, 921:citric acid & lime juice, 799:citric acid & salt)

Sample code	N	Estimated Median	Sum of Rank
853	10	7.500	12.9
921	10	7.500	14.0
799	10	8.000	19.7

4.1.4.2 The effect of flavour enhancement combination on the Taste of the herbal drink

The probability value (P=0.004) of the test was lower than the minimum probability value (P=0.05) reveled that there was significant difference in all three samples. On the other hand, sample code 799, which contained citric acid and salt gained highest sum of ranks with the highest, estimated median for taste. Therefore this samples comes under the category of "Like very much" according to the 9-point hedonic scale (See.App.07).

Table 4.13 Effect of flavour enhancement combination on Taste

(Sample 853:citric acid, 921:citric acid & lime juice, 799:citric acid & salt)

Sample code	N	Estimated Median	Sum of Rank
853	10	6.500	10.5
921	10	7.000	13.4
799	10	8.000	22.6

4.1.4.3 The effect of flavour enhancement combination on the Smell of the herbal drink

The probability value (P=0.280) of the test was higher than the minimum probability value (P=0.05) reveled that there was no significant difference in all three samples (See.App.07).

Table 4.14 Effect of flavour enhancement combination on Smell

(Sample 853:citric acid, 921:citric acid & lime juice, 799:citric acid & salt)

Sample code	N	Estimated Median	Sum of Rank
853	10	8.000	16.1
921	10	7.500	12.5
799	10	8.000	17.9

4.1.4.4 The effect of flavour enhancement combination on the Appearance of the herbal drink

The probability value (P=0.687) of the test was higher than the minimum probability value (P=0.05) reveled that there was no significant difference in all three samples.

Table 4.15 Effect of flavour enhancement combination on Appearance

(Sample 853:citric acid, 921:citric acid & lime juice, 799:citric acid & salt)

Sample code	N	Estimated Median	Sum of Rank
853	10	8.000	15.6
921	10	8.000	14.2
799	10	8.000	16.8

4.1.4.5 The effect of flavour enhancement combination on the Overall acceptability of the herbal drink

The probability value (P=0.002) of the test was lower than the minimum probability value (P=0.05) reveled that there was significant difference in all three samples. On the other hand, sample code 799, which contained citric acid and salt gained highest sum of ranks with the highest, estimated median for taste. Therefore this samples comes under the category of "Like very much" according to the 9-point hedonic scale (See.App.07).

Table 4.16 Effect of flavour enhancement combination on Overall acceptability

(Sample 853:citric acid, 921:citric acid & lime juice, 799:citric acid & salt)

Sample code	N	Estimated Median	Sum of Rank
853	10	6.500	9.9
921	10	7.000	13.7
799	10	8.000	23.0

4.1.5 Formulation of natural Iramusu Herbal drink

Formulation was done by conducting number of trials and sensory evaluations. Finally the formulation was completed for the concentrated natural herbal drink with Brix value 25°.

Table 4.17 Ingredient of formulated Natural Iramusu Herbal drink. Sun of the Rank o f sensory attribute

Ingredient	Amount
Extracted Iramusu Juice	40ml(40%)
Sugar	20ml(20%)
Water	40ml(20%)
Citric acid	250mg(0.25%)
Salt	250mg(0.25%)
Stabilizer (CMC)	500mg(0.5%)
SMS	15mg(0.015%)

Fig 4.1 Final product of the Concentrated Herbal drink

4.1.6 Shelf life evaluation

4.1.6.1 Chemical Storage Studies

Final selected sample stored for 1 ½ month at room temperature and chemical storage studies (Titratable acidity, pH, TSS) were observed in this storage period at room temperature (27°C±3°C). There were no changes in the pH, TSS, Titratable acidity with in these 6 weeks. The result revealed that product was acceptable for 1 ½ month.

Table 4.18 Chemical storage studies

Storage week	Parameters		
	pH	TSS	Acidity
0	4.2	25	0.05
1	4.2	25	0.05
2	4.2	25	0.05
3	4.2	25	0.05
4	4.2	25	0.05
5	4.2	25	0.05
6	4.2	25	0.05

4.1.6.2 Microbiological Evaluation

(A) Coliform Test

After 24 hrs, and 48 hrs the observations were done to determine the presence of Coliform.

There was no gas formation in the Durham tubes inserted in the test tube, which were kept in the incubator. It was confirmed that the product does not contain any coliforms. Presence of Coliform indicates generally poor sanitation condition.

