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Fruits & vegetables are an important nutritional requirement of human beings as these foods not only meet the quantitative needs to some extent but also supply vitamins & minerals which improve the quality of the diet & maintain health. Fruit, vegetables & oil seeds processing is one of the pillars of the food & edible oil industry. India is the second largest producer of both fruits and vegetables. Fruits and vegetables are the reservoir of vital nutrients. Being highly perishable, 20 to 40% of the total production of fruits and vegetables goes waste from the time of harvesting till they reach the consumers. It is, therefore, necessary to make them available for consumption throughout the year in processed or preserved form and to save the sizeable amount of losses. At present, about 2% of the total produce is processed in India mainly for domestic consumption. Fruits and vegetables have great potential for value addition and diversification to give a boost to food industry, create employment opportunities and give better returns to the farmers. Oil seeds also play an important role in the food sector & daily life. Edible oils constitute an important component of Indian households. Domestic edible oil consumption in India is increasing. Self sufficiency in edible oils today stands at in recent years, availabilities of non conventional oil, rice bran oil, soybean oil, palmolein oil and cottonseed have increased. Oils are essential components of all plants. However, commercial oil production facilities only utilize plants that accumulate large amounts of oil and are readily available In order to improve the nutritional status of the people & also to exploit the export potential of processed products there is need to increase the productivity of processed food in the country. Currently, India accounts for 7.0% of world oilseeds output; 7.0% of world oil meal production; 6.0% of world oil meal export; 6.0% of world veg. oil production; 14% of world veg. oil import; and 10 % of the world edible oil consumption.

Some of the fundamentals of the book are preservation of pineapple, mango and papaya chunks by hurdle technology, effect of boiling on beta-carotene content of forest green leafy vegetables consumed by tribals of south India, process development for production of pure apple juice in natural colour of choice, physical refining of rice bran and soybean oils, anti nutrients and protein digestibility of fababean and ricebean as affected by soaking, dehulling and germination, quality changes in banana (musa acuminata) wines on adding pectolase and passion fruit, essential oil composition of fresh and osmotically dehydrated galgal peels, development of cold grinding process, packaging and storage of cumin powder, bakery products and confections, etc.

This book deals completely on the basic principles & methodology of fruits, vegetables, corn & oilseed processing & its preservation. This will be very resourceful to readers especially to technocrats, engineers, upcoming entrepreneurs, scientists, food technologists etc.

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Sample Chapter:
Preservation of Pineapple, Mango and Papaya Chunks by Hurdle Technology

Shelf stability of minimally processed products can be enhanced by using a combination of treatments such as blanching-osmo-dehydration, addition of chemical preservatives, modification of pH and appropriate packaging. This technology is known as Hurdle Technology. The different treatments called as hurdles are used in preventing the chemical as well as microbiological deterioration of the food product. A combination of these hurdles is very effective, while individually each hurdle is ineffective in protecting food from deterioration.

Hurdle technology has also been used for the preservation of minimally processed fruit slices. A combination of mild heat treatment, reduction of water activity, lowering of pH and addition of potassium sorbate and sodium bisulphite for producing shelf stable pineapple slices was reported by Alzamora. High moisture products from peach, pineapple, mango, papaya, chicozapote and banana treated with a combination of mild heat treatment, water activity reduction, addition of antimicrobials and packed in glass flasks or high density polyethylene bags with syrup containing preservatives, had a shelf stability ranging from 4 to 8 months. They observed that pineapple slices blanched in saturated vapour for 2 min, cooled in water and immersed in a glucose syrup containing 150 mg/kg sodium bisulphite and 1000 mg/kg potassium sorbate were acceptable up to 120 days of storage.

However, in all these studies, the fruit chunks were stored in syrup containing high concentrations of preservatives. The usage of covering syrup not only increases the bulk but also increases the cost of the product. Therefore, there is a need to explore the possibility of preserving fruit chunks of tropical fruits such as pineapple, mango and papaya in a cost effective method. The main objective of the present investigation was to study the effect of different barriers or hurdles viz. osmo-blanching, reduction of pH, addition of preservative and vacuum packaging on the shelf stability of fruit chunks of pineapple, mango and papaya packed in polypropylene pouches, without covering syrup.

Materials and Methods

**Fruit chunks**: Mature, firm ripe pineapples, mangoes (C. V. ‘Neelum’), and papayas were procured from the local fruit market, washed, manually peeled, cored and cut into 1 inch x 1 inch x 0.5 inch dimensions. The cut fruit chunks were used for different treatments. 20^0Brix [a] and 40^0Brix [b] sucrose syrup with 0.2% citric acid at 85^0C for 5 min using 1:2 fruits to syrup ratio. The blanched fruit chunks were further dipped in 20^0 Brix syrup containing 0.2% citric acid, 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg for 8 h at ambient temperature, drained and packed in 150 gauge polypropylene pouches and sealed:

The fruit chunks were analysed at 0.30 and 60 days for the chemical, microbiological and sensory qualities.

