PRACTICAL MANUAL FOR EXPERIENTIAL LEARNING PROGRAM

ON

FOOD

PROCESSING TECHNOLOGY

(EL-AGP-809)

B.Sc. Agriculture, 4th Year, 8th Semester

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Index

1. Business idea: Production of flavored soymilk

Introduction

Soy milk (also known as soya milk), is a plant-based drink produced by soaking and grinding soybeans, boiling the mixture, and filtering out remaining particulates. It is a stable emulsion of oil, water, and protein. Its original form is an intermediate product of the manufacture of tofu. Originating in China, it became a common beverage in Europe and North America in the latter half of the 20th century, especially as production techniques were developed to give it a taste and consistency more closely resembling that of dairy milk. Soy milk may be used as a substitute for dairy milk by individuals who are vegan or are lactose intolerant.

A cup (243 ml) serving of a generic unsweetened commercial nutrient-fortified brand of soy milk provides 80 calories from 4 g of carbohydrates (including 1 g of sugar), 4 g of fat and 7 g of protein. This processed soy milk contains appreciable levels of vitamin A, B vitamins, and vitamin D in a range of 10 to 45% of the Daily Value, with calcium and magnesium also in significant content.

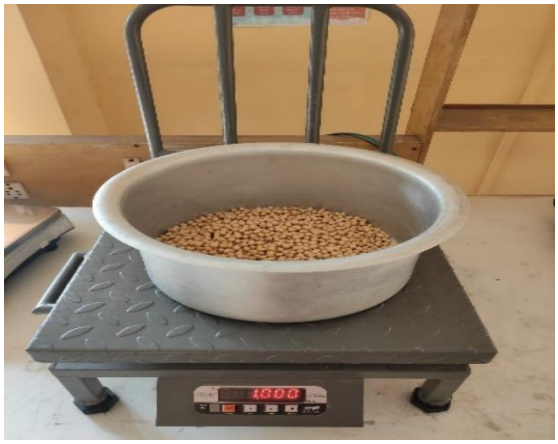
Processing of raw material.

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

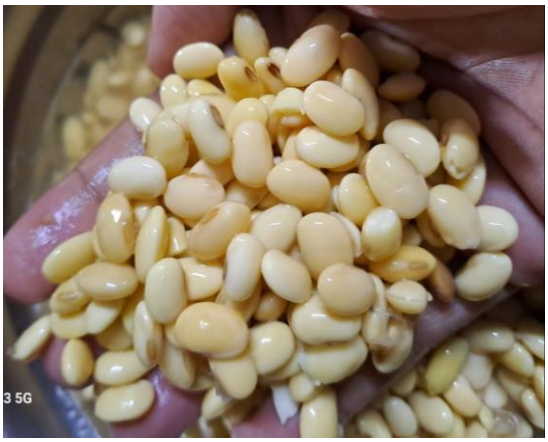
when the importance of cleaning and grading grain developed. As the operating capacities of mills increased, the use of oscillating or vibratory screeners expanded to keep up with the increased capacity requirements. Aspiration was incorporated into these designs to remove the dust and light impurities 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 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 chakki) or vertical, and the gap between the stones can be adjusted to the seed size to optimize dehulled seed yields. A lot of work is being done on dehusking 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.

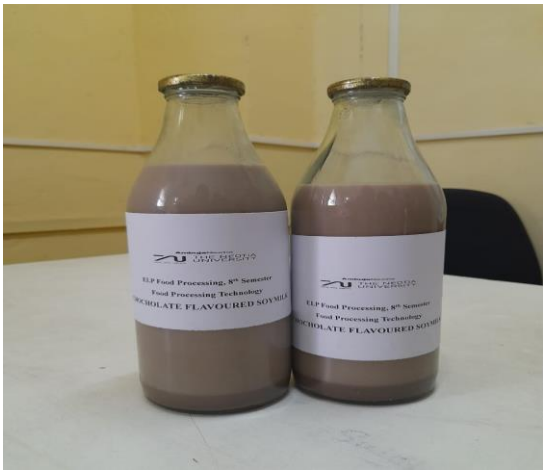


Weighing of soyabeans



Soaked soyabeans

Soymilk



Flavored soymilk

Objective

To learn the process of preparation of flavoured soy-based drinks.

Ingredients

Soybean seeds, water, fruit flavours

Equipments and miscellaneous items

Pressure cooker, stainless steel pan, thermometer, muslin cloth, gas stove, packaging material (glass)

Procedures

The procedures for making Flavoured Soymilk are as follows:

1. Clean and sort out the soybean seeds to remove the defected seeds, pebbles and other foreign materials.

2. Wash the cleaned soybean seeds with clean tap water.

3. Soak the soybeans (1 kg) overnight for 8 hours and drain off the residual water.

4. Steam the cleaned soybean seeds quickly for easier removal of its seed coat.

5. Eliminate the soybean seed coat by hand rubbing with flowing tap water. The beans are ready for milk extraction.

6. Blend the cleaned soybean seeds for five minutes or until smooth. For One kilogram of soybean seeds, twelve litters of drinking water can be added.

7. The soymilk is strained into a casserole through a very fine sieve and re-strain using double-layered cheese cloth to ensure the separation of the okara from the milk.

8. Boil the soymilk boiled to 90^{o}C centigrade for 15 minutes. Make sure to stir occasionally to prevent scorching.

9. Remove the soymilk from heat and keep for cooling. When the temperature dropped to 50^{o}C, gradually add melted powdered fruits.

