Recent Advances In Production Of Non-Alcoholic Naturally Carbonated Beverages

Sweety Kaur[a,ł], Richa Arora[a*]

[a]School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

[ł]Present address: Nestle India Limited, Rajarhat, Kolkata, India

Abstract: India produces about 50 million tonnes of fruits per year but only 2% of this goes for processing, while over 25% is spoiled due to improper handling and storage. Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. The fermentation debitters the beverage, retains nutrients and additionally CO_2 produced adds tangy taste and sparkle to beverage. Several studies and development has been done in the area of production of high alcoholic beverages, a little study has been done on non-alcoholic naturally carbonated beverages. This review investigates characterization of yeast employed for fermentation, factors influencing fermentation procedure, chemical characterization of the fermentated product, analysis of the CO_2 production along with its antimicrobial mechanism and different types of non-alcoholic beverages with their characteristic properties and various studies based on them. In conclusion, compared to fruit juices the formulation of naturally carbonated fruit beverage offers more variety of flavors, nutrients, long shelf life and other physiological benefits with the greater margin of safety in a drink with a lower inherent cost.

Keywords: Fermentation, organic compounds, yeast, alcoholic and non-alcoholic beverages, antimicrobial mechanism of CO_2, shef-life and carbonated juices/beverages.

Introduction

India stands second in the world for production of fruits and vegetables owing to the remarkable diversity of its geographical conditions. The country produces about 50 million tonnes of fruits per year but only 2% of this goes for processing, while over 25% is spoiled due to improper handling and storage, which results in quantitative and qualitatively losses (flavor, texture, nutritional value and safety) (Singh and Goswami 2006).

With commercially important citrus crops like mandarins, sweet orange, limes and lemons, citrus industry of India is among the leading and major citrus producing countries contributing in several million tons of turnovers. Altogether approximately 17.35 million tons of lime and lemon fruit are cultivated over world-wide covering 1.08 million hectare of cultivated field including maximum producing countries like like India, Mexico, China, Argentina and Brazil with gross annual production of about 61.32% (FAOSTAT, **2018**). However, when the crop is harvested, most of the perishable fruits are lost during their journey through the agric-food chain, due to microbial spoilage, physiological decay, water loss, mechanical damage during harvesting, packaging and transporting thereby creates a serious problem for their storage, transport, marketing and contamination by mycotoxin producing fungi (Aniche 2003).

To make seasonal fruits available throughout the year in the form of beverage and safeguard the interest of progressive horticulturists, a reliable, controllable and reproducible technology has been developed for the production of Non-

alcoholic naturally carbonated beverage with retention of all the nutrients. Compared to fruit juices the formulation of naturally carbonated fruit beverage offers more variety of flavors, nutrients, long shelf life and other physiological benefits with a greater margin of safety in a drink with a lower inherent cost.

Lemon [(*Citrus limon (L) Burman*] belonging to family Rutaceae is a cultivated hybrid derived from wild species such as citron and mandarin. Gopalan et al (2002) reported that one hundred gram edible portion of lemon fruit contains ascorbic acid (39 mg), minerals (K-270 mg, Ca-70 mg, and P- 10 mg), carbohydrates (11.1g) and 57 Kcal of energy. Lemon juice is reported to have antiseptic, antiscorbutic, antioxidative, anti-inflammatory effects, dietic and medicinal values and reduces cholesterol. Hill lemon (*Citrus pseudolimon Tan.*) also called as Galgal is a well known cultivar of lemon grown extensively in the mountains and sub-mountaineous regions of North-Western Himalayan ranges of the country including Himachal Pradesh, Punjab and some parts of Uttar Pradesh. Citrus fruits are excellent source of vitamin C comprising of trace elements such as iron, copper, potassium and calcium with desirable amount and presence of ascorbic and tannic acids within juice are a great source of antioxidants (J. Singh, 2017). The nutritional and therapeutic value of lemon provides ample scope for processing into a value added fermented product with retention of organoleptic properties, nutritional attributes, characteristics sensory properties flavor, aroma and texture and long shelf life. The fermentation debitters the beverage, retains nutrients and additionally CO_2 produced adds tangy taste and sparkle to beverage.

Though a lot of work has been done in the area of production of high alcoholic beverages, a little study has been done on non-alcoholic naturally carbonated beverages. Coupled with increasing demand for soft drinks, there is considerable scope for developing non-alcoholic naturally carbonated beverages. The work related to present study investigates characterization of yeast employed for fermentation, factors influencing fermentation procedure, chemical characterization of the fermentated product, analysis of the CO_2 production along with its antimicrobial mechanism and different types of non-alcoholic beverages with their characteristic properties and various studies based on them.

Yeast characterization

Lachance *et al* (2000) studied the ribosomal DNA of the cactophilic yeast species *Clavispora opuntiae* in order to clarify the global distribution of the yeast. Over 500 strains, including isolates from several new localities worldwide, were characterized by rDNA restriction mapping. An unusual restriction pattern previously encountered only in one strain from Conception Island (Bahamas) was found in several Brazilian isolates. Sequences of the D1/D2 and D7/D8 divergent domains of the large subunit (LSU) and of the intergenic spacers (IGS) confirmed that these strains represent a genetically distinct variety of *Clavispora opuntiae*.

Fonesca *et al* (2000) isolated an undescribed anamorphic yeast species of ascomycetous affinity, *Candida tartarivorans*, from dried wine lees in Portugal using a selective medium with L (+)-tartaric acid as the sole source of carbon and energy. The single isolate (IGC 4854) showed the following characteristics: sympodial holoblastic conidiogenesis, absence of asci with ascospores, a negative colour reaction with Diazonium Blue B (DBB), production of elaborate pseudomycelium, and ability to grow with inositol as sole source of carbon. Analysis of the physiological data pointed to a close relationship with other inositol-assimilating taxa, namely the genera *Arxula*, *Stephanoascus*, *Sympodiomyces*, *Zygoascus* and selected *Candida* species. The comparative analysis of the D1/D2 variable domain of the 26S rRNA gene of all available sequences for ascomycetous yeasts showed that strain IGC 4854 did not match with any other species in the database. The closest relative was *Candida auringiensis* Santa Maria but the two species differed in 24 nucleotide positions.

Escalante-Minakata *et al* (2008) identified eleven different micro-organisms by restriction and sequence analysis of the amplified region, between 18S and 28S rDNA and 16S rDNA genes in the mezcal fermentation from Agave salmiana. Three of them were the following yeast: *Clavispora lusitaniae, Pichia fermentans* and *Kluyveromyces marxianus*. The bacteria found were *Zymomonas mobilis* subsp. *mobilis* and *Zymomonas mobilis* subsp. *pomaceae*, *Weissella cibaria*, *Weissella paramesenteroides*, *Lactobacillus pontis, Lactobacillus kefiri*, *Lactobacillus plantarum* and *Lactobacillus farraginis.* The phylogenetic analysis of 16S rDNA and ITS sequences showed that microbial diversity present in mezcal is dominated by bacteria, mainly lactic acid bacteria species and *Zymomonas mobilis*. *Pichia fermentans* and *K. marxianus* could be micro-organisms with high potential for the production of some volatile compounds in mescal.

The yeasts with similar physiological properties has been examined using recently published phylogenetic analyses of 26S domain D1/D2 rDNA nucleotide sequences from all currently recognized ascomycetous yeasts. Unique metabolic pathways were examined, a relationship between physiology and rDNA phylogeny was evident. Yeasts subject to the petite mutation, resulting in respiratory deficiency, belong to three different clades, viz. a *Saccharomyces* clade delimited by *S. cerevisiae* and *S. rosinii*, the *Dekkera/Brettanomyces* clade, and some *Schizosaccharomyces* species (*'Archiascomycete'* clade). However, petite mutants were also found in *Zygosaccharomyces fermentati* and some other more distantly related species. Yeasts able to assimilate n-hexadecane, uric acid or amines as sole carbon source are broadly distributed over the ascomycetous phylogenetic tree. However, species that assimilate adenine as sole carbon source are closely related. Most of these species also assimilated glycine, uric acid, n-hexadecane, putrescine and branched-chain aliphatic compounds such as isobutanol, leucine and isoleucine. Among the *Saccharomycetales*, species utilizing all or the great majority of these eight compounds are in the *Stephanoascus/Arxula/Blastobotrys* clade. *Candida blankii*, which is distantly related to this clade, proved to be an exception and assimilated six of eight of these compounds (Middelhoven and Kurtzman 2003).

