M.Sc. FOOD SCIENCE & NUTRITION
LAB MANUAL
3rd Semester

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PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Food Science & Nutrition Third Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non-teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

Laboratory Practice Safety Rules

1. Use safety glass when dealing with fire and chemical.
2. Should use front cover clothes during biochemistry practical.
3. Always use hand wash after dissection and any type of chemical use.
4. Carefully handle needles , forceps, microscope and any other dissecting instrument.

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FSN – 395 BIOSTATISTICS AND COMPUTER APPLICATION LAB

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d. Students t-test – a) for Independent group b) paired group
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Experiment on Computer application

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FSN – 396 **FOOD PROCESSING LAB AND FOOD INDUSTRY VISIT**

1. Preparation of cookies-different types:

A cookies is a baked or cooked food that is typically small and taste sweet. It usually contain flour, sugar and some type of oil or fat. It may include other ingredients such as oats, chocolate nuts etc. In most English speaking countries cookies are called biscuits.

Ingredients required:

i. 1 cup butter, room temperature
ii. 1 cup sugar
iii. 1 egg
iv. Few drops of vanilla essence
v. 2.5 cup flour
vi. 1 teaspoon baking powder
vii. ½ tea spoon salt

Procedure:

i. Preheat the oven at 200^0C
ii. Mix the dry ingredients by mixing the flour, salt and baking powder in a bowl.
iii. Cream the wet ingredients in a separate bowel. Place the butter, sugar, egg and vanilla in a bowel and beat until the ingredients are incorporated and the mixture is light and fluffy.
iv. Mix the dry and wet ingredient. Pour the flour mixture into the bowl at wet ingredient. Use a long handed spoon to stir the batter.
v. Drop the mixture into a baking tray; use a spoon or a small ice cream scoop. Place the baking dough in small quantities on a sheet and placed in a oven
vi. Spread sugar powder on the top of the mixture.
vii. Bake the cookies for 2-3 minutes or until a golden colour occurs at the top of the cookies.
viii. Let the cookies cool down, remove them from the oven and place them on a cooking rack.

Nutrient analysis:

Food stuff	Amount (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (Kcal)
Butter	100	-	81	-	729
Sugar	100	0.1	-	99.4	398
Flour	250	27.5	2.25	173.5	870
Egg	50	6.65	6.65	-	86.5
Baking powder	5	0.005	0.02	2.35	97
Total		34.25	89.92	275.25	2180.5

So, 100g product contains 34.25 g protein, 89.92g fat, 275.25g carbohydrate and 2180.5 kcal energy.

Cost analysis

Food stuff	Amount (g)	Cost(Rs.)
Butter	100	46
Sugar	100	4
Flour	250	16
Baking powder	5	0.93
Egg	50	6
Total cost		74.18

Nutritional significance:

Cookies differ on their taste and nutritional value based on the method of preparing. Cookies are a healthy meal one should not just imagine of sugar and butter but when cookies are prepared it is more acceptable. There are healthy costumed prepared cookies to fit our body. Several cookies are healthy with excellent nutritional benefits because they are made up of natural ingredients.

2. Preparation of chocolates:

Chocolate is usually sweet preparation of roasted cocoa seeds that is made in the form of liquid paste. Chocolate is very sensitive to temperature and humidity. Ideal storage temperature for chocolate is between 15- 17^0c. The chocolates normally supplies 540 kcal/ 100 g.

Ingredients:

- 1/3 cup of cocoa powder
- 1/3 cup of milk powder
- 1/3 cup of sugar
- 70 g of butter
- Silicone mold
- Nuts or dry fruits

Procedure:

- Put 1/3 cup of cocoa powder in a bowl
- Added 1/3 cup of milk powder
- Add 1/3 cup of sugar to make it sweet
- Mixed all the ingredients very well and then 70 g of melted butter was added to it.
- Taken silicon molds to make chocolate
- Use roasted almonds, walnuts cashew and raisins to his mixture.
- Wait for consisting of thin batter.
- Do not make the batter thick as it will be difficult while filling the mold.
- After ready the thin batter is was transferred into the mold one by one using a spoon.
- Now the roasted dry fruits can be added at the top of the mixture.
- Keep the silicon mold separately so that it can spread evenly.
- Keep the silicone mold in a refrigerator and leave it for an hour to set the chocolates.
- After one hour take out the chocolates from the molds and the chocolate is ready to serve**.**

Nutritional composition:

Food stuff	**Amount (g)**	**Protein(g)**	**Fat (g)**	**Carbohydrate (g)**	**Energy (Kcal)**
Buter	100	-	80	-	722
Cocoa powder	100	23	11	35	331
Sugar	100	-	-	99.4	398
Milk powder	100	22	18	50	450
Total	400	45	109	184.6	1901

Cost analysis:

Food stuff	Amount (g)	Rate (Rs)
Buter	100	45
Cocoa powder	100	80
Sugar	100	5
Milk powder	100	50
Total	400	80

Nutritional significance:

Chocolate comes from cacao, which is a plant with high levels of minerals and antioxidants. The higher the cocoa content, as in dark chocolate, the more benefits there are. The fatty acid profile of cocoa and dark chocolate is also excellent.

3. Assessment of some important nutraceuticals in foods:

Qualitative phytochemical screening for nutraceuticals present in foods:

Introduction:

A nutraceutical is a naturally nutrient rich or mrdically active food, such as garlic or soya beans, or may be any specific compounds of a food such as omega 3 fatty acid that can derivred from many sea fishes. The term nutraceutical combines two words- nutrient (a nourishing food component) and pharmaceutical (a medical drug). The philosophy behind nutraceuticals is to focus on prevention, according to a Greek physician said 'let food be your medicine'.

On the basis of type, the nutraceuticals are as follows-

Prebiotics, probiotics, omega 3 fatty acids, selenium, carotenoids, dietary fibre, phytochemicals and plant extracts, and others.

Requirements:

Sample (ethanol extract from green tea and spinach)

Test tubes, beaker, Chemicals

Procedure:

Extract preparation:

10 g food powder was added to 100ml ethanol and kept in a shaker incubator for ver night at room temperature.

Filtered and filtrate was collected and dried to get the crude ethanol extract (about 200mg).

Nutraceuticals assessment:

Test	Observation	Result
2 ml of distilled water was added to the plant extracts in a test tube and shake for 15 minutes.	Formation of 1 cm foam	Presence of saponins
Alkaline reagent test: 2ml of 2% NaOH solution was mixed with plant crude extract and 2 drops of diluted acid was added to that solution	Intensive yellow colour was formed Turned into colour less.	Presence of flavonoids
0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was mixed.	Blue colour observed Green black colour observed	Indicates presence of gallic tannins Indicates catecholic tannins
Libermann- Burchard test: about 20 mg of extract was dissolved in 2 ml acetic anhydride and 1 or 2 drops of concentrated H2SO4 was added slowly along the sides of the test tube.	Formation of blue green colour	Indicate the presence of phytosterols
0.5 ml of the plant extract was added with equal volume of chloroform and a few drops of concentrated H2SO4 was added.	