CHAPTER 2

EXPERIMENTAL DETAILS
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This chapter is discussed in following order

I) Apparatus and Chemicals

II) Instruments

III) Sampling

IV) Methods of analysis of various parameters.

1) GLASS WARES: All the glass apparatus used were of 'BOROSIL' make. Burettes, pipettes, volumetric flask etc. were calibrated by standard method\textsuperscript{1}. Borosil glass bottles were used to preserve the ash of the sample and polythene bottles with screw cap were used to preserve the standard solutions of AAS. All the apparatus were washed and cleaned before use, as per the standard method

2) WATER: Distilled water obtained from metal distillation plant was redistilled over alkaline permanganate in an all quick fit assembly and was used to prepare all solutions.

3) CHEMICALS: Chemicals used for the determination of various parameters in the present research work were obtained from S. D. Fine, Fischer, Merck and Loba Chemie and all the chemicals were of A. R. Grade

II) INSTRUMENTS:

1) Balance

2) Electric Oven

3) Hot Plate
4) Muffle furnace
5) Refractometer
6) pH meter
7) Spectrophotometer
8) Atomic Absorption Spectrometer (AAS)

1) **BALANCE:**
A single pan balance manufactured by Contec was used for weighing during research work. The balance had a sensitivity of 0.1 mg and was used to weigh chemicals and the sample.

2) **ELECTRIC OVEN:**
For drying the sample, for finding out the loss on drying at 105°C, an electrically heated oven was used. The oven with digital temperature controller, manufactured by Sunflame was used. The oven was electrically heated and thermostatically controlled up to 280°C ± 1°C.

3) **HOT PLATE**

4) **MUFFLE FURNACE:**
Muffle furnace with temperature regulator was used for the analysis. The maximum temperature for ashing the sample was 520°C ± 1°C

5) **REFRACTOMETER:**
Refractometer supplied by Toshniwal insts & Engg. Co, New Delhi, S.No 1955 was used to record the Refractive Index of the sample at appropriate temperature.
6) **pH METER:**

Digital pH meter supplied by "Elico Ltd", Model no LI 120 was used for pH measurement of different samples. The pH meter was standardized using buffer solution of 4.0, 7.0 and 9.2 pH at 25°C by dissolving the respective tablets separately in 100cc decarbonised distilled water. The accuracy of the instrument was pH ± 0.02. Samples were also dissolved in decarbonised distilled water by warming wherever required and pH of 5% w/v solution in water was recorded at 25°C.

7) **SPECTROPHOTOMETER:**

A UV - VISIBLE RECORDING Spectrophotometer manufactured by "Elico Ltd", model no SL 159 was used for estimating the tannin content in different samples of jelly.

The spectrophotometer used, was a double beam, most modern general purpose ultraviolet / visible spectrophotometer which covers the range between 200 and 800 nm.

**PRINCIPLE:**

In double beam spectrophotometer, the monochromatic beam of radiation from tungsten and deuterium lamp split into two identical beams. One passes through the reference cell and other through the sample cell. The signal for the adsorption of the contents of the reference cell is automatically subtracted from that for the sample, giving a net signal corresponding to absorption for the components in the sample solution.
Schematic diagram for the double beam spectrophotometer is as follows:

According to Beer's law, when the monochromatic light is passed through the transparent solution, the intensity of radiation decreases exponentially, as the concentration of the absorbing species increases arithmetically. Lambert Beer's law can be expressed mathematically as:

\[ A = \log \frac{I_0}{I} = a \cdot c \cdot t \]

Where,

- \( a \) = Modified proportionality constant
- \( c \) = Concentration
- \( t \) = Thickness of the medium

Calibration of the instrument was done by standard method, using potassium dichromate solution.

8) **ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS):**

Atomic absorption Spectrophotometer supplied by M/s Chemito, AA-203 was used with individual Hollow Cathode Lamp of element of interest.