Ten different versions of the D1/D2 divergent domain of the large-subunit ribosomal DNA were identified among interbreeding members of the yeast species *Clavispora lusitaniae* (Lachance *et al* 2003). One major polymorphism, located in a 90-bp structural motif of the D2 domain, exists in two versions that differ by 32 base substitutions. Three other polymorphisms consist of a two-base substitution, a two-base deletion, and a single-base deletion, respectively. The polymorphisms are independent of one another and of the two mating types, indicating that the strains studied belong to a single, sexually active Mendelian population.A total of 194 bacterial isolates and 187 yeast isolates from the surfaces of four Irish farmhouse smear-ripened cheeses were identified at the midpoint of ripening using pulsed-field gel electrophoresis (PFGE), repetitive sequence-based PCR, and 16S rRNA gene sequencing for identifying and typing the bacteria and Fourier transform infrared spectroscopy and mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP) analysis for identifying and typing the yeast. The yeast microflora was very uniform, and *Debaryomyces hansenii* was the dominant species in the four cheeses. *Yarrowia lipolytica* was also isolated in low numbers from one cheese (Jérôme Mounier *et al* 2005).

Factors affecting Fermentation:

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. Yeasts respond to changes in environment, such as glucose concentration, availability of other nutrients, osmotic pressure, heat, and other stresses by regulatory mechanism. An environmental signal is sensed by a receptor in the cell membrane, being forwarded through a signaling pathway to

the nucleus, where it results in altering the transcription of genes, eventually manifested in response of activation, induction and repression or derepression of enzymes or other proteins. In the cytoplasmic membrane, various G (guanine nucleotide binding) proteins are the most frequent signal receptors; substrate carriers and other transporters (Boles and Andre 2004).

Rolland *et al* (2002) reported that in responding to nutrients, stresses and other environmental signals, signaling pathways such as protein kinase A-cyclic AMP and the mitogen-activated protein (MAP) kinase pathways play important role in diverse cellular functions, such as cell cycle, budding, flocculation, differentiation and mating in yeasts. Those parameters that influence fermentation process are as follows:

(i) Effect of Substrate Concentration:

In a fermentative process, the variable used to establish the process kinetic has been usually the variation of biomass (synthesis) in the system, variation of substrate content, variation of a product (metabolite), determination of O_2 consumed or CO_2 evolved, heat evolved etc. Among these variables, biomass synthesis during the process and substrate consumption determination usually represent kinetically any fermentation process (Pandey *et al* 2001).Considering biomass variation during running time in the process it can be defined as a general manner that this variation depends on several factors, so:

$$dx/dt = f(x,s,T) \quad (1)$$

where; x = biomass concentration (g biomass/l); t = time (h) ; s = substrate (g substrate/l);
T = temperature (^oC)

First term in expression (1) is a velocity or kinetic term. It represents a unit change (biomass concentration) by time unit which can be used to determine parameters such as maximum cell concentration obtained in the fermentation, time delay in the rise of biomass content in the reactor, substrate consumed in the process at any time, yield obtained in the process. A mathematical model that related biomass synthesis with substrate consumed. The model is:

$$\mu = \mu_{max} . S/ (K_s + S) \quad (2)$$

where; μ = specific growth rate (h^{-1}); μ_{max} = maximum specific growth rate (h^{-1}); S = substrate concentration (g/l); K_s = affinity constant biomass/substrate (g/l)

Specific growth rate (μ) is related with velocity or rate (dx/dt) as well as the intensity relating the velocity or rate term with the quantity of biomass present at a particular time in the fermenter. The parameters μ, μ_{max} and K_s determine values for a particular process. At the same time it must be pointed out that specific growth rate is a definition and so is independent of the process. This definition is expressed as :

$$\mu = (1/X) (dX/dt) \quad (3)$$

where; X=biomass concentration at a particular time t (g/l); t=time (h); μ= specific growth rate (h^{-1})

Specific growth rate (μ) defined through expression (3) had permitted the representation of kinetic pattern of microbial growth through different phases known as growth phases, expressed as lag phase ($\mu \sim 0$), accelerated growth phase ($\mu > 0$), exponential growth phase ($\mu = \mu_{max}$), desacelerated growth phase ($\mu_{max} > \mu > 0$), stationary growth phase ($\mu = 0$) and negative growth rate phase ($\mu < 0$). Applying to equation (3), the statement that in the exponential phase $\mu = k$, where k is a constant, it can be stated that:

$$\ln X/X_0 = \mu t \quad (4)$$

where; X=biomass concentration at time t of the exponential growth (g/l); X_0 = biomass concentration at time t = t_0 (g/l); μ= specific growth rate at the exponential phase (h^{-1}); t=time that corresponds to the biomass concentration X (h)

From equation (4), doubling time value can be obtained i.e. the time that takes the biomass concentration to achieve its double value:

$$t_d = \ln 2/\mu_{max} = 0.693/\mu_{max} \quad (5)$$

where t_d is the doubling time (h).

Yield based on substrate consumption ($Y_{x/s}$) and is defined as: $\mathbf{Y_{x/s} = S_0\text{-}S_t / X_t\text{-}X_0}$

where; $Y_{x/s}$ = yield based on substrate consumption (dimensionless)**;** S_0 = initial substrate concentration (g/l)**;** S_t = final substrate concentration (g/l)**;** X_0 = initial biomass concentration (g/l)**;** X_t = final biomass concentration (g/l).

Devine and Slaughter (1980) studied the effect of medium composition on ethanol production. Panchal and Stewart (1980) reported that when the substrate concentration is increased beyond 25 per cent, the effect of osmotic pressure becomes pronounced which seriously affects fermentation efficiency and leads to decreased ethanol production.

Lafourcade (1983) observed that the ability of yeast to ferment sugar decreases with increase in sugar concentration at initial stages. In the musts with elevated levels of sugars, the final part of fermentation is conducted by cells in the decline phase which leads to premature cessation of fermentation. During batch processes, the influence of sucrose concentration on the specific production rate using *S.cerevisiae* was studied by Richter and Becker (1985). It was observed that the decrease of fermentation activity of the cells caused by both sucrose and ethanol have additional relation to each other.

Bertolini *et al* (1991) reported new yeast strains for alcoholic fermentation at higher sugar concentration. Joshi and Sharma (1994) reported that apricot musts having initial TSS 30^0B showed slightly lower rate of fermentation, more residual TSS and reducing sugar, better appearance and overall quality as compared to musts having initial TSS 24 ^{o}B. Optimum TSS 24 ^{o}B for the production of kinnow wine has been reported by Singh *et al* (1998).

(ii) Effect of Temperature:

Biological processes are characterized by the fact that they are developed in relatively very narrow range of temperature. High temperature limits for development could be found at values not higher than 60-80^{0}C and with particular strains utmost at 120^{0}C. The significance of temperature in the development of a biological process is such that it could determine significant effects as protein denaturization, enzymatic inhibition, promotion or inhibition on the production of a particular metabolite, cells death etc. Importance of temperature in the growth of micro-organisms classifies them into extremothermophiles (100-250^{0}C), thermophiles (45-70^{0}C), mesophiles (30-45^{0}C) and psychrophiles (10-20^{0}C)

Temperature has many other effects besides its direct effect on yeast growth and activity. These are due to loss of alcohol and aromatic constituents, formation of by-products at higher temperatures as well as to direct effects on the efficiency of fermentation. Low temperatures increased the length of fermentation, the yeast viability along the process, modified the lipid composition of yeast cells, increasing the membrane fluidity, and improved the aromatic composition of the beverage, increasing the flavor-active compounds and decreasing the unpleasant ones such as acetic acid and fusel alcohols.

In addition to the influence on fermentation ecology and growth rate, temperature also affects biochemical activities of yeast and, as a result, the production of ethanol, secondary metabolites such as glycerol, acetic acid, succinic acid, and aromatic compounds such as fusel alcohols, acetate esters and fatty acid ethyl esters. These changes could determine the

chemical composition and sensory quality. The main groups of compounds that form the "fermentation bouquet" are the organic acids, higher (fusel) alcohols and esters, and to a lesser extent, aldehydes (Rapp and Versini 1991). Fermentation increases the chemical and flavor complexity, and producing a substantial amount of yeast metabolites. The formation of aroma compounds by yeast, i.e. short and medium-chain fatty acids and their corresponding ethyl esters, higher alcohols and their corresponding acetate esters, is intrinsically linked to the metabolism of yeast cells.

Lee *et al* (1980) have reported the effect of temperature on kinetics of ethanol production by *S. cerevisiae*. Optimum temperature for growth of yeast was 34ºC while for maximum ethanol production rate, the optimum temperature was 37-43ºC. Hang *et al* (1981) observed that sugar consumption and ethanol production were higher at 30ºC during fermentation of apple pomace.

Freezing does not cause immediate death of yeast cells, although the number of survivors in a frozen state decreases with time. The degree and rate of death caused by freezing depend on a number of factors, such as the temperature of freezing, the rate of temperature decrease, the time spent in the frozen state. The faster the rate of freezing and thawing, and the lower the temperature of the frozen state, the higher the rate of survivors due to the formation of ice microcrystals that cause less destruction of cells. Under these conditions, the cell membranes of yeast cells are exposed for a shorter time to the destructive effect of increased osmotic pressure (Gelinas *et al* 1991).