10. Pack the cooled flavoured soya milk in transparent bottle and seal tightly.

Observation

Table 1. Evaluation of some physical and chemical properties

Sl. No	Sample Weight (l)	Flavours added (g)	TSS (ºBrix)	Colour	Moisture content (% w. b)	pH/acidity
1						
2						
3						

Table 2. Analysis of profit and loss

Sl. No	List of items	Cost price (expenses)	Selling Price (sales)	Profit/Loss
1	Raw materials			
2	Equipment/utilities			
3	Packaging/Service			
	Total			

Results/Conclusion

2. Business idea: Production of Tofu from soymilk

Introduction

Tofu (also known as bean curd in English), is a food prepared by coagulating soy milk and then pressing the resulting curds into solid white blocks of varying softness; it can be *silken*, *soft*, *firm*, *extra firm* or *super firm*. Beyond these broad textural categories, there are many varieties of tofu. It has a subtle flavor, so it can be used in savory and sweet dishes. It is often seasoned or marinated to suit the dish and its flavors, and due to its spongy texture, it absorbs flavors well.

Nutritionally, tofu is low in calories, while containing a relatively large amount of protein. It is high in iron, and can have a high calcium or magnesium content depending on the salt coagulants (e.g. calcium chloride, calcium sulphate, magnesium sulphate) used in manufacturing. Acids such as vinegar and lemon can be used as a tofu coagulant but the resulting tofu is gritty and sour. Many of the health benefits of tofu includes reduced risk of cancer, diabetes, and heart disease which are attributed to its high isoflavone contents.

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.

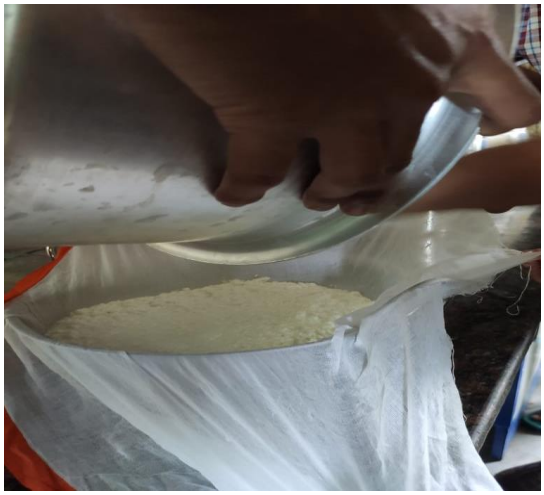
Objective

To learn the process of preparation of tofu from soymilk

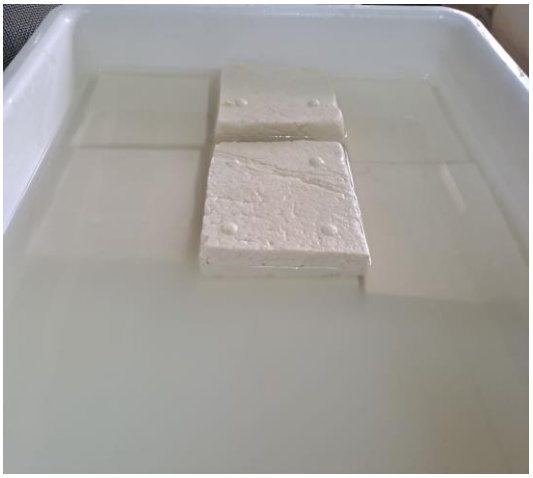
Ingredients

Soybean, citric acid/vinegar, water

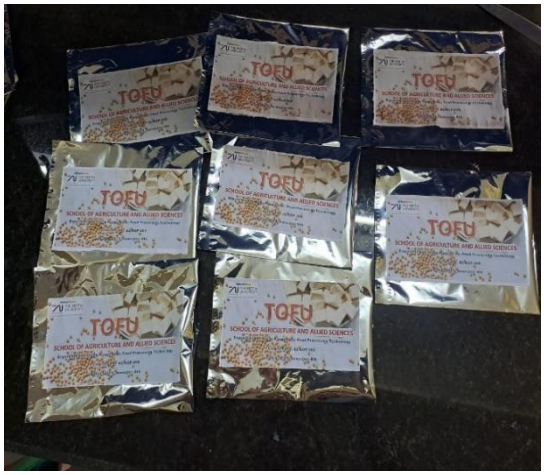
Soaking of soyabeans



Straining of tofu after coagulation



Pressed tofu kept in chilled water



Packaged tofu

Equipments and miscellaneous items

Pan, Blender, pressing machine, gas stove, muslin cloth, packaging material

Procedures

The procedures for making tofu are as follows:

1. Soak 1 kg of soybeans in water overnight. This will soften the soybeans and get them ready for blending.
2. Discard the water and rinse soybeans. Remove the skins as best as you can.
3. Add soybeans and water to a blender for blending until smooth.
4. Strain the blended mixture using muslin cloth.
5. Once the soymilk base is extracted, it is pumped into curding vats or pan. Heat the strained soymilk in the pan at 100^{o}C for 15-20 minutes.

