Chapter 3
Materials & Methods

Certain honey based fruits and vegetables products like honey aonla murabba, honey carrot candy, honey mixed fruit jam, honey aonla squash and honey toffee were developed by replacing white sugar with honey. Experimental studies were carried out to examine the effects of different packaging materials and storage temperature on various physico-chemical, textural, microbiological and organoleptic characteristic of these honey based food products. Shelf life studies of different developed products were also carried out. This chapter presents the details of material and methods used in present investigation.

3.1 Materials (Honey and fruits & vegetables)

Honey and large sized aonla (Varity: Banarsi) were procured from the K.V.K. Aligarh and orchards of the Agricultural Faculty of A.M.U., Aligarh respectively. Carrot, papaya and guava were procured from the local fruits and vegetable’s shops. Compositional constituents of honey and fruits and vegetables used in this study were determined before preparation of the honey based food products.

3.2 Equipment and Apparatus

A number of equipments and Apparatus were required to conduct the present study. These included Digital pH meter for pH measurement (Thermo Orion USA), Soxhalate apparatus for fat estimation (Borosil Glass), Laminar flow for microbial studies (Yarco, India), B.O.D. cum humidity chamber (Yorco, India), Autoclave (Pooja Scientific instrument, New Delhi), High Speed Tissue Homogeniser (Yorco, India), Hot Air Oven for moisture content (Tanco, India), Electronic Balance (Anamed, India), Spectrophotometer for optical density (Digital Spectrophotometer Model 310E, India), Atmospheric Packaging Machine (Quick Seal, Sevana, India), and Texture Analyzer for textural properties (THAD Stable Micro system, England) etc in addition to glassware’s and electronic balances.

3.3 Methods

3.3.1 Preparation of Honey based food Products

3.3.1.1 Honey Aonla Murabba: One kg honey was used for the preparation of murabba. The recipe included following:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aonla fruit</td>
<td>1.00 kg</td>
</tr>
<tr>
<td>Honey</td>
<td>1.00 kg</td>
</tr>
<tr>
<td>Water</td>
<td>150 ml</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>Citric acid</td>
<td>2-3 gm</td>
</tr>
</tbody>
</table>

Fruits were washed with cold water and after the damaged ones were discarded, they were properly cleaned and pricked with stainless steel fork/knife and immersed in two percent NaCl solution at room temperature. Concentration of the solution was increased by two percent/day and the operation was continued for four days. Fruits were taken out from the NaCl solution after four days and washed thoroughly and dipped in fresh water for 1-2 days. The cleaned fruits were blanched in 1-2% potash alum solution for 4-5 minutes or until separation of segments was observed when the fruits were hand-pressed. After the blanching fruits were washed thoroughly to remove the traces of alum. The blanched fruits were transferred in honey syrup of 55-60° Brix and kept in it for one night. Next day fruits were taken out from the syrup and the syrup was boiled. The syrup was cooled and added again with the fruits. The product was kept again for 24 hours. On third day, the process was repeated with addition of the fruits in hot syrup and the product was kept again for two days at ambient temperature. After two days, the fruits and syrup were boiled together till syrup obtained 68-70° Brix corresponding to temperature of 105-106°C. The product was allowed to cool and packed in clean and sterilized dry glass and PET jars, which were stored in cool and dry place. The flow chart for the preparation of honey-aonla murabba is in fig 3.1:
Washing several times to remove alum

Soaking in honey syrup (55-60 ° Brix)

Raising of strength to 68-70 ° Brix (3-4 Days)

Packaging

Storage

Fig. 3.1 Flow sheet for the preparation of Aonla Murabba in honey syrup

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**Fig. 3.2 Honey aonla Murabba**

**3.3.1.2 Honey Carrot Candy:** 750 gm of honey was used for the preparation of one kg carrot candy. The recipe included following:

- Carrot: 1.00 kg
- Honey: 750 gm

After washing, peeling and removing inedible portion, the carrots were pricked with stainless steel fork and cut into pieces of 1.25-1.5 cm. lengthwise. The pieces were blanched in boiling water for 5 minutes and blanched pieces were placed on a dry cloth and excess water was allowed to drain off. The pricked and blanched pieces were soaked in honey syrup at room temperature overnight. Next day, the carrots were taken out from the syrup and syrup was boiled. The syrup was cooled and added again with carrots. The product was kept again for 24 hrs. On third day, the process
was repeated with addition of carrots in hot syrup and product was kept again for 24 hrs. Next day, the carrots and syrup were cooked together till the candies obtained 68° Brix. The pieces were dried at room temperature till non-sticky. The prepared candies were packed and stored. A flow chart for the preparation of candies is given in fig 3.3:

Fig. 3.3 Flow sheet for the preparation of honey carrot candy in honey syrup
3.3.1.3 **Honey aonla Squash:** 600 ml Aonla fruit juice and 400 ml of honey were used in the preparation of one litre honey aonla squash. The recipe included following:

- Aonla fruit juice — 600 ml
- Honey — 400 ml
- Citric acid — 2 – 3 gm
- Kms — 350 ppm

Fruits were washed with cold water and after the damaged ones discarded, they were properly cleaned and heated in boiling water for 15 mins. The seeds were removed and water added in 1:1 ratio. The separated segments were passed through a pulping machine to get pulp. The juice was strained and mixed with honey, citric acid and kms. Now bottling, capping and labeling was done and stored in cool and dry place. The flow chart for the preparation of squash is given in fig 3.5:
Fig. 3.5 Flow sheet for the preparation of honey aonla squash

Fig. 3.6 Honey aonla Squash
3.3.1.4 Honey Mixed Fruit Jam: 750 gm of honey was used for the preparation of one kg mixed fruit jam. The recipe included following:

- Papaya — 500 gm
- Guava — 500 gm
- Honey — 750 gm
- Citric acid — 10.0 gm

Fully matured, sound and uniform sized fruits were cleaned, washed with tap water and manually peeled and cut into small pieces. The seeds were removed and pieces were passed through mixer to get homogenized pulp. The pulp was concentrated and other ingredients (honey, citric acid) were added. The cooking of pulp was continued till the jam obtained 68.5 °Brix. Now bottling and cooling of jam in glass bottles was done and stored in room temperature or in refrigerator. The flow chart for the preparation of jam is given in fig 3.7:

![Flow chart for the preparation of jam](image)

**Fig. 3.7 Flow sheet for the preparation of jam**
3.3.1.5 Honey toffee

Honey (200g) was taken into a boiling pan and heated up to 1 min. then milk powder was added with continuous thorough mixing and heating continued at low flame for 12-15 min. now 24g hydrogenated fat was added and heating was continued for 4-5 min. at low flame. After heating was stopped, the mass thus obtained was spread over stainless steel tray and allowed to cool down for 5 min. The semi solid mass was now fed to the moulding machine for moulding the toffee into the desired shape and size. The toffee thus obtained mass was allowed to dry for 1.5hr. After drying, toffees are individually wrapped in metalized polypropylene sheets manually. These wrapped toffees were then packed in LDPE bag of 500g capacities. The flow chart for the preparation of honey based toffee is given below:
Fig. 3.9 Flow sheet for the preparation of honey toffee

Fig.3.10 Honey and Honey Chicory Toffee

3.3.2.1 Determination of Physico–Chemical Properties Moisture content, pH, browning index, fat content, ascorbic acid content, sugar content, TSS, β-carotene contents, acidity values etc. of fresh products and during storage period were evaluated as per details given below:
(i) Moisture Content

10 g of finely crushed sample was weighed in a flat bottom dried tared dish. The dish and its content were placed in hot air oven which was thermo statistically controlled at 65±5°C and heated until successive weighing showed no further weight loss. At the end, the dish was removed from the oven and placed in a dessicator and allowed to cool and then again weighed. Following formula was used for the estimation of moisture content of honey based food product's sample.

\[
\text{Moisture content (\%) = } \frac{\text{Loss in weight}}{\text{Initial weight of sample}} \times 100
\]

(ii) Total soluble solids (TSS)

Total soluble solid value is defined as the amount of sugar and soluble minerals present in fruits and vegetables. TSS of final products was determined with the help of hand refractometer, which is based on the principle of total refraction. A drop of sample was placed on the prism and the percentage of dry substance in it read directly. Mean value was expressed as percent total soluble solids in °brix.