An isolate of *Kluyveromyces marxianus* from fermented molasses grew upto 48^0C, and a few strains of exceptional thermotolerance were found, able to grow and ferment at 52^0C (Banat and Marchant 1995). However, temperatures above 50^0C are usually lethal for yeast cells.

Yamamura *et al* (1988) studied the effects of elevated temperature on growth, respiratory deficient mutation, respiratory activity and ethanol production in yeast. Charoenchai *et al* (1998) reported that with increasing temperature, growth rate increased with most strains of yeast giving fastest growth at 25ºC. Maximum cell biomass produced during fermentation increased substantially between 10ºC and 15ºC but *Saccharomyces cerevisiae* shows similar growth at 15-25ºC.

(iii) Effect of pH:

pH is the most important factor for any fermentation process. Each microorganism possesses a pH range for its growth and activity with an optimum value in between the range. pH strongly influences fermentation properties such as color, oxidation, biological and chemical stability. Lower pH values are known to improve the stability by inhibiting bacteria and causes sugar fermentation to progress more evenly. High pH values allow bacteria to grow rapidly. This condition causes less biological and chemical stability, and poorer color.

Fleet and Gao (1988) studied the effects of temperature and pH on the ethanol tolerance of the wine yeasts, *Saccharomyces cerevisiae*, *Candida stellate* and *Kloeckera apiculata*. Sensitivity of the yeast cells to ethanol was marginally increased on decreasing the pH from 6.0-3.0.

The combined effects of pH, acetic acid and ethanol on intracellular pH of fermenting yeast have been studied by Pampulha and Loureiror-Dias (1989). They observed that for all external pH values tested, the internal pH was 7.0-7.2 in absence of inhibitors.

Aono (1990) reported that yeasts tolerate acidic conditions better than alkali ones; strains belonging to *Dekkera* (*Brettanomyces*), *Saccharomycodes* and *Schizosaccharomyces* were especially alkali sensitive and could not grow above pH 8.

(iv) Effect of Fermentable sugars:

Natural habitats of yeasts were examined for the presence of strains able to produce ethanol from d-xylose (Nigam *et al* 1985b). Black knots, insect frass, and tree exudates were screened by enrichment in liquid d-xylose-yeast extract medium. Among the 412 isolates examined, 36 produced more than 1 g of ethanol liter from 20 g of d-xylose liter, all under aerated conditions. Some strains produced more biomass than ethanol, and among these, ethanol may or may not be assimilated rapidly after depletion of d-xylose. Ethanol production appeared best at low pH values and under mild aeration. Possible correlations between the nutritional profiles of the yeasts and their ability to produce ethanol from d-xylose were explored by multivariate analysis. d-Xylose appeared slightly better utilized by yeasts which rate poorly in terms of fermentation. The fermentation of d-glucose had no bearing on d-xylose fermentation. No specific nutritional trait could discriminate well between better d-xylose fermentors and other yeasts.

Nigam *et al* (1985a) studied eleven strains of an undescribed species of *Clavispora* fermented D-xylose directly to ethanol under aerobic conditions. Strain UWO(PS)83-877-1 was grown in a medium containing 2% D-xylose and 0.5% yeast extract, and the following results were obtained: ethanol yield coefficient (ethanol/D-xylose), 0.29 g (57.4% of theoretical); cell yield coefficient (dry biomass/D-xylose), 0.25 g; maximum ethanol concentration, 5.9 g liter; maximum volumetric ethanol productivity, 0.11 g liter h.

The growth parameters of *Debaryomyces hansenii* with respect to the utilization of pentoses and hexoses in mixtures and as single carbon sources were studied by Nobre *et al* (1999). Growth on pentoses was slower than on hexoses, but the values obtained for biomass yields were very similar in both types of sugars. Glucose and xylose were transported by cells grown on glucose, via a specific low- affinity facilitated diffusion system. Cells derepressed by growth on xylose exhibited two distinct high-affinity transport systems for glucose and xylose.

Chemical characteristization of fermented juices

(i) Juice content:

Citrus juices are an excellent source of potassium, vitamin C, folic acid, inositol and bioflavonoids that not only give citrus juice its flavor and color but are potent antioxidants. The percentage of juice is the character found to be markedly varying with varieties. In India, Prasad *et al* (1999) reported a range of 31.18 – 46.21% for juice content of eight lemon cultivars. In physico-chemical analysis of lemon cultivars, Muthukrishnan *et al* (1966) recorded that juice recovery was highest (58.18 %) in case of Tahiti lime whereas the least (18.71%) was in Rahahmundary.

Ziena (2000) reported that Fruits of Seedless lime (*Citrus latifolia Tan*) has juice yield of 55.6% when fruit is dark green and it increases to 59.4% when it is light greenish yellow. Mature 'Kagzi' and Tahiti limes yielded 61 and 51.2 % juice respectively (Rao *et al* 1977). Sandhu *et al* (2000) reported that the juice content in three species of Kagzi lime, Baramasi lemon and Hill lemon was more than 40%.

In an evaluation trial of lemons in Hissar, juice content varied from 21.33 % in cultivar Eureka to 42.33 percent in Seedless and Baramasi cultivars (Arora and Daulta 1981). Suddamath and Iyer (1982) reported that juice content of Nepali round and Tahiti lime was quite high (more than 46 %) whereas fruits of Nepali oblong had only 32 % juice.

(ii) Total Soluble Solids:

Total Soluble Solids (TSS) of citrus juices constitutes mainly sugars (80-85 per cent). Citric and other acids and their salts, nitrogenous compounds, and other minor substances such as water soluble vitamins constitute the remaining

composition of TSS. Soluble solids can be measured from the refractive index and refractometers are calibrated to give ^{o}B or % total soluble solids values directly. Refractometer reading changes with temperature as the refractive index of the sugars changes with the temperature. TSS and TSS/acid ratio are the reliable indices for assessing the maturity in citrus. Total soluble solids content in Hill lemon was found to be lower as compared to Baramasi lemon and Kagzi lime selections (Sandhu *et al* 2000).

Prasad *et al* (1999) studied eight lemon cultivars for variability and reported that TSS content of cultivars varied from 5.98-6.90 percent. However, the genotypic coefficient of variation and genetic advance was lower for this character, indicating the role of environmental factors in this variation.

Singh and Govind (2000) reported that TSS content was the maximum (8.2 %) in Gol neembu followed by Elaichi Lebu (8.0 %) and was the maximum in Jaintia and Assam lemon (5.3 %).

(iii) Acidity:

Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars, which are used as the main index of maturity and one of the major analytical measures of flavor quality. Acidity contributing to the tartness in most citrus fruits is largely due to citric acid accounting from 85 to 95 % of total acids in various cultivars (Ting and Rouseff 1986). In addition, traces of malic acid tartaric, benzoic, oxalic and succinic acids have also been reported (Kale and Adsule, 1995).

In a trial on selections of lime and lemon under Ludhiana, conditions in Punjab, Sandhu *et al* (2000) reported that maximum acidity was recorded in Kagzi lime followed by Baramasi lemon and Hill lemon. In addition, acid content in January harvested fruits of Baramasi lemon and Kagzi lime was lower than July harvested fruits.

Selection from existing natural variability led to identification of sour mutant of sweet lime which had much higher acid percentage in juice (5.40 %) than common sweet lime (Govind and Singh 2000).

Prasad *et al* (1999) studied variability for chemical characters in lemon cultivars and reported that acidity varied from 0.47 g/100 ml to 0.55 g/100 ml. Singh and Govind (2000) reported that acidity percentage was minimum (4.2 %) in Hill lemon and was maximum (5.8 %) in Gol neembu.

The respective value of citric acid percent and pH found in beverages were 0.14 - 0.23 % and 2.10 -3.02 in lime, 0.12 % and 3.07 in lemon, 0.19 % and 3.39 in orange, 0.12 % acid and pH value of 3.10 in grape flavored carbonated beverages (Ranganna 1977).

(iv) Ascorbic acid

Ascorbic acid, the most abundant vitamin in citrus fruits plays a vital role in human nutrition. It is an important antioxidant that protects against cancers, heart disease and stress. It is part of the cellular chemistry that provide energy, involved in collagen synthesis, bone and teeth calcification, involved in building cartilage, joints, skin and blood vessels (Champe and Harvey 1994). It helps in absorption of dietary iron by keeping it in the reduced form (ferrous form). The recommended daily dose for children is 40 mg and for men and women, 50-60 mg. Heating or drying of fresh fruits usually leads to destruction of most of ascorbic acid originally present.

Sandhu *et al* (2000) evaluated certain strains of lime and lemon under Ludhiana conditions and reported that Baramasi lemon II and III had ascorbic acid content of 41.7 and 42.6 mg/100ml juice in July, and 49.5 and 48.4 mg/100ml juice in January harvested fruits, which was much higher than other lime/lemon selections.

Ahmed *et al* (1986) observed that coloured bottles retained more ascorbic acid than non coloured bottles in apple juice, citrus squashes and raspberry, cherry and blackcurrant juices and beverages, respectively at ambient temperature.

