PRACTICAL MANUAL FOR EXPERIENTIAL LEARNING PROGRAM ON FOOD PROCESSING TECHNOLOGY (EL-AGP-809)

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Business idea: -Production of Soybean Based Food Products (Flavoured Soy Milk, Tofu, Soya Curd).

1. Topic- Processing of raw material.

The production of quality soymilk begins with effective cleaning of soybean. Critical quality characteristics of soymilk like sensory properties, odour, nuttiness/consistency, colour, etc are impacted by the cleanliness of the soybean grains. Consistency and texture of the soymilk base depends on effectively removing dust, foreign grains and other impurities from the raw material.

The soybean processing industry continues to develop and incorporate new technology to improve soy products' quality and consistency and to maximize equipment utilization by reducing downtime and improved energy utilization. Recent advances in the process to remove the unwanted impurities from the raw soybeans reflect the industry's desire to become more efficient at producing consistent, quality products.

Screening or sieving to separate good quality soybean from foreign grains, sand, stones and many other types of coarse and fine impurities has been the principal method of cleaning soybean from the beginning of soymilk and derivatives processing history. Over time, various machines have been introduced and improved upon to increase sieving efficiencies. Sifters and sifting reels to remove impurities and classify soybean by size were commonly used as primary cleaning equipment when the importance of cleaning and grading grain developed.

As the operating capacities of mills increased, the use of oscillating or vibratory screeners expanded to keep up with the increased capacity requirements. Aspiration was incorporated into these designs to remove the dust and light impurities before the screening to improve effectiveness of the sieving as well. As grain cleaning technology continued to advance, machines that combined multiple cleaning principles were introduced that incorporated sieving, density separation and aspiration into one machine. Combination machines allowed higher cleaning capacity to be installed in a smaller space, helping to reduce the capital cost of new mills and mill expansions. New innovations in grain cleaning reintroduced the advantages of sifting and grading soybean by size while removing impurities. These more recent advancements in soybean cleaning integrate modern technology and materials to meet the growing demand for higher operating capacity, improved cleaning efficiency and lowering the cost of operation and maintenance.

Soybean hulls contain unwanted substances and the same are also an obstruction to processing, especially in the decanter. Soil bacteria are present in the soybean hulls and therefore hulls should be removed to reduce bacteria count in the soymilk, resulting in better flavour and shelf-life. Soybean hulls contain polysaccharides that should be removed to avoid off-flavours and processing problems caused by foaming. Notably, the holding time for heat treatment of the

soybeans to inactivate undesired enzymes can be shortened when using de-hulled soybeans. This will decrease protein denaturation and browning of the soymilk. Further, dehulled soybeans produce a white, attractive and appetizing soymilk.

For dehulling, traditional stone chakki design was used as a template for the attrition-type mills as commercial-scale dehulling of soybean emerged. The two-stone principle was retained and the much larger stones were rotated using the energy of harnessed animals (such as bullocks) or running water. The mills were adapted as electricity became an available power source, and automation increased. Now, the stones are artificial and coated with carborundum (derived from silicon carbide) of various abrasive grades (grit size). These new improved attrition-type mills are often called under runner disk shellers (URD Shellers). The orientation of the stones can be either horizontal (as in the original chakkis) or vertical, and the gap between the stones can be adjusted to the seed size to optimize dehulled seed yields.

The other modern mill type is a carborundum roller mill. It has a cylindrical carborundum stone that is tapered and rotates inside a perforated metal casing so that the gap between the roller and casing decreases from the inlet to the outlet. The stone or casing can sometimes be moved to adjust the gap depending on the seed size. These mills are often mounted horizontally or on a slight downward angle to facilitate passage of the seed. A lot of work is being done on de-husking of seed coat. New Generation de-hullers in market are able to scratch seed coat more effectively with a proper control on emery Speed, Pressure, and Appropriate selection of emery grit size vis-à-vis typical soybean variety. New hullers are capable of handling all types of seeds with equal ease. Efficient De-husking means removal of seed-coat with minimum damage which in turn helps to increase yield.

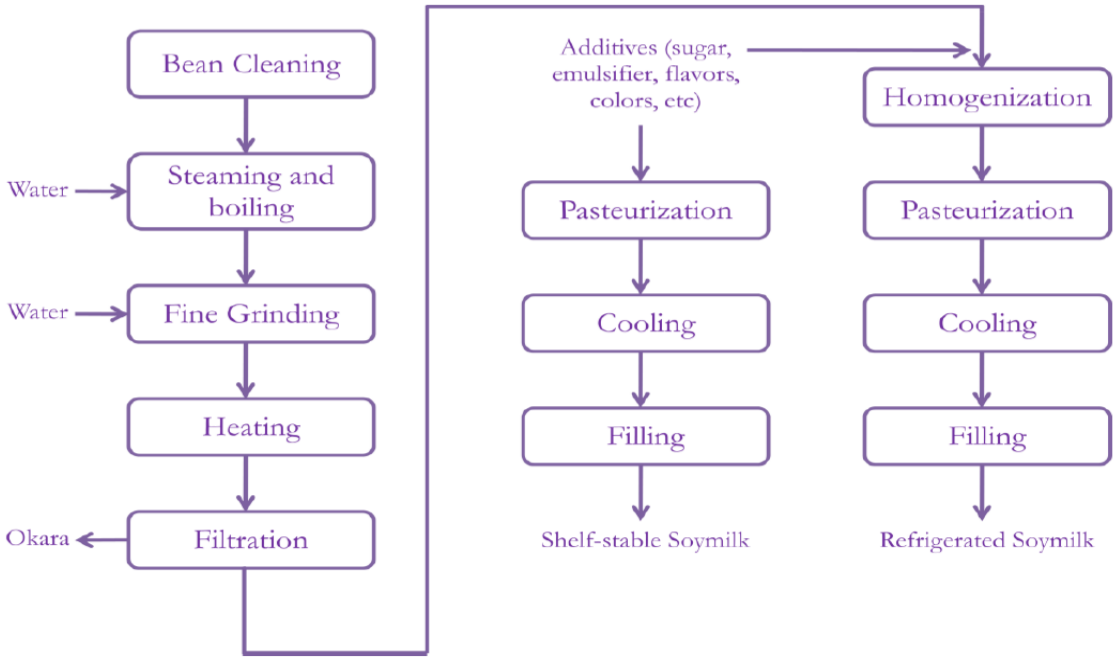
Drying technology was another technological gap where milling industry was struggling to get food safe solution and efficient de-hulling. Dryers introduced in the food grain industry efficiently remove surface moisture and core moisture of seed. This makes seed coat scratching easy. Drying technology helps in getting higher yield and higher productivity. New drying technologies are helping processors to avoid sun drying process which was not food safe. Dryers with better temperature and process controls help soybean seed to retain its original properties. With ever increasing focus on colour properties of products, naturally the demand for the colour consistent product has increased even in the soymilk market. Hence, colour sorting (i.e., surface and colour grading) of soybean before processing for milk base has become a standard practice. The technology in colour sorters has advanced from basic monochromatic versions to bi-chromatic and now to the advanced Tri-chromatic/RGBS models.

2. Topic- Preparation of soy milk.

Soymilk production has three stages of processing. The first stage involves proper pre-cleaning and main cleaning of the raw material produce. The second stage involves production of soymilk base and third stage involves conversion of soymilk base to tofu or yogurt or other value-added foods.

In the first stage, raw material soybean from farm is transported to unit for processing. The material passes through several cleaning machines like reel machines, gravity separators, destoners, etc. Cleaned and graded material is later processed in a dehusker machine which is used for cracking the husk layer and for scratching of clean soybean passing through it. This is done for loosening the husk from sticking to the grains. During this operation soybean grains are de husked and often split which are separated by sieving and the husk is aspirated off. For complete de-husking the whole process may also be repeated. The grains are further moved to colour sorting section to remove any colour and surface level defects.

