PREPARATION AND STORAGE STABILITY OF YACON (*Smallanthus sonchifolius*) JUICE

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Preparation and Storage Stability of Yacon (*Smallanthus sonchifolius*) Juice

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *Preparation and Storage Stability of Yacon (Smallanthus sonchifolius) Juice by* Abisikha Regmi has been accepted as a partial fulfillment of the requirements for the B. Tech degree in Food Technology.

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Abstract

The main aim of this study was to prepare yacon juice and study its storage stability. Yacon brought from Uttarpani, Dhankuta which was used for the preparation of yacon juice. The fruit was sorted, washed, disinfected by sodium hypochloride solution and peeled for juice extraction. Yacon juice was analyzed for TSS, pH, titrable acidity and juce yield. Yacon juice was prepared maintaining optimum TSS (15°Bx) with addition of table sugar and optimal acidity was maintained with the help of citric acid. Pasteurization was carried out by holding at 93°C for 5 min. Then the juice was aseptically filled to sterilized plastic bottles followed by sealing with aluminum foil seal. Prepared juice was then stored for 45 days at room temperature (22±3°C) and chemical changes occurring during storage of yacon juice were analyzed. Effect of storage time on sensory attributes of the juice was evaluated using five point Hedonic scale rating test to identify the best product. The data were analyzed by two-way ANOVA using Genstat at 5% significance level.

The pH, acidity(as citric acid), TSS (°Bx), vitamin C, reducing sugar (as dextrose), total sugar(as dextrose), glucose, fructose and TPC of juice on 0 day were found to be 3.82, 0.33% m/v, 15°Bx, 21.06 mg/100 ml, 4.03 % m/v, 7.98 % m/v, 160.33 mg/100 ml, 241.33 mg/100 ml and 0 respectively. Sensory analysis showed that there was significant difference among all the products with respect to smell, taste, flavor, color, consistency and overall acceptance. Increasing storage period affected the physiochemical properties of yacon juice. From sensory evaluation the juice stored for 15 days was found to be superior and had 15.33°Bx TSS, 0.34% acidity (as % citric acid), 3.59 pH, 16.61 mg/100 ml ascorbic acid and TPC of 5×10^{3} cfu/ml.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
BI	Browning index
FOS	Fructooiligosacharide
LSD	Least significance difference
NDOs	Non digestible oligosaccharides
NFC	Not from concentrate
PCA	Plate count agar
PDA	Potato dextrose agar
PEF	Pulsed electric field
PME	Pectin methyl esterase
POD	Peroxidase
PPOs	Polyphenol oxidases
PVCs	p-vinyl guaiacol
RTS	Ready to serve
SCFA	Short chain fatty acids
TPC	Total plate count
TSS	Total soluble solids
UI	Uncertainty interval
YMPC	Yeast mold plate count

Part I

Introduction

1.1 General introduction

Yacon (*Smallanthus sonchifolius*) is a perennial herbaceous plant of the family Asteraceae, native to the Andean regions of South America. Yacon yields starchy, fruit-like roots of different shapes and sizes that are usually consumed raw and taste sweet. Their crunchy texture very much resembles that of an apple. Water content of yacon root usually exceeds 70% of the fresh weight while the major portion of the dry matter consists of fructooligosacharides (FOS) .It has gradually received more attention due to its abundant content of fructooligosaccharides (FOS) and phenolic compounds. FOS content ranges from 6.4% to 70% of the dry matter (0.7% to 13.2% of the fresh weight) depending upon the specific crop and location (Caetano *et al.*, 2016).

There is a variety of common names for yacon around the world. These include aricoma and aricuma in Bolivia, jicama, chicama and shicama in Ecuador, and arboloco in Colombia. However, the Spanish term yacon, derived from the Quéchua word "yaku" which means "watery", is the most used worldwide. Interestingly, water is the most abundant component of the yacon root (Caetano *et al.*, 2016).

Yacon is an underutilized and scientifically neglected root crop that is native to the Andean region. Interest in this little known crop has increased recently, since it has become known that it is the plant source with the largest content of fructooligosaccharides (FOS). FOS is a type of sugar that has a lower caloric value than other sugar types (approximately 25 to 35% of the calories of normal carbohydrates). The consumption of FOS is also known to promote better health of the intestinal tract (Manrique *et al.*, 2005).

Fruit juice is the unfermented but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means including post harvest surface treatments applied in accordance with the applicable provisions of the Codex Alimentarius Commission (Anon., 2005). In other words, Juice is defined in the most general sense as "the extractable fluid contents of cells or tissues". Botanically, fruit is a plant organ, the principal biological purpose of which is to protect and eventually nourish the seed(s) as part of the natural plant propagation. The fleshy component, which is normally the edible part of the mature/ripe fruit, contains mainly parenchyma cells, possessing thin cell walls and a vacuole that occupies most of the cell volume. Thus, the cell sap found inside the vacuole represents the major component of the fruit juice (Mihalev *et al.*, 2018). Juice wasn't really possible until 1869, when a dentist in New Jersey, a Dr Thomas Welch, developed a process to pasteurize bottles of Juice to stop the Juice from fermenting into alcohol. Before then, you couldn't just have Juice, unless you made it and drank it right away (Randal, 2004).

Juice consumption has been documented since time immemorial, with the first reference being mentioned in Vedic or ancient Greek, Egyptian, or pre-Roman scripts, but the word “juice” meaning “the watery part of fruits” was first recorded in the early 14th century. However, in the most general sense, fruit juice can be defined as an extract or an extractable fluid content of cells or tissues made by mechanically squeezing or pressing out the natural liquid contained in ripe fruits without using any heat or solvent. Fruit juices are therefore a popular choice of beverage amongst consumers, they are natural and considered healthy, and play an important role in human diet. Apart from macronutrients and micronutrients, fruit juices are also a rich source of nutraceutical compounds which can provide better immunity and various other health benefits. According to the National Health Service (UK), a person should consume five recommended portions of a variety of fruits and vegetables a day (five-a-day), wherein a 150 ml glass of unsweetened 100% fruit or vegetable juice counts as one portion (one of your five-a-day) (Rajauria and Tiwari, 2018).

1.2 Statement of the Problem and Justification

Yacon is an underutilized fruit crop but it has great potential to become a profitable product in small scale farming using organic cultivation. In Nepal, yacon is being commercially produced in various parts of the country but still they aren’t being efficiently utilized in the market. Yacon itself is an abundant source of Fructooligosaccharide (FOS). FOS is a type of sugar that has a lower calorie value than other sugar types and its intake favors the growth of health-promoting bacteria while reducing pathogenic bacteria populations. The main objective of this dissertation work is to utilize the yacon fruit for juice production and its commercialization.

1.3 Objective

1.3.1 General objective

The general objective of the work is to prepare juice from locally cultivated yacon in Nepal and study its storage stability.

1.3.2 Specific objective

The specific objectives are as follows:

- Extraction of yacon juice and preparation of yacon juice beverage.
- Sensory and physicochemical analysis of the prepared juice.
- To study storage stability of prepared yacon juice at room temperature.

1.4 Significance of study

This study can be beneficial to identify the possible utilization of yacon as the raw material for juice preparation so that loss occurring from its underutilization can be minimized. This work mainly focus on preparation of optimized yacon juice, its packaging, also studying its storage stability without added preservative. Hence, this study can provide information and general guide to the cultivators to produce yacon juice in domestic scale, so that they can produce a value added product from yacon. It might also be useful to the juice industries in Nepal for producing complete new product with beneficial qualities health wise as the current world is super conscious about diet and nutrition of every food. The result generated from this study may be an initiation for further study to make a good quality yacon juice. Yacon is underutilized fruit despite of its high health benefits. Hence, this work might also provide enthusiastic market for yacon which would also help the economy of people involved in its cultivation, production and marketing, ultimately uplifting their living standards.

1.5 Limitations of the study

1. Juice filling was done at normal condition i.e. aseptic condition couldn’t be maintained.
2. Fructooligosaccharide (FOS) content of the prepared juice was not analyzed.

Part II

Literature review

2.1 Yacon

Yacon (*Smallanthus sonchifolius*) is a perennial herbaceous plant of the family Asteraceae, native to the Andean regions of South America. Yacon yields starchy, fruit-like roots of different shapes and sizes that are usually consumed raw and taste sweet. Their crunchy texture very much resembles that of an apple. Yacon (*Smallanthus sonchifolius*) is a perennial plant which forms sweet-tasting underground tuberous roots. The roots vary greatly in shape and size; commonly they are 15-20 cm long and 10 cm thick. They come in different colors, brown, pink, purple and cream. One plant can produce more than 10 kg of roots (Ojansivu *et al.*, 2011). Yacon root's water content usually exceeds 70% of the fresh weight while the major portion of the dry matter consists of fructooligosacharides (FOS). It has gradually received more attention due to its abundant content of fructooligosaccharides (FOS) and phenolic compounds. FOS content ranges from 6.4% to 70% of the dry matter (0.7% to 13.2% of the fresh weight) depending upon the specific crop and location (Caetano *et al.*, 2016).

Yacon is an Andean plant that is attracting global attention for its prebiotic advantages and benefits that are due to its high content of non-digestible oligosaccharides (NDOs), such as fructooligosaccharides and inulin, as well as phenolic compounds. Therefore, yacon's tuberous roots have been used as natural sweeteners and syrups for digestive problems, particularly for balancing the intestinal microbiota. Inaddition to prebiotics, yacon contains flavonoids, phenolic acids and tryptophan, which display antioxidant, anti-inflammatory, antimicrobial and anticancer activities. The phenolic compounds in yacon protects biomolecules such as DNA, lipids and proteins against damaged caused by free radicals (Delgado *et al.*, 2013).

The yacon tuber contains carotenoids that confer its yellow color (Quinteros, 2000). It also contains chlorogenic acid, ferulic acid, and caffeic acid which make the tubers susceptible to enzymatic browning reactions caused by polyphenol oxidases (PPOs). To inhibit these reactions, PPOs are inactivated by the heat or by the use of reducing agents, such as sulphites and organic acids (ascorbic, malic, citric acids) (Manrique *et al.*, 2005).

Nowadays, Companies have also developed novel products such as yacon syrup and yacon tea. Both products are popular among diabetics and dieters. Besides this, Yacon juice treated with active carbon powder, Yacon vinegar, yacon wine, chocolate cake, and Yacon juice blended with peach or lemon juice, are some other products that have been developed (Granato *et al.*, 2011).

There are reports on yacon cultivation in other countries, including EUA, Europe, New Zealand and Brazil. Yacon has been cultivated in south-eastern Brazil as a crop since 1991 yielding up to 100 t/ha. In folk medicine, yacon tuberous roots and infusions from dried leaves are consumed by people suffering from diabetes or from various digestive or renal disorders. Its tuberous roots are consumed fresh or cooked and it has been considered a functional food because of the large amounts of fructans (i.e., inulin and fructooligosaccharides). Fructans are carbohydrates reserve which contains up to 70 fructose units linked or not to a terminal sucrose molecule, may have linear or branched structure held together by frutosil-fructose bonds. Studies have shown that the best period to harvest yacon in tropical regions is between the 31st and 35th week after cultivation, regarding the concentration of fructans and their proportion in relation to mono- and disaccharides. Taxonomic classification of yacon (Ojansivu *et al.*, 2011) is given in Table 2.1. The yacon plants present a high hydrolytic activity at maturation phase of the tuberous roots, contributing to the predominance of a low degree of polymerization such as FOS rather than fermentable long-term fraction fructans (Moura *et al.*, 2012).

Table 2.1 Taxonomic classification of yacon

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Compositae = Asteraceae

Ojansivu *et al.* (2011)

2.1.1 Historical background and current situation of yacon

Yacon *(Smallanthus sonchifolius*) is the name commonly given to the plant and its storage root. Yacon is native to the Andean region and is known to have been cultivated and consumed since pre-Inca times. Yacon belongs with 23 *Smallanthus* species to the family of *Asteraceae* (Vitali *et al*., 2015). The origin of yacon and its relatives are the humid slopes in the Andean region of Latin America. In Peru seven *smallanthus* species are found, from which only yacon is domesticated species (Polreich, 2003). The first written record of yacon was in 1615 when Felipe Guaman Poma de Ayala included it in a list of 55 native crops cultivated by the Andeans. Attempts to establish yacon outside the Andes have been mixed. An early experiment with yacon in Italy was successful, but discontinued with the onset of World War II. A single variety from Ecuador was introduced to New Zealand in 1979. In the 1980s and 1990s, yacon was established in Japan, Brazil, South Korea, and the Czech Republic (Manrique *et al.*, 2005). It has since spread around the world and is grown on a small scale in many countries in Europe, North America and Asia. Yacon is believed to have entered Nepal from Japan since almost four decades ago, Sudarshan Karki named for first commercial cultivar and promotor of yacon in Nepal (Karki, 2013).

Despite this, and unlike other Andean root crops such as potato and sweet potato, yacon remains relatively unexploited. Until now yacon has generally been cultivated only as a subsistence crop by Andean farmers, and more recently for sale in small provincial market towns. It is only in the last few years that the health benefits of yacon have become known and it has reached the market places of the big cities where efforts have begun to commercialize it and to experiment with processing techniques. Therefore, also be grown in many other regions of the world. It has been successfully cultivated in several different regions with varying climates including: Brazil, Czech Republic, China, Korea, Japan, New Zealand, Russia, Taiwan and United States (Manrique *et al.*, 2005). It is likely that in a very early stage the Andean peasants discovered yacon properties and changed its status from weed to a managed plant, and later to a cultivated plant. The most probable area where these early events took place is the eastern humid slopes of the Andes, in the region extending from northern Bolivia to central Peru, the area with the largest clone diversity, and where native Quechua and Aymara names are used. Diversity of clones is more reduced in Ecuador, where modifications of the Mexican word *xicama* dominate. Both facts may indicate that the species was introduced there at later stages, perhaps with the Inca conquest of Ecuador, only decades earlier than the Spanish invasion (Grau and Rea, 1997).

From the humid mountain forests of Peru and Bolivia, yacon may have expanded to the north and south along the humid slopes of the Andes, to the dry inter-Andean valleys and to the Peruvian coast. It is in the coastal archeological sites of Nazca (500-1200 AD), Peru that the oldest phytomorphic representations of yacon have been identified, depicted on textiles and ceramic material. Further south, putative remains of tuberous yacon roots have been recovered at a site of the Candelaria culture, which developed between 1 and 1000 AD in the Salta province, south from the present area of cultivation in Argentina (Grau and Rea, 1997). The first written record on yacon *(llacum)* is by Felipe Guaman Poma de Ayala (1615) in a list of 55 native crops cultivated by the Andeans, including eight crops introduced from Spain (Grau and Rea, 1997).

2.1.2 Botanical characterization of yacon

Yacon (*Smallanthus sonchifolia*), syn. *Polymnia sonchifolia*, a native of the Andes closely related to the sunflower, is a vigorous, herbaceous perennial plant (family Compositae or Asteraceae – sunflower family). The plant produces large tuberous roots similar to sweet potatoes in appearance, but they have a much sweeter taste and crunchy flesh. The plants are extremely hardy and are able to grow under hot or cold conditions. Yacon grows up to a height of two meters, has large opposite sagittate leaves with serrate margins, and multiple yellow-orange flowers 3 cm in size. The plant is distinguished by having two kinds of tuberous roots, a central rhizome with "eyes" for producing new stems, and multiple edible tuberous roots radiating from the rhizome. The brittle, tan to purple, smoothly tapered edible tuberous roots are actually fattened roots that can be up 40 cm in length and weigh two kilos. The edible tuberous roots are crunchy like a crisp, sweet, and juicier than any pear. Like the sunflower, the yacon presents distributed big leaves of to even along very little ramified shafts. The plant grows in warm, temperate Andean valleys, but can be found at altitudes up to 3200 m. It represents the typical inflorescence – grouping of flowers in a called structure chapter. Tuberous root crops, in which tuberous roots are formed after cessation of stem growth, seem to have a similar mechanism of tuberous root formation to potato. On the other hand, the similarities with potato seem to be low in tuberous root crops, in which tuberous roots thicken from the base of the stem. Smallholders in the Andes cultivate yacon fairly commonly for subsistence (Pazos *et al.*, 1997-1998).