Okunowo *et al* (2005) observed that vitamin-C was reduced significantly in orange juice by the yeast strains *S. cerevisiae* (isolated from yam), *S. cerevisiae* (from sugarcane molasses), *S. carlsbergensis* (from sugarcane molasses) and *S. cerevisiae var. ellipsoideus* (from orange).Vitamin C level was highest (9.02 mg/100 g) with *S. cerevisiae var. ellipsoideus* and lowest (6.65 mg/100 g) with *S. carlsbergensis.*

Antimicrobial mechanism of CO_2 :

The protective role of CO_2 is especially important to prevent the mould growth as it penetrates microbial membranes resulting in acidification of cytoplasm and thus interferes with cytoplasmic enzymes and influences cellular metabolism by extending the lag phase of microbial growth and decreasing the growth rate. Since CO_2 is more soluble than oxygen it displaces oxygen and may minimize degradation reactions such as rancidity.

Carbonation is a process of saturating the beverage with CO_2 for preservation and to give sparkling appearance to beverage along with thirst quenching properties. The effectiveness of carbonation is the main factor in determining the quality and consumer acceptance of final beverage. According to Ranganna (1977) gas volume found in various carbonated beverages were 3.7 to 4.0 in lime, 3.2 in lemon, 2.3 in orange and 1.3 in grape flavored carbonated beverages.

Higher CO_2 pressure of about 3000 KPa is required to stop the fermentation and increased CO_2 pressure enhances the fermentation lag-time and maximum specific growth rate of yeasts (Cahill *et al* 1980). Flocculation of yeast cells with particulates brings about an entrapment of CO_2 gas, agitating the medium with a stimulation of fermentation. In the secondary fermentation, the CO_2 produced is proportional to the sugar fermented (Amerine *et al* 1980).

Markides (1986) reported that yeasts ferment the sugar to alcohol and producing CO_2 as the by-product having the bottle pressure of about 500-600 KPa (5-6 atmospheres) at 10^0C; after the completion of secondary fermentation and for each 100 KPa of pressure rise, approximately 4g/l of sugar was required.

According to Cahill *et al* (1980) retention of CO_2 in a beverage in the form of foam is a desirable characteristic. In carbonated beverages, total soluble solids have been found to inversely affect the retention of CO_2 content and also exert their influence independently on the CO_2 absorbance capacity. The CO_2 absorbancy of sparkling wines was found to be influenced by their ethyl alcohol content also. However, 12 to 20% ethyl alcohol had lesser effect of the CO_2 absorption than its high concentration. A better quality product is obtained with a longer fermentation period at a lower than at the higher temperature due to less satisfactory absorption of the carbon dioxide in the wine.

Developmental and Characteristic study of different forms of beverages

(i) Carbonated Beverages:

According to Philips (1992) the popularity of carbonated drinks is due to their unique taste, zest and sparkling imparted by dissolved CO_2. Jairath (2009) prepared non-alcoholic naturally carbonated beverage from amla var. Francis with 14ºB, 0.73 (% v/v) alcohol and Co_2 pressure of 1.20 Bar. On the basis of organoleptic evaluation, all the sensory attributes varied non-significantly throughout storage period of three months under refrigerated conditions.

Sahota *et al* (2009) prepared low alcoholic self carbonated blended beverages from Carrot var. selection-21 and Amla var. Chakaiya in the ratio of 75:25, 50:50, 25:75 after optimizing the inoculum concentration and TSS adjusted to 16°B. On the basis of organoleptic evaluation carrot-amla (50:50) was rated the highest.

Kaushal et al (2004) reported that total soluble solids (TSS) and CO_2 gas pressure for carbonation are the key parameters that affect the organoleptic quality of carbonated beverages. Apple juice beverage as well as pear juice beverage with 14°B TSS carbonated at 80 psi CO_2 gas pressure was adjudged to be best from overall sensory point of view.

Ranganna (1994) reported that commonly used concentration of CO_2 in carbonated fruit juice beverages (0.1 to 0.8 %) is lower than that required for complete inhibition of microbial activity (14.6 g/L), yet the level is significant in supplementing the lethal effect of acidity on pathogenic bacteria.

Zimzik (1982) developed carbonated beverage by blending various additives including fruit juices, natural or artificial aromas, essential oils, mineral and herbs into lactic acid fermented whey before fermentation with lactic acid bacteria. Finally natural or mineral water carbonated or still was added and the product was used as a beverage. Jayaprakasha *et al* (1986) developed a whey drink by deproteinating and clarifying whey from cheese, channa or acid casein, adding 1-12% sugar 0.02-0.4% citric acid and flavoring @ 0.15-0.45 ml/l of whey followed by in bottle pasteurization with or without carbonation.

Kumar (1997) standardized the technology and evaluation of carbonated citrus fruit juices and their blends with synthetic beverages. Carbonated pure mandarin juice at 100 psi of carbonation and in case of blends of juice with synthetic concentrates, 5 per cent mandarin juice, 20 per cent synthetic concentrate and 2.5 per cent galgal juice, 22.5 per cent synthetic orange concentrate blend at 100 psi pressure were adjudged the best having highest sensory qualities characteristics as well as acceptable storage life for period of six months.

Kaushal (2002) prepared carbonated beverage from apple juice, pear juice and reconstituted apple juice, TSS of 14°B and CO_2 gas pressure of 80 psi were found to be optimum. Carbonated beverage showed good storage stability for a period of 9 months from nutritional, microbiologically and sensory quality point of view.

Singh (2002) prepared dietetic beverages from bitter gourd and found an increase in total soluble solids, reducing sugars and decrease in titratable acidity on storage.

(ii) Non-alcoholic beverages

The flavor of alcoholic beverages is a consequence of a complex mixture of many compounds (higher alcohols, aldehydes, esters) including small concentrations of some volatile metabolites known as congeners, which are produced by the yeast during the fermentation. The more important compounds are those that can be found in all the alcoholic beverages in different concentrations, and they can be grouped on the following chemical species: higher alcohols, esters, and carbonyls (M del 1999).

Sima *et al* (1990) had prepared a low alcoholic carbonated beverage by alcoholic fermentation of grape juice and other fruit juice containing refined sugar. The final alcohol level reached 3-7% v/v and CO_2 level of 10 %.

Ihle (1991) manufactured a high nutritional value, non-fermented alcohol free beer by mashing at 15^0 C, slow heating to saccharification, temperature and adjustment of wort pH to 4.0- 4.3 before boiling and addition of brewer's yeast as a nutrient supplement during wort boiling, CO_2 flushing in boiled wort to eliminate O_2 and wort flavor, followed by clarification, filteration, carbonation and bottling.

Iresel *et al* (1995) produced a non-alcoholic beer by limited fermentation with immobilized cells of *Saccharomyces cerevisiae* in a packed bed reactor. Under combined stress factors such as low temperature 2 – 4^0C and anaerobic conditions, only a small amount of glucose is metabolized resulting in low concentration of the ethanol (<0.08%).

Alobo (2002) studied physicochemical changes associated with the fermentation of lemon juice and analyzed that TSS decreased from 22.5°B to 4.0°B and acidity increased from 0.634% to 0.82%. Fermentation was 139% efficient when inoculated with 3% (v/v) yeast and fermented for 24 days.A fermented whey beverage prepared by clarification of whey and fermentation using *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at 39^0C, pH 3.0, sugar concentration 14 % and incubated for 12 hrs (Shaikh *et al* 2001).

Eglintun *et al* (2002) found that glycerol is a major fermentation product of *Saccharomyces cerevisiae* that contributes to the sensory characteristics of wine and produce less ethanol than wild-type strains.

Munene *et al* (2002) found that the ratio of glycerol to bioethanol could be altered in favour of glycerol by adjusting fermentation parameters as osmotic pressure, pH, temperature and yeast cell inoculum.

Navratil *et al* (2002) produced a non-alcoholic beer using 5 mutant strains of *S. cerevisiae* defective in synthesis of TCA cycle enzyme by batch or continuous fermentation and compared the beer produced with standard *Saccharomyces cerevisiae* for parameters like fermentation time, color, pH, and concentration of total nitrogen, ethanol, free amino nitrogen and polyphenol. Results showed that beer prepared using mutant cells produced ethanol concentration between 0.07 – 0.31% (w/v).

Durojaiye *et al* (2003) studied refrigeration and pasteurization delayed and decreased the decline of pH, increased the shelf life of the kunu, a non-alcoholic beverage from millet and reduced the sedimentation of suspended particles.

Pandove (2007) prepared low alcoholic self carbonated beverages from carrot and amla after optimising inoculum concentration of. *Saccharomyces cerevisiae* var. *ellipsoideus* culture at the rate of 0.5% v/v. On the basis of organoleptic evaluation, low alcoholic self carbonated carrot-amla (50:50) beverage was rated the best, with shelf life of three months.

Gokavi *et al* (2005) developed an oat-based symbiotic non-alcoholic beverage to get the combined benefit of the probiotic property of cultures, isolated from the traditional Bulgarian cereal-based beverages and the prebiotic property of dietary fibre oats.