(iii) Fat Estimation

The soxhlet method suggested by Association of Official analytical Chemist (AOAC) was used for food product's fat extraction as described below:
20g of crushed sample was taken and a thimble was made with the help of porous paper. The thimble was placed in the extracting tube and this tube was connected with the weighed flask and also the condenser.

The heat vaporized the volatile solvent, which passed up the side arm and was condensed in the condenser. The condensed solvent fell drop by drop on to the thimble. When sufficient amount of solvent had thus been transferred to the extracting tube to fill the siphon arm, it siphoned back over in to the weighed flask. This process was continued for 20 hours until the extraction was completed. Then the bottom flask was removed, the volatile solvent was evaporated and fat extracted was obtained as residue. The following formula was used to express fat content of sample.

\[
\text{Fat content (\%) = } \frac{\text{Wt of the residue left after evaporation of solvent}}{\text{Wt. of sample taken}} \times 100
\]
(vi) **pH Measurement**

pH is the measurement of the inverse log of hydrogen in the solution. It is expressed as

\[ pH = -\log [H^+] \]

where, \( H = \) Hydrogen ion concentrations (g/lit.)

The electronic pH meter (Digital pH meter, Metzer model) was calibrated using 7 pH and 9 pH standard buffer solutions. Then the electrode was dipped in the test solution and the temperature knob was adjusted to the temperature of test solution. The function selector switch was set to 'pH' and reading of digital display was allowed to stabilize before it was noted.

(v) **Determination of Browning Index**

Browning index of honey based food products was determined in terms of optical density (O.D.) by method recommended by Srivastava and Kumar (1994). A brief description is given below

10 g of sample was taken in a beaker and 10 ml of distilled water and 30 ml of 60% ethyl alcohol was added. It was thoroughly mixed and the sample was filtered using Whatman filter paper and filtrate was collected. The filtrate was taken in cuvette and optical density of filtrate at 440 nm was measured by spectrophotometer using 60% ethyl alcohol as a blank. The recorded value gives the browning index of the sample.
(vi) Estimation of Reducing sugars and Total sugars

Reducing and total sugars of honey based products were determined by using Lane and Eynon method suggested by Ranganna (2002), as described below:

**Reagents**

1. **Fehling's Solution A**: 69.28 g of copper sulphate was dissolved in water and made upto 1000 ml.
2. **Fehling's Solution B**: 346 g of Rochelle salt (potassium sodium tartrate) and 10 g NaOH was dissolved in water and made upto 1000 ml.
3. **Methylene Blue Indicator**: 1 g of methylene blue was dissolved in 100 ml of water.
4. **45% Neutral Lead Acetate Solution**: 225 g of neutral lead acetate was dissolved in water and made upto 500 ml.
5. **22% Potassium Oxalate Solution**: 110 g of potassium oxalate was dissolved in water and diluted to 500 ml.
6. **Standard Invert Sugar Solution**: 9.5 g of sucrose (analytical grade) was added to 100-ml water and 5 ml. of conc. HCl. Allowed to stand for 3 days at 20-25 °C for inversion to take place and then made upto mark of the one litre volumetric flask with distilled water.

25 ml of the standard invert sugar solution pipetted into a 100 ml volumetric flask and about 50 ml water was added and neutralized with 20% NaOH using phenolphthalein as indicator until the solution turned pink. Acidified with 1N HCl by adding it dropwise until one drop cause the pink colour to disappear. Made upto mark with water (1 ml = 2.5 mg of invert sugar).