Storage root growth is caused by the proliferation of parenchymatous tissue in the root cortex and particularly in the vascular cylinder. The parenchyma accumulates sugars and, in some cases, pigments typical of certain clone groups. According to pigments, flesh color varies considerably: white, cream, white with purple striations, purple, pink and yellow. The tuberous root bark is brown, pink, purplish, cream or ivory white, thin (l-2 mm) and contains resin conduits filled with yellow crystals (Grau and Rea, 1997).

2.1.3 Chemical composition of yacon

A major portion of root biomass is composed of water that usually exceeds 70% of fresh weight. Due to high water content, the root energy value is low. The tuberous roots contain only 0.3–3.7% protein, but 70–80% of dry matter is composed of saccharides, mainly fructooligosaccharides. The underground storage organs of yacon accumulate over 60% (on dry basis) of inulin type β (2→1) fructans, mainly oligomers. They found that during summer the amount of fructans accumulated in each part was minimum despite of the existence of relatively high specific activities of sucrose: sucrose fructosyltransferase and fructan:fructan fructosyltransferase in the stems, tuberous roots, and rhizomatous stem. Pazos *et al.* (1997-1998) reported that yacon fructans are of low molecular mass and yacon has a significant fructose (3–22% of root dry matter) and glucose (2–5% of root dry matter) content. The calculated yacon food energy 619–937 kJ/kg of fresh matter is very low and has similar properties like dietary fibre. The highest dry matter and fructan yields were observed in dodecaploid lines as compared with octoploid ones. Cisneros-Zevallos *et al.* (2002) evaluated three accessions of yacon from Huanco (Peru) for their saccharide distribution and stability after 0, 15, 30, 45 and 90 days of storage at 4°C and room temperature (25°C). The results indicated high variability in fructooligosaccharide content (2.1–70.8 g/100 g dry matter) and a reverse relationship between fructooligosaccharide content and reducing sugars (Lachman *et al*., 2003a). The chemical composition of yacon root (Lachman *et al.*, 2003) is given in Table 2.2.

Table 2.2 Chemical composition of yacon root

Compound	Amount
Water (%)	70
Ash (%)	2.40
Proteins (%)	2.22
Lipids (%)	0.13
Fiber (%)	1.75
Saccharides (%)	19.67

Source: Lachman *et al.* (2003)

2.1.4 The functional effects of yacon

Yacon administered as a dietary supplement is well tolerated and produces no negative response, toxicity or adverse nutritional effects. In addition, low glucose content and high concentrations of fructooligosaccharides of yacon allow the study of possible effects in patients with metabolic diseases, such as diabetes and metabolic syndrome. In this regard, yacon has been proven to reduce levels of glucose in normal and diabetic rats with hyperglycemia (Delgado *et al.*, 2013).

Because of its inulin and FOS contents, yacon has shown important prebiotic characteristics. It resists digestion in the upper digestive tract and is hydrolyzed and fermented by colonic bacteria, such as lactobacillus and bifidobacteria. It has been shown that the association between prebiotics and probiotics has beneficial consequences for the intestinal tract. Bifidobacteria inhibit the growth of putrefactive bacteria in the colon and promote the absorption of Ca^{2+} and PO^{4-} ions as well B vitamin synthesis. These bacteria can also stimulate the immune system. In this regard, the consumption of yacon root leads to an increase in short-chain fatty acids (SCFA) that protect against colon cancer (Delgado *et al.*, 2013).

Certain investigations have shown that yacon promotes several important aspects of health. An experiment using a diabetic rodent model showed that FOS consumption could improve insulin release and/or insulin-like activity. Some authors state that the saccharides present in yacon, particularly β-(2$\rightarrow$1) fructooligosaccharides, could modulate the metabolic syndrome that occurs in type 2 diabetes and dyslipidemia, which are considered to be risk factors for atherosclerosis (Delgado *et al.*, 2013).

Low pH and the production of SCFA due to the consumption of prebiotics result in hypertrophy of the mucosal cells, enlargement of the intestinal surface and enhanced solubility of mineral ions. Yacon consumption for relatively short periods also resulted in increased intestinal absorption of minerals and bone mass, favoring the biomechanical properties of bone in rats. The enlargement of the cecal wall observed after yacon consumption appears to contribute to the increased mineral absorption in those animals. In fact, some studies show that daily consumption of a combination of short- and long-chain inulin type fructans significantly increases calcium absorption and bone mineralization during pubertal growth. The effects of these dietary factors on calcium absorption appear to be modulated by genetic factors, including genetic polymorphism of a specific D-vitamin receptor. It has been observed that the consumption of inulin type fructans also reduces osteoporosis progression by increasing the bioavailability of calcium, with a significant increase in bone density and bone mineral mass (Delgado *et al.*, 2013).

2.1.5 Health benefits of yacon

Some of the most popular yacon health benefits include the control of blood sugar levels, control of cholesterol level, boosting immune system and helping in weight loss. But the most important is that yacon rich with carbs which is benefit to bring energy for daily activities. To get to know more brief information, below are several health benefits of yacon fruit. Yacon is a multifunctional food because it contains several bioactive compounds, including: phytoalexins which have antimicrobial activity, phenolic compounds that exert antioxidant activity, such as chlorogenic acids and high contents of fructans (inulin and FOS) that have prebiotic properties (Paula *et al.*, 2013).

2.1.5.1 Bioactivity and Potential Health Benefits

FOS are able to escape enzymatic digestion in the upper gastrointestinal tract, reaching the colon intact before undergoing microbial fermentation. FOS intake elicits a bifidogenic effect by selectively stimulating the proliferation of bifidobacteria, a group of beneficial bacteria naturally found in the human colon. Short chain fatty acids (SCFA), the endproducts of FOS fermentation by the intestinal microbiota, can also favor the growth of health-promoting bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp., while reducing or maintaining pathogenic populations (e.g., *Clostridium* spp. and *Escherichia coli*) at low levels. Thus, FOS are small soluble dietary fibers that exhibit prebiotic activity (Caetano *et al.*, 2016).

2.1.5.2 Control of type II Diabetes

Yacon contain inulin, which is a type of fructose found in the yacon tuber. Some researchers have claimed that it’s useful in insulin independent diabetes, such as type II diabetes for regulating blood sugar levels. Research is still being continued and researchers are striving to document any importance of this plant in diabetes, which could be revolutionizing in the field of medicine as a therapeutic agent (Anon., 2009-2015).

2.1.5.3 Used as natural sweetener

They can be used in the preparation of sugar free sweeteners for diabetic patients and those who are motivated to lose weight. Yacon sweetener can also be added in coffee and tea (Anon., 2009-2015).

2.1.5.4 Cancer prevention

It is a potent anticancer agent for it inhibits the proliferation of mutant cells by initiating apoptosis (programmed cell death). It is found effective against skin, colon and blood cancer (Anon., 2009-2015).

2.1.5.5 Prevention of fatty liver

Yacon is an important regulator of body fat and prevent excessive cholesterol accumulation, thus protecting liver function by assisting it in cholesterol metabolism. It helps in metabolic processes of body (Anon., 2009-2015).

2.1.5.6 Reduced triglycerides

Yacon contain fructooligosaccharide that lowers bad cholesterol (triglycerides and low density lipoprotein). Low level of triglyceride is associated with reduce risk of heart attacks and stroke (Anon., 2009-2015).

2.1.5.7 Prebiotic effects

It is associated with correction of digestion by increasing intestinal flora and preventing colitis (Anon., 2009-2015).

2.1.5.8 Cure for constipation

It is a used to increase intestinal motility thus reducing constipation (Anon., 2009-2015).

2.1.5.9 Antioxidant activity

Chemical analysis of yacon has shown antioxidant activity which prevents the body from inflammatory and chronic diseases. This is one of the chief yacon root benefits. These antioxidants were extracted by methanol from the yacon plant. Caffeic acid, ferulic acid and chlorogenic are the antioxidants found in the leaves of yacon (Anon., 2009-2015).

2.1.5.10 Antifungal

Yacon leaves have reported important antifungal effects. The yacon leaf may be used in the treatment of fungal diseases like athlete’s foot (Anon., 2009-2015).

2.1.5.11 Tribal and herbal medicine

In South America, yacon plants are used as tribal medicines for kidney and bladder problems and also for cystitis, nephritis and externally for myalgia (Anon., 2009-2015).

2.1.5.12 Manage blood pressure

Consuming yacon is also good to manage the blood pressure. Therefore, it is good for people with symptoms of hypertension. By keeping the blood pressure, it can also help to avoid various diseases related to hypertension such as heart attack. One of the killer disease in the world (Anon., 2017).

2.1.5.13 Control cholesterol

Another health benefit of yacon is to manage cholesterol level inside the blood. It can work to manage the HDL and LDL level inside the blood and help to avoid blood cod. Through a better blood circulation, it can produce a better health and body system. This is the same health benefits accedes that can control the cholesterol level too (Anon., 2017).

2.1.5.14 Avoid cardiovascular diseases

Yacon is good to manage the cardiovascular health. Therefore, it can work to avoid cardiovascular diseases such as the early symptoms of stroke. By frequent consume of the fruit, the body will manage a better cardiovascular condition and can avoid a heart attack too. Furthermore, it can help to balance the blood cells and avoid any fat inside the blood (Anon., 2017).

2.1.5.15 Avoid hypertension

The fruit is able to lower down the blood pressure. It can work to manage the blood pressure level. Therefore, it can bring a huge benefit for people with symptoms of hypertension. A proper portion of the fruit daily can help to stabilize the blood pressure. This is the same health benefits of sword beans that can avoid high blood pressure too (Anon., 2017).

2.1.5.16 Rich of fiber

Yacon is rich of fiber therefore; it can help to ease the digestive system. It will manage intestine bowel movement to be optimized and work faster. By fasten digest, the body will absorb important nutrient and work to avoid fat formation (Anon., 2017).

2.1.5.17 Ease digestive

Due to the fiber content inside the fruit, it can benefit to ease the digestive system. A better digestive will lead to a better body metabolism rate. Through a better metabolism rate, it can help to optimize the blood circulation and bring the oxygen level into the whole part of the body. Furthermore, it can optimize the brain function and nerve stimulation (Anon., 2017).

2.1.5.18 Source of minerals

The fruit is rich with vitamins and minerals. Therefore, it is really good for children to support nutrition for child development. Furthermore, it can bring a good source of calcium and phosphorus that beneficial for the bone strength in children. To get children interest eating the fruit, try to preserve it as a cake, syrup or ice cream (Anon., 2017).

2.1.6 Use of yacon and its potential in Nepal

Yacon can be eaten raw or cooked and have traditionally been used in fruit salads, jams, puddings, and juices. Their peeled skin, once dried, can also be used to make nutritious organic tea. Farmers in Brazil and Japan produce a number of processed yacon products, such as air-dried tuber slices unrefined yacon syrup that has a consistency of honey and can be marketed as a dietetic sweetener or a juice without addition of sweeteners, synthetic colorants and preservatives, with only small additions of vitamin C. The yacon tuberous roots serve as a source of raw material for the production of sweet pastries, fermented vegetables and ethanol; they can be used as “chips” in dehydrated form. Another product is yacon juice treated with active carbon powder to obtain its clarification, decolorisation and deodorization, acetic acid fermentation of yacon juice with *Acetobacter pasteurianus* for production of improved yacon vinegar containing natural fructooligosaccharides. Yacon slices and stripes retain crunchiness during cooking and could be used in Asian stir-fried dishes (Manrique *et al.*, 2005).

One of the main yacon properties is medicinal. Antidiabetic medicinal properties were attributed mainly to yacon leaves. Dried yacon leaves were used to prepare a medicinal infusion or mixed with common tea leaves in Japan. The yacon tuberous roots as well as stems and leaves containing a high level of proteins could be used as a food for cattle and other domestic animals (Lachman *et al.*, 2003) .

Geographically, the climate and agricultural conditions of Nepal are quite similar to those of the Andes in South America. Unfortunately, due to their resemblance to a vegetable and the general lack of knowledge, yacóns are not as popular as initially imagined. However commercial production of yacon began in Nepal. As for now (2018), yacon is produced in around 40 districts of Nepal. However of late, farmers are getting upset with slow sales and have reportedly ended up using yacon as cattle fodder or in liquor (alcoholic) making (Khatiwada, 2018). In Nepal works on yacon and its possible utilization has been started Shrestha (2015) conducted a study on preparation and quality analyses of yacon ready to serve (RTS) and wine. The commercial production of yacon syrup also started in Nepal. Perhaps in the future, innovative farmers will adopt newer crops from Latin America to Nepal as well and commercial farming and processing will take its place slowly (Karki, 2013).

2.1.7 Products of yacon

2.1.7.1 Yacon syrup

This product is primarily used as a natural sweetener, and is extracted from the yacon plant. The tuberous roots are where the syrup is actually found, and they are indigenous in Bolivia, Brazil, and Peru. Yacon is considered one of the most preferred natural sweetener because it contains about 50% FOS or Fructooligosacharides, which do not in any way increase blood glucose levels (a fact that diabetics should be aware of). As far as taste goes, Yacon syrup is quite similar to caramel or molasses but some connoisseurs would argue that it does have a different layer of flavor (Marshall, 2014).

Yacon syrup is one of the natural supplements that can help you lose weight. From fat diets to so called revolutionary fitness regimens, people these days are no strangers to new discoveries in the field of health and wellness. For the most part, there have been promising products that bring about positive results (Marshall, 2014).

2.1.7.2 Yacon RTS

RTS is a fruit beverage prepared from juice of yacon mixing with sweeteners, acidulants and coloring and flavoring materials that are optional. Such beverage which includes not less than 10% juice content is known as RTS (ready-to-serve). Yacon RTS was also prepared and optimization was carried out by (Shrestha, 2015).

2.1.7.3 Yacon biscuit

Demand for health oriented bakery products with low sugar and high fiber contents are increasing. Incorporating dietary fiber and replacing sugar in biscuit dough tends to require some cares. Biscuits are consumed daily by the majority of population, especially by the teenagers and for years, the food industry has focused in increasing the nutritional value of these products. Enhancing the dietary fiber and replacing sugars of baked products is challenging and to this day such products are not widely accepted by the consumers. As the new sources of fiber become available, and consumer are moving towards healthier diets, research on the use of fiber as functional ingredient in baking is becoming more extensive (Silva *et al.*, 2014).

Yacon flour incorporated biscuit turns out to be a substitute food and therefore, due to the growing population concern about health and physiological effects of the soluble fiber, and new product developments like biscuits containing functional compounds, this is promising food source carrying health benefits (Silva *et al.*, 2014).

2.1.7.4 Yacon wine

The term ‘wine’ is applied to the product made by alcoholic fermentation of grapes or grape juice, with an aging process. However, products of fermentation of others berries, fruits, and honey are also called wines and the resultant wine is normally named after the substrate used. Wine is one of the God’s choicest gift to man and history is almost a romance. The oldest testament is the Bible gives evidence of wine existing but there is a definite evidence of its use in China and Egypt in 2000 and 3000 B.C. respectively (Andrew, 1980). With various optimization yacon wine of TSS 9.2°Bx and acidity of 3.24% was said to be optimal (Pokhrel, 2018).

2.1.8 Advantages of yacon

Yacon possesses an attractive set of features advantageous to producers, processors, consumers and the environment.