Sahota and Sunil (2006) developed a reliable, controllable and reproducible technology for preparation of non-alcoholic naturally carbonated plum beverage by inoculating *Saccharomyces ellipsoideus* and optimized the fermentation condition, such as TSS 14°B, alcohol content 0.7 % (w/v), CO_2 pressure 3psi.

Ade-Omowaye *et al* (2006) carried out the development and quality evaluation of non-alcoholic beverages from maize based product, titrable acidity was found to be 0.22, 0.18, 0.05 and 0.30 % for plain, fruit flavored, soy fortified and soured beverage respectively. Fruit flavored ranked highest in preference followed closely by the plain beverage, while soy-fortified samples was the least acceptable.

Ilamara and Amutha (2007) prepared carbonated beverage from banana and sapota fruits pulp and treated it with pectinase at 0.5% concentration (w/v), incubated for 2-3 hrs at ambient temperature and could be stored for 6 months at ambient temperature and low temperature (3-5^0C) and found acceptable for color, flavor and taste.

Temelli (2004) developed a formulation and processing procedure for a functional orange-flavoured beverage incorporating the barley β-glucan and WPI at pilot plant level and found it to be stable during the 8-week refrigerated storage period.

Singh and Nath (2004) showed that bael fruit beverages may be fortified with the protein-acidic polysaccharide complexes, viz. CMC-WPC complex and pectin-WPC complex. The bael fruit beverage with 16°B and 1.75% CMC-WPC was rated the best; fortification to a higher protein level reduced its acceptability.

(iii) Blended beverages:

Fruits which are rich in nutrients but are not accepted due to poor taste and flavor can be blended with other fruits to improve their acceptability and make use of available nutrients.

Shukla *et al* (2003) prepared beverages by blending juice/pulp from apples, bananas, guavas, litchis and mangoes with separated and reconstituted skim milk. Organoleptic evaluation of the beverages showed that apple juice and guava pulp could be blended at up to 300 and 100 g/L in milk products, respectively. Banana and mango pulp could also successfully be used at up to 200 g/L in separated milk and reconstituted skim milk. Litchi juice could be blended up to 300 g/L in separated milk and 200 g/L in reconstituted skim milk.

Shrera (2005) utilized Hill lemon (*Citrus pseudolimon* Tan) and Tulsi (*Ocimum sanctum* L) for the development of RTS and appetizer. RTS beverages were prepared from 5% Hill lemon juice, 10% tulsi extract, 14°B TSS were found better than RTS beverages prepared from other combination and showed good storage stability for a period of six months on the basis of nutritional, microbiological and sensory attributes.

Saxena *et al* (1996) prepared RTS drinks from mixed juices from grapes-mango and grape-pineapple in different ratio and reported that all the ratio were acceptable but superior one was 1:1 due to balanced taste and flavor.

Tiwari (2000) prepared RTS beverages from guava-papaya blends with 15 per cent pulp, 14°B and 0.3 per cent acidity and found that RTS prepared from guava-papaya blended in the proportion of 70:30 was organoleptically better.

Deka and Sethi (2001) studied the preparation of mixed fruit juice spiced RTS beverages and observed that quality of RTS beverages improved with the addition of spice extracts and spice drops. They showed that RTS prepared from blended lime-amla (95:5) juice containing ginger (5%), salt (1%) and other spices was superior over the other combinations.

Kumar and Manimegalai (2001) prepared blended RTS beverages from pineapple-pear and pineapple-pomegranate (1:1), pomegranate-pear-pomegranate (1:1:1) and pineapple juice alone and concluded on the basis of sensory properties that blend of pineapple-pear-pomegranate (1:1:1) was most acceptable as compared to other.

Saravana Kumar and Manimegalai (2005) prepared a whey-based papaya fruit juice blended ready-to-serve (RTS) beverage by blending papaya juice at 10% with whey. The TSS and acidity were maintained at 15^0B and 0.3%. During storage there was an increase in acidity and reducing sugar and a decrease in pH, total sugars and ascorbic acid but TSS did not change.

(iv) Clarified beverages

Clarification of fruit juices by fining treatment with bentonite or gelatin and silica sol rests on unspecific binding of haze-active polyphenols and proteins and capture of sediments and cloud substances during subsequent sedimentation of the resulting colloidal structure (Versari *et al* 1999).

Beveridge *et al* (1986) clarified natural apple juice with bentonite together with gelatin and studied the storage stability of juice as well as concentrate. Durr *et al* (1988) examined the use of gelatin/bentonite before UF and polyvinylpolypyrrolidone (PVPP) after UF for the prevention of haze formation in apple juice. Samples treated with gelatin/bentonite remained clear, while those treated with PVPP developed haze.

Bentonite which is negatively charged attracts the positively charged proteins Protein molecules get absorbed on the bentonite particles and the complex settles down. Other soluble cationic species also get adsorbed by the bentonite clay. 0.6% w/v bentonite (sodium form) slurry was used for clarifying wine by adsorbing the proteins in a model wine solution (Blade and Boulton 1988).

According to Rodriguez *et al* (1977), the adsorption of proteins and a number of other soluble cationic constituents in wine by bentonites is primarily due to the action of cation exchange. The extent to which exchange is possible is determined by the cation exchange capacity (CEC), and this is dependent on the amount of displacement of aluminium ions by sodium, calcium, or magnesium ions when the clay is formed. The ions also influence the interlayer spacing of the bentonite and its swelling properties.

Vardin and Fenercioglu (2003) studied the effects of clarification agents and methods on pomegranate juice quality. Juice was clarified with gelatin, polyvinylypolypyrrolidone (PVPP) and natural sedimentation. The most effective method was the application of 1 g/L gelatin for clarification.

Rai *et al* (2006a) studied the efficacy of various pretreatment processes in comparison to pectinase treatment and explored the possibility of substitution of pectinase for juice pretreatment. Various pretreatment methods investigated were centrifugation, centrifugation followed by addition of gelatin, centrifugation followed by addition of bentonite, centrifugation followed by addition of bentonite and gelatin, and finally, enzymatic treatment using pectinase. The performance of UF of mosambi juice after each of this pretreatment method was evaluated in terms of the permeate flux and permeate quality.

Rai *et al* (2004) used response surface methodology to optimize the enzyme treatment of mosambi juice. Pectinase from *Aspergillus niger* with activity 3.5–7 units/mg (proteins Lowry) was used for enzymatic treatment of mosambi juice. The experimental variables were the time of reaction (40–141 min), temperature (32–49°C), and concentration of enzyme used (0.0004–0.0014 %w/v). The response variables were viscosity, clarity and pectin concentration in terms of alcohol insoluble solids (AIS). A three-variable (five levels of each variable) second order central composite rotatable experimental design was employed. The response functions were calculated from the final polynomial after deletion of all the nonsignificant terms.

Chatterjee et al (2004) clarified different fruit juices with chitosan at low concentration which was made partially water-soluble by hydrolyzing with 7% acetic acid. Appearance and acceptability of the juices significantly increased after treatment with chitosan on a nine point Hedonic scale.

Alvarez *et al* (2000) produced clarified apple juice by integrated membrane process and recovered the aroma compounds from the preconcentrated juice as apple juice aroma concentrate by pervaporation.

Rai *et al* (2007) studied the effect of various pretreatment methods on permeate flux and quality of mosambi *(Citrus sinensis (L.) Osbeck)* juice during ultrafiltration The various pretreatment methods attempted were centrifugation, fining by gelatin, fining by bentonite, fining by bentonite followed by gelatin, enzymatic treatment, enzymatic treatment followed by centrifugation and enzymatic treatment followed by fining by bentonite. Maximum permeate flux was observed with enzymatic treatment followed by adsorption using bentonite. The resulting juice after filtration had more than 93% clarity without deterioration of important quality index in fruit juice e.g. pH, citric acid and total soluble solid. The process variables were determined using contour plot and response functions and these were time of reaction (100 min), temperature of enzyme treatment (42°C) and enzyme concentration (0.0004 %w/v).

Rai *et al* (2006 b) selected the membrane for clarification of depectinized mosambi *(Citrus sinensis* (L.) Osbeck) juice juice. The experiments were conducted in a stirred cell in continuous mode of operation and the operating pressure and stirring speed during the experiment were fixed at 414 kPa and 1200 rpm respectively.

Youn *et al* (2004) clarified reconstituted apple juice using membrane filtration and found that pH and total acidity were not changed significantly during pretreatment of apple juice.

UF has also been investigated for the clarification of pear (Kirk *et al* 1983), orange and lemon (Capannelli *et al* 1994), starfruit (Sulaiman *et al* 1998), kiwifruit (Wilson and Burns 1983), pineapple (Jiraratananon *et al* 1997) and apple (Bruijn *et al* 2003).

