DETERMINATION OF THE EFFECT OF CARBONATION ON BETA CAROTENE LEVEL IN CARROT (*Dacus carota*) DRINK

By

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In

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DECLARATION

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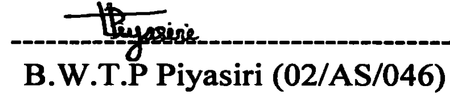

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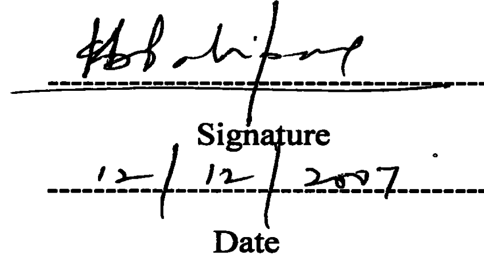

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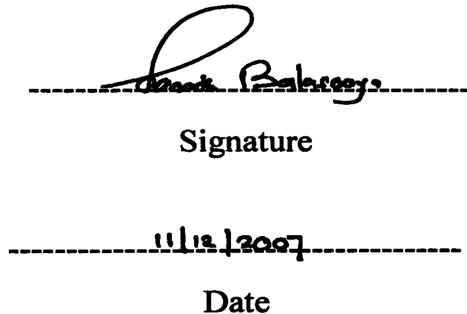

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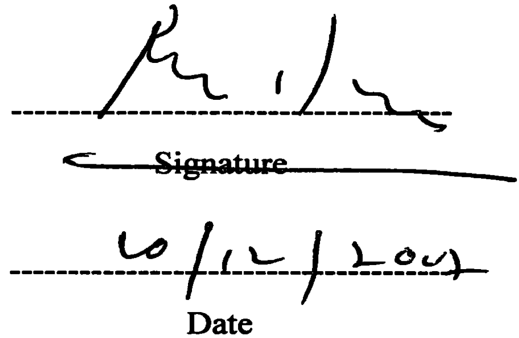

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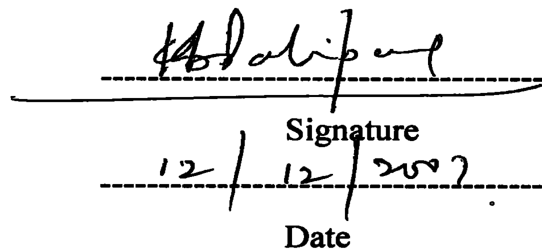

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Affectionately Dedicated To My Parents And Brother

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Abstract

Carrot (*Dacus carota*) is one of the promising sources of beta carotene the precursor of vitamin A. Recently, the nutritional content of food after preparation has become an important concern to health & food professionals. However, during several processing phases, the ultra structure of carotenoids and other complexes can be broken, exposing the pigments to adverse factors that can be lead to their destruction. This study aims to develop a carbonated carrot drink & to analyze the effect of carbonation on beta carotene level.

The optimum carbonation level of reformulated carrot drink was assessed through 9 point hedonic test organoleptically. Shelf life evaluation was performed physiochemically and microbiologically using the filter membrane technique. Separation & identification of Beta carotene were done by using column chromatography (Alumina activity III column) & thin layer chromatography (TLC) respectively. Quantification was done spectrophotometrically by using UV/Visible spectrophotometer set wave length at 450 nm.

Optimum ingredient amount to be added for preparing 200ml of drink was found to be 34 ml Carrot juice, 28 g Sugar, 0.3 g Citric acid, 0.02 g SMS & 0.6 g NaCl with good flavor enhancement capacity. Chemical analysis reveals that the best sample has pH 3.5, Brix 16.5 ^{0} & titratable acidity 0.15%. According to the sensory analysis results, 1.56 CO_2 volume was the best with acceptable mouth feel & effervescence. No significant change in physiochemical properties were observed during the two months storage period in both samples stored under ambient & refrigerated conditions. Drink was microbiologically safe for 2 months storage period. (Coliform, Yeast & Mold – Nil, Bacteria -7 per 1ml.)

Beta carotene amount in non-carbonated & carbonated carrot drinks at four different carbonation levels 0 , 1.48 ,1.56 ,1.86 ,2.09 Vol were 266.3 ± 26.1, 236.0 ±19, 290.7 ± 52.1, 263.3 ± 43.61 , 277.7 ± 58 (µg / 100ml) respectively. It was statistically proved that carbonation has no significant effect on beta carotene level in carrot drink ($p > 0.05$). Total carotenoids in non carbonated & carbonated carrot drinks at four different carbonation levels were 369.33 ± 9.45, 364.67 ± 9.87, 372.00 ± 15.10, 360.67 ± 6.11 & 374.70 ± 23.40 (µg /100ml) and there was no significant difference among those samples($p > 0.05$).The best sample selected from sensory analysis was at a carbonation level of 1.56 Vol and contained 290.7 ± 52.1 µg/100ml of beta carotene & 372.00 ± 15.10 µg/100ml of total carotenoids.

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LIST OF ABREVIATIONS

et al.	And others
FAO	Food and Agriculture Organization
g	Gram
H_2O	Water
HCl	Hydro Chloric Acid
mg	Mili gram
ml	Mili liter
N	Nitrogen
NaOH	Sodium Hydroxide
WHO	World Health Organization
^0C	Degree of Celsius
%	Percentage
SMS	Sodium metabi sulphate

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CHAPTER 01
INTRODUCTION

1.1 Introduction

Vitamin A deficiency is one of the more serious micronutrient problem in developing countries, specially their younger generation (Chawla et al,2005). According to Simpson many programmes have been drawn up to combat this deficiency and among the elements included are preservation, distribution practices based on reduction of substantial losses of beta carotene the precursor of vitamin A(Milgros *et al* ,1985).The most inexpensive and best way of ensuring adequate intake of vitamin A is to include Carotene rich vegetables to our diet.

Vegetables are an important constituent in diet and provide significant nutrients depending upon their nature, specially vitamins, minerals and fiber. Among vegetables Carrot is one of the best sources of carotenoids which contains relatively high amount of beta carotene, the precursor of vitamin A and α carotene. The main carotenoids in carrot of the "Orange " variety are α and β carotene, ranking from 80% to 90% of total carotenoids(Maria *et al* 1998). The remaining consists of lutein and ε carotene.

Carbonation is defined as the impregnation of liquid with carbon dioxide gas. Artificial inclusion of carbon dioxide gas to a soft drink product imparts to beverage which sparkles and foams as it is dispensed and consumed. This escape of CO_2 during consumption of the drink should complement and enhance the flavor and will add an exiting tingle that stimulates the palate(Mitchell, 1990). Organoleptic quality of carbonated drinks is greatly affected by total soluble solids (TSS), acidity, CO_2 volume gas pressure and water quality.

Carotenoids have been extensively studied due to their important biological functions for human and also as a natural pigment. Nowadays, the nutritional content of food after preparation has become an important concern to health and food professionals. It is also a concern for consumer and manufacturer who, without doubt, have contributed to better food quality, and consequently, to consumer health. Although the effect of carbonation on beta carotene level is not much investigated, the effect of reduction of PH in this process should take in to concern. This study is mainly

focused on the determination of the effect of carbonation on beta carotene level which is the main active component present in carrot drink.

1.2 Objectives

Development of a Ready To Serve (RTS) carbonated carrot drink and determination of the effect of carbonation on beta carotene level in the product

1.2.1 Specific objectives

1.1.2.1 Development of Ready To Serve (RTS) carrot drink as a carbonated beverage.

1.1.2.2 Determination of the best blanching conditions to develop cloud stable Carrot drink

1.1.2.3 Determination of the shelf life of the product

1.1.2.4 Determination of the carbonation shelf life of the product

1.1.2.4 Determination of the effect of carbonation on beta carotene level of the product

CHAPTER 02
LITERATURE REVIEW

2.1 Carrot (Dacus carota)

2.1.1 Introduction

Carrot (Dacus carota) is a vegetable blessed with relatively high content of vitamin A which contains 11000 IU in raw form & 25000 IU in liquid form. (Ensminger *et al* 1994).

A carrot is a root vegetable usually orange or white in color with a woody texture in which taproot is the edible part. It is a biennial plant which grows a rosette of leaves in the spring and summer while building up the stout taproot, which stores large amounts of sugars for the plant to flower in the second year.

2.1.2 Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	Daucus
Species	carota

2.1.3 Vernacular name

German Name: Karotte,Mohre,

Latin Name: Daucus

English Name: Cultivated Carrot

Sanskrit & Indian Names : Shikha-mula ,Garijara

2.1.4 Origin

They are believed to have originated in the Near East & Central Asia, where they were cultivated for thousands of years. However the ancient ancestors of the modern form of this vegetable were not Yellow - Orange but had Purplish color ranging from Lavender to almost Black. The Yellow – Orange root is the mutant variety which lacked the purple pigment. (Ensminger *et al* 1994).

2.1.5 Distribution

California is the leading producer of both fresh and processing Carrot, followed by Texas and Michigan for fresh market and Washington for processing. In the 14th century Carrot were taken to China. Beginning with the 17th century European agriculturists worked in the development of the Yellow Orange Carrot & finally Purple type production is discontinued. The total estimated value of the crop in 1983 was dollars 206,802,000. (Peirce C.L, 1987)

2.1.6 Properties:

Sweet in flavor, mild (raw carrot is slightly cool) in nature, it is related to the channels of the spleen, liver and lung

2.1.7 Description

2.1.7.1 Plant

Embryophyta (plants); Angiospermae (flowering plants); Eudicotyledons

As the plant emerges, it is characterizes by two strap like seed leaves followed by the typical tight rosette of finely cut leaves. The flowering stem grows to about 1 m tall, with umbels of white. (Wikipedia)

Figure 2.1 Carrot plant

2.1.7.2 Inflorescence

Figure 2.2 umbels of Carrot

2.1.7.3 Root

The predominant root is a tap with fine, fibrous side branches. The tap root enlarges gradually and at first contains two areas each of primary xylem and phloem. Cambium fragments between xylem and phloem eventually join, becoming circular, and produce secondary xylem towards the center of the root, secondary phloem towards the outside. At maturity, the two tissues are distinguishable as a true root. "peel" is periderm, of which suberin and wax like substances are constituents.The periderm functions in a manner similar to the cuticle of a leaf. (Peirce C.L ,1987)

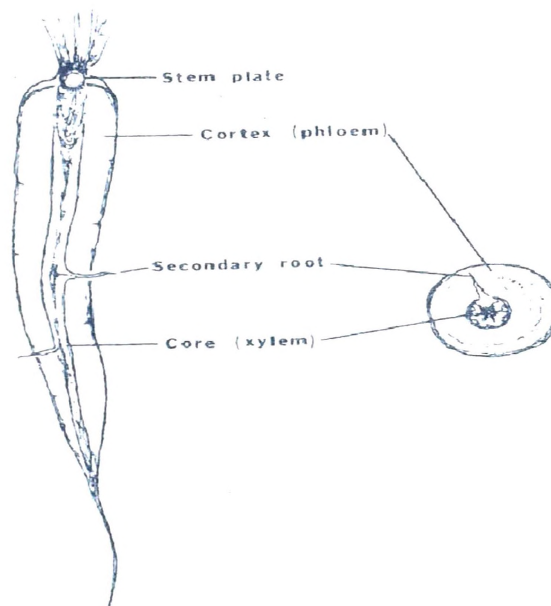


Figure 2.3 Longitudinal and cross sections of carrot root during vegetative growth

2.1.7.4 Composition

Per 100 g

Moisture	59.0 g
Calorie	42.0 g
Protein	1.2 g
Fat	0.2 g
Carbohydrates	9.7 g
Fiber	1.0 g

Vitamin Fat soluble

Vitamin A	11000.0 IU
Vitamin D	0 mg
Vitamin E	0.45 mg

Minerals

Macro Mineral

Calcium	37 mg
Phosphorus	36 mg
Sodium	47 mg
Magnesium	18.5 mg

Micro minerals

Potassium	341 mg
Iron	0.7 mg
Zinc	0.4 mg
Copper	0.01 mg

Vitamins Water soluble

Vitamin C	8 mg
Thiamin	0.06 mg
Riboflavin	0.05 mg
Niacin	0.6 mg
Vitamin B_{12}	0 mg
Vitamin B_6	0.15 mg

Considering nutrients of major root crops, Carrot bears markedly high amount of vitamin A, 11000 IU per 100g sample. (Appendix I)

2.1.7.5 Uses

The whole herb, collected in July; the seeds and root are widely use in medicine. The whole herb is the part now more generally in use.

2.2 Processing of vegetables (Carrot) as value added product

2.2.1 Carrot processing.

Carrot were among the first vegetable to be canned following the development of the process by the French food technologist Appert in the early 1800s.British developed some high carotene carrot during the world war 11 .So their aviator can might see better at night.(Ensminger *et al* 1994).

A little less than the half of US carrot crop is processed. Leading type of processing is freezing (over 148000 metric tones annually) followed by the canning, except freezed canning there's steadily growing utilization of other processes like dehydration, juice production & ready to serve salads. There are an abundant supply of raw carrot from fresh market rejects as roots that are off sized or shape ,but otherwise not in high quality

No high production of Carrot in Sri Lanka. The production is centralized to the central province where the suitable climatic conditions are existing. Carrot price is highly fluctuates within the harvesting period. In some seasons price of a one kilo may Rs 20. In such a situations carrot processing for the development of new food products may be one of the wise decision.

2.2.2 Carrot juice

Vegetable juice is defined (FAO, 1992) as: "the liquid unfermented but fermentable product or lactic acid fermented product intended for direct consumption obtained from the edible part of one or more sound vegetables and preserved exclusively by physical means. The juice shall be free from skins, seeds and other coarse parts of the vegetables. It may be clear, turbid, or pulpy. It may have been concentrated and reconstituted with water..." "Vegetables for the purpose of the Standard are: the parts of edible plants including roots, corms, tubers, stems and shoots, leaves and flowers and legumes." Although the standard applies to edible plants, herbs, botanicals and possibly medical plants may also fit the definition provided that the plant part(s) is edible and not toxic

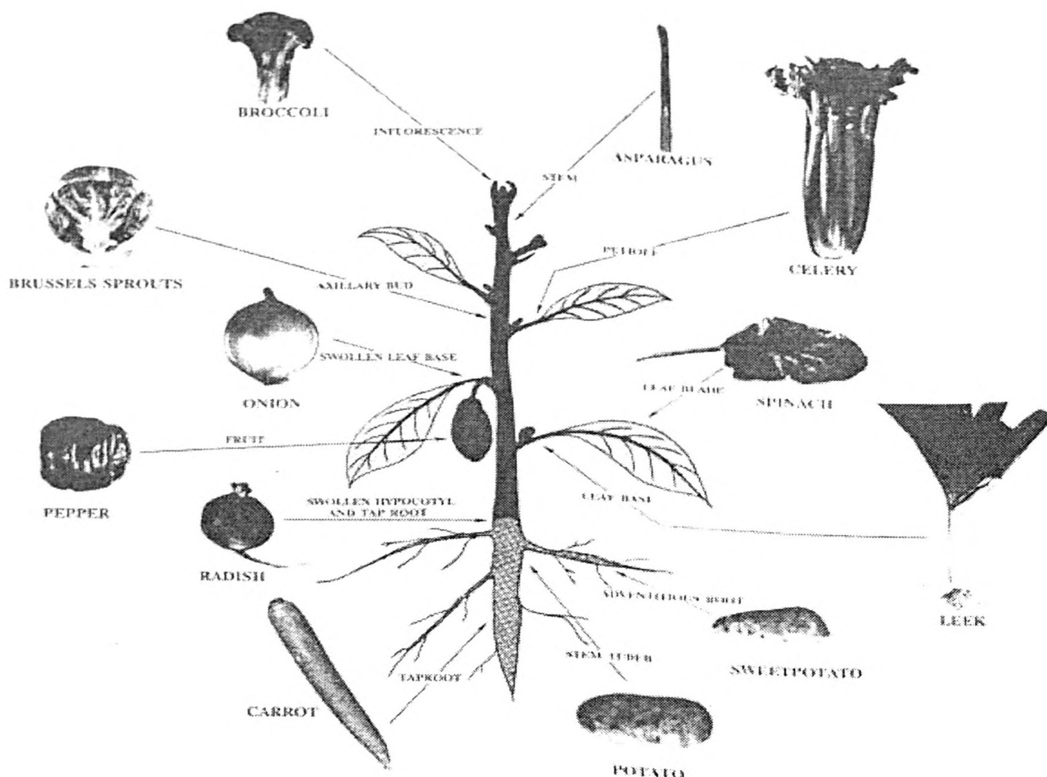


Figure 2.4 Plant parts as vegetables

Vegetable juices are attracting more attention due to the nutritional and phytochemical value of many vegetables. Carrot juice (Daucus carota L.) is one of the most popular vegetable juices and represents a rich source of natural β-carotene. (Savas Bahceci *et al*)However, other vegetables are becoming more acceptable.

High quality carrot juice is defined as a cloud stable juice with fresh taste, appealing intense color, high carrot content & a healthy dietary fiber balance. (R.P Bates *et al.).*Carrot juice also typified the current appeal the health conscious consumer .The health image of carrot is strongly promoted while the blending juices, citrus & tropical fruits provide flavor & reduced pH.

2.2.2.1 Benefits

The benefit of carrot juice exceeds crunching through a bunch of carrots by far. When consuming carrots, increase body's absorption of the valuable nutrients in them almost hundredfold, by simply employing a juicer, rather than teeth.

Night vision improvement

High carrot intake is associated lowered risks of many cancers

Stronger, healthier nails and hair.

