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Impact of enzymatic treatment on physicochemical properties of various vegetable juices

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Abstract
This study was aimed to compare Juice yield and Physico chemical properties of seven different Vegetable juices (Carrot, beet root, Pumpkin, Cucumber, Tomato, Ivy Gourd and Bottle Gourd), which were prepared both in conventional pressing & liquefied enzymatic treatment (Pectinase) process. Physico chemical properties like Total soluble solids, Titratable acidity, p^H, Viscosity, and Yield of seven vegetable Juices were determined in both liquefied enzyme and non-enzyme treated juices. Yield of Juice and significant favor of Physico chemical properties was high in Enzyme Treated Juices when compared to Non-Enzyme Treated vegetable Juices.

Keywords: Liquefied enzyme treatment, yield, comparison, vegetable juices.

1. Introduction
Vegetables contribute nutrients like vitamins, minerals, proteins, dietary fibers, fats and energy. Vitamin C and B complex vitamins are found to be major source in Vegetables. The USDA's Food Guide Pyramid recommended at least five servings of vegetables daily consumption due to their immense health benefits. Vegetables not only provides nutrition but also contributes texture, color and flavor of the final food. Vegetables are good source of minerals like K, Fe, Na, Ca and Mg and vitamins like Provitamin A, Vitamin C and B complex vitamins along with vitamin K. All most all vegetables are important for their non-nutritive compounds like carotenoids, flavonoids, phenolics, anthocyanin, chlorophyll, indoles and sulforaphane and which are not only contribute colour, flavor and aroma but also act as anti-oxidants to help in prevention of carcinogenic health problems (Nirmal K. Sinha, 2011) [15]. Vegetables are a rich source of phytochemicals, such as carotenoids, chlorophylls, anthocyanins, and flavonoids (Andersen and Jordheim 2006).

Carrot and Pumpkin are rich in β-Carotene Antioxidant, anti-inflammatory perspectives, inhibits arthritis and prostate cancer, improves skin tone, carrot especially effective in diabetes mellitus, lowers cholesterol, and reduces colon cancer. Cucumber is rich in Antioxidants, carotenoids, and Vitamins and which are Antioxidant potential, improves the skin tone. Tomatoes are rich in Lycopene which are Potent antioxidants, lowers cholesterol/glucose, anticancer potential (Nirmal K. Sinha, 2011) [15]. Beet roots are rich in phenolic compounds, betalains consisting of red violet coloured betacyanins and yellow colored betaxanthins and the concentration 300-600mg/Kg of betanin, whereas isobetanin, betanidin, and betaxanthins were present at lower concentrations (Kanner *et al*. 2001) [11]. A 100 g of edible portion of the bottle gourd contains ascorbic acid 12.0 mg, potassium 87.0 mg, calcium 12.0 mg, phosphorus 37.0 mg and niacin 0.3 mg (Rumeza *et al*., 2006; Sawate *et al*., 2009) [18, 19]. In the treatment of acidity, indigestion, ulcers, pectoral cough and in other bronchial disorders bottle gourd is used. (Deore *et al*., 2009) [6].

Ivy gourd is rich in β-carotene and also a good source of fibre, protein and a moderate source of calcium and can consume by all age groups hence it is being selected for several studies in Thailand. (Xu *et al*., 2003) [23].

Vegetable juice and blends are among the major processed vegetable products. They are liquid foods prepared from vegetables as the major raw material. Juices can be classified into three types, namely clear juice, cloudy juice and pulpy juice, based on the appearance which is reflected by the content and size of insoluble solids. Clear juice contains no insoluble solids. Cloudy juice is translucent, containing homogeneously suspended tiny insoluble particles. Pulpy juice contains coarse particles that may float on the surface, suspend in the liquid, or precipitate to the bottom. Cloudy juice is the most popular form of vegetable juice and blends on the market (Nirmal K. Sinha, 2011) [15].