Mosambi juice was clarified in stirred continuous mode and stored at both the room and refrigerated temperature. The ultra filtered juice was stored in amber-colored and transparent vials for one month and its quality parameters (total soluble sugar, pH, clarity, color and ascorbic acid) were monitored at five-day interval. The juice stored at room temperature was spoiled in three days. The soluble sugar and pH of juice stored at refrigerated temperature in both the vials did not change with time. The clarity of juice decreased marginally with time and after storage period of thirty days, about 3.2 and 4% decrease occurred for amber-colored and transparent storage vials respectively and even after one month storage period, clarity of the juice was quite high. The decrease in ascorbic acid concentration was about 18 and 20% respectively, for amber and transparent vials. The degradation kinetics of ascorbic acid and nonenzymatic browning followed the zero-order and first-order kinetics respectively, for both the storage vials. The increase in color was 32% for transparent vial and 28% for amber vial after 30 days of storage (Rai *et al* 2008).

Rai and De (2009) carried out clarification of pectin-containing juice using ultrafiltration and storage study of the filtered juice and it was observed that a typical pectin-rich juice, clarified by ultrafiltration (cold sterilization) can have adequate shelf-life without any heat treatment or addition of preservatives.

According to Grassin *et al* (1996), Enzymatic depectinization in fruit juice clarification is assumed to work by pectinase-catalyzed electrostatic destabilization of suspended, cloud-causing pectin particles.

(v) Ready to serve beverages

Kotecha and Kadam (2003) prepared RTS beverage from tamarind juice containing 10% juice, 15°B TSS and 0.50% acidity. Sahu *et al* (2005) developed RTS beverage from paneer whey and mango pulp with the addition of lemon grass distillate. Fresh beverage having the properties of 15.2°B total soluble solids (TSS), pH 4.35, 23.29% total sugars, 4.53% reducing sugars, 0.19% acidity and 1.5% lemon grass distillate obtained the average sensory score of 8.58, which was highest among the other beverages prepared with different concentrations of lemon grass distillate.

The RTS beverage prepared from custard apple contained 20% juice, 15ºB TSS and 0.25% acidity (Kotecha et al 1995). Saini et al (1996) prepared RTS beverage from 'Dusehri' mango of 15% pulp and 0.4% acidity.

Saikia and Dutta (1995) processed outenga (*Dillenia indica*) fruit for developing RTS beverage with increased recovery of fruit juice by application of blanching in steam followed by pressing in screw press. Increase in blanching time significantly increased the yield of juice, reducing sugar, TSS and acidity.

Doodnath and Badriel (2000) processed water melon var. Crimson sweet to form RTS pasteurized beverage. Sensory evaluation indicated that beverage with 20-25ºB, 0.20% xanthum gum, 0.15% citric acid or pH 3.75-3.87 was adjudged the best.

Saklani *et al* (2008) investigated the beverage development potential of seabuckthorn fruit. Beverage formulation containing 10 and 13%, 12.5 and 13 % and 15 and 15 % pulp and sugar were rated most acceptable.

Conclusions

To safeguard the interest of progressive horticulturists, the utilization of fruits for the production of non-alcoholic naturally carbonated beverage is economical and a viable technology. Compared to fruit juices the formulation of naturally carbonated fruit beverage offers more variety of flavors, nutrients, long shelf life and other physiological benefits with the greater margin of safety in a drink with a lower inherent cost. The results indicated the feasibility of such a technology, although challenges remain.

Future Perspectives

Other factors that may be of interest in continued studies include increasing the shelf life stability, determination of total antioxidant capacity and the effect of fermentation on bitter components like naringin and hesperidin. The abilities to stabilize both the functional and microbiological characteristics of juice products and to prepare new functional ingredients consisting of juice constituents will provide the beverage industry with new possibilities. Economic feasibility and marketing research will also need to be performed to ensure the success of the product.

References

1. Ade-Omowaye B I O, Olaniyan S A, Adeyem I A and O O Isola (2006) Development and quality evaluation of non-alcoholic beverages from maize based products. *Nut Food Sci* **36**(3): 183-90.
2. Ahmed M, Chaudhry M A, Khan I (1986) Technological studies on citrus and other fruit drinks. Nucleus **23** (3/4): 41-45.
3. Alobo A P (2002) Some physicochemical changes associated with the fermentation of lemon juice for wine production. *J Sustainable Agri Environ* (Original not seen. Abstr in *CAB Abstracts* Ac No20043048284).
4. Alvarez S, Riera F A, Alvarez R, Coca J, Cuperus F P, Bouwer S, Boswinkel G, van Germert R W, Veldsink J W, Giorno L, Donato L, Todisco S, Drioli E, Olsson J, Tragardh G, Gaeta S N and Panyor L (2000) A new integrated membrane process for producing clarified apple juice and apple juice aroma concentrate. *J Food Engineering* **46**: 109-125.
5. Amerine M A, Kunkee R E, Ough C S, Singleton V L and Webb A D (1980) Technology of Wine making. AVI Publishing Company, Inc. Westport, Connecticut. pp 794.
6. Aniche O K (2003) The Need for Postharvest Physiology Application in Food Security Approach in Nigeria. Food Science and Technology, Federal University of Technology. Owerri. 4pp.
7. Aono R (1990) Taxonomic distribution of alkali-tolerant yeasts. *System Appl Microbiol* **13**: 394- 97.
8. Arora R K and Daulta B S (1981) Evaluation of some lime cultivars (*Citrus limon* Burm) for their physico-chemical composition and yield under Hissar conditions. *Haryana J Hort Sci* **10**: 182-85.

9. Banat I M and Marchant R (1995) Characterization and potential industrial application of five novel, thermotolerant, fermentative yeast strains. *World J Microbiol Biotechnol* **11**: 304- 06.
10. Bertolini M C, Ernandes J R and Laluce C (1991) New yeast strains for alcoholic fermentation at higher sugar concentration. *Biotechnol Lett* **13** (3): 197-02.
11. Beveridge T, Franz K and Harrison J E (1986) Clarified natural apple juice: production and storage stability of juice and concentrate. *J Food Sci* **51**: 411–414.
12. Blade W H and Boulton R (1988) Adsorption of protein by bentonite in a model wine solution. *Am J Enol Vitic* **39**: 193-199.
13. Boles E and Andre B (2004) Role of transporter-like sensors in glucose and amino acid signalling in yeast. *Top Curr Genet* **9**: 121-53.
14. Bruijn J P F de, Venegas A, Martínez J A and Bórquez R (2003) Ultrafiltration performance of Carbosep membranes for the clarification of apple juice. *Lebensm-Wiss Technol* **36**: 397-406.
15. Cahill J T, Carroad P A and Kunkee R E (1980) Cultivation of yeast under carbon dioxide pressure for use in continuous sparkling wine production. *Am J Enol Vitic* **31**(1): 46-52.
16. Capannelli G, Bottino A, Munari S, Lister D G, Maschio G and Becchi I (1994) The use of membrane processes in the clarification of orange and lemon juices. *J Food Eng* **21**: 473–483.
17. Champe P C and Harvey R A (1994) Lippincott's *Illustrated Reviews, Biochemistry 2nd Ed.* Lippincott-Raven Publishers Inc, USA. pp 328-50.
18. Charoenchai C, Fleet G H and Henschke P A (1998) Effects of temperature, pH and sugar concentration on the growth rates and cell biomass of wine yeasts. *Am J Enol Vitic* **49** (3) 283-88.
19. Chatterjee S, Chatterjee S, Chatterjee B P and Guha A K (2004) Clarification of fruit juice with chitosan. *Process Biochem* **39** (12): 2229-2232.
20. Deka B C and Sethi V (2001) Preparation of mixed fruit juice spiced RTS beverages. *Indian Food Packer* **53:** 58-61
21. Devine S I and Slaughter J C (1980) The effect of medium composition on the production of ethanol by *S.cerevisiae*. *FEMS Microbiol Letter* **9** (1): 19-21.
22. Doodnath and Badriel (2000) Processing and quality evaluation of Ready-to-Serve watermelon nectars. *Indian Food Packer* July-August: 71-78.
23. Durojaiya A B, Akingbala and Uzo-Peters (2003) Effect of refrigeration and pasteurization on storage stability of kunu. *J Food Sci Technol* **3:**162- 68.
24. Durr P, Schobinger U and Waldvogel R (1988) Tests with zirconium oxide membranes in connection with problem of haze formation of ultra-filtered apple juices. *Flüssiges Obst* **55**: 621-640.
25. Eglintun J M, Heinrich A J, Pollnitz A P, Langridge P, Henschke P A and Lopes M D B (2002) Decreasing acetic acid accumulation by a glycerol over producing strain of *Saccharomyces cerevisiae* by deleting the ALD6 aldehyde dehydrogease gene. *Yeast* **19:** 295-301.
26. Escalante-Minakata P, Blaschek H P, Barba de la Rosa A P, Santos L, De León-Rodríguez A (2008) Identification of yeast and bacteria involved in the mezcal fermentation of Agave salmiana**.** *Lett Appl Microbiol* Apr 9:18489025 (P,S,E,B,D).
27. FAOSTAT, "Food and Agriculture Organization of the United Nations", http://www.fao.org/faostat/, **(2018)**
28. Fleet G H and Gao C X (1988) The effect of temperature and pH on the ethanol tolerance of the yeasts, *S. cereisiae*, *Candida stellata* and *Kloeckera apiculata*. *J Appl Bacteriol* **65**(5):405-09.
29. Fonseca A, Fell J W, Kurtzman C P and Spencer-Martins I (2000) *Candida tartarivorans* sp. nov., an anamorphic ascomycetous yeast with the capacity to degrade L(+)- and meso-tartaric acid. *Int J Syst Evol Microbiol* **50**: 389-94.
30. Gelinas P, Toupin C J, Goulet J (1991) Cell water permeability and cryotolerance of *Saccharomyces cerevisiae*. *Lett Appl Microbiol* **12**: 236-40.
31. Gokavi S, Zhang L, Huang M K, Zhao X and Guo M (2005) Oat-based symbiotic beverage fermented by *Lactobacillus plantarum, Lactobacillus paracasei* ssp. *casei*, and *Lactobacillus acidophilus*. *J Food Sci* **70**(4): 216-23.
32. Gopalan C, Ramasastri B V and Balasubramanian S C (2002) Nutritive value of Indian Foods. National Institute of Nutrition (ICMR), Hyderabad, India.
33. Grassin C, Fauquembergue F Fruit Juices (1996) In *Industrial Enzymology*, 2nd ed.; Godfrey T, West, S., Eds. MacMillan Press: London, U.K.: 225-264.
34. Hang Y D, Lee C Y, Woodams E F and Cooley H J (1981) Production of alcohol from fruit waste Apple pomace. *Appl Environ Microbiol* **42** (6): 1128-29.
35. Ihle G (1991) Process for manufacture of alcohol free beer. German patent DE 3920506A1 (c.f. FSTA 1991 **23:** 9 -31.