1) High fresh weight productivity

2) Adaptability to a wide range of climates and soils

3) Potential good fit in agro forestry systems

4) Erosion control

5) Potential use as forage for both underground and aerial parts

6) Wide range of processing alternatives

7) Good post-harvest life, if managed properly

8) Exceptional qualities for low calorie diets

9) Medicinal properties (Grau and Rea, 1997).

2.2 Browning

Browning reaction occurs widely in food material. The color produced range from pale yellow to dark brown or black, depending on the type of product and extent of the reaction. In some food browning is considered desirable, e.g. honey, chocolate, brown crust of baked products, etc., while in others it is detrimental, as in darkening of dehydrated fruits, vegetables, etc. (Srivastava and Kumar, 2007).

Browning reactions may be either enzymatic or no enzymatic. Many of the enzymatic reactions are seen in fruits and vegetables, and involve the oxidation of polyphenolic compound by oxidative enzyme in plant cells. The non-enzymatic browning reactions frequently involve sugars or sugar related compounds (Srivastava and Kumar, 2007).

2.2.1 Enzymatic browning

Many fruits and vegetables have the tendency to turn brown when damaged or when cut surfaces are exposed to air, e.g., apples, bananas, potatoes, etc., and this is due to enzymatic reaction. The formation of brown color is due to action of enzyme phenolase (also known as polyphenol oxidase, tyrosinase or catecholase) or phenolic substances. Normally the phenolic substrates are separated from phenolase in intact tissues and browning does not occur. When foods containing such substances are cut and exposed to air rapid browning of cut surfaces takes place.

Polyphenols +	Oxygen	$\xrightarrow{\text{oxidase}}$	Brown
(In cells)	(In air)	(In cells)	

The enzymatic reaction is due to oxidation of phenols into orthoquinones, which in turn rapidly polymerize to form melanin (the brown pigment). When the substrate is phenol, it is first converted by hydroxylation into orthodiphenol and then oxidized to orthoquinone. Tyrosine is the major phenolic substrate for phenolase action in foods. Other phenolic substances are caffeic acid, protocatechuic acid, and chlorogenic acid. The reaction occurs in several steps and are catalyzed by several enzymes e.g., phenolases, peroxidases and others (Srivastava and Kumar, 2007). Some fruits do not contain these enzymes and do not darken on exposure of cut surfaces to air. Orange, lemon, grapefruit, strawberry, tomato, etc. seem to be free from these enzymes (Srivastava and Kumar, 2007).

Melanin formation is undesirable during processing of fruits and vegetables. Pigment formation can be eliminated by inhibiting enzyme action. Heat treatment, addition of sulphur dioxide or sulphites and ascorbic acids are commonly used methods of inactivating these enzymes. Both sulphur dioxide and ascorbic acids are strong reducing agents. They consume oxygen and destroy the browning activity of enzymes and either of them may be added to products that tend to darken. The enzyme is irreversibly inactivated at pH of 3 or less and this can be achieved by addition of sufficient acidulants such as citric, malic or phosphoric acids. Browning can also be prevented by excluding oxygen from the reaction site or protecting the phenolic substrate. The exclusion of oxygen is achieved by immersing the tissues in brine or syrup, or by processing under vacuum and the phenolic substances are protected by the use of certain enzymes which modify ortho phenolic substrates (Srivastava and Kumar, 2007).

2.2.1.1 Prevention of enzymatic browning

Some of the methods for controlling browning are:

1. Use of chelating agents (e.g., EDTA) to bind and remove copper

2. Vacuum or inert gas packaging to avoid the effects of oxygen
3. Inactivation of polyphenolase by heat treatment (blanching)
4. Storage at low temperature to reduce rate of enzyme action
5. Avoiding injury to foods during handling, storage and transportation
6. Drying by physical or chemical (e.g., use of salts) means to reduce water activity below that required for enzyme action.
7. Irradiation
8. High pressure treatment
9. Use of browning inhibitors like SO_2, ascorbic acid, phenolic antioxidants, acidulants (e.g., citric acid) (Rai, 2006).

2.2.2 Non enzymatic browning

Non enzymatic browning reactions are responsible for the color and flavor of foods. Roasting of potatoes, like toasting of biscuits and baking of breads and cakes, produces a golden brown color. Sometimes this reaction produces a desirable flavor (the chocolate flavor of cocoa beans), at other times the reaction is undesirable (dark brown of potato chips). Because this does not involve enzymes, it is distinguished from enzymatic browning by being termed non-enzymatic browning. The presence of reactive reducing sugars is responsible for browning in foods. On heating the sugars undergo ring opening, enolisation, dehydration and fragmentation. The unsaturated carbonyl compounds that are formed react to produce brown polymers and flavor compounds. Heat-induced non enzymatic browning reaction can be divided into two groups: Maillard reaction and caramelization while others are ascorbic acid browning, lipid peroxidation etc (Srivastava and Kumar, 2007).

2.2.2.1 Maillard reaction

The Maillard reaction has been named after the French chemist Louis Maillard who first described it but it was only in 1953 that the first coherent scheme was put forward by Hodge (Martins *et al.*, 2001). Maillard reaction also known as maillard browning is a color, flavor, odor and sometimes texture change which results from a chemical reaction between proteins and carbohydrates. It is named after Frenchman Maillard who discovered it. The maillard reaction denotes a number of complex reactions between (i) nitrogenous compounds and sugars, (ii) nitrogenous compounds and organic acids, (iii) sugars and organic acids, and (iv) among organic acid themselves. The carbonyl group of acyclic sugars readily combines with the basic amino groups of proteins, peptides and amino acids, resulting in sugar amines. The set of various reactions that sugar-amines undergo resulting in browning is known as maillard reaction. The sugar amines have a brown color at a low temperature. It has been found that histidine, threonine, phenylalanine, tryptophan, and lysine are the most reactive amino acids. The initial reaction is thought to be between the aldehyde of the sugar and the amino group of amino acid (Srivastava and Kumar, 2007).

From the fact that 'we also eat with our eyes', the significance of maillard browning in processed foods, in consumer acceptance is obvious. The degree of browning (usually measured via absorbance at 420 nm) is often used analytically to assess the extent to which the Maillard reaction has taken place in foods. Nevertheless, it has been stated that fluorescent compounds are formed prior to brown compounds. In the final stage of the reaction, coloured intermediates and other reactive precursors (enaminol products, low-molecularweight sugar analogues, and unsaturated carbonyl products) condense and polymerise to form brown polymers, under acceleration by an amine catalyst. Some of their known properties are brown, high molecular weight, furan ring-containing and nitrogen-containing polymers; they may contain carbonyl, carboxyl, amine, amide, pyrrole, indole, azomethine, ester, anhydride, ether, methyl and/or hydroxyl groups. Studies on melanoidins formation have been summarized in different review articles. The isolation and identification of coloured maillard products has so far been achieved only with model systems, mostly for low molecular weight (<500 Da) products. Hashiba concluded that browning was directly proportional to the reducing power of the sugar and to the amounts of glycine consumed, by comparing different sugars with one single amino acid (Martins *et al.*, 2001).

Light does not accelerate maillard browning but does increase other color changes. This problem is addressed commercially by packaging foods in opaque materials. Householders need to store their glass jars and plastic bags in dark places. Avoid a sunny window in the pantry or leaving a light on to decrease humidity or discourage pests (Srivastava and Kumar, 2007)

Hence, maillard reaction products predominate in browned foods. The condensation product of sugar and amine undergoes enrolization and rearrangement and then condensation and polymerization to form red-brown and dark-brown compounds. The brown to black amorphous, unsaturated heterogeneous polymers are called “melanoids”. And the rate of melanoid production dependent on temperature (Hodge, 1996).

The final products of nonenzymic browning are called melanoidins to distinguish them from the melanins produced by enzymic browning. Theoretically, the distinction is clear; however, in practice, it is very difficult to classify the dark brown products formed in foods, since they tend to be very complex mixtures and are chemically relatively intractable (Nursten, 2005).

Inhibition of maillard reaction can be accomplished by keeping the pH below the isoelectric pH of the amino acids, peptides and proteins and by keeping the temperature as low as possible during processing and storage. Use of non-reducing sugars such as sucrose, under conditions not favoring inversion, also helps to bring down Maillard browning. Sulphur dioxide and sulphites used in extending the storage life of dehydrated foods, fruit juices and wines also inhibit the reaction (Srivastava and Kumar, 2007).

2.2.2.2 Caramelization

Caramelization is defined as the thermal degradation of sugars leading to the formation of volatiles (caramel aroma) and browned colored products (caramel colors), caramelization entails a series of complex reactions, some of which are still not well understood. The reactions, in sequence, include intramolecular arrangement of sugar molecules, dehydration, degradation, condensation, and polymerization (Rai, 2006). According to the directive, the term caramel relates to products of a more or less intense brown colour, which are intended for colouring. It does not correspond to the sugary aromatic product obtained from heating sugars and which is used for flavouring food (e.g., confectionery, pastry, and alcoholic drinks). Caramel is the only colour permitted in malt bread, vinegar, and alcoholic drinks, such as beer, whisky, and liqueurs. Clearly, Class III and IV caramels are close to melanoidins produced by the Maillard reaction, but detailed structures for the coloured components cannot be given for any of these commercial caramels (Nursten, 2005).

2.2.2.3 Ascorbic acid browning

Ascorbic acid is a reductone and therefore can participate in the browning reaction as in maillard reaction. The molecule is first oxidized to dehydro ascorbic acid and then transformed into diketogluconic acid. This acid is eventually decomposed to furfuraldehyde or related compounds which then polymerize or react with nitrogen (as in maillard reaction) to form brown pigments (Rai, 2007).

2.2.2.4 Lipid peroxidation

These are oxidized to form aldehydes and ketones which then react with amino acids to form brown pigments, as in the maillard reaction. It is possible that peroxidation products induce the browning reaction of the Amadori products (Rai, 2007). Lipid peroxidation is a chain reaction and is created by free radicals influencing unsaturated fatty acids in cell membranes, leading to their damage. Free radicals are initiators and terminators of lipid peroxidation processes. Once activated, reaction continues auto catalytically, it has a progressive course, and its final result is structural and functional changes of substrate (Cole and Freeman, 2010).

2.2.3 Browning Index

Browning index in the literature may mean one of two things: a simple indicator of a chemical change (often characterized by the optical density at a given wavelength or the ratio of the reflectance at 570 and 650 nm) or the color change due to oxidation of a freshly cut fruit or vegetable surface, during storage or drying, or the baking of bread (Hirschler, 2012).

Since quality is supremely important in food, deterioration has to be controlled during storage. In concentrated juices, one of the main causes of deterioration is non enzymatic browning, since enzymatic browning is eliminated by heat treatment during processing. So, kinetic modeling of nonenzymic reactions is very important for fruit juice storage. By measuring rate and temperature-dependence of these reactions, it is possible to determine the period of storage at a given temperature without quality deterioration (Burdurlu and Karadeniz, 2003).

2.3 Fruit Juices

Fruit juice is defined as the fermentable but unfermented product obtained from the edible part of fruit which is sound and ripe, fresh or preserved by chilling or freezing of one or more kinds mixed together having the characteristic color, flavor and taste typical of the juice of the fruit from which it comes, i.e., the juice obtained directly from fruit. This product is often described as "direct juice" or "not from concentrate (NFC) juice" although these names are not controlled by the regulations (Mihalev *et al.*, 2018).

2.3.1 Types of fruit juices

According to their dispersion system composition, fruit juices can be divided into the following four main types:

2.3.1.1 Clear/clarified (transparent) juice

This represents a water solution of the so called soluble solids (sugars, organic acids, salts, free amino acids, water-soluble vitamins, and pigments, etc.) with particle sizes under 0.001 μm. It could be approximated with the cell sap found inside the plant cell vacuole. Clear juice is obtained by technological processing (clarification) of freshly pressed fruit juice (Mihalev *et al.*, 2018).

2.3.1.2 Opalescent (translucent) juice

In addition to the soluble solids (clear juice), contains colloidal substances with a distribution spectrum of 0.10.001 μm. This dispersed phase includes pectin, hemicelluloses, proteins, protein polyphenol complexes, and dissolved starch (Mihalev *et al.*, 2018).

2.3.1.3 Cloudy (turbid) juice

This is actually an unclarified juice, i.e., pressed juice that is not subjected to clarification treatments. To improve cloud stability, coarse particles, which are generally unstable and prone to rapid sedimentation, can be partly removed (e.g., by centrifugation). With cloudy apple juice, 95% of all particles are of a smaller size than 2.5 μm; the most frequent diameter is 0.60.8 μm. These are relatively stable fine cloud particles, which consist of proteins, polysaccharides, lipids, and polyphenols. Fine cloud particles probably arise from cell membranes/walls, but appear not to be simply cell debris. There seems to be an association between cell membrane/ wall fragments and colloidally dissolved macromolecules, with native adsorbed pectin being an important factor for the cloud stability (Mihalev *et al.*, 2018).

2.3.1.4 Pulp-enriched juice

This contains a distinct amount of coarse cloud particles (sometimes termed pulp particles) with diameters of over 100 μm, which are mostly fruit flesh fragments, e.g., juice sacs of the citrus fruit endocarp. Pulp-enriched juice can be obtained by blending of cloudy juice with fruit purée. Smoothies, comprising blended beverages of mashed fruits and/or purées and juices, could also be categorized under this type of dispersion system. The highest amount of coarse cloud particles can be found in fruit/vegetable purées (Mihalev *et al.*, 2018).

2.3.2 The history of the juice industry

The beginnings of juice production date back to antiquity. In fact, juices can be considered as old as (or even older than) agriculture itself, because transporting fruits at an adequate maturity stage for consumption might have easily resulted in the creation of juice. Later, tool-making skills and trial-and-error assays improved the manufacture and storage of juices. However, the shelf life of these products strongly limited their consumption until preservation methods such as natural chilling and fermentation were discovered. Some key findings, such as the acidification of grape juice produces vinegar, also provided new conservation methods for other fruits and vegetables, some of which could be stored and squeezed later. Nonetheless, even with such discoveries, there was limited time for the consumption of fruit juices (Falguera and Ibarz, 2014).

According to some researchers, the first “modern” juice seems to have been lemonade, which was invented in the Middle East and imported to Italy during the sixteenth century. As with many other industrial sectors, World War II represented a major boost for juice production and distribution (Falguera and Ibarz, 2014).

In the early 1970s, aseptic processing was introduced and commercialized on a large scale, which was an essential breakthrough that allowed the juice market to expand worldwide, thereby ensuring the safety of juices and reducing their production and marketing expenses. In addition, aseptic storage and packaging allowed new modes of transport, with a reduced risk of contamination and cost per unit. During that decade, another important issue also changed the direction of the juice industry. The aforementioned advantages of aseptic processing that made packaging and distribution easier and cheaper, along with consumer demand for quality and convenience, led to increased sales of chilled ready-to-serve (RTS) juice. However, this price gap narrowed when glass packaging was progressively replaced by paper envelopes. The chilled RTS market share growth was especially significant for orange and grapefruit juices (Falguera and Ibarz, 2014)

In the 1980s, not-from-concentrate (NFC) juices grabbed consumers' attention, although they were significantly more expensive than the RTS reconstituted juices. The ability of NFC juices to better conserve the flavor of fresh fruit because they had never been subjected to evaporation made them increasingly popular. From then on, one of the major challenges of the current fruit industry has been (and still is) the search for new, exotic juices, blends, or juice-based products that provide new flavors and new healthy or functional benefits, according to the current expectations of consumers and the issues that are nowadays included in the definition of quality (Falguera and Ibarz, 2014).

