PREPARATION OF READY TO SERVE NATURAL BEVERAGE FROM YOUNG COCONUT WATER

By

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Declaration

The work described in the thesis was carried out by me at Kelani Valley Canneries (Pvt) Ltd., and Faculty of Applied Sciences under the supervision of Mr. Shermith Jinadasa and Mr.Jagath Wansapala. A report on this has not been submitted to any other university for another degree.

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AFFECTIONATELY DEDICATED TO

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INTRODUCTION

Coconut (*Cocos nucifera)* is a perennial plant is grown successfully in Sri Lanka, and it is a one of the major exporting crop. It belongs to the family palmaceae and one of the most important families of micotyledons.(Purseglove, 1972)

The liquid endosperm of coconut water (seven months old) makes a delicious and nutrious drink. Because of the presence of proteins, fats, sugars, vitamins, amino acids and growth hormones. Nutritive value almost entirely in its sugar content. (Thampan, 1993)

The coconut water also is used for medicinal purposes due to its neutrality and nutrious value (CISIR, Report 1982). It has caloric value of 17.4 /100g of water (FAO 2000), and the amino acid percentage of arginine, alanine, cystine and serine in the protein are higher than these in the cow's milk.(Thampan, 1993)

The coconut water preferred not only by Sri Lankan's but also by others in specially Japan, Hong Kong, Singapore and Middle East countries.

Coconut has high potential for export to the overseas markets. Sri Lanka has initiated the export of coconut water as semi process nuts under low temperature storage condition. But it is very expensive method, and problems have been involved for that. These were packing and handling problems cost of sales and shelf life of the product also very low .(only 21 days)

Solving these problem ready to serve natural beverage can be prepared from coconut water. Then increase the shelf life easily handling and packing problem and there is a cost effectiveness.

Browning of coconut water will reduce the nut water, and that will effect for the quality of the beverage. This discolouration has been prevented using antioxidants and preservatives. In modern world consumer preference for the natural products. Because of this reason this product also prepared without adding any artificial flavours or colours.

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ABSTRACT

Coconut (*Cocos nucifera*) belongs to the family palmaceae. Sri Lanka has high export potential of young coconuts to the overseas markets. The majior problem observed bottling of coconut water during ambient temperature storage, was deiscolouration or browning of water and flavour also changed.

This study was conducted to preparing a Ready to Serve Natural Beverage from young coconut water, preventing browning/ discolouration reaction and enhancing flavour.

Different concentration of sugars were mixed to enhance the flavour and to take overall flavourability of every samples to become equally.

The browning reaction of coconut water has been prevented by adding lime-juice as an antioxidant. Further this antioxident has prevented the formation of pink colouration.

Product can be kept at 2 months maintiaining the quality of beverage, reducing the pH of samples up to 4.0 and adding KMS (70 ppm) as a preservative.

Ready to serve natural beverage with different formulations were subjected to sensory survey to identify a suitable formula. All the formulations were not varying significantly for overall acceptability. According to the sensory results, formulations (119) is the best sample for consumer preference. Identifying the problems and developing the process this product can be used for further developments.

LIST OF CONTENT

LIST OF FIGURES

LIST OF TABLES

The present study was carried out with the following objectives.

- Preparation of fresh flavoured natural beverage.
- Prevention of browning reaction of coconut water.
- To maintain the quality of the product during storage.

CHAPTER II

2.0 Literature Review

2.1 Coconut water

Coconut water is the liquid endosperm of the coconut. It is formed within the cavity internal to the kernel or endosperm protected by the hard shell and the husk.

Table 2.1 Composition of coconut water of the tender nuts

Composition	Amounts
Water	95.5%
Protein	0.1%
Fat	<0.1%
Mineral matter	0.4%
Carbohydrates	4.0%
Calcium	0.02%
Phosphorus	<0.01%
Iron	0.5%

Source: Thampan, P. K. 1993

The nut water contains sugars, which reach a maximum concentration of about 6% at about the seventh month after flowering. Sugars in the invert form.

Tender coconut water is delicious and nutritious drink and no other tree provides a ready to serve beverage. The tender coconut water in its natural state is sterile and is commonly as an oral rehydration medium for children suffering from gastro-enteritis and as a useful substitute for saline glucose in intravenous infusions. It is also prescribed in serious cases of diarrhoea and vomiting against dehydration of body tissues and causes profuse diuresis. It is also a urinary antiseptic and eliminates poisons through the kidneys in case of mineral in microbiological work and is useful in lotions. (Thampan, 1993)

PH of the water varies from 4.8 to 5.3 and the water contains both ascorbic acid (Vitamin c) vitamin B groups. The concentration of ascorbic and ranges from 2.2 to 3.7 mg/100ml, which gradually decreases as the kernel begins to harden.

Table 2.2 The values for the vitamins of the B group

Vitamin B group	Percentage (µg/ml)
Nicotinic acid	0.64
Pantothenic acid	0.52
Biotin	0.02
Riboflavin	< 0.01
Folic acid	0.003
Thiamin	Traces
Pyridoxine	Traces

Source: Thampan P.K. 1993

The tender nut water also contains varies minerals of which potash is the major constitute, concentration of potash depend upon the nature of the potash manuring.

Table 2.3 The mineral composition of the tender nut water

Minerals	Amount (in mg /100 ml)
Sodium	105.0
Potassium	312.0
Calcium	29.0
Magnesium	30.0
Iron	0.10
Copper	0.04
Phosphorus	37.0
Sulphur	24.0
Chlorine	183.0

Source: Thampan, P.K. 1993

2.2 Composition changes with maturity

Table: 2.4 Changes in chemical composition of coconut water maturation

Stage of maturity	Total nut water g/ 100g nut	Titrable acidy as citric acid mg/100g	pH	Total solids %	Total sugars %	Reducing sugars %	Total nitrogen mg/ 100g	Non protein nitrogen mg/100g	Fat mg/100g	Ash %
I	44.62	112	4.80	5.82	4.80	4.00	10.8	9.0	4.5	0.58
II	47.16	118	4.85	6.50	5.71	4.40	10.9	8.0	1.5	0.53
III	41.58	116	5.05	6.56	4.60	3.20	14.3	10.7	7.2	0.56
IV	30.23	144	4.50	6.03	3.80	2.40	21.4	15.1	12.6	0.55
V	23.46	144	4.45	5.52	3.10	2.00	30.1	22.8	28.7	0.56
VI	17.04	144	4.55	5.92	2.80	0.60	35.9	23.2	53.6	0.65
VII	15.49	64	5.10	5.40	2.00	0.20	37.6	25.5	83.0	0.54