In the second stage, the cleaned grains are moved to soymilk processing section. The process details are as under:



Soymilk Manufacturing Process

Cleaned and graded soybean is required for processing and preparation of soya milk, and subsequently to prepare flavoured soya milk, soya yogurt and tofu. The soybeans are steamed and split in half. This loosens the hull on the bean. A vacuum suck off the hulls. Next, soybeans must be cooked in order to invalidate, or counteract, a specific enzyme which makes them indigestible to humans. This cooking occurs in the Enzyme Invalidator, in which the de-hulled soybeans are cooked using high pressure, Water, and high temperature (creating very hot live steam) to invalidate that enzyme. The cooked soybeans then are transferred to the first rough

grinder or mill. Water is added to the machine and the bean pieces are roughly ground in this first milling. Although they have been ground once, the cooked soybeans are still rather coarse. Thus, the fine grinder further pulverizes the bean pellets into small particles. The hot slurry is white in colour with minuscule particles of insoluble soybean particles.

A large centrifuge (or muslin cloth in micro units) is then used to extract the tiny bits of soybean that are insoluble and cannot be included in the finished product. These particles are separated from the soy milk slurry. A rubber roller presses the soy milk slurry against the surface of a drum within the centrifuge, forcing the liquid inside the drum while the fibres remain on the outside of the drum. The drum is then scraped off these fibers.

These soybean fibres are physically removed from the production process at this time. This soy fibre is called okara and almost resembles mashed potatoes. A separate process dries the okara for use other than human consumption. The fibre-less soy liquid is raw soy milk at this point and is referred to in the industry as jun.

The jun is injected into large tanks and flavourings, sugar, and vitamins are mixed separately in smaller tanks. Ingredients of the smaller tank are infused into the larger tanks, thus blending the flavours with the raw milk. At this point, it is essential that the jun be sealed within the equipment until the end of the manufacturing process (including packaging) in order to keep out air and ambient bacteria and germs that can grow in low-acid soy milk. Sterilization occurs with pressure and very hot temperatures within a vacuum for a short period of time. From the sterilizer, the hot milk is sent to the homogenizer. This breaks down the fat particles and prevents them from separating from the rest of the mixture. In the homogenizer, which is essentially a high-pressure piston pump, the milk is blended as it is drawn into the pump cylinder and then forced back out in a repetitive motion. Next, the hot milk is piped to the cooling tank. Here, the hot milk passes next to cold plates that lower the temperature of the soy milk to room temperature. The cooled milk is sent to the aseptic (sealed) tanks and held here in preparation for packaging. Here, the soy milk is refrigerated, pressurized, and sealed to ensure no bacteria thrive in the milk.

<u>The Procedure of Making Flavoured Soymilk</u>

1. All necessary materials were prepared.
2. The soybean seeds were cleaned and sorted out, the defected seeds, pebbles and other foreign materials were removed.
3. The soybean seeds were washed with clean tap water.
4. The soybeans were soaked overnight for 8 hours, after soaking, the water was drained.
5. The cleaned soybean seeds were quickly steamed for easier removal of its seed coat.
6. The soybean seed coat was eliminated by rubbing using hands with flowing tap water. The beans were ready for milk extraction.
7. Cleaned soybean seeds were blended for five minutes or until smooth. One kilogram of soybean seeds, twelve litters of drinking water was added. The soymilk was strained into a casserole through a very fine sieve and re-strain using double-layered cheesecloth to ensure the separation of the okara from the milk.
8. The soymilk was boiled to 90 ^{0}C centigrade for 15 minutes, stirred occasionally to prevent scorching.

9. The soymilk was removed from heat and cooled with running water, when the temperature dropped to 50 ^{0}C, melted powdered fruits were gradually added.
10. Cooled flavoured soya milk was packed in the transparent bottle and sealed tightly.

3. Topic- Production of tofu using soy milk.

Once the soymilk base is extracted, it is pumped into curding vats. A coagulating agent is mixed in, such as calcium sulphate, magnesium chloride, etc. The coagulant alters the pH and curds the soymilk much like the process for making cottage cheese. This step takes about 20 minutes. Traditionally the obtained curd like output is pressed with hand-turned screw presses or simple lever presses. The tofu may be pressed in cheesecloth-lined boxes. Modern systems use centrifuges or hydraulic presses. The (residual) water in pressing is drained off, leaving soft blocks of pressed curds. Tofu can be produced in a variety of textures, from a dense cheese-like texture to a softer or liquid form.
Automated cutters slice the cake tofu into desired weight blocks. The tofu blocks are washed in vats of water where they firm up and are stored until they are ready to be processed further. Tofu may be packaged into shrink-wrapped blocks or continuous thermo-form packages. Water may be added to the packages, or tubs, and then they are sealed, weighed, and dated.
The packaged tofu is pasteurized at about 180°F (82°C). Pasteurization extends the shelf life of tofu to about 30 days. The tofu is then chilled in water until it is ready to be placed into boxes and shipped to distributors. Tofu must be refrigerated at below 45°F (7°C) to keep it fresh.
Formulation is also possible with tofu. The addition of herbs and spices and other ingredients can make the product more ready to eat and easier to prepare at home. The addition of other ingredients, before coagulation, requires careful testing since some ingredients will negatively affect the coagulation or pressing. Silken tofu, which is very soft and pudding-like, is made without removing the whey water or pressing.

4. Topic- Production of curd/yogurt using soy milk.

After obtaining the soymilk base, the same is allowed to cool down to a lower temperature (though keeping it warm). Later, curd/yogurt culture (curd/yogurt bacteria) is added and the same is incubated for few hours to make yogurt. Once the yogurt is ready, it is kept in cool temperature and packed in transparent food grade pouch at the time of sale. Cup filling systems are also used in some modern soy yogurt plants. Alternatively, some further value-added products like yogurt desserts, flavoured curds, etc are prepared for sale.
Yogurt and its derivative foods and drinks are some of the easiest products to understand and produce for people with dairy food experience. The processing of soy yogurt is almost identical to that made of cow milk, but with some very important differences. Formulation is required, primarily to add a simple carbohydrate such as sugar, to compensate for the missing lactose found in cow milk. The bacterial culture, which can be of the same type as for cow milk, as well as others, needs the additional carbohydrate to feed and develop. Another ingredient may

be required to reduce the surface separation of water, which occurs somewhat more than that with cow milk. Flavoured yogurt and drinks are easily formulated and processed for packing the same way that traditional yogurt products are packed. The flavoured products, when properly produced, are almost identical in taste to cow milk versions. Incubation time and temperature are also adjusted compared to traditional yogurt. Soy yogurt can require 6-10 hours or more of incubation at temperatures near 40º C. This is also partly determined by the type of finished yogurt and the desired or neutral taste.
It is important to note that the soymilk base for making soy yogurt products, does not have to be absolutely bland tasting and that vacuum deodorization may not be necessary. This allows a less complex system and also one with a medium or lower production volume. Nevertheless, a good quality soymilk base is a must.

5. Topic-Preservation of soy milk-based product.

The nutritious nature of soymilk makes it prone to microbial attack as the nutrients it contains are also required for the growth of most spoilage organisms if not properly processed and stored. A large number of microorganisms such as coliforms, mesophilic aerobic bacteria, yeasts and moulds are responsible for the spoilage of soymilk and it can produce undesirable changes in the milk.

The preservatives in food preparations inhibit or retard the growth of microorganisms which reduces the deteriorative effects of microorganism on food. The soymilk is stable up to two days at room temperature without addition of any preservatives and by using different types of preservatives in different levels the shelf life can be increased up to 14-17 days at refrigeration temperature. Potassium-meta-bi-sulphite (KMS), Sodium-benzoate and potassium sorbate can be used individually or in a mixture of the three for preservation of soymilk. Sodium benzoate and Potassium meta bi-sulphite are acceptable then potassium sorbate because these two preservatives not only preserve soymilk up to 17 days but also increase the colour, flavour, taste and overall acceptability of soymilk. The mixed preservatives can give shelf life of 17 days but decreases the colour, flavour, taste and overall acceptability of soymilk. The soy milk can be kept for up to 13 days at refrigerated condition without no multiplication of mesophilic aerobes above 3x103 cfu/mL when preserved with 700-800 parts per million (ppm) of sodium benzoate, pasteurization and refrigeration while a combination of 175 ppm of sodium metabisulphite and 400 ppm of sodium benzoate can achieve a preservation of the milk for about 11 days.