2.3.3 Demand and consumption of fruit juices

The global juice market is expanding and it is likely driven by the fitness conscious consumer and the demand for healthy food products. Nowadays juice manufacturers are customer centered and focus on introducing different juices varieties, flavors, and mix juices along with innovative packaging and detailed nutrition and health claims (Report, 2015). The global juice market is predicted to witness strong growth at a compound annual growth rate of 3% during the period 2016-20 and is expected to be adding $128.74 billion to the world economy by the end of 2017. However, the growth is entirely dependent on the production, availability and geographical distribution of fruits. The global juice market is segmented into Latin America, the Middle East and Africa, Europe, Asia Pacific, and North America, but the leading markets for juice in these regions are Japan, China, France, Germany, the United Kingdom, and the United States (MarketResearch.com Report, 2017).

The key juice manufacturers in the global market are Nestlé, Coca-Cola, Welch's, Dr. Pepper Snapple, Citrus World, PepsiCo, and Del Monte, among several others. On average, fruit juice consumption is directly related to a country's income level and lifestyle. Across geographic regions, the average consumption of fruit juice was recorded to be from 0.66 (95% uncertainty interval (UI): 0.36, 1.13) servings/day to 0.013 (95% UI: 0.011, 0.017) servings/day, with the highest intake in Australasia (especially in New Zealand) and the lowest in East Asia (Rajauria and Tiwari, 2018).

2.3.4 Juice processing and preservation

The perishable nature of fruit juices poses significant challenges associated with production and preservation. Unless the juice was consumed fresh, storage at chilling or freezing temperatures was the only alternative to protect the organoleptic properties of juice. The fermentation of juice soon after squeezing was the biggest challenge until preservation techniques were developed (Correa *et al.*, 2010). Initially fermentation was the only choice to prolong the shelf life of juices, but juices emerged as popular drinks after the development of pasteurization techniques. Studies have shown that the amounts of nutraceuticals in fruit juices are dependent on how they are produced, processed, and preserved. Thus, there is a need not only to document the traditional ways of extracting and preserving juices, but also to explore how novel processes can help to reduce the challenges encountered by the juice industry. Traditional techniques including canning, pasteurization, concentrating, freezing, evaporation, and spray drying have resulted in significant extensions in shelf life also the high pressure processing has significant effect on microbial, physical and chemical properties of fruit juice (Bull *et al.*, 2004) but at the cost of nutritional or health attributes. Thermal treatments sometimes fail to produce a quality, high-nutrition, and microbiologically stable product. In recent decades, the emphasis has been on employing novel approaches to enhance the safety and shelf life whilst retaining the nutritional quality of fruit juices. Numerous emerging technologies including high-pressure processing, pulsed electric field (PEF) processing, ultrasound, ozone processing, light-based technologies, irradiation, and non-thermal plasma have been applied for fruit juice preservation, processing, and packaging. These novel techniques are rapidly acquiring the juice market as they are efficient in shelf life extension, enzymatic activity reduction, and microorganism inactivation, while maintaining the quality of the original, fresh pressed produce (Mohamed and Eissa, 2012).

2.3.5 Challenges associated with fruit juices production

As fruit juices are a healthier choice among consumers, the quality and the safety of juice products are always a worry, and they are always subject to very detailed legislation ensuring all necessary information on their nutritional benefits and compositions (Rajauria and Tiwari, 2018).

Apart from strict regulations, there are some other factors that pose challenges in the production of fruit juices and inhibit the growth of the global juice market. One of the main challenges is associated with the constant supply of fruits, as most of fruits are seasonal and this affects the overall production. Other challenges include: manufacturing challenges (homogenization, extraction, filtration, processing, preservation, packaging, and storage); ingredients challenges (fruit components, sweeteners, flavors, colors, preservatives, nutraceutical ingredients, and miscellaneous additives); quality issues (color and flavor deterioration, appearance changes, packaging material, storage conditions, microbiological problems, shelf life, water quality and bottling issues); and most recent are new product development and marketing challenges (cost constraints, marketing brief, consumer assessment and complaints) However, availability of substitutes such as carbonated soft drinks, sports and energy drinks, and other hybrid drinks pose the prime challenges to the juice industry and inhibit the growth of the global fruit juice market(Rajauria and Tiwari, 2018).

2.3.6 Microbiological background and target microorganisms of fruit juices

2.3.6.1 *Escherichia coli, Salmonella,* and *Listeria monocytogenes*

Pasteurization is a treatment that can increase the safety of fruit juices. In choosing the target microorganism to calculate the lethality of a pasteurization treatment, juice processors may consider either *E. coli* O157:H7 or *Salmonella*, due to the numerous outbreaks that have been associated with them in unpasteurized juices, or *L. monocytogenes* due to its ubiquitous nature. The target microorganism should be the most heat-resistant pathogen likely to occur in the juice because, in inactivation conditions that are applied for the most heat-resistant pathogen, other microorganisms are also eliminated (Agcam *et al.*, 2018).

E. coli O157:H7 is not part of the endogenous microflora of fruits and, therefore, its presence derives from some fecal contamination prior to consumption. *E. coli* O157:H7 is responsible for the disease syndromes of hemorrhagic colitis, hemolytic uremic syndrome, and thrombocytopenic purpura in humans. Because of its acid tolerance, *E. coli* 0157:H7 has already been associated with outbreaks caused by juice. On other hand, *Salmonella* is another important pathogen that has been associated with outbreaks caused by juices in the last three decades. Salmonella is also associated with poor hygiene of the food handlers (Agcam *et al.*, 2018).

L. monocytogenes is not well established as a relevant fruit-juice-borne pathogen in the literature, as compared to *Salmonella* and *E. coli* 0157:H7. However, this pathogen can be considered to be of concern in fresh fruits and fruit juices, because it has a remarkable ability to survive under a variety of adverse conditions (Agcam *et al.*, 2018).

In recent years, different research groups all over the world have reported that microorganisms are able to improve thermal tolerance by a mechanism calling acid adaptation. In the other words, acid adaptation or acid tolerance is a phenomenon by which microorganisms show an increased resistance to environmental stress after exposure to a moderate acid environment. Some food borne pathogens can develop acid adaptation systems that induce cross-protection, and make them more resistant against other environmental stresses, thus increasing their ability to survive in juice. *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and *C. parvum* can tolerate low pH values and survive in fruit juices and juice concentrates longer than cells that are unable to adapt. The acid adaptation of *Salmonella spp*., *L. monocytogenes*, and, *E. coli* O157:H7 also increases the heat resistance of these bacteria in apple, orange, white grape juices, apple cider, juice blends, cantaloupe, and watermelon juice (Agcam *et al.*, 2018).

2.3.6.2 *Alicyclobacillus*

Presently, more than 20 species have been described to belong to *Alicyclobacillus* genus but only four species (*A. acidoterrestris, A. pomorum, A. herbarius,* and *A. acidiphillus*) have been reported to be responsible for fruit juice or beverage spoilage. However, A. *acidoterrestris* is considered to be the most important, due to the number of spoilage episodes and its incidence. *Alicyclobacillus* spoilage is characterized by a phenolic offflavor as a result of its ability to produce guaiacol, 2,6-dibromophenol and 2,6-dichlorophenol (Agcam *et al.*, 2018).

2.3.6.3 Molds and yeasts

The growth in fruit juices of the fungus that can produce mycotoxins should be prevented for public health. Spores and vegetative cells of most molds are inactivated upon exposure to 60ºC for 5 min to avoid fungal growth and mycotoxin formation in foods. Notable exceptions are the ascospores of certain strains of the molds *Byssochlamys nivea, Byssochlamys fulva, Neosartorya fischeri, Talaromyces flavus,* and *Eupenicillium javanicum* in high-acid fruit pulps/juices (Agcam *et al.*, 2018).

Fermentation caused by yeasts and molds can be a problem in the juice industry, but the main problem in apple juice is patulin, a mycotoxin produced by various species of mold. Patulin have been reported as mutagenic, carcinogenic, and teratogenic (Agcam *et al.*, 2018).

According to a survey on the yeast flora of frozen fruit juice concentrates, the isolates recovered represented 12 genera and 21 species of yeast. The five most frequently isolated yeast species included *S. cerevisiae* (24.7%), *Candida stellata* (22.1%), *Z. rouxii* (14.3%), *T. delbrueckii,* and *R. mucilaginosa* (Agcam *et al*., 2018).

2.3.7 Preservation of juice

Freshly extracted juices are highly attractive in appearance and possess good taste and aroma but deteriorates rapidly, if kept for some time. To retain the natural taste and aroma of juice it is necessary to preserve it soon after extraction without allowing it to stand for any length of time. Various method of preservation are employed and each has its own merits (Parajuli, 2010). The methods generally used are:

(i) Pasteurization

(ii) Addition of chemicals

(iii) Addition of sugar

(iv) Freezing

(v) Drying

(vi) Filtration.

2.3.7.1 Pasteurization

The term ‘pasteurization’ was originally named after the French scientist, Louis Pasteur, who invented the process of heating at a mild temperature for a short time to extend the shelf life of liquids (wine and beer). Thermal pasteurization is a relatively mild form of heat treatment that is used to inactivate relatively heat-sensitive microorganisms, such as vegetative bacteria, yeasts, and molds, which are responsible for food spoilage or food poisoning. In addition to microbiologic inactivation, thermal pasteurization is successfully used to inactivate fruit juice enzymes such as polyphenoloxidase (PPO), lipoxygenase (LOX), peroxidase (POD), and pectinmethylesterase (PME), which are responsible for decaying quality. Thus, the shelf life of thermally processed fruit juices can be extended for several months without safety concerns or important quality losses at low or room temperatures (Agcam *et al.*, 2018).

Pasteurization is relatively mild heat treatment, in which food is heated to below 100ºC. In low acid foods (pH > 4.5, for example milk) it is used to minimize possible health hazards from pathogenic microorganisms and to extend the shelf life of food for several days. In acidic foods (pH < 4.5, for example bottled fruit) it is used to extend the shelf life for several months by destruction of spoilage microorganism (yeasts or moulds) and/or enzyme inactivation. In both type of food, minimal changes are caused to the sensory characteristics of nutritive value (Parajuli, 2010).

Minimum processing condition with the purpose of pasteurization for different foods are illustrated in the Table 2.3

Table 2.3 Purpose of pasteurization for different foods

Food	Main purpose	Subsidiary purpose	Minimum processing conditions
pH<4.5 (Fruit juices)	Enzyme inactivation (Pectin esterase and polygalacturanase)	Destruction of spoilage micro-organisms(yeast, fungi)	65ºC for 30 min;77ºC for 1 min:88ºC for 15 s

Beer	Destruction of spoilage micro organisms (wild yeasts, *Lactobacillus* species and residual Yeasts		65°C-68°C for 20 min (in bottle); 72-75°C for 1-4 min a 900-1000 kPa.
pH>4.5 (Milk)	Destruction of pathogens(*Mycobacterium tuberculosis*, *Coxiella burnetti*)	Destruction of spoilage organisms, enzymes.	63°C for 30 min; 71.5°C for 15 s
Ice cream	Destruction of pathogens	Destruction of spoilage organisms.	65°C for 30 min; 71°C for 10 min; 80°C for 15 s.

Source: Fellows (2000)

Fruit juices are pasteurized at such temperatures and for such periods as would render them sterile, without impairing their flavor. Usually, the juices are pasteurized according to the nature of juice and the size of the container. Acid fruit juice requires lower temperature and less time for pasteurization than the less acid ones. Pectin enzymes, which cause changes in the flavor and also bring about the clotting of particles in the juice, can be destroyed by heating the juice at the temperature mentioned above also enzymes requires air for their action and can, therefore be destroyed at a moderate temperature by removing the air from the juice. To obtain satisfactory results, it is essential to keep all equipment perfectly clean and to carry out the work under hygienic conditions. There are a number of methods used for the pasteurization of fruit juice. Some of the common methods are described in the following paragraphs (Parajuli, 2010).

2.3.7.1.1 Bottle method or 'holding' pasteurization

This is used commonly in home scale. The extracted juice is strained, filtered, as required and filled into bottles leaving proper head spaces for the expansion of the juice during heating. The bottles are then sealed airtight and pasteurized (Parajuli, 2010).

2.3.7.1.2 Pasteurization by over flow method

In this method the juice is heated to temperature about 2.5°C higher than the pasteurization temperature and filled into hot sterilized bottles up to the rim, taking care to see that during filling and sealing, the temperature of the juice does not fall below the pasteurization temperature. The bottles should be hot at the time of filling to safeguard against fall of temperature of the juice and to prevent breakage of bottles are pasteurized at a temperature 2.5°C lower than the filling and sealing temperature. After pasteurization, the bottles are cooled. On cooling, juice contracts leaving a small head space, which does not contain any air (Parajuli, 2010).

2.3.7.1.3 Flash pasteurization

This is a process in which the fruit is heated for only a short time at a temperature higher than the pasteurization temperature for the juice. The juice is heated to a temperature 5.5°C higher than the pasteurization temperature and is held at that temperature for about a minute and then filled into container, which are sealed airtight under cover of stream to sterilize the seal, and then cooled (Parajuli, 2010).

2.3.8 Relation of fruit juice acidity and thermal treatment.

The hydrogen-ion concentration of a food is a controlling factor in regulating many chemical, biochemical, and microbiological reactions, and is symbolized by the term pH. Hydrogen-ion concentration is expressed in moles and pH is the negative log ion concentration. The pH value of foods is a deterministic factor of growth and activity of microorganisms. Thus, pH is also important in determining adequate heating requirements. There are three pH groups of foods: (1) high acid foods (pH , 3.7); (2) acid or medium acid foods (3.7 , pH , 4.5); and (3) low acid foods (pH . 4.5) (Agcam *et al.*, 2018).

The most important factor affecting microbial spoilage is acidity, and thermal processing requirements for various foods depend mainly on pH. For example, the main purpose of thermal treatment is destruction of pathogenic bacteria in low-acid foods (pH . 4.5) such as mango, banana, or watermelon, and destruction of spoilage microorganisms or inactivation of specific enzymes for protecting food quality in medium- or high-acid foods (pH, 4.5) such as orange, lemon, or apple juice. The growth or presence of spore-bearing bacteria is not the key risk in acidic foods and killing the spore bearing microorganisms is not the target of the pasteurization process. Thus, pasteurization is applicable for highly acidic foods. The spoilage can be caused by generally non spore forming *Lactobacillus* and *Leuconostoc,* yeast, or molds, in high acid foods. On the other hand, in acidic products such as tomatoes (pH 4.0-4.4), spore-forming bacteria can be a risk factor, especially *Bacillus coagulans*, *Clostridium pasteurianum*, and *Clostridium thermosaccharolyticum*. High-acid fruits contain many enzymes such as catalase, POD, PPO, and some of them (mainly PODs) have higher resistance to heat than the spoilage organisms. Thus, enzyme inactivation can be the target of pasteurization in some cases especially in canned fruit products (Agcam *et al.*, 2018).

2.3.9 Types of pasteurization according to intensity

Nowadays, people tend to consume not only safe and shelf-stable foods, but also foods that are rich in nutrients and are favorable in appearance, while food processors demand high speed, minimum cost and energy lost through food processing techniques. For these reasons, different pasteurization types are developed by researchers. According to intensity of applied heat treatment, there are four groups of conventional pasteurization:

• High-temperature long time (HTLT);

• High-temperature short time (HTST);

• Mild temperature-long time (MTLT); and

• Mild temperature-short time (MTST) (Petruzzi *et al.*, 2017).

HTLT pasteurization with temperatures in the range of 80°C-100°C and duration of less than 30 sec is the most commonly used method in the processing of juices. Low-acid juices with pH 4.5 need stronger treatments to have protected food quality. This treatment type could affect some bioactive compounds (phenolic compounds, flavonoids, and anthocyanins) in a positive way, and can reduce the activity of some enzymes, while other bioactive compounds with health benefits are affected negatively (Petruzzi *et al.*, 2017).