36. Ilamaran M and Amutha S (2007) Effect of total soluble solids and CO_2 pressure on physico-chemical and sensory qualities of carbonated banana and sapota beverages. *J Food Sci Technol* **44:**178-82.
37. Iresel M F M Van, Meerman E, Swinkles W, Abee T and Rombouts F M (1995) Continuous production of non-alcohol beer by immobilizied yeast at low temperature. *J Ind Microbiol* **14:** 495-01.
38. J. Singh (2017), "Effect of surface coatings on storage life and quality of Baramasi lemon (Citrus limon L. Burm) fruits", Doctoral dissertation, Punjab Agricultural University, Ludhiana.
39. Jairath S (2009) *Microbiological preparation of Non-alcoholic naturally carbonated beverage from amla and lemon.* M.Sc. thesis, Punjab Agricultural University, Ludhiana, India.
40. Jayaprakasha H M, Anantakrishnan C P, Antmaram and Natarajan A M (1986) Preparation of soft drinks from the clarified and deproternized whey. Chevon. **15:** 16-19 (cited from *Dairy Sci* Abstr. **49:** 202.
41. Jérôme Mounier, Roberto Gelsomino, Stefanie Goerges, Marc Vancanneyt, Katrien Vandemeulebroecke, Bart Hoste, Siegfried Scherer, Jean Swings, Gerald F. Fitzgerald, and Timothy M. Cogan. (2005) Surface Microflora of Four Smear-Ripened Cheeses. *Appl Environ Microbiol* **71**(11): 6489–500.
42. Jiraratananon R, Uttapap D and Tangamornsuksun C (1997) Selfforming dynamic membrane for ultrafiltration of pineapple juice. *J Membr Sc*i **129**: 135-143.
43. Joshi V K and Sharma S K (1994) Effect of method of must preparation and initial sugar levels on the quality of apricot wine. *Res Ind India* **39**(4):255-57.
44. Kale P N and Adsule P G (1995) Citrus. In: Handbook of fruit science and technology. Production, composition, storage and processing. (Eds.: D.K. Salunkhe and S.S. Kadam), Marcel Dekker, New York, pp. 39-65.
45. Kaushal (2002) *Studies on juice extraction, clarification and preparation of carbonated beverages from apple and peer fruits.* Ph.D. Dissertation. Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni –Solan (HP), India.
46. Kaushal (2004) Optimization of total soluble solids and carbon dioxide gas pressure for the preparation of carbonated beverages from apple and pear juices. *J Food Sci Technol.* **41**: 142-149.
47. Kirk D E, Montgomery M W and Kortekaas M G (1983) Clarification of pear juice by hollow fibre ultrafiltration. *J Food Sci* **48**: 1663–1666.
48. Kotecha and Kadam (2003) Preparation of Ready-To-Serve beverage, syrup and concentrate from tamarind. *J Food Sci Technol* **40**(1): 76-79.
49. Kotecha P M, Adsule R N and Kadam S S (1995) Processing of custard apple: Preparation of ready-to-serve beverage and wine. *Indian Food Packer* **49** (4): 5-11.
50. Kumar S (1997) *Standardization of technology and evaluation of carbonated citrus fruit juices and their blends with synthetic beverage*. M Sc thesis, Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni-Solan, (HP), India.
51. Kumar S R and Manimemegalai G (2001) Storage stability of mixed fruit juice RTS beverage in different storage conditions. *Bev Food World* **28:** 28.
52. Lachance M A, Daniel H M, Meyer W, Prasad G S, Gautam S P, Boundy-Mills K (2003) The D1/D2 domain of the large-subunit rDNA of the yeast species *Clavispora lusitaniae* is unusually polymorphic. *FEMS Yeast Res* **4**(3):253-58.
53. Lachance M A, Starmer W T, Bowles J M., Phaff H J, and Rosa C A (2000) Ribosomal DNA, species structure, and biogeography of the cactophilic yeast *Clavispora opuntiae*. *Can J Microbiol* **46** (3): 195-210.
54. Lafourcade L S (1983) Wine and Brandy. Biotechnology edited by H J Rehim and G Reed **5**: 81-163.
55. Lee J H, Williamson D and Roger P L C Sch (1980) The effect of temperature on kinetics of ethanol production by *S. cerevisiae. Biotechnol Lett* **2**: 141.
56. M del C, Santillán-Valverde and García-Garibay M (1999) Congeners biosynthesis during alcoholic fermentations (In Spanish). *Rev Latinoam Microbiol* **40** (1-2): 109-19.
57. Markides A J (1986) The microbiology of methode Champenoise. In Proceedings of 6th Australian wine industry Technical Conference, Adelaide, 14-17, 1986. Australian Industrial Publishers, Adelaide. pp 232-36.
58. Middelhoven W J and Kurtzman C P (2003) Relation between phylogeny and physiology in some ascomycetous yeasts. *Antonie van Leeuwenhoek* **83**: 69-74.
59. Munene C N, Kampen W H and Njapav Henry (2002) Effects of altering fermentation parameters on glycerol and bioethanol production from cane molasses. *J Sci Food Agri* **82:** 309-17.
60. Muthukrishan C R, Palaniappan S P and Palaniswamy K P (1966) Physico-chemical analysis of some lemon varieties (Citrus limon Burm) grown in South India. *South Indian Hort* **14**: 1-8.
61. Navratil M, Domeny Z, Sturclik E, Smogrovicova D and Germenier P(2002) Production of non-alcoholic beer using free and immobilized cells of *Saccharomyces* deficient in the tricarboxylic acid cycle. *Biotechnol Appl Biochem* **35:**133- 40.