6. Topic: - Packaging of soymilk.

A very important part of the production is the aseptic packaging of the product. Packaging machines have been developed for soymilk and similar liquid products that are able to mechanically package the product without exposing it to air. The cooled milk is sent to this packaging machine which has a ribbon of flat packaging (cardboard) threaded into it. As the milk runs through the machine, the packaging surrounds the milk and a cutter cut through the cardboard packaging and the milk, simultaneously folding the package and sealing the milk within it. A machine glues a plastic spout onto the sealed package. From here, the product is sent to an automatic sorter that packs a case and places it on a pallet. Alternatively, soymilk is also filled in bottles (generally of 200 ml capacity) and crown caps are fixed on bottles. Bottles are then fed in Autoclave unit and treated (retorted- controlled method to reduce microbial load) for 15 minutes at 121 Degree Celsius. Packaged soymilk is also often kept in cold storage for enhanced shelf life.

Business idea: Processing of different fruits for the production of ready to drink beverages.

7. TOPIC: - PREPARATION OF GRAPE BASED RTD

Ready to drink beverages, as the name suggests, are drinks that are packed in single-use containers for immediate consumption. Due to the rising consumer demand, ready to drink beverages, also known as just RTD, are taking over the market shelves. Nowadays, all kinds of drinks can be found in this type of packaging, from juices, coffees, and teas, to alcoholic drinks, milk, and yogurt, and even some health beverages, too. To prepare grape based RTD beverages the required ingredients are as follows;

Ingredients:
- Liquid Glucose
- Invert Sugar
- Salt
- Milk
- Flavours
- Fruit essences
- Peel oil
- Sugar
- Water

Machines:
• Cleaner- To remove adhering dirt, to remove latex strains, to remove surface organism if any, Wash with 50 ppm chlorine to prevent the microbial contamination

• Peeler/ Pulper - Graded grapes are washed in the bubble washing machine then transferred through the conveyer belt for peeling and stone removal, fruits passed to pulping machine to get puree.

1. Slice and scoop out flesh from the cheek halves.
2. Peel and scrape the remaining flesh from the seed.
3. Liquefy flesh in a continuous pulping blender. Strain.

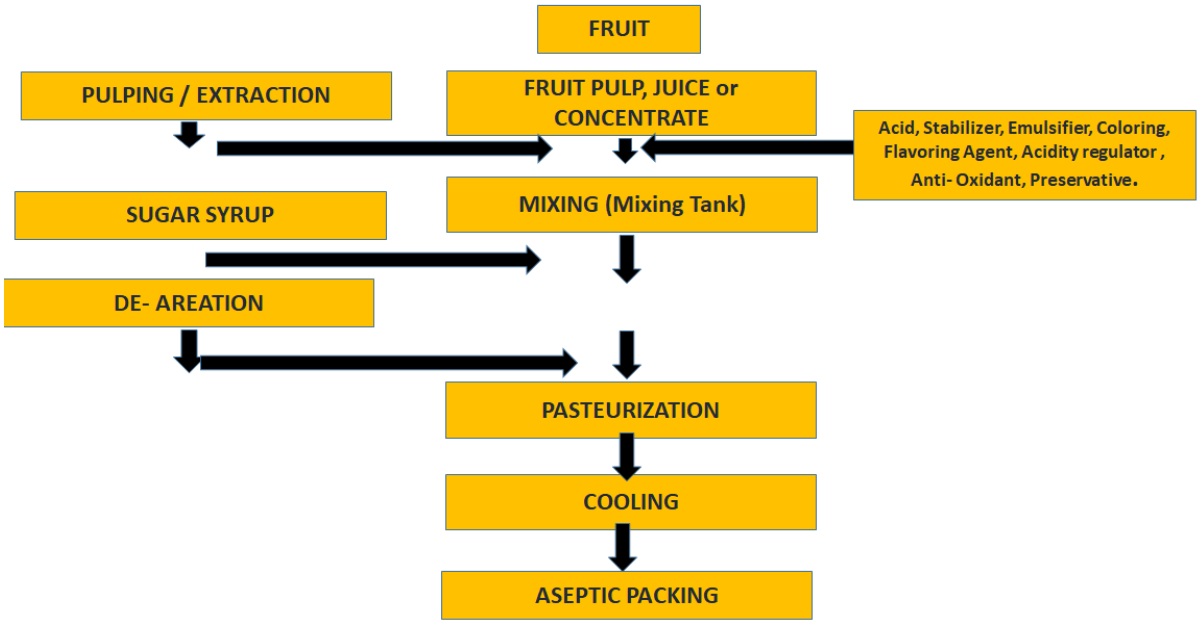
• Homogenizer –

1. The grapes juice will be homogenized under 20-25 MPa pressure for 2-3 times in a homogenizer.
2. The 1n the big particles in grapes juice are crushed to under the size of 5 μm.
3. Homogenization can unify the particles in fruit juice, regulate sediments and improve drink taste .

- Pasteurizer-
    1. Sterilization is necessary to ensure the shelf life of grapes juice.
    2. The sterilization temperature can be adjusted from 62 ℃ to 85 ℃ for 1 minute.
    3. It can eliminate bacteria, yeast and inhibit enzyme activity in fruit juice.

- Packaging machine-
    1. Bottling filling and sealing machine.
    2. Pouch packaging.

Process Flow Chart



Business idea: Processing of different vegetables for the production of value-added products.

8. Production of Mushroom Pickle: -

Under our conditions, especially in rural area, it may not be possible to do canning of mushrooms. A locally accepted alternative is to make pickle. In fact, some mushrooms like milky mushroom are more accepted as pickle than as fresh product. Pickling of mushrooms is an easy home scale process for preservation of mushrooms to a value-added product of high market acceptability.

Ingredients: -

For 1 kg mushroom you need various spices viz., turmeric powder (20g), black mustard seed powder (35g), red chilli powder (10g), cumin seed powder (1.5 g), carom seed (10 g), nigella seed (kalonji) (10 g), fennel seed powder (1.5 g), salt (90 g) and mustard oil (200 ml). You may also use Acetic acid (upto 100 ml) and sodium benzoate within the permitted limits as preservatives.

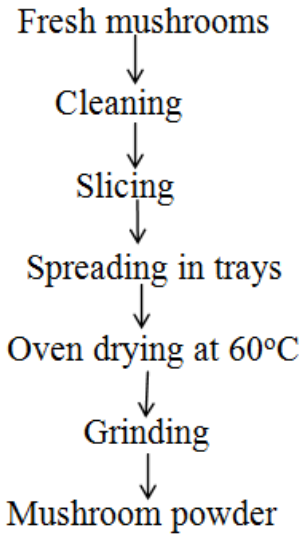
For preparing mushroom pickle

- Take one kg mushrooms are washed in 0.03 to 0.05% KMS solution and then blanched for 5 min at 85°C.
- The blanched mushrooms are washed in cold water 2-3 times and the excess water is drained off.
- Then mushrooms are cut in halves or quarters according to the size. Then the mushrooms are subjected to salt curing process, in which 20 g sodium chloride per kg of mushroom is added and kept overnight.
- The excess water that oozes-out of mushroom is removed on the next day.
- Mushrooms are allowed to dry for 2-3 hours and spices, salt and preservatives are mixed to the desired taste and quality of mushroom pickle.
- We add heated mustard oil from the top to the mixture and blend with both hands, leave overnight for imbibing of spices and flavour development, fill in plastic/glass jars and again top-fill with mustard oil, close the caps and seal the jars and store pickle at shaded ambient place.
- This pickle can be stored up to one year in the airtight bottles.

9. Production of Mushroom flour: -

As you are aware that drying is one of the most important methods to decrease the water content of the produce. The dried produce can be further utilized for preparing a number of value-added products. Button mushroom cannot be easily sundried. However, mushrooms like oyster, shiitake, wood ear mushroom, etc can be easily sundried (Fig. 10.1). Besides sun drying, mushrooms can be dried in cabinet dryers at a drying temperature of 55-60ºC which gives dehydrated final product of lower moisture content with longer shelf life and better quality.