Health Functions of Vegetable Juice

Fruits and vegetables are found to be protective against the risks of arthritis, cardiovascular diseases (CVD), cancers and chronic inflammation (Chen *et al*. 2006) [4]. The presence of carotenoids, Vitamin E, vitamin C, minerals and Fiber (Roy *et al*. 2007) [17]. Deep colored vegetables juices supplementation demonstrated immunomodulatory potential via the regulation of Th1/Th2 cytokine secretions, especially Th1 cytokines (Lin and Tang 2008) [12]. Vegetable juice also play an important role in delaying the onset of Alzheimer's disease, particularly among those who are at high risk for the disease (Dai *et al*. 2006) [5].

2. Material and Methods

2.1. Materials

2.1.1 Chemicals

All chemicals and reagents used in the experiments were of analytical grade and purchased

Mostly from SD Fine, India and SRL private Limited, India

2.1.2. Selection of raw materials.

Carrot, Beet root, Pumpkin (yellow skin), Tomato, Ivy gourd, Cucumber and Bottle gourd procured from single vendor for uniqueness in the local market of Mysore.

2.1.3. Unit operations involved in juice extraction

Unit operations such as Sorting, Cleaning, Peeling, slicing, puree preparation-Enzyme treatment, filtration, pasteurization and packaging were carried out in the present research. The unit operations are briefly explained in the following sections;

2.1.3.1. Sorting and Cleaning

- Procured high grade raw materials and sorted out for uniformity in raw material based on visual inspection (color, size and ripening).
- Carrots were selected in uniform Orange color with medium size.
- Beet roots were selected in uniform dark color with medium size.
- Bottle gourds were selected with medium size and tender seeds.
- Tomatoes were selected with reddish color medium sized.
- Ivy gourds were selected in medium size, which were unripened one.
- Cucumbers were selected in medium size and without bitter taste,
- Pumpkins were selected in yellow colored pomace one with medium size.

All vegetables were washed properly under potable running tap water and allowed drain the excess water after washing.

2.1.3.2. Peeling & Seed removing

Peeling of carrot, beet root, bottle gourd, Ivy gourd and cucumber was done to remove unwanted skin on vegetables with the help of knife and hand peeler and seeds of tomatoes, pumpkin and bottle gourd were removed with the help of knife.

2.1.3.3. Blanching

Blanching of Carrots, beet root and tomatoes were done to improve tenderness of vegetable and inactivation of enzymes in boiling water for 1min.

2.1.3.4. Chopping

Blanched vegetables and remaining vegetables were chopped to easy pulp in the home level grinder (Ultra Choice Plus).

2.1.3.5. Enzyme treatment, Puree preparation & Incubation

Chopped vegetables were taken into home scale mixer of the common type (Ultra Choice + RX, 1000 watts) for puree preparation. Liquefied Enzyme was added before puree preparation starts. To improve the yield of juice, while puree preparation all the vegetables were treated with different percentages of liquefied Pectinase enzyme, but finally optimized with 2% liquefied Pectinase treatment for carrot & 1% for remaining all vegetables based on the juice yield calculation. Enzyme treated puree kept for incubation at room temperature for min 2 hours to get enhanced juice. Pectinase will active at the temperature around 40^0 C and that required temperature will be attained while grinding to make puree.

Prepared puree was kept for incubation at room temperature for 2 hours in closed vessel.

In case of Non-enzyme treated juices, puree was prepared without adding liquefied Enzyme.

2.1.3.6. Filtration

Filtration was done using muslin cloth to remove the residual pomace material in the enzyme treated juice.

2.1.3.7. Pasteurization & Storage

The filtered juice was stored under freezing condition (-18 ^{0}C) after inactivation of enzyme at 70 ^{0}C for 5 min.

2.1.4. Juice Yield

Juice yield was measured based on puree made & juice extracted from the puree.

$$\text{Juice Yield (\%)} = \frac{Extracted\ Juice}{Puree} * 100$$

2.1.5. Physico Chemical analysis

2.1.5.1. Total Soluble Solids (T.S.S, ^{0}Brix)

The brix or total soluble solid of juices was measured using electronic refractometer (Model: ATAGO RX-5000, Tokyo, Japan). The measuring prism of the refractometer must be kept clean. The working surface of the prism should be free from scratches. Adjusted refractometer to 25 °C to determine °Brix / TSS of the sample. The TSS of the juices were taken in triplicates and expressed as ^{0}Brix.