(B) Total Plate Count (Aerobic plate count)

After 24hrs of incubation, there were number of colonies appeared in the Petri dishes of Standard Method Agar. All the colonies were counted in the Petri dishes and TPC was counted as According to the SLS Standards as follows.

Number of microbial cell per ml=Number of colonies*dilution factor

Colonies per plate=x

Dilution factor=10^y

Volume of dilution added to the plate=1ml

So, Microbial count=$x*10^y$ Cells/1ml

Table 4.19 shows the amount of colonies appeared in the Petri dishes after 24 hrs.

Table 4.19 Microbial count

Microbial count	Added dilution to the plate	No: of colonies per plate	Microbial count per 1g of sample
10^0	1ml	26	26
10^{-1}	1ml	3	$3*10^1$
10^{-2}	1ml	No detected	_
10^{-3}	1ml	No detected	_
10^{-4}	1ml	No detected	_
10^{-5}	1ml	No detected	_
10^{-6}	1ml	No detected	_

The total plate count of the product can be identified as $3* 10^1$ cells per 1 ml, which shows the least number of viable cells among the Standard Method Agar Petri dishes.

4.2 Discussion

Concentrated herbal drink was prepared by using Dry maha iramusu root and incorporation with Sugar, Water, CMC, SMS, Citric acid and Salt.

Among the 8 samples best relationship for time and amount of water was determined by using TSS. Sample Number 7 has highest value o the TSS and so that sample No.7, which has best relationship for time and amount of water, used for the boiling. So that can be concluded optimum extraction condition was 100g of dry Iramusu root with 500ml water for 30 min boiling period.

To determine the best-extracted Iramusu amount among the three samples of herbal drink with 30ml, 40ml, and 50ml of extracted iramusu juice respectively. The sample number 514 which contain 40ml of extract Iramusu juice gained the highest rank for Appearance, Flavour, Colour, Smell, Overall acceptability.

Sensory evaluation was conducted to determine the Optimum sugar level among the four sample of herbal drink with 10g, 15g, 20g, 25g sugar level respectively, the sample number 174 which contain 20g of sugar gained the highest rank for Appearance, Flavour, Colour, Smell, Overall acceptability.

Best flavour enhancement combination was determined among the 3 samples of herbal drink with citric acid, citric acid and salt, citric acid with limejuice respectively. The sample number 799 which contain citric acid and salt gain highest rank for Taste and Overall acceptability. There was no significant difference in Colour, smell, Flavour and in three treatments.

Finally formulated sample was stored in 1 ½ month at room temperature and measured the pH, Titratable acidity, TSS once a week with in 6 weeks. Results showed no change in the pH, TSS, Titratable acidity with in these 6 weeks of storage. The result revealed that product was acceptable for 1 ½ months. Generaly herbal drinks available in the market like ginger cordial have shelf life around one year.

After the 6 weeks of storage microbiological analysis (Total plate Count, Coliform test) was done to determine the microbiological quality of the product. Total plate count of the product was $3*10^1$ per 1 ml. It was below the maximum acceptable level. Presence of bacteria due to contamination during

preparation by air, water, equipment and person involvement. There was negative for Coliform.

CHAPTER 05

CONCLUSTION AND RECOMMENDATION

5.1 Conclusion

After considering the observation and results from beginning to end of the research period, the final conclusion can express as follows.

1) The Optimum extraction condition is 100g of Iramusu roots in 0.5L of water for 30min boiling period.
2) Optimum sugar level is 20g for 100ml of final volume.
3) Optimum Iramusu amount is 40ml for 100ml of final volume.
4) Flavor Combination of salt and citric acid is best for the Herbal drink preparation.
5) The quality parameters (Titratable acidity, pH and TSS) of the concentrated herbal drink retained constant during the study period (1 ½ month).
6) Microbial evaluation revealed that sample was negative for Coliform. It was confirmed the absence of pathogens.
7) According to the results of total plate count of the product was $3*10^1$ per 1 ml. Viable microorganism that can grow in acidic media.

5.2 Recommendations

1) Chemical composition of concentrated herbal drink should be studies.
2) It is better to evaluate the changes of sensory attributes of concentrated herbal drink during the expected storage period.
3) Chemical change occurred in the concentrated herbal drink which leads to Color variation should be further studied.
4) Should be evaluated which quantity of active compound that retain to concentrated herbal drink using HPLC (High Performance liquid chromatography) method.