Mushrooms have about 90% water and hence after drying these become very light. Care has to be taken while sun-drying. Mushrooms should be dried in dust free area and in containers where these don't get dispersed with wind. Additional advantage of sun drying is the increase in Vitamin D content in the mushroom. Mushrooms having open gills should be firstly dried with gill side upwards preferably in shade and then dried in sun. You may use solar based heater. But it is important to monitor the temperature of drying. Temperature above 60°C is not desirable as it leads to charring of sugars, loss of flavour and also decrease in rehydrability (that is gain of weight on rehydration of the dry product). You may powder the dried mushrooms and use it to make various products. Up to 10% powder can be added to flour that may be used to make biscuits, bread, cake or any other bakery product.



Method: -

1. Fresh mushrooms are cleaned and cut into slices (about 3 mm thickness).
2. Drying is carried out at 60°C for 8 hr in a tray drier.
3. Afterwards dried mushroom sample are ground separately in an electric grinder/ mortal pestle.
4. Grounded mushroom powder then sifted through an 80-mesh screen to obtain fine powders.
5. The obtained powder is then cooled and hygienically packed and stored in airtight container for further use.

Business idea: Processing and preservation of raw fish for the production of value-added food products.

10. Production of fish pickle: -

Traditionally, pickles made of vegetables like lime; gooseberry, ginger, garlic etc. are used as an important side dish along with meals. Though such pickles made of fish or meats were practically unknown in the past, such products have now become very popular and products under several brand names are now available in the market.

Ingredients

- Fish dressed and cut into small pieces: 1 kg
- Mustard: 10 g
- Green chilli (cut into pieces): 50 g
- Garlic (peeled): 200 g
- Ginger (peeled and chopped): 150 g
- Chilli powder: 50 g
- Turmeric powder: 2 g
- Gingerly oil: 200 g
- Vinegar (acetic acid 1.5%): 400 ml
- Salt: 60g
- Pepper (powdered): 2.5g
- Sugar: 10g
- Cardamom, clove, cinnamon (powdered): 1.5 g

Method of Preparation

1. Mix the fish thoroughly with 3% of its weight of salt and keep for two hours. Light salted and partially dried fish also may be used.
2. Fry the fish in minimum quantity of oil. Set apart the fried fish.
3. Fry the ingredients (mustard, green chillies, garlic, ginger) in the remaining quantity of oil and then add chilli powder, pepper powder and turmeric powder and mix well over low flame for a few minutes.
4. Remove from fire, add fried fish and mix well.
5. When cooled, add vinegar, powdered cardamom, clove, cinnamon, sugar and remaining salt and mix thoroughly.
6. Sufficient quantity of boiled and cooled water may be added to cover the ingredients well.
7. Transfer to clean, sterile g1ass bottles and seal with acid proof caps. Take care to see that there is a layer of oil over the contents in the bottle.

8. Flexible pouches made of 12-micron polyester laminated with l 18micron LD-HD co-extruded film can also be used for packing the pickle.

11. Topic-Shelf-life study of food products.

Shelf life is defined as "the period of time during which the food product will remain safe; be certain to retain its desired sensory, chemical, physical, microbiological, and functional characteristics; where appropriate, comply with any label declaration of nutrition data, when stored under the recommended conditions."
Both food safety and quality are important aspects of acceptable shelf life. Although pathogens are usually monitored during shelf-life studies.

● A guide for the consumer of the period for food can be kept before it starts to deteriorate, provided any stated storage conditions have been followed.

Describes how long a food will retain its quality during storage.

- Period during which food
  1. Remains safe to eat.
  2. Keeps its appearance, texture and flavour.
  3. Meets nutritional claims provided on the label, if any.

● Shelf-life is multifaceted property that is enormously important to food manufacturers and processors as well as consumers. The food safety and desired quality are the two main aspects of an acceptable shelf-life.

Shelf Life of Food: -

- It Begins from the time the food is prepared or manufactured.
- It is Indicated by labelling the product with a date mark.
- It is Dependent on many factors for example types of ingredients, manufacturing process, type of packaging and storage conditions.

Declaration about shelf life of food: -
Any packaged food with a shelf life of less than two years to be labelled with a date mark. Food to be safe up to, and including, the date marked.
One of the following options must be used:

1. "Use by" date.
   - Used for highly perishable foods and present a safety risk if consumed after this date.
   - A food must not be sold if it is past its —Use by date, nor should it be consumed.

2. "Best before" date.
   - This is used for foods other than those specified above.

- It is not illegal to sell food that has reached its Best before date.
- Storage conditions should be such that they are achievable in the distribution, retail systems and in the home. The seller should store the food according to stated storage instructions.

What is a Shelf-Life Study?
Many food products have some variation of open shelf-life dating marked on their containers. These dates help the consumer to decide how long the product may be stored prior to consumption. Food manufacturer conduct studies to determine the shelf-life of their product.

Direct Method of shelf life of food: -
1. Storing the product under pre-selected conditions for a period of time longer than the expected shelf life
2. Checking the product at regular intervals to see when it begins to spoil.

The Direct method involves
- Identification of causes for spoilage of food
- Selection of suitable tests for determining spoilage of food
- Planning of shelf-life study
- Running the shelf-life study
- Determination of the shelf life
- Monitoring.

The shelf-life Identification of causes for spoilage of food Three main categories of food spoilage are
- Physical.
- Chemical.
- Microbiological.

Microbiological examination of food: -

i. **Principle**

Direct Microscopic Count (DMC)
This method consists of examining under a compound microscope-stained films of a measured volume of milk or milk products spread and dried onglass slides over a specified area. The major advantages are:

- The rapid estimation of the total bacterial population of a sample ofmilk/ milk products.
- Recognition of distinctive shapes and arrangements of bacteria andsomatic cells in films as facilitated by staining.
- Revelation of useful information regarding the tracing of possible sources of contamination.

Standard Plate Count Method (SPC)

This method employs universal standardization of equipment, materials and incubation methods. It aims at determining the population of viable bacteria in the sample of milk/ milk products. A small quantity of the sample is mixed with the appropriate nutrient agar medium and poured into a Petri dish. The agar is allowed to set and plates are incubated at specific temperature for a definite period of time. The bacterial colonies grown on the agar surface during incubation are counted presuming each colony to have grown from one bacterium or bacterial clump present in the inoculum. The standard plate count is estimated by multiplying number of colonies with dilution factor. This method is specifically suitable for following purposes:

- Estimation of number of bacteria in pasteurized milk or milk products.
- In-line testing of products at various stage of processing.
- Detection of the sources of contamination.

Cultured dairy products or dairy products, to which a bacterial culture has been added, however are not tested ordinarily by this method.

The ratio of the standard plate count to direct microscopic count has been reported to be 1:4.

Count of Thermoduric, Thermophilic and Psychotropic bacteria

Thermoduric count – In the dairy industry those bacteria that survive pasteurization but do not grow at this temperature are considered as thermoduric bacteria. The major sources of contamination are poorly cleaned and sanitized utensils and equipment on farms and in processing plants. Their undesirable growth in milk and milk products results in spoilage of dairy products such as acids/ rennet coagulation, peptonisation and off flavour. The thermoduric count in dairy industry is used as

- A test of sanitization of dairy utensils.
- A means of detecting sources of organisms responsible for high counts in pasteurized products.

This test is carried out by determining the bacterial count in raw milk subjected to laboratory pasteurization (heating of milk at 63.5°C for 30 min in a water bath) with the standard plate count technique.

Thermophilic count – The term thermophilic bacteria in dairy industry applies particularly to those bacteria which grow in milk held at elevated temperature (55°C or higher) including pasteurization. These organisms enter milk from various sources on the farm or from poorly cleaned equipment in the processing plant. When milk is held at high temperatures for longer duration, these bacteria rapidly multiply in number and may cause flavour defects or problems with respect to bacterial standards. Thermophilic count is obtained by the SPC method with incubation temperature of 55°C.