2.1.5.2. Titratable Acidity (%)

The Acidity of the product samples taken in triplicates was estimated by following method suggested by Ranganna (1999) [16]. It is expressed in percentage.

Reagents**:** 0.1 NaOH, Phenolphthalein indicator

Procedure: 10mL of homogenate sample was weighed, dissolved in dist. water and filtered. The filtrate was made up to 100ml with distill water. 10ml aliquot was taken in titration flask and 2- 3 drops of phenolphthalein indicator was added to this. Then it was titrated against 0.1N NaOH till the permanent pale pink colour end point appeared. Three consecutive readings for each sample were taken and the acidity was calculated by using the following formula in terms of citric acid:

$$\text{Titratable acidity (\%)} = \frac{\text{titre} * \text{N of NaOH} * \text{volume made up} * \text{Eq.wt of acid}}{\text{Wt.of the smaple (g)} * \text{Aliquot taken (ml)} * 1000}$$

2.1.5.3. P^H
The pH of juice was measured using EUTECH INSTRUMENTS pH TUTOR. The PH relates to the free hydrogen ions in sample indicating the alkaline/acidity balance. The meter was calibrated with standard buffers of 4, 7 & 9 pH before commencement of the sample analysis.

2.1.5.4. Viscosity
Viscosity is a principal parameter when any flow measurements of fluids, such as liquids, semi-solids, gases and even solids are made. Viscosity measurements are made in conjunction with product quality and efficiency. Anyone involved with flow characterization, in research or development, quality control or fluid transfer, at one time or another gets involved with some type of viscosity measurement. In this experiment Viscosity was measured using Brookfield Viscometer. Viscosity was measured in terms of percentage of Torque Vs Time & Spindle number 2 with 100rpm for 30 seconds was measured and viscosity was expressed as mPa.s.
Following formula and expressed in terms of mg/100mL.

$$\text{Total carotene } (\mu g/100mL) = \left(\frac{3.857 * OD \text{ at } 452\ nm * volume\ made\ up * dil}{weight\ of\ the\ sample}\right) \times 100$$

3. Results and Discussion
The consumer demand for fresh ready-to-use products has led, over recent years, to increasing interest in minimally processed fruits and vegetables, as these products combine freshness and convenience (Yamada H, 1996) [22]. Treatment of fruit & vegetable pulp with appropriate enzyme preparations is a general practice in juice processing. Most enzymatic preparations for juice processing are composed of cellulase, hemicellulases and pectinases. These preparations hydrolyse protopectin of the middle lamella, which connects the flesh cells, leading to the release of cell wall contents, which have been envisioned to be composed of cellulose fibres embedded in an amorphous mixture of polysaccharides and glycoproteins (El-Zoghbi M *et al.*, 1992 and Borowska J, 2000) [7, 3]. In this work we have studied liquefied Enzyme (Pectinase) treatment effect on Carrot, Beetroot, Pumpkin, Tomato, Bottle Gourd, Ivy Gourd and Cucumber juice extraction.

3.1. Yield
The results of both Non Enzyme Treated (NET) & Enzyme Treated (ET) juices were presented in table 1 and the comparison of results were shown in Fig 1.

Table 1: Juice Yield (%).

Juices	NET	ET
Carrot	58.14±1.22	74.34±1.08
Beetroot	58.06±0.98	75.84±1.16
Pumpkin	61.12±1.57	75.79±0.98
Cucumber	79.99±1.18	86.01±1.50
Bottle gourd	76.3±0.53	84.91±1.06
Ivy gourd	75.29±1.02	84.27±0.63
Tomato	78.56±1.50	85.55±0.98