Many of the aches and pains associated with getting older will be improved when ingesting carrot juice on a daily basis.

Improving the liver function, a benefit of carrot juice, is one of the best things can do to enhance the quality of life.

In fact, digestive tract as a whole will benefit from the healing power of carrot juice.

Improvement in skin infections, as well as a variety of other infections.

A myriad other nutrients add to the benefit of carrot juice.

It also contains important minerals such as calcium and potassium. Body can battle to absorb calcium from sources such as dairy. However, the calcium in carrot juice can be assimilated in full. Iron, phosphorous, and sulphur will also be included in carrot cocktail.

2.3 Blanching

Scalding the vegetables in water or steam for a short period of time is known as blanching. Microwave blanching is the latest blanching method which introduced recently. Blanching is the most hygienic and acceptable method used in destroying enzymes which cause unnecessary changes at cellular and tissue level.

2.3.2 Purpose of blanching

Thermal inactivation of enzymes basically polyphenol oxidase is considered as the main purpose of blanching which are responsible for brown colour pigment formation in living cells.

Furthermore enzymes responsible for chemical changes that lead to cloud formation of carrot drink are controlled to the appreciable extent by blanching. Proper blanching brightens colour, helps to prevent to nutritional losses and cleanses the surface from dirt and micro organisms to certain extent. It also softens cell walls and makes them easier to pack.

Blanching is important as a thermal processing method ,due to it makes some nutrient more available to human body than in raw form(Granada *et al*,1992).Pro vitamin A carotenoid availability is increased by cooking up to300% or more to human body.Retinol equivalent is also increased.This was attributed to increase extractability of these compounds as a result of cooking .In compiling a data base on the carotenoid content of a large number of fruits and vegetables.(Mangels *et al*,1993)Also noted that substantially higher contents in cooked than in raw vegetables.

2.3.3 Blanching time determination

Blanching time may vary depending on several factors

1. Specification s of the vegetable species
2. Varietial specifications like size, shape, and thickness of the edible part to be blanch.
3. Heat conductivity
4. Susceptibility to heat treatment
5. Natural level of enzymes

Blanching is very delicate processing step where the time, temperature and other conditions are carefully monitored. Few minutes blanching are enough for satisfactory inactivation of enzymes.

Under blanching - stimulates the enzyme activity worsening the quality of treated parts than the with no blanching.

Overbalancing – loss of flavour, colour, vitamin and minerals, strike together on the drying trays.

Catalase and peroxidase are the two most heat resistance enzymes in vegetables. If these two enzymes are destroyed, it can conclude that other enzymes have been destroyed. So peroxidase activity is used as an indicator of determining the extent of enzyme inactivation.When blanching plant materials reacting with 4% potoluidine solution and few amount of hydrogen peroxide, it gives brown colour if peroxide enzyme is active.

2.4 Carbonation

2.4.1 Definitions

1. Dissolving CO_2 gas in water utilizing temperature and pressure (Woodroof J.G 1974)
2. Impregnation of a liquid with gas (Mitchell A.J, 1990)

2.4.2 History

Popularity of natural occurring mineral waters which are discharged in a slightly carbonated form from rock formation s in many of the well known spa resorts around the world is being the foundation for the artificial inclusion of the dissolved gas in a soft drink beverage.

The medicinal advantage of spa products have been panegyrized to the point of exaggeration and the ingestion of the dissolved CO_2 has always been considered an important part of the therapeutic process.

2.4.3 Benefits

Addition of CO_2 makes any soft drink more palatable and visually attractive. When applied to a soft drink product, the result is a beverage, which sparkles and foams as it is dispensed and consumed. This escape of CO_2 during consumption of the drink should complement and enhance the flavor and will add an exciting tingle that stimulates the palate (Mitchell A.J, 1990).

The organoleptic effects are not the only benefits of the CO_2 content. CO2 gas has preservative property at carbonation above 3.0 volumes. The extent of which is dependent upon pH, sugar, initial microbial load and the nature of the microorganisms. This desirable attribute of carbonation should be considered as an added 'Bonus' and must not be a substitute for other precautions taken to ensure the safety and extended shelf life of the product. Correct carbonation of a soft drink is most important because of the pungent taste it gives the beverage and the beneficial effect it has on the digestive system (Woodroof D.J, 1974)

2.4.4 Principle

2.4.4.1 Effect of Temperature

Carbon dioxide gas is readily soluble in cool or cold water. A given volume of water will absorb an equal volume of CO_2 gas at 60^0F and under 1 atm (14.7 lb psi) of pressure. The CO_2 gas is relatively insoluble in boiling water. The solubility of CO_2 gas in water decreases with increasing temperature. (Jacob M.B, 1959)

2.4.4.2 Effect of pressure

According to the Henry's law, the solubility of CO_2 in water at constant temperature is dependent only on the pressure of the CO_2 gas and independent of the pressure and presence of other gases such as air. The solubility of CO_2 gas in water increase with the increasing CO_2 pressure (Phillips G.F, 1974).

2.4.4.3 Pressure Temperature relationship

Pressure in the carbonated beverage will goes up with increase in temperature. As the pressure goes up there may be leakage in trough the crown of bottled carbonated beverages. These relationships are shown in table

Table 2.1

Increase of pressure with increase of Temperatures in Carbonated Beverages

Temperature	Pressure in psi developed with Volumes of CO_2					
^{0}F	3.0	4.0	5.0	6.0	7.0	8.0
40	16	26	36	46	56	66
50	23	40	46	59	72	84
60	30	44	59	74	86	103
70	37	56	72	86	107	124*
80	46	66	86	106	126*	146*
90	56	79	102	125*	148*	172*
100	65	91	118*	144*	170*	197*
110	75	104	137*	163*	193*	223*

- Pressure at which there is danger of crown leakage.

Source Jacob B.M ,1959

In the case of cans increase in pressure beyond permissible limits may cause the can to be strained or distorted. If the manufacturer wishes to use an in can process of pasteurization this hazard is increased.

2.4.4.4 Equilibrium pressure

In a system composed of a soluble gas and a liquid the equilibrium pressure is defined as the pressure exerted by the gas when the rate of gas absorb by the liquid is exactly equal to the rate at which the absorbed gas leaves the liquid (Phillips F.G ,1974).

Carbonation of the product will remain completely constant and uniform if the above pressure and temperature are not changed. The equilibrium pressure for a gas liquid system will vary with temperature and the amount of gas and liquid present. At equilibrium pressure the gas liquid mixture is just stable but any decrease in pressure or increase in temperature will render the mixture supersaturated. In that case the pressure, temperature combination is insufficient to keep the CO_2 in solution.

In this circumstance, gas will be spontaneously released. This condition is known as foaming or fobbing and usually apparent when a bottle of carbonated product is opened to atmospheric pressure. The inability of a carbonated beverage to retain its full CO_2 content in solution at atmospheric pressure gives rise to the attractive ebullience observed during the act of pouring the drink in to a glass and the liberation of further CO_2 during the actual consumption.

Carbonated product held in a container that is open to the atmosphere will gradually lose carbonation as the gas is liberated and escapes from the liquid. In a closed container, this evolution of gas proceeds to fill the headspace volume. This causes to gradual increment of the pressure quickly at first and then more slowly as the equilibrium condition is approached. The CO_2 gas leaves the beverage and collects in the headspace volume to provide the necessary equilibrium pressure to keep the remaining gas in the solution .This condition applies to all bottles and cans that have been filled with carbonated beverage and then sealed with the appropriate closure.

2.4.4.5 Carbo – Cooler

Type of carbonator that cools and carbonates at the same time is the carbocoolaer. In this type of equipment, warm, uncarbonated water enters at the top going into a distribution pan from which it flows down over stainless steel cooling plates becoming carbonated with the carbon dioxide being admitted from the side. The cooled carbonated water flows to the reservoir from which it can be conducted to the filler, at about 34^0F.

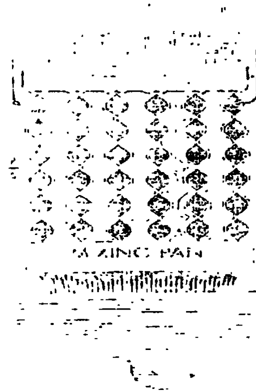


Figure 2.5 Mojonnier Carbo – Cooler

2.4.5 Carbonation methods

2.4.5.1 Pre mix method

Pre sterilized bottles are filled with carbonated water and then juice is introduced. Loss of 23% of beverage is noticed due to overflowing under gas pressure. (Khurdiya, D.S 1989)

2.4.5.2 Post mix method

Juice is introduced to pre sterilized bottles and then filled with carbonated water. Drink loss can be minimizing through this method.

2.4.6 Carbonation Measurements

Availability of a standard form of carbonation measurement is imperative due to the degree of carbonation is such an important factor in the formulation of a soft drink.

1. Volumes Bunsen

Gas volume is measured at atmospheric pressure at 760 mm of Mercury) and the freezing point of H_2O (32^0F or 0^0C)

2. Volumes Ostwald

Gas volume is measured at atmospheric pressure but any temperature adjustment is ignored.

3. European Method

Carbonation is measured in grams per liter and since 1 Vol (Bunsen) is equivalent to 1, 96 g CO_2 per liter

2.4.6.1 CO_2 Volumes

The volumes of the CO_2 in the finished beverage is a most important factor, for it is the quantity of dissolved CO_2 in the beverage that gives it is sparkle and governs the length of time, along with other factors, such as temperature, that the beverage will continue to effervesce.

Henry's law states that the amount of a gas dissolved by a given volume of a solvent at constant temperature is directly proportional to the pressure of the gas with which it is in equilibrium. According to this law, the amount of carbon dioxide dissolved by water at a given temperature is proportional to the pressure of the CO_2 on the water. This law is however, conditioned by the nature of the molecule as it exists in the gaseous state and as it exists in the solution. In the instant of CO_2, as far as carbonated drinks are concerned, variations from Henry's law are not large.

The amount of CO_2 gas producing the carbonation effect is usually specified as volumes. In broad terms, the number of times the total volume of dissolved gas may be divided by the volumes of the liquid.

e.g. 1. Pepsi 2l at3.4 vol

= 2l beverage * 3.4 CO_2 vol

= 6.8 l of CO_2 at 0^0C and 1 atm

2. A 3 volume drink will contains CO_2 to the extend of 3 times the vol of the beverage

Each soft drink formulation requires a particular degree of carbonation so that the effervescence is appropriate to the flavor and nature of the beverage. Fruit drink – such an orange, bitter lemon etc should contain low level of carbonation whereas juice-based drinks colas, ginger beer and cream soda-should be in the medium to high range of CO_2 content. Mixer drinks, so called as they are used mainly to mix with spirits, requires high carbonations since the addition to still non carbonated liquors dilutes the original carbonation level. Drinks in this category would be tonic water, ginger ale and soda water. Soda water filled in to siphons contains the maximum degree of carbonation usually encountered in the industry. These particular containers rely upon internal pressure to dispense the contents and, as the siphon empties, this pressure is replenished from the CO_2 dissolved in the product.(Appendix II)

In practical terms, carbonation levels vary between 1 volumes of CO_2 in fruit juices to 4.7 volumes in mixers and up to 6 volumes in soda syphons. Figure2.6 shows typical carbonation values for a range of well-known drinks. A degree of latitude is indicated since individual recipes require their particular carbonations. The increasingly popular large PET bottles constitute a special case; not only does the gradually escape through the permeable polymer material producing a marked reduction the carbonation of the content over a period of time, but the repeated opening and closing of the container for occasional consumption can result in the final 25/30% of the content having an unacceptably low carbonation. This latter problem arises because each time the bottle is resealed CO_2 gas escapes from the residual product to press rise the headspace volume and most of this gas then escâpes to atmosphere on the next occasion the bottle is opened. These large containers are not really intended for intermittent consumption and to compensate for the future loss of carbonation, the product is carbonated to a slightly higher than would be appropriate for the particular drink. (Mitchell A.J, 1990)

2.4.7 The carbonation test

Various carbonation test procedures have been devising to minimize or correct the error caused by the presence of air in bottled carbonated beverages. Carbonation tester is used for carbonation measurements. (Figure 2.7)

The most common test procedures used in the carbonated beverage industry employ a temperature pressure measurement, either with or without first snifting the head space gases of the test bottle.The purpose of snifting before testing is to expel the air present. This possible because air is only slightly soluble in water and most of it is present in the headspace of the bottle. This test procedure with a snift of the headspace gases is generally used, but the test procedure without snifting is some times recommended for use with certain types of filling equipment which do not permit much air to remain in the close product container. (Philips *et al*, 1974)

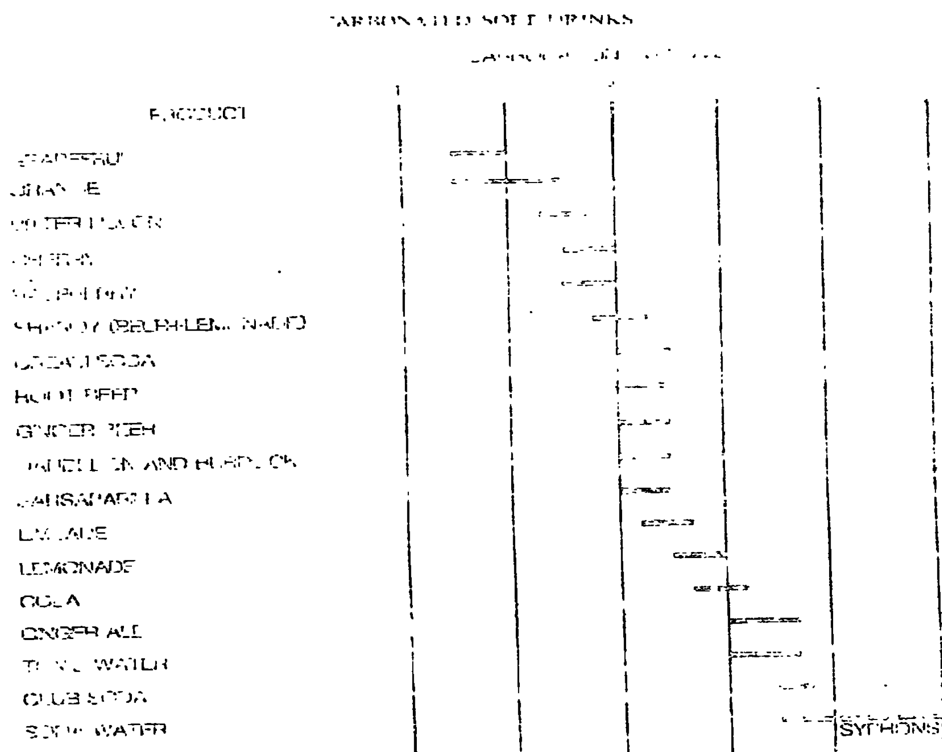


Figure2.6 Typical carbonation levels for well – known products

Source Mitchel A.J ,1990

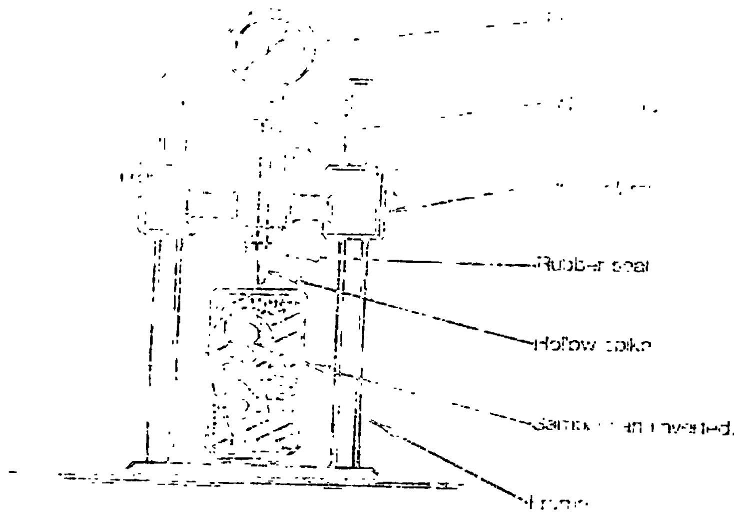


Figure 2.7 Pressure testers for cans and plastic bottles

To estimate the volumes of CO_2 in a bottle of a carbonated drink, it is necessary to make awareness the temperature of the beverage and the pressure of the contents of the bottle. The data are determined with guages and thermometers. Finally gas volume is obtained by referring the gas volume test chart. (Appendix III)

2.4.8 Loss of carbonation

One most serious problem in manufacture and sale of carbonated beverages is the loss of carbonation at the filler or after bottle has been opened.

2.4.8.1 Boiling

sudden loss of carbonation is termed boiling when it occurs at the filler. Cooling the water and the syrup prior to carbonation prevents boiling at the filler.

2.4.8.2 Gushing

Rapid loss of carbonation is termed gushing when it occurs as the bottle cap is removed. Gushing of beverage is mainly due to following reasons.

1) Presence of discharge nuclei
2) Excess air in the drink
3) Unnecessary agitation
4) Improper storage

Nuclei in the beverage act as discharge points for CO_2 gas. Rough irregular shape particles are much more likely to act as nuclei for the release of CO_2 than smooth round particles. While dirty bottles are the principle source of such disturbing nuclei, dusty and moldy crowns, protozoa and algae and oil droplets are other common sources. Scratches on the interior bottle walls provide places which are equivalent to nuclei in causing release of CO_2.

Proper soaker operation and maintenance will overcome and avoid this source of trouble. To avoid scratching the brushes in such soakers must be inspected carefully periodically and replaced when necessary. Since moldy and dusty crowns can contribute particles which will causes gushing. Care must be taken to store these items in such manner that they will not become moldy or dusty. In addition the hopper for crown delivery should be equipped with an air line to blow away any dust in crowns and the hopper itself should be covered.