Psychotropic count - In the dairy industry the term psychotropic indicates organisms capable of appreciable growth in milk and milk products at commercial refrigeration temperature irrespective of their optimum growth temperature. The number of psychotropic bacteria in raw milk depends on sanitary conditions prevailing during production and on time and temperature of milk storage before processing. These bacteria are generally non-pathogenic, but in dairy products they may be held responsible for.

- Production of off-flavours,
- Loss of flavour in cultured milk products.
- Discoloration of milk products.
- Decrease in the yield of cheese.
- Problem in meeting bacterial standards.

The SPC method with low temperature incubation is used to enumerate psychotropic organisms.

Coliform Test

The coliform group of bacteria comprises all aerobic and facultative anaerobic, gram-negative, non-spore forming rods able to ferment lactose with production of acid and gas at 37^{o}C within 48 h. One source of theseorganisms is the intestinal tract of human and animals. Their presence in milk and milk products is indicative of possible fecal contamination althoughsome species (e.g. *Enterobacter aerogens*) may be derived from feedingmaterials and soil. As these organisms are heat labile, their presence in pasteurized milk is considered to indicate post-pasteurization contamination. For testing presence of coliforms in milk and milk product, a small quantityof the product (1.0, 0.1 or 0.01 ml) is added to liquid or solid media containing lactose and bile salt with a suitable indicator. Production of acid and gas in liquid media and appearance of typical coliform colonies on theplates is taken as evidence of coliform contamination. A few other bacteria, such as those belonging to the genus *Clostridium* and *Bacillus* and certain yeasts also produce acid and gas under these conditions giving rise to false positive result. Hence, the test commonly employed to detect the presence of coliform bacteria in milk is called presumptive coliform test and in theevent of doubt the confirmed test is conducted to ascertain presence ofcoliforms in dairy products.

Yeast and Mould count

Yeasts and moulds are special class of microorganisms belonging to groupfungi. Yeasts are single cell organisms larger than bacteria. They reproduceby budding and also by formation of spores. They are commonly found insoil, fruits, and dairy products e.g. butter & cheese. Yeasts are used asstarter organisms in manufacture of fermented milk products e.g. Kefir and Koumiss. Moulds occur in filamentous forms and are larger than bacteria. Moulds are often present in air and cause contaminations and subsequent spoilage of dairy products e.g. cream, butter, cultured milk products, indigenous milk products, and condensed milk. Their presence in dairy products indicates improper pasteurization and poor sanitary conditions. Moulds are also known to produce mycotoxins e.g. aflatoxins. Some of themoulds are used for ripening of certain varieties of cheese.

ii. Requirements

a) Direct microscopic count method

i) Clean grease-free slides with one square centimetre area clearly markedon each of them.

ii) Breed's pipettes calibrated to deliver 0.01 ml of milk.

iii) Needle with bent point for spreading milk.

iv) Compound microscope.

v) Stage micrometre slide ruled in 1 mm.

vi) Newman's strain.

b) Standard plate count method

i) Incubator.

ii) Bacteriological delivery pipettes (1.0 and 1.1 ml).

iii) Dilution blanks (9 or 99 ml).

iv) Tryptone glucose agar or milk agar.

v) Petri dishes (outside diameter 98 mm; inside diameter – 94 mm; depth – 15 mm).

c) Thermoduric, Thermophilic and Psychotropic count

i) Water bath maintained at 63.5-o-C.

ii) Test tubes. 10 ml and 1 ml bacteriological pipettes.

iii) Petri dishes.

iv) Dilution blanks (9 and 99 ml).

v) Tryptone glucose agar/ milk agar.

vi) Thermometer; stopwatch, ice cold water.

vii) Incubator

d) Coliform Count

i) Bacteriological pipettes (1, 1.1, and 10 ml).

ii) MacConkey's broth tubes with Durham's fermentation tubes.

iii) MacConkey's agar

iv) Eosine Methylene blue agar

v) Endo agar

vi) Dilution blanks.

vii) Test tubes

viii) Petri dishes.

ix) Inoculation needle

e) Yeast and Mould Count

i) Dilution blanks.

ii) Potato dextrose agar.

iii) Pipettes (1.0 and 10.0 ml).

iv) Petri dishes

v) Incubator

iii. Procedure

a) DMC Method

i) Determination of Microscopic Factor

- Place the stage micrometer on the stage the microscope and focuson the scale first with the 16 mm objective and then with the oilimmersion objective.
- Count the number of small divisions (0.01 mm each) in the maximumdiameter of the field and thus determine the diameter of the field.
- The microscopic factor (MF) is calculated as follows:

$$MF = \frac{\text{Area f smear (100 sqmm)}}{\text{Area of microscopic field}} \times \frac{1}{\text{Volume of milk (0.01ml)}} = \frac{10{,}000}{3.1416 \times r}$$

ii) Preparation of Milk Spear

- Mix the sample of milk thoroughly by shaking.
- Draw milk into the Breed's pipette above the graduation mark,and adjust the volume of the sample to exactly 0.01 ml mark.
- Touch the tip of the pipette to the center of a one square centimeterarea on a slide and expel the entire volume of milk.
- Spread the portion of milk uniformly over the centre of one square centimeter area on the slide with the help of flamed bent pint needle.
- Dry the smears at 40^{o} – 45^{o}C within 5 minutes.

***iii)* Staining the Films**

- Dip the slides in Newman's strain (in a jar) for ½ to 1 minute.
- Remove excess strain by allowing water to run over from one endto another end.
- Air-dry the smear.

***iv)* Microscopic Examination**

- Observe the smear under oil immersion objective.
- Count the single organisms or well-isolated clumps of cells on anumber of microscopic fields.

The field for counting should be so selected to represent all parts of the film asfollows:

Average number of clumps/ fields	Number of fields to be counted
0.5	50
0.5 – 1.0	25
1.0 – 10.0	10
10.0– 30.0	5

b) Standard Plate Count Method

***i)* Preparation of Dilutions**

- Prior to removal of the sample from its container, thoroughly andvigorously mix contents to ensure the sampling of representative portion.
- Before opening a sample container, wipe the top of container with asterile cloth or cotton saturated with 70% alcohol.
- Immediately before transferring test portion of milk or cream, shakecontainer, making 25 complete up-and-down/ back-and-forth movements of about one foot in 7 seconds.
- Select dilution(s) in a manner that the total number of colonies on aplate will be between 30 and 300.
- Remove 1 ml of the sample of milk or milk products having viscositysimilar to milk e.g. cream with a sterile bacteriological pipette andtransfer it to the first tube of diluents (9 ml). Allow about 2 – 4 seconds for the content of the pipette to drain and gently blow out thelast drop. Rotate the test tube between palms of the hand to completethe mixing. This makes a dilution of 1:10.

- Similarly, a series of dilutions can be prepared by transferring 1 ml ofthe first dilution (1:10) into another 9 ml dilution blank to get 1:100dilution and so on.
- Where the solids content or viscosity of samples exceeds that of whole milk e.g. dried milk, condensed milk, ice cream, cultured dairyproducts, prepare the initial 1:100 (or 1:1000) dilution by weighing 1g (or 11 g) aseptically into dilution bottles containing 99 ml of dilutionblank.

ii) **Preparation and Incubation of Plates**

- Use of fresh pipette and transfer 1 ml of each required dilution intosterile Petri dishes in duplicate.
- Allow 2-4 seconds for the pipette to drain, touch the top of the pipette to a dry place in the Petri dish to drain out the last drop.
- Add 10-15 ml of standard milk agar previously melted and cooled to45^{o}C.
- Mix the contents of the plate thoroughly while the medium is still liquidby gently rotating the Petri dishes and allow the agar to cool and set. Invert the plates and incubate at 37^{o}C for 48 h.

iii) **Counting of Colonies**

- Remove the plates after 48 h and select the pair of plates having colonies between 30 and 300 on each plate.
- Count the number of colonies with the help of a colony counter anddetermine the average of the counts in the two plates and multiply thisby the dilution factor and report as SPC/ ml or g.