6. Add citric acid/vinegar while still hot. Curdling will start and make sure the liquid is stirred frequently while adding citric acid.
7. Cool the mixture and strain the curdled soy in a muslin cloth.
8. Cover the muslin cloth and press under a pressing machine at 2-4 kg/cm^2 for 25-30 minutes. Traditionally the obtained curd like output is pressed with hand-turned screw presses or simple lever presses at around. The tofu may be pressed in cheesecloth-lined boxes. Modern systems use centrifuges or hydraulic presses.
9. Drain off the (residual) water in pressing leaving soft blocks of pressed curds.
10. Cut the pressed at the desired size and pack.
11. Store in a refrigerator.

Observation

Table 1. Evaluation of yield and some physical and chemical properties

Sl. No	Fresh sample (g)	Citric acid/Vinegar (g)	Yield (g)	Colour	Moisture content (% w. b)	pH
1						
2						
3						

Table 2. Analysis of profit and loss

Sl. No	List of items	Cost price (expenses)	Selling Price (sales)	Profit/Loss
1	Raw materials			
2	Equipment/utilities			
3	Packaging/Service			
	Total			

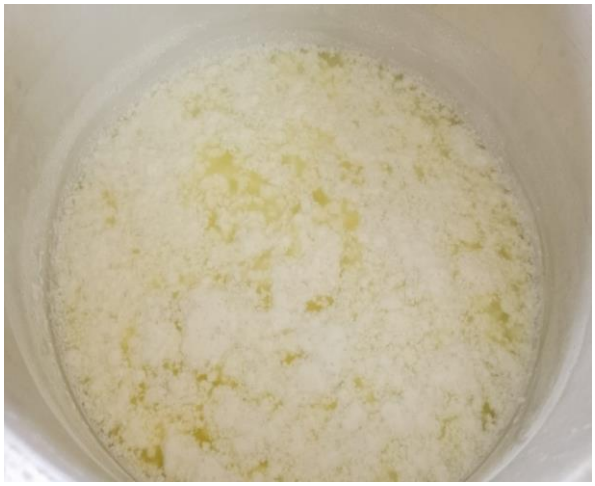
Results/Conclusion

3. Business idea: Production of Paneer from cow milk

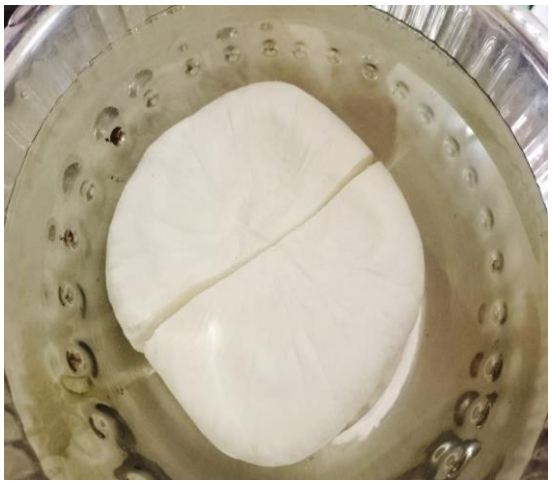
Introduction

Paneer, also known as Indian cottage cheese is a fresh acid-set cheese common in the Indian subcontinent (Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka) made from cow or buffalo milk. It is a non-aged, non-melting soft cheese made by curdling milk with a fruit- or vegetable-derived acid, such as lemon juice.

Paneer is a good choice for proteins, especially in a vegetarian diet. It is sometimes wrapped in dough and deep-fried or served with either spinach (palak paneer) or peas (mattar paneer). Paneer can be sweet, like the shahi paneer, or spicy, like the chilli paneer. With a biological value of protein being 80-86%, it contains all the nine essential amino acids. As we all know, proteins are the building blocks of our cells; they are also essential for maintaining growth, repairing tissue, keeping our immunity in top shape, maintaining our blood volume and are an essential part of almost every hormone, and enzyme. Indian diets lack proteins and are largely a cereal-based diet. Paneer can replace dals. Fats in paneer are about 20% and it is a rich source of saturated fats. But it also has healthy Monounsaturated fats (MUFA). MUFA is associated with lowering the LDL (bad cholesterol) in the blood. Oleic acid, the main MUFA in paneer, has been associated with lowering BP. Paneer also contains a fair amount of alpha linoleic acid, an omega-3 polyunsaturated fat, associated with a moderate lowering of risk of cardiac diseases. Paneer contains lower amounts of carbohydrates, as compared to dals which help in the Indian diets where carbs are very high as it is. Being a milk product, paneer is a rich source of calcium and phosphorus, both of which are crucial for our musculoskeletal health. Calcium alone may not be able to improve bone health; it needs phosphorus for optimum use, so if there is a food that gives you both together then it's amazing. Paneer is also healthy food choice for diabetics. It is low in carbs and a rich source of protein, which makes it perfect for controlling post meal sugar surges.



Coagulation of milk



Paneer after pressing

Objective

To learn the process of preparation of paneer from cow milk

Ingredients

Milk (full fat), vinegar/citric acid, chilled water

Equipments and miscellaneous

Pan, pressing machine, gas stove, muslin cloth

Procedures

The procedures for making paneer are as follows:

1. Take 1 litre full fat milk and boil in medium flame for 2-3 minutes.
2. Stir the milk occasionally to prevent from burning.
3. Add vinegar water (65 ml water+30 ml vinegar) while still hot.
4. Milk will start curdling as soon as vinegar water is added.
5. Strain out the whey from the curdled milk (Coagulum) using a muslin cloth.
6. Cover the cloth and keep for pressing in the paneer pressing machine for 1-2 hours.
7. Pressed paneer blocks are removed from pressing machine and cut into desired piece size (15×15 cm)
8. Immerse the paneer blocks in chilled water (4^oC) for 2-3 hours to assist in developing texture and speed up the cooling process.
9. Drain the water and wipe the surface.