Air- Acetylene flame was used for all of the elements.
Arrangement of an Atomic Absorption Spectrophotometer:

PRINCIPLE:

When salt solution is introduced into the flame in the form of aerosole, ultimately atomic vapour is formed. Due to thermal energy, fraction of atoms gets excited to higher energy levels. However, majority of the atoms remain in ground state. Such vaporized atoms in ground state can absorb resonance frequency and get excited to higher energy states. The frequency whose energy exactly corresponds to the energy gap between ground state and excited state of the element is known as resonance energy. In atomic absorption spectroscopy radiations of suitable frequency are allowed to pass through a flame. Sample to be analysed is introduced into the flame. The intensity of radiation transmitted through flame is measured before and after the introduction of the sample in the flame. In presence of sample, due to absorption there is a decrease in intensity of transmitted radiation. The decrease in intensity is proportional
to the concentration of element in the flame. A direct relation is observed between decrease in such intensity and concentration. Trace elements such as iron, copper, zinc, lead were analyzed in processed food such as jam and jelly, by atomic absorption spectroscopy. This estimation was done at parts per million (ppm) levels.

III) SAMPLING:

i) Location

ii) Sampling technique

i) Location:

Variety of samples of vinegar, jams and jellies were collected from City Chowk, Gulmandi, Cidco area in Aurangabad. All the samples of vinegar and jam were manufactured in India whereas only few samples of jelly brands were manufactured in Malaysia.

ii) Sampling technique:

Different batches of vinegar, jam and jelly were selected at random from shop. Different types of vinegar that were selected were White Synthetic Vinegar, Black Berry Vinegar, Grapes Vinegar, Red cooking Vinegar, Sugarcane Vinegar, Non Fruit White Vinegar.

Jam samples that were selected were of different flavours such as, mix fruit, pineapple, raspberry, apple, strawberry, mulberry, mango. Brands like Warana, Pic up, Manama and Mala jams were selected.

Jelly samples that were selected were of different colours, like, yellow,
green, red, orange, violet, pink, for brands like Mougli ice tube, Gold Fish and Hi-Po jelly, where as different flavours like, Coconut, Mixed fruit, Blue berry, Mango, Jack fruit, Strawberry, Pineapple, Orange, Lemon, Grape were selected for Mougli fiber jelly.

IV) METHODS OF ANALYSIS OF VARIOUS PARAMETERS:

ANALYSIS OF VINEGAR

In case of vinegar sample, following parameters were analysed:\n
1) Total acids
2) Non Volatile Acids
3) Volatile Acids
4) Ester Number
5) Oxidation number
6) Iodine number
7) Alkaline Oxidation number
8) Nitrogen
9) Total solids
10) Ethanol
11) Reducing sugar
12) Mineral acid
13) Formal titration
1) **TOTAL ACIDS:**

Procedure:

10 ml of the sample was pipetted into a beaker and dilute to 100 ml and titrated with 0.1 M Sodium hydroxide solution using phenolphthalein indicator.

2) **NON VOLATILE ACIDS:**

Procedure:

10 ml of sample was evaporated to dryness. 10 ml water was added and then evaporated just to dryness, the procedure was carried out for five times. Then the sample was diluted to 200 ml with boiled water and titrated with 0.1 N Sodium hydroxide solution with phenolphthalein as an indicator.

3) **VOLATILE ACIDS:**

The volatile acids was obtained form the difference between total acids and non volatile acids.

4) **ESTER NUMBER:**

Ester number can be defined as milliters of 0.01 N potassium hydroxide solution required to saponify the ester in 100 ml of sample under standard conditions.

Procedure:

100 ml of the sample was pipetted and distilled slowly until 30 ml of distillate are collected, phenolphthalein was added as an indicator and
1.0 N potassium hydroxide was added to the distillate until pink colour appeared. 0.02 N hydrochloric acid was added dropwise until the colour was discharged, then 10 ml of 0.1 N potassium hydroxide solution was added and saponified by refluxing on water bath for 2 hours. It was cooled add more phenolphthalein was added and titrated with 0.02 N hydrochloric acid. Blank determination was also made by refluxing 30 ml of water with 10 ml of 0.1 N potassium hydroxide solution and titrated with 0.02 N hydrochloric acid as in actual analysis. Ester number was calculated as;

\[ E = 2(B - A) \]

5) **OXIDATION NUMBER:**

Oxidation number can be defined as milliliters if 0.01 N Potassium permanganate required to oxidize 100 ml of the sample in 80 minutes under standard conditions.