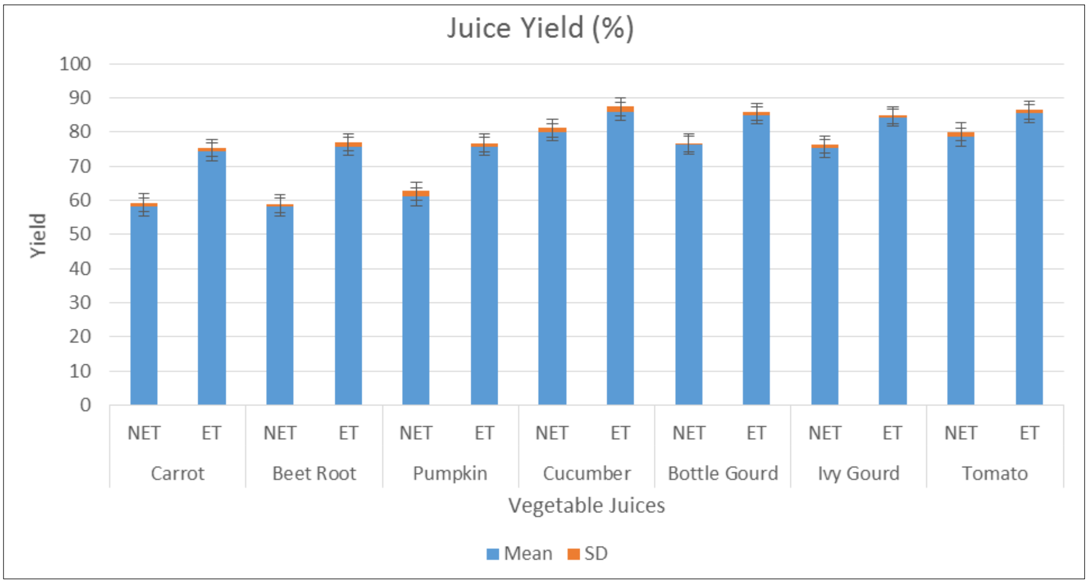

Fig 1: Yield of Juices

Among all the juices it was observed that enzyme treated pulp was given more yield when compared to conventional crushing & pressing method. The increase in juice yield was ranged from 6 to 18%. Among all seven vegetables Enzyme treated beetroot pulp was given increase in yield of juice. i.e. 18% and minimum effect was observed in cucumber and shown graphically in figure 1. The increase in yield of juice was due to degrade cell wall polysaccharides and thereby releasing soluble compounds and enhanced yield of juice (Grassin, C & Fauquembergue, P., 1996) [8].

3.2. Total Soluble Solids
Results of Total Soluble solids of both non-enzyme treated (ET) and Non-Enzyme Treated were presented in Table 2.

Table 2: Total Soluble Solids (^{0}Brix)

Vegetable Juices	NET	ET
Carrot	5.73±0.05	6.5±0.02
Beet Root	6.57±0.12	7.4±0.07
Pumpkin	6.53±0.05	7.46±0.02
Cucumber	2.82±0.02	2.88±0.01
Tomato	4.03±0.09	4.7±0.03
Ivy gourd	3.8±0.08	4.26±0.01
Bottle Gourd	5.23±0.05	5.98±0.20