2.4.9 Carbon dioxide

2.4.9 Introduction

The sparkle of a carbonated soft drink is due to the carbon dioxide it contains. This component adds to the " life " of the beverage and contributes, in some measure to tang (Moriris B.J, 1959).

2.4.9.1 Carbon dioxide

CO_2 is a colorless, nontoxic gas with slight pungent odor when dissolved in water. The resultant carbonic acid mixture has an acidic and biting taste which is not unpleasant (Mitchell A.J, 1990). It is distributed in cylinders under high pressure, as a liquid in tank trucks or rail cars under low pressure or solid in insulated containers or trucks at atmospheric pressure.

CO_2 is widely distributed in nature both as the free gas in normal air and as salts and compounds such as sodium carbonate (Na_2CO_3), sodium bicarbonate (Na_2HCO_3), Calcium Carbonate ($CaCO_3$) and the like. Many rocks are composed in whole or in part of carbonates. Among the more common are marble, limestone, chalk and Magnesite which are principally composed of calcium carbonate and dolomite, a Calcium Magnesium Carbonate (Morris B.J, 1959)

CO_2 is one of the very few gasses stable for providing the effervescence in soft drink. It is non toxic ,inert virtually tasteless readily available at moderate dost and may be liquefied at reasonable temperatures and pressures allowing convenient bulk transportation and storage. At atmospheric pressure and room temperature the solubility of CO_2 in both water and soft drink product allows an acceptable retention of gas in solution. Although slight agitation will promote an evolution of gas bubbles from the body of the drink, which creates the attractive sparkling, effect (Mitchell A.J, 1990).

4.9.1.1.1 Properties

CO_2 exist in three status. Solid (dry Ice), liquid and gas. Existing state is determined by the temperature and pressure. CO_2 is usually present in atmosphere at a level of approximately 300 ppm by volume. It is dangerous to breathe atmosphere containing more then 5 % by volumes has been postulated that workers may be safely exposed to a maximum concentration of 5000ppm CO_2 by volume for 8 hours per day.

Since the gas is 1.53 times heavier than air at 70^0F, great care must be taken when entering vessels that have contained CO_2 and may not have been sufficiently vented and purged. In these circumstances, residual CO_2 will lay in the base of the tank to trap- the unwary entrant.

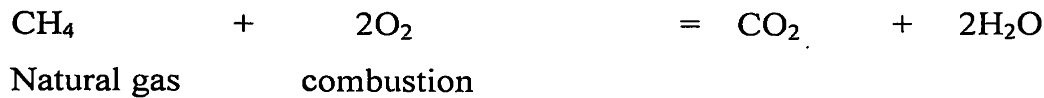
When dissolved in water, CO_2 produces Carbonic acid and the solution is chemically active due to its acid properties. Only a small part of the dissolved CO_2 unites chemically with the water to form the acid .It's pH value is in the range of 3.2 to 3.7.

2.4.9.1.2.1 Sources of Carbon dioxide

The supply of CO_2 and O_2 in the air is kept fairly stable by an important exchange of gasses between animals and plants.CO_2 is formed naturally in the earth by the decomposing of rocks and vegetable matter. But the commercially used co2 is derived from the following principle sources.

1) Burning of Carbon compounds. (Coke, Oil, gas etc)
2) Heating limestone – forms lime and CO_2
3) Fermentation process – produces alcohol and CO_2
4) CO_2 gas wells.

The chemistry of these production process as follows.



$$CH_4 + 2O_2 = CO_2 + 2H_2O$$

Natural gas combustion

$$CaCO_3 + \text{Heat} = CaO + CO_2$$

Limestone Lime

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

Dextrose (Sugar) Fermentation Alcohol

$$CaCO3 + H_2SO_4 = CaSO4 + CO_2 + 2H_2O$$

In order to recover the CO_2 from the raw gas produced by combustion and limestone processes which is a mixture of CO_2 ,N_2 and various products of combustion a reversible chemical process is used. In this process, a special chemical which rejects impurities and CO_2 in the raw gasses is absorbed. Finally the impurities passing on and being discharged to the out side air. Two commonly used chemicals for the absorption steps are Sodium carbonate and monoethanolamine.These accept CO_2 chemically cool and expel it by chemical decomposition on dieing heated.Absorpton process can be eliminated for CO_2 produced from fermentation process and natural CO_2 wells.

The gasses produced as in either case are still considered raw CO_2 requiring further purification. This additional purification generally consist of

1) Scrubbing with water
2) Chemical treatment to remove sulfurous elements if present
3) Odor removal either chemically or by passing through activated carbon
4) Drying to remove water

The purified CO_2 is still a gas .It must be liquefied before being stored or packaged in cylinders or produced as solid.(Phillips *et al,* 1974)

2.5 Nutritional value

Fruits and vegetables play an important role in providing essential nutrients to human. In fact half of our vitamin A requirement and about 90% of Vitamin C requirements are covered through consumption of vegetables. (Rizek,et al,1974,Senti and Rizek,1975,Goddard and Matthews,1979,Wills et al,1981,IFT,) Other than that following other nutrients are also provided by vegetables. Folacin 40%, (Wills *et al* ,1981)Thiamin 17-20%,Vitamin B6 25-30%, riboflavin 18%,Magnesium 26% and Iron 19%.

Scientists pay more attention due to vitamin A and C is the main nutrients provided by vegetables. Vitamin A activity is resides in carotenoids in vegetables. Hence carotenoids are among the one of the mostly studied compounds.

2.5.1 Carotenoids in foods

Carotenoids are notable for their wide distribution, structural diversity, and various functions. More than 600 carotenoids are, not including cis and trans isomers,have been isolated and characterized from natural sources This impressive figure includes the enormous array of arytenoids in algae, bacteria ,yeast and fungi. Only a fraction of the carotenoids recorded to date are found in food, nonetheless the carotenoid composition of foods can still be complex.

Carotenoids in foods are symmetric molecules and C_{40} tetraterpenoids.Generally formed from eight C_5 isoprenoid units joined head to tail, except at the center where a tail to tail linkage reverse the order. (Figure 2.8) Centrally located extended conjugated double bond system is an important feature which constitutes the light – absorbing chromophore that gives carotenoids their attractive color and provides the visible absorption spectrum that serves as a basis for their identification and quantification. Multitude structures are formed by cyclization, hydrogenation, dehydrogenation, introduction of oxygen functions, rearrangement, and chain shortening like modifications in basic skeleton. Hydrogen carotenoids (eg, Beta carotene, lycopene) are known as carotens, and oxygenated derivativesare known as xanthophylls. Hydroxy (as in beta cryptoxanthin),Keto (as in canthaxanthin),epoxy (as in violaxanthin)and aldehyde(as in citraurin) groups.Carotenoids can be mono

cyclic (e.g γ carotene) or dicyclic as in α and β carotene or acyclic as in lycopene..In nature carotenoids exist primilarly in the more stable all –trans form, but small amount of cis or z isomers do occur. (Kimura *et al*, 2004)

2.5.1.1 Beta carotene

Structurally, vitamin A (retinol) is essentially one half of the molecule of beta carotene with an added molecule of water at the end of the lateral polyene chain. Thus beta carotene is the potent pro vitamin A to which 100% activity is assigned. An unsubstututed Beta ring with a C_{11} polyene chain is the minimum requirement for vitamin A activity. α Carotene, γ carotene, β cryptoxanthin, α cryptoxanthin and β-caroten-5, 6-epoxide, all of which have one unsubstituted ring would have about half the bioactivity of β carotene. The acyclic carotenoids, which are devoid of β rings and the xanthophylls other those mentioned above, in which the β rings have hydroxy, epoxy and carbonyl substituents, are not provitamins A. (Rodriguez-Amaya B, 1999)

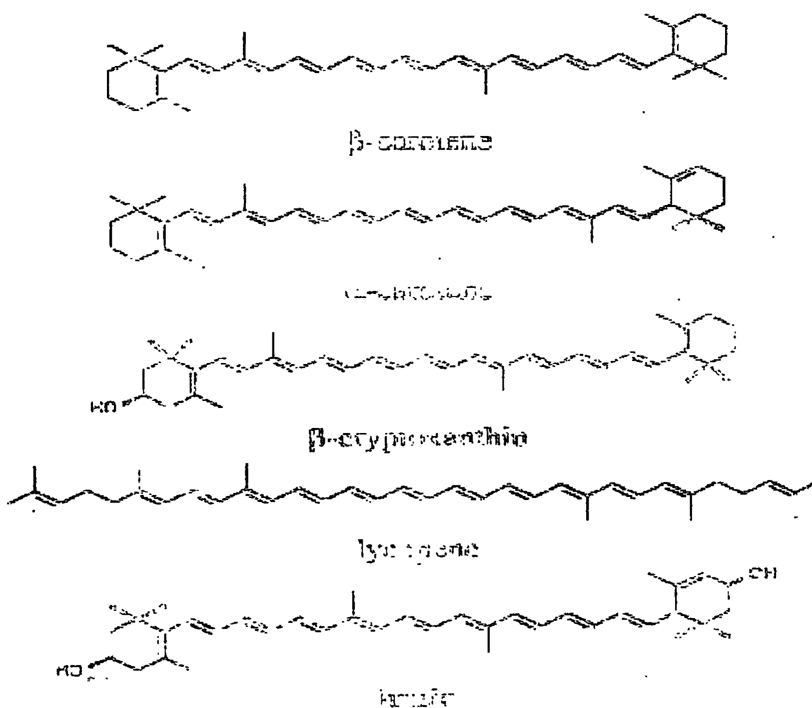


Figure 2.8 Structures of the principal carotenoids in foods

2.5.2 Health benefits to human

β Carotene,α carotene, β cryptoxanthin, lutein and lycopene are the carotenoids most commonly found in human plasma. These carotenoids together with Zeaxanthin have been shown to have health promoting effects.Carotenoids, whether provitamin A or not have been credited with other beneficial effects on human health.

Enhancement of the immune response, Reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, and cataract. The action of carotenoids against diseases has been attributed to an antioxidant property, specially their ability to quench singlet oxygen and interact with free radicals. The ability to carotenoids to quench singlet oxygen has been linked to the conjugated double bond system. How ever other mechanisms have been reported as modulation of carcinogen metabolism, inhibition of cell ploriferation, enhancement of cell defferenciation, stimulation of the cell to cell communination, and filtering the blue light. (Kimura m *et al*, 2004.)

2.5.3 Effect of processing

Alterateration or loss of carotenoids during processing and storage of food occurs through physical removal,geometric isomerization and enzymatic or non enzymatic oxidation.(Rodrigez Amaya,1990).Natural carotenoids in fruits and vegetables are usually stable during blanching , retorting and freezing, although heat causes isomerization.(Weckletal,1962,de Ritter,1976,Simpson,1983),drying and extrusion cooking are particularly destructive steps.(Mackinney et al ,1958).The canning of vegetables causes little or no degradation at all of naturally occurring carotenoid-protein complexes(Borenstein and Bunnell,1966)

As carbonation of vegetable juices is one of the new trends, it is not surprising to find that very little information is available on the mechanism and the rate of changing beta beta carotene in carbonated condition.

2.5.4 Analytical method for carotenoid analysis

Carotenoid analysis usually consist of 1)sampling 2)extraction 3)partition 4)saponification and washing 5)evaporation the solvent 6)chromatographic separation 7)identification and 8)quantification. Chromatographic techniques applicable in

carotenoid analysis can be classified by ranging from open column (OCC) to high performance liquid chromatography.(HPLC) (Rodriquez-Amaya 1999)

2.5.4.1 Sample preparation

Carbonated Carrot drink contains approximately 70% of water per 100ml.Freeze drying the sample is minimize the interference of water for the further analytical steps.

2.5.4.1.1 Freeze-drying

Freeze drying or process works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water to in the material to sublime directly from solid phase to gas. It brings the system around the triple point, avoiding the direct liquid gas transition seen in ordinary drying. (Figure 2.9). There are three stages in freeze-drying. Process: Freezing, primary drying and secondary Drying

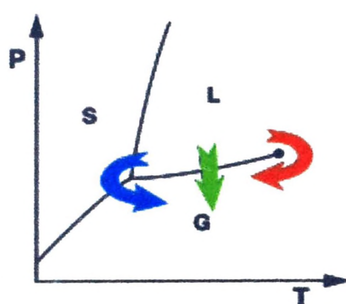


Figure 2.9 Phase diagram

2.5.4.1.2 Evaporation

Evaporation or concentration is done by using rotary evaporator which is a device used in chemical and biochemical laboratories for the efficient and gentle evaporation of solvents. The system works because lowering the pressure lowers the boiling point of liquids including that of the solvent. That allows solvent to be removed without excessive heating. Main components are a vacuum system, consisting of a vacuum pump and controller, a rotating evaporation flask which can be heated in heated fluid bath, and a condenser with a condensate collecting flask.

2.5.4.2 Chromatographic separation by Alumina (activity 111) column

In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column and the mobile phase, a liquid, is added to the top and flows down through the column by either gravity or external pressure.

Column chromatography is generally used as a purification technique. It isolates desired compounds from a mixture. The mixture to be analyzed by column chromatography is applied to the top of the column. The solvent (the eluent) is passed through the column by gravity or by the application of pressure

2.5.4.2.1 Principle

An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column.

2.5.4.2.2 Types of column chromatography

Column chromatography is separated into two categories, depending on how the solvent flows down the column.

Gravity column chromatography - solvent is allowed to flow down the column by gravity, or percolation.

Flash chromatography - The term "flash chromatography" was coined by Professor W. Clark Still because it can be done in a "flash. Solvent is forced down the column by positive air pressure; it is called a "state of the art" method currently used in organic chemistry research laboratories.

2.5.4.2.3 The Adsorbent

Silica (SiO_2) gel and Alumina (Al_2O_3) are two adsorbents commonly used by the organic chemist for column chromatography. These adsorbents are sold in different mesh sizes, as indicated by a number on the bottle label: "silica gel 60" or "silica gel 230-400" is a couple of examples. Alumina is used more frequently in column chromatography than it is in TLC. Alumina is quite sensitive to the amount of water

which is bound to it. The higher its water content, the less polar sites it has to bind organic compounds, and thus the less "sticky" it is. This stickiness or activity is designated as I, II, or III, with I being the most active. Activity refers to Brockmann activity. Relative activity is directly related to the water content of the sorbent, most active being I and thereafter decreasing to the least active III.Alumina is usually purchased as activity I and deactivated with water before use according to specific procedures. Alumina comes in three forms: acidic, neutral, and basic. Aluminum oxide chromatography sorbents will adsorb water from the air. It must be stored in air-tight containers or its Brockmann activity can change.

2.5.4.2.4The Solvent

The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique. TLC is generally used to determine the system for a column chromatography separation. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute a less-polar compound. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compound

2.5.4.2.5 Analysis of Column Eluants

If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. More commonly, the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labeled tubes and the composition of each fraction is analyzed by thin layer chromatography.

2.5.4.3 Identification – Thin layer chromatography (TLC)

This technique is classified under planar chromatography in which the stationary phase is a solid and the mobile phase is a liquid. Thin layer of adsorbent is bound to an inert planar support. The support may be glass plates .plastic sheets and aluminium foil.Different layer thickness, pre coated plates are available in commercially in a wide variety of sorbant, including chemically modified silicas.Silica gel; Alumina and cellulose are the frequently used TLC sorbents.

2.5.4.4 Quantification – Ultraviolet-visible Spectrophotometer

Instrument used in ultraviolet-visible spectroscopy is called a UV/vis spectrophotometer. Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometer (UV/ VIS) involves the spectroscopy of photons and spectrophotometer. It uses light in the visible and adjacent near ultraviolet (UV) and near infrared (NIR) ranges. In this region of energy space molecules undergo electronic transitions. UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds. Organic compounds especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water soluble compounds, or ethanol for organic-soluble compounds.

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the solution's concentration. Thus UV/VIS spectroscopy can be used to determine the concentration of a solution.

It measures the intensity of light passing through a sample (I),and compares it to the intensity of light before it passes through the sample (I_0).The ratio I/I_0 is called the transmittanmce,and is usually expressed as a percentage(T%).The absorbance ,A, is based on the transmittance.

It measures the $A = -\log(\%T)$

1) A light source (often an incandescent bulb for the visible wavelengths, or a deuterium arc lamp in the ultraviolet),

2) A sample holder,

3) A diffraction grating or monochromator to separate the different wavelengths of light,

4) A detector.

A spectrophotometer can be either single beam or double beam

In a single beam instrument (such as the Spectronic 20), all of the light passes through the sample cell. I_o must be measured by removing the sample.

In a double beam instrument the light is split in to two beams before it reaches the sample .One beam is use as reference, the other beam passes through the sample. Some double beam instruments have two detectors. (Photodiodes), and the sample and the reference beam is measured at the same time. In other instrument, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam. (Wikipedia)

2.5.4.5 Statistical analysis

As a Non-parametric procedure Kruskal Wallis determines how much the "Average Ranks" of each of the samples differs from the "Over All" average rank for all samples combined as one! If the sample average ranks differ by a lot the H statistic will be accompanied by a low probability for its degrees of freedom and IF that $p<=0.05$ we infer that at least one sample Median differs from at least one other. The minitab output provides some indication of where that difference may lie by providing a Z-value for each sample. Z shows how far the average rank for the sample deviates from the overall average rank for all the samples.