10. Pack and store at 4^{o}C.

Observation

Table 1. Evaluation of yield, some physical and chemical properties

Sl. No	Fresh sample weight (g)	Citric acid/Vinegar (g)	Yield (g)	Colour	Moisture content (% w. b)	pH
1						
2						
3						

Table 2. Analysis of profit and loss

Sl. No	List of items	Cost price (expenses)	Selling Price (sales)	Profit/Loss
1	Raw materials			
2	Equipment/utilities			
3	Packaging/Service			
	Total			

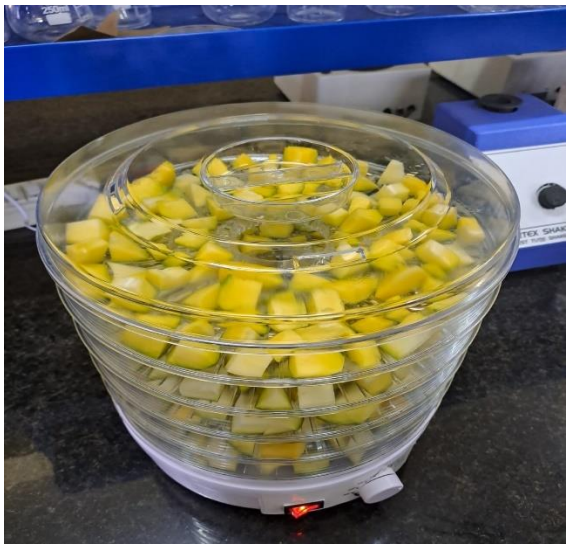
Results/Conclusion

4. Business idea: Production of pickle (Mango)

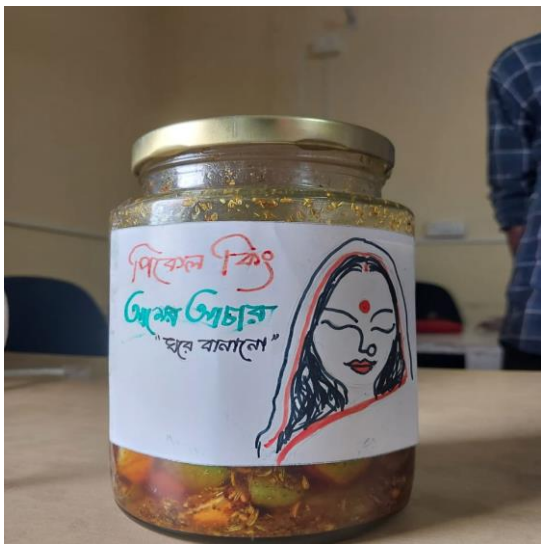
Introduction

Processing of fruits and vegetables offers immense scope for wastage minimization and value addition; thus, can generate significant income and employment in countries of agrarian economy. Pickle making is a global culinary art; you can find variety of pickles around the world. It is one of the oldest methods of preserving food. Traditionally, people used to make pickles at home and use to preserve it in ceramic jars or earthern pots, but with due course of time its popularity and demand increased which made people to use it commercially. The optimization of pickle quality depends on maintenance of proper acidity, salt concentration, temperature and sanitary conditions.

A mango pickle is a variety of pickle prepared using mango. It is a very popular South Asian pickle. Sometimes spicy pickles are also available commercially.



Drying of mango slices in dehydrator



Packaged mango pickle

Objective

To learn how to make pickle (e.g, mango)

Ingredients

Raw mangoes (2kg), rai (50 g) (mustard seeds), saunf (100 g) (fennel seeds), Kashmiri red chilli powder (100 g), haldi powder (50 g), salt (250 g), crushed methi (100 g), mustard oil (1 litre), hing (1 tbsp)

Equipments and miscellaneous

Knife, gas stove, kadhai, bowl, glass bottles

Procedures

The procedures for making pickle are as follows:

1. Select the right variety of raw mangoes you want to prepare
2. Wash the mangoes thoroughly with plain water and wipe them dry
3. Cut the mangoes into desired size as per your choice (may be 1 inch cubes)
4. Dry them by spreading in a single layer in the sun/dryer for 6-7 hrs.
5. Collect the dried mangoes and keep aside for making pickle
6. Grind the mustard seeds into powder and keep aside
7. Grind the fennel powder only about 15-20%.
8. Take a big bowl and add the mustard powder, crushed fennel seeds, red chilli powder, turmeric powder, salt, crushed methi, 400 ml of mustard oil and mixed together with the dried mangoes.
9. Heat 100 ml of mustard oil separately in a frying pan until its smoke releases. Once the smoke starts to release, switch off the flames and allow the oil to cool down a little bit and add hing.
10. Cool down the oil completely and then later pour this oil over the mango and mix well.
11. After mixing the mangoes with the ingredients, bottle them using airtight container or ceramic jar or glass jars provided it is precleaned and dried completely.
12. In the bottle, pour raw mustard oil and seal the jar
13. Keep the bottled mangoes in direct sunlight for maturing and to get its distinct flavour.
14. After a minimum of 3 days, the pickle is ready to consume.