**Chemical analysis**: Moisture, total solids, reducing sugars, total sugars and carotenoids were analyzed as per the procedure described by Ranganna. pH was measured using a digital pH meter, Model No. APX 175, Control Dynamics, Bangalore, India. Sulphur dioxide content of the sample was distilled in the
presence of acid in an inert atmosphere and absorbed by iodine, which converts sulphurous acid to sulphuric acid and titrated against standard sodium thiosulphate solution. The SO2 was reported as free and combined. The sample was saturated with NaCr solution and neutralized by excess NaOH. The benzoic acid present in the sample was converted into water soluble sodium benzoate, the solution was acidified with excess HCl, water insoluble benzoic acid was formed, and extracted with chloroform. The chloroform was removed by evaporation and the residue containing benzoic acid was dissolved in alcohol and titrated against standard NaOH.

Sensory evaluation: The samples were assessed for colour, flavour, texture and overall quality by a 15 member trained panel on a 10 point scale, where 1-2 = poor, 3-4 = fair, 5-6 = good, 7-8 = very good, and 9-10 = excellent. Samples receiving an overall quality score of 7 or above were considered acceptable and those receiving below 7 were considered unacceptable. The sensory data were subjected to two way analysis of variance and the difference between the means was analyzed by Duncans multiple range test.

Results and Discussion
The pHs of mango and papaya fruit chunks got reduced to below 4.0 after the syrup treatment as compared to the pH of fresh chunks (Table 1). The reduction of pH and penetration of sugar into the treated fruit chunks also improved the sensory quality by establishing sugar acid ratio in addition to the preservative effect. King also observed that reduction of pH in case of fruits having a pH of 4.5 and above also functioned as an effective hurdle in improving the shelf stability of minimally processed products. Lower pH caused unfolding of the protein molecule by disruption of the hydrogen bonds, thereby reducing the enzyme activity and increasing its sensitivity to denaturation. There was no significant change in pH in all the treatments during storage. The SO2 concentration in the treated pineapple and mango chunks ranged from 65 mg/kg to 80 mg/kg and the benzoic acid from 150 mg/kg to 200 mg/kg. The treated papaya chunks had higher amount of SO2 (135 mg/kg) and benzoic acid (135 mg/kg).

In case of papaya where initial total soluble solids content was very low i.e. 10 Brix the syrup treatment improved colour, flavour and acceptability. The pH of the fruit slices adjusted to 3.5-3.8 had not effected the natural taste of the product.

Effect of storage on chemical changes in pineapple chunks: Reducing sugars of the pineapple chunks showed a steady increase during storage in both [a] and [b] treatments after 30 days and 60 days storage and the increase was found to be maximum in the samples stored at 27°C (Table 2). This could be explained by the higher rate of inversion under acidic conditions at high temperature. There were no significant differences in total sugars in both the treatments stored at 20°C and 27°C. The retention of total carotenoids was found to be maximum in the samples stored at 2°C, indicating the adverse effect of temperature on the samples stored at 270C. A marginal increase in total solids was observed in 60 days storage of both the treated samples, which could be due to the loss of moisture during storage.

Effect of storage on chemical changes in mango chunks: Reducing sugar contents of mango chunks stored at 270C increased during storage. Total carotenoid contents decreased in the mango chunks stored at 270C, whereas, there were no significant changes in the samples stored at 20C, indicating better storage stability at low temperature. The changes in total sugars were not appreciable. Total solids content increased marginally during storage, indicating loss of moisture (Table 2).

Effect of storage on microbiological quality of pineapple chunks: Pineapple chunks subjected to the blanching treatment of (a] and stored for 30 and 60 days showed mesophilic aerobic counts of 640 and 1000 cfu/g, respectively. In case of chunks subjected to treatment [b], the count was 500 cfu/g both at 30 and 60 days of storage at 270C. There were significant increases in yeast and mold counts after 60 days of storage.
storage in both [a] and [b] treatments (Table 3). The pineapple chunks had an acceptable quality up to 30 days storage at 270C. Coliform count was found to be negative in all the treatments of pineapple chunks during the entire period of storage. Alzamora also reported that pineapple slices preserved by combined methods and stored for 4 months at 270C exhibited no growth of microorganisms during 30 days storage. The lower microbial growth rates in their study could be due to the higher level of preservatives used and packing the fruit slices along with the syrup containing preservatives.

Carotene contents of papaya chunks decreased with increase in temperature (270C) during storage. It was a first order reaction rate constant ‘k’. The reaction rate (k) was higher at higher temperature, indicating faster degradation of carotenes at 270C than at 20C. Also, the product packed under vacuum had lower degradation of carotenes, as compared to ordinary packing. This might be due to the fact, that carotene was more sensitive to oxygen in the storage atmosphere (Fig. 2).