Source: Jayalakshmi, A. et al., 1983

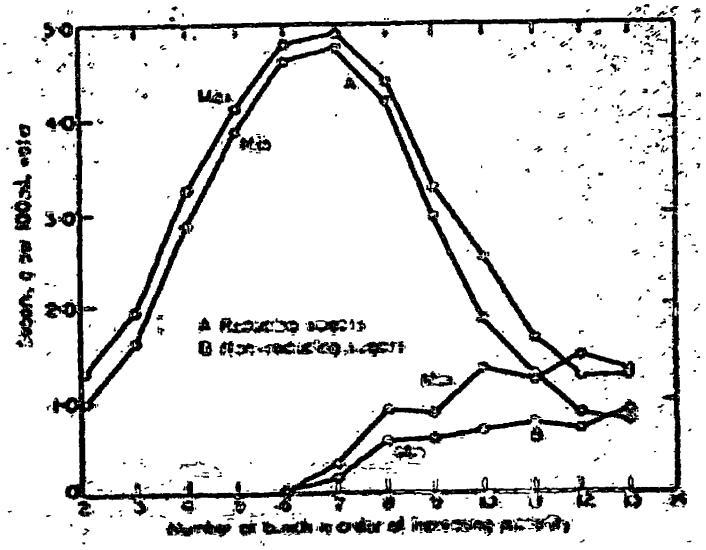
I Six months maturity stage
II seven months maturity stage
III Eight months maturity stage
IV Nine months maturity stage
V Ten months maturity stage
VI eleven months maturity stage

The amount of water decreased steadily with increasing maturity due to absorption by developing endosperm and also to evaporation. However there are no reports on the exact mature of the loss of water from the cavity.

The acidity of water due to organic acids, free from amino acids and dissolved CO_2 The titrable acidity was comparatively low at the 6-8 months, from which it increased to the maximum during 8-11 months, the significant drop in the final stage.

Total solids showed very little variation on percentage basis. The changes in the total solids per nut however recorded a steep decline on maturation. The reduction in the total solids could be partly attributed to the conversion of some compounds to endosperm constituents and partly to simple absorption by developing endosperm.

Sugars are the most important constituents. Changes in the sugars were marked than other constituent. The fall in the sugars content of nut water and accompanying rise in the fat accumilated in the solid endosperm on maturation. Developing endosperm utilizes these sugars as precursors for fat synthesis. At the earliest stage only invert sugar is present and the concentration increases to a maximum in the fifth and sixth months. There after sucrose appears, but the concentration of total sugars falls rapidly with maturity.



Figyre 2.1 concentration of sugars in coconut water

Total nitrogen and non-protein nitrogen show a progressive and rapid increase with increasing maturity. Non-protein nitrogen is comprised of free amino acids and some growth stimulating factors the major contribution being free from amino acids. When the proportion of non-protein decrease, utilized for protein synthesis by the developing endosperm due to that increase the protein percentage.

Total fat increase with increasing maturity. Fatty acid composition of fat changes during maturation. On the whole, increase in the relative proportion of fatty acids up to 14:0 and corresponding decrease in the higher unsaturated fatty acids were the major changes observed. The fatty acid 6:0 was absent in the first three stages and it appeared only at the later stages. Other acids were recorded a progressive increase with maturity. 12:0 is the most abundant acid. Fat samples from early stages contained very high levels of 18:2 and 18:3, which declined significantly at later stages. Relatively greater amounts of higher saturated fatty acids were presence.

Table 2.5: Changes in fatty acid composition of coconut water

Stage of maturity	Fatty acid composition (Area%)															
	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	17:0	18:0	18:1	18:2	20:0	18:3	22:0
I	-	0.8	0.5	7.9	2.5	2.2	2.3	4.7	4.7	4.2	1.1	14.3	7.5	15.2	26.2	5.9
II	-	1.4	1.1	15.3	8.8	-	0.6	14.4	-	2.5	2.2	13.4	9.4	10.9	16.4	3.6
III	-	3.0	1.9	25.0	13.2	-	0.8	10.2	-	1.7	2.0	10.4	7.2	8.3	13.6	2.7
IV	0.3	6.7	4.1	41.3	20.4	-	-	10.1	-	-	1.4	8.5	4.0	1.9	0.9	0.4
V	0.5	7.1	4.6	43.4	18.8	-	-	8.1	-	-	1.1	7.7	2.2	2.4	3.3	0.8
VI	0.2	6.5	4.5	47.1	19.4	-	-	7.2	-	-	1.8	5.9	1.8	3.3	2.1	0.2
VII	0.5	9.5	5.4	46.9	18.7	-	-	7.0	-	-	1.6	4.2	1.5	3.0	1.4	0.2

Source: Jayalakshmy, A., et al 1983.

I, II, III, IV, V, VI, VII denotes as in table 2.4

The total ash content showed very little variation on percentage basis potassium for about half of the ash content. Potassium decreased on maturation whereas sodium and sulphur showed an increase.

Table 2.5 Changes in mineral constituents of coconut water

Mineral content per 100 g									
Stage of maturity	K (mg)	Na (mg)	Cl (mg)	Ca (mg)	Mg (mg)	S (mg)	P (mg)	Fe (µ)	Cu (µ)
I	324	21	100	48	16	58	9.2	106	26
II	291	42	75	44	10	58	9.2	106	26
III	290	42	91	53	11	60	9.6	105	26
IV	272	52	85	54	11	65	8.3	132	26
V	282	52	72	57	17	70	7.1	79	26
VI	275	52	130	51	15	90	8.9	79	26
VII	247	48	108	40	15	80	6.3	79	26

Source: Jayalakshmy,A., et al., 1983.

I, II, III, IV, V, VI, VII, numbers denotes as in table 2.4

Major chemical constituents of coconut water, therefore, are sugars and minerals and minor ones are fat and nitrogenous substances. The pleasant taste of tender coconut water could be attributed mainly to the sugars and mineral contents. The minor constituent such as fat, free amino acids nucleic acids, organic acids and dissolved gasses might also contribute to the overall flavour and mouth feel. Coconut water functions as a reservoir of precursors for the developing endosperm, which utilizes them for biosynthesis of endosperm constituents.

2.3 Browning Reactions

Browning reactions occur very widely in food materials. The colours produced range from pale yellow to dark brown or black, depending on the type of product and the extent of the reaction.