Observation

Table 1. Evaluation of some physical and chemical properties

Sl. No	Fresh sample weight (g)	Final sample weight (g)	Colour	Moisture content (% w. b)	pH
1					
2					
3					

Table 2. Analysis of profit and loss

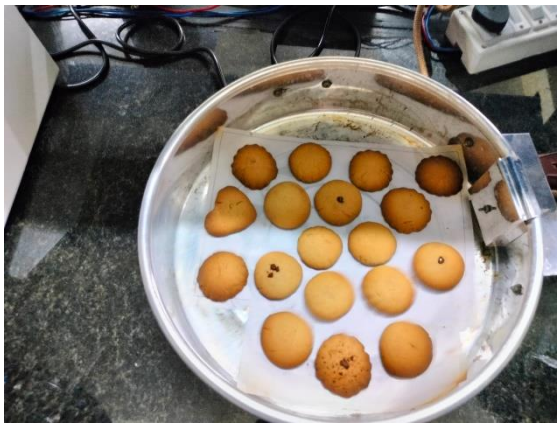
Sl. No	List of items	Cost price (expenses)	Selling Price (sales)	Profit/Loss
1	Raw materials			
2	Equipment/utilities			
3	Packaging/Service			
	Total			

Results/Conclusion

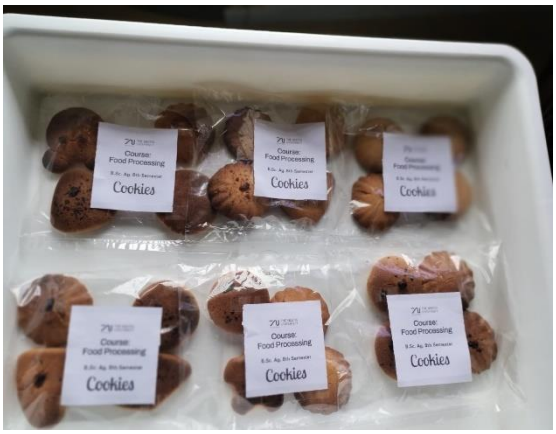
5. Business idea: Production of cookies

Introduction

A cookie is a baked or cooked snack or dessert that is typically small, flat and sweet. It usually contains flour, sugar, egg, and some type of oil, fat, or butter. It may include other ingredients such as raisins, oats, chocolate chips, nuts, etc. Biscuit or cookie variants include sandwich biscuits, such as custard creams, Jammie Dodgers, Bourbons and Oreos, with marshmallow or jam filling and sometimes dipped in chocolate or another sweet coating. Cookies are often served with beverages such as milk, coffee or tea and sometimes "dunked", an approach which releases more flavour from confections by dissolving the sugars. Cookies are most commonly baked until crisp or else for just long enough to ensure soft interior. Other types of cookies are not baked at all, such as varieties of peanut butter cookies that use solidified chocolate rather than set eggs and wheat gluten as a binder. Cookies are produced in a wide variety of styles, using an array of ingredients including sugars, spices, chocolate, butter, peanut butter, nuts, or dried fruits.



Cookies baked in baking oven



Packaged cookies

Objective

To obtain the skill of baking cookies to start a bakery unit.

Ingredients

Wheat flour (maida) (100 g), millet flour (50 g), sugar (100 g), egg (1-2), milk (1-2 cup), butter (150 g), baking powder (1 g), raisins, vanilla essence and flavours (2-3 drops)

Equipments and miscellaneous

Baking oven, mixing bowls, egg beater, spoon, trays, packaging material

Procedures

The procedures for making cookies/biscuits are as follows:

1. Sift the wheat flour along with the baking powder.
2. In a separate bowl, whisk together both the flours, baking powder and egg.
3. Beat butter until smooth and creamy in a bowl.
4. Add the flour mixture to the batter and mix thoroughly. Add enough milk to make a soft batter. Then add the raisins and beat until combined.
5. Then grease the tray with oil/butter.
6. For each cookie, drop about one table-spoon of batter onto the prepared baking tray, spacing the cookies about 2 inches apart.
7. Switch on the oven and when the temperature reaches up to 180^{o}C then place the tray into the oven.
8. Bake the cookies for about 15 to 20 minutes.
9. Remove from oven and transfer the cookies onto a wire rack to cool.

Observation

Table 1. Evaluation of some physical and chemical properties

Sl. No	Batter weight (g)	Baking temperature (^{o}C)	Baking time (min)	Colour	Moisture content (% w. b)	pH
1						
2						
3						

Table 2. Analysis of profit and loss

Sl. No	List of items	Cost price (expenses)	Selling Price (sales)	Profit/Loss
1	Raw materials			
2	Equipment/utilities			
3	Packaging/Service			
	Total			

Results/Conclusion

6. 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. It describes how long a food will retain its quality during storage such as period during which food remains safe to eat, keeps its appearance, texture and flavour and 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.

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 on glass slides over a specified area. The major advantages are:

- The rapid estimation of the total bacterial population of a sample of milk/ milk products.
- Recognition of distinctive shapes and arrangements of bacteria and somatic 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 Petridish. 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 63.5oC 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 (55oC 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^{o}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 these organisms is the intestinal tract of human and animals. Their presence in milk and Milk products is indicative of possible faecal contamination although some species (e.g. Enterobacter aerogens) may be derived from feeding materials 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 quantity of 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 the plates 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 the event of doubt the confirmed test is conducted to ascertain presence of coliforms in dairy products.

