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Deactivation of Bromelain in Pineapple Juice and analysing its Physico-Chemical and Organoleptic properties for successful commercialization via FMCG Industries in Indian Subcontinent.

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Abstract: The plan of this investigation was formulated to study the biochemical and physical attributes of juice extracted from the pulp which has high sugar (glucose and fructose) content and adequate moisture. Pineapple confers health benefit on the host as it contains huge quantities of vitamin C and other antioxidants. Deactivation of bromelain was also performed so that there are no hindrances in the juice manufacturing process. Sodium meta-bisulphite was also added to increase the shelf life of the product. During the production period, the pH, temperature and acidity was monitored. The pineapple juice was then subsequently characterized for sugar analysis, gas chromatography, UV spectral analysis and FT-IR. The amount of volatile compounds was quantified by HPLC with differential solvent system. The juice sample was also subjected to sensory evaluation and their colour stability was also checked. This research component would be a key technique to unlock the problems and tap the potential of pineapple juice manuafcturing in India.

Keywords: pineapple juice, volatile composition, sensory evaluation, gas chromatography, UV Spectroscopy, FT-IR, HPLC.

Full Length Original Research Paper

Introduction

Pineapple is a tropical fruit which has a good pallatibility and has a great acceptance among the global population. More than 25 cultivars of pineapple are cultivated commercially [12] in various regions of India. Pineapple has in itself a class of enzymes called bromelain [13]that are responsible for interfering with the juice making process[14]. It binds with the essential proteins and hinders with the sugar metabolism[15] or in the alcohol conversion process[16]. There is subsequent froth formation[17] and therefore efficiency is low[18]. Consequently, the yield is low and therefore the companies suffer a loss and eventually there is a decrease in Pineapple Juice production[19]. Our research will emphasize on the removal of bromelain so that there is an increased yield of juice and therefore a possible incrementation in the market turnover.

Materials and Methods

Collection of Pulp and Juice Extraction and subsequent Clarification of Juice sample

Pineapple **(Figure 1)** commonly available and grown in Vellore City, Tamil Nadu, was procured from a local market for this study. For processing, the pineapple was washed, peeled and macerated using a kitchen applicant grinder. The obtained juice sample is now clarified with addition of 5 ml of pectinase P50 enzyme

commercially available. From one kilogram of pineapple initial juice content was 750 ml and after treatment with pectinase the juice yield percentage imporved upto 7 %. The juice obtained in this manner was then subjected for analysis for various physio-chemical characteristics. Filtration was done so that the juice components can be segregated from the residual solid wastes which are not a part of the juice making process in FMCG Companies.

Figure 1

Deactivation of Bromelain and addition of preservative to increase shelf life

100 ml of pineapple juice was now treated with 50 mg Zinc Gluconate so that the enzyme gets precipitated with gradual stiring with a pH 5.5. Zinc is a metal ion and it gets itself ionized when it comes in contact with an electrolytic media[22]. Zinc ions break from the gluconate anions and binds on to the enzymes and increases its precipitation coefficient[22]. As a result the turbidity is removed indicating a possible occurrence of a precipitation reaction. Now the juice is filtered through a mesh cloth and the filtrate is collected and is heated at subsequest temperatures of 90° C for 4 seconds, 65° C for 3 seconds and then 72° C for 5 seconds and is immediately cooled to 4° C so that the quality does not degrade and the nutrients are not lost. Now to preserve the juice, 2 mg of Sodium Bisulphite is added in the final product so that it remains fresh and can be consumed.

Effect of different conditions on the storage of juice

Pineapple juice was packed in bottles of different colours, viz. brown, green and white which was stored at a temperature of 0°C, 4°C, 37°C, 55°C and at room temperature. The changes in optical density at 420nm were monitored.

Estimation of glucose

These were quantitatively estimated continuously by conventional glucose assay at an interval of 10 days for a total of 40 days, the assay was carried out by utilizing 10% $K_2Cr_2O_7$ and commercially available 100% ethanol and 98% H_2SO_4.

Physico-chemical and sensory analysis

The concentration of reducing sugars was estimated by Shaffer & Somogyi method[23]. The specific gravity was determined at 20°C with densitometer. Total acidity was determined by titration with 0.1N NaOH expressed in tartaric acid and volatile acidity within the distillate samples expressed in acetic acid mg/100ml. Phenols of the pineapple juice and juices were estimated by Folin-ciocalteau method[24]. Effect of storage, colour stability and browning index of stored juices was measured as juice colour OD at 420 nm.