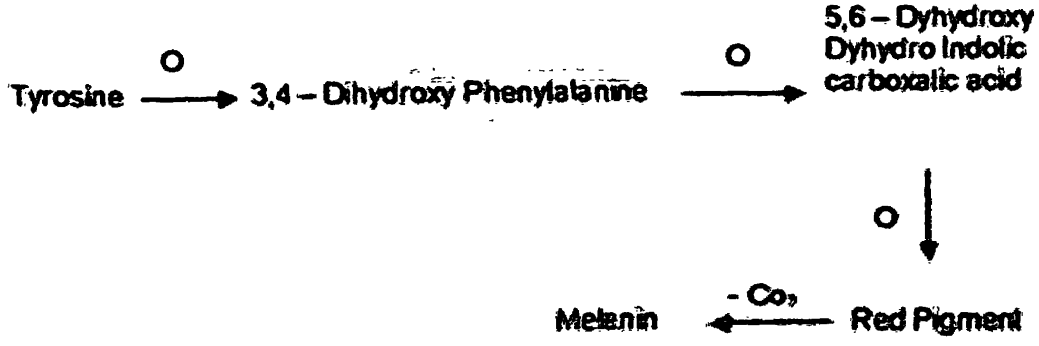
Browning reactions may be either enzymatic or non-enzymatic.

(I) Enzymatic Browning

Many fruits and vegetables have a tendency to turn brown when damaged or cut surfaces are exposed to air e. g., apples, bananas, potatoes etc., The formation of brown colour is due to the action of the enzyme phenolase (also known as polyphenol oxidase, tyrosine or catacholase.) on phonolic substances. Normally, the phonolic substrates are separated from phenolic when such substrates are cut and exposed to air rapid browning of cut surface takes place.

$$\underset{\text{(in cells)}}{\text{Polyphenols}} + \underset{\text{(air)}}{\text{Oxygen}} \xrightarrow[\text{(in cells)}]{\text{Oxidase}} \text{Brown}$$

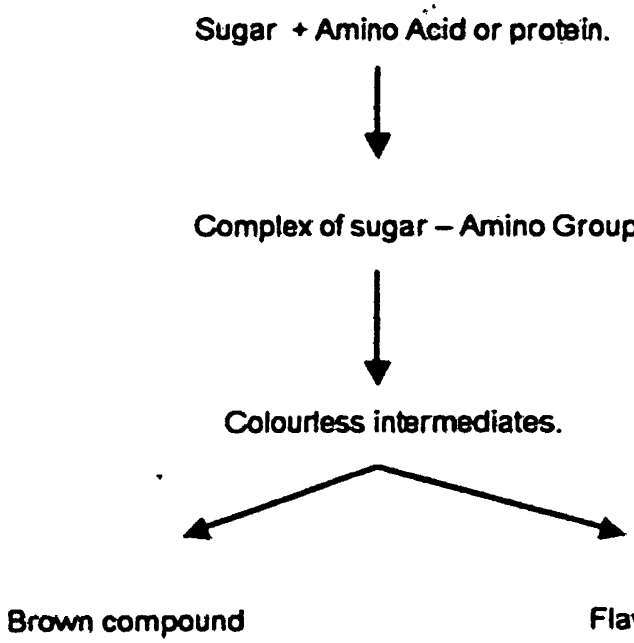
The enzymatic browning due to oxidation of phenols to orthoquinone, and which is rapidly polymerize to form melanin. (The brown pigment) When the substrate is a phenol, it is first converted by hydroxylation into orthodiphenol and then oxidized to orthoquinone. Tyrosine is the major phenolic substrate for phenols action in foods. Other phenolic substances are caffeic acid, protochatachuic acid and chlorogenic acid. The reactions occur in several steps and are catalyzed several enzymes.
eg. Phenolases, peroxidases.



(ii) Non enzymatic browning.

Non-enzymatic browning reactions are responsible for the colour and flavour of foods. Some times this reaction produces desirable flavour (the chocolate flavour) at other time this reaction is undeliverable (dark brown of potato chips). This does not involve enzymes. The presence of reactive reducing sugars is responsible for browning of foods. On heating the sugars undergo ring opening, idolization, dehydration and fragmentation. The unsaturated carbonyl compounds that are form react to produce brown polymers and flavour compounds. Heat induce browning can be divided in to two groups.

This denotes a group of money complex reactions between (i) nitrogen compounds and sugars, (ii) nitrogenous compounds and organic acids, (iii) sugars and organic acids, (iv) among organic acids themselves. The carbonyl group of cyclic sugars readily combines with the basic amino groups of proteins, peptides and amino acids, resulting in sugar- amines. Set of various reactions that sugar – amines undergo resulting in browning is known as the maillard reaction. The sugar – amines have a brown colour at a low temperature. It has been found that histidine; thereonine, phenytalanine, tryptophane and lysine are the most reactive amino acids. The initial reaction is between the aldehyde group of the sugar and amino group of the amino acid.

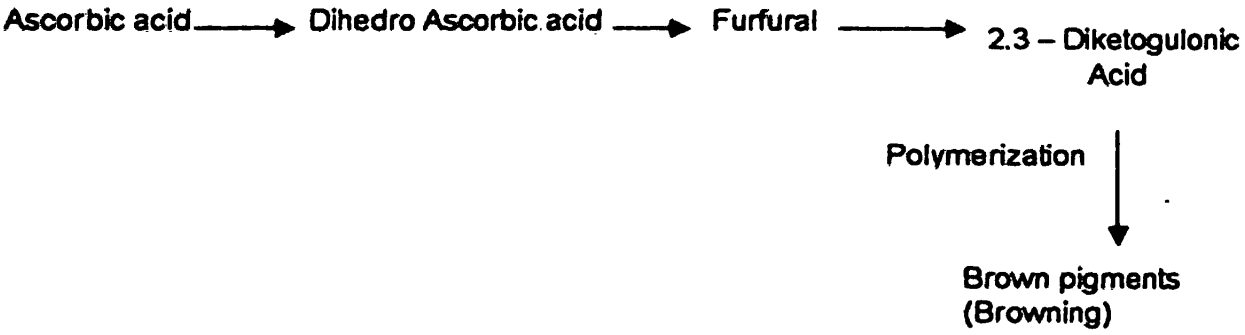


Maillard reaction products predominate in browned foods. The condensation products of sugar and amine under goes enolysation and rearrangement and the condensation and polymerization to form red brown and dark brown compounds. The brown to black, amorphorus, unsaturated heterogeneous polymers are called "melanoids"

(iii) Caramalyzation.

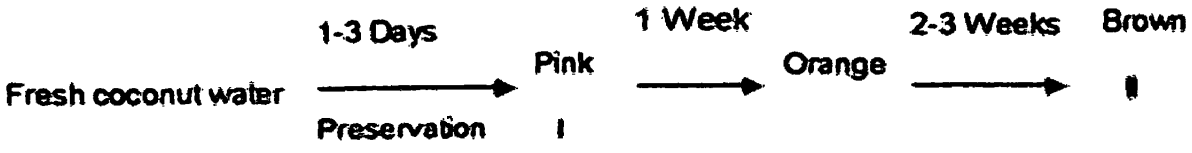
Sugars in dry condition when heated beyond their melting point decomposed and form brown mass known as caramel, which has a bitter, astringent taste. The process of caramalyzation occurs at a high temperature.