Yeast and Mould count

Yeasts and moulds are special class of microorganisms belonging to group fungi. Yeasts are single cell organisms larger than bacteria. They reproduce by budding and also by formation of spores. They are commonly found in soil, fruits, and dairy products e.g. butter & cheese. Yeasts are used as starter 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 the moulds 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 marked on 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 in1mm.

vi) Newman's strain.

b) Standard plate count method

i) Incubator.

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

iii) Dilution blanks (9 or 99ml).

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. 10ml 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

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 focus on the scale first with the 16 mm objective and then with the oil immersion objective.

- Count the number of small divisions (0.01 mm each) in the maximum diameter of the field and thus determine the diameter of the field.
- The microscopic factor (MF) is calculated as follows:

$$MF = \frac{Area\ of\ smear\ (sq.mm)}{Area\ of\ microscopic\ field} \times \frac{1}{Volume\ of\ milk\ (0.01\ ml)} = \frac{10000}{3.1416 \times r}$$

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 centre of a one square centimeter area 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 – 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 end to 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 a number of microscopic fields.

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

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 and vigorously mix contents to ensure the sampling of representative portion.
- Before opening a sample container, wipe the top of container with a sterile cloth or cotton saturated with 70% alcohol.
- Immediately before transferring test portion of milk or cream, shake container, 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 a plate will be between 30 and 300.
- Remove 1 ml of the sample of milk or milk products having viscosity similar to milk e.g. cream with a sterile bacteriological pipette and transfer 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 the last drop. Rotate the test tube between palms of the hand to complete the mixing. This makes a dilution of 1:10.
- Similarly, a series of dilutions can be prepared by transferring 1 ml of the 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 dairy products, prepare the initial 1:100 (or 1:1000) dilution by weighing 1g (or 11 g) aseptically into dilution bottles containing 99 ml of dilution blank.

ii) Preparation and Incubation of Plates

- Use of fresh pipette and transfer 1 ml of each required dilution into sterile 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 to 45^{o}C.
- Mix the contents of the plate thoroughly while the medium is still liquid by 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 48handselect 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 and determine the average of the counts in the two plates and multiply this by 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 10mlofmilk 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 milk reaches 63.5^{o}C start your stopwatch.
- Terminate the incubation exactly after 30 minutes by taking out the tubes from the water bath and immediately chilling the milk by immersing in ice-cold water.
- Prepare appropriate dilutions and perform the standard plate count method with incubation at 37^{o}C for 48 h.
- Multiply the average number of colonies with dilution factor and report as 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 at 55^{o}C for 24 h.
- Report the results as thermophilic bacterial count/ml or g (TBC/ml or g).

iii) Psychotropic Bacterial Count

- Prepare dilutions and plates as per the method of standard plate count.
- Incubate plate at 7^{o}C for 10 days.
- After determining the colony count, report as psychrotrophic bacterial count 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 37oC and observe for the production of acid 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 and orange to pink in the case of Andrade's indicator. Production of gas is observed in the Durham's tubes, which maybe partially or completely filled 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 melted and cooled to 45^{o}C.
- Mix the contents by rotating the plates.
- Allow the 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^{o}C for 24 h.
- Once incubation is over, examine the plates for presence of typical dark red colonies measuring at least 0.5 mm in diameter.
- Count such colonies and express the results as coliform count per ml of milk.

Confirmation Test

- Pour10 to15ml 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 streak gently 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 agar surface. Such colonies will appear pink with dark centre and metallicsheen on EMB agar. Endo agar produces red colonies.

e) Yeast and Mould Count

- Prepare 1:10 dilution.
- Transfer 1ml of dilution to duplicate Petri dish for plating.
- Adjust the pH of potato dextrose agar to 3.5 by adding calculated amount of sterile tartaric acid solution at the time of pouring plates.
- Pour the melted agar cooled to 25^{o}C and mix the contents well. Allow the agar to set.
- Invert and incubate the plates at 25^{o}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 per cells 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 count	Average	DMC/ml or g
1			
2			
3			
4			

Table 4. Thermophilic bacterial count method

Milk/Milk product sample	Dilution colony count	Average	TBC/ml or g
1			
2			
3			
4			

Table 5. Psychotropic bacterial count method

Milk/Milk product sample	Dilution colony count	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 or 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 plate		Colony 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 plate		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 products on the basis of Microbiological standards (BIS & PFA) furnished in the textbook.

7 Physicochemical analysis of food

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 condition 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^{o}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 starch indicator towards endpoint. Carry out the blank, side-by-side, using 10ml DW instead of a sugar solution. The difference between the two readings i.e., blank and sample reding, gives the thiosulphate value corresponding to the aldose present.

1 ml of (N/10) $Na_2S_2O_3$ = 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) $Na_2S_2O_3$ = 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 reaction mixture.