CHAPTER 03
MATERIALS AND METHODOLOGY

3.1. Material

3.1.1. Development of carbonated carrot drink

Mature carrot root

Refined sugar (New Krung Thai factory co.Ltd)

Sodium chloride

Sodium metabisulphite

Citric acid

Carbonated water (Ceylon oxygen Ltd)

Treated water

3.1.2 Carbondioxide purity test

10% Sodium or potassium hydroxide solution

3.1.3 Carbonation measurements

Treated water for cleaning

Distilled water for check standard

3.1.3.1Pressure gauge calibration

Light machine oil SAE (20)

Standard 5, 10 psig weights

3.1.4 Carbonation shelf life test

CO_2 gas volume chart

3.1.5 Chemical tests

3.1.5.1 Degassing beverage

Pressurized clean air or nitrogen at constant pressure

Timer

3.1.5.2 Brix

Distilled water

Analytical grade sucrose (Avonchem Ltd, UK)

3.1.5.3 PH

Three buffer solutions of PH 3, 7 & 10

Distilled water

3 molar Kcl

3.1.5.4 Titratable acidity (TA)

0.1000 N sodium Hydroxide

Distilled water

0.5% Phenolphthalein solution

0.1000 sulphuric solutions

3.1.6 Microbiological test

Tryptone glucose Extract (TGE) Broth (Millipore corporation, USA)

m- Green yeast & mold Broth (Millipore corporation, USA)

m-Endo Total Coliform Broth (Millipore corporation, USA)

Media pads (Millipore corporation ,USA)

Filter membrane (White gridded, 0.45 µm.47 mm) (Millipore corporation, USA)

70% Ethanol

Forceps

Gloves

Face mask

3.1.7 Identification and quantification of carotenoids in Carrot root & Carrot drink

Cold acetone (99.5% purity, refrigerated for 2 hours)

Pet ether (BDH chemicals Ltd)

Anhydrous sodium sulphate

Celite

Alumina activity (III)

Glass wool

Beta-carotene standard (Sigma chemicals)

Toluene

Methanol

3.2 Apparatus

3.2.1 Development of carbonated carrot drink

Blender

Hot plate

Grater

Knife

Spoons

Muslin cloth

3.2.2 Carbondioxide purity test

CO_2 purity tester (Zahm & Nagel co.inc)

18"length of ¼ or 3/8"rubber or plastic tubing

¼ or 3/8 in line CO_2 sampling valve

3.2.3 Carbonation measurements

Handshake tester (Zahm & Nagel co.inc)

Calibrated 0-60 pressure gauge

Calibrated thermometer (0-50^0C)

Carbonation volume chart

Plastic mesh or leather safety shield

3.2.3.1 Pressure gauge calibration

Dead weight tester (Terris consolidated industries)

3.2.4 Carbonation shelf life test

Carbonation tester

3.2.5 Chemical tests

3...2.5.1 Degassing beverage

Degassing instrument

Sample insertion tube or diffuser

Air line filter & water trap

Pressure regulator

3..2.5.2 Brix

Bench refractometer (Bellingham Stanley Ltd)

Eye dropper

Balance

3.2.5.3 PH

PH meter & electrode

250 ml beaker

Magnetic Stirrer

Magnetic Stirring bar

3.2.5.4 Titratable acidity (TA)

PH meter & electrode

50 ml calibrated self zero burette with titrant reservoir

250 ml beaker

Magnetic Stirring bar

3.2.6 Microbiological test

Media pads holder

1 ml pipette

Pre sterilized Petri dishes (47 mm, Millipore Corporation)

Autoclave (Sturdy Industrial Co.Ltd), Taiwan)

Incubator -bacteria, Coliform (Boekel Industries Inc)

BOD incubator – Yeast & Mold (Jindal Scientific Industries, Delhi)

3.2.7 Identification and quantification of carotenoids in Carrot root & Carrot drink

Mortar and pestle

Separatory funnel (500 ml)

Volumetric flask (50, 25 ml)

Rotary evaporator (Heizbad WB, speed 10-270 rpm, Tem 20-100°C)

Sonicator

Vacuum oven (Heraeus instruments, Germany)

Freeze dryer

Funnel

TLC sheets

Pasture pipettes

Spectrophotometer

Micropipette

3.3 Procedure

3.3.1 Development of carbonated carrot drink

3.3.1.1 Determination of the best blanching condition

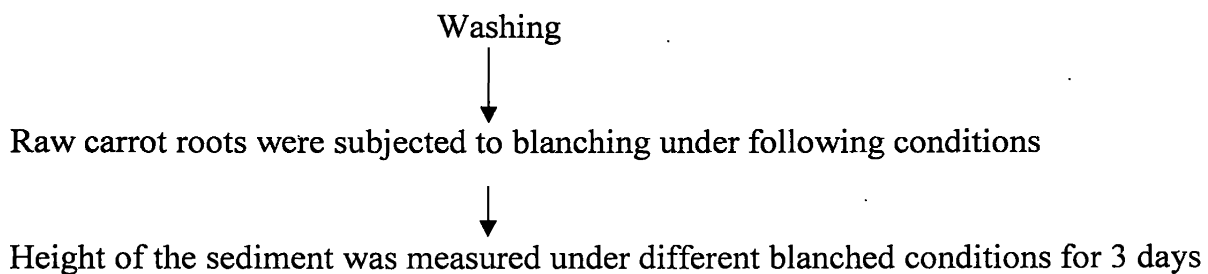


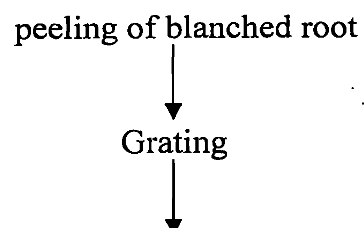
Table 3.1 Blanching conditions 01

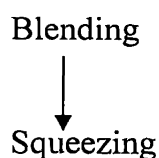
Sample code	Temperature (C^0)	Time (Min)
153	80	1
428	80	2
102	80	3

Table 3.2 Blanching conditions 02

Sample code	Temperature (C^0)	Time (Min)
328	90	1
562	90	3
137	90	5

3.3.1.2 Juice extraction





3.3.1.3 Reformulation the recipe

Previously prepared recipe was reformulated according to preliminary sensory analysis.

Table 3.3 Determination of the best juice amount per 100ml

Sample code	juice	Sugar	Citric acid	NaCl	Water
321	13	10	0.1	0.5	77
453	15	10	0.1	0.5	75
182	17	10	0.1	0.5	73

Table 3.4. Determination of the best sugar amount per 100ml

Sample code	juice	Sugar	Citric acid	NaCl	Water
324	17	10	0.1	0.5	73
173	17	14	0.1	0.5	79
897	17	16	0.1	0.5	67

Table 3.5 Determination of the best citric acid amount per 100ml

Sample code	juice	Sugar	Citric acid	Nacl	Water
231	17	14	0.10	0.5	69
563	17	14	0.15	0.5	69
976	17	14	0.20	0.5	69

Table 3.6 Determination of the best NaCl amount per 100ml

Sample code	juice	Sugar	Citric acid	NaCl	Water
153	17	14	0.15	0.5	69
325	17	14	0.15	0.3	69
451	17	14	0.15	0.1	69

Figure 3.1 Developed carbonated carrot drink

3.3.2 Carbonation

Post mix method was used

Juice base was places in a bottle

Chilled treated water was added

Carbonated water was added up to the 200 ml mark of the bottle

3.3.3 Carbon dioxide purity test

Purity tester was positioned in upright position

Pressure was regulated to below 10 psi

Narrow tapered tubular section of the purity tester was connected in to in line CO_2 sampling valve using 18" plastic tubing

both valves of the purity tester was opened

inline CO_2 sampling valve was opened slowly and uniform gas flow was obtained

gas flow was allowed to flow through purity tester for 2-3 minutes

inline sampling valve was closed

both valves of purity tester was closed immediately after closing the sampling valve

purity tester was disconnected from plastic tubing & sampling valve

↓

purity tester was placed in a sampling position & upper reservoir of tester was filled with 10% K or NaOH solution to level indicated on bulb

↓

the valve below reservoir containing NaOH solution was opened slowly until fully opened

↓

after flow was ceased to lower reservoir tester was gently agitated to assure the lower bulb is filled to 100% capacity

↓

the valve below the caustic reservoir was closed

↓

tester was carefully turned until 90^0

↓

% purity was taken on vertical calibration position of purity tester using bottom of Meniscus

3.3.4 Pressure gauge calibration

Certified weights Oil reservoir pressure gauge

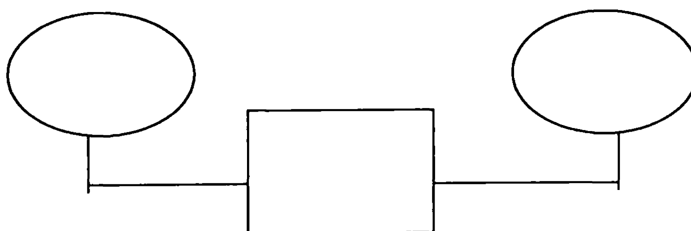


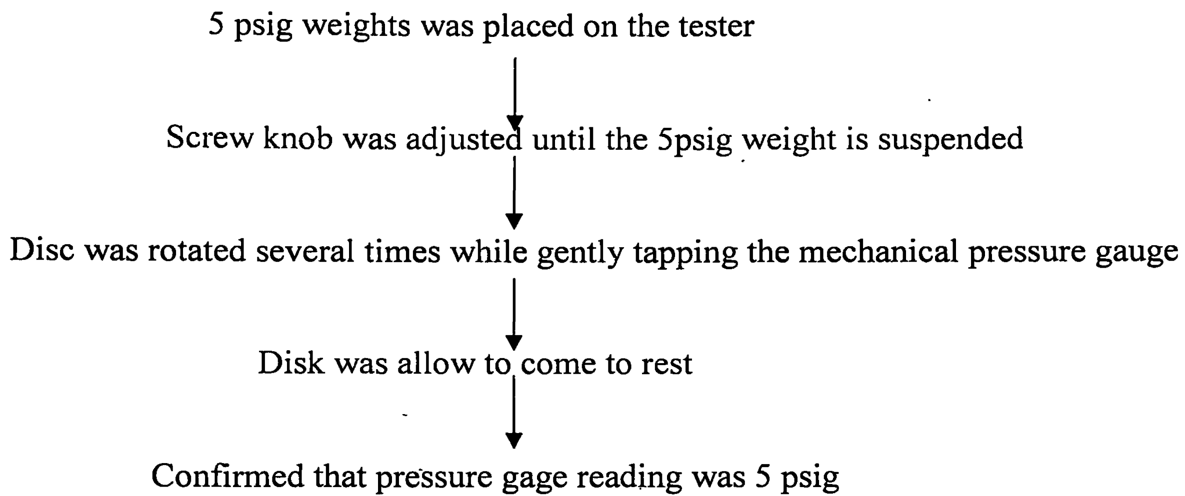
Figure 3.2 Dead weight tester

Reservoir was completely filled with oil

↓

Screw knob was turn in and out several times pressure gauge was tightly screwed into the gauge receiver.

↓



3.3.5 Carbonation measurements

calibrate the pressure gauge

↓

Calibrate the thermometer

↓

sample bottles was placed at refrigerator to adjust temperature to $40^0F \pm 3^0F$ ($4^0C \pm 2^0C$)

↓

Gently invert the samples 15 times in 30 seconds

↓

Bottle was placed under tester

↓

Snift valve was closed

↓

Thermo meter was kept above the liquid

↓

Head space pressure was released by carefully unscreaming the snift valve

↓

Pressure was allowed to drop rapidly to 2 psi

↓

Valve was closed quickly

↓

Thermometer was pushed in the liquid as far as it'll go

↓

bottle was placed on the shaking machine in a horizontal position & vigorously shaken in a horizontal motion until the pressure gauge reads constant maximum pressure

pressure and temperature were noted down

they were converted in to gas volume

snift valve was opened and vent until pressure is zero before removing the bottle from the tester

Sensory evaluation to find the best carbonation level

Table 3.7 Recipes for sensory evaluation (per 200 ml)

Sample code	**832**	**256**	**587**
Carrot juice (ml)	34	34	34
Sugar (g)	28	28	28
Citric (g)	0.3	0.3	0.3
SMS (g)	0.02	0.02	0.02
Nacl (g)	0.6	0.6	0.6
Chilled carbonated water	30	50	80
Chilled treated water(ml)	108	88	58
Final volume (ml)	200	200	200
Pressure (PSI)	5.5	7.0	7.0
Temperature (F^0)	56	57.5	45
CO_2 volume	1.48	1.56	1.90

30 untrained panelists in Ole Spring Bottlers (pvt) Ltd were provided with ballet of 9- point hedonic scale papers (Appendix IV)

data were analyzed by using Minitab 14 statistical package

3.3.7 Carbonation shelf life test

Place the samples in refrigerator before testing

Measure the carbonation volume as carbonation measurement test with time

3.3.8 Shelf life study

Shelf life study was carried out for 2 months time period for the best sample from the sensory analysis test stored under ambient temperature and refrigerator. Shelf life was evaluated according to result obtained from both chemical & microbiological tests

3.3.8.1 Chemical tests

3.3.8.1.1 Degassing study

Beverage was poured in to cup in degassing instrument

Degassed for 5 minutes by inserting air tube in to cup

Immediately after time has been passed check the titratable acidity

These steps were followed for 10, 15, 20 25 & 30 minutes intervals

Draw the graph titratable acidity Vs time

Select the time relevant to the minimum point in graph

3.3.8.1.2 Brix

3.3.8.1.2.1 Calibration of bench refractometer

3.3.8.1.2.1.1 Preparation of the sucrose standard (12 Brix)

clean dry 100 ml beaker was tared to 0.00g

12 ± 0.01 of dry sucrose was Weighed out in to beaker

Exactly 88.00 ± 0.01distilledwater was added to beaker

magnetic stirring bar was added and sealed the beaker with parafilm

mixture was mixed for 10 minutes until the mixture remain clear

3.3.8.1.2.1.2 Calibration

Prism was Cleaned with distilled water & dried

distilled water was applied

zero setting was adjusted

Prism was Cleaned with distilled water & dried

Prepared sucrose standard solution was placed on the prism

12.00 reading was taken

3.3.8.1.2.2 Brix reading

prism was Cleaned with distilled water & dried

degassed beverage sample was placed directly on the prism by using eye dropper

top was closed without trapping any air bubbles

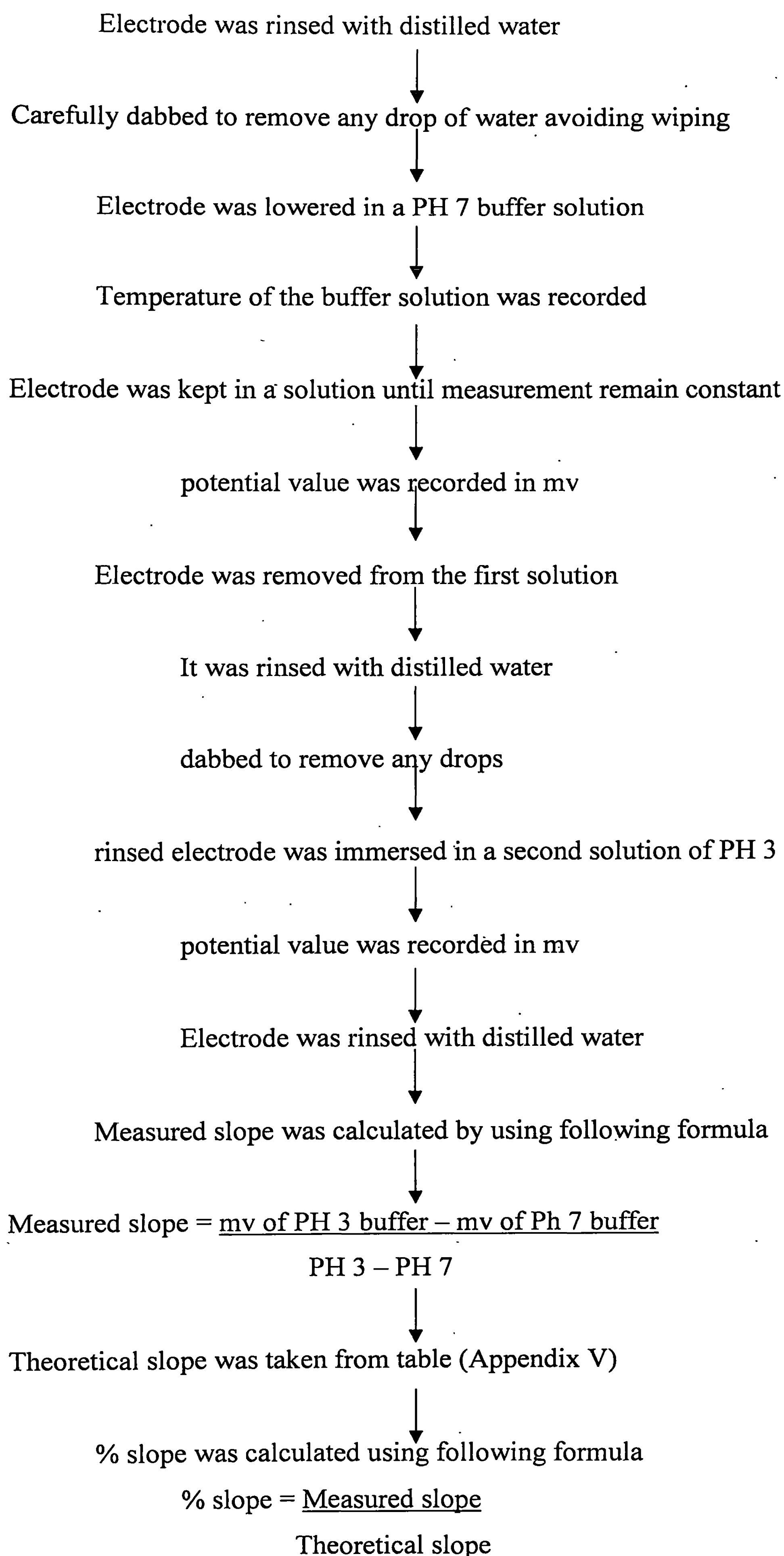
sample was kept 20 sec for temperature stabilization