Instrumental Analysis of Phytochemical Compounds

Estimation of Phytochemical Compounds using Gas Chromatography

The samples were centrifuged at 5000xg for 10min. Nitrogen was used as carrier gas and 2 m, 2 mm ID1/4 mm quantity with a flow of 20ml/min was applied. The analysis of total volatile compound composition was carried out on a Hewlett-Packard series 6890gas chromatograph linked to an HP-5973 mass-selective detector, with a 30m×0.25mm i.d., 0.25um film thickness HP-5MS (Agilent, Palo Alto, CA) fused silica capillary column. The compounds were preliminarily

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identified by the use of NIST, Wiley, NSB mass libraries, as well as mass data from literature.

Estimation of the Desired Functional groups in the phytochemical compounds using Fourier Transformed Infrared Spectroscopy

The samples were subjected to FTIR for confirmation of the functional groups in the pineapple juice. Notably all the compounds present in the juice medium may have different functional groups but the corresponding wave numbers suggest the type of bonds and the gradual shift of peaks in the spectral data.

Quantification of Desired phytochemical compounds using High Performance Liquid Chromatography- Reverse Phase

The phyto-chemical compounds were subjected to High performance liquid chromatography so that we can find out the purity of the compounds using the most appropriate solvent system. Reversed phase HPLC was used for analysis of the sample. The flow volume for apparatus is 1ml. Methanol was used as mobile phase. 20 ul of the compound was injected into the apparatus. The run time was 50-100minutes.

Finding the Nature and Type of Pigments using Ultraviolet Visible Spectroscopy

The refined juice has some pigments and this is the reason why the colour of the juice changes when it is stored in the different bottles[5]. To understand the type of pigments present in the juice, we use UV Spectroscopy to find the desired peak positions at corresponding wavelengths. Still preliminary studies[20] suggest the possibility of the presence of carotene, chlorophyll a, b and huge quantities of xanthophyll.

Sensory Evaluation Using Bax-Baum Plot

Sensory evaluation of juice samples were performed by 4 well trained panellists. Different attributes of produced pineapple juice included were: visual, aroma, taste and harmony. The system used was Bax-Baum that contains total 20 points including, 2 points for colour, 2 points for clarity, 4 points for smell and 12 points for taste.

Temperature Optimization of the Juice Sample

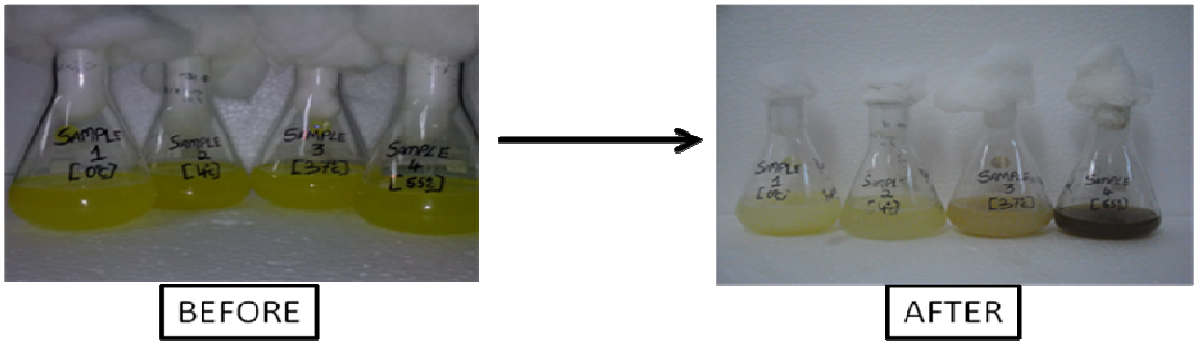


Figure 2

Juice samples were kept at various temperatures and it was optimized **(Figure 2).**

pH Optimization of the Juice Sample

The pH of the juice samples were regulated in addition to acetic acid, phosphoric acid and increasing amounts of 0.1 N NaCl (**Figure 3)**.

Figure 3

Results and Discussion

The following results have been obtained after the experimental procedures.