These studies have indicated the effect of storage conditions in particular temperature and the method of packing, on the changes in the reducing sugars and carotenes during storage. By determining the ‘k’ value, the rate at which these quality parameters deteriorate over the storage period can be evaluated. To retain the maximum nutrients, appropriate conditions can be applied.

Effect of storage on microbial quality of papaya chunks: Microbial study during 3 months storage at 270C indicated no significant growth of microorganisms, indicating the stability of the product during storage. The aerobic counts increased marginally at 60 days storage and decreased at 90 days storage. There was no growth of yeast and mold and coliform count was found to be negative during the entire period of storage (Table 6). Guerrero reported that aerobic plate count increased by two logarithmic cycles and then decreased, reaching values similar to initial one during storage of banana puree by combined methods.

Effect of storage on sensory quality of papaya chunks: Table 7 shows the overall quality of papaya chunks during storage. Two way ANOVA indicated significant differences among the treatments at the end of 30 days storage. The overall quality of papaya chunks packed without vacuum was significantly superior to other treatments. The vacuum-packed papaya had slightly lower overall quality scores as compared to papaya chunks packed without vacuum because of leaching of juice and partial softening of the tissue during vacuum packing. The papaya chunks packed without vacuum and stored at 20C had superior overall quality, followed by the papaya chunks stored at 270C. The overall quality ratings reduced at the end of 90 days storage but the papaya chunks were still acceptable.

Lopez-Mayo reported blanched papaya slices equilibrated in syrup for 3 days with continuous circulation of syrup packed along with the syrup containing, pH 3.5, 1000 mg potassium sorbate/kg and 150 mg sodium bisulphite/kg in the ‘glass jars had a storage life of 5 months at 250C. The longer storage life in their study was attributed to the higher level of preservatives and packing of slices along with the syrup. In all the earlier reported studies involving the preservation of fruit slices by combined methods, the fruit slices were packed with syrup containing preservative either in glass flasks or high density polyethylene pouches. The fruit slices were also blanched with saturated steam before the treatment. However in the present study, the fruit chunks were blanched in sugar syrup, followed by a dip in syrup containing preservatives. The significance of blanching in sugar syrup was to inactivate the enzymes, reduce microbial load, as well as partial reduction in water activity due to osmotic dehydration during syrup treatment. The flavour of the fruit chunks can also be retained during osmo blanching as compared to the steam blanching where volatile flavour compounds may be lost. The preservative levels in the treated fruit chunks are also comparatively lower than those reported in earlier studies.

It may be concluded that pineapple and mango fruit chunks, blanched in syrup at 850C for 5 min, dipped in syrup containing 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg for 8 h and packed in 150 gauge polypropylene pouches have a shelf life of up to a period of 30 days at 270C and 60 days at 20C, whereas the papaya chunks treated with increase in level of preservatives 680 mg potassium
metabisulphite/kg and 826 mg sodium benzoate/kg have exhibited good storage stability up to 90 days at 20°C and ambient temperature.

Process Development for Production of Pure Apple Juice in Natural Colour of Choice

Consumers always prefer food material free from any chemical preservative or additive. This is especially true in case of food products like fruit juices which are expected to be additive free and close to natural characteristics. From a limited survey in the local markets of Mumbai, it was observed that many additives including preservatives and colour were being used in apple juice. These additives many times would mar the natural quality of juice mainly the taste and appearance. This observation prompted to develop a process in which use of any additive was completely eliminated, while at the same time retaining the colour and other attributes in their original form to the maximum possible extent.

Apple juice can be preserved in very pure state without having any additive in it. Its colour also can be controlled by regulating the enzymatic browning of the juice. The regulation is achieved by physical techniques. In these techniques, the enzymatic browning reaction is temporarily avoided by extracting the juice in the absence of oxygen, under inert atmosphere of nitrogen, which is immediately followed by permanent prevention of the reaction by inactivation of the polyphenol oxidase by thermal energy. Since in apple juice, colour development is due to enzymatic browning and to some extent leaching of colour from skin into juice, regulation of the two phenomena was attempted. The technique developed was very effective in controlling colour of apple juice, as a result of which, the juice could be obtained as a drink in three different colours, free from any additive in it.

Materials and Methods

**Apple:** Apples mainly 'Red delicious' available in Mumbai were used in this study. In few trials a small sized variety locally known as 'Saharangpur' variety were also used. The apples were stored at 0-40°C before use.

