

QUANTITATIVE AND QUALITATIVE ANALYSIS OF SPICES

An Industrial Training Report submitted
For the partial fulfillment of the Degree of Master of Science

By

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[M.Sc. (Microbiology), Semester IV]



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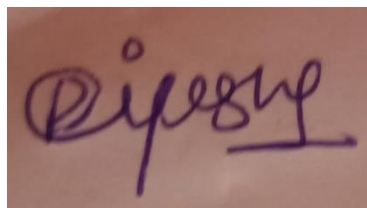
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C E R T I F I C A T E

This is to certify that this training report entitled “**QUANTITATIVE AND QUALITATIVE ESTIMATION OF SPICES**” was successfully carried out by **Ms. Megha P. Patel** towards the partial fulfillment of requirements for the degree of Master of Science in Microbiology of Shree M. & N. Virani Science College, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of **Mr. Dipesh pipaliya** for a period of 2 Months. During the academic year of 2019-20. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

Mr. Dipesh Pipaliya



Signature of the Head of the
Department

Name & Signature of the
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DECLARATION

I hereby declare that the work incorporated in the present internship report entitled “**QUANTITATIVE & QUALITATIVE ANALYSIS OF SPICES**” is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Megha P. Patel

Date: 04/05/2021

(Name and signature of Student)

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Abbreviation:

ETO: Ethyl Tetra Oxide

TPC: Total Plate Count

PDA: Potato Dextrose Agar

T7: Tergitol-7 Agar

TTC: 2,3,5-Triphenyl Tetrazolium Choride

XLD: Xylose Lysine Deoxycholate Agar

BSA: Bismuth Sulfite Agar

EMB: Eosin Methylene Blue Agar

O.D: Optical Density

CFU: Colony-Forming Unit

CU: Color Units

SHU: Scoville Heat Unit

QC: Quality Content

Abstract

The present study is aimed to equal quality maintain and control by lab testing in include study of physical, chemical, microbiological parameters in different types of spices.

Here, particular study on different types of the spices for quality control further more include the physical in test, Odour, color, particle size, appearance, etc. In chemical color value pungency, total ash, insoluble ash is mainly create hazards problems, other microbiological testing in spices it must need for the stability of color, test, and smell some organism spoil the spices and also pathogenic organism is harmful for human health.

The Microbiological load controlling and decreasing by ozone sterilization treatments (which combine with grinding machine).

Over Microbiological load controlling by ETO (Ethyl Tetra Oxide) Sterilization treatment.

By this overall quality controlling process for provide good manufacturing and service to consumers or customers.

This company is India's leading manufacturers and exporters of spices/blended spices. That is ISO 9001:2008 and ISO 22000:2005 certified company, certified by TUV South Asia.

CHAPTER 1: INTRODUCTION

Quality assurance of spices directly corresponds to fetching best rates and sure shot growth of business, export and trade for the spice industry. Quality assurance comes into play right from procurement, then processing to packaging of the spices. All throughout the supply chain, from sourcing to final production, quality standards are set as per specifications.

MICROBIOLOGICAL ANALYSIS

Microbiological testing is conducted to check for pathogens and microorganisms in the spices. The spice testing parameters include evaluating the presence of yeast, mold, Coliform, E.Coli, Salmonella to name a few. This test gives an indication of the cleanliness of spices microbiologically.

PHYSICAL ANALYSIS

Whatever form of the product is as per the form of the spices product, the particle size is studied to help understand the quality. It also measures the amount of foreign organic and inorganic matter. In addition to this, the spice density is also tested, which further helps in decisions of storage and packaging.

CHEMICAL ANALYSIS

Spices testing also includes chemical residue testing that analyses the use of harmful pesticides on spices that include the likes of solvent residues or ethylene oxide residues. Chemical analysis also measures the moisture content, the amount of water insoluble steam volatile oil, the amount of carbon-free ash and the amount of acid insoluble ash.

INTRODUCTION OF COMPANY & THEIR PRODUCTS:

- Adani food products were established in the year 1955. Adani food products Pvt.Ltd. located at metoda.
- Adani food product Pvt.Ltd. is india's leading manufactures and exporters of different type of spices.
- The stimulating force that led to their success is the inspiration from their parents Late Shri Anantray Adani & Late Smt. Lakshkunvarben Adani.

- Adani Food Products Pvt. Ltd. is a medium scale unit and their spices are exported in USA, UK, Poland, Greece, Germany, Sweden etc.