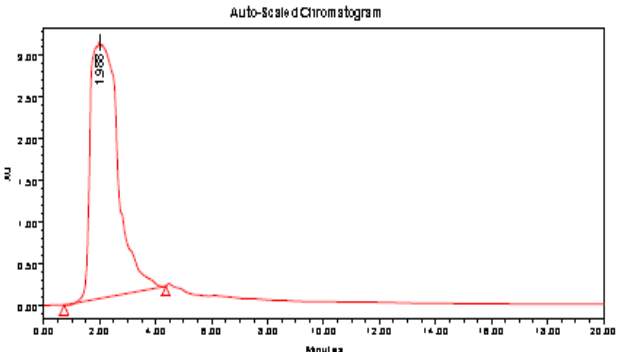
Glucose Estimation

The glucose levels **(Figure 4)** have also been recorded.

Figure 4

HPLC Results

(Figure 5) shows quantification of relevant biochemical compounds given for analysis



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.988	211072638	3085327		

Figure 5

FTIR Results

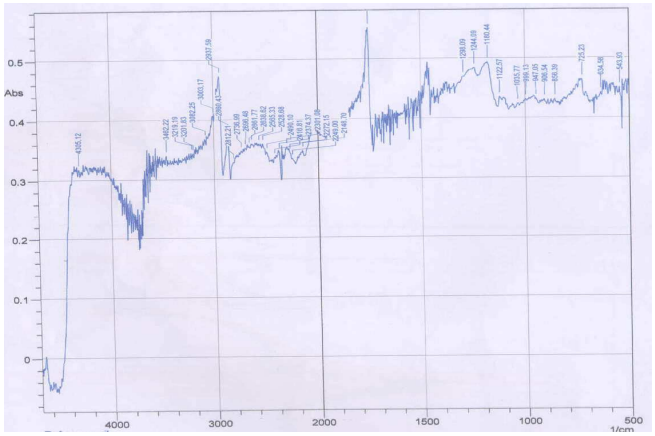


Figure 6

(Figure 6) corresponds to the fundamental functional groups at specific wavenumbers (Table 1)

SL. No	Peak	functional group
1	808.17 & 825.53	alkyl halides
2	927.76 & 1751.36	carboxylic acids
3	952.84 & 995.27	alkenes
4	1043.49, 1058.92, 1093.64, 1182.36 &1247.94	aliphatic amines
5	2150.63 & 2216.21	alkynes
6	2862.36, 2939.52, 2962.66	alkanes
7	3365.78	alcohols, phenols

Table 1

UV Anaylsis of pigments

Figure 7 shows the UV Peak absorbance for the pigments present in the system.

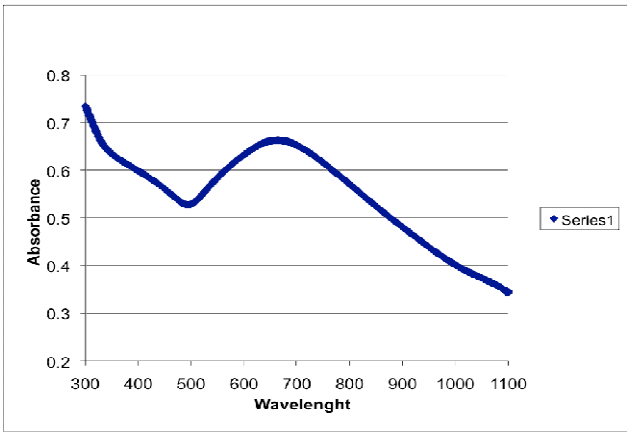


Figure 7

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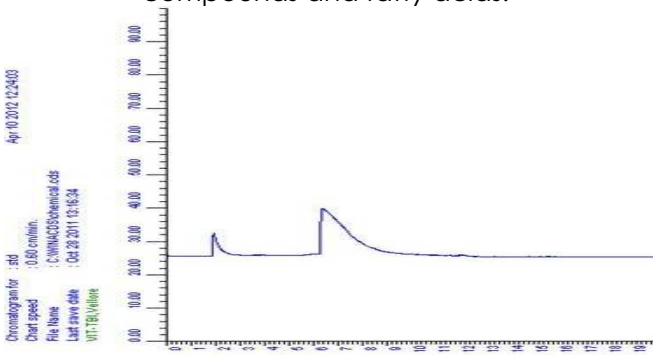
Gas Chromatogram

Figure 8 shows the chromatogram for volatile compounds and fatty acids.