c) Count of Thermoduric, Thermophilic and Psychotropic Bacteria

i) **Thermoduric Bacteria**

- Arrange the water bath at 63.5^{o}C.
- Perform the proper mixing of milk samples.
- Transfer 10 ml of milk into test tubes aseptically.
- Insert a thermometer into one of the test tubes under observation.
- Lower the test tubes in the water bath. When the temperature of milkreaches 63.5^{o}C start your stopwatch.
- Terminate the incubation exactly after 30 minutes by taking out thetubes from the water bath and immediately chilling the milk by immersing in ice-cold water.
- Prepare appropriate dilutions and perform the standard plate countmethod with incubation at 37^{o}C for 48 h.
- Multiply the average number of colonies with dilution factor and reportas Laboratory pasteurization count per ml or 9 (LPC/ ml or g).

ii) **Thermophilic Bacterial Count**

- Perform the standard plate count method with incubation of plates at55^{o}C for 24 h.
- Report the results as thermophilic bacterial count/ ml or g (TBC/ ml org).

iii) Psychotropic Bacterial Count

- Prepare dilutions and plates as per the method of standard plate count.
- Incubate plate at 7°C for 10 days.
- After determining the colony count, report as psychrotrophic bacterialcount per ml or g (PBC/ ml or g).

d) Coliform Test

Presumptive Test

Liquid Media

- Prepare serial dilutions of the sample of milk or milk products.
- Transfer 1 ml of required dilution into MacConkey's broth tubes in triplicate.
- Incubate the tubes for 24 h at 37°C and observe for the production ofacid and gas. The production of acid is exhibited by change of color of medium from purple to yellow in the case of bromo cresol purple andorange to pink in the case of Andrade's indicator. Production of gas is observed in the Durham's tubes, which may be partially or completelyfilled with gas.
- In case of no change, further incubate for another 24 h and record the observation.

Solid Media

- Prepare serial dilutions of the sample.
- Incubate 1 ml portions of the required dilutions into sterile Petri plates in duplicates.
- Add to each plate 10-15 ml of MacConkey's agar previously meltedand cooled to 45°C.
- Mix the contents by rotating the plates.
- Allow to agar to solidify.
- Pour additional layer (3-4 ml) of the medium completely over the surface of the solidified medium.
- Invert and incubate the plates at 37°C for 24 h.
- Once incubation is over, examine the plates for presence of typicaldark red colonies measuring at least 0.5 mm in diameter.
- Count such colonies and express the results as coliform count per mlof milk.

Confirmation Test

- Pour 10 to 15 ml of melted Eosine Methylene Blue Agar or Endo agarinto Petri dish and allow the media to set.
- Introduce the sterile inoculating needs to the depth of 0.5 cm below the surface of the positive tube. In case of positive agar plates, transfer portion of typical colonies to the EMB/ Endo agar's plates.

- Place the curved section of the needle on the agar surface and streakgently to avoid tearing of the medium.
- Invert the plates and incubate at for 24 h.
- Observe the appearance of typical colonies of coliform on the agarsurface. Such colonies will appear pink with dark center and metallicsheen on EMB agar. Endo agar produces red colonies.

e) Yeast and Mould Count

- Prepare 1:10 dilution.
- Transfer 1 ml of dilution to duplicate Petri dish for plating.
- Adjust the pH of potato dextrose agar to 3.5 by adding calculated amountof sterile tartaric acid solution at the time of pouring plates.
- Pour the melted agar cooled to 25° C and mix the contents well. Allow theagar to set.
- Invert and incubate the plates at 25°C for 3-5 days.
- Count the number of colonies.

iv. Observations

We should record the following observations from the following tables.

Table 1. Direct Microscopic Count

Sample	Number of Clumps or Cells Per Microscopic Field 1, 2, 3, 4, ...10	Average	DMC/ ml
1			
2			
3			
4			

Table 2. Standard Plate Count

Sample Dilution	Counts in Plate 1, 2, 3, 4, ……10	Average	SPC/ ml or g
1			
2			
3			
4			

Table 3. Thermoduric Bacterial Count Method

Milk/ Milk Product Sample	Dilution Colony Counts	Average	LPC/ ml or g
1			
2			
3			
4			

Table 4 Thermophilic Bacterial Count Method

Milk/ Milk Product Sample	Dilution Colony Counts	Average	TBC/ ml or g
1			
2			
3			
4			

Table 5. Psychotropic Bacterial Count Method

Milk/ Milk Product Sample	Dilution Colony Counts	Average	PBC/ ml or g
1			
2			
3			
4			

Table 6. Presumptive Coliform Test (MacConkey's Broth)

Sample	Dilution	Observation at the end of 24 h and 48 h
1		
2		
3		
4		

Table 7. Presumptive Coliform Test (MacConkey's agar)

Sample Dilution	Presence of Coliforms Colonies (±)	Number of Colonies in the Plates		Coliform Count/ ml or g
		1	2	
1				
2				
3				
4				

Table 8. Confirmation test for coliform

Sample	Medium	Color of Medium	Colonies	
			Typical	Negative
EMB Agar				
Endo Agar				

Table 9. Yeast and Mould Count

Sample	Dilution	Number of yeast and Mould Colonies in Plates		Yeast and Mould Count/ ml or g
		1	2	
1				
2				
3				
4				

v. Results/Interpretation

Interpret the results obtained by microbiological analysis of milk and milk productson the basis of Microbiological standards (BIS & PFA) furnished in the textbook.

12. Topic: - Physicochemical analysis of food products.

1. Estimation of Total Reducing and Non-Reducing Sugars

Estimation of glucose and lactose by Willstatter's method

Principle:

Free aldehydic group can be readily oxidized by a mild-oxidizing agent to form corresponding acid. But free ketonic groups are more resistant to oxidation and require stronger oxidizing agents which oxidize ketoses and cleaves them into smaller fragments. The difference in the susceptibility towards oxidation can be used to advantage in the estimation of aldose when present alone or in a mixture with ketoses. In this method, iodine in alkaline codition is the oxidizing agent.

Reaction:

$RCHO + I_2 + 3NaOH = RCOONa + 2NaI + 2H_20$
$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6$

Reagents:

1) N/10 Sodium Thiosulphate.
2) N/10 Iodine.
3) N/2 NaOH
4) 2N H_2SO_4
5) 1% Starch solution.
6) Dextrose anhydrous (2mg/ml) & Lactose monohydrate (2mg/ml). Prepare 1000ml stock solutions of each.

For unknown solution, give sample containing 4% glucose.

All the above reagents, except for N/10 Iodine, are prepared by usual method.

Preparation of N/10 Iodine solution:

Solubility of iodine in water is 0.335g/lit at 25°C. so to dissolve iodine in water. KI is used in combination with iodine crystals.

Vogel's methods:

Dissolve 20g of iodate-free KI in 30-40ml DW in a glass stoppered 1 lit graduated flask. Weigh out about 12.7gm of resublimed iodine on a watch glass on a rough balance (never on an analytical balance due to iodine vapor) and transfer it by means of a small dry funnel into conc. KI solution. Insert glass stopper onto flask and shake in cold until all iodine has dissolves. Allow solution to acquire RT ant make up volume with DW. Store in a cool, dark place in an amber coloured glass stopped bottle. The bottles must be completely filled to prevent contact of air.

Ampule method:

For preparation of N/10 Iodine solution, Titrisol ampules (0.1 N iodine or 0.05 m/L iodine; 50ml) were used. Each ampule was opened into a 500ml volumetric flask and the volume was made up with DW. The resultant solution was N/10.

Procedure:

Part I: Estimation of glucose or dextrose

Take 10ml of sugar solution in a glass stoppered bottle. Add 5 ml of N/2 NaOH and 20ml of I_2 (N/10) solution. Allow to stand in ice for 20-30 min in dark cupboard. Acidify with 5ml H_2SO_4 (2N) and titrate the liberated iodine against N/10 $Na_2S_2O_3$ using strach indicator towards endpoint. Carry out the blank, side-by-side, using 10ml DW instead of a sugar solution. The difference between the two redings i.e., blank and sample reding, gives the thiosulphate value corresponding to the aldose present.