**Standardization of the Fehling's Solution**

Equal quantities of Fehling's solution were mixed and 10 ml of mixed solution was pipetted into 250 ml conical flask and 25 to 40 ml of water was added. Standard invert sugar solution was taken in a 50 ml burette and added to the mixed Fehling's solution, almost the whole of the standard invert sugar solution required to effect the reduction of all the copper, so that no more than 1 ml was required later to complete the titration. The flask containing the cold mixture was heated. When the liquid began to boil, 3 drops of methylene blue indicator were added and boiling was continued during titration. The end point was indicated by the discoloration of the indicator. The
volume of sugar solution required for completely reducing 10 ml of Fehling’s solution was noted.

\[
\text{Titration volume} \times 2.5 = \frac{\text{Factor for Fehling’s solution}}{(\text{g of invert sugar})} \times \frac{1000}{1000}
\]

**Preparation of Sample**

50g of finely crushed sample was weighed and 400 ml of water was added to it. The solution was neutralized with 1N NaOH using phenolphthalein indicator followed by gentle boiling with occasional stirring. Boiling water was added to maintain the original level. The solution was cooled and transferred to a 500 ml volumetric flask, made up to the mark by distilled water and solution was filtered through a Whatman filter paper. 100 ml aliquot was pipetted into a 500 ml volumetric flask. To this, 2 ml of neutral lead acetate solution and about 200 ml of water was added. It was allowed to stand for 10 min, then excess of lead was precipitated with potassium oxalate solution. It was made up to the mark and filtered.

**Procedure for determination of reducing sugars**

**The incremental method of titration:** 10 ml of the mixed Fehling’s solution was pipetted into a 250 ml flask and 50 ml water was added to it. Burette was filled with the clarified sugar solution. Sugar solution from burette sufficient to reduce almost completely the fehling’s solution used was added. It was mixed and heated to boiling point on hot plate / burner covered with a clean asbestos, filled wire gauze. It was boiled for 15 sec. if the colour remained blue, 2-3 ml of the sugar solution was further added. The solution was boiled for few seconds after each addition until only a faintest perceptible blue colour remained. 3 drops of methylene blue indicator was added to it and the titration was completed by adding the sugar solution drop wise until the indicator was completely discoloured. The volume of the solution used was recorded.

**Procedure for determination of total sugars**

50 ml of the clarified solution was pipetted into a 250 ml conical flask. 5 ml of citric acid and 50 ml of water were added to it and boiled gently for 10 min to complete the inversion of sucrose, and then it was cooled. It was transferred to a 250 ml volumetric flask and neutralized with 1 N NaOH using phenolphthalein as indicator, and made up to the volume.
An aliquot was taken and the total sugars as invert sugars was determined by the incremental method of titration.

Calculations

% Reducing Sugars = \( \frac{\text{mg of invert sugar} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{wt. or volume of sample} \times 100} \)

% Total sugars = \( \frac{\text{Mg of invert sugar} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{wt. or volume of sample} \times 100} \)

(vii) Acidity

Titrable acidity was determined as described by Ranganna (2002). To prepare the sample, the sample was pulped with the help of a pestle and mortar, 5 g of pulped sample was then boiled in 100 ml of distilled water for one hour, replacing the water lost by evaporation. It was then cooled, filtered and transferred to a volumetric flask and made up to 100 ml with distilled water. 10 ml of aliquot was pipetted out and titrated against 0.1 N NaOH with few drops of phenolphthalein as indicator.

The titre value was noted and percent total acid was calculated as percent anhydrous citric acid using the following formula

\[ \text{% Total acid} = \frac{\text{Titre} \times \text{normality of NaOH} \times \text{Vol. Made up} \times \text{Eq. Wt. of Citric acid} \times 100}{\text{Wt. of sample} \times \text{vol. of aliquot} \times 1000} \]

(viii) Estimation of Vitamin C content

The vitamin C content of honey based products was determined by using 2,6-dichlorophenol-Indophenol Visual Titration Method suggested by the Ranganna (2002).

Reagents

(1) 3% Metaphosphoric acid (HPO₃): The sticks or pallets of HPO₃ were dissolved in glass distilled water.

(2) Ascorbic acid standard: 100 mg of l-ascorbic acid was weighed and made up to 100 ml with 3% HPO₃. 10 ml of this was taken and diluted to 100 ml with 3% HPO₃ (1 ml = 0.1 mg of ascorbic acid).
Dye Solution: 50mg of the sodium salt of indophenol was dissolved in approximately 150ml of hot glass distilled water containing 42mg of sodium bicarbonate. The dye was cooled and diluted with glass distilled water to 200ml.