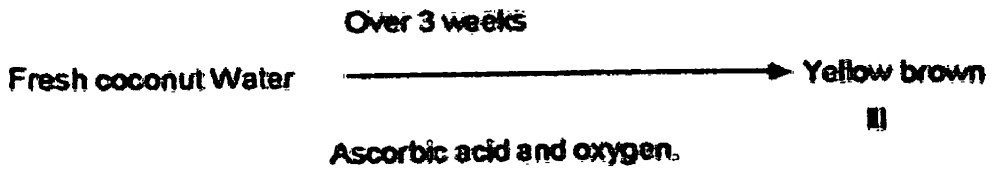
Besides reaction of sugars and amino acids, brake down of ascorbic acid during storage of the products may be effect for the development of browning.



2:4 Colour reactions of coconut water

Young coconut water maintain under sterile conditions develops a pink coloration.





A mixture of the pink and brown formed the orange colour. Maximum development of the pink colour was in water from nuts 6 to 7 months old, while no such colour developed in nuts 5 months old or younger. (Jeya Raj and Jansz, 1969)

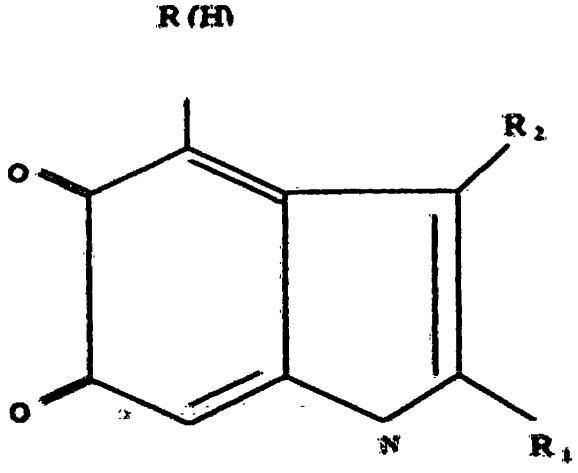
2: 4: 1. Formation of red pigment

The coloured compound can be formed in two ways.

(a) By enzymatic reactions of the tyrosine type amino acids,

(b) By the reaction of oxidized polyphenols with amino acids. (Rinderknecht and jurd, 1958, James et al. 1948)

The red coloring substance and intermediate in the formation of melanins. (Cromartie et al.1953), Bu' Lock and Harley masons (1951), Bruce (1954), Lineus (vennet 1966).



Red pigment

Coconut water has been shown to be comparatively rich in tyrosine type amino acids (Peters, 1952). Melanin can be formed from tyrosine without the action of tyrosinase in the presence of ferric ions and tetrahydropholic acid type nucleus compounds (visontini 1973). The present investigations have shown the presence of phenols and phenol oxidase in coconut water.

Change to brown II

The formation of a brown derivative from the red pigment can be explained by oxidative coupling at position 7 (Bu'lock and Harley, 1951).
Oxidation of the polyphenols can also contribute to the final brown colour.

Change to yellow brown III

The formation of yellow-brown products in the presence of ascorbic acid and oxygen appears to be due to ascorbic acid preventing the reaction to form the compound (Rostin 1962) instead a complex with oxidized.

2.4.2 Factors effect on formation of Red pigment

PH, temperature, maturity, freshness of the nut and also oxygen availability and also other undefined factors such as reactions, competing for the precursor or an intermediate.

Effect of pH
PH optimum was between 4.5 to 5.0

Effect of temperature
Maximum colour development at 50^0 C

Effect of time
The colour had reached nearly the full intensity and remained approximately constant up to four days. If nuts have not been harvested on the same day, colour development can be quicker.

Effect of maturity

Colour development by coincides with at a maximum at the best stage of maturity for drinking purposes.

Amino acid also effect for the formation of pink colour. Free tyrosine concentration is in young coconut water is low and that this is no significant decline of free tyrosine when the pink colour has formed. Thus free tyrosine as the precursor of the red compound.

2.5 Heat treatment as pasteurization and sterilization

Pasteurization

The process of heating at boiling point of slightly below it for a sufficient length of time to kill micro organisms which cause spoilage and destruction of enzymes. It does not kill the thermophiles, which cannot grow in acid media, and consequently their presence is of no practical significance. The main purpose of pasteurization is to increase the shelf life of product for several days. Liquid foods are pasteurized after filling in to containers (Gupta, 1993).

Heating for a few minutes at 60^0C- 65^0C kills yeast and resistant mold spores required in most cases a temperature of 80^0C for 20 minutes.

Molds require O_2 for growth. Removal of air from the juice by filling the containers completely or de-aeration of the air with CO_2, therefore, facilitates the destruction of molds at low temperatures. Most uncarbonated drinks must be pasteurized at 80^0C. Juices of high acidity may be pasteurized at lower temperatures 70^0C - 72^0C.

Pasteurization can be done in two ways.

(a) By heating at a low temperature for a long period.

(b) By heating at a high temperature for a short period (HTST method).

- Bottle or "Holding" pasteurization.

  The extracted juice is strained or clarified as the case may be, and filled in to bottles, leaving sufficient head space for the expansion of the juice during heating. The bottles are then sealed airtight and pasteurization.

  - Overflow method

    Juice is heated to a temperature about 2.5°C than the pasteurization temperature, and then filled in hot sterilized bottles. Upped the brim, taking care that during filling and sealing the temperature of juice does not fall below the pasteurization temperature. The sealed bottles are pasteurized at a temperature 2.5°C lower than the filling and sealing temperature and then cooled.

  - Flash pasteurization

    The juice is heated rapidly to temperature of about 5.5°C higher than the pasteurization temperature and kept at this temperature for about a minute.

Sterilization

Sterilization refers to the complete destruction of microorganisms. Because of the resistance of certain bacterial spores to heat, this frequently requires a treatment of least 121°C wet heat for 15 minutes, or its equivalent. It is also mean that every particles of food must receive this heat treatment.

If a can or a bottle of food is sterilized, then immersing it into a 121°C pressure cooker or retort for 15 minutes, will not be sufficient because of the relatively slow rate heat transfer through the food to container.

Steam pressure of approximately 10, 15 and 20 psi are required for heating 116°C, 121°C and 127°C in autoclave for sterilization.