1 ml of (N/10) $Na_2S_2O_3$ Ξ 9mg of glucose

Part II: Estimation of lactose

Take 10ml of sample and estimate sample for lactose by above described method. For milk sample, it is customary to dilute the sample for analysis. Take 10ml of milk sample and dilute to 100ml in a volumetric flask. Take the diluted sample for analysis of lactose by Willstatter's method.

1ml of (N/10) $Na_2S_2O_3$ Ξ 17 mg of lactose

2. ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Aim: To estimate the amount of protein in the given sample by Lowry's method.

Principle: The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteay phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10 - 10.5.

The Lowry method is sensitive to low concentrations of protein. Dunn [1992] suggests concentrations ranging from 0.10 - 2 mg of protein per ml while Price [1996] suggests concentrations of 0.005 - 0.10 mg of protein per ml. The major disadvantage of the Lowry method is the narrow pH range within which it is accurate. However, we will be using very small volumes of sample, which will have little or no effect on pH of the reactionmixture.

A variety of compounds will interfere with the Lowry procedure. These include some amino acid derivatives, certain buffers, drugs, lipids, sugars, salts, nucleic acids and sulphydryl reagents [Dunn, 1992]. Price [1996] notes that ammonium ions, zwitter ionic buffers, nonionic buffers and thiol compounds may also interfere with the Lowry reaction. These substances should be removed or diluted before running Lowry assays.

Reagents
A. 2% Na_2CO_3 in 0.1 N NaOH
B. 1% NaK Tartrate in H_2O
C. 0.5% $CuSO_4.5H_2O$ in H_2O
D. Reagent I: 48 ml of A, 1 ml of B, 1 ml C
E. Reagent II- 1 part Folin-Phenol [2 N]: 1 part water

BSA Standard - 1 mg/ ml

Procedure:
• 0.2 ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water.
• The test tube with 1 ml distilled water serves as blank.
• Add 4.5 ml of Reagent I and incubate for 10 minutes.
• After incubation add 0.5 ml of reagent II and incubate for 30 minutes
• Measure the absorbance at 660 nm and plot the standard graph.
• Estimate the amount of protein present in the given sample from the standard graph.

Tabulation:

S.No	Vol. of BSA (ml)	Conc of BSA (mg/ml)	Vol. of Distilled water (ml)	Vol. of reagent I (ml)	Incubation for 10 min	Vol. of Reagent II (ml)	Incubation for 30 min	OD At 660nm

Result: The amount of protein present in the given sample was found to be ……

3. **Determination of pH Value: -**

- pH is the measurement of H+ ion activity; It measures active acidity. pH may be determined by measuring the electrode potential between glass and reference electrodes; pH meter is standardised using standard pH buffers. Use homogenized sample for the determination of pH.

4. Determination of acidity: -

Titrable acidity can be expressed conveniently in gms acid per 100 gm or per 100 ml as appropriate, by using the factor appropriate to the acid as follows:

- 1 ml of 0.1 N NaOH equals
- Malic acid - 0.0067 gms
- Oxalic acid - 0.0045 gms
- Citric acid monohydrate - 0.0070 gms
- Citric acid anhydrous - 0.0064 gms
- Tartaric acid - 0.0075 gms
- Lactic acid – 0.0090 gms
- Acetic acid – 0.0060 gms
- Oleic acid – 0.00282 gms

Take 10 gm well mixed juice, dilute to 250 ml with neutralised or recently boiled water. Titrate with 0.1 N NaOH using 0.3 ml phenolpthlein for each 100 ml of the solution to pink end point persisting for 30 seconds. Report acidity as ml 0.1 N NaOH per 100 gm or 100 ml as required.

5. Determination of total Sugars: -

The presence of added sucrose can be detected by determining sugars before and after inversion by copper- reduction methods.
Standardization of Fehling's solution: Prepare standard dextrose solution into a 50ml. burette. Find the titre (volume of dextrose solution required to reduce all the copper in 10 ml. of Fehling solution) corresponding to the standard dextrose solution (Refer table below).

Pipette 10 ml of Fehling's solution into a 300 ml of conical flask and run in from the burette almost the whole of the standard dextrose solution required to effect reduction of all the copper, so that more than one millilitre will be required later to complete the titration.

Heat the flask containing mixture over wire gauze. Gently boil the contents of the flask for 2 minutes. At the end of two minutes of boiling add without interrupting boiling, one ml. of methylene blue indicator solution.
While the contents of the flask begin to boil, begin to add standard dextrose solution (one or two drops at a time) from the burette till blue colour of indicator disappears [The titration should be completed within one minute so that the contents of the flask boil together for 3 minutes without interpretation.

Note the titre (that is total volume in ml. of std. dextrose solution used for the reduction of all the copper in 10 ml. of Fehling's solution). Multiply the titre (obtd. by direct titration) by the number of milligrams of anhydrous dextrose in one millilitre of standard dextrose solution to obtain the dextrose factor. Compare this factor with the dextrose factor and determine correction.

Dextrose factors for 10 ml. of Fehling's Solution		
Titre (ml)	Dextrose factor	Dextrose content per100 ml of solution (mg)
15	49.1	327
16	49.2	307
17	49.3	289
18	49.3	274
19	49.4	260
20	49.5	247.4
21	49.5	235.8
22	49.6	225.5
23	49.7	216.1
24	49.8	207.4
25	49.8	199.3
26	49.9	191.8
27	49.9	184.9
28	50.0	178.5
29	50.0	172.5
30	50.1	167.0
31	50.2	161.8
32	50.2	156.9
33	50.3	152.4
34	50.3	148.0
35	50.4	148.9
36	50.4	140.0
37	50.5	136.4
38	50.5	132.9
39	50.6	129.6
40	50.6	126.5
41	50.7	123.6
42	50.7	120.8
43	50.8	118.1
44	50.8	115.5
45	50.9	113.0
46	50.9	110.6
47	51.0	108.4
48	51.0	106.2
49	51.0	104.1
50	51.1	102.2
Milligrams of anhydrous dextrose corresponding to 10 ml of Fehlings solution		

Transfer test sample representing about 2- 2.5 gm sugar to 200 ml volumetric flask, dilute to about 100 ml and add excess of saturated neutral Lead acetate solution (about 2 ml is usually enough). Mix, dilute to volume and filter, discarding the first few ml filtrate. Add dry Pot. or Sod. Oxalate to precipitate excess lead used in clarification, mix and filter,

discarding the first few ml filtrate.

Note: Use of Potassium Ferrocyanide and Zinc acetate is preferable instead of Lead acetate and Sodium oxalate, due to safety issues.

Take 25 ml filterate or aliquot containing (if possible) 50 – 200 mg reducing sugars and titrate with mixed Fehling A and B solution using Lane and Eynon Volumetric method.

(1) Fehling A: Dissolve 69.28-g copper sulphate ($CuSO_4.5H_2O$) in distilled water. Dilute to 1000 ml. Filter and store in amber coloured bottle.

(2) Fehling B: Dissolve 346 g Rochelle salt (potassium sodium tartrate) (K Na $C_4H_4O_6$. $4H_2O$) and 100 g NaOH in distilled water. Dilute to 1000 ml. Filter and store in amber coloured bottle.

For inversion at room temperature, transfer 50 ml aliquot clarified and diluted solution to a 100 ml volumetric flask, add 10 ml HCl (1+ 1) and let stand at room temperature for 24 hours. (For inversion, the sample with HCl can be heated at 70^0 C for 1 hr. This saves time and makes the whole process shorter). Neutralize exactly with conc. NaOH solution using phenolphthalein and dilute to 100 ml. Titrate against mixed Fehling A and B solution (25 ml of Fehlings Solution can be considered for the purpose) and determine total sugar as invert sugar (Calculate added sugar by deducting reducing sugars from total sugars).