**Juicer:** A juicer, Mixer, Grinder Model No HL 3298 [Philips (India) Ltd.] was used to extract juice.

**Modification of juicer:** Under normal conditions, when juice is extracted from apple, it instantly turns brown due to enzymatic reaction. When this juice is bottled, the final product gets a brown shade. Fig. 1 shows the laboratory scale experimental set up to preserve apple juice, in three different colour shades, without adding any external ingredient to it. The above unit had capacity to process 10-kg apples per hour to produce 5 liters of apple juice and pomace, which after drying can yield 1.5kg dry apple powder. The limiting factor of this unit was that the capacity of compartment collecting the waste material and sedimentation of fine powdery sediments in juicer assembly, causing obstacle to rapid discharge of juice from juicer housing into outlet duct. Due to this, after processing of 4 kg, the debris and sediments were removed and the process was restarted.

To overcome the phenomenon of enzymatic browning, the air inside the juicer was replaced with nitrogen and an inert atmosphere was maintained in the juicer throughout the period of operation. Such situation was achieved by modifying the open outlet duct of the juicer by covering it with detachable a box type cover termed as an attachment having a nozzle fitted to it at the base. The attachment was designed and fabricated from 'Perspex' sheets. The attachment can easily be attached to or detached from the juicer outlet duct simply by pushing or pulling. Once the attachment was fixed, air could not enter through the juicer outlet duct as long as a positive pressure of nitrogen gas existed inside the juicer.

Two 'Tygon' tubing of 1/32" ID and 3/32" OD well fitting into the hole, were inserted through the two holes provided on the attachment to introduce nitrogen gas into the attachment and juicer.

The attachment was connected to hot water bath through the flexible PVC tubing. The upper end of the
PVC tubing is connected to the nozzle of the attachment, while the other end to the SS tubing passed through the hot water bath.

Method of flushing air inside the juicer with nitrogen: Nitrogen gas was distributed using a 5-point gas distribution tubing as detailed in Fig. 1. Using this device, nitrogen was introduced at four critical points A, B, C and D as depicted in the figure. This ensured complete nitrogen atmosphere at i) just below the cutter sieve disc of the juicer ii) inside the attachment iii) just above the cutter sieve disc and iv) at the base of waste material collection chamber.

Hot water bath: The function of this unit was to increase apple juice temperature in shortest possible time. The drop of juice just after release from apple tissue entered into heat exchanger and before coming out of the unit it instantly attained a temperature of 80 - 85°C. Polyphenol oxidase (PPO), which catalyses oxidation of phenol leading to browning got totally inactivated and the juice never turned brown.

The hot water bath consists of a thin walled cylindrical tank of 26 cm inside diameter and 26 cm height. A thermostatically regulated heating coil was provided at the center of the circular base (Fig. 1).

Stainless steel tubing of 6 mm inside diameter, 0.1(thickness having 5 meter length was rolled into a helical. The coil was having 25 cm mean diameter of the helical with a descending slope, 5 cm per turn. The total turns 5 in number. The two ends of the SS tubing were brought about 10 cm through the wall of the cylindrical tank. While upper end was connected to the attachment, the lower was connected to a similar tank through which chilled was circulated instead of the hot water.

Cold water bath: This water bath was identical in do to the hot water bath but having no heating coil and cooling coil was shorter in length. This bath was meant to down the juice to a temperature around 20-300C.

Filtration: The juice coming out from the chilled bath, contained some amount of fine particles which need to be filtered out. In laboratory scale, because of volume, the juice was first filtered through cheesecloth remove coarse particles, followed by removal of very fine particles by centrifugation.

Bottling and sterilization: The clear juice thus obtained was filled under nitrogen in 200 ml capacity bottles. The bottles were sterilized by keeping them in boiling water for 15 min. Storage of the bottles at room temperature did not show any microbial growth.

Spectrum of brown pigment formed in apple juice and measurement of degree of browning: Apple juice was extracted and kept in presence of air until brown colour was developed. The juice was centrifuged to remove insoluble solids and the spectrum of the brown pigment was recorded against the reference prepared as follow. Apple juice was extracted from the same lot of apples in absence of air or oxygen. The juice was collected in a conical flask (with stopper) flushed with nitrogen and containing pre-weighed quantity of the antioxidant sodium erythorbate to bring the final concentration to 25 mM. The clear solution obtained after centrifugation was used as a reference. The reference sample was prepared first and then the experimental. The reference sample shows 0.3 O.D. at 390 nm with respect to distilled water. Hence, in absence of any reference sample, browning of apple juice was measured by its absorbance at 390 nm with reference to distilled water minus 0.3.

Determination of the temperature required for inactivation of PPO: About 50-60 ml of apple juice was quickly extracted and collected under nitrogen in a stoppered conical flask having a thermometer and sample withdrawing arrangement. Immediately after extraction the flask was quickly transferred in boiling water bath. Samples (3 ml) were withdrawn from the flask at regular time intervals after noting its temperature at the time of withdrawing. The sample under test was transferred in a test tube kept in a water bath at 300C After allowing the full development of brown colour the test sample was centrifuged and the OD of the brown pigment formed in the clear fluid was read against distilled water at 390 nm using a Shimatzu spectrophotometer.
**Sensory evaluation**: The three varieties of apple juice prepared in laboratory along with sample of two well-known commercial brands of apple juice procured from local market were simultaneously served under stipulated conditions to a taste panel containing 36 judges. The above five apple juice test samples were simultaneously evaluated for five characteristics namely, appearance, colour, flavour, taste and overall acceptability on a 9-point Hedonic scale.