Ground spices	Blended spices	Whole & seed spices
Chilli powder	Garam masala	Coriander seeds,
Clove powder	Tea masala	Cumin seeds
Coriander powder	Chat masala	Fennel seeds
Cumin powder	Panipuri masala	Fenugreek seeds
Ginger powder	Chole masala	Mustard seeds
Fenugreek powder		Clove seeds
Black pepper powder		
Turmeric powder		

Table 1.1.1 Products of company

CHAPTER 2: MATERIALS AND METHOD

Materials:

- Test tube
- Flask
- Petri plates
- Caps
- Micro-pipettes
- Beaker
- Pipette tip
- Wire loop
- Aluminum foil
- Electronic balance
- Burner
- Microscope
- Autoclave
- Incubator
- Laminar air flow
- Measuring cylinder
- Moisture meter
- Heating mental
- Muffle furnace
- Silica Crucible
- Whatman Filter paper
- Volumetric flask
- Spectrophotometer

2.1. MICROBIOLOGICAL TEST

How to collect sample?

Make every effort to use aseptic technique when sampling the spices to minimize its contamination from hands, nearby materials and air. Prior to opening swab the exterior area of the container with 70% ethanol to destroy microorganisms that might contaminate the sample. After sampling spices materials, sterilize the sampling tools and equipment to avoid contaminating subsequent samples. Sterilization is done either dry sterilization or wet sterilization. Dry samples should be aseptically stirred with a sterile spoon, spatula, or other utensil to ensure a homogenous sample.

2.1.1. Total plate count:

- Total plate count test is useful in the estimation of number of viable microorganisms in the spices. TPC test includes bacteria, yeast, molds and fungi that grow in plate count agar. Enumeration of TPC by pour plate Method. Test employs the serial dilution technique.

Procedure:

- Take 1gm sample in 9ml peptone water.
- Perform serial dilution (10^{-2}).
- Take 1ml sample from dilution, placed in petri plate and pour the agar in petri plate, mixed well.
- After the solidification of agar, the petri plate is incubated at 37°C for 24 hours. Next day observe the result.

2.1.2. Fungal test:

- Potato dextrose agar is used for the cultivation of fungi. Enumeration of fungal test by pour plate method.

Procedure:

- Take 1 gram sample in 9 ml peptone water.
- Perform serial dilution (10^{-2}).
- Take 1ml sample from dilution, placed in petri plate and pour the PDA agar in petri plate, mixed well.
- After the solidification of the agar, the petri plate is incubate at 27 °C for 2 to 3 days. After 2-3 days observe the result.

2.1.3. Coliform test:

- Tergitol-7 agar used for selective isolation of *coliform* bacteria in sample. Tergitol-7 was originally designed by Chapman and later on modified by incorporating 2,3,5-Triphenyl Tetrazolium Chloride (TTC) in to the medium.

Procedure:

- Take 1 gram sample in 9 ml peptone water.
- Perform serial dilution (10^{-2}).
- Take 1ml sample from dilution, placed in petri plate and pour the T7 agar in petri plate, mixed well.
- After the solidification of the agar, the petri plate is incubated at 37 °C for 24 hours.
- Next day observe the result.

2.1.4. Salmonella test:

- Xylose Lysine Deoxycholate agar (XLD) used for isolation of *Salmonellae*.
- Bismuth sulfite agar (BSA) is recommended for the selective isolation and preliminary identification of *Salmonellae*.
- FSC – recommended as an enrichment medium for isolation of *Salmonellae* from foods.
- Lactose broth – used for pre-enrichment for *Salmonella*.

Procedure:

- Day 1:- Take 25 gram sample add in Lactose broth.
- Incubate at 37 °C for 24 hour.
- Day 2: -Take 1 ml from Lactose broth and add in to 10 ml FSC.
- FSC incubate at 37°C for 24 hours.
- Day 3: -Take loopful culture from FSC and streak on Xylose lysine Deoxycholate agar (XLD) and bismuth sulfite agar (BSA).
- Incubate both at 37°C for 48 hours and next day observe the result.

2.1.5. Escherichia coli test:

- We used Eosin Methylene Blue Agar (EMB) for isolation of *E.Coli*. EMB agar is combination of two dyes eosin and Methylene blue that

inhibits most gram positive bacteria but allows many gram negative organisms to grow.

Procedure:

- Take 25 gram sample add in Lactose broth.
- Incubate at 37 °C for 24 hours.
- Next day, take loopful culture from Lactose broth and streak on EMB plate.
- Incubate EMB plate at 37 °C for 24 hours. Next day observe the result.