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, non-ionic 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% $CuSO_4$. 5 H_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:

Sl 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 660 nm

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 g acid per 100 g 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 g
- Oxalic acid - 0.0045 g
- Citric acid monohydrate - 0.0070 g
- Citric acid anhydrous - 0.0064 g
- Tartaric acid - 0.0075 g
- Lactic acid – 0.0090 g
- Acetic acid – 0.0060 g
- Oleic acid – 0.00282 g

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 phenolphthalein 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 (obtained 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 filtrate 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) ($K\ NaC_4H_4O_6.\ 4H_2O$) 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 700 C for1 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

$$\textbf{\textit{Reducing sugar}}\ (\%) = \frac{\textbf{\textit{mg.of invert sugar x vol.made up x}}\ \mathbf{100}}{\textbf{\textit{TR x Wt.of sample x}}\ \mathbf{1000}}$$

$$\textbf{\textit{Total Reducing sugar}}\ (\%) = \frac{\textbf{\textit{mg.of invert sugar x final vol.made up x original volume x}}\mathbf{100}}{\textbf{\textit{TR x Wt.of sample x aliquot taken for inversion x}}\mathbf{1000}}$$

$$\boldsymbol{Total\ sugar\ (as\ sucrose)(\%) =}$$
$$\boldsymbol{(Total\ reducing\ sugar - Reducing\ sugar)x\ 0.95 + Reducing\ sugar}$$

6. Determination of Vitamin C (Ascorbic Acid):

The ascorbic acid content in fruits and vegetables can be estimated by macerating 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 up to 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 and standardize before use.

Blank correction: Dissolve 50 mg 2,6-dichloroindophenol Na salt that has been stored in desiccator over soda lime, in 50 mL H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, 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 reduced solution is not practically colorless, discard, and prepare new stock solution. If dry dye is 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 titrations should check within 0.1 mL). Similarly titrate 3 blanks composed 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 solution as mg ascorbic acid equivalent to 1.0 mL re agent. Standardize indophenol solution daily with freshly prepared 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 a volumetric 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 dye solution = 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. Titrate with indophenol solution until a faint pink color persists for 15 seconds.

Calculation

Vitamin of Vitamin C per 100g/ml = Titre 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.

8 Sensory analysis of food products

Introduction

Food is consumed because it can stimulate human sensing organs to consume it. Human sensing organs serve as a measuring instrument for detecting whether a food is favored or not. We can measure color of food using colorimeter and measure milk temperature to pasteurize using thermometer. However, we can't measure food preference (like or dislike) using any instrument.

Sensory evaluation is an instrument used to measure food preference using human sensing organs. It is used the senses for measuring texture, sighting, the scent and flavour of food product. Humans have five senses: taste, smell, touch, sight, and hearing. All of the senses are important when eating a food. There is no instrument that can replace or replicate the human senses, making the sensory evaluation subject is important to learn.

Sensory evaluation is one of an area that generally well-known and important for food industry. However, many people considered that application of sensory food evaluation only needed to the research and development department in food industry. In fact, sensory evaluation can be used in many areas such as:

- To evaluate or improve quality of food product
- To provide information for decision making (launching a new lamb steak or a new chicken steak in steak restaurant).
- To determine shelf-life of a product
- To test the taste of new recipe to others people
- To test the taste of modified recipe
- To compare our new product to competitor's product

GENERAL RULES OF SENSORY EVALUATION

Sample food (carrier, size and temperature)

All foods presented in sensory evaluation test must be safe to eat. Food that has become moldy or has been treated causes microbiological or chemical contamination, then only the odor and appearance attributes of the food can be evaluated.

Some foods are not easily evaluated on their own, e.g. fat spreads, cereal, jam, sauces, etc. They are required an additional product to be presented as a carrier. Some carriers are unsalted cracker, bread, milk, and pasta.

Foods presented in sensory evaluation must be in adequate size (adequate but not excessive). The recommendation size for solid sample is 30 grams and for liquid sample is 15 ml (The American Society for Testing and Materials STP 434, 1968).

Technically, based on some recommendations from literature, frozen dessert should be served between -18^{o}C and -10^{o}C, cold beverages served between 5^{o}C and 9^{o}C, hot food should be present on 60^{o}C- 66^{o}C and hot tea and coffee present on 66^{o}C-71^{o}C. While there are many foods can be served in ambient temperature, e.g. snack, cereal, bread, and so on.

Preparation of samples

Samples for presentation must be from homogenous lot. Careful sampling of the food is necessary for sensory evaluation. Samples to be tested should be prepared by identical methods. All samples should be at the same temperature, optimum level and kept constant during the test. Stainless steel forks and spoons can be used for tasting the samples.

Samples are presented with 3-5 digits code markings to obscure the identity of the samples. The order of presentation should also be randomised within east test session.

Panellists

People who test the food in sensory evaluation are named "Panellists". Panellists can identify the sensory properties that will help to describe the product. The sensory qualities, particularly the flavour attributes are essential to be measured subjectively. From early times this judging has been the preserve of experts who used to evaluate tea, coffee and wine. With the development of sensory evaluation techniques on scientific lines, the experts are being replaced by panels whose sensitivity and consistency has been established by training and repeated test. The panel members analyse food products through properly planned experiments and their judgements are quantified by appropriate statistical analysis.

Selection of panel of judges: Actually, one extremely discriminating pain taking and unbaised individual would suffice for tasting. Furthur one individual may not be able to discriminate different aspects of food quality. Hence. A panel of judges may be used.

The requirement for an ideal panel member are as follows.

- He should be able to discriminate easily between samples and should be able to distinguish appreciable differences in taste and smell.
- He should have good health. If he is suffering from cold his sensitivity may be affected. A sick patient cannot judge the food correctly. He should not be habituated to chewing pan or supari.
- He should be experienced in the particular field.
- He should have high personal integrity. He should not be prejudiced. He should be able to evaluate objectively.
- Willingness to spend time for the sensory evaluation work is required.
- He should have interest in sensory analysis of samples and intellectual curiosity.
- He should have ability to concentrate and derive proper conclusion.