**Operational procedure**: The product can be obtained in three colour shade (1) Brown (2) Slightly pink and (3) Pale yellow, the natural shade of apple juice. Whenever browning is to be eliminated, the attachment is placed at its proper position. The nitrogen carrying tubes were inserted properly up to the points A, B, C and D as shown in Fig. 1. Processing was initiated by switching on the hot water bath and raising the temperature of water in it to 95-96°C. Once the required temperature was attained nitrogen gas was flushed vigorously for about 1 or 2 min, till the air inside the juicer space is flushed out completely. Once this stage was reached, the flow rate of nitrogen gas was reduced to such a level, which was just sufficient to maintain some positive level in the juicer so that no air from outside entered into the space inside juicer. Once such conditions were established, apples (stored either in freezer at 0-4°C or at room temperature) were cut one by one into pieces of the size, which could enter in the juicer. Within a minute or shorter time after cutting, the pieces were crushed in juicer after removing the seeds with help of knife or scoop so that there was very little chance for browning to occur.

Once the juice got released from tissues by the action of speedily rotating cutter sieve, the same was collected in juice collecting compartment filled with nitrogen. Because of non-availability of oxygen in it, browning reaction could not proceed and due to the slope in juicer housing, the juice automatically flowed under gravity and fell in the juicer attachment then through nozzle of attachment, into the inlet port of the hot water bath. As it started flowing due to gravitational force through the helical tubing submerged under water at 95-96°C, its temperature started increasing very rapidly with its further advancement. Once juice entered hot water bath it came out of it in just 30-40 sec with its temperature being increased from 30°C to 80-85°C.

Because of non-availability of small size, constant flow rate pump, gravitational force was utilized to circulate juice through the heat exchanger. However, pump was a better choice.

The hot water bath was designed in such a way that juice once entered in it, flowed continuously without resting in between, and under steady conditions, when juice was continuously fed without any break, the temperature of the juice coming out from outlet port would not deviate from 80-85°C. The hot juice was cooled to 20-30°C, while passing through chilled water bath.

Cleaning of tube in water baths: After completion of operations, water was flushed through tubing which removed all deposits inside the tubing. Holding hot NaOH solution in the SS tubing for some time, followed by flushing with water could carry out harsh cleaning, whenever found necessary.

Apple powder: Apple waste left after juice extraction was dried in an oven at 70°C, the dry mass was pulverized and then passed through a screen of proper mesh size.

**Results and Discussion**

Fig. 2 shows the spectrum of the brown pigment formed when apple juice is exposed to air, showing the maximum absorbance at 390 nm. The residual PPO caused brown pigment formation in the juice after keeping it at 30°C vs the highest temperature to which the juice was subjected during heating treatment to inactivate PPO. Mono phenol and diphenolases (PPO) have been reported to be quite heat stable. Temperature reported here was in full agreement with the data presented. Based on this study, the hot water bath was designed in such a way that under normal operating conditions where juice was expected to flow uninterruptedly at a rate of about
80-90 ml per min, each drop of juice attained a temperature of 80-850C before coming out of the outlet. The various steps involved in processing apple juice to preserve it in three different colour shades. The brown shade of the juice was mainly due to enzymatic browning and to some extent due to the colour leached out from the peel. The leached colour subsequently changed its shade due to heat during sterilization process. The colour due to non-enzymatic browning during sterilization also got added to it and thus final shade was formed.

The pinkish coloured apple juice lacked totally the enzymatically formed brown pigment in it. However, it contained the colour that was leached out from peel into juice (which changed its shade during sterilization) and colour, which developed due to non-enzymatic reactions during sterilization operation.

The third category juice lacked both the enzymatic, formed brown pigment and the pigment, which got extra from peel. The pale but bright colour of the juice was due to natural shade of apple tissue fluid with slight red tinge, which got added due to the non-enzymatic brown, taking place during sterilization. The three samples could stored at room temperature but low temperature was prefer for a longer shelf life.

Organoleptic evaluation: The data on organoleptic evaluation of the three products stored for three months at 40C, along with two-apple juice samples of known common brands are presented in Table 1. As far as the appearance and colour were concerned, two apple juice types brown and pinkish scored the same points as those of market sample 1 (MKS-1) and market sample 2 # (MKS-2). The third type the apple juice in its natural colour, scored marginally less than MKS-2. However, all three varieties of apple juice scored between 6 and 7 between the quality mark "Good" and "below good but all fair" on the Hedonic scale. With respect to the characteristic flavour, apple juice in which browning was allowed showed highest score among all the samples, followed by coloured, the natural coloured juice and then MKS-1 with little margin. However, MKS-2 showed very low score compared with the scores of the four samples.