Procedure

Standardization of dye: 5ml of standard ascorbic acid and 5ml of HPO₃ was taken in a 100ml conical flask. It was titrated with the dye solution to a pink colour. The dye factor was determined, i.e. mg of ascorbic acid per ml of the dye, using the formula:

\[
Dye \ factor = \frac{0.5}{Titration \ value}
\]

Preparation of Sample

10gm of sample was taken. It was blended with 3% HPO₃ and made up to 100ml with HPO₃ and filtered the solution with whatman filter paper.

Assay of Extract: 2-10ml aliquot of the HPO₃ extract of the sample was taken and titrated with the standard dye to the pink end point which should persist for at least 15 sec. It was titrated rapidly and made a preliminary determination of the titre. In the next determination, added most of the dye required and it was titrated accurately. The aliquot of the sample taken should be such that the titre should not exceed 3 to 5ml.

Elimination of Interference due to Sulphur dioxide

10ml of the filtrate was taken in a test tube, 1ml of 40% formaldehyde and 0.1ml of HCl were added. It was allowed to stand for 10 minutes and titrated as before.

Calculations:

\[
mg \ of \ ascorbic \ acid = \frac{Titre \times Dye \ factor \times vol. \ Made \ up \times 100}{Aliquot \ of \ extract \ taken \times Wt. \ or \ vol. \ of \ sample \ for \ estimation}
\]

(ix) Estimation of β-carotene content

Reagents

1. Acetone
2. Anhydrous sodium sulphate
3. Petroleum ether

Procedure

5 gm of fresh sample was taken and crushed in 10-1 ml acetone, few crystals of anhydrous sodium sulphate were added, with the help of pestle and mortar. The supernatant was decanted into a beaker. The process was repeated twice and the combined supernatant was transferred in a separatory funnel. 10-15ml petroleum
ether were added and mixed thoroughly. Separation of two layers was found on standing. The lower layer were discarded and upper layer was collected in a 100 ml volumetric flask, made up to the 100 ml with petroleum ether and optical density was recorded at 452 nm using petroleum ether as a blank.

**Calculation:**

\[
\beta\text{- carotene (µg /100g)} = \frac{\text{O.D.} \times 13.9 \times 10^4 \times 100}{\text{Wt. of sample} \times 560 \times 1000}
\]

\[
\text{Vitamin A (I.U.)} = \frac{\text{Beta- carotene (µg /100g)}}{0.6}
\]

**3.3.2.2 Microbiological quality**

Microbial analysis was done aseptically to determine the total plate count of the samples on Nutrient Agar (NA) for bacterial count, Potato dextrose Agar (PDA) for yeast and mold count and Mac Conkey Agar for coliform count.

**Procedure**

**Preparation of media:** The various compositions are as follows

**Nutrient Agar Media (NA)**

- Peptone: 5 g
- Agar-Agar: 20 g
- Beef extract: 1.5 g
- Yeast extract: 1.5 g
- NaCl: 5 g
- Distilled water: 1000 ml

**Potato Dextrose Agar (PDA)**

- Potato infusion from: 200 g
- Dextrose: 20 g
- Agar: 15 g
- Streptomycin: 3 mg
- Distilled water: 1000 ml

**Mac Conkey Agar**

- Peptic digest of tissue animal: 20 g
- Lactose: 10 g
- Sodium chloride: 5 g
- Bile salts: 1.5 g
Neutral red  - 0.05 g  
Crystal violet  - 0.001 g  
Agar  - 15 g  
Distilled water  - 1000 ml  

**Normal Saline Solution (NSS)**  
Distilled water  - 1000 ml  
NaCl  - 8.6 g

**Sterilization**  
All the necessary glass wares and media such as, required number of petridishes, NA media for TPC, PDA and Mac Conkey Agar media for yeast and mold count and for coliform count respectively, 9 ml of NSS distributed in 7 test tubes for TPC and 7 test tubes for yeast and mold count and 7 test tubes for coliform count, microbial tips (1ml, 0.5ml), spreader were heated for 20 min. in an autoclave maintained at 15 psi for sterilization. The autoclave was then switched off and the steam was allowed to escape.