HTST pasteurization is developed to ensure high product quality by minimizing the intensity of heat treatment. HTST treatment, the thermal treatments with temperatures above 80°C for duration lower than 30 s, could reduce enzymes such as PME, PPO, and POD in some juices. Increasing total phenolic, nutritional value, viscosity, and color hue of some juices or nectars were reported in HTST treatment (Petruzzi *et al.*, 2017).

The pasteurization process with a combination of temperature less than 80°C and duration greater than 30 s is called MTLT pasteurization. It is a process that is applied for improving minimally processed food products with longer shelf life (Petruzzi *et al.*, 2017).

MTST pasteurization with norms less than 80°C and 30 sec is a lighter process than the other pasteurization process types. Nevertheless, MTST treatment can affect the physicochemical, sensory, and functional properties of juices (Petruzzi *et al.*, 2017).

2.3.10 Storage stability of fruit juices

All food products are inherently unstable and quality retention depends upon a number of factors, including storage time and temperature. This is recognised in all new product work, in changing or improving existing products, and in modifying process. Storage stability technologies have come a long way in a few short years. This can be seen visually in the evolution of systems for holding products to study storage stability, first in desiccators, to programming storage cabinets, to storage rooms which can be programmed to duplicate the climate of any place on earth (Desrosier and Desrosier, 1977).

2.3.10.1 Effect on colour: The product kept at -17°C or -28°C has a slightly lighter appearance whereas the product stored at 21°C and 37°C tend to darken. The degradation in colour may be due to maillard reaction or may be some other reasons as well (Li *et al.*, 2018). Fruit juices appear darker due to browning. Browning of fruit juice during storage is result of a non-enzymatic chemical reaction between amino acids and reducing sugars called as maillard reaction. HMF (hydroxymethylfurfural), formed in the maillard reaction as well as during caramelization (pyrolysis of sugar), is the main product during storage which cause browning of food (Singh and Sharma, 2017).

2.3.10.2 TSS variation in fruit juices during storage: A gradual rise in TSS value during storage of fruit juice has been reported under all storage conditions which might be associated with continuous increase in hydrolysis of polysaccharides and acids (Bhardwaj and Pandey, 2011). Bhardwaj (2013) proposed about the gradual passage of storage time as a function of increase in TSS which may be due to greater hydrolysis of polysaccharides. Also in in preparation of mixed fruit RTS the slight increase in TSS was observed (Deka and Sethi, 2001). However, this rise in TSS is functional to storage temperature and a direct relation has been reported between increase in TSS and storage temperature. This might be correlated with lower rate of hydrolysis of sugars, polysaccharides and organic acids at lower temperature following the La Chatelier Principles of chemical reactions (Singh and Sharma, 2017).

2.3.10.3 Ascorbic acid (Vitamin C) variation in fruit juices during storage: Vitamin C is important nutrient that possesses antioxidant ability and provides the protection against free radicals (Esteve *et al.*, 2005). Ascorbic acid degradation is common in all consumable items during storage and can occur aerobically as well as anaerobically. However rate of aerobic degradation is 100 to 1000 times faster than anaerobic degradation (Krishnaveni *et al.*, 2001). Vitamin C is light and heat sensitive (Davey *et al.*, 2000), the concentration of vitamin C follows first order kinetics and thus storage time affects vitamin C content (Singh and Sharma, 2017). On normal room temperature storage the degradation of thermo sensible nutrients present in fruit juices such as, ascorbic acid occurs which when may oxidized may result in off flavor (Oliveira *et al.*, 2012).

2.3.10.4 Acidity variation in fruit juices during storage: The titratable acidity of fruits or fruit juice includes the organic acids predominantly present in fruits. These organic acids are of high nutritional values and are useful in extending shelf life of fruit juice during storage. However, these are highly sensitive to temperature, storage condition and duration. The organic acids undergo degradation during storage which might be due to conversion of acids into sugar and salt by invertase enzymes (Singh and Sharma, 2017). Mehmood *et al.* (2008) reported that the acidity can also increase due to the formation of acid by sugars or the breakdown of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances and similar pattern was also found in a juice blend of bottle guard and basil leaves juice by (Majumdar *et al.*, 2011).

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials

3.1.1.1 Yacon (ground apple)

Mature and sound yacon fruit was bought from the farm of Uttarpani, Dhankuta.

3.1.1.2 Table sugar

The table sugar was purchased from the local market of Dharan, Nepal.

3.1.1.3 Citric acid

The citric acid was added to control the browning of the juice as well as for antioxidant property. It was provided from campus laboratory.

3.1.2 Other materials

All Other required materials were obtained from local market of Dharan while the aluminium foil seal was bought from JBS, Itahari. List of other materials used for this work is shown in Table 3.1

Table 3.1 List of other materials used

Material	Material
Muslin cloth	Electric Iron
Plastic bottle (HDPE)	Aluminium foil seal

3.1.3 Equipment

All equipments required for the experiment were obtained from laboratory of Central campus of Technology. List of equipment used for this work is shown in Table 3.2.

Table 3.2 List of equipment used

Physical apparatus	Physical apparatus
Stainless steel vessels	Weighing arrangement
Hand refractometer (0-30ºBx)	Mixer grinder
pH meter	Handheld potato peeler
Autoclave	Knives
Thermometer	Heating arrangement
Titration apparatus	Other routine glassware
Spectrophotometer (Labtronics, India)	Centrifuge (Remi R 24)

3.1.4 Chemicals

All the chemicals required for the experiment were obtained from laboratory of Central campus of Technology. List of chemicals used for this work is shown in Table 3.3.

Table 3.3 List of chemicals used

Chemicals	Chemicals
Potassium metabisulfite (KMS)	Sodium hypochlorite solution
Sodium hydroxide (NaOH)	Phenolphthalein
Buffer solution (4 and 7)	60% ethanol
2,6-Dichlorophenol Indophenols dye	Carrez-I solution
Metaphosphoric acid (HPO_3)	Carrez-II solution
Ascorbic acid	Fehling B solution
Oxalic acid	Fehling A solution
Sodium carbonate	Methylene blue indicator
Hydrochloric acid	

3.2 Methodology

The total work was based on preparation and study of storage stability of yacon juice.

3.2.1 Experimental procedure

The general flow sheet of preparation of yacon juice is given in Fig. 3.1

3.2.1.1 Selection of raw material

Yacon was brought from Uttarpani, Dhankuta where one of the very few commercial yacon farming have been initiated since few years. Due to the harsh journey during the transportation of raw materials, some of the yacon tubers were damaged on arrival at work place but then the damaged tubers were segregated from the good ones.

3.2.1.2 Washing and disinfecting the raw material

Washing was done using plenty of water, rubbing the roots against one another and with the use of a scrubbing brush which easily remove the soil that adheres to the surface of the root. After washing, the roots were submerged in a solution of sodium hypochloride and water with a concentration of 200 ppm. This reduces the microorganism content which is still present on the yacon surface. Sodium hypochloride is one of the most effective, economical and easy to use disinfectants available (Manrique *et al.*, 2005).

3.2.1.3 Peeling of roots

The roots were peeled manually using hand held potato peeler and they were submerged in clean water in order to control browning. The concentration of sugars found in the root increases from center to surface of root. For this reason care was taken not to remove an excessive amount of flesh when peeling since this is where the highest concentration of sugar is found. On the other hand, the skin contains the highest concentration of substances that are catalysts for the oxidation of the juice, must be completely removed (Butler and Rivera, 2004).

3.2.1.4 Extraction of juice and oxidation control

For the extraction of yacon juice, electric mixer grinder was used. Since browning is a major problem, calculated amount of citric acid was added while grinding the juice. In this way the juice came into immediate contact with antioxidants, which prevented yacon juice from oxidation.

3.2.1.5 Straining of juice

The grated pulp obtained from the grinder contains significant amount of suspended insoluble solid that should be eliminated. For this purpose juice was allowed to strain through double folded muslin cloth and analysis of TSS, pH, acidity, total sugar and reducing sugar content was carried out.

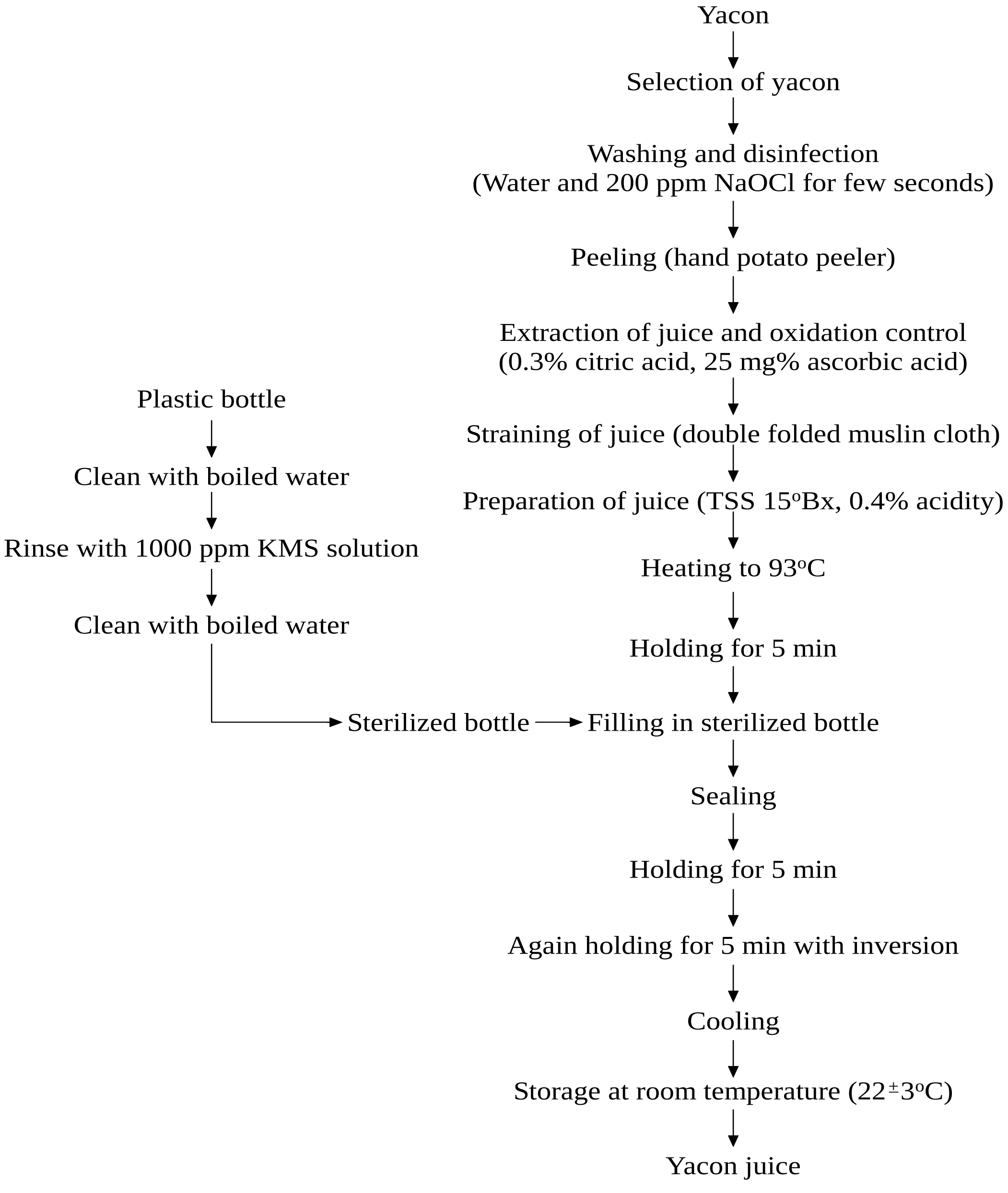

Fig. 3.1 Flow sheet of yacon juice preparation

3.2.1.6 Preparation of juice

After analysis of yacon juice, the acidity of juice was maintained at 0.4% by adding calculated amount of citric acid in the pulp while grinding. Similarly ascorbic acid was also added as per 25 mg% of the total juice which also helps to control browning. The final TSS of 15ºBx was maintained by addition of table sugar.

3.2.1.7 Pasteurization, filling, sealing and cooling

Pasteurization of juice was performed in a stainless steel vessel at 93ºC. When the temperature reached 93ºC, holding of juice was done for 5 min in the vessel.

Soon after the first holding, juice was then filled into the sterilized plastic bottle and again held for 5 min in the bottle. And then the sealing process was performed using aluminum seal along with electric iron i.e. heat sealing. Then the sealed bottles were inverted and holding for 5 min. Later the bottles were cooled and stored at room temperature.

3.2.1.8 Storage stability

Pasteurized juice was filled in pre-sterilized bottles and was stored in room temperature (22±3°C) and the storage stability according to change in total soluble solids, acidity, retention of vitamin C and microbiological changes were studied at 15 days of interval upon 45 days of storage.

3.2.2 Analytical procedure

Although different authors have described different methods and parameters to analyze juice, only those parameters and related methods, which were feasible in the laboratory, were determined in this study. The determination was conducted in triplicates.

For juice TSS, pH, acidity, reducing sugar, total sugar, glucose and fructose were analyzed. The final product was then kept at room temperature for 45 days and analysis on every 15 days was conducted which include determination of TSS, acidity, vitamin C, browning index and microbial analysis including Total plate count (TPC) and Coliform count.

3.2.2.1 Determination of total soluble solid (TSS)

Total soluble solids were determined with hand refractometer (0-30) and values were expressed as °Brix according to Ranganna (2001).

3.2.2.2 Determination of titrable acidity

It was measured by titrating 10 ml clear juice with standard N/10 NaOH and the result are expressed as percent citric acid K.C. and Rai (2007).

3.2.2.3 Determination of reducing sugar and total sugar

The reducing sugar and total sugar of yacon juice was determined as per Lane and Eynon method as described in (Ranganna 2001).

3.2.2.4 Determination of Vitamin C

Ascorbic acid was determined by 2-6-dichloro-indophenol visual titration method as per (Ranganna 2001).

3.2.2.5 Fructose content

The sample was diluted to tenfold from which one ml was taken in test tube. 1 ml of resorcinol reagent and 7 ml of dilute hydrochloric acid were added into the test tube. Pipette out 0.0 (blank), 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard and make up the volume to 2 ml with water. 1 ml of resorcinol reagent and 7 ml of dilute HCl were added as above. All the tubes were added in a water-bath at 80ºC for exactly 10 min. The tubes were cooled by immersing in tap water for 5 min. Color was red at 520 nm within 30 min. Standard graph was drawn and calculated the amount of fructose present in the sample was determined using the standard graph (Sadasivam and Manickam, 1996).

3.2.2.6 Glucose content

In the test tube, 0.5 ml sample was taken 0.5 ml distilled water and 1 ml glucose oxidase peroxidase reagents were added. Into a series of test tubes 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard glucose solution were pipette out and made up the volume to 1 ml with distilled water. Then 1 ml of glucose oxidase peroxidase reagent was added in each test tube. All the tubes were incubated at 35ºC for 40 min. Finally, the color intensity at 540 nm was read. Standard graph was drawn and calculated amount of glucose present in the prepared sample was determined from the standard graph (Sadasivam and Manickam, 1996).

3.2.3 Sensory analysis

The prepared 4 juice samples by varying storage time were subjected to sensory evaluation for consumer's acceptability. Sensory was done using four varieties of products. The samples were served in clean glass beakers at silent environment around 12:00 pm and room temperature was 25ºC. Sensory attributes (such as taste, smell, flavor, color, consistency (Mouth feel),and overall acceptance) were evaluated using 5 points hedonic rating test ranging from faulty (1) to exceptional (5) as described by Ranganna (2001) with the help of 12 semi- trained panelist who were teachers and students of Food Technology Bachelor at CCT and they were familiar with the beverage.