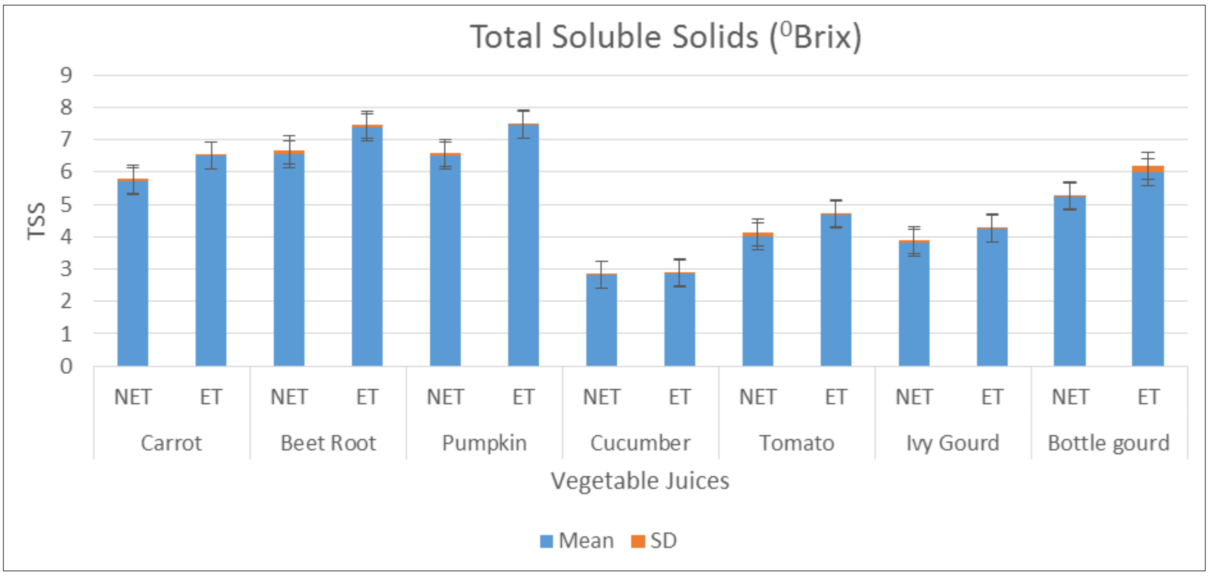

Fig 2: Total Soluble Solids (^{0}Brix)

Significant increase in Totals Soluble Solids was observed in all enzyme treated vegetable juices and the compared results were shown in figure 2. The increase in Total Soluble Solids of enzyme treated juices were resulted might be action of liquefied pectinase enzyme on polysaccharides by producing soluble sugars (Schobinger *et al.*, 1981) [20].

3.3. Acidity

Obtained results of both Enzyme Treated (ET) and Non-Enzyme Treated (NET) were presented in table 3.

Table 3: Acidity of vegetable Juices (%)

Juices	NET (%)	ET (%)
Carrot	0.1±0.01	0.12±0.01
Beet Root	0.14±0.01	0.17±0.01
Pumpkin	0.16±0	0.18±0.01
Cucumber	0.05±0	0.06±0.01
Tomato	0.25±0	0.27±0.00
Ivy gourd	0.08±0	0.09±0.01
Bottle Gourd	0.14±0	0.19±0.01

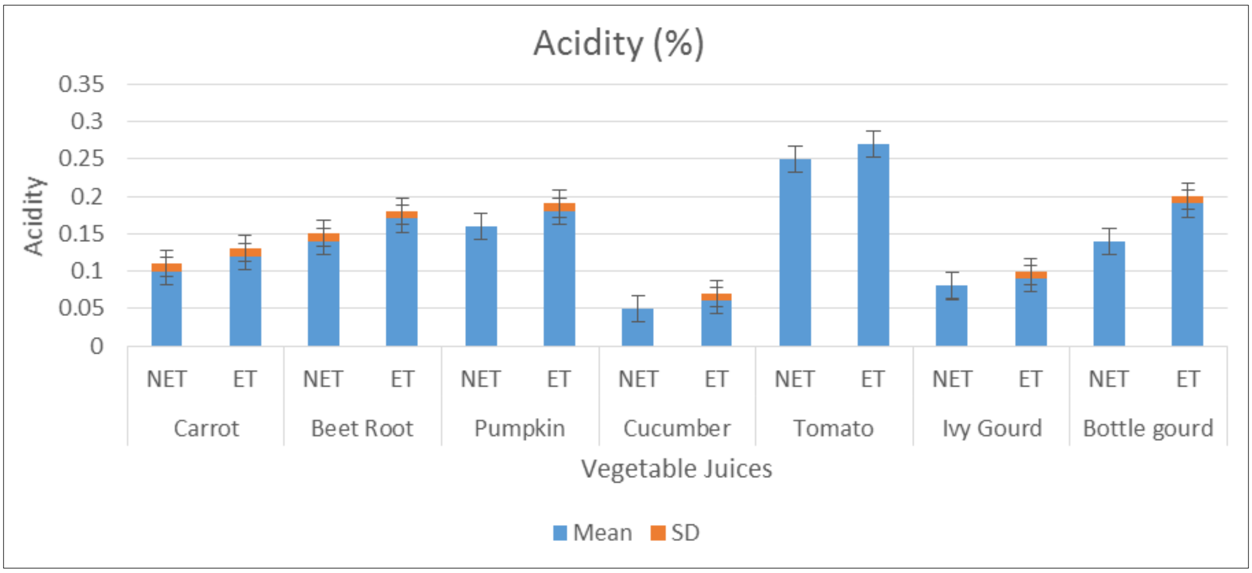

Fig 3: Acidity of Vegetable Juices.