2.6 Improvement of keeping quality using preservatives

There are two important chemical preservatives.

i) Sulphur dioxide (SO_2)

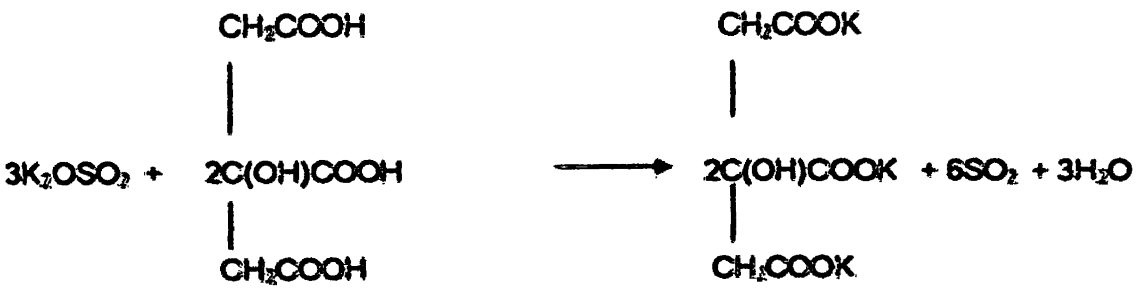
ii) Benzoic acid

SO_2 is widely used throughout the world in the preservation of the juice, pulp, nectar, squash, crush, cordial and other products. It has good preserving action against bacteria and molds and inhibits enzymes etc. In addition, it acts as an anti-oxidant and leaching agent. These properties help in the retention of ascorbic acid, carotene and other oxidizable compounds. It also retards development of non-enzymatic browning or discoloration (after killing the enzyme) of the product. It is generally use in the form of its salts such as sulphite, bisulphite and metabisulphite.

Potassium metabisulphate ($K_2O.2SO_2$ or $K_2S_2O_5$) is commonly used as a stable source of sulphur dioxide. Being a solid, it is easier to use than liquid or gaseous sulphur dioxide. It is fairly stable in neutral or alkaline medium but decomposes by weak acid like carbonic, citric, tartaric and malic acids.

In acidic media react with acid forming potassium salt and sulphurdioxide, which is liberated and form sulphurous acid with the water present in the media.

The reactions involved are as,



Potassium metabisulphite + Citric acid ⟶ Potassium citrate + Sulphur dioxide + Water

SO_2 has a better preservation action than Sodium benzoate against bacteria and molds. It retards the development of yeast in juice, but cannot arrest their multiplication, once their number has reached a high value.

Permitted level for sweetened nearly to serve beverage: - 70 ppm (parts per million) .(Srivastava, Sanjeev, 1994).

Table 2.7: The concentration of sulphur dioxide required to prevent the growth of microorganism at different pH levels.

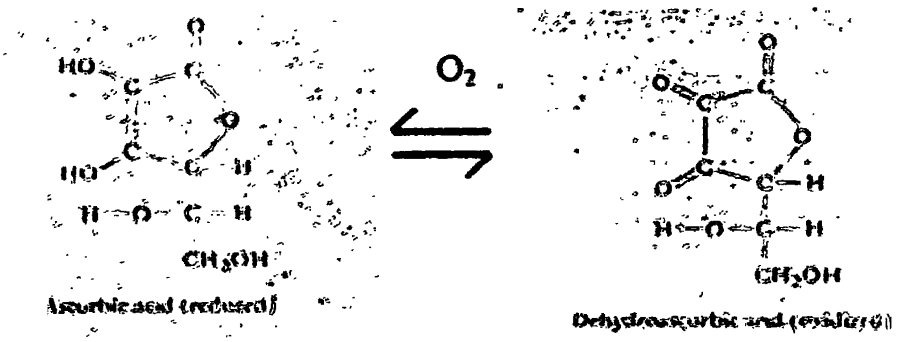
pH	Organisms and Sulphur dioxide concentration (ppm)			
	Saccharomyces Ellipsoideus (Yeast)	*Mucor* (Mould)	*Penicillium* (Mould)	Mixed bacteria
2.5	200	200	300	100
3.5	300	600	600	300
7.0	Above 5,000	Above 5,000	Above 5,000	Above 1,000

Source: Srivastava, R. P., Sanjeev Kumar, 1994.

2.7 Lime juice used as an antioxidant

An antioxidant is a substance which when added to fat and fat containing foods to prevent their oxidation and thus prolongs their shelf life, wholesomeness and palatability. Antioxidant function by interrupting the free radical chain reaction involved in lipid oxidation. An antioxidant haven't any harmful physiological effect and showed not impart an objectionable flavour, odour or to the food in which it presents. It should be effective in low concentration (0.01% to 0.02%) and be fat-soluble. Some antioxidants used in foods are butylated hydroxyanisole (BHA), bytylated hydroxytolune (BHT), tertiary-butylhydroquinone (TBHQ), propylgallate (PG), thiodipropionic acid, dilauryl thiodipropionate, stannous chloride, tocopherols, sulphur dioxide, and ascorbic acid (Srivastava, Sanjeev, 1994)

Lime- juice is used as an antioxidant. It contains vitamin C 50 mg/100g of fruit. Vitamin C exists in a reduced form, called ascorbic acid, and in an oxidized form, called dehydroascorbic acid. The two forms are interchangeable, and both are biologically active (Mayer, 1960).



CHAPTER III

3.0 Materials and Methods

3.1 Production of Ready to Serve Natural Beverage from coconut water

3.1.1 Materials

Coconut water (Seventh month maturity level) was used as main ingredient. Sugar, limejuice, potassium metabysulphite and citric acid were used as rest of the ingredients for ready to serve beverage from coconut water.

3.1.2 Equipments

The main equipment used are analytical balance, electronic balance, thermometer, bottle sealing machine, retorter, pH meter, hand refracto meter and bottles.

3.2 Methodology

3.2.1 Determination of optimal level amount of lime juice to prevent browning reaction

Coconut water was taken from same maturity stage from one tree, and samples were prepared following table.

Table 3.1: Required amount of lime juice to prevent to browning reaction.

Sample	Amount of lime-juice g/kg
A	140g
B	120g
C	100g
D	80g
E	60g

3.2.2 Preparation of Ready to serve natural beverage.

Young coconut nuts (7 months old) were cut using stainless steel knife and water was collected to the stainless steel saucepans and weighted the amount of water. PH of the samples was adjusted to 4.0, mixing with lime- juice and trace amount of citric acid.

Potassium metabisulphite was added according to the recommended level (70 ppm).

Sugar also mixed according to that four varieties were made (S_1, S_2, S_3, S_4) and four control samples (without preservatives) were kept for above four varieties.

S_1- (452)

S_2- (873)

S_3- (781)

S_4- (119)

Then filtered the mixture using net cloth and filled into hot sterilized bottles and then sealing operation was done. Sealed bottles were pasteurized under water bath (80^0C for 30 minutes). Sealed bottles were stored under room temperature.