Reducing and total reducing sugar can be calculated as, Reducing sugar (%)

$$\frac{\text{mg. of invert sugar x vol. made up x 100}}{\text{TR x Wt. of sample x 1000}}$$

$$\text{Total reducing sugar (\%)} = \frac{\text{mg. of invert sugar x final vol. made up x original volume x 100}}{\text{TR x Wt. of sample x aliquot taken for inversion x1000}}$$

Total sugar (as sucrose) (%) = (Total reducing sugar – Reducing sugar) x 0.95 + Reducing sugar

6. Determination of Vitamin C (Ascorbic Acid):

The ascorbic acid content in fruits and vegetables can be estimated bymacerating the sample with stabilizing agents such as 20 % metaphosphoric acid.

Principle:

2, 6 -dichlorophenol indophenol is reduced to a colorless form by ascorbic acid. The reaction is specific for ascorbic acid at pH 1 to 3.5. The dye is blue in alkaline solution and pink in acid.

Reagents:

1. Standard Indophenol Solution – Dissolve 0.05 gm 2, 6 dichlorophenol indophenol in 50 ml. water, to which 42 mg. sodium carbonate is added, and make upto 200 ml. with water and filter. Sodium carbonate is added for stability purpose. The dye solution keeps for a few weeks if stored in refrigerator. Prepare fresh if possible andstandardize before use.

Blank correction: Dissolve 50 mg 2,6-dichloroindophenol Na salt that has been stored indesiccator over soda lime, in 50 mL H_2O to which has been added 42 mg $NaHCO_3$; shakevigorously, and when dye dis solves, dilute to 200 mL with H_2O. Filter through fluted paper into amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make end point in distinct occur in some batches of dry indophenol and also develop with time in stock solution. Add 5.0 mL extracting solution containing excess ascorbic acid to 15 mL dye re agent. If reducedsolution is not practically colorless, discard, and prepare new stock solution. If dry dyeis at fault, obtain new supply.)

Transfer three 2.0 mL aliquots ascorbic acid standard solution to each of three 50 mL Erlenmeyers containing 5.0 mL HPO_3–CH_3COOH solution, B(a)(*1*). Titrate rapidly with indophenol solution from 50 mL burette until light but distinct rose pink persists $^{3}5$ s. (Each titration should require ca 15 mL indophenol solution, and titrationsshould check within 0.1 mL). Similarly titrate 3 blanks com posed of 7.0 mL HPO_3– CH_3COOH solution, B(a)(*1*), plus volume H_2O ca equal to volume indophenol solution used in direct titrations. After subtracting average blanks (usually ca 0.1 mL) from standardization titrations, calculate and express concentration of indophenol solutionas mg ascorbic acid equivalent to 1.0 mL re agent. Standardize indophenol solution dailywith freshly pre pared ascorbic acid standard solution.

2.Standard Ascorbic acid solution – Dissolve 0.05 gm pure ascorbic acid in 60 ml of 20 % metaphosphoric acid (HPO_3) and dilute with water to exactly 250 ml in avolumetric flask.

3.Metaphosphoric acid - 20 %
4.Acetone

Standardization of Dye:

Pipette 10 ml of standard Ascorbic acid solution in a small flask and titrate with indophenol solution until a faint pink color persists for 15 seconds. Express the concentration as mg Ascorbic acid equivalent to 1 ml of dye solution i.e 10 ml of Ascorbic acid solution = 0.002 gm ascorbic acid

If 0.002 gm ascorbic acid requires V ml dye solution to neutralize it then 1 ml dyesolution = 0.002 / V gm ascorbic acid.

Procedure

Pipette 50 ml of unconcentrated juice (or the equivalent of concentrated juice) into a 100 ml volumetric flask, add 25 ml of 20 % metaphosphoric acid as stabilizing agent and dilute to volume. Pipette 10 ml in a small flask and add 2.5 ml acetone. Titratewith indophenol solution until a faint pink color persists for 15 seconds.

Calculation-

Vitamin of Vitamin C per 100g/ml = Titer value x Dye factor X Vol made up X 100

Where, Aliquot x: is wt. or vol. of sample

mg Ascorbic acid /g, tablet, ml, etc. = (X – B) x (F/E) x (V/Y)

Where, X = average ml for test solution titration,

B = average ml for test blank titration,

F = mg ascorbic acid equivalent to one ml iodophenol standard solution,

E = no. of g, tablets, ml, etc. assayed

V= volume initial test solution and

Y= volume test solution titrated

Note: -

Acetone may be omitted if sulphur dioxide is known to be absent. Its function is to form the acetone bisulphate complex with sulphur dioxide which otherwise interferes with the titration. Sometime a small proportion of the ascorbic acid in foods becomes reversibly oxidized during aging and forms dehydroascorbic acid. If this is suspected, first estimate the ascorbic acid as above, then through another portion of the solution pass a stream of Hydrogen sulphide for 10 minutes. Stopper the flask and allow it to stand overnight in a refrigerator. Then remove hydrogen sulphide by bubbling nitrogen through the mixture and titrated as before. The difference between the two titrations gives a measure of the dehydroascorbic acid. One international unit of vitamin C = 50 µg ascorbic acid.

13. Topic: - Sensory evaluation of food products.

The food samples were analysed for colour, creaminess, taste, and overall liking after overnight storage at 4-5 °C using two methods: the rating test (method 1) and the preference-ranking test (method 2). Twenty-five untrained panellists, who declared themselves regular consumers of that particular food products were asked to rate the samples on the basis of a 7-point hedonic scale anchored by: 1 = 'Strongly disliked'; 2 = 'Moderately disliked'; 3 = 'Slightly disliked'; 4 = 'Indifferent'; 5 = 'Slightly liked'; 6 = 'Moderately liked', and 7 = 'Strongly liked'. The samples were presented monadically following a completely randomized design. Mineral water and cream cracker biscuits were available as neutralizers between samples in order to avoid carryover effects. Taste preference was evaluated using the ranking test according to the subjects' degree of liking. A sample ballot for a ranking test of five products was adopted. A variation on this ballot lists the ranks from 1 = most preferred to 5 = least preferred. The subjects were instructed to taste food samples and rank them according to the first sensory impression and re-test to be certain of the correct ranking. Prior to each assessment, the subjects were informed about the task. In addition to the oral information, a detailed set of written instructions on the testing methods was available in each booth. A total of 50 mL/gm of each food sample at ambient temperature was served to each subject in coded opaque plastic tumblers. The tests were performed in the sensory laboratory under conditions of standard light and temperature (20 °C).

14. Topic: - Important video links.

- Production of soybean-based food products: -
  I. https://www.youtube.com/watch?v=D97s0voyvX8.
  II. https://www.youtube.com/watch?v=oLafN2XcZZw.
  III. https://www.youtube.com/watch?v=i_ezoUGN0Z4.
  IV. https://www.youtube.com/watch?v=kfFTcseAwNE.

- Production of fruits-based food products: -
  I. https://www.youtube.com/watch?v=kxmHEbAI3HQ.
  II. https://www.youtube.com/watch?v=MP2EAgiO5vk.
  III. https://www.youtube.com/watch?v=5JxnoAdGRNc.
  IV. https://www.youtube.com/watch?v=o3O-9YF09FU.

- Production of vegetable-based food products: -
  I. https://www.youtube.com/watch?v=oYDCTK_UlgA.
  II. https://www.youtube.com/watch?v=Sh1iMBrN-hQ.
  III. https://www.youtube.com/watch?v=YeGduu2-bVg.
  IV. https://www.youtube.com/watch?v=ADuEgp8-IYo.

- Production of fish-based food products: -
  I. https://www.youtube.com/watch?v=6tcmhdz9ea4.
  II. https://www.youtube.com/watch?v=9z8oRVlKfuM.

III. https://www.youtube.com/watch?v=FFYti5PHxE8.
IV. https://www.youtube.com/watch?v=J1z4Myvbkmg.

- Analysis of food: -
  I. https://www.youtube.com/watch?v=Nj1WDMWrtA0.
  II. https://www.youtube.com/watch?v=NpH5W7y8qp8.
  III. https://www.youtube.com/watch?v=FOPcDR0TKt4.
  IV. https://www.youtube.com/watch?v=1PNDss9DB3I.
  V. https://www.youtube.com/watch?v=bb6eeKOPiSo.