The elimination of enzymatic browning reduced the score with respect to flavour from 7.49 to 6.63 and further for elimination of phenolic constituents, which were associated with peel, by the action of removal of peel reduced the score further down 6.63 to 6.53. Here the loss in flavour could be due to the one extra heat treatment apart from sterilization the pinkish and the natural coloured juices were undergo for PPO inactivation. Apple is rich source of phenol compounds (90-100 Mg/l00g). These compounds include catachin, pyrocyanadin, epicatechin, 4-methyl catechol, phenol chlorogenic acid, naringin and rutin. The latter two concentrated in peel. The obtained suggest the possibility of involvement of compounds and the associated browning reaction development of flavour. PPOs and phenolic compounds known to be involved in development of flavour during coffee processing and in potato.

Taste is the most crucial characteristic and all the varieties under test showed the score around and above 7('Good') on the Hedonic scale, whereas the market samples were lacking this quality as the scoring was 5.97 (MKS-1) and 4.6 (MKS-2). Many panelists felt that the market apple juice samples were sour in taste, especially (MKS-2) was too much acidic although the claim on was 100 % natural. All the three test samples were far better than the two market samples with respect to taste.

As a result, in respect of the overall acceptance, the highest, score was shown by the brown coloured juice (7.22), followed by pinkish sample (6.75), followed by the natural coloured juice (6.51), then MKS-1 (6.2) the least accepted was MKS-2 (4.78). MKS-1 contains lot of additives including caramel colour while MKS-2 has been labelled as 100 % pure and 100% natural.

Among the three-apple juice samples prepared in our laboratory, the best sample was the brown coloured juice, followed by pinkish one and the last the natural coloured apple juice. However, all these samples were superior to the two market samples MKS-1 and MKS-2 mainly due to taste and flavour. They were not in any way significantly inferior to the market samples with respect to the appearance and colour.

Anthocyanins from Indian Varieties of Grapes
Anthocyanins comprise the largest group of naturally occurring pigments, which are responsible for the red, blue, purple, violet and magenta colouration of most species in the plant kingdom. Notable exceptions are tomatoes and beets, which owe their red-orange and red-purple colouration to the pigments, lycopene and betanin, respectively. These polyphenolic substances are glycosides of anthocyanidins; which are polyhydroxy and polymethoxy derivatives of 2-phenyl benopyrrillum or flavylium salts. The large numbers of glycosyl and acyl groups, which may bind to six different naturally occurring anthocyanins, have contributed to more than 225 different anthocyanin pigments.

Grapes owe their attractive red to purple colouration to these water-soluble flavonoids. Generally, the colouring matter of grapes is found only in the cells of the skin and is a good source of anthocyanins. Grape pomace is a relatively inexpensive source of anthocyanins, since it is a by-product of the wine industry.

However, grape pomace has been reported to be useful in preparation of carbonated beverage with 0.168 mg/ml of anthocyanins. In another report, stability of anthocyanin in model squash and juice systems was studied. The physico-chemical evaluation with respect to berry weight and juice volume, Brix, total acids, soluble acids and sugar rate has been studied in Indian varieties of grapes. Since there are no reports in literature on identification of anthocyanin in Indian varieties of grapes, the present studies were undertaken to identify the same in Indian grapes.

**Materials and Methods**

Grapes, used for the analysis of anthocyanins were procured from the Agricultural Products Marketing Committee Market, Navi Mumbai, India.

Indion 652, a weakly acidic cationic exchange resin, an equivalent of Amberlite IRC 50 was procured from Ion Exchange (p) Ltd., Mumbai, India. All other chemicals were of Analar grade procured from Sisco Research Labs, Mumbai, India.

**Extraction:** The skin was separated from the fruit, and washed with water to remove adhering matter. The grape skin was blended with 0.1% conc. HCl in methanol in a Waring blender. The mixture was left for 15 h at 100°C. It was then filtered through Whatman No. 1 filter paper, using a Buchner funnel. The residue was extracted repeatedly with the same solvent until the extract became colourless. The combined filtrates were concentrated using a Rotary vacuum evapourator (vacuum 700 mm, temperature 350°C).

**Purification:** The extract was purified by passing through weakly acidic ion exchange resin, Indion 652 using the method described by Fuleki.

**Total anthocyanins:** The total anthocyanin content was calculated using the formula:

\[
C = \frac{A}{e} \times \frac{529 \times 1}{L}
\]

expressed with respect to malvidin-3-glucoside, the major pigment found in grapes.