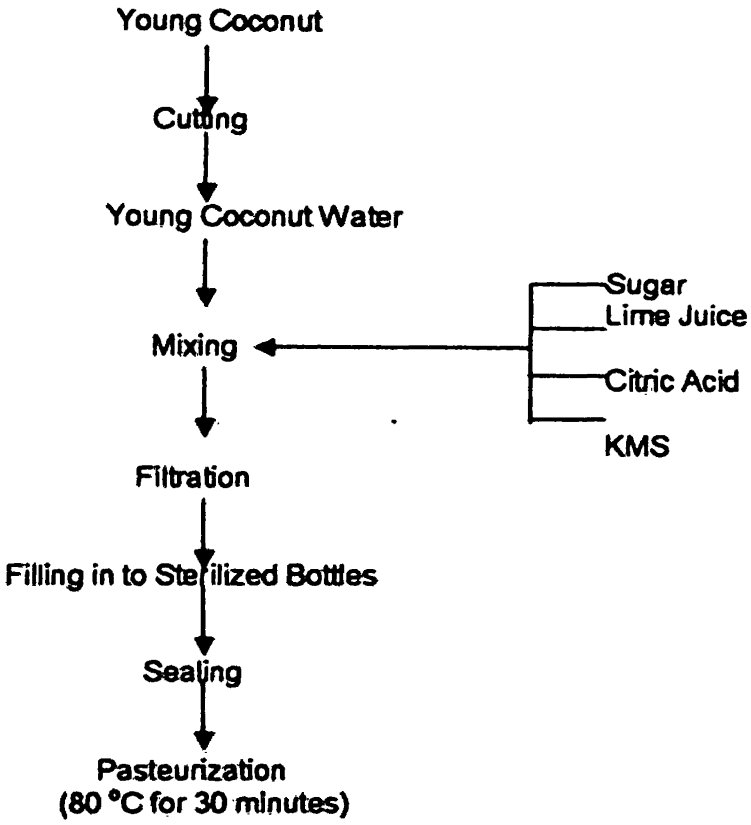


Figure 3.1: - Flow diagram for Ready to serve natural beverage preparation from young coconut water

3.3 Determination of basic chemical parameters of coconut water

3.3.1 Determination of total soluble solid content (Brix value)

Methodology: -

The brix value was recorded using "Erma", 0-25% hand refract meter. Brix values were recorded, initially and finally in every sample.

3.3.2 Determination of pH value

Methodology

A portion of coconut water was taken and the pH values were recorded using "Orian Model 410 A" pH meter, initially and finally.

3.3.3 Determination of Titrable acidity

Methodology: -

Reagents

- 0.1M NaOH Solution
- Phenolphthalein indicator

Titration procedure

10 ml of coconut water taken into titration flask, and 3 drops of phenolphthalein indicator added and titrated against NaOH Solution.

Calculation

$$\text{Acidity} = A \times \text{Factor}$$

Where, A ——→Burette reading.

3.4 Microbiological tests

3.4.1 Determination of Yeast, Mould and Bacteria in Ready to serve natural beverage from coconut water

Autoclave, petri dishes, 1ml pipettes, Conical flask incubator, Analytical balance Water bath, Bunsen burner were used as apparatus.

Potato dextrose agar medium was used (PDA) for the determining the mould and yeast Nutrient agar was used for determining the bacteria.

3.4.1.1 Determination of yeast and moulds presents in the samples,

Methodology

Agar medium was put in to a conical flask and closed the orifice by cotton and covered with an aluminum paper. After it was autoclaved (20 minutes in 121^{0}C) under 15 1b/in^2. After that PDA was added to all petri dishes and 1ml of coconut water samples was added.

3.4.1.2 Determining the bacteria present in the samples,

Methodology

Nutrient agar medium was sterilized by using an autoclave as in 3.3.1. After that nutrient agar was added into all petri dishes (as about 15 ml for one plate). Nutrient agar and coconut water beverage sample mixed well separately. After the medium because solid stage petri dishes were placed in incubation at 35^{0}C for growth of bacteria.
All microbiological testing were done under aseptic conditions.

3.3 Sensory evaluation: -

Ready to serve natural beverage from coconut water samples were given to untrained panelists, which consist of 14 members with a questionnaire shown in appendix. Sensory properties appearance, flavour and overall acceptability were investigated using a ranking method. It is generally used to select one or two of the best samples from a group of rather than to test all samples.

CHAPTER IV

4.0 Results and Discussion

4.1 Results

4.1.1 optimal level of lime juice to prevent to browning

Table 4.1 Required amount of limejuice to prevent browning reaction

Beverage Varieties	Amount of lime juice g/kg	Appearance	Flavour
A	140g	Colourless	Undesirable
B	120g	Colourless	Undesirable
C	100g	Colourless	Desirable
D	80g	Fairly pink	Desirable
E	60g	Pink	Desirable

Table 4.2 Determination of pH value

Beverage varieties	Initial pH value	Final pH value
S_1	4.8	4.0
S_2	5.0	4.0
S_3	5.0	4.0
S_4	4.9	4.0

Letters denoted o the samples as in 3.2.2

Table 4.3 Determination of total soluble solid content

Beverage Samples	Initial brix value (%)	Final brix value (%)
S_1	5.0	6.0
S_2	5.2	7.0
S_3	5.2	8.0
S_4	4.8	9.0

Letters denoted to the samples in 3.2.2

Table 4.4 Determination of titrable acidity

Beverage Samples	Initial titrable acidity as citric acid mg/100g	Final titrable acidity mg/100g
S_1	116.0	116.5
S_2	119.0	120.0
S_3	118.5	118.0
S_4	118.0	118.25

Letters denoted to the samples as in 3.2.2

4.1.2 Results of the microbiological tests

Table 4.5 Determination of yeast and mould

Beverage Samples	With preservatives	Without preservatives
S_1	0	X
S_2	0	X
S_3	0	X
S_4	0	X

0- Absence of mould and yeast patches After 1 1/2 weeks period
X- Presence of mould and yeast patches After 1 1/2 weeks period

Letters denoted to the samples as in 3.2.2

Table 4.6 Determination of Bacteria

Beverage Samples	With preservatives	Without Preservatives
S_1	0	X
S_2	0	X
S_3	0	X
S_4	0	X

0- Absence of Bacteria After 1 1/2 Weeks
X- Presence of Bacteria After 1 1/2 Weeks

Letters denoted to the samples as in 3.2.2

4.1.3.Results of the Sensory evaluation

Table 4.7 Ranking of samples

Judges	Samples			
	452	873	781	119
1	4	3	1	2
2	4	3	1	2
3	3	2	1	4
4	4	2	1	3
5	4	2	1	3
6	4	3	1	2
7	1	3	4	2
8	4	2	3	1
9	3	4	2	1
10	3	4	2	1
11	4	3	2	1
12	4	2	3	1
13	3	2	4	1
14	4	1	3	2
Total	49	36	29	26