**Pouring**  
This is the transfer of media into petridishes. This was done in the laminar flow chamber. The flame was lighted and petridishes were slightly opened near the flame and the media was poured in the petridishes. The petridishes with media then kept undisturbed for solidification.

**Preparation of sample (Serial dilution)**  
1g of sample was transferred to the test tubes with 9 ml of NSS. It was marked as $10^{-1}$ and others as $10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}$ and $10^{-6}$. The test tube containing sample was homogenized with the help of cyclomixer. 1 ml of sample suspended in saline solution from $10^{-1}$ test tube was transferred in test tube marked as $10^{-2}$ with the help of micropipette and homogenized. 1 ml of sample from $10^{-2}$ marked tube was transferred to $10^{-3}$ with a sterilized 1 ml micropipette. Similarly the sample was transferred till the test tube marked as $10^{-6}$. Same procedure was followed for the yeast and mold count and coliform count.

**Inoculation of sample**  
This was also done aseptically in the laminar flow chamber 0.5 ml of the sample suspended a saline solution from $10^{-1}$ was taken with micropipette and transferred to Petri dish marked as $10^{-1}$ of NA media. The microbial tip was discarded and another sterilized tip was used to transfer sample from $10^{-2}$ saline solution to $10^{-2}$.
Precautions were taken in inoculation that contamination should not take place. Similarly all the samples suspended in saline solution were transferred to the respective petridishes of NA media. For each dilution two replicate were taken. A control of NA media was also kept without inoculation. The inoculated petridishes were incubated in a B.O.D. incubator for 48 hours, where the temperature was maintained at 37°C. After 24 and 48 hours, total plate count was taken for NA plates. Same procedure was followed for the yeast and mold count and coliform count.

\[ TPC \text{ (cfu/g)} = \text{No. of colonies} \times \text{dilution factor} \times 10 \]

### 3.3.2.3 Evaluation of Sensory Characteristics

Sensory attributes such as colour, aroma, texture, taste, juiciness and mouth feel of the honey based products were evaluated as recommended by Ranganna (1994) by Hedonic rating test. A semi-trained panel consisting of 14 judges was selected to evaluate the sample through properly planned experiments. The panelists were selected from the staff and students of Department of Post Harvest Engg. and Technology, Faculty of Agricultural Sciences, AMU, Aligarh.

The requirement for panel membership are (i) good health (ii) average sensitivity (iii) high degree of personnel integrity (iv) intellectual curiosity and interest in sensory evaluation (v) ability to concentrate and learn; and (vi) availability and willingness to spend time in evaluation and submission to periodic test for acuity and consistency. Candidates possessing these qualities are indexed with details of age, sex; specific likes and dislikes etc. Laboratory panels are then carefully trained for specific product. These tests aim at finding differences in specific quality of characteristics between different stimuli and also direction and/or intensity of the differences. Periodically the panel is given refresher training.

Samples were served to the panelists and they were asked to rate the acceptability of the product through the sense of their organs. Different attributes viz. colour, aroma, texture, taste, juiciness and mouth feel of honey based products were rated as scale of the 9 points of the hedonic scale ranging from 1\textsuperscript{st} point (extremely dislike/ most undesirable) to 9\textsuperscript{th} point (extremely like/most desirable) as shown in Table 3.1.

A test proforma was also made and supplied to panelists at the time of evaluation, which is given below
Table 3.1: Performa on 9 Point Hedonic

<table>
<thead>
<tr>
<th>Attributes</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Like extremely</td>
<td>9</td>
</tr>
<tr>
<td>Like very much</td>
<td>8</td>
</tr>
<tr>
<td>Like moderately</td>
<td>7</td>
</tr>
<tr>
<td>Likeslightly</td>
<td>6</td>
</tr>
<tr>
<td>Neither like nor dislike</td>
<td>5</td>
</tr>
<tr>
<td>Dislike slightly</td>
<td>4</td>
</tr>
<tr>
<td>Dislike moderately</td>
<td>3</td>
</tr>
<tr>
<td>Dislike very much</td>
<td>2</td>
</tr>
<tr>
<td>Dislike extremely</td>
<td>1</td>
</tr>
</tbody>
</table>

3.3.2.4 Instrumental Texture Analysis

Texture profile analysis (TPA Test)

(i) Fracturability

Fracturability is defined as the force required to rupture the material and is measured as the force at the first significant break in the first positive bite area.