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Last save date : Oct 28 2011 13:16:34
VIT-TBI,Vellore

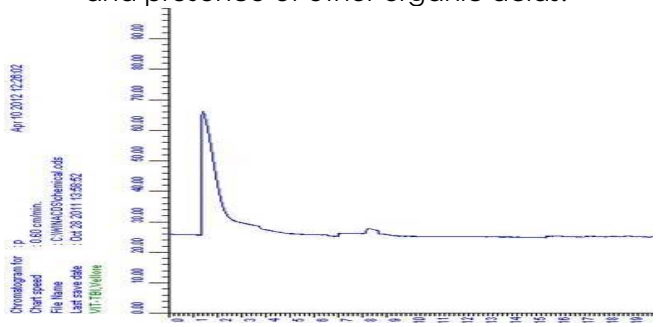
Peak No	RT min:sec	Height mV	Area mV-sec	Amount % Area	Name
1	01 : 56	6.766	106.188	9.822	
2	06 : 24	13.891	927.329	85.775	
3	08 : 29	1.505	31.601	2.923	
4	10 : 57	0.437	16.000	1.480	

Total Area : 1081.118 mV-Sec

Checked:GSU
Approved:LA

Figure 8

Figure 9 shows the chromatogram for the esters and presence of other organic acids.



RAN Report for : p Apr 10 2012 12:27:03
File Name : C:\WINACDS\chemical.cds
Last save date : Oct 28 2011 13:58:52
VIT-TBI,Vellore

Peak No	RT min:sec	Height mV	Area mV-sec	Amount % Area	Name
1	01 : 24	40.660	1823.498	92.843	
2	07 : 17	0.999	30.709	1.564	
3	08 : 00	0.827	27.694	1.410	
4	08 : 16	2.452	82.164	4.183	

Total Area : 1964.065 mV-Sec

Checked:GSU
Approved:LA

Figure 9

Screening of pineapple cultivars:

The total soluble solids (TSS) of the pineapple must range from 14.2 to 20.5 percent. The sugar content of musts range from 15 to 18% (w/v) and the pH was 3.8 to 5. The concentration of esters in juice varied from 15 to 35 mg/l and it was affected by factors like clarification conditions. The result obtained confirmed the previous published reports[20]. The quantity of phenolic compounds present in pineapple juice is comparable with commercial white juices[19]. Phenolic compounds play a pivotal role in determining the quality of juice, because they greatly influence the colour, bitterness, astringency and chemical stability of juice[10].

Characterisation of pineapple juice by Gas Chromatography:

Preformed alcohols, esters and volatile organic acids were already present in different proportions. The fruity alcohols present are isoamyl alcohol, isobutyl alcohol, n-propanol, ethyl acetate, and phenyl ethanol. The organic acids detected in the juice sample are acetic acid, propanoic acid and benzoic acid.

Effect of storage temperature and glass surface color on pineapple juice color:

The changes in the color of pineapple juice stored at different temperatures (8, 16 and 25 degree centigrade) in bottles of various colors were monitored by the browning index. Juice that was stored in the darker brown bottles at low temperature showed low browning indices when compared to juice stored in white bottle. Juices stored in the brown bottles have the lowest browning index. These findings are in accordance with previous report. The pineapple juices in clear white bottles had an undesired color and a higher browning index **Table 2.** This shows how glass surface color can alter and change the colour of the juice pigmentation to a certain extent. The plausible reasons might be due to acute absorption of wavelengths through the glass interiors and hence no outgoing of it from the system.

Type of effect	OD at 420nm
Temperature	
8	0.18
16	0.21
26	0.26
Bottle color	
Brown	0.21
Green	0.25
White	0.28

Table 2

Conclusions

The sensory evaluation has indicated that the juice possesses novel characteristics in aroma and taste and good market acceptability. Classified data on an adequate process for juice making from pineapple and the findings regarding pectinase treatment, formation of major volatile compounds, GC, FTIR and HPLC characterisation and sensory evaluation can be of utmost importance to the FMCG industries for a better global profitability and creating generic products with high value propositions and benchmarking the safety standards.

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