Reading was taken

Prism was washed with distilled water & dried with lense tissues

3.3.8.1.3 PH

3.3.8.1.3.1Calibration



Electrode was rinsed with distilled water
Carefully dabbed to remove any drop of water avoiding wiping
Electrode was lowered in a PH 7 buffer solution
Temperature of the buffer solution was recorded
Electrode was kept in a solution until measurement remain constant
potential value was recorded in mv
Electrode was removed from the first solution
It was rinsed with distilled water
dabbed to remove any drops
rinsed electrode was immersed in a second solution of PH 3
potential value was recorded in mv
Electrode was rinsed with distilled water
Measured slope was calculated by using following formula
Measured slope = mv of PH 3 buffer – mv of Ph 7 buffer
PH 3 – PH 7
Theoretical slope was taken from table (Appendix V)
% slope was calculated using following formula
% slope = Measured slope
Theoretical slope

3.3.8.1.3.2 PH reading

100 ml of degassed beverage was placed in a cleaned and dried beaker

↓

cleaned and dried stirring bar was introduced carefully in to a beaker

↓

beaker was placed in on the cleaned and dried

↓

stirrer was turned on until the sample is gently agitated

↓

bottom one –third of the electrode was immersed in the sample

↓

After stabilizing the PH reading it was recorded

↓

Electrode was rinsed with distilled water & dried

↓

It was placed in the 3M KCl solution

3.3.8.1.4 Titratable acidity (Percent acid)

Approximate weight of beverage was chosen based on the approximate target percent acid (Appendix VI)

↓

Beaker was tare on the balance

↓

Appropriate weight of sample was added to the beaker

↓

Weight was recorded

↓

Distilled water was added up to 100 ml

↓

Self zeroing buret was rinsed twice with NAOH & then filled completely

↓

Beaker was placed in a stir plate & stir bar was added

↓

stir plate was turned on to was well mix the Sample

Beaker was placed over the beaker

PH electrode was placed in the solution

NaOH was added carefully to the sample until the PH is 8.3

Exact amount of PH used was recorded

Calculate the percent acid by using following formula

$$\% \text{ Citric Acid} = \frac{(\text{ml of NaOH}) * (0.06404) * N * 100}{\text{Sample weight in grams}}$$

N = Normality of the NaOH solution

3.3.8.2 Microbiological test

1 ml pipette was sterilized in an autoclave set at 121 ^{0}C for 20 minutes

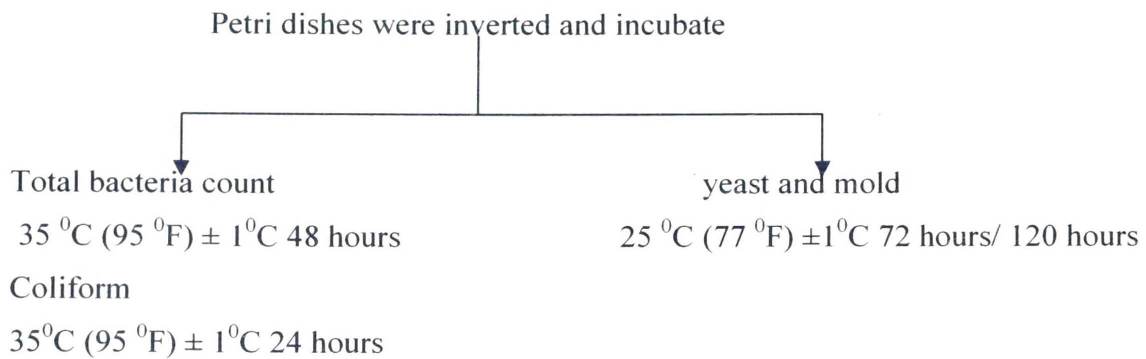
Media pad was placed in a petridish aseptically

Content in ampoules was dispensed aseptically on to media pad (micro media)

membrane filter sheet was placed grid side up on the pre saturated media pad using sterile forceps

Sample beverage bottle was sprayed with 70 % ethanol solution

Bottle was opened and 1ml of beverage was introduced on to the filter sheet

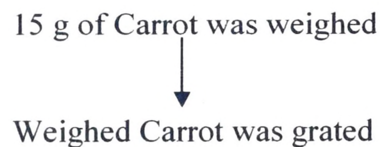


3.3.9 Identification & quantification of carotenoids in Carrot root & Carrot drink

Total carotenoids and beta carotene quantification was done in carrot root Carbonated Carrot drink samples were prepared by varying carbonation level. Effect of carbonation on beta carotene level was determined according to modified method of Rodriguez- Amaya 2001.

3.3.9.1 Sample preparation

3.3.9.1.1 Carrot root



3.3.9.1.2 Carbonated carrot drink

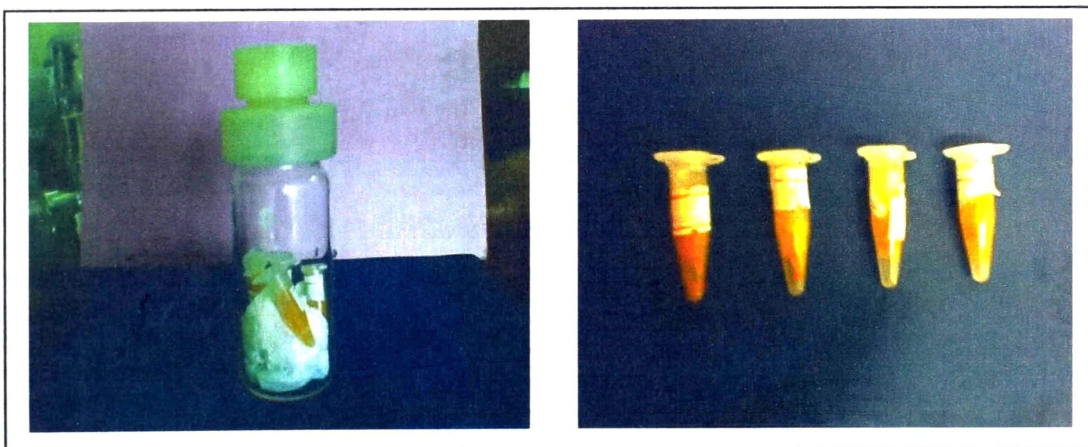


Figure 3.3 Freeze dried samples

1 ml from each five samples were placed in apendrof by using micro pipette

↓

Triplicates from each sample were produced

↓

All samples were freeze dried

↓

They were placed in a freezer until carotenoid analysis column

↓

Samples were mixed with methanol separately

↓

solvent & sample mixture was sonicated for five minutes (T < = 35°C)

↓

Solution was filtered through a Celite column mounted in a pasture pipette

↓

Filtrate was vacuum dried overnight

3.3.9.2 Extraction to acetone

3.3.9.2.1 Carrot root

Grated sample was ground using mortar and pestle with added celite (3g) & cold acetone (50 ml)

↓

Macerated mass was filtered through funnel with filter paper containing anhydrous sodium sulphate

↓

Residue in mortar & filter paper was macerated again with added acetone

Macerated mass was filtered again

↓

Above procedure was repeated until the residue color remain colorless

3.3.9.2.2 Carbonated carrot drink

Vacuum dried sample was dissolve in acetone (10 ml)

↓

sample was sonicated for five minutes

↓

2ml of methanol was added to sonicated solution

3.3.9.3 Partition to pet ether

3.3.9.3.1Carrot root

Pet ether was placed in a 500 ml separatory funnel (40 ml)

↓

Acetone extract was added in to a separatory funnel got from the previous step

↓

150 ml of distilled water was added until 2 layers were performed

↓

Pet ether phase was recovered

↓

Bulked ether extract was washed further with distilled water

↓

Washing with distilled water was repeated for 3-4 times

↓

Ethereal phase was collected in to 50 ml volumetric flask

↓

It was passed through a funnel with anhydrous sodium sulphate

↓

Funnel was washed with a small amount of pet ether, collecting the washing in to the volumetric flask

↓

Pet ether was added to make up to the volume

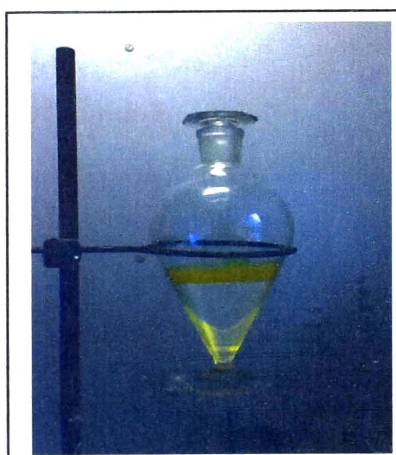


Figure 3.4 Partition of carotenoids to pet ether

3.3.9.3.2 Carbonated carrot drink

Pet ether was places in a 500 ml separatory funnel (20ml)

↓

Acetone methanol extract was added

↓

150 ml of distilled water was added until 2 layers were performed

↓

Ethereal phase was recovered

↓

Bulked ether extract was washed further with distilled water

↓

Washing with distilled water was repeated for 3-4 times

↓

Ethereal phase was collected in to 25ml volumetric flask

↓

It was passed through a funnel with anhydrous sodium sulphate

↓

Funnel was washed with a small amount of pet ether, collecting the washing in to the volumetric flask

↓

Pet ether was added to make up to the volume

3.3.9.4 Calculations (Total carotenoids)

3.3.9.4.1 Carrot root

10 ml from pet ether layer was taken

↓

Absorbance was taken by spectrophotometer at 450 nm wave length

↓

Total carotenoid content was calculated by using following formula

$$\text{Total carotenoids } (\mu g/g) = \frac{A_{total} * vol\,(ml) * 10^4}{A^{1\%}_{1cm} * \text{sample wheight (g)}}$$

A_{total} = Absorbance

Vol = Total volume of extract (50ml)

$A^{1\%}_{1cm}$ = Absorption co-efficient of 2500 which is recommended for mixtures

3.3.9.4.2 Carrot drink

5 ml from pet ether layer was taken

Absorbance was taken by spectrophotometer at 450 nm wave length

Total carotenoid content was calculated by using following formula

$$\text{Total carotenids } (\mu g/ml) = \frac{A_{total} * vol\,(ml) * 10^4}{A^{1\%}_{1cm} * \text{sample volume (ml)}}$$

A_{total} = Absorbance

Vol = Total volume of extract (25ml)

$A^{1\%}_{1cm}$ = Absorption co-efficient of 2500 which is recommended for mixtures

3.3.9.5 β carotene separation (Carrot root / carrot drink)

remaining carotenoid extract was concentrated in a rotary evaporator up to 1 ml

($T <= 35^0C$)

sample was kept in a refrigerator until run through column

3.3.9.5.1 Preparing the column adsorbent

Neutral Alumina (activity I)

6% water (w/w) was added

adsorbent and water was vigorously shaked in a closed container until no lumps are observed

↓

mixture was kept 12 hours to equilibrate

↓

Alumina (activity III)

↓

container was well closed until column packing

3.3.9.5.2 Column packing

Minicolumn was mounted in a Pasteur pipette with tip cut to 2 cm

↓

Small glass wool lug was placed at the bottom of the column

↓

1 g of neutral Alumina activity III was added

↓

better accommodation of the adsorbent in a column was ensured by tapping the side the column 3 or 4 times

↓

column was tapped with a 0.5 cm layer of anhydrous sodium sulphate

3.3.9.5.3 Developing the column

concentrated carotenoid solution was added in to the column by using a pipette

↓

rinsing of round bottom flask (1ml of PE) was added to the column after sample layer go down almost to the surface of the sodium sulphate layer

↓

Pet ether addition was continued until first band is collected

3.3.9.6 Identification of β carotene

3.3.9.6.1 Thin Layer Chromatography (TLC)

5% methanol in toluene solution was added to the TLC tank

↓

TLC tank was closed hermetically to saturate the chamber with the solvent system

β Carotene standard and sample was spotted on pre prepared TLC plate just 1cm above the base of the silica plate

Silica plate was placed in the TLC chamber and allowed to run (sample spot should be just above the level of the solvent system)

Co TLC was also run

TLC plate was taken out, air dried and observed for spot traveling path to confirm Whether the sample contains β carotene or not

Retardation factor values were measured by using following equation

$$R_f = \frac{\text{Distance travel by compound (x)}}{\text{Distance travel by mobile phase (y)}}$$

R_f = Distance travel by compound with respect to the mobile phase

If $R_f = 1$ low polar compound

$R_f < 1$ high polar compound

3.3.9.6 Calculation of beta carotene

Absorbance values were taken for each triplicate samples by using spectrophotometer at 450 nm

Beta carotene concentration was calculated by using following formula

$$X\,(\mu g/ml) = \frac{A * y(ml)\, 10^4 * 1.25}{A^{1\%}_{1cm} * \text{sample vol (ml)}}$$

A = Absorbance

Y= Eluted amount

$A^{1\%}_{1cm}$ = Absorption co-efficient of beta carotene in pet ether (2592) (Appendix VII)

Multiplying by 1.25 = account for the use of 20 ml aliquot taken from a total extract of 25 ml (From carrot drink)

Use of 40 ml aliquot taken from a total extract of 50ml (From carrot root)

3.3.9.8 Statistical analysis

Statistical analysis has been done by using Minitab 14 statistical package.Non parametric kruskal Wallis test was used to find whether there is any significant difference of beta carotene and total carotenoid level between carrot drink samples with carbonation level.

CHAPTER 04
RESULTS AND DISCUSSION

4.1 Determination of the best blanching conditions

The levels and relative distribution of deteriorative enzymes vary in different varieties of carrot. The activities of three deteriorative enzymes peroxidase, catechol oxidase and pectinesterase in juices prepared from whole carrot as well as superficial tissues, core, root tip and stem end result sedimentation or cloudy drink.. Catechol oxidase was found to be the least stable and pectinesterase the most stable in each of the varieties. In most cases, effective inactivation was achieved within 2 min at 85 °C. (M. V Harshul *et al,* 1999). Heating whole carrots to 93°C prior to milling and pressing improved juice color, but reduced juice yield compared to heating milled carrots to 93°C. (C.A Sims *et al,* 1993)

cloud can be classified as coarse and fine clouds. Fine particulate is shown to be the true stable cloud and to contain considerable protein, carbohydrate, and lipid components. Often, tannin is present as well. The fine cloud probably arises from cell membranes and appears not to be simply cell debris. Factors relating to the stability of fruit juice cloud, including particle sizes, size (Tom Beveridge, 2002)

In this study thermal inactivation of deteriorative enzymes assessed over a range of temperatures and raw carrot roots blanched at 90 ^{0}C for 5 minutes found to be the sediment free or cloud stable carbonated carrot drink. Same was observed by Harshul M.V ,William S A Kyle & Darryl M Small that pectin esterase required treatment at 90 °C to ensure rapid inactivation. It is concluded further that pectin esterase should be used as the indicator enzyme in the assessment of blanching sufficiency for processing of carrots. Blanching at 80 ^{0} C was not sufficient to develop cloud free carrot juice.

4.2 Reformulation of the recipe

According to preliminary sensory analysis following recipe was selected as the best recipe (per 100 ml)

Carrot juice	17 ml
Refined sugar	14 g
Citric acid	0.15 g
Sodium metabisulphite	0.01 g
Sodium chloride	0.3 g
Water	69 ml

4.3 Sensory evaluation to find the best carbonation level

4.3.1 Statistical analysis

Table 4.1 Sensory evaluation results to find the best carbonation level

Color Carbonation level/code number	Means	P value
1.56 Vol 256	7.567	0.150
1.90 Vol 587	7.200	
1.48 Vol 832	7.733	

Mouthfeel **Carbonation volum/Sample code**	**Mean**	**P-value**	**Carrot taste** **Carbonation volum/Sample code**	**Mean**	**P-value**	**Overall acceptability** **Carbonation volum/Sample code**	**Mean**	**P-value**
1.56Vol 256	**6.433**	**0.029**	**1.56Vol 256**	**6.967**	**0.070**	**1.56Vol 256**	**7.100**	**0.004**
1.90 Vol 587	**5.433**		**1.90 Vol 587**	**6.267**		**1.90 Vol 587**	**5.733**	
1.48 Vol 832	**6.033**		**1.48 Vol 832**	**6.933**		**1.48 Vol 832**	**6.333**	

Analysis of variances (ANOVA) results for RTS samples (256,587,832) reveals that p value for color(0.150) indicate there are sufficient evidence that all the means are equal when alpha is set at 0.05.This implies that there is no statistically significant different in color among three samples. (Appendix VIII)

Analysis of variances (ANOVA) results for RTS samples (256,587,832) reveals that p value for mouth feel (0.029) ,for carrot taste (0.070),for overall acceptability (0.004) indicate there are sufficient evidence that all the means are not equal when alpha is set at 0.05. This implies that there is a statistically significant different in above characteristicsl among three samples. Since sample coded 256 had the highest mean value it could be considered as the sample got the best CO_2 taste and the best with overall acceptability. (Appendix IX ,X , XI)

4.4 Carbonation shelf life test

Provision of adequate protection by the packaging material to maintain the carbonation above the specified minimum level over course of the distribution & use cycle is assured by carbonation shelf life test.