Where, \(e\) - Molar extinction co-efficient of malvidin-3-glucoside = 28,000 M.W. of malvidin-3-glucoside = 529, \(L\), path length = 1 cm, \(A\) = absorbency.

**Separation:** The purified concentrate was subjected to descending chromatography on Whatman No. 3 filter paper. The solvent system used was butanol: acetic acid: water (4:1:5). Each run took 15 h. The paper was air-dried and each band was cut. The pigment on the band was eluted with methanol: acetic acid: water (85:5:5) into 0.1% HCl in methanol. Each fraction was concentrated using rotary vacuum evaporator followed by nitrogen flushing. This concentrate was then used for identification of anthocyanins. For this, the different experiments included (a) spectral analysis, (b) chromatographic mobility, (c) determination of the sugar moiety, (d) aglycone, (e) acyl group.

**Chromatography of anthocyanins:** Table 1 shows the solvent systems used for the extraction of the pigment.

**Partial hydrolysis of anthocyanin:** To about 2.0 mg pigments in methanol, 2.0 ml of 2N HCl was added and
placed in a boiling water bath. An aliquot was withdrawn at 5-min. and spotted on TLC plate. This test distinguishes the type of glycosides.

**Aglycone and sugar:** The pigment (about 1.0 mg in 2.0 ml methanol) was hydrolysed with 2.0 ml of 2N HCl as described above. The mixture was cooled and the aglycone extracted with 1 ml of amyl alcohol. The aqueous phase was extracted thrice with 1 ml of 10% di-N octyl methylamine in chloroform to remove traces of HCl. Finally, traces of amines were removed by extraction with 1.0 ml of chloroform. The sugar solution was dried in a water bath and dissolved in a drop of water. The sample was spotted along with authentic sugars (glucose, galactose, arabinose, rhamnose and xylose). The paper was developed with BBPW. The chromatogram was air-dried and visualised by spraying with aniline hydrogen phthalate followed by heating at 1050°C for 5 min.

The amyl alcohol solution containing the aglycone was spotted on Whatman No. 1 papers or TLC plates and developed independently with solvent systems for aglycone detection.

**Acyl moieties:** To 1.0 mg pigment in 1.0 ml methanol, 1.0 ml of 2N NaOH was added. The test tube was flushed with nitrogen; closed and left at 300°C (room temperature) for 2 h. The mixture was neutralised with 1.5 ml of 2N HCl. For extraction of the acyl moiety, the mixture was extracted thrice with ethyl ether using 2.0 ml each time and evaporated with nitrogen flushing. The residue was dissolved in a few drops of ether and spotted on Whatman No. 1 papers along with authentic compounds (p-coumaric, ferulic and caffeic acid). The papers were developed independently with BAW and 2% acetic acid. The air-dried chromatograms were visualised under UV light at 254 and 366 nm.

**Spectral measurements:** The absorption spectra of anthocyanins and anthocyanidins were measured in 0.1% HCl containing methanol using the Genesys 5 UV-Visible spectrophotometer. Methanol containing 0.1% HCl was used as blank. The AlCl₃ shift was measured by recording the spectrum after addition of 3 drops of 10% AlCl₃ in 95% ethanol.

**Thin layer chromatography:** This was carried out using 20 x 20 cm glass plates with precoated 0.2 mm thick layer of cellulose (E-Merck). The plates were heated at 1200°C for 15 min, before use for activation. Chromatograms were developed in solvent systems.

**Absorption spectra of pigments:** The absorption maxima of the individual pigments in the visible and ultraviolet regions were measured in methanol with 0.1% HCl (Tables 4, 5 and 6). It has been reported that (O.D at 440 nm/visible maximum) of 5-substituted anthocyanin was approximately half of the corresponding anthocyanin in which the 5-hydroxyl group is free. The ratio provides a means of distinguishing between 3- and 3,5- diglucosides. In the present study in pigments of 'Sharad seedless' extract it was observed that these values for monoglucosides were much higher as compared to diglucosides. Acylation of the glucose moiety resulted in reduction in ratio as can be seen with 3,5 diglucoside (46%) and the feruloyl derivative (30%) (Table 4.) Additional peak at 310-325 nm has been reported to indicate the presence of acyl group. This was observed in acylated anthocyanins of 'Sharad seedless' extract.

**Shift in visible lmax** with few drops of AlCl₃ indicated presence of ortho dihydroxyl groups as seen in Tables 4 and 5 for derivatives of delphinidin, and cyanidin. This was, however, not as prominent in pigments of 'Sharad seedless'. However, in the different extracts, it was observed that there was no shift in visible lmax for malvidin derivatives as reported earlier.

**Partial hydrolysis of anthocyanins:** On partial hydrolysis, it was possible to distinguish monoglucosides from diglucosides. In pigments of 'Sharad seedless', which were acylated, the acyl groups separated from the pigments and could be observed under UV with Rf greater than 0.95 in the solvent systems used for identification of aglycones.