Procedure:

I) **Distillation:**

60 ml of the sample was mixed with 15 ml of water in a 100 ml distilling flask. Pumic stones were added and distilled slowly until 60 ml of distillate have been collected.

II) **Oxidation:**

25 ml of the distillate was added 10 cc of 0.1 N potassium permanganate solution. It was kept for 30 minutes, then 5 ml of 10% potassium iodide
was added and titrated the liberated iodine with 0.02 N sodium thiosulphate solution using starch as an indicator. Blank determination was made using 25 ml of water. Oxidation number was calculated as

\[ O = 8 (B - A) \]

6) **IODINE NUMBER:**

Iodine number is milliliters of 0.01 N iodine solution absorbed by 100 ml of the sample under standard conditions.

Procedure:

25 ml of the distillate was neutralize to litmus paper with 10 N potassium hydroxide solutions; 10 ml of 0.1 N iodine was added and allowed to stand in dark for 15 minutes, then 10 ml of 9 N sulphuric acid was added and titrated with 0.02 N Sodium thiosulphate solution. Blank determination on 25 ml of water was carried out.

Iodine number was calculated by the formula;

\[ \text{Iodine number} (I) = 8 (B - A) \]

7) **ALKALINE OXIDATION NUMBER:**

Alkaline oxidation value\(^6\) is the number of parts by weight of oxygen required to oxidize 100 000 parts of sample under standard condition.

Procedure:

2 ml of the distillate (distilled vinegar), 100 ml water, 10 ml of 10% sodium hydroxide solution and exactly 10 ml of 0.02 M potassium permanganate was taken. It was allowed to stand for 30 minutes and then acidify with 10
ml dilute sulphuric acid (1+3). 0.5 g potrassium iodidewas added and the liberated iodine was titrated with 0.02 M thiosulphate using starch as an indicator. The blank titration was carried out at the same time.

Alkaline Oxidation Number was calculated by the formula;

Alkaline Oxidation Number = 8 (B – A)

8) NITROGEN:

Kjeldhal Method was used to find out the amount of nitrogen present in the sample.

Procedure:

25 ml of sample was taken into a Kjeldhal flask. 0.7 g of anhydrous copper sulphate, 15 g powdered potassium sulphate and 40 ml of concentrated sulphuric acid was added into it. It was heated in an inclined position for 2 hours.

It was cooled and approximately 200 ml water, 25 ml of Sodium thiosulphate and NaOH about 110 ml (450 g/ l) was added. The flask was connected to a distillation apparatus. To the condenser a delivery tube was fitted which just dips below the surface of a pipetted volume of standard acid in a conical flask receiver. It was boiled until at least 150 ml have distilled into the receiver. 5 drops of methyl red indicator was added and titrated with 0.1 M hydrochloric acid.

The amount of nitrogen present was calculated by the formula

1 ml of 0.1 M HCl or 0.05 M H$_2$SO$_4$ = 0.0014 g Nitrogen
9) **TOTAL SOLIDS:**

10 ml of the sample was evaporated, in a tared flat bottom dish continuing the drying in a boiling water oven for 2.5 hours. It was cooled in a desiccator and weighed. Calculate the result as grams per 100 ml.

10) **ETHANOL:**

Martin and Nourisson Dichromate Iodometric method:

Vinegar was diluted to 5 to 10 times its volume with water and 10 ml of the liquid was pipetted into the distillation flask. 20 ml of 0.01667 N Potassium dichromate solution and 10 ml of sulphuric acid was placed into the receiving tube. The liquid was transferred into the receiving tube to a 500 ml standard flask and was diluted to 250 ml. 10 ml of 5% Potassium Iodide solution was added and titrated with .01 N thiosulphate solution until green yellow colour changed to green blue.

The percentage of ethanol (E) is obtained by:

\[ E = (20 - N) \times 0.00144 \times 50 \]

Where N is the number of milliliters of thiosulphate solution used in titration.