Letters denoted to the samples as in 3.2.2

4.2 Discussion

4.2.1 Production of natural beverage from coconut water

At seven month maturity level (3.1.2) total solid content (6%) and nutritive value high. Therefore coconut water from this maturity level can be used as a medicinal drink. (Thampan, 1993) pH of this stage ranges between 4.8- 5.0 (2.1). The maximum colour development has been observed at pH 5.5. (2.4.2)

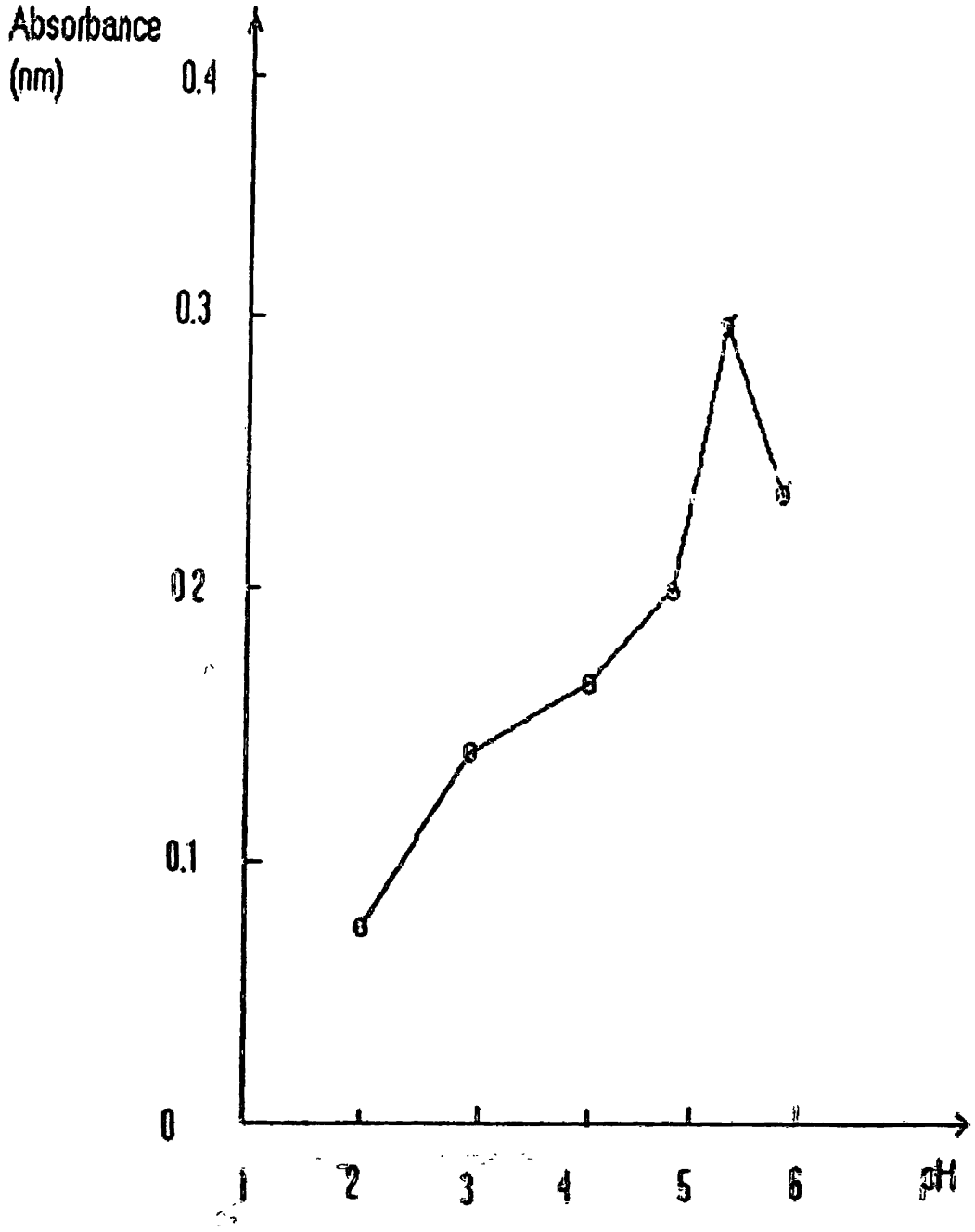


Figure 3.1 Effect of PH on colour development

According to the literature no such colour developed in nuts five months old or younger. Although nutritive value and flavour of this level have been lower. (2.4)

Browning of coconut water due to enzymatic reaction of tyrosine type amino acids and the reaction of oxidized polyphenols with amino acids. (2:4:1) In all control samples (V_1, V2, V3, V4) colour reaction were occurred and has given an undesirable flavour. This oxidation could be prevented using lime juice as an antioxidant. Ascorbic acids present in limejuice oxidize to dehydro ascorbic acid and prevent the oxidation of polyphenols and amino acids. (Srivasta, and Sanjeev, 1994). Present content (12mg/l) of ascorbic acid (Srivasta and Sanjeev, 1994) not enough to prevent the browning reaction. There fore additional amount should be added. That is shown in the table (4.1). This can act as antioxidant as well as the antibrowning agent.

According to the table (4.1) results intensity of colour has directly proportional to the added amount of antioxidant (lime juice). When adding limejuice in sample A, B (140g, 120g) has given an undesirable (more acidic) flavour. But when adding 100g of lime juice it has given a desirable flavour. When adding 80g and 60g (sample C, D) flavour has given a desirable but pink colour has formed.

Lime juice also affect for the reducing the pH value. According to the table (4.2) pH of water has adjusted to 4.0 using limejuice and citric acid. Then microbial growth will reduce.

Sugar was added as a sweetening agent. It enhance the sweetness and total soluble solid content of the product (4.3)

Potassium metabisulphite was used as a preservative. This inhibits the growth of microorganisms and browning reaction. According to the pH value, of media (pH 4.0) is acidic. In acidic media react with acid forming potassium salt and sulphurdioxide, which is liberated and form sulphurous acid with the water present in the media.

When adding SO_2 to beverage it undergoes association dissociation reaction in the aqueous phase and equilibrium is set up between SO_2, H_2SO_3, HSO_3, and SO_3. The equilibrium depend both on the pH of the product and other reactive species present in food matrix such as free carboxyl groups in sugar or disulphide groups in proteins

After adding all the ingredients was filtered through net cloth, because dissolved and unwanted particles that contain in raw materials.