It has been established that a 15% loss of carbonation from initial levels can be discerned by the consumer. Once a carbonated beverage product loses too much carbonation (e.g. 17.5%), it is unacceptable to sell to a consumer It is an essential component of the package performance to minimize the loss of carbonation, throughout the shelf life of the product. (Quality manual PepsiCo international, 2004)

It 's practicing that the addition of carbonating agent to the carbonated beverage that undergoes slow hydrolysis in the acidic aqueous medium and releases the CO_2 to constantly replenish CO_2 that is lost through the walls or top of the container (e.g. plastic container), thereby extending the shelf life of the beverage. U.S. Pat. No. 3,441,417 to Feldman et al. discloses a dry beverage composition adapted to be reconstituted with water to form an effervescent beverage. An essential carbonating ingredient of the composition is an organic compound having a carbonic acid anhydride group capable of controlled hydrolysis in water to release CO_2 over a period of 30 seconds to 3 minutes.

In this study rapid CO_2 loss was observed in samples stored in sunlight than samples stored in air condition. (Appendix XII)

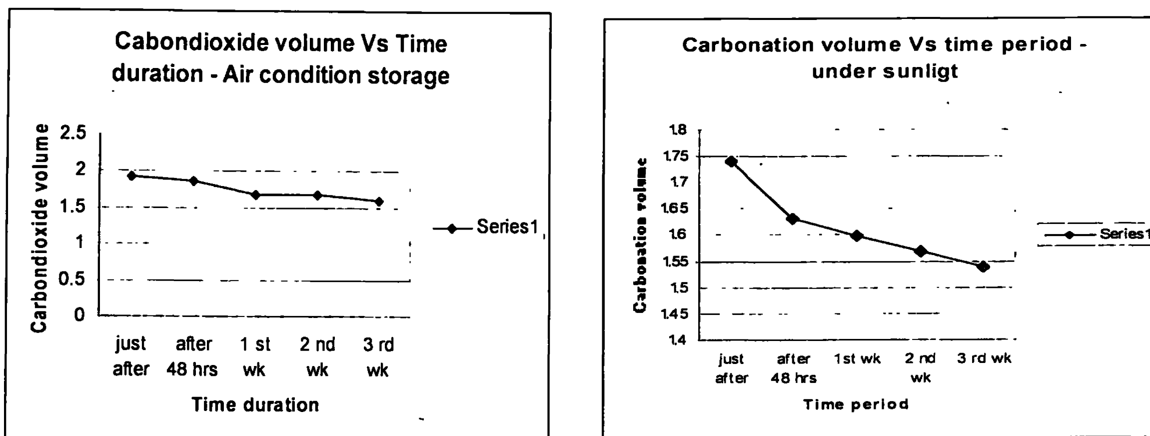


Figure 4.1 carbon dioxide volumes with time duration in carbonated carrot drink

4.5 Shelf life study of carbonated carrot drink

4.5.1 Chemical tests

4.5.1.1 Degassing study

Carbonation must be completely removed from finished beverage in order to obtain accurate pH and titratable acidity results. Any dissolved CO_2 remaining in the sample will convert to carbonic acid and will be titrated to falsely inflate the TA & lower the pH. Furthermore, high level of carbonation will interfere with the brix measurements.

The optimum degassing time for the TA test would be the time necessary to remove all of the CO_2 from the beverage. Time period shorter than this optimum time will have CO_2in the beverage resulting in high TA values. Time period longer than this optimum time could result in evaporation of some the water from the beverage result in high TA. (CO2 quality manual -Pepsi cola manual 2005).

According to degassing study 20 minutes was found to be the best time duration for degas the beverage. (Appendix XIII)

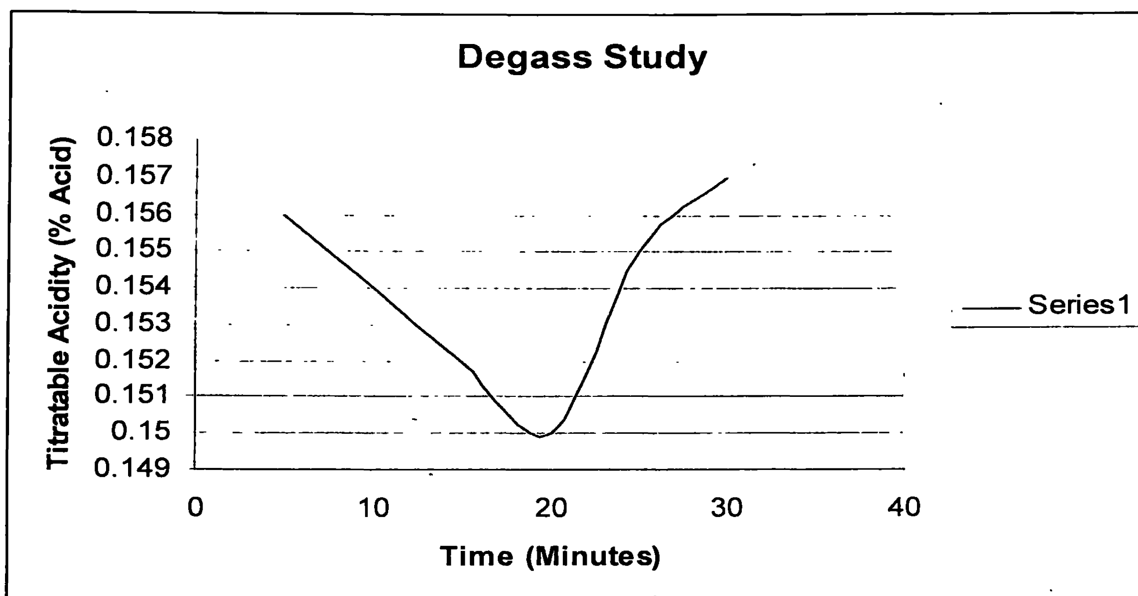


Figure 4.2 Degassing study of carbonated carrot drink

4.5.1.2 PH

4.5.1.2.1 PH calibration

1st buffer solution pH 7 +6 mV

2nd buffer solution pH 3 + 239 mV

$$\text{Measured slope} = \frac{239\ \text{mV} - 6\text{mV}}{\text{pH } 3 - \text{pH } 7} = -58.25$$

Theoretical slope at 20 ^{0}C = -58.17

$$\%\ \text{slope} = \frac{-58.25}{-58.17} = 100.1\%$$

Calibration is within the tolerance range

Physio – chemical analysis result reveals that there's no significant change in pH ,Brix and Titratable acidity throughout the 2 months storage time period both in ambient temperature and refrigerated temperature storage.This result is tally with the Saldana.G et.al. & Elena *et al.* Their Analytical determinations was clearly explained that Storage time had no effect on pH, Acid & Brix in carrot drink stored for 9 months at 20 ^{0}C & 10 month period respectively. pH , Brix and titratable acidity was within the range of 3.6 – 3.98, 16.00 – 16.93 & 0.148% to 0.158% respectively (Appendix XIV)

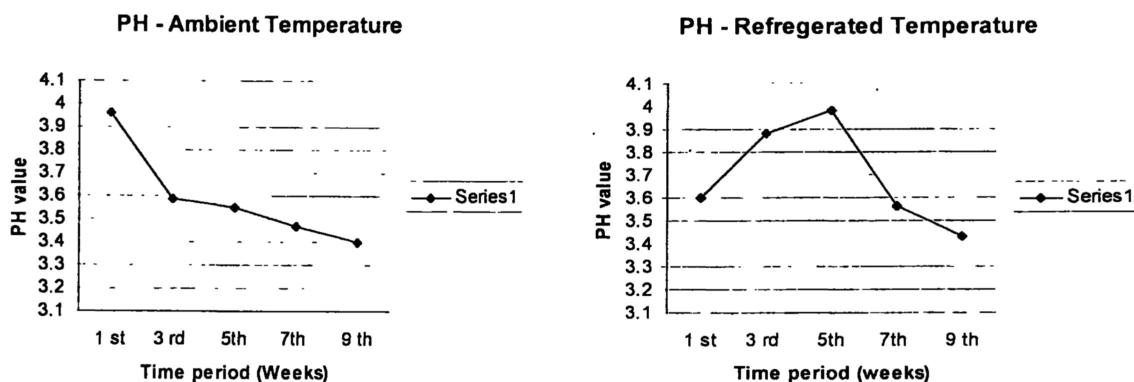


Figure 4.3 PH variations through the shelf life study of carbonated carrot drink

4.5.1.3 Brix

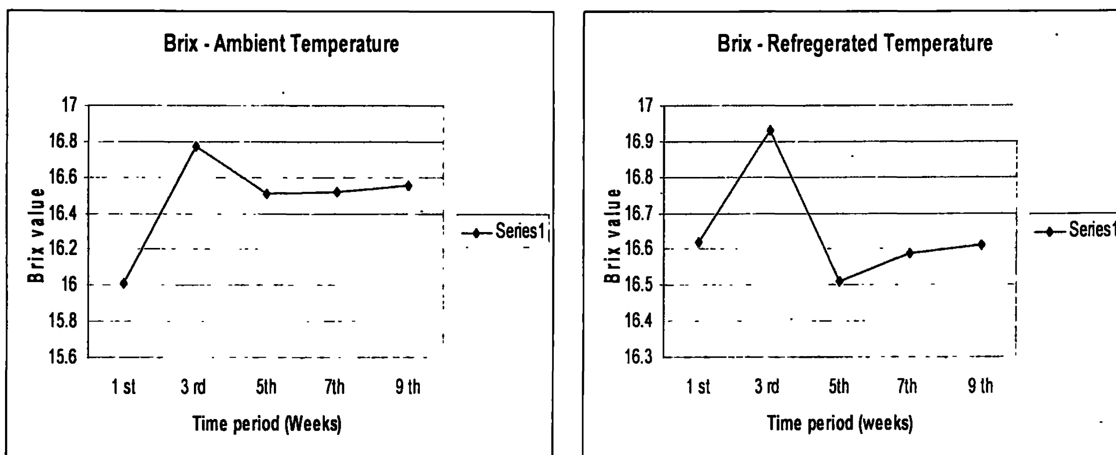


Figure 4.4 Brix variations throughout the shelf life study of carbonated carrot drink

4.5.1.3Titratable acidity

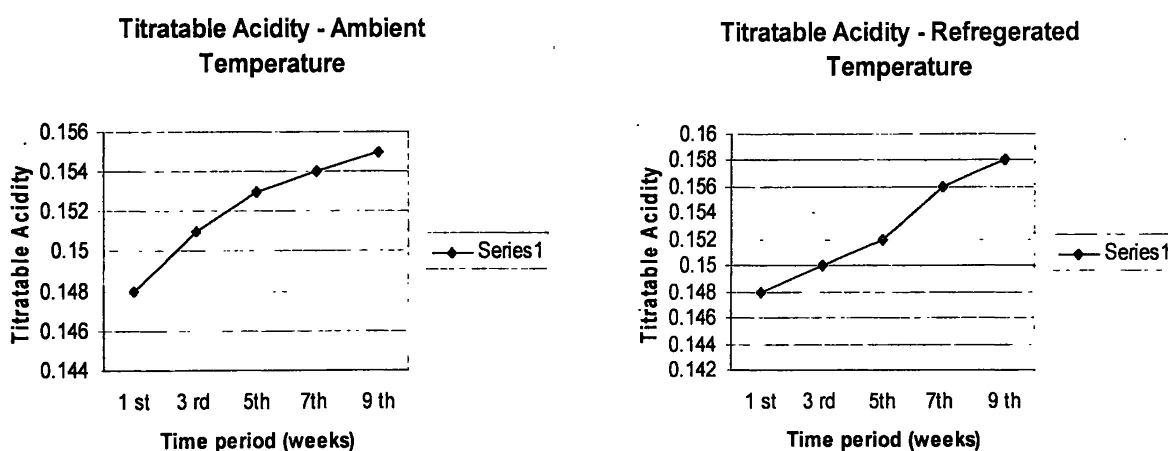


Figure 4.5 Titratable acidity variations throughout the shelf life study of carbonated carrot drink

Specification	value
Titratable acidity	0.150 %
PH	3.5
Brix	16.5 ^{0}

Table 4.2 Specifications of formulated carbonated carrot drink RTS drink

4.5.2 Microbiological test

Compared with storage under air, deaeration of carrot juice by helium or Nitrogen bubbling, followed by flush packaging had no effect on shelf life. Similar treatment with CO_2 ,giving a carbonated carrot drink, prolong the shelf life by up to 250 %.(Alklint C,2003)

In this study bacterial colony counts yeast & mold in both of carbonated carrot drinks stored at ambient temperature and refrigerated temperature had lower number per 1 ml of drink than the colony count should present in carbonated dinks in SLS standards. Also free from coliform was tally with the SLS standards.

Table 4.3 Microbiological colony counts – Ambient temperature storage

Time period (weeks)	Per 1ml of drink			
	Bacteria	**Yeast**	**Mold**	**Coliform**
1 st	Nil	Nil	Nil	Nil
3 rd	20	Nil	Nil	Nil
5 th	27	Nil	Nil	Nil
7 th	23	Nil	Nil	Nil
9 th	7	Nil	02	Nil

Table 4.4 microbiological colony counts – Refrigerated storage

Time period (weeks)	Per 1ml of drink			
	Bacteria	Yeast	Mold	Coliform
1 st	Nil	Nil	Nil	Nil
3 rd	06	Nil	Nil	Nil
5 th	08	Nil	Nil	Nil
7 th	04	02	Nil	Nil
9 th	05	Nil	Nil	Nil

Compared with storage under air ,deaeration of carrot juice by helium or Nitrogen bubbling ,followed by flush packaging had no effect on shelf life. Similar treatment with CO_2, giving a carbonated carrot drink, prolong the shelf life by up to 250 %.(Alklint C, 2003)

In this study bacterial colony counts yeast & mold in both of carbonated carrot drinks stored at ambient temperature and refrigerated temperature had lower number per 1 ml of drink than the colony count should present in carbonated dinks in SLS standards. Also free from coliform was tally with the SLS standards.

4.6 Total carotenoids

Carrots are known as one of the best source of α and β carotene, carrots are therefore high in provitamin A (Britton G, 1992). Other than α and β carotene carrots also contains ζ carotene and lutein (Khachik.F et al 1991, Rodriguez Amaya 2002).

4.6.1 TLC for Total carotenods

TLC plate spotted with standard beta-carotene & carrot drink samples shown below reveals that the presence of another compounds other than the beta-carotene

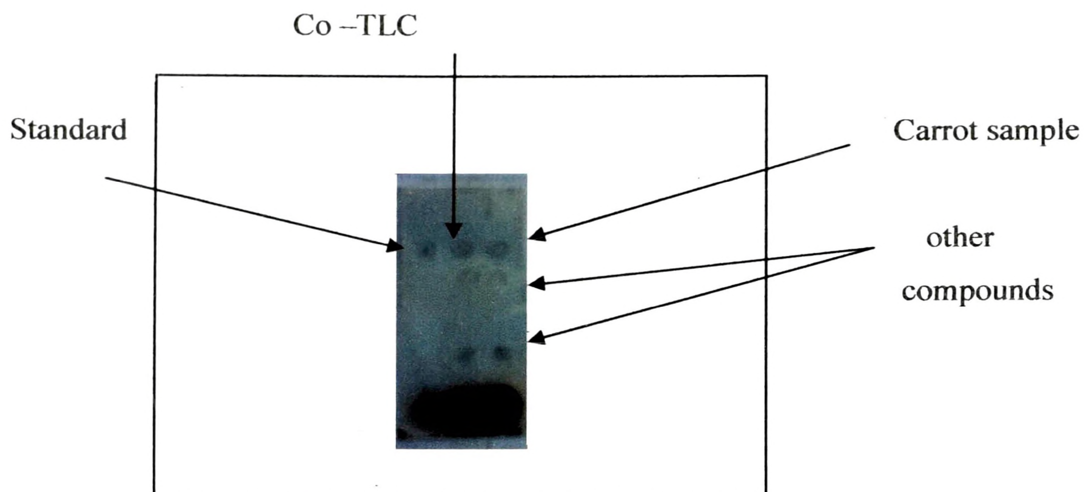


Figure 4.6 TLC plate spotted with standard beta-carotene & carrot drink samples

4.7 Identification of β carotene (Carrot root / carrot drink)

4.7.1 TLC for Beta carotene.

TLC plate spotted with standard beta carotene and eluent from Alumina column shown below illustrates the presence of beta carotene in carrot drink samples

$$R_f \text{ value} = \frac{3.6 \text{ cm}}{3.6 \text{ cm}} = 1$$

R_f value equal to 1 reveals that the compound is low in polarity.

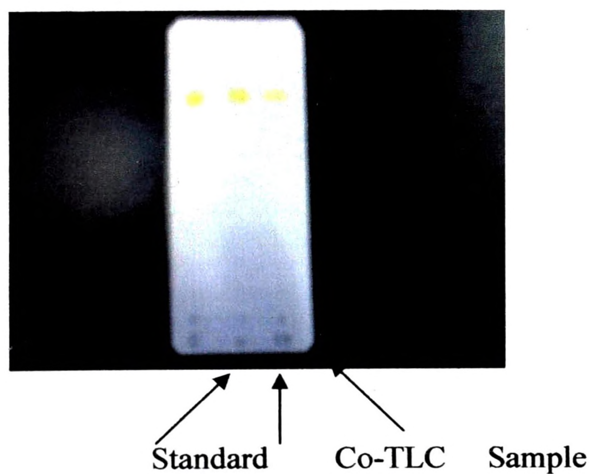


Figure 4.7 TLC plate spotted with standard beta carotene and eluants from Alumina Column

4.8 Quantitative analysis of carotenoids

4.8.1 Carrot root (calculation of concentrations)

Total carotenoids

$$\text{Total carotenids } (\mu g/g) = \frac{A_{total} * \text{vol (ml)} * 10^4}{A^{1\%}_{1cm} * \text{sample weight (g)}}$$

β carotene

$$\beta \text{ carotene } (\mu g/g) = \frac{A * \text{y(ml)}\ 10^4 * 1.25}{A^{1\%}_{1cm} * \text{sample weight (g)}}$$

The carotenoids composition of foods are affected by factors such as cultivar or variety, part of the plant consumed, stage of maturity, climate or geomatic site of production, harvesting, post harvest handling, processing and storage (Rodriguez-Amaya, 2001) Cultivar or varietial differences can be only in terms of the quantitative composition., because essentially the same carotenids are found in the different varieties. (Appendix XV).

The main carotenoids in raw carrots of the "Orange" variety are α and β carotene, ranking from 80 to90% of total carotenoids (Maria H et al,1998). 41.95% beta carotene was presence in raw carrot. This result is tally with Ramos 1991 observed that the main carotenoids in raw carrots of the Nantes variety are beta carotene (51.3%), alpha carotene (29.5%) and ζ carotene (5.1%).

Table 4.5 Total carotenoids content in carrot root

Sample No	weight of sample	Absorbance at 450nm	Concentration (μg/g)	Concentration ± SD (μg/g)
1	15.01	0.810	53.96	
2	15.12	0.800	52.91	53.643 ± 0.637
3	15.02	0.812	54.06	

Table 4.6 Beta carotene content in carrot root

Sample No	weight of sample	Absorbance at 450nm	Concentration (µg/g)	Concentration ± SD (µg/g)
1	15.01	1.403	22.54	
2	15.12	1.400	22.33	22.507 ± 0.163
3	15.0	1.411	22.65	

4.8.2 Carrot drink (Appendix XVI)

Total carotenoids

$$\text{Total carotenids } (\mu g/g) = \frac{A_{total} * \text{vol (ml)} * 10^4}{A^{1\%}_{1cm} * \text{sample vol(ml)}}$$

β carotene

$$X\ (\mu g/ml) = \frac{A * y(ml)\ 10^4 * 1.25}{A^{1\%}_{1cm} * \text{sample vol (ml)}}$$

Processing enhance the susceptibility of carotenoids to isomerization and oxidation. Loss of color and biologic activity and the formation of volatile compounds that imparts desirable or undesirable flavor in some foods are the practical consequences. The occurrence of oxidation depend on the severity of treatment like destruction of the ultra structure that protects the carotenoids, increase of surface area and duration and temperature of the heat treatment, packaging material and storage conditions. (Rodriguez-Amaya , 1997).

Carotenoids are present in nature in the trans configuration, which is more stable. However, the cis- isomers may occur and increase during industrial processing and cooking method.(Maria et al, 1998).

4.9 Statistical Analysis

Very few reports dealt with the effects of various processing methods on color and carotenoid stability in carrot juice. According to the statistical analysis for beta carotene by using kruskal Wallis test the test statistic (H) had a p-value of 0.598, (adjusted for ties), ($p > 0.05$) indicating that beta carotene content was not significantly affected by carbonation level. (Appendix XVII)

According to the statistical analysis for Total carotenoids by using kruskal Wallis test the test statistic (H) had a p-value of 0.394 (adjusted for ties), ($p > 0.05$) indicating that total carotenoid content was not also significantly affected by carbonation level. (Appendix XVIII)

Table 4.7 Variation of total carotenoids and beta carotene content in carrot drink samples with carbonation level

Carbonation volume	Concentration (μg / 100ml) ± SD	
	β carotene	Total carotenoids
0	266.3 ± 26.1	369.33 ± 9.45
1.48	236.0 ±19.1	364.67 ± 9.87
1.56	290.7 ± 52.1	372.00 ± 15.10
1.86	263.3 ± 43.6	360.67 ± 6.11
2.09	277.7 ± 58.1	374.70 ± 23.40

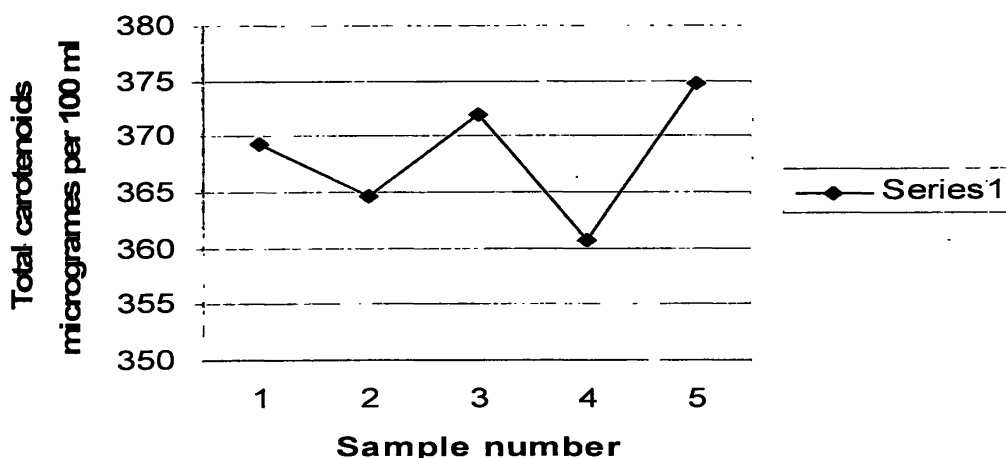


Figure 4.8variation of total carotenoid content in carrot drink samples with carbonation level

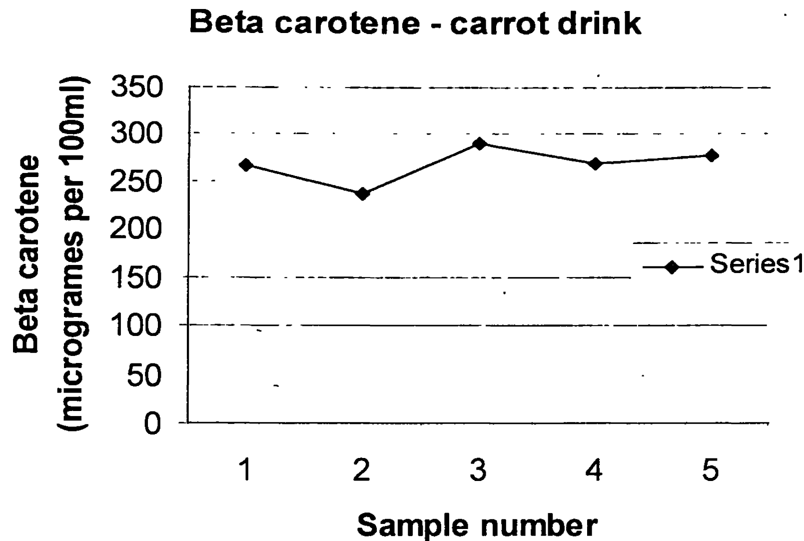


Figure 4.9 variation of beta carotene content in carrot drink samples with Carbonation level

Color is a very important quality attribute of foods .It was also observed that the color of four different carbonated carrot drink samples were not significantly different comparing to non-carbonated carrot drink sample. This result is apparently tally with the Munch and Simard, 1983, demonstrated that the color change of carrot juice during processing correlated well to carotenoid content.

As carrot are low acid foods (PH 5.5-6.5) sterilization of carrot juice under high temperature is often required.(Chen B.H et al, 1995)To minimize loss of color and carotenoid ,the raw carrot juice is often acidified with before processing. So the sterilization temperature can be lowered. The vitamin A content decreased along with increasing temperature and heating time. In this study carrot drink was not subjected to any heat treatment because it may interfere to determination of the effect of carbonation on beta carotene level. PH of developed carbonated carrot drink samples lays in-between 3-4 .So it is enough to ensure the microbiological safety of the drink. Other than that used water has been well treated and tested for free of coliforms.

Acidification plus 105 ^{0}C heating for 25 seconds and acidification showed the highest destruction of carotenoids in carrot juice.(B.H Chen *et al* ,1995)In general beta carotene content decreased whereas acidity and ascorbic acid content increased.(Bajaj

K.L and Mahajan R ,2005)Although introduced co2 in to carrot drink donates bicarbonate ions .it was not significantly affect the acidity of the carrot drink .It may mainly due to maintaining the carbonation level as minimum(1 – 2 Vol)specially in fruit and vegetable juices.

CHAPTER 05
CONCLUSION

5.1 Conclusions

A carbonated carrot drink was formulated & developed with the specifications: Carrot juice 34 ml, Sugar28 g, Citric acid 0.3 g, SMS 0.02 g, NaCl 0.6 g.

Chemical analysis reveals that the best sample has PH 3.5, Brix 16.5 ^{0} ,titratable acidity 0.15% & CO_2 volume 1.56. Throughout the two months storage time Significant change in physio chemical properties was not observed in samples stored under ambient & refrigerated temperatures.

According to Microbial analysis, the drink was free from total bacterial count, yeast, and mold & coliform. Microbial analysis reveals that the Bacterial colony count 7, mold 2, & yeast and coliform remain 7 , 2 ,0 and 0 respectively in samples stored in ambient temperature.The samples stored in refrigerated temperature bears Bacterial colony count , mold , yeast and coliform 5, 0 ,0 and 0 per 1ml respectively. Coliform count was remaining 0 throughout the 2 months samples stored under ambient & refrigerated temperatures.

Beta carotene amount in non-carbonated & carbonated carrot drinks at the four different carbonation levels 0 ,1.48 ,1.56 ,1.86, and 2.09 were 266.3 ± 26.1, 236.0 ±19, 290.7 ± 52.1, 263.3 ± 43.61 , 277.7 ± 58 (μg / 100ml) respectively. It has been statistically proved that carbonation has no significant effect on beta carotene level in carrot drink ($p > 0.05$).Total carotenoids in non carbonated & carbonated carrot drinks at the four different carbonation levels were 369.33 ± 9.45, 364.67 ± 9.87, 372.00 ± 15.10, 360.67 ± 6.11 & 374.70 ± 23.40 (μg /100ml.) and there was no significant difference among those samples($p > 0.05$).Best sample selected from sensory analysis contained 290.7 ± 52.1 μg/100ml of beta carotene & 372.00 ± 15.10 μg/100ml of total carotenoids.Raw carrot root contains 22.5531 μg / g of beta carotene & 54 μg / g of total carotenoids

5.1.2 Further studies

Determination of the bioavailability of beta carotene by doing invitro analysis

Determination of the effect of carbonation on beta carotene level with storage.

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Appendix I

Nutritional composition in major root crops

Appendix II

CO_2 content of commercial carbonated beverages

Name of product	Pressure	Temperature	Volume of CO_2 in 1 volume
	(Psi)	(F^0)	
Coca Cola	26 –30	45	3.6 - 4.0
Carbonated waters	28	40	4.2
Canada dry ginger ale	30	50	3.6
Sevem up	32	50	3.7
Crown cola	34	50	3.9
Pepsi cola	34	50	3.9

Appendix III

Gas volume chart

°F, in bottle	Gage pressures in bottle, pounds per sq. in																								
	16[a]	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64
45	2.7	2.9	3.1	3.3	3.4	3.6	3.8	4.0	4.1	4.3	4.5	4.7	4.8	5.0	5.2	5.4	5.6	5.7	5.9	6.1	6.2	6.4	6.6	6.8	6.9
46	2.7	2.8	3.0	3.2	3.4	3.5	3.7	3.9	4.0	4.2	4.4	4.6	4.7	4.9	5.1	5.3	5.4	5.6	5.8	6.0	6.1	6.3	6.4	6.6	6.8
47	2.6	2.8	2.9	3.1	3.3	3.5	3.6	3.8	4.0	4.1	4.3	4.5	4.6	4.8	5.0	5.2	5.3	5.5	5.7	5.9	6.0	6.2	6.3	6.5	6.7
48	2.6	2.7	2.9	3.1	3.2	3.4	3.6	3.7	3.9	4.1	4.2	4.4	4.6	4.7	4.9	5.1	5.2	5.4	5.6	5.7	5.9	6.1	6.2	6.4	6.6
49	2.5	2.7	2.8	3.0	3.2	3.3	3.5	3.7	3.8	4.0	4.1	4.3	4.5	4.6	4.8	5.0	5.1	5.3	5.5	5.6	5.8	6.0	6.1	6.3	6.4
50	2.5	2.6	2.8	2.9	3.1	3.3	3.4	3.6	3.7	3.9	4.0	4.2	4.4	4.5	4.7	4.9	5.0	5.2	5.4	5.5	5.7	5.9	6.0	6.2	6.3
51	2.4	2.6	2.7	2.9	3.1	3.2	3.4	3.5	3.7	3.8	4.0	4.2	4.3	4.5	4.6	4.8	5.0	5.1	5.3	5.4	5.6	5.7	5.9	6.1	6.2
52	2.4	2.5	2.7	2.8	3.0	3.2	3.3	3.5	3.6	3.8	3.9	4.1	4.2	4.4	4.5	4.7	4.9	5.0	5.2	5.3	5.5	5.6	5.8	5.9	6.1
53	2.3	2.5	2.6	2.8	2.9	3.1	3.3	3.4	3.6	3.7	3.8	4.0	4.2	4.3	4.4	4.6	4.8	4.9	5.1	5.2	5.4	5.5	5.7	5.9	6.0
54	2.3	2.4	2.6	2.7	2.9	3.0	3.2	3.3	3.5	3.6	3.8	3.9	4.1	4.2	4.4	4.5	4.7	4.8	5.0	5.2	5.3	5.4	5.6	5.7	5.9
55	2.3	2.4	2.6	2.7	2.8	3.0	3.1	3.3	3.4	3.6	3.7	3.9	4.0	4.1	4.3	4.4	4.6	4.7	4.9	5.1	5.2	5.3	5.5	5.6	5.8
56	2.2	2.4	2.5	2.6	2.8	2.9	3.1	3.2	3.4	3.5	3.7	3.8	3.9	4.1	4.2	4.4	4.5	4.7	4.8	5.0	5.1	5.2	5.4	5.5	5.7
57	2.2	2.3	2.5	2.6	2.7	2.9	3.0	3.2	3.3	3.5	3.6	3.7	3.9	4.0	4.1	4.3	4.4	4.6	4.7	4.9	5.0	5.2	5.3	5.4	5.6
58	2.1	2.3	2.4	2.6	2.7	2.8	3.0	3.1	3.3	3.4	3.5	3.7	3.8	3.9	4.1	4.2	4.4	4.5	4.6	4.7	4.9	5.1	5.2	5.3	5.5
59	2.1	2.2	2.4	2.5	2.7	2.8	2.9	3.1	3.2	3.3	3.5	3.6	3.7	3.9	4.0	4.2	4.3	4.4	4.6	4.7	4.8	5.0	5.1	5.3	5.4
60	2.1	2.2	2.3	2.5	2.6	2.7	2.9	3.0	3.1	3.3	3.4	3.5	3.7	3.8	3.9	4.1	4.2	4.3	4.5	4.6	4.7	4.9	5.0	5.2	5.3
61	2.0	2.2	2.3	2.4	2.6	2.7	2.8	3.0	3.1	3.2	3.3	3.5	3.6	3.7	3.9	4.0	4.1	4.3	4.4	4.5	4.7	4.8	4.9	5.1	5.2
62	2.0	2.1	2.3	2.4	2.5	2.6	2.8	2.9	3.0	3.2	3.3	3.4	3.6	3.7	3.8	4.0	4.1	4.2	4.3	4.4	4.6	4.7	4.8	5.0	5.1
63	2.0	2.1	2.2	2.4	2.5	2.6	2.7	2.9	3.0	3.1	3.2	3.4	3.5	3.6	3.8	3.9	4.0	4.2	4.3	4.4	4.5	4.6	4.8	4.9	5.0
64	1.9	2.1	2.2	2.3	2.4	2.6	2.7	2.8	2.9	3.1	3.2	3.3	3.5	3.6	3.7	3.8	3.9	4.1	4.2	4.3	4.4	4.6	4.7	4.8	4.9
65	1.9	2.0	2.2	2.3	2.4	2.5	2.6	2.8	2.9	3.0	3.1	3.3	3.4	3.5	3.6	3.8	3.9	4.0	4.1	4.2	4.4	4.5	4.6	4.7	4.8
66	1.9	2.0	2.1	2.2	2.4	2.5	2.6	2.7	2.8	3.0	3.1	3.2	3.3	3.5	3.6	3.7	3.8	3.9	4.1	4.2	4.3	4.4	4.5	4.7	4.8
67	1.8	2.0	2.1	2.2	2.3	2.4	2.6	2.7	2.8	2.9	3.0	3.2	3.3	3.4	3.5	3.6	3.7	3.8	4.0	4.1	4.2	4.3	4.4	4.6	4.7
68	1.8	1.9	2.0	2.2	2.3	2.4	2.5	2.6	2.7	2.9	3.0	3.1	3.2	3.3	3.5	3.6	3.7	3.8	3.9	4.0	4.2	4.3	4.4	4.5	4.6
69	1.8	1.9	2.0	2.1	2.2	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.2	3.3	3.4	3.5	3.6	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5
70	1.7	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.5
71	1.7	1.8	1.9	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.9	4.0	4.1	4.2	4.3	4.4
72	1.7	1.8	1.9	2.0	2.1	2.2	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3
73	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2
74	1.6	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2
75	1.6	1.7	1.8	1.9	2.0	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1
76	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.4	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1
77	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0
78	1.5	1.6	1.7	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0
79	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9

Appendix IV

Preference test for carbonated carrot drink samples

Name: ....   Date ....

Instructions:

1. You have been given three samples of carbonated carrot drink in random order.
2. Please assess the characters of the samples given below in the given order using the following scale.
3. At the beginning and in between samples wash your mouth with water.
4. Don't compare the samples.

Character	Code number		
	256	587	832
Color			
Mouth feeling			
Carrot Taste			
Overall Acceptability			
Sourness			

9- Like extremely

8- Like very much

7- Like moderately

6- Like slightly

5- Neither like nor dislike

4- Dislike slightly

3- Dislike moderately

2- Dislike very much

1- Dislike extremely

Comments: ....

....

....

THANK YOU!

Appendix V

Theoretical slope values for PH

Temperature		Nernst slope
^0C	^0F	Dmv / DPH
10	50.0	-56.18
15	59.0	-57.16
18	64.4	-57.77
19	66.2	-57.97
20	68.0	-58.17
21	69.8	-58.37
22	71.6	-58.57
25	77.0	-59.16
30	86.0	- 60.15

Appendix VI

Percent acid chart

Approximate target percent citric acid	Approximate target percent Malic acid	Approximate weight (g) to be added to the beaker
25	25	0.3
20	20	0.4
15	15	0.5
10	10	0.8
7.5	7.5	1
4	4	2
1.5	1.5	5
1.0	1.0	10
0.6	0.5	20
0.2	0.2	50

Appendix VII

Absorption coefficients ($A^{1\%}_{1cm}$) of common food carotenoids.

carotenoid	Solvent	λ max, nm	$A^{1\%}_{1cm}$
Antheraxanthin	Ethanol	446	2350
Astaxanthin	Hexane	470	2100
Auroxanthin	Ethanol	400	1850
Bixin	Petroleum ether	456	4200
Canthaxanthin	Petroleum ether	466	2200
Capsanthin	Benzene	483	2072
Capsorubin	Benzene	489	2200
α-carotene	Petroleum ether Hexane	444 445	2800 2710
β-carotene	**Petroleum ether** **Ethanol** **Choloroform**	**450** **450** **465**	**2592** **2620** **2396**
β-carotene-5,6-epoxide	Hexane	444	2590
β-carotene-5,6,5`6`-di epoxide	Hexane	440	2690
δ-carotene	Petroleum ether	456	3290

γ- carotene	Petroleum ether	462	3100
	Hexane	462	2760
ζ- carotene	Hexane	400	2555
Crocetin	Petroleum ether	422	4320
α-cryptoxanthin/zeinoxanthin	Hexane	445	2636
β -cryptoxanthin	Petroleum ether	449	2386
	Hexane	450	2460
Echinenone	Petroleum ether	458	2158
Lutein	Ethanol	445	2550
	Diethyl ether	445	2480
	Diethyl ether	445	2600
Litein-5,6-epoxide	Ethanol	441	2400
	Ethanol	441	2800
Lycopene	Petroleum ether	470	3450
Lycoxanthin	Acetone	474	3080
Mutatochrome	Diethyl ether	428	2260
Neoxanthin	Ethanol	438	2470
	Ethanol	439	2243
Neurosporene	Hexane	440	2918
Phytoene	Petroleum ether	286	1250
Phytofluene	Petroleum ether	348	1350
	Hexane	348	1577

Appendix VIII

Normality test (sample codes 256 ,587, 832)

H_0: data set is normal H_1: data set is not normal

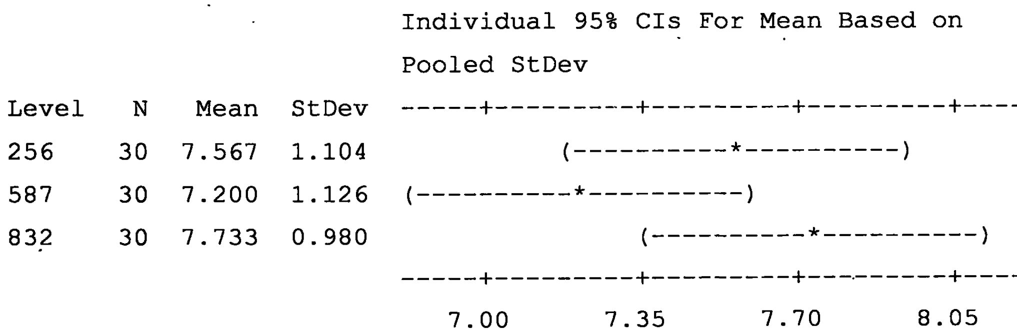
Interpretation

The p value 0.150 provide by performed Kolmogorov Smirnov test for each four samples (**256 ,587, 832**) separately provide enough evidence to that data are normal when alpha is set at 0.05. Hence it can conclude that all the samples are drawn from normal populations.

One-way ANOVA: Color versus Sample

Source	DF	SS	MS	F	P
Sample code	2	4.47	2.23	1.94	0.150
Error	87	100.03	1.15		
Total	89	104.50			

S = 1.072 R-Sq = 4.27% R-Sq(adj) = 2.07%



```
                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level   N   Mean  StDev  -----+---------+---------+---------+----
256    30  7.567  1.104            (----------*----------)
587    30  7.200  1.126  (----------*----------)
832    30  7.733  0.980                 (----------*----------)
                         -----+---------+---------+---------+----
                            7.00      7.35      7.70      8.05
```

Pooled StDev = 1.072

Interpretation

In the ANOVA table, the p-value (0.150) for samples indicates that there is sufficient evidence that all the means are equal when alpha is set at 0.05..So can conclude that there's no much significant difference among colour of the samples.

Appendix IX

Normality test (sample codes 256 ,587, 832)

H_0: data set is normal H_1: data set is not normal

Interpretation

The p value 0.150 provide by performed Kolmogorov Smirnov test for each four samples (**256 ,587, 832**) separately provide enough evidence to that data are normal when alpha is set at 0.05. Hence it can conclude that all the sample are drawn from normal populations .

One-way ANOVA: Mouth feel versus Sample

```
Source       DF      SS    MS     F      P
sample code   2   15.20  7.60  3.68  0.029
Error        87  179.70  2.07
Total        89  194.90

S = 1.437   R-Sq = 7.80%   R-Sq(adj) = 5.68%

                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level   N   Mean  StDev  --------+---------+---------+---------+-
256    30  6.433  1.165                   (-------*--------)
587    30  5.433  1.654  (--------*-------)
832    30  6.033  1.450           (--------*-------)
                         --------+---------+---------+---------+-
                              5.40      6.00      6.60      7.20

Pooled StDev = 1.437

Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 1.94

Intervals for level mean minus largest of other level means
Level   Lower  Center  Upper  --+---------+---------+---------+-------
256    -0.320   0.400  1.120                    (--------*--------)
587    -1.720  -1.000  0.000  (---------*-----------)
832    -1.120  -0.400  0.320          (--------*--------)
                              --+---------+---------+---------+-------
                              -1.60     -0.80     -0.00      0.80
```

Interpretation

In the ANOVA table, the p-value (0.029) for samples indicates that there is sufficient evidence that not all the means are equal when alpha is set at 0.05.

Hsu's MCB (Multiple Comparisons with the Best) compares each mean with the best (largest) of the other means. Minitab compares the sample means of 587,& 832 to the sample code 256 mean because it is the largest No evidence exists that sample 587 is the best because the upper interval endpoints are 0, the smallest possible value. So it can be concluded that the sample code 256 is the sample with the best mouth feel

Appendix X

H_0: data set is normal H_1: data set is not normal

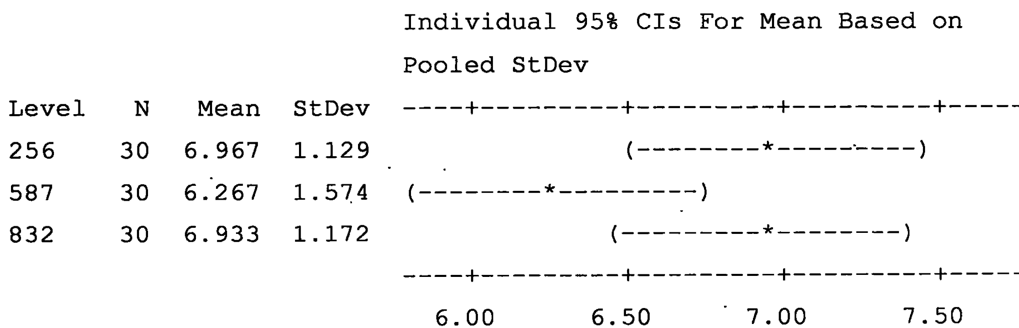
Interpretation

The p value 0.150 provide by performed Kolmogorov Smirnov test for each four samples (**256 ,587, 832**) separately provide enough evidence to that data are normal when alpha is set at 0.05. Hence it can conclude that all the samples are drawn from normal populations.

One-way ANOVA: Carrot Taste versus sample

Source	DF	SS	MS	F	P
sample code	2	9.36	4.68	2.74	0.070
Error	87	148.70	1.71		
Total	89	158.06			

```
S = 1.307   R-Sq = 5.92%   R-Sq(adj) = 3.76%
```



```
                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level   N   Mean   StDev   ----+---------+---------+---------+-----
256    30  6.967   1.129               (--------*---------)
587    30  6.267   1.574   (--------*---------)
832    30  6.933   1.172              (---------*--------)
                           ----+---------+---------+---------+-----
                             6.00      6.50      7.00      7.50
```

```
Pooled StDev = 1.307
```

Interpretation

In the ANOVA table, the p-value (0.070) for samples indicates that there is sufficient evidence that all the means are equal when alpha is set at 0.05..So can conclude that there's no much significant difference among colour of the samples.

Appendix XI

Normality test (sample codes 256 ,587, 832)

H_0: data set is normal H_1: data set is not normal

Interpretation

The p value 0.150 provide by performed Kolmogorov Smirnov test for each four samples (**256 ,587, 832**) separately provide enough evidence to that data are normal when alpha is set at 0.05. Hence it can conclude that all the samples are drawn from normal populations.

One-way ANOVA: Overall Acceptability VS Sample

```
Source       DF      SS     MS     F      P
Sample code   2   28.16  14.08  6.03  0.004
Error        87  203.23   2.34
Total        89  231.39

S = 1.528   R-Sq = 12.17%   R-Sq(adj) = 10.15%
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level   N   Mean  StDev  ------+---------+---------+---------+---
256    30  7.100  1.094                      (------*-------)
587    30  5.733  1.837  (-------*-------)
832    30  6.333  1.561           (------*-------)
                         ------+---------+---------+---------+---
                            5.60      6.30      7.00      7.70

Pooled StDev = 1.528

Hsu's MCB (Multiple Comparisons with the Best)
Family error rate = 0.05
Critical value = 1.94
Intervals for level mean minus largest of other level means

Level   Lower  Center  Upper   -+---------+---------+---------+--------
256     0.000   0.767  1.533                         (-------*------)
587    -2.133  -1.367  0.000   (------*-------------)
832    -1.533  -0.767  0.000         (------*-------)
                               -+---------+---------+---------+--------
                              -2.0      -1.0       0.0       1.0
```

Interpretation

In the ANOVA table, the p-value (0.004) for samples indicates that there is sufficient evidence that not all the means are equal when alpha is set at 0.05.

Hsu's MCB (Multiple Comparisons with the Best) compares each mean with the best (largest) of the other means. Minitab compares the sample means of, 587,& 832 to the sample code 256 mean because it is the largest. Sample code 256 is the best because the corresponding confidence intervals contain positive values. No evidence exists that sample 587 or 832 is the best because the upper interval endpoints are 0, the smallest possible value. So it can be concluded that the sample code 256 is the sample that overlay accepted as the best.

Appendix XII

Carbonation shelf life test data

Air condition storage (AC)

Time period	Pressure (psi)	Temperature (F^0)	Gas volume
Just after preparation	7.0	44	1.93
After 48 hours	5.0	40	1.86
1st week	4.0	43	1.69
2 nd week	7.0	52.5	1.68
3 rd week	6.0	52.5	1.60

Ambient Temperature Storage

Time period	Pressure (psi)	Temperature (F^0)	Gas volume
Just after preparation	7.5	51.5	1.74
After 48 hours	4.0	45	1.63
1st week	6.5	54.5	1.60
2 nd week	5.5	52	1.57
3 rd week	5.5	53.5	1.54

Appendix XIII

Degassing Study

Time period	Titratable acidity
5	0.156
10	0.154
15	0.152
20	0.150
25	0.155
30	0.157

Appendix XIV

Shelf life study –Chemical test data

Time period (week)	PH value	
	Ambient Temperature	Refrigerated Temperature
1 st	3.96	3.60
3 rd	3.59	3.88
5 th	3.55	3.98
7 th	3.47	3.56
9 th	3.40	3.43

Time period (week)	Total soluble solids(Brix)	
	Ambient Temperature	Refrigerated Temperature
1 st	16.01	16.62
3 rd	16.77	16.93
5 th	16.51	16.51
7 th	16.53	16.59
9 th	16.55	16.61

Time period (week)	Titratable Acidity	
	Ambient Temperature	Refrigerated Temperature
1 st	0.148	0.148
3 rd	0.151	0.150
5 th	0.153	0.152
7 th	0.154	0.156
9 th	0.155	0.158

Appendix XV

Carotenoids of carrot cultivars in Finland

[illegible]	Site	n	Concentration, μg/g			
			α-Carotene	β-Carotene	γ-Carotene	Lutein
[illegible]	B	[illegible]	25	66	8	16
[illegible]	B	3	25	56	12	1[illegible]
[illegible]	C	3	23	60	8	40
[illegible]	C	3	45	85	24	56
[illegible]	B	3	35	69	12	[illegible]
[illegible]	C	[illegible]	25	6[illegible]	6	45
[illegible]	C	6	22	55	6	21
[illegible]	C	3	27	65	10	34
[illegible]	D	[illegible]	27	55	15	13
[illegible]	B	3	27	45	25	12
[illegible]	B	3	30	60	27	1[illegible]
[illegible]	B	2	30	60	9	36
[illegible]	A	2	39	84	17	20
[illegible]	B	3	42	79	15	15
[illegible]	B	3	42	74	16	26
[illegible]	A	3	35	48	12	19
[illegible]	B	3	48	103	15	35
[illegible]	B	3	49	90	15	22
[illegible]	B	3	34	66	12	15

Appendix XVI

Carbonated carrot drink

Total carotenoid concentration

Carbonation level (Vol)	Absorbance at 450 nm			Total carotenoid concentration (μg/100 ml) ± SD
0	0.183	0.190	0.181	369.33 ± 9.45
1.48	0.180	0.188	0.179	364.67 ± 9.87
1.56	0.178	0.187	0.193	372.00 ± 15.10
1.86	0.181	0.183	0.177	360.67 ± 6.11
2.09	0.200	0.177	0.185	374.70 ± 23.40

β carotene concentration

Carbonation level(Vol)	Absorbance at 450 nm			β carotene concentration (μg/100 ml)±SD
0	0.372	0.381	0.169	266.3 ± 26.1
1.48	0.376	0.380	0.373	236.0 ± 19.1
1.56	0.393	0.381	0.379	290.7 ± 52.1
1.86	0.360	0.364	0.370	269.3 ± 42.0
2.09	0.329	0.377	0.390	277.7 ± 58.1

Appendix XVII

Kruskal-Wallis Test: beta carotene versus sample number

H0: sample medians are equal
H1: sample medians are not equal

Kruskal-Wallis Test on beta carotene

Sample number	N	Median	Ave Rank	Z
1	3	269.0	8.5	0.22
2	3	238.0	4.5	-1.52
3	3	294.0	10.3	1.01
4	3	268.0	8.0	0.00
5	3	273.0	8.7	0.29
Overall	15		8.0	

H = 2.76 DF = 4 P = 0.599
H = 2.76 DF = 4 P = 0.598 (adjusted for ties)

- NOTE * One or more small samples

Interpretation

The test statistic (H) had a p-value of 0.598, (adjusted for ties), ($p > 0.05$) indicating that the null hypothesis can be accepted. There are no significant differences between Medians. So it can conclude that there's no any effect of carbonation on beta carotene level in carrot drink samples

Appendix XVIII

Kruskal-Wallis Test: Total carotenoids versus Sample number

H0: sample medians are equal

H1: sample medians are not equal

```
Kruskal-Wallis Test on Total carotenoids

Sample
number     N  Median  Ave Rank      Z
1          3   366.0       7.5  -0.22
2          3   392.0      11.7   1.59
3          3   374.0       8.3   0.14
4          3   362.0       4.3  -1.59
5          3   370.0       8.2   0.07
Overall   15               8.0

H = 4.09  DF = 4  P = 0.394
H = 4.14  DF = 4  P = 0.388  (adjusted for ties)

* NOTE * One or more small samples
```

Interpretation

The test statistic (H) had a p-value of 0.394, (adjusted for ties), ($p > 0.05$) indicating that the null hypothesis can be accepted. There are no significant differences between Medians. So it can conclude that there's no any effect of carbonation on total carotenoids in carrot drink samples

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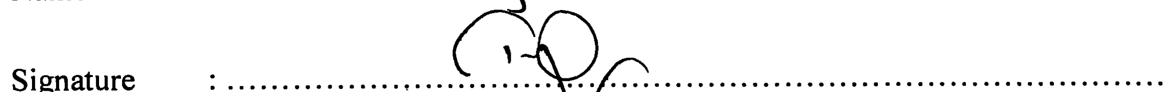

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