11) **REDUCING SUGAR:**

Munson And Walker Unified Gravimetric Method;

Procedure:

i) Copper Reduction:

In a 400 ml beaker 15 ml of Fehling-Soxhlet solution 1 & 2 was mixed, from a pipette 50 ml of sugar solution was runed into it. It was heated to
boiling over asbestos gauze so that boiling begins in exactly 4 minutes and boiling was continued for exactly 2 minutes longer, keeping the beaker covered with a watch glass.

ii) Filtration:
Without delay the precipitate was filtered on Gooch Crucible. Which was in meantime had been heated, cooled and weighed. After pouring off the clear supernatant liquid onto the crucible, remove the clear filtrate was removed so that if any cuprous oxide runs through later it will be evident. It was washed with water at about 60°C, then with 10 ml of ethanol and finally with 10 ml of ether.

iii) Weighing As Cuprous Oxide:
Precipitate was dried for 30 minutes in a boiling water oven, cooled in a desiccator, and weighed as cuprous oxide.

iv) Calculation:
From the weight of cuprous oxide, the amount of sugar present from the Munson and Walker-Hammond table was found out.

12) MINERAL ACID:
Qualitative test for mineral acid:
10 ml of the vinegar was diluted with an equal volume of water and 2 to 5 drops of 0.1% aqueous methyl violet solution was added. If a blue or green colour appears, the presence of mineral acid is indicated.
13) **FORMAL TITRATION:**

Phenolphthalein was added to 10 ml sample, acid was neutralised with 0.5 M Sodium hydroxide and then sample was made exactly neutral with 0.05 M Sodium hydroxide. 5 ml formalin was added (previously made exactly neutral to phenolphthalein), it was mixed well and after standing for 5 minutes, the acidity produced was titrated with 0.05 M or 0.1 M sodium hydroxide.

**ANALYSIS OF JAM AND JELLY**

1) **PHYSICAL PARAMETERS:**

i) Moisture content in the form of Loss On Drying at 105°C  
ii) pH of 5% w/v solution in deionised water  
iii) Acidity calculated as citric acid in g / 100 g  
iv) Refractive index at 20°C / 24°C  
v) Content of ash at 520°C  
vi) Content of Tannin in ppm

2) **CHEMICAL PARAMETERS:**

Estimation of trace elements such as  

i) Iron  
ii) Copper  
iii) Zinc  
iv) Lead
1) PHYSICAL PARAMETERS:

i) Moisture content in the form of Loss On Drying at 105°C
About 2.0 g sample was accurately weighed in a tared loss on drying bottle and dried in an oven at 105°C for 24 hours. Then it was removed from the oven, kept in a desiccator to attain the room temperature and weighed till constant weight.

ii) pH:
Exactly 5 gm of jam / jelly sample was weighed in a 100 ml dry beaker. It was dissolved in small amount of boiled and cooled distilled water, by little warming. The volume was made to 100 ml by using decarbonised distilled water. The pH of this solution was recorded on a pH-meter at room temperature.

iii) Acidity:
Accurately weighed about 2 g sample was dissolved in 50 ml decarbonised distilled water. Warmed it for about 15 – 20 minutes. Collected to room temperature and titrated against 0.1 N sodium hydroxide solution using phenolphthalein solution as an indicator. From the titration, burette reading was noted as 'B.R'. Acidity was calculated as citric acid by applying following formula:

\[
\text{Acidity calculated as citric acid} = \frac{\text{B.R} \times \text{Normality of NaOH} \times \text{Eq. Wt} \times 100}{\text{Weight of sample in (g) x 1000}} \quad \text{in (g / 100 g)}
\]
iv) Refractive Index:

Throughly mixed sample of jam / jelly was subjected to attain the appropriate temperature (24°C / 20°C). then the little homogenous sample was placed on the refractometer prism. Determined the direct refractometer reading on Refractive Index scale.

v) Content of Ash:

Jam sample was mixed properly with a glass rod and it was removed out with the wooden spoon. For weighing jelly, scoops of jelly were directly opened and weighed the entire amount.

About 2 g of jam / jelly sample was accurately weighed in a tared dry silica / platinum crucible. It was dehydrated on water bath and then dried in an oven at about 200°C for 3 hours. Subsequently it was heated and charred on hot plate at about 300°C for few hours. The charred mass was then incinerated at about 520°C in muffle furnace under controlled temperature.