**Aglycones:** Identification of aglycones was carried out by comparison of Rf in different solvent systems with reported values. In 'Kalisahebi' extract the Rf values for anthocyanidins were comparable to reported values in Forestal solvent system. The Rf values for anthocyanidins in 'Sharad seedless' were comparable to
values reported in Formic and Forestal solvents systems. However, in case of ‘Pinot Noir’ extract the Rf values for anthocyanidins did not correspond exactly with reported values.

Sugar identification: While glucose was the only sugar found in 'Kalishebi' and 'Pinot Noir' grapes, 'Sharad seedless' grapes contained rhamnose also as sugar group (Table 7).

The genetic relationship among V. vinifera with regard to anthocyanin pigments in grapes has been discussed. It has been concluded that V. vinifera varieties contain only monoglucosides of anthocyanidins. The present study indicates that 'Sharad seedless' variety of grapes is a hybrid variety since diglucoside anthocyanins were present in the extract.

Pigment 1 of 'Sharad seedless' extract contained no acyl or sugar group. It can therefore be concluded that the pigment was the aglycone derived from pigment 2. This band was found to be in very low concentration (Table 4) also indicating that it could be the degradation product of pigment 2.

Physical and Functional Properties of Mucilages from Yellow Mustard (Sinapis alba L.) and Different Varieties of Fenugreek (Trigonella foenum-graecum L.) Seeds

Traditionally, starch and gelatin have been used to provide the desired textural properties to foods. Large scale processing technology places greater demands on the thickeners and gelling agents employed. Recently, mucilaginous substances from oilseeds (mustard, canola and flax) and fenugreek seeds have shown to possess polymerase rheological behaviour of gummy substances. These mucilages are known to have certain functional properties in food applications such as thickeners, emulsifiers, stabilizers and foaming agents.

Among the several sources of mucilages the seeds of fenugreek (Trigonella foenum-graecum L.), a leguminous herb, commonly cultivated as fodder and condiments as well as for culinary and medicinal purpose, seems to be a potential source of mucilaginous substance. India ranks first in the production of fenugreek; during the year 1997-98 India produced about 40000 tonnes of fenugreek. This occupies the third place in area and fourth place in production among the spices grown in India. India occupies first position in the world, both with regard to acreage and production of rapeseed and mustard. In India, the Brassica crops occupy the second largest position after groundnut, with 7.06 million hectares, producing about 7.2 lakh tonnes of seeds annually. Therefore, it is pertinent to exploit the use of mucilages from the seeds of fenugreek and yellow mustard. This may help in promoting new food additive and result in increased economic incentives to producers besides helping in by-product utilization and value addition to food.

Materials and Methods

Yellow mustard seeds: The seeds of yellow mustard (Sinapis alba L) were procured from Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.


Reagents: Analytical grade reagents were used.

Mucilage extraction from the seeds of yellow mustard and fenugreek: The method of Cui was standardized
for the extraction of mucilage from seeds of yellow mustard and fenugreek. The seeds were dispersed in boiling water (1: 10 w/v for yellow mustard and fenugreek). The dispersion was incubated at 750C for 25 min, followed by stirring on a magnetic stirrer for 12 h at 230C and the dispersion was filtered through muslin cloth. Mucilage was precipitated from filtrate by adding 95% alcohol (1 : 1 v/v). Precipitated mucilage was either stored in refrigerator at 40C or oven dried at 450C, followed by grinding and finally storage at room temperature (250 10C). The yield of mucilage was calculated for selecting the highest yielding varieties.

**Physical properties**: Specific gravity of the mucilage aqueous solutions (0.1 and 1% w/v) was determined at 200C using specific gravity bottle. The pH of the mucilage solution in distilled water (0.1 and 1% w/v) were determined using a digital pH meter. Viscosity of mucilage solution in distilled water (0.1 and 1% w/v) was determined by Brookfield Synchrolectric Rotary Viscometer (Model LVT) using appropriate spindle. The corresponding factor was noted from the standard table to convert the dial reading to centi poise.

\[
\text{Viscosity (Cp)} = \text{Dial reading of meter} \times \text{speed factor}
\]

**Functional properties**: Water holding capacity was determined according to the method of Quinn. Fat binding capacity was determined by the method described by Lin. Emulsion activity and emulsion stability, as the indices of emulsifying properties, were measured by the modified method of Cui. Whipping properties were measured by the method of Yatsumatsu, at They were expressed as foam expansion and foam stability. Solubility was determined using the method described by MA.

**Statistical analysis**: Data were analysed for ANOVA as described on factorial Combined Randomised Design (CRD).

Results and Discussion

**Mucilage yield**: Mucilage yield from yellow mustard and different varieties of fonugreek is presented in Table 1. Effect of variety on mucilage yield was highly significant (P