3.2.4 Statistical analysis

The data were analyzed for two way ANOVA, mean ANOVA (No blocking at 5% level of significance), LSD and interaction effects using Genstat (Genstat Discovery Edition 12, 2009) at 5% significance level were obtained to determine whether the sample were significantly different from each other and to determine which one is superior among them. The specimen evaluation card used for the sensory test appears in Appendix A. The mean is compared using LSD method. Standard deviation and means were also analyzed from the same statistical tool.

3.2.5 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48 h) (AOAC, 2005).

3.2.6 Storage studies

Yacon juice was aseptically filled in the plastic bottles. The bottles containing beverages were stored at normal condition (22-25°C) for 45 days. Samples were drawn at intervals of 15 days and evaluated for physicochemical properties (TSS, acidity, ascorbic acid, and browning index) and microbiological qualities (TPC and coliform).

Part IV

Results and discussion

The yacon was collected from Uttarpani, Dhankuta and different physical and chemical analyses were carried. Yacon juice was extracted the acidity was maintained at 0.4% by adding citric acid and TSS was maintained at 15ºBx by adding table sugar. Pasteurization was done at 93°C for 5 min and the product was stored at room temperature for 45 days. The chemical and microbiological changes during storage were studied at every 15 days till 45 days.

4.1 Chemical analysis of yacon (*Smallanthus sonchifolius*) fruit juice.

Chemical composition of yacon fruit juice is given in Table 4.1.

Table 4.1 Chemical composition of yacon fruit (raw) juice

Parameter	Value
Moisture (%)	88% (0)
TSS (ºBx)	11 (0)
Total acidity (% as citric acid)	0.09 (0.01)
pH	5.68 (0.13)
Juice yield (% total fresh weight)	56.67 (2.88)

* Values are the means of three determinations. Figures in the parentheses are the standard deviations.

Above parameters for yacon fruit juice resembled to the data obtained by Shrestha, (2015) in terms of TSS (10°Bx) and also within the range as stated by Pazos *et al.* (1997-1998) (9-12.6°Bx) but slightly different in terms of acidity (0.074%), pH (6.25) and juice yield (60%) were found and this variation could be due to addition of citric acid during juice extraction to prevent juice from oxidation and the variation in juice yield could be due to the maturity and different variety of yacon.

4.2 Chemical analysis of yacon (*Smallanthus sonchifolius*) formulated juice beverage

The chemical composition of the yacon juice beverage was analyzed and the results are shown in Table 4.2

Table 4.2 Chemical composition of yacon juice beverage.

Parameter	Values*
TSS (ºBx)	15(0)
Total acidity (% as citric acid)	0.33(0.01)
pH	3.82(0.01)
Reducing sugar (% dextrose)	4.03(0.15)
Total sugar (% dextrose)	7.98(0.03)
Glucose (mg/100 ml)	160.33(0.57)
Fructose(mg/100 ml)	241.33(0.57)
Vitamin C (mg/100 ml ascorbic acid)	20.86(0.12)

* Values are the means of three determinations. Figures in the parentheses are the standard deviations.

The TSS of the yacon beverage was similar that reported by Shrestha, (2015) (15°Bx) but the total acidity was a bit lower compared to that reported by Shrestha, (2015) (0.33%). The reducing and total sugars of yacon juice beverage were reported to be 4.03 % and 7.98% respectively. Vitamin C (mg/100 ml) of the yacon beverage was found to be higher than that found by Shrestha, (2015) (6 mg/100 ml).

4.3 Storage study

Prepared yacon juice was stored for 45 days' time period at ambient temperature (22±3°C). Chemical and microbiological analyses were carried out at 15 days of intervals.

4.3.1 Microbiological analysis

In microbiological analysis TPC and coliform were performed and the changes in microbial counts during storage are given in Table 4.3.

Table 4.3 Microbiological analysis of product

Days/parameter	Day 0	Day 15	Day 30	Day 45
TPC (cfu/ml)	0	5×10^3	11×10^3	21×10^3
Coliform count (cfu/ml)	0	0	0	0

No coliforms were observed during entire storage period of 45 days.

4.3.2 Chemical changes during storage of formulated yacon juice beverage.

The chemical characteristics of the yacon beverage were analyzed in terms of TSS, acidity, pH and Vitamin C at 15 days intervals and the result are shown in Fig. 4.3.2.1 to 4.3.2.4.

4.3.2.1 Effect on TSS

The effect of storage time on the TSS of the beverage is shown in Fig. 4.1.

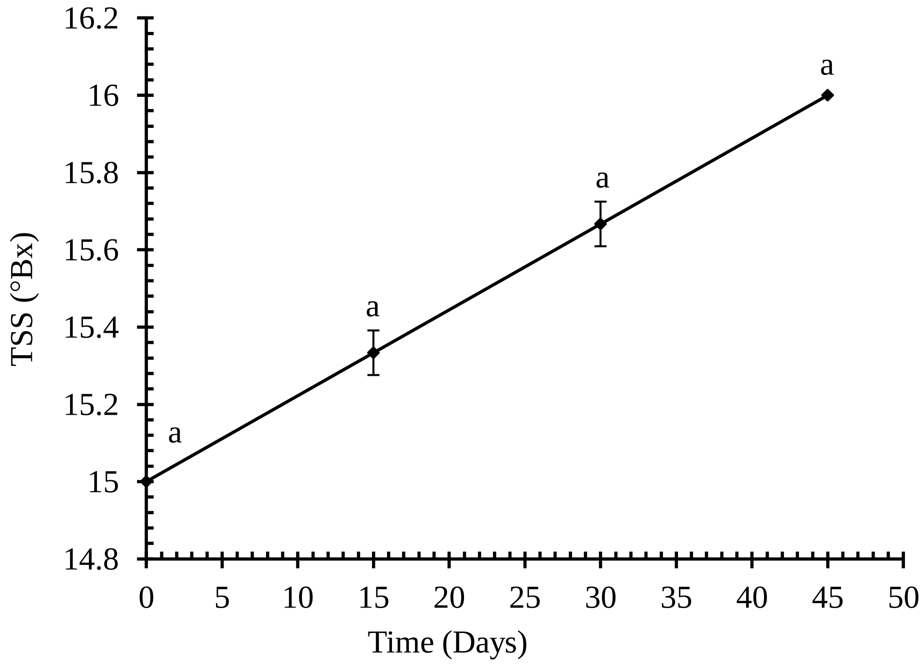

Fig. 4.1 Effect of storage time on TSS of yacon juice*

*****Values are the means of three determinations. Means having similar alphabets are not significantly different by LSD at p>0.05

The TSS values of the beverage were found to be 15, 15.33, 15.66 and 16°Bx for 0, 15, 30 and 45 days of storage respectively. Statistical analysis showed that the storage time doesn't have a significant effect (p>0.05) on the TSS of the beverage. However the values were not significantly different.

Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj and Pandey, 2011). Similar results were observed in the preparation of mixed fruit RTS (Bull *et al.*, 2004; Deka and Sethi, 2001). A gradual rise in TSS value during storage of fruit juice has been reported under all storage conditions which might be associated with continuous increase in hydrolysis of polysaccharides and acids. The gradual passage of storage time as a function of increase in TSS may be due to greater hydrolysis of polysaccharides. However, this rise in TSS is functional to storage temperature and a direct relation has been reported between increase in TSS and storage temperature. This might be correlated with lower rate of hydrolysis of sugars, polysaccharides and organic acids at lower temperature following the La Chatelier Principles of chemical reactions (Singh and Sharma, 2017).

4.3.2.2 Effect on titrable acidity

The effect of storage time on the acidity of the beverage is shown in Fig. 4.2.

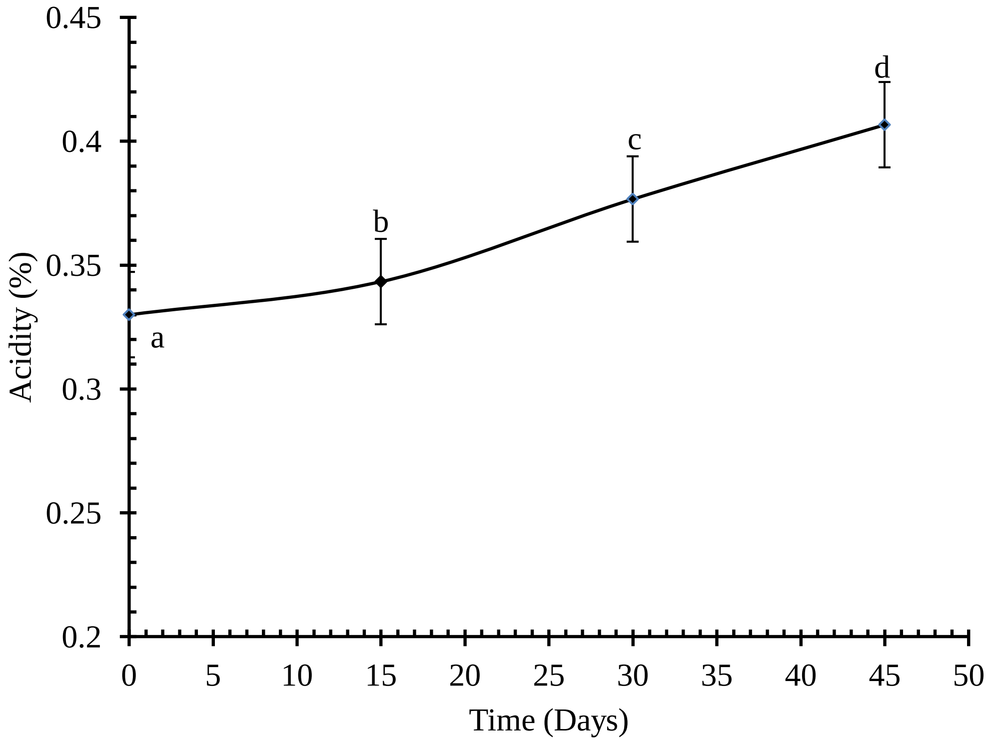

Fig. 4.2 Effect of storage time on titrable acidity of yacon juice*

> ***Values are the means of three determinations. Means having similar alphabets are not significantly different by LSD at $p>0.05$

The acidity values of the beverage were found to be 0.33, 0.34, 0.37, 0.40% for 0, 15, 30, 45 days of storage respectively. The acidity increased steadily with increase in storage time. Statistical analysis showed that storage time had a significant effect ($p<0.05$) on the acidity of the beverage. LSD indicated that the acidity contents of all the samples were significantly different from each other.

The increasing trend in acidity plotted in Fig. 4.2 might be due to the formation of acid by sugars or by breakdown of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances (Mehmood *et al.*, 2008). Similar results were reported for a juice blend of bottle guard and basil leaves juice by Majumdar *et al.* (2011).

4.3.2.3 Effect on pH

The effect of storage time on the pH of the beverage is shown in Fig. 4.3.

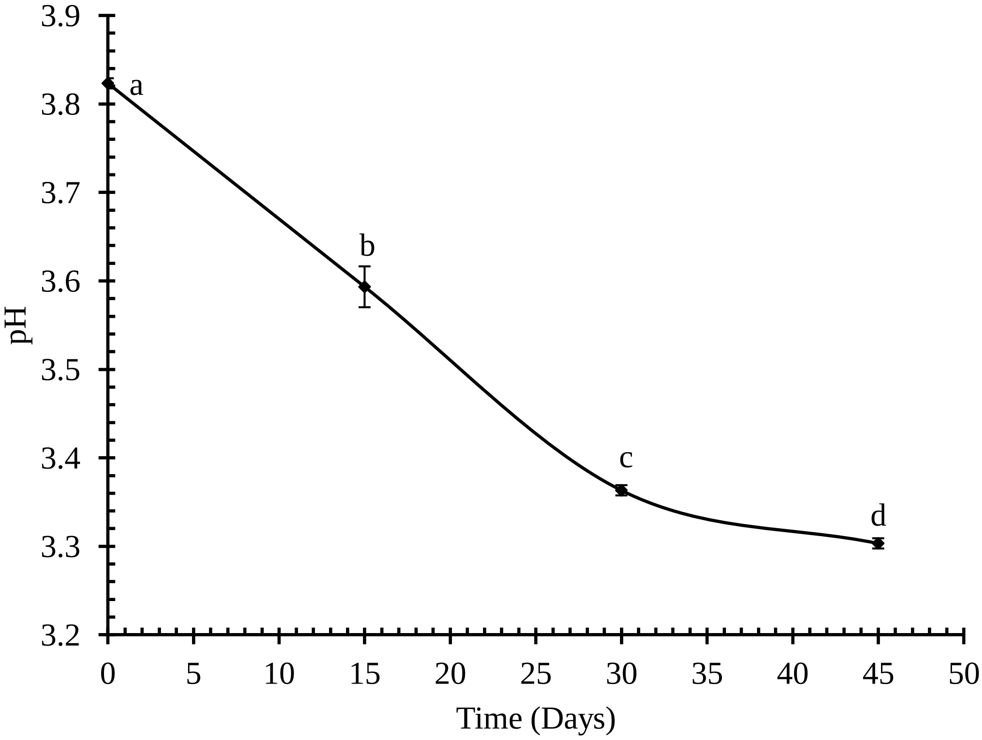

Fig. 4.3 Effect of storage time on pH of yacon juice*

*****Values are the means of three determinations. Means having similar alphabets are not significantly different by LSD at p>0.05

The pH values of beverage were found to be 3.82, 3.59, 3.36 and 3.30 for 0, 15, 30 and 45 days of storage respectively. The pH decreased sharply at first and steadily later in storage time. Statistical analysis showed that storage time had a significant effect ($p<0.05$) on the pH of the beverage. LSD indicated that the pH of all the samples were significantly different from each other.

The decrease in pH of juice might be due to increase in titrable acidity, as acidity and pH are inversely proportional to each other. Similar results were reported for a juice blend of bottle guard and basil leaves juice by Majumdar *et al.* (2011).

4.3.2.4 Effect on Vitamin C

The effect of storage time on the vitamin C of the beverage is shown in Fig. 4.4.

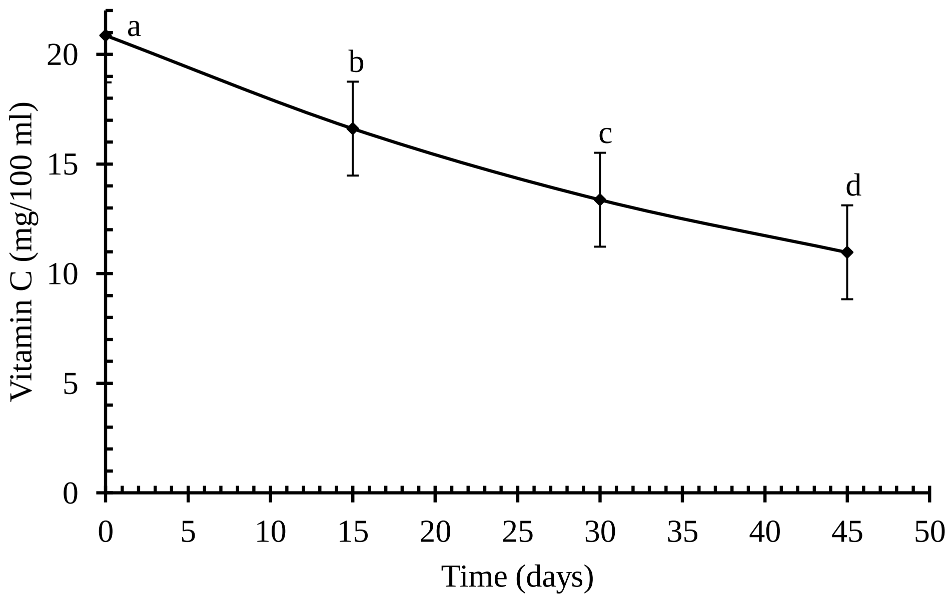

Fig. 4.4 Effect of storage time on Vitamin C content of yacon juice*

*****Values are the means of three determinations. Means having similar alphabets are not significantly different by LSD at p>0.05.