The ash obtained was then cooled to room temperature in a desiccator and weigh till constant weight. It was stored properly in small clean borosil glass bottles for further estimation.

vi) Content of Tannin:

These are phenolic compounds that have several hydrolyzable groups. They are classified as: a) Hydrolyzable, yielding phenols such as gallic acid in the presence of acid and heat b) condensed, obtained from the extract of Oak trees and not hydrolyzable.
Content of tannin was estimated in jelly samples by colorimetric method\(^9\).

**Reagents:**

Folin-Denis reagent:- 150 cm\(^3\) of distilled water, 20g of sodium tungstate (Na\(_2\)WO\(_4\).2H\(_2\)O), 4g of phosphotungstomolybdic acid and 10cm\(^3\) of 85% phosphoric acid (H\(_3\)PO\(_4\)) was refluxed for 2 hours. Cooled to 25°C and diluted to 200cm\(^3\) with distilled water.

Saturated sodium carbonate solution:- To 100cm\(^3\) of distilled water 35g of anhydrous sodium carbonate was added and dissolved at 70-80°C. Cooled overnight. Next day the solution was filtered and used.

Tannic acid standard solution:- 100 mg of Tannic acid dissolved in 100 cm\(^3\) distilled water, was shaken and 10 cm\(^3\) from this was diluted to 100 cm\(^3\). (1 cm\(^3\) = 0.1 mg of tannic acid)

**Preparation of standard curve:-**

Pipetted out 0 to 10 cm\(^3\) aliquots of the standard tannic acid solution into 100 cm\(^3\) volumetric flasks containing 75 cm\(^3\) of distilled water. 5 cm\(^3\) Folin-Denis reagent and 10 cm\(^3\) with distilled water. After mixing, the colour of the solution was measured after 30 minutes., at 760 nm against experimental blank adjusted to 0 absorbency.

**Sample preparations:-**

Appropriate quantity about 1 to 3 g of jelly sample was dissolved in about 50 cm\(^3\) of distilled water by heating on water bath. after cooling to the room temperature, entire solution was used for colour development.
Determinations:-

Absorbance of sample reading was plotted on standard curve and the concentration of tannin in the sample was calculated in ppm by considering the standard concentration and dilution factor.

Calculations :-

\[
\text{Tannin as} = \frac{\text{mg of tannic acid} \times \text{Dilution}}{\text{ml of sample taken} \times \text{wt of spl taken}} \times 100
\]

For colour development

Concentration of Tannin in ppm = % tannin \times 10^4

2) CHEMICAL PARAMETERS:

Content of trace elements such as Iron, Copper, Zinc and Lead was found out by AAS.

In case of jam samples, jam in the bottle or plastic pack was stirred properly with the clean dry glass rod. The homogenous mixture of jam sample was removed with the glass rod or with the wooden spoon, for weighing. About 2 g jam sample was taken for ashing.

In case of jelly samples, jelly packs were hygienically opened and was directly taken for weighing, whereas, jelly in the cup form and cylinder form was mixed properly and homogenous mixed sample was removed with plastic spoon for weighing the sample. Different colours of the same brand were weighed separately for trace metal estimation. Similarly, jelly in the form of pieces was mixed and ashed. The ash of jam/ jelly obtained by the method mentioned previously, was dissolved in 40 cm³ aqua regia.
It was digested at low temperature on the hot plate. Concentrated to about 20 cm$^3$ and diluted to appropriate dilution. After attaining the room temperature, it was filtered through filter paper No. 1. The absorbance reading was taken on the Atomic Absorption Spectrophotometer (AAS) at respective wavelength by using appropriate Hollow Cathode Lamp. Blank solution was aspirated before each sample solution. Working standards were prepared from 1000 ppm stock standards of individual metal ions, supplied by M/s Merck India Ltd. Absorbance of different concentration of working standards of each element was recorded and the standard curve was drawn. For each element the calibration curve was constructed using standard solution of each element in the appropriate range. The instrument reading directly displayed the concentration of metal ion in the unknown solution on the basis of previously recorded calibration curve. The concentration of element in the sample was calculated in ppm.
REFERENCES


5) Winton and Winton, Techniques of Food Analysis