Acidity of all enzyme treated vegetable juices were significantly high when compared to non-enzyme treated vegetable juices and shown graphically in figure 3. The acidity of non-enzyme treated vegetable juices were lower than Enzyme Treated vegetable juices. The significant high acidity of enzymatic vegetable juices might be due to enzymatic breakdown of polysaccharides to galacturonic acids (Acar J *et al.*, 1999) [2].

3.4. Viscosity

The results of both enzyme treated (ET) and non-enzyme treated (NET) vegetables were presented in Table 4.

Table 4: Viscosity of Vegetable Juices

	NET	ET
Carrot	7.37±0.12	6.67±0.55
Beet Root	8.37±0.12	7.90±0.17
Pumpkin	8.63±0.09	7.58±0.99
Cucumber	7.13±0.12	6.83±0.15
Tomato	7.77±0.09	7.17±0.21
Ivy gourd	7.93±0.12	7.33±0.15
Bottle Gourd	8.2±0.16	8.03±0.12

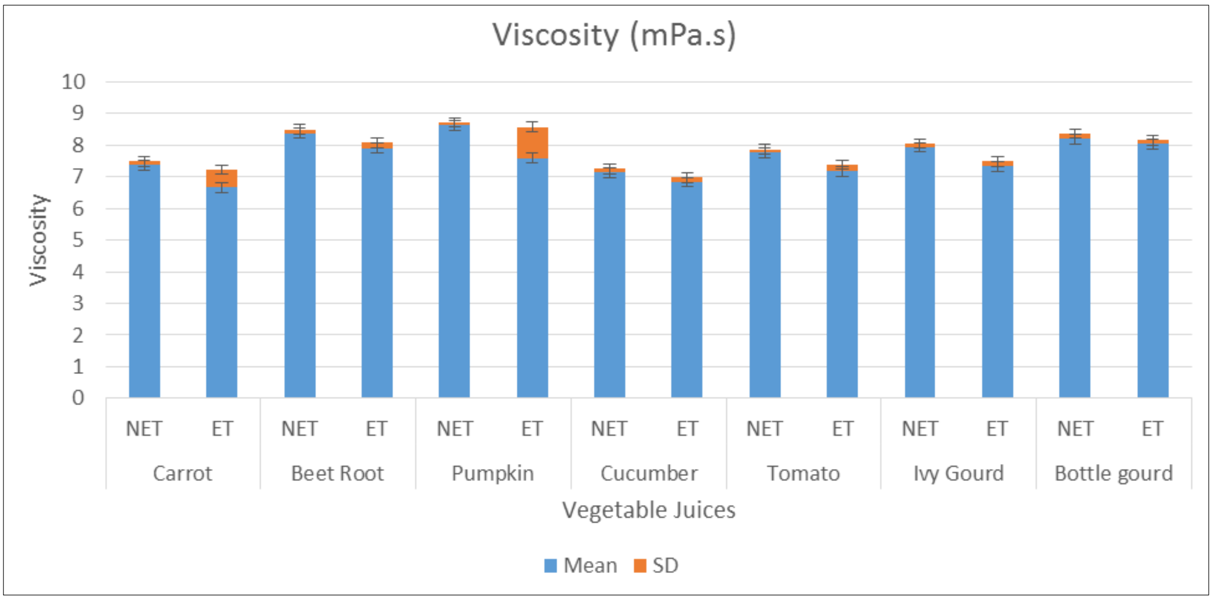

Fig 4: Viscosity of Vegetable Juices.

Lower values of viscosity were observed in all liquefied enzyme treated juices compared to Non-enzyme treated vegetable juices and shown the comparison graphically in figure 4. In general pectic substances or polysaccharides shows string affinity to retain in their cohesive structure and the lower values of viscosity in enzyme treated vegetables might be due to degradation of pectic substances or polysaccharides reduces the affinity of retention of water in their structures there by increasing free water in the enzyme treated juice (Lee *et al*., 2006; Sin *et al*., 2006; Abdullah *et al*., 2007; Maktouf *et al*., 2014) [13, 21, 1, 14].