62. Nigam J N, Margaritis A, Lachance M A (1985a) Aerobic Fermentation of D-Xylose to Ethanol by *Clavispora* sp. *Appl Environ Microbiol* **50** (4): 763-66.
63. Nigam J N, Ireland R S, Margaritis A, Lachance M A (1985b) Isolation and Screening of Yeasts That Ferment d-Xylose Directly to Ethanol. *Appl Environ Microbiol* **50**(6): 1486-89.
64. Nobre A, Lucas C and Leão C (1999) Transport and utilization of hexoses and pentoses in the halotolerant yeast *Debaryomyces hansenii*. *Appl Environ Microbiol* **65**:3594-3598. *Appl Environ Microbiol* **50**(6): 1486-89.
65. Okunowo W O, Okotore R O, Osuntoki A A (2005) The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice. *Afr J Biotechnol* **4** (11): 1290- 96.
66. Pampulha M E and Loureiror-Dias M C (1989) Combined effect of acetic acid, pH and ethanol on intracellular pH of fermenting yeast. *Appl Microbiol Biotechnol* **31** (5-6): 547-50.
67. Panchal P J and Stewart G G (1980) The effect of osmotic pressure on the production and excretion of ethanol and glycerol by a brewing yeast strain. *J Inst Brew* **86**: 207-10.
68. Pandey A, Soccol C R, Rodriguez-Leon J A, Nigam P (2001) *Solid-State Fermentation in Biotechnology*: *Fundamentals and Applications*. Asiatech Publishers, Inc. New Delhi. pp 32-37.
69. Pandove G (2007) *Preparation of low alcoholic self carbonated beverages from carrot and its blends.* M.Sc. thesis, Punjab Agricultural University, Ludhiana, India.
70. Phillips G F (1992) Carbonated beverages In: Encyclopedia of Food Science and Technology Hui Y H (ed) Vol I. John Wiley and Sons. Inc. New York. p286-93.
71. Prasad M B N V, Rao G S P and Rekha A (1999) Studies on variability and correlation in some physico-chemical characters of fruits in lemon. *Proc Nat Symp Citriculture*, Nov 17-19, 1997, Nagpur pp: 33-35.
72. Rai P and De S (2009) Clarification of pectin-containing juice using ultrafiltration. *Current Sci* **96** (10): 1361-1371.
73. Rai P, Majumdar G C, Das Gupta S and De S (2007) Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. *J Food Engineering* **78** (2007): 561-568.
74. Rai P, Majumdar G C, DasGupta S and De S (2004) Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology. *J Food Engineering* **64** (3): 397-403.
75. Rai P, Majumdar G C, Jayanti V K, DasGupta S and De S (2006a) Alternate pretreatment methods to substitute enzyme treatment for clarification of mosambi juice using ultrafiltration. J *Food Process Eng* **29**: 202-218.
76. Rai P, Majumdar G C, Sharma G, DasGupta S and De S (2006b) Effect of various cutoff membranes on permeate flux and quality during filtration of mosambi *(Citrus sinensis* (L.) Osbeck) juice.*Food Biol Process* **84**: 213-219.
77. Rai P, Rai C, Majumdar G C, DasGupta S and De S (2008) Storage study of ultrafiltered mosambi (*Citrus sinensis* (L.) Osbeck) juice. *J Food Process Preserv* **32**: 923-934.
78. Ranganna S (1977) *Manual of Analysis of fruit and vegetable products*. Tata McGraw-Hill Pub. Co. Ltd. pp. 634.
79. Ranganna S (1994) *Handbook of Analysis and Quality Control for fruit and vegetable products*. 2nd edn. Tata Mc. Graw-Hill pub. Co. Ltd. New Delhi, India.
80. Rao M M, Hittalmani S V, Bojappa K M (1977) Impact of cultural practices on production and quality of Indian lime. Proc Int Citrus Symp, Bangalore, 17-22 Dec, p 211-16.
81. Rapp A and Versini G (1991) Influence of nitrogen compounds in grapes on aroma compounds of wines. *In Proceedings of the International Symposium on Nitrogen in Grapes and Wine*; Ranz J M Ed.; Am. Soc. Enol. Vitic. Davis C A.
82. Richter K and Becker U (1985) Appearance of inhibition in the ehanol fermentation II. Influence of substrate concentration on the specific rate of ethanol production of *S. cerevisiae. Acta biotechnol* **5**(2): 145-52.
83. Rodriguez J L P, Weiss A and Lagaly G (1977) A natural clay organic complex from Andalusian black earth. *Clay and Clay Minerals* **25**: 243-51.
84. Sahota P P and Sunil (2006) Preparation of Non-alcoholic naturally carbonated plum beverage after optimizing the fermentation conditions. *Bev Food World* **32**: 52-58.
85. Sahota P, Pandove G and Jairath S (2009) Preparation of Low-Alcoholic Self Carbonated beverage from carrot:amla juices. Bev Food World **36**(4): 921-26.
86. Sahu C, Choudhary P L and Patel S (2005) Techno-economic feasibility of ready-to-serve whey based mango-herbal (lemongrass) beverage. *Indian J Dairy Sci* **58** (4): 258-263.
87. Saikia L and Dutta R (1995) Processing of ou-tenga (Dillenia indica) fruit for RTS beverages. *Indian Food Packer* Sept-Oct: 25-32.
88. Saini S P S, Bawa A S and Ranote P S (1996) Thermal process for Ready-to-Serve mango beverage. *J Food Sci Technol* **33**(5): 434-35.
89. Saklani S, Sharma H R, Singh V, Tangying Y and Xiufeng W (2008) Chemical Constituents and Sensory Acceptability of Ready to Serve Seabuckthorn Beverage. The Global Seabuckthorn Research and Development; 2008 **(1):** 40-44.

90. Sandhu A S, Kaur Harminder and Chanana Y R (2000) Studies on variability in physico-chemical characteristics of lime and lemon selections. *Proc Nat Symp Citriculture*, 17-19 Nov, 1999, NRCC, Nagpur pp: 537-39.
91. Saravana Kumar R and Manimegalai G (2005) Studies on storage stability of whey-based papaya juice blended RTS beverage. *J Fd Sci Technol* **42**(2): 185-88.
92. Sexana A K, Teotia M S and Berry S K (1996) Studies on the development of grape-mango and grape-pineapple beverage blends. *Indian Food Packer* **50:** 26-29
93. Shaikh S Y, Rathi S D, Pawar V D and Agarkar B S (2001) Studies on development of process for preparation of fermented carbonated whey beverage. *J Food Sci Technol* **38:** 519-21.
94. Shrera S K (2005*) Utilization of Hill lemon and tulsi for the development of RTS and appetizer*. M Sc Thesis Dr. Y.S. Parmar University of Horticulture and Forestry. Nauni-Solan (HP), India
95. Shukla F C, Sharma A, Singh B (2003) Studies on the development of beverages using fruit juice/pulp, separated milk and reconstituted skim milk. *Int J Dairy Technol* **56** (4): 243-246.
96. Sima J, Mikeska E, Bacikova O, Janostik S, Zboril M and Voldrich R (1990) Low alcohol carbonated beverage. Czech Patent CS 267876.
97. Singh A K and Goswami T K (2006) Controlled atmosphere storage of fruits and vegetables: A review. J Fd Sci Technol **43**(1): 1-7.
98. Singh A K and Nath N (2004) Development and Evaluation of whey protein enriched Bael fruit (Aegle marmelos) beverage. *J Food Sci. Technol* **41**(4): 432-36.
99. Singh I P and Govind Sheo (2000) Variability in physico-chemical characteristics of indigenous Citrus species/land races of NEH region of India. *Proc Nat Symp Citriculture*, 17-19 Nov, NRCC, Nagpur pp: 91-96.
100. Singh M, Panesar P S and Marwaha S S (1998) Studies on the suitability of kinnow fruits for the production of wine. *J Food Sci Technol* **35** (5): 455-57.
101. Singh T K (2002*) Processing the bittergourd for the production of dietetic beverages*. M Sc Thesis Dr. Y. S. Parmar University of Horticulture and Forestry. Nauni-Solan (HP), India.
102. Suddamath V V and Iyer C P A (1982) Preliminary evaluation of some lemon (*Citrus limon* Burm) cultivars for growth yield and quality. *South Indian Hort* **30**: 69-71.
103. Sulaiman M Z, Sulaiman N K and Shih L Y (1998) Limiting permeate flux in the clarification of untreated starfruit juice by membrane ultrafiltration. *J Chem Eng* **68**: 145-148.
104. Temelli E, Bansema C and Stobbe K (2004) Development of an orange-flavored barley β-Glucan beverage with added whey protein isolate. *J Food Sci* **69**(7): 237-42.
105. Ting S V and Rouseff R L (1986) *Citrus fruits and their products*: Analysis and Technology Marcel Dekker, Inc, New York.
106. Tiwari R B (2000) Studies on blending of grape and papaya pulp for RTS beverages. *Indian Food Packer* **52:** 68.
107. Vardin H and Fenercioglu H (2003) Study on the development of pomegranate juice processing technology: Clarification of pomegranate juice. *Nahrung/Food* **47** (5): 300-303.
108. Versari A, Barbanti D, Potentini G, Parpinello G P, Galassi S (1999) Preliminary study on the interaction of gelatin-red wine components. *Ital J Food Sci* **11**: 231-239.
109. Wilson E L and Burns D J W (1983) Kiwifruit juice processing using heat treatment techniques and ultrafiltration. *J Food Sci* **48**: 1101-1105.
110. Yamamura M, Nagami Y, Vongsuvanlert V, Kumnuanta J and Kamihara T (1988) Effects of elevated temperature on growth, respiratory deficient mutation, respiratory activity an ethanol production in yeast. *Can J Microbiol* **34** (8): 1014-17.
111. Youn K S, Hong J H, Bae D H, Kim S J and Kim S D (2004) Effective clarifying process of reconstituted apple juice using membrane filtration with filter-aid pretreatment. *J Membrane Sci* **228** (2): 179-186.
112. Ziena H M S (2000) Quality attributes of Bearss Seedless lime (*Citrus latifolia Tan*) juice during storage. *Food Chem* **71** (2): 167-172.
113. Zimzik H (1982) Method for extending the use of whey. German Federal Republic Patent Application DE 3018087(cited from Abstr in Food Sci Technol Abstracts 15:303).