2.2. PHYSICAL TEST:

2.2.1. Appearance:

- With naked eyes.

2.2.2. Color:

- With naked eyes.

2.2.3. Odour:

- Smell of the products.

2.2.4. Taste:

- Characteristics of product.

2.2.5. Extraneous matter:

- Hair, roots, leaf, thread, etc.

2.2.6. Particle size:

- Make fine powder of sample.
- Pass the sample through appropriate sieves size.

2.2.7. Moisture:

- Moisture content define as the weight loss of mass that occurs as the sample is heated.
- Moisture of chilly and clove are measure at 95°C and moisture of other spices are measure at 115°C.

Procedure:

- Make fine powder of given sample.
- Cover the plate of moisture meter with sample powder.
- Close the lid of moisture meter and set the parameters according to sample.
- Range :- Maximum 12%

2.3. CHEMICAL TEST:

2.3.1. Volatile oil:

- The most commonly test run on spices is volatile oil. The method consist of boiling in water and collecting the condensed water and volatile oil. The amount of oil measured by volume. The volatile oil measurement is a fairly reliable indicator of flavor content for those spices. The measurement of volatile oil is spices as also a good measurement of the age.

Procedure:

- Grind the sample and make fine powder.
- Weigh accurately 50 gram sample and add in to round bottom flask.
- Add water in round bottom flask up to the surface of heat plate.
- Set the apparatus and place in suitable electric heating mantle and heat the flask to boiling.

- Adjust refluxing rate to 1 drop per second by adjusting the regulator of the mantle.
- After few hour, note down the amount of oil collected in the trap in ml.

2.3.2. Total ash:

- The total ash content of spices is a measure of the amount of sand and grit in the spices. The total ash determination is performed by heating the sample until the organic matter has been burned off. It is important to remember that a perfectly clean spice does contain some inorganic minerals that are measured as acid insoluble ash.

Procedure:

- Take pre weight of crucible.
- Weigh accurately 5 gram of sample in the tared crucible.
- Place the crucible in the muffle furnace and set temperature at 600°C for 2.5 hours.
- Cool the crucible at room temperature and take post weight of crucible.

2.3.3. Color value & Scoville heat unit in chilli:

- The Scoville scale is a measurement of the pungency of chilli. Scoville heat unit is based on the concentration of capsaicin. Coloring compounds are capsanthin and capsorubin.

Procedure:

❖ Color value of chilli:

- Take 100 ml volumetric flask.
- Put 0.1 gm sample in it.
- Make up with acetone up to 100 ml.
- Incubate at room temperature for 16 hours.
- Take O.D at 460 nm.

❖ **SHU of chilli:**

- Take approx, 10 gm of chilli powder in to flask
- Add 100 ml 1,2-Dichloroethane and mix It properly and cover it with aluminum foil.
- Keep it for 16 hours in dark.
- Filter the solution using simple filter paper and give 2 wash of 1,2-Dichloroethane to the residue.
- Evaporate 1,2-Dichloroethane at low temperature on heat plate. Evaporate till 1,2-Dichloroethane smell is removed. Residue is oleoresin.
- Prepare 70% methanol, add pinch of charcoal and put magnet in beaker.
- Cover the surface properly.
- Put on magnetic stirrer for 30 min at 800 to 900 rpm.
- Filter the solution with double filter paper.
- Make 4 solutions in 25 ml volumetric flask as follow.
- Put base in reference cell and acid in standard cell, and measure the absorbance at 248 nm & 296 nm.
- Make 4 solutions in 25 ml volumetric flask as follow, and make up 25ml with methanol.

Blank O.D

Acid	1N HCL – 0.25 ml	4.75 ml water
Base	1N NaOH – 0.5 ml	4.50 ml water

Table no. 2.3.3(a) blank O.D

Sample O.D

Acid & Base	Water	Sample
1N HCL - 0.25 ml	4.45 ml	1 ml
1N NaOH – 0.5 ml	4.20 ml	1 ml

Table no. 2.3.3(b) sample O.D

CHAPTER 3: RESULT AND DISCUSSION

3.1. MICROBIOLOGICAL TEST:

3.1.1. Total plate count:

- So, the observation of the qualitative estimation of TPC Plate's (Chilli powder) there is too numerous to count growth observed.
- So, the observation of the qualitative estimation of TPC Plate's (Clove powder) colony- forming unit is 1000 CFU/gm.