- He should be available and willing to submit to periodic test to get consistent results.

There are different types of panels:

1. Trained panel: The number of members in the trained panel should be small varying from 5 to 10.
2. Discriminative, communicative or semi-trained panels: Panels of 25-30 are used to find the acceptability or preference of final experimental products prior to large scale consumer trials.
3. Consumer panels: Such panels are made up of untrained people chosen at random. A group of not less than 100 is considered the minimum.

Environment

In general, the facilities for sensory analysis must meet the basic requirements. The basic requirements for

sensory analysis is:

1. A food preparation area

2. A separate panel discussion area

3. A quiet panel booth area

4. A desk or office for the panel leader

5. Supplies for preparing and serving samples

Testing time

Testing should be done at a time when the panel members are fresh. The test time is generally between 10 to 12 in the morning. Too many samples should not be given as the may produce fatigue and lead to errors in the result (not more than 4-5 samples at a time).

Utensils

Utensils used for sensory evaluation method are the vessel and cutlery if needed. The vessel or container for sample should give no additional sensory characteristic to the sample. We can use glass, but it is more expensive than plastic, and can be hazard. Clear or plain white containers are recommended to use in food sensory evaluation.

Evaluation card

The questionnaire or score card should be prepared carefully for each test. The card should be clearly typed or printed. It should be simple and use unambiguous terms and directions in the desired sequence of action as a guide to the evaluation.

No single score cards fit all experiments. Instead, the score needs to be developed for the specific experiment. All score cards should contain the date and name of the judge.

Types of tests

Different sensory tests are employed for food evaluation. The tests are grouped into four types

a. Difference tests
    - **Paired comparison test**
    - **Duo-trio test**
    - **Triangle test**
b. Rating tests
    - Ranking test
    - Single sample test
    - Two sample difference tests
    - Multiple sample difference test
    - **Hedonic rating test**
    - Numerical scoring test
    - Composite scoring test
c. Sensitivity tests
    - Sensitivity-threshold test
    - Dilution test
d. Descriptive tests

Difference tests

1. **Paired comparison test**

- The panel members receive several pairs of samples. These may be different or the. same samples in each pair. Samples are always given in code numbers.
- Different samples are given in each pair which differ in the intensity of one characteristic, e.g., sweetness, bitterness or rancidity. In each pair, the sample with more or less intense taste will have to be picked out.

2. **Duo-trio test**

- This test employs three samples, two identical and one different. The panel is first given one of the pair of identical samples as known reference sample R and then the other two successively in random order, and asked to match one of these with the first. A positive answer is required even if it is a guess. The chance probability of placing the samples in a certain order is one-half.

3. **Triangle test**

- This test employs three samples, two identical and one different, presented simultaneously to the panel. The judge is asked to determine which of the three is the

odd sample. A positive answer is required even if it is a guess. Since all three samples are unknown, the chance probability of placing the samples in a certain order is one-third. Two samples A and B can be presented in two combinations AAB and BBA and for replication in six different arrangements-AAB, ABA, BAA, BAB, ABB and BBA.

Hedonic rating test

Hedonic rating relates to pleasurable or unpleasurable experiences. The hedonic rating test is used to measure the consumer acceptability of food products. From one to four samples are served to the panellist at one session. He is asked to rate the acceptability of the product on a scale, usually of 9 points, ranging from ‘like extremely’ to ‘dislike extremely’. Scales with different ranges and other experience phrases could also be used. Semi-trained panels in smaller number are used to screen a number of products for selecting a few for consumer preference studies.

Specimen evaluation card

Paired Comparison Test

Name: ……. **Date: …….**

Product: …….

You are given one or several pairs of samples. Evaluate the two samples in the pair for……*.

Is there any difference between the two samples in the pair?

Code no. of pairs	**Yes**	**No**
……	……	……
……	……	……
……	……	……
……	……	……

Signature

Note: The less preferred sample need not be of poor quality and may still fall in the acceptable category and this should be decided by a separate test.

***Mention the specified sensory characteristic to be studied e.g., sweetness, texture, flavour or overall quality and use separate cards for each characteristic.**

Specimen evaluation card

Duo-trio Test

Name: ……. **Date: …….**

Product: …….

The first sample 'R' given is the reference sample

Taste it carefully.

From the pair of coded sample next given, judge which sample is the same as 'R'

Set No	Code no of pairs	Same as 'R'
I	……	……
II	……	……
III	……	……
IV	……	……

Signature

Specimen evaluation card

Triangle Test

Name: …… **Date: …….**

Product: ……

Two of the three samples are identical. Determine the odd sample.

Set No	**Code**	**Code**	**Code**
I	**……**	**……**	**……..**
II	**……**	**……**	**……..**
III	**……**	**……**	**……..**
IV	**……**	**……**	**……..**

Signature

Specimen evaluation card

Hedonic Rating Test

Name: …… **Date: …….**

Product: ……

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feelings about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us.

	Code	Code	Code
Like extremely	……	……	……..
Like very much	……	……	……..
Like moderately	……	……	……..
Like slightly	……	……	……..
Neither like nor dislike	……	……	……..
Dislike slightly	……	……	……..
Dislike moderately	……	……	……..
Dislike very much	……	……	……..
Dislike extremely	……	……	……..
Reason	……	……	……..

Signature

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