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Appendix 01

Sensory Evaluation Ballot Paper

Name: - .....

Date: -.....

This is a product based on Iramusu

Instructions: -

- Please taste the samples according to the following order.

Sample Codes: -

- Rank the samples according to data given below

Like extremely 9

Like very much 8

Like moderately 7

Like slightly 6

.....

Neither like not dislike 5

Dislike slightly 4

Dislike moderately 3

Dislike very much 2

.....

Dislike extremely 1

.....

	359	514	188
Taste			
Color			
Smell			
Appearance			
Overall Acceptability			

Your comments .....

Thank you.

Appendix 02

Sensory Evaluation Ballot Paper

Name: .....

Date:-.....

This is a product based on Iramusu

Instructions: -

- Please taste the samples according to the following order.

Sample Codes: -

- Rank the samples according to data given below

Like extremely 9

Like very much 8

Like moderately 7

Like slightly 6

.....

Neither like not dislike 5

Dislike slightly 4

Dislike moderately 3

Dislike very much 2

.....

Dislike extremely 1

.....

	897	195	174	192
Taste				
Color				
Smell				
Appearance				
Overall Acceptability				

Your comments .....

Thank you.

Appendix 03

Sensory Evaluation Ballot Paper

Name: .....

Date:-.....

This is a product based on Iramusu

Instructions: -

- Please taste the samples according to the following order.

Sample Codes: -

- Rank the samples according to data given below

Like extremely 9

Like very much 8

Like moderately 7

Like slightly 6

.....

Neither like not dislike 5

Dislike slightly 4

Dislike moderately 3

Dislike very much 2

.....

Dislike extremely 1

.....

	853	921	799
Taste			
Color			
Smell			
Appearance			
Overall Acceptability			

Your comments .....

Thank you.

Appendix 04

Calculation for acidity (As citric acid).

Acidity (as any hydrous citric acid), percent by mass= 6.404 V C/M

Where,

V = Volume in ml of standard NaOH required for titration

C = Concentration is mcl/1, of the standard NaOH solution.

M = Mass in g of the sample taken for test

Appendix 05

Statisticaly Analysis data for finding Optimum Iramusu amount

Kruskal-Wallis Test: For Apperance

C1	N	Median	Ave Rank	Z
1	30	5.000	34.0	-2.96
2	30	7.000	64.4	4.84
3	30	6.000	38.2	-1.89
Overall	90		45.5	

H = 23.85 DF = 2 P = 0.000
H = 24.93 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test:For Colour

C1	N	Median	Ave Rank	Z
1	30	5.000	32.4	-3.37
2	30	7.000	64.9	4.98
3	30	6.000	39.2	-1.61
Overall	90		45.5	

H = 25.80 DF = 2 P = 0.000
H = 27.12 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: For Overall Acceptability

C1	N	Median	Ave Rank	Z
1	30	5.000	31.2	-3.66
2	30	7.000	64.3	4.84
3	30	6.000	40.9	-1.17
Overall	90		45.5	

H = 25.45 DF = 2 P = 0.000
H = 26.77 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: For Smell

C1	N	Median	Ave Rank	Z
1	30	5.500	33.6	-3.05
2	30	7.000	62.9	4.46
3	30	6.000	40.0	-1.41
Overall	90		45.5	

H = 20.79 DF = 2 P = 0.000
H = 22.08 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: For Taste

C1	N	Median	Ave Rank	Z
1	30	6.000	32.1	-3.45
2	30	7.500	65.5	5.14
3	30	6.000	39.0	-1.68
Overall	90		45.5	

H = 27.42 DF = 2 P = 0.000
H = 28.71 DF = 2 P = 0.000 (adjusted for ties)

Appendix 06

Statistically Analysis data for finding Optimum Sugar level

Kruskal-Wallis Test: consider about Appearance

C1	N	Median	Ave Rank	Z
1	30	6.000	39.7	-3.78
2	30	7.000	54.5	-1.09
3	30	7.000	75.1	2.65
4	30	7.000	72.7	2.21
Overall	120		60.5	

H = 20.52 DF = 3 P = 0.000
H = 21.90 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: for colour

C1	N	Median	Ave Rank	Z
1	30	6.000	35.9	-4.47
2	30	7.000	52.4	-1.47
3	30	7.000	81.6	3.84
4	30	7.000	72.1	2.10
Overall	120		60.5	