3.5. P^H^

The pH results of both Enzyme Treated (ET) and Non-Enzyme Treated Vegetable juices were presented in table 6.

Table 5: pH

	NET	ET
Carrot	6.13±0.09	5.75±0.02
Beet Root	5.82±0.02	5.71±0.01
Pumpkin	6.48±0.02	6.34±0.02
Cucumber	4.97±0.05	4.87±0.06
Tomato	4.1±0.08	3.86±0.00
Ivy gourd	4.23±0.05	4.00±0.01
Bottle Gourd	4.2±0.08	4.02±0.01

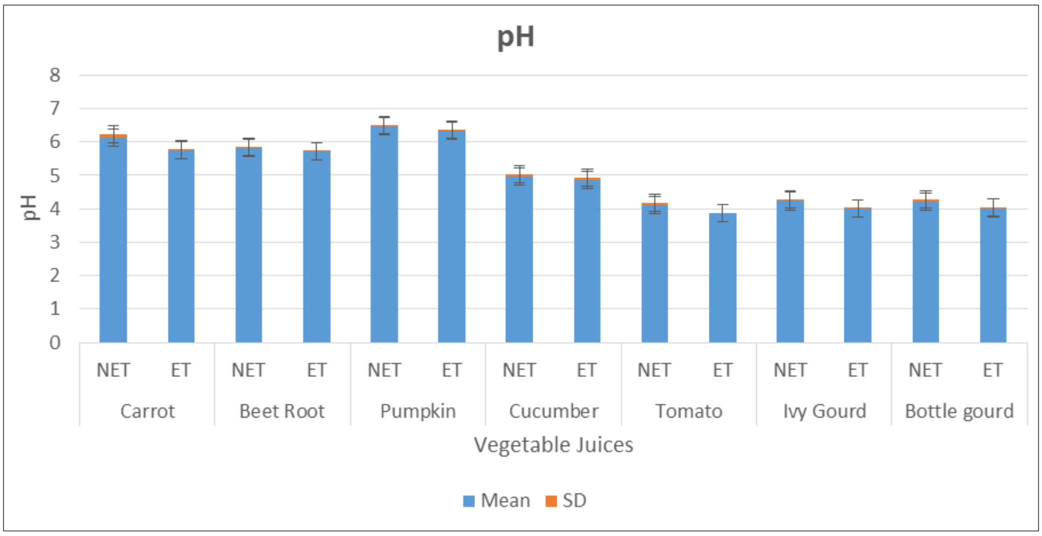

Fig 5: p^H of Vegetable Juices.

P^H was lower in enzyme treated vegetable juices than that of non-enzyme treated vegetable juices and the comparison was shown graphically in figure 5. The lower pH was might be due to release of carboxyl groups and galacturonic acid from polysaccharides and pectin (Gurrieri S, *et al*., 2000) [9].

4. Conclusion

In the present study Yield, Total Soluble Solids, Colour, Acidity, Viscosity and pH of both Enzyme Treated and Non-Enzyme Treated Vegetable Juices were evaluated. In all enzyme treated purees the yield of juice was extremely high (14-18%). Among all seven vegetables, the yield of Juices was high in Carrot, Beetroot and pumpkin where as in remaining enzyme treated vegetables juices the yield was not that much high (6-9%). Total soluble solids content was increased in enzyme treated juices, the increase of Total

Soluble Solids of enzyme treated juices was ranged from 0.06 to 0.93^0Brix. Acidity was increased in enzyme treated juices and the increase was ranged from 0.007 to 0.05%. The increment was more in bottle gourd (0.05%) and lower in Cucumber (0.007%). Viscosity of enzyme treated vegetable juices was lower compared to non-enzyme treated juices and the lowering was ranged from 0.17 to 1.05mPa.s. Compared to non-enzyme treated vegetables juices, Enzyme treated juices pH was lower and that ranged from 0.1 to 0.38. The reduction was high in carrot (0.38) and lower in cucumber (0.1). Liquefied enzyme treated puree was given more yield of juice when compared to non-enzyme treated puree and all the remaining studied parameters were also shown some significant difference but not that much variation.

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