(ii) Cohesiveness

Cohesiveness is the property of the material, which determines the extent of deformation. The material withstand file before it ruptures. It is evaluated as the ratio of the positive force area during the second compression cycle to the positive force area during the first compression cycle.

(iii) Hardness

It is defined as the force necessary to attain a given deformation and is evaluated as the peak force during the first compression cycle. The hardness of any biological material is important parameter for its textural evaluation and quality control in terms of maturity, ripeness and storability.

Fracturability = Not all products fracture; but when they do fracture the Fracturability point occurs where the plot has its first significant peak (where the force falls off) during the probe's first compression of the product.

Cohesiveness = \( \frac{P_{A_2}}{P_{A_1}} \) \ (\( P_{A_1} \) and \( P_{A_2} \) are the areas of first and second bite)

Hardness = \( h_1 \) (Peak force) during first compression

Springiness = Height to which the food recovers between end of the first byte and start of the second byte

Gumminess = hardness x Cohesiveness

= \( h_1 \times \frac{P_{A_2}}{P_{A_1}} \) \ (Where \( h_1 \) is the hardness)
Stickiness = -ve peak force during first compression

Figure 3.12 TAHD Type texture analyzer
TPA Test Setting:

Texture analysis setting version : 07.13H
Load Cell : 50 kg
Test Mode and Option
Measure force in compression
Repeat until count
Parameters:

Pre test speed : 2.11 mm/s
Test speed : 1.00 mm/s
Post test speed : 2.00 mm/s
Rupture test distance : 4.00%
Distance : 50.0 %
Force : 100 g
Time : 5.00 Sec
Count : 2
Load Cell : 50 kg
Temp : 25°C
Trigger Type : Auto
Force : 5g
The probe compression platen (100mm) was used for TPA test and it was performed by two-bite compression. The compression platen (100mm) was attached to the crosshead of Texture Analyzer and the sample was placed on plate form. After making TA setting, the test was run and a graph was created on texture expert. The texture properties were calculated from the graph as follows.

3.3.2.5 Statistical analysis

1. Standard deviation

The best and most commonly used statistical evaluation of the precision of analytical data is the standard deviation. The standard deviation measures the spread of the experimental values and gives a good indication of how close the values are to each other.

Samples were prepared in three replications and data obtained for selected quality parameters were analyzed for mean and standard deviations using following formula:

\[
SD = \pm \sqrt{\frac{\sum (X_i - X)^2}{n}}
\]

Where,
- \(X_i\) = individual sample values
- \(X\) = mean of individual samples
- \(n\) = total population of sample

2. Analysis of Variance

To test the significance of effect of storage period on quality parameters, analysis of variance (ANOVA) was carried out as applicable to experiments of randomized designs, outlined by Mandal and Nambiar (2002). The critical difference (CD) of mean values were also calculated using formula:

\[
CD = \sqrt{\frac{2 \times EMSS}{r}} \times t
\]

Where,
- \(EMSS\) = Errors mean sum of sq.
- \(r\) = No. of replications
- \(t\) = Value at 5% and 1% of confidence

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3.3.2.6 Economic Feasibility Study

Analysis of economics of manufacturing of honey based food product, taking several assumptions into consideration. Following economic indicators were worked out.

(1) Pay back period
\[ \frac{\text{Total capital investment} + \text{Working capital}}{\text{Net annual profit} + \text{Depreciation}} \]

(2) Return on investment
\[ \frac{\text{Net annual profit}}{\text{Total capital investment} + \text{Working capital}} \times 100 \]

(3) Benefit cost ratio
\[ = \frac{\text{Annual benefit}}{\text{Total annual cost}} \]

(4) Break even point
For x to be break even point in days
Fixed cost per year + variable cost per day \( X \) = Revenue per day \( X \) x