3.1.2. Fungal test:

- There is fungal growth present in PDA plate of Chilli powder.
- There is fungal growth present in PDA plate of Clove powder.

3.1.3. Coliform test:

- There is no pink color colony observed on the T7 Agar plate of Chilli powder.
- There is no growth observed on the T7 Agar plate of Clove powder.

3.1.4. Salmonella test:

- There is no black centre colony observed on XLD Agar plate of Chilli powder and Clove powder.
- There is not Observe black colony with metallic sheen on BSA plate of Chilli powder and Clove powder.

3.1.5. Escherichia coli test:

- There is Observe green metallic sheen colony on EMB Agar plate of Chilli powder.
- There is not Observe green metallic sheen colony on EMB Agar plate of Clove powder.

3.2. PHYSICAL TEST:

Sr. No.	Test	Chilli powder	Clove powder
1	Appearance	Fine ground powder	Fine ground powder
2	Color	Orange red	Dark brown
3	Odour	Pungent	Aromatic, Strong spicy
4	Taste	Pungent, spicy	Aromatic, pungent, bitter & spicy
5	Extraneous Matter	Free from Extraneous Matter	Free from Extraneous Matter
6	Particle size	Sieve size 500 μ m	Sieve size 500 μ m
7	Moisture	9.7%	12%

Table 3.2.1(a) Result of physical test

3.3 CHEMICAL TEST:

3.3.1. Volatile oil:

Sample	%Volatile oil
Chilli powder	18.9%
Clove powder	16.8%

Table 3.3.1(a) Result of Volatile oil

3.3.2. Total ash:

Sample	%Acid insoluble ash
Chilli powder	6.12%
Clove powder	4.7%

Table 3.3.2(a) Result of total ash

3.3.3. Color value & Scoville heat unit of chilli powder:

- Calculate the color value of chilly by following way :

$$Cu = \frac{0.D \times 16.4 \times 0.98}{0.1}$$

- Calculate the SHU of chilly by following way :

$$248\% = \frac{[-blank(248) + sample(248)] \times 2500}{(314 \times 10)}$$

$$296\% = \frac{[-blank(296) + sample(296)] \times 2500}{(127 \times 10)}$$

$$Avg. = \frac{P(248) + P(296)}{2}$$

$$SHU = Avg. \times 150000$$

Sample	%Color unit (Cu)	SHU
Chilli powder	230.26 Cu	13127.35

Table 3.3.3 (a) Color value & Scoville heat unit of chilli powder

CHAPTER 4: CONCLUSION

During this training period, we can conclude that spices which we eat at our home are not just spices; they reach to us after passing through a lot of tests. There are so many physical, chemical and microbiological tests through which spices pass before they reach the consumers.

We can modify certain physiological, chemicals and microbiological parameters of products using different technical method and QC process conditions in company. Here, we were optimized different condition of products and maintain quality of products by testing them for *Salmonella*, Yeast and Mold, TPC, E.Coli, Fungal, Moisture , SHU, Total Ash, Color Value, Total Ash, and Volatile Oil testing of chilli powder and clove powder from the QC lab of the company. All this is done to maintain consistent quality of the products and provide good service to consumers, agencies and market.

If spices are not tested for such pathogenic organisms, it can prove injurious to health. There are many methods to reduce the microbiological load of the spices, ozone sterilization being one of them. Other methods include ETO treatment, Steam sterilization. Etc. The spices are fit for consumption only after they pass through all the tests and if not, reesterilization is done.

The tests which we have learnt have minute details to be taken care of starting from collection of sample to interpretation of result. Every stage is important and should be performed with utmost care.

CHAPTER 5: TABLE & FIGURES:

5.1. MICROBIOLOGICAL TEST:

5.1.1. Total plate count:



Fig. 5.1.1(a) Chilli powder

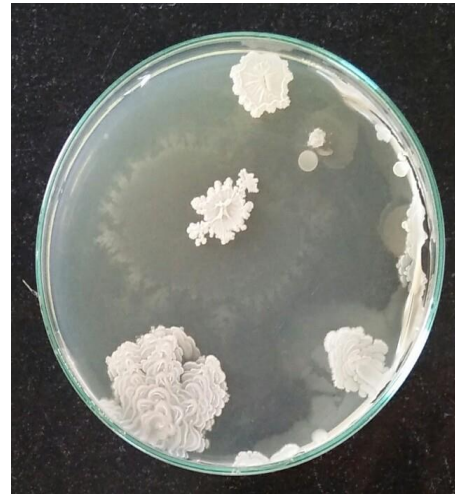


Fig. 5.1.1(b) Clove powder

5.1.2. Fungal test:



Fig. 5.1.2(a) Chilli powder



Fig. 5.1.2(b) Clove powder

5.1.3. Coliform test:

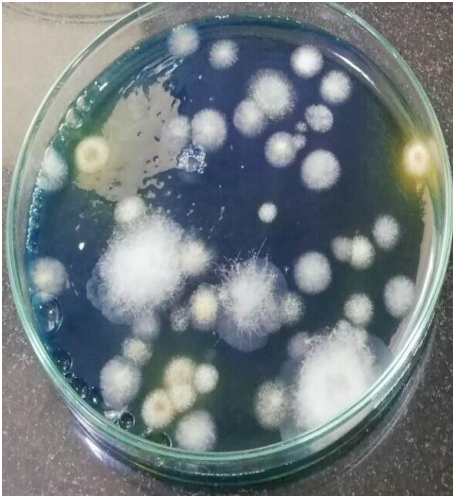


Fig. 5.1.3(a) Chilli powder

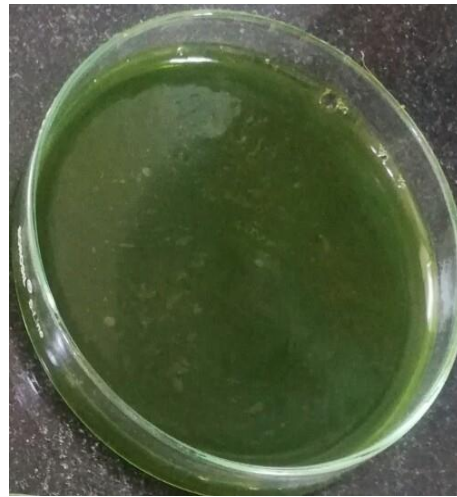


Fig. 5.1.3(b) Clove powder

5.1.4. Salmonella test:



Fig. 5.1.4(a) XLD Agar plate of Chilli powder & Clove powder



Fig. 5.1.4(b) BSA plate of Chilli powder & Clove powder

5.1.5. Escherichia coli test:



Fig. 5.1.5(a) Chilli powder

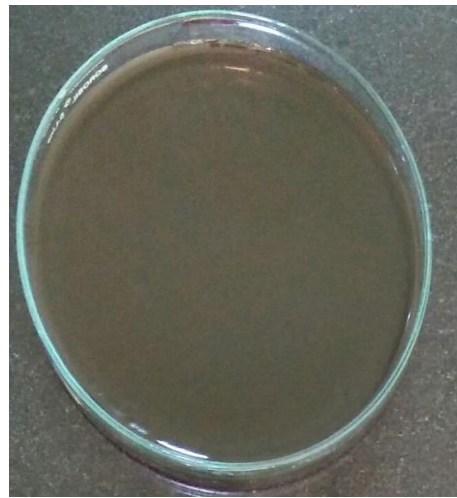


Fig. 5.1.5(b) Clove powder

5.2. PHYSICAL TEST:

5.2.1. Appearance:

➤ Chilli:



Fig. 5.2.1(a) Good chilli



Fig. 5.2.1(b) Broken part



Fig. 5.2.1(c) Yellow chilli



Fig. 5.2.1(d) Seed%



Fig. 5.2.1(e) loose seed



Fig. 5.2.1(f) stacks



Fig. 5.2.1(g) Infected chilli



Fig. 5.2.1(h) Ex. matter

➤ **Clove:**



Fig. 5.2.1(i) Good clove



Fig. 5.2.1(j) without head



Fig. 5.2.1(k) Immature



Fig. 5.2.1(l) Stacks



Fig. 5.2.1(m) Ex. Matter



Fig. 5.2.1(n) Ex. Matter

5.2.6. Particle size:



Fig. 5.2.6(a) particle size

5.2.7. Moisture:



Fig. 5.2.7(a) Moisture meter

5.3. CHEMICAL TEST:

5.3.1. Volatile oil:



Fig. 5.3.1(a) Apparatus for determination **Fig. 5.3.1(b) volatile oil**
Of volatile oil

5.3.2. Total ash:



Fig. 5.3.2 (a) Muffle furnace



Fig. 5.3.2 (b) Total ash of chilli & clove powder

5.3.3 Scoville heat unit of chilli powder:



Fig. 5.3.3 (a) SHU of chilli powder

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