The Vitamin C content values of the beverage were found to be 20.86, 16.61, 13.36, 10.97 mg/100 ml for 0, 15, 30 and 45 days of storage respectively. The vitamin C decreased gradually with increase in storage time. Statistical analysis showed that storage time had significant effect ($p<0.05$) on the vitamin C of the beverage. LSD indicated that the vitamin C contents of all the samples were significantly different from each other.

Vitamin C is an important nutrient that possesses antioxidant ability and provides the protection against free radicals (Esteve *et al.*, 2005). It is also considered an indicator of the nutritional quality of juices. Storage temperature, type of processing and packaging materials affect the rate of ascorbic acid degradation during storage (Bull *et al.*, 2004). This decrease was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst (Davey *et al.*, 2000). Similar results of decrease in ascorbic acid content with the increase in storage intervals in storage stability of jack fruit RTS beverage (Krishnaveni *et al.*, 2001) was reported. Storage temperature is one of the measure contributing factors for ascorbic acid degradation during storage as it is highly thermal sensitive. Pasteurization of fruit juice produces p-vinylguaiacol (PVG) and induces ascorbic acid degradation (Singh and Sharma, 2017).

4.3.2.5 Effect on browning index (BI)

Effect of storage time on browning index of yacon juice is shown in Fig. 4.5. Increase in browning index (BI) at both zero hour and six hour time was observed. Initially at zero day BI at zero hour was 0.35 and six hour reading of same day was 0.38 while that of 45th day were 0.71 and 0.75 respectively.

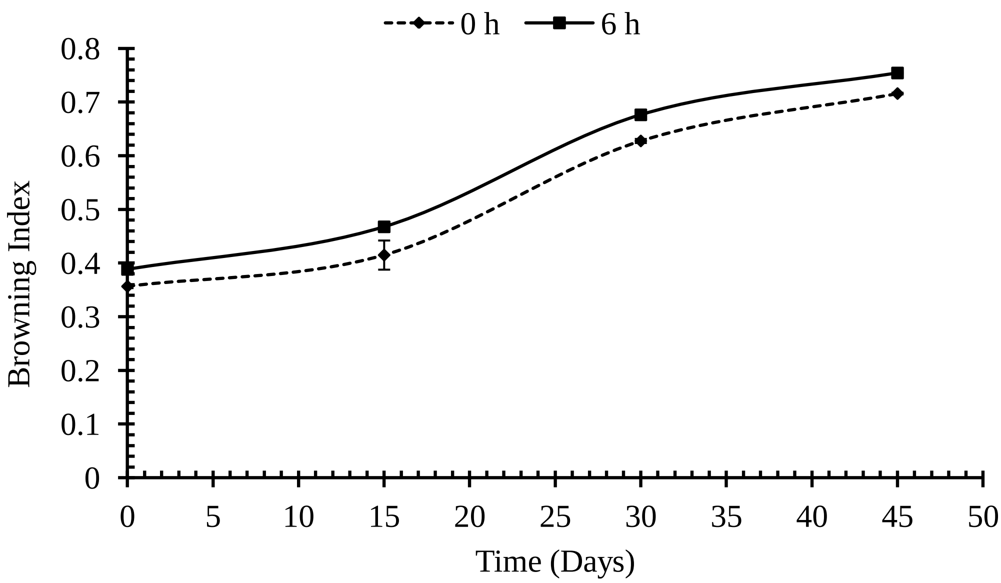

Fig. 4.5 Effect of storage time on browning index (BI) of yacon juice*

*****Values are the means of three determinations. 0 h= immediately after juice preparation and 6 h= after 6 hours of juice preparation.

Browning of fruit juice during storage is result of a non-enzymatic chemical reaction between amino acids and reducing sugars called as maillard reaction. HMF (hydroxymethylfurfural), formed in the maillard reaction as well as during caramelization (pyrolysis of sugar), is the main product during storage which cause browning of food. The formation of HMF is accelerated by storage time and temperature. R. L. Bhardwaj (2013) had reported a liner increase in non-enzymatic browning during storage of blended juice of Kinnow for 6 months.

4.4 Effect of storage time on sensory quality of yacon juice.

Four different samples of different storage time were taken and coded as A, B, C and D respectively. The final product were kept at room temperature for 45 days and beginning from zero day on every 15 day interval the product were taken for chemical and microbiological analysis. The samples were provided to the semi trained panelist in order to evaluate the product on the basis of various sensory parameter namely, taste, smell, flavor, color, consistency (mouth feel) and overall acceptability. The scores were provided in the score sheets as per their perception and was analyzed using Genstat 12.1 using two way ANOVA.

Where, sample A= juice stored for 30 days, sample B= juice stored for 15 days, sample C= juice stored for 45 days and sample D= juice stored for 0 day.

The statistical analysis of the final product on the basis of effect of variation of storage time is shown from 4.4.1 to 4.4.6.

4.4.1 Color

The similar alphabets in the bar diagram Fig. 4.6 indicates that the samples are not significantly different ($p>0.05$). The mean sensory score for samples A, B, C and D were found to be 3.09, 3.81, 3 and 2.72 respectively. The statistical analysis showed significant effect on the product ($p<0.05$) at 5% level of significance. LSD shows that sample A, C and D were significantly different from sample B while sample A, C and D were not significantly different from one another. From sensory evaluation and statistical analysis, it showed that sample B had high mean sensory score.

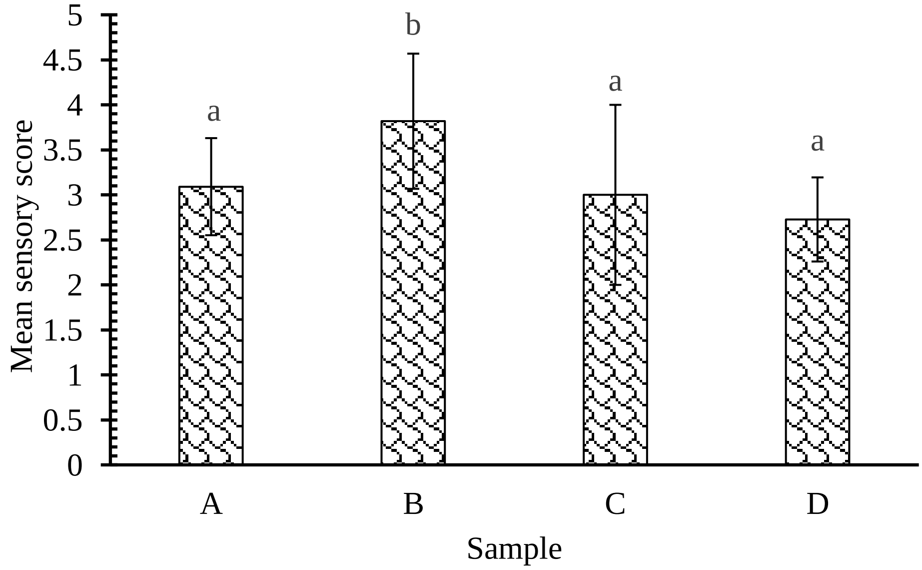

Fig. 4.6 Effect of storage on the color of the yacon beverage*.

* Values are the means of three determinations. Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by panelist.

The product stored at 21°C and 37°C tend to darken. The degradation in colour may be due to maillard reaction or may be some other reasons as well. (Li *et al.*, 2018) Fruit juices appear darker due to browning. Browning of fruit juice during storage is result of a non-enzymatic chemical reaction between amino acids and reducing sugars called as maillard reaction. HMF (hydroxymethylfurfural), formed in the maillard reaction as well as during caramelization (pyrolysis of sugar), is the main product during storage which cause browning of food (Singh and Sharma, 2017). The presence of air causes a marked increase in destruction of ascorbic acid and has some effect upon the change in color (Beattie *et al.*, 1943). These color changes may have been caused by non-enzymatic oxidative degradation of nutrients, such as carotenoids, vitamin C, and other phenolic compounds, which leads to the formation of dark pigments in the product, thus affecting quality and limiting the shelf-life (Oliveira *et al.*, 2012).

4.4.2 Smell

The similar alphabets in the bar diagram Fig. 4.7 indicates that the samples are not significantly different (p>0.05). The mean sensory score for samples A, B, C and D were found to be 3.45, 3.81, 2.27 and 2.36 respectively. The statistical analysis showed significant effect on the product (p<0.05) at 5% level of significance. LSD shows that the product A & B and sample C & D were significantly different from one another while sample A and B and sample C and D were not different from each other. From sensory evaluation and statistical analysis, it showed that sample B had high mean sensory score.

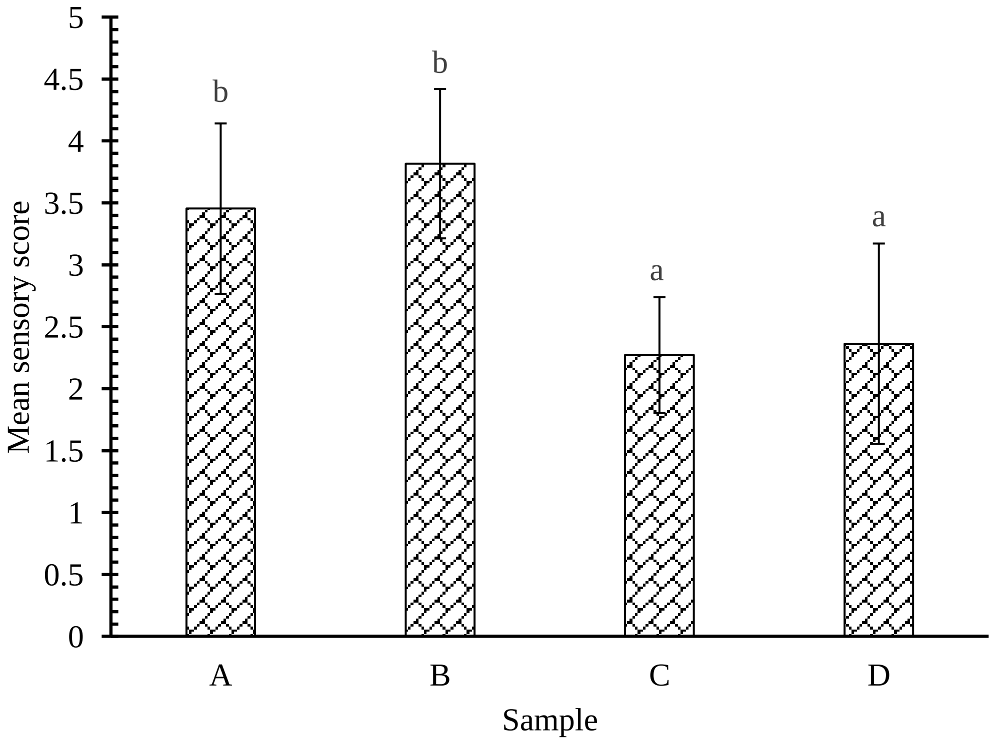

Fig. 4.7 Effect of storage on the smell of the yacon beverage*.

* Values are the means of three determinations. Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by panelist.

In clarified apple juice, it was observed that there was an increase in hexanal concentration during 90 days of storage under room temperature. However the deterioration of odor in apple juice resulted due to non enzymatic oxidation of saturated compounds (Correa *et al.*, 2010).

4.4.3 Taste

The similar alphabets in the bar diagram i.e. Fig. 4.8 indicates that the samples are not significantly different ($p>0.05$). The mean score for taste of the samples A, B, C and D are 3.45, 4.09, 2.18 and 2.54 respectively. Statistical analysis showed that there is significant difference ($p<0.05$) within the samples at 5% level of significance. Samples A and B were significantly different from one another while sample C and D were not different from each other but sample A and B are significantly different from sample C and D. From sensory evaluation and statistical analysis, it showed that sample B had high mean sensory score.

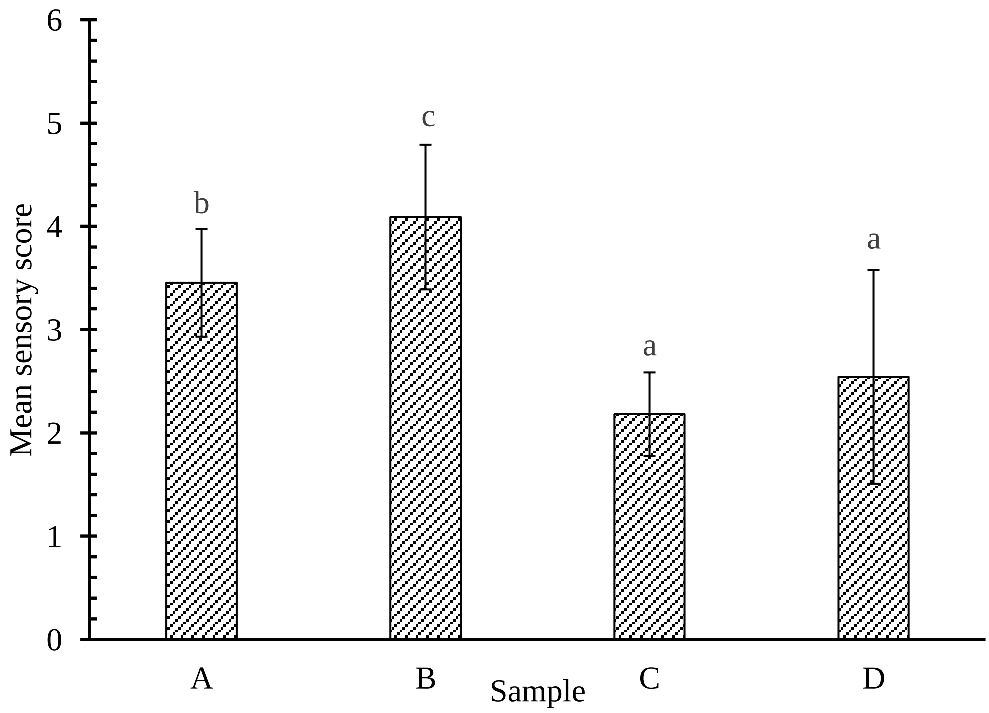

Fig: 4.8 Effect of storage on the taste of the yacon beverage*.

* Values are the means of three determinations. Bars sharing similar letters are not significantly different by LSD at $p>0.05$. Vertical error bars represent standard deviation of scores given by panelist.

A gradual rise in TSS value during storage of fruit juice has been reported under all storage conditions which might be associated with continuous increase in hydrolysis of polysaccharides and acids (Singh and Sharma, 2017). High amount of sugar ratio in the sample was not preferred by the panelists (Parajuli, 2010).

4.4.4 Flavor

The similar alphabets in the bar diagram Fig. 4.9 indicates that the samples are not significantly different ($p>0.05$). The mean sensory score for samples A, B, C and D were found to be 3.09, 3.72, 2.27 and 2.27 respectively. The statistical analysis showed significant effect on the product ($p<0.05$) at 5% level of significance. LSD shows that samples A and B were significantly different from one another while sample C and D were not different from each other but sample A and B are significantly different from sample C and D. From sensory evaluation and statistical analysis, it showed that sample B had high mean sensory score.