H = 31.02 DF = 3 P = 0.000
H = 33.07 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: For Overall Acceptability

C1	N	Median	Ave Rank	Z
1	30	5.000	34.3	-4.77
2	30	6.000	43.4	-3.11
3	30	8.000	85.8	4.60
4	30	8.000	78.6	3.28
Overall	120		60.5	

H = 48.31 DF = 3 P = 0.000
H = 50.34 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: For Smell

C1	N	Median	Ave Rank	Z
1	30	6.500	49.6	-1.98
2	30	6.500	50.1	-1.89
3	30	7.500	77.1	3.01
4	30	7.000	65.3	0.86
Overall	120		60.5	

H = 13.00 DF = 3 P = 0.005
H = 13.63 DF = 3 P = 0.003 (adjusted for ties)

Kruskal-Wallis Test: For Taste

C1	N	Median	Ave Rank	Z
1	30	4.500	33.6	-4.89
2	30	5.000	41.0	-3.55
3	30	7.500	87.2	4.85
4	30	7.500	80.3	3.59
Overall	120		60.5	

H = 54.76 DF = 3 P = 0.000
H = 56.27 DF = 3 P = 0.000 (adjusted for ties)

Appendix 07

Statistically Analysis data for finding suitable flavour

Kruskal-Wallis Test: For Appearance

C1	N	Median	Ave Rank	Z
1	10	8.000	15.6	0.02
2	10	8.000	14.2	-0.59
3	10	8.000	16.8	0.57
Overall	30		15.5	

H = 0.45 DF = 2 P = 0.797
H = 0.75 DF = 2 P = 0.687 (adjusted for ties)

Kruskal-Wallis Test: For Colour

C1	N	Median	Ave Rank	Z
1	10	7.500	12.9	-1.17
2	10	7.500	14.0	-0.68
3	10	8.000	19.7	1.85
Overall	30		15.5	

H = 3.49 DF = 2 P = 0.174
H = 4.13 DF = 2 P = 0.127 (adjusted for ties)

Kruskal-Wallis Test: For Overall acceptability

C1	N	Median	Ave Rank	Z
1	10	6.500	9.9	-2.49
2	10	7.000	3.7	-0.81
3	10	8.000	23.0	3.30
Overall	30		15.5	

H = 11.82 DF = 2 P = 0.003
H = 12.92 DF = 2 P = 0.002 (adjusted for ties)

Kruskal-Wallis Test: for Smell

C1	N	Median	Ave Rank	Z
1	10	8.000	16.1	0.26
2	10	7.500	12.5	-1.32
3	10	8.000	17.9	1.06
Overall	30		15.5	

H = 1.95 DF = 2 P = 0.377
H = 2.55 DF = 2 P = 0.280 (adjusted for ties)

Kruskal-Wallis Test:for Taste

C1	N	Median	Ave Rank	Z
1	10	6.500	10.5	-2.20
2	10	7.000	13.4	-0.92
3	10	8.000	22.6	3.12
Overall	30	15.5		

H = 10.30 DF = 2 P = 0.006
H = 11.01 DF = 2 P = 0.004 (adjusted for ties)

Appendix 08

MPN Table

Number of positive tubes for the tree dilution factors retained			MPN	Confidence limits			
0	0	0	0.3				
0	1	0	0.3	0.1	2.3	0.1	1.7
1	0	0	0.4	0.1	2.8	0.1	2.1
1	0	1	0.7	0.1	3.5	0.2	2.7
1	1	0	0.7	0.1	3.6	0.2	2.8
1	2	0	1.1	0.2	4.4	0.4	3.5
2	0	0	0.9	0.1	5.0	0.2	3.8
2	0	1	1.4	0.3	6.2	0.5	4.8
2	1	0	1.5	0.3	6.5	0.5	5.0
2	1	1	2.0	0.5	7.7	0.8	6.1
2	2	0	2.1	0.5	0.8	0.8	6.3
3	0	0	2.3	0.4	17.7	0.7	12.9
3	0	1	4	1	25	1	18
3	1	0	4	1	29	2	21
3	1	1	7	2	37	2	28
3	2	0	9	2	52	3	39
3	2	1	15	3	66	5	51
3	2	2	21	5	82	8	64
3	3	0	20	10	190	10	140
3	3	1	50	10	320	20	240
3	3	2	110	20	640	30	480
3	3	3	110				

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