In addition to the sulphuring again preserved by pasteurization. Pasteurization was a relative mild heat treatment, usually performed below 100°C, which is used to extend the shelf life of the product for several months. It preserves beverage by inactivation of enzymes and destruction of relatively heat sensitive microorganism (non spore forming bacteria, yeast and mould). But it causes minimal changes in the sensory characteristics or nutritive value of this beverage. Using water bath did Pasteurization.

There was no change in final pH, titrable acidity and brix value of the sample S_1, S_2, S_3 and S_4 (452,873,781,119).
PH of control varieties were changed and flavour also undesirable (V1, V2, V3, and V_4) thus titrable acidity also increased.

Cloudy appearance has been observed after pasteurization, and this was the major problem of the product.

.2.2 Microbiological testing

According to the microbiological testing (4.5) and (4.6) S_1, S2, S3, S4 (452,873,781,119) samples had no colonies. It mean yeast mould and bacterial colonies have not being present on S_1, S2, S3, S4 samples, PH of the medium, due to preserving action of SO_2 and pasteurization microorganisms have been death. In control samples yeast, mould and bacterial colonies were present.

4.2.3 Sensory evaluation

The rank totals are compared with the values in appendix. When there are four varieties and fourteen judges, the tabular entries are 26-44. The lowest insignificant rank sum is 26 and the highest insignificant rank sum is 44. If one or more rank sums are higher than the upper left value in the block (26) or higher than the upper right value in the block (44) statistical significance at the 5% level of significance is indicated. Since one rank total in table 4.1 are laid within 26-44, there is a significance difference among the treatments. All the rank sums are compared to the lower pair of values in block to determine which samples are significantly low or high. Sample S_4 (119) which received a rank sum of 26, is significantly better than sample S_1 (452) which received a rank sum of 49. This (119) can be used for further developments.

CHAPTER V

5.0 Conclusion and Recommendation

5.1 Conclusion

It is evident from the present study that 50 ppm of ascorbic acid (100g of lime juice) has been prevented the browning reaction (formation of red pigment) of young coconut water. Therefore this can be act as an antioxidant as well as the antibrowning agent. Lime- juice was reducing the pH level of the samples this also effect for the prevention of microbial growth.

Ready to serve natural beverage from coconut water have been kept for a period of six weeks without any flavour change or microbial changes.

5.2 Recommendations

The cloudy precipitate was appeared after pasteurization of the samples. Further studies should be planned to identify this process and thereby take the preventive measures for it.

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APPENDIX I

Statistical Chart

Rank Totals

Rank totals required for significance at the 5% level (P<=0.05). The four figure blocks represent; lowest insignificant rank sum, any treatment-highest insignificant rank sum, any treatment. Lowest insignificant rank sum, predetermined treatment-highest insignificant rank sum predetermined treatment.

No of reps.	Number of treatments or samples									
	2	3	4	5	6	7	8	9	10	11
2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	3-9	3-11	3-13	4-14	4-16	4-18	5-19
3	.....	.....	.....	4-14	4-17	4-20	4-23	5-25	5-28	5-31
	.....	4-8	4-11	5-13	6-15	6-18	7-20	8-22	8-25	9-27
4	.....	5-11	5-15	6-18	6-22	7-25	7-29	8-32	8-36	8-40
	.....	5-11	6-14	7-17	8-20	9-23	10-26	11-29	13-31	14-34
5	.....	6-14	7-18	8-22	9-26	9-31	10-35	11-39	12-43	12-48
	6-9	7-13	8-17	10-20	11-24	13-27	14-31	15-35	17-78	18-42
6	7-11	8-16	9-21	10-26	11-31	12-36	13-41	14-46	15-51	17-55
	7-11	9-15	11-19	12-24	14-28	16-32	18-36	20-40	21-45	23-49
7	8-13	10-18	11-24	12-30	14-35	15-41	17-46	18-52	19-58	21-63
	8-13	10-18	13-22	15-27	17-32	19-38	22-41	24-46	26-51	28-58
8	9-15	11-21	13-27	15-33	17-39	18-46	20-52	22-58	24-64	25-71
	10-14	12-20	15-25	17-31	20-36	23-41	25-47	28-52	31-57	33-63
9	11-16	13-23	15-30	17-37	19-44	22-50	24-57	26-64	28-71	30-78
	11-16	14-22	17-28	20-34	23-40	26-46	29-52	32-58	35-64	38-70
10	12-18	15-25	17-33	20-40	22-48	25-55	27-63	30-70	32-78	34-86
	12-18	16-24	19-31	23-37	26-44	30-50	33-57	37-63	40-70	44-76
11	13-20	16-28	19-36	22-44	25-52	28-60	31-68	34-76	36-85	39-93
	14-19	18-26	21-34	25-41	29-48	30-55	37-62	41-69	45-76	49-83
12	15-21	18-30	21-39	25-47	28-56	31-65	34-74	38-82	41-91	44-100
	15-21	19-29	24-36	28-44	32-52	37-59	41-67	45-75	50-82	54-90
13	16-23	20-32	24-41	27-51	31-60	35-69	38-79	42-88	45-98	49-107
	17-22	21-31	26-39	31-48	35-56	40-64	45-72	55-80	54-89	59-97
14	17-25	22-34	26-44	30-54	34-64	38-74	42-84	46-94	50-104	54-114
	18-24	23-33	28-42	33-51	38-60	44-68	49-77	54-86	59-95	65-103
15	19-26	23-37	28-47	32-58	37-68	41-79	46-89	50-100	54-111	58-122
	19-26	25-35	30-45	36-54	42-63	47-73	53-82	59-91	64-101	70-110
16	20-28	25-39	30-50	35-61	40-72	45-83	49-95	54-106	59-117	63-129
	21-27	27-37	33-47	39-57	45-67	51-78	57-87	63-97	69-107	75-117
17	22-29	27-41	32-53	38-64	43-76	48-88	53-100	58-112	63-124	68-136
	22-29	28-40	35-50	41-61	48-71	54-82	61-92	67-103	74-113	81-123
18	23-31	29-43	34-56	40-68	46-80	51-93	57-105	62-118	68-130	73-143
	24-30	30-42	37-53	44-64	51-75	58-86	65-97	72-108	79-119	88-130
19	24-33	30-46	37-58	43-71	49-84	55-97	61-	67-	73-	78-
	25-32	32-44	39-56	47-67	54-79	62-90	110	123	136	150
							69-	76-	84-	91-
							102	114	125	137

Source: Modified from Larmond, 1977

APPENDIX II

QUESTIONNAIRE FOR RANKING

Name ; ..... **Date :** .....

Product : .....

INSTRUCTIONS

- Please check the appearance, flavour for each sample.
- After that, rank the sample you like best as first and the sample you like least as last.
- Taste the samples in the following order.

119 781 873 452

First	
Second	
Third	
Fourth	

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