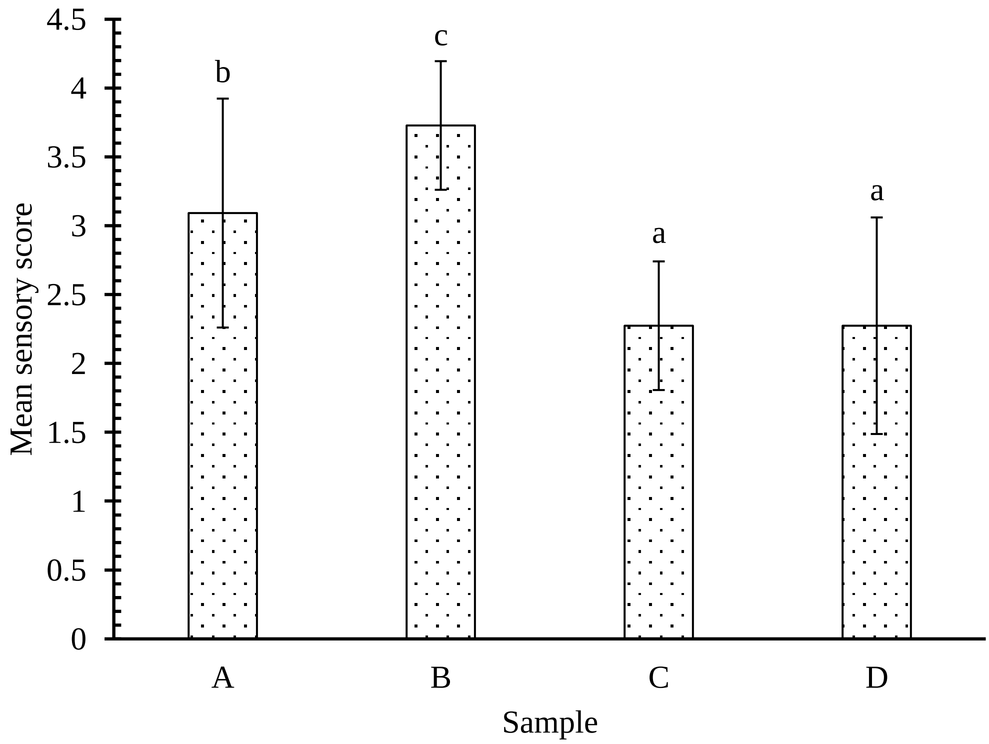

Fig. 4.9 Effect of storage on flavor of the yacon beverage*.

* Values are the means of three determinations. Bars sharing similar letters are not significantly different by LSD at $p>0.05$. Vertical error bars represent standard deviation of scores given by panelist.

The maintenance of flavor in juices depends mainly on the destruction of certain enzymes during heat treatment. Thus, the fact that the product was previously pasteurized probably contributed to the maintenance of flavor at room temperature. However, the storage temperature might have caused an increased rate of degradation of thermo sensible nutrients present in juice, such as vitamin C, which, when oxidized may result in off-flavor (Oliveira *et al.*, 2012).

4.4.5 Consistency (Mouth feel)

The similar alphabets in the bar diagram Fig. 4.10 indicates that the samples are not significantly different ($p>0.05$). The mean sensory score for samples A, B, C and D were found to be 3.36, 3.09, 2.54 and 2.27 respectively. The statistical analysis showed significant effect on the product ($p<0.05$) at 5% level of significance.LSD shows that the product A&B and sample C&D were significantly different from one another while sample A and B and sample C and D were not different from each other. From sensory evaluation and statistical analysis, it showed that sample B had high mean sensory score.

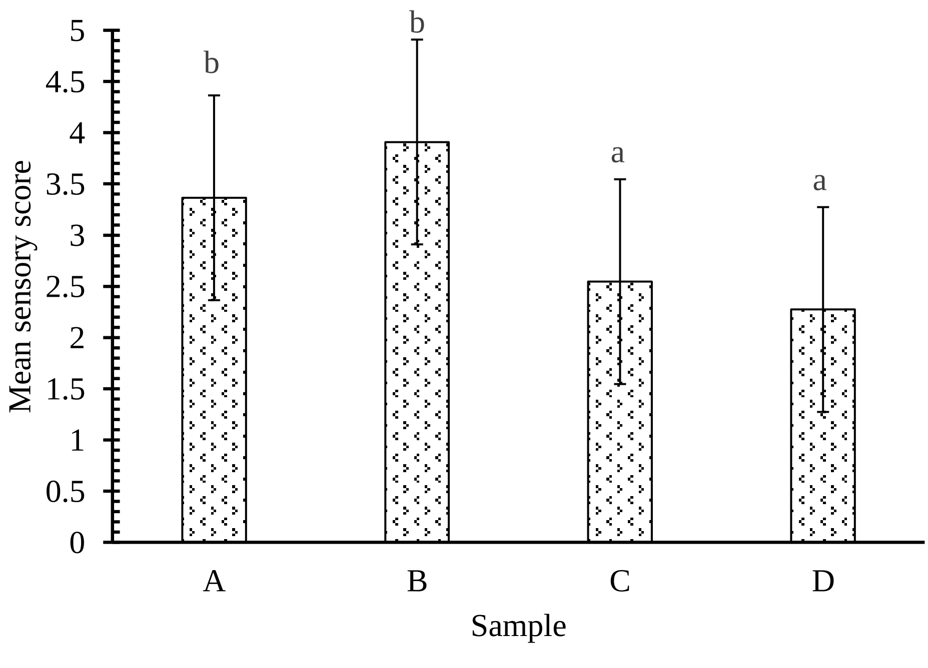

Fig. 4.10 Effect of storage on the consistency (mouth feel) of the yacon beverage*.

* Values are the means of three determinations. Bars sharing similar letters are not significantly different by LSD at $p>0.05$. Vertical error bars represent standard deviation of scores given by panelist.

Consistency maintenance is an indication that the heat processing of the product was effective in inactivating the enzymes responsible for changes in consistency in fruit juices, such as pectinesterase, which catalyze the degradation of pectin to pectic acid and methanol (Oliveira *et al.*, 2012).

4.4.6 Overall acceptance

The similar alphabets in the bar diagram Fig. 4.11 indicates that the samples are not significantly different (p>0.05). The mean sensory score for samples A, B, C and D were found to be 3.182, 4, 2.63 and 2.27 respectively. The statistical analysis showed significant effect on the product (p<0.05) at 5% level of significance. LSD shows that sample A, B, C and D were significantly different from one another. From sensory evaluation and statistical analysis, it showed that sample B had high mean sensory score.

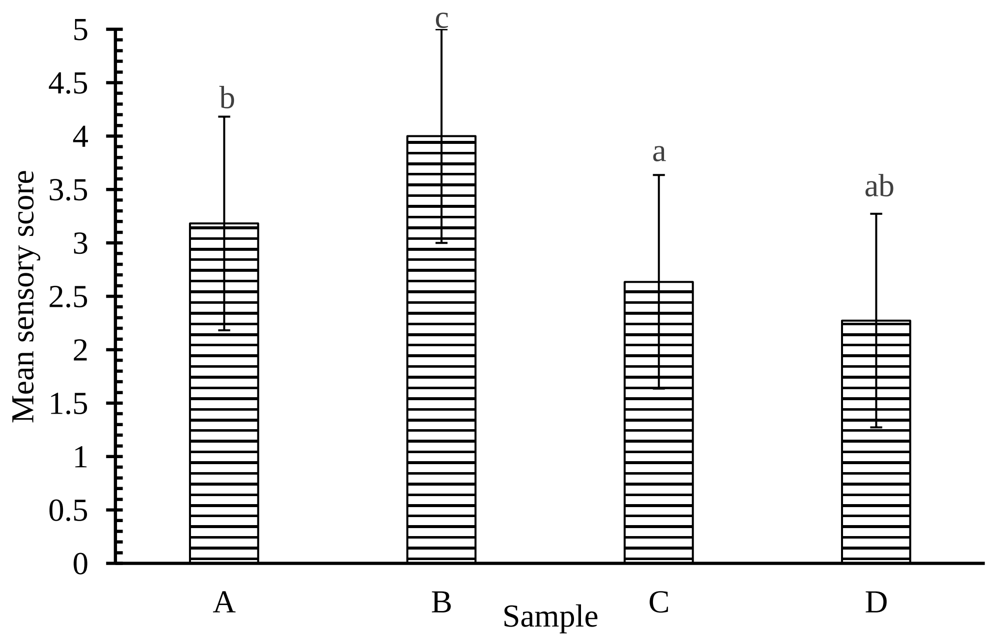

Fig. 4.11 Effect of storage on the overall acceptance of the yacon beverage*.

* Values are the means of three determinations. Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by panelist.

Statistical analysis showed that there was significant difference in terms of taste, smell, flavor, color and consistency of the product at 5% level of significance. Hence, Sample B was found superior through sensory analysis.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the results and discussion of the study, the following conclusions were drawn:

1. Addition of citric acid during the pulping prevented enzymatic browning in the extracted juice.
2. The TSS, acidity, browning index and microbial load increased while pH and ascorbic acid decreased during storage.
3. From sensory analysis, yacon juice stored for 15 days was found to be superior with respect to color, smell, taste, flavor, consistency and overall acceptance.
4. The microbial count was within the range up to 30 days of storage.

5.2 Recommendations

Based on the present study, the following recommendations have been made:

1. Yacon juice can be prepared by using 0.4% acidity, 15ºBx TSS and 25 mg% vitamin C at large scale.
2. Shelf life of juice (storage stability) can be studied using different chemical preservatives, packaging materials and storage conditions.
3. FOS (fructooligosaccharide) content of prepared juice can be measured.

Part VI

Summary

In this study, yacon was taken from Dhankuta, which is one of the districts for commercial cultivation of yacon in Nepal. And other essential materials (citric acid, sugar and juice bottle) and other chemical and apparatus were obtained from local market of Dharan and campus laboratory. First, yacon was subjected to preliminary operations like sorting, washing with plenty of water and scrubbing brush, disinfecting them by 200 ppm sodium hypochloride solution, peeling them with potato peeler and immediately submerged to clean water inorder to prevent it from browning. After this yacon were subjected to mixer grinder for extraction of yacon juice which is susceptible to oxidation hence to preserve it calculated amount of antioxidant i.e. both citric acid and ascorbic acid were added before grinding the pulp. The juice obtained was filtered through muslin cloth. Thus obtained juice was then filtered through muslin cloth and kept in stainless steel vessel. To the juice obtained calculated amount of table sugar was added in order to maintain the TSS of juice at 15°Bx. After that the juice was heated at 93°C holding for 5 min. Then the pasteurized juice was aseptically filled in sterilized plastic bottles and again held for 5 min, later the bottles were sealed with aluminum seal using electric iron and the bottles were inverted again holding for 5 min. Lastly the sealed bottles were cooled to room temperature and stored for 45 days.

Physicochemical analysis of raw juice of yacon (without addition of citric acid) included TSS 11°Bx, 0.09% (% citric acid) acidity, 5.68 pH and 56% juice yield. The pH, acidity, TSS, vitamin C, reducing sugar, total sugar, glucose, fructose and TPC of juice on 0 day were found to be 3.82, 0.33%, 15°Bx, 21.06 mg/100 ml, 4.03 % dextrose, 7.98 % dextrose, 160.33 mg/100 ml, 241.33 mg/100 ml and nil respectively. The prepared juice was stored at room temperature (22±3°C) and physicochemical as well as microbial changes of juice during storage time of 45 days period were observed on every 15 day interval starting from 0 day to 45th day. During storage study TSS, browning index (BI) and acidity increased while pH and vitamin C content decreased. From sensory evaluation the juice stored for 15 days was found to be superior and had 15.33°Bx TSS, 0.34% acidity (as % citric acid), 3.59 pH, 16.61 mg/100 ml ascorbic acid and from microbiological analysis TPC 5×10^3 cfu/ml.

The present study concludes that the juice can be stored at room temperature (22±3°C) without adding any chemical preservative with desirable acceptability up to 45 days. Consequently, yacon juice holds a lot of promise from commercial point of view.

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Appendices

Appendix A

SPECIMEN CARD FOR SENSORY EVALUATION

Name of the product: *Yacon juice*

Dear panelist, you are given 4 samples of *Yacon juice* on each proportion with variation on *storage time.* Please taste the sample and score how much you prefer the each one. Please give points for your degree of preference for each parameter as shown below using the scale given.

Parameter	Sample A	Sample B	Sample C	Sample C
Taste				
Smell				
Flavor				
Color				
Consistency (mouthfeel)				
Overall acceptance				

Give points as follows:

1=Poor 2=fair 3=Satisfactory 4=Good 5=Excellent

Comments (If any)………………………………………………………………………

Signature………………

Appendix B

ANOVA result for sensory analysis of yacon juice

Table B.1 ANOVA (no blocking) for taste of yacon juice

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	24.7955	8.2652	16.04	<.001	0.625
Panelist	10	4.5455	0.4545	0.88	0.559	1.036
Residual	30	15.4545	0.5152			
Total	43	44.7955				

Table B.2 ANOVA (no blocking) for smell of yacon juice

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	19.8864	6.6288	13.84	<.001	0.603
Panelist	10	2.7273	0.2727	0.57	0.825	0.999
Residual	30	14.3636	0.4788			
Total	43	36.9773				

Table B.3 ANOVA (no blocking) for flavor of yacon juice

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	7.1591	2.3864	4.45	0.011	0.638
Panelist	10	4.6364	0.4636	0.86	0.575	1.058
Residual	30	16.0909	0.5364			
Total	43	27.8864				

Table B.4 ANOVA (no blocking) for color of yacon juice

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	16.4318	5.4773	10.73	<.001	0.622
Panelist	10	2.1364	0.2136	0.42	0.927	1.032
Residual	30	15.3182	0.5106			
Total	43	33.8864				

Table B.5 ANOVA (no blocking) for consistency of yacon juice

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	18.6136	6.2045	9.24	<.001	0.713
Panelist	10	2.2273	0.2227	0.33	0.965	1.183
Residual	30	20.1364	0.6712			
Total	43	40.9773				

Table B.6 ANOVA (no blocking) for overall of yacon juice

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	18.6136	6.2045	11.9	<.001	0.629
Panelist	10	2.7273	0.2727	0.52	0.86	1.043
Residual	30	15.6364	0.5212			
Total	43	36.9773				

Appendix C
ANOVA result for chemical changes during storage of yacon juice

Table C.1 ANOVA (no blocking) for acidity of yacon juice.

Source of variation	d.f	s.s	m.s	v.r	F pr.	l.s.d
Sample	3	0.01069	0.00356	71.28	<.001	0.01331
Residual	8	0.0004	0.00005			
Total	11	0.01109				

Table C.2 ANOVA (no blocking) for TSS of yacon juice

Source of variation	d.f	s.s	m.s	v.r	F pr.	l.s.d
Sample	3	1.6667	0.5556	3.33	0.077	0.769
Residual	8	1.3333	0.1667			
Total	11	3				

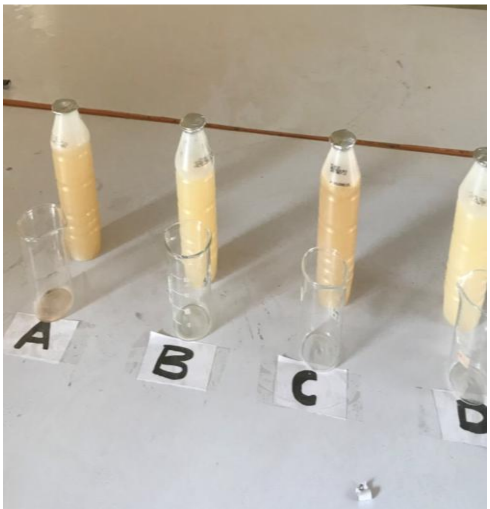
Table C.3 ANOVA (no blocking) for pH of yacon juice

Source of variation	d.f	s.s	m.s	v.r	F pr.	l.s.d
Sample	3	0.50662	0.168875	1066.58	<.001	0.02369
Residual	8	0.00126	0.000158			
Total	11	0.50789				

Table C.4 ANOVA (no blocking) for Vitamin C of yacon juice

Source of variation	d.f	s.s	m.s	v.r	F pr.	l.s.d
Sample	3	173.058	57.68614	874.03	<0.001	0.4837
Residual	8	0.528	0.066			
Total	11	173.5864				

Color Plates



P1: Samples

P2: Microbiological analysis

P3: Sensory evaluation

P4: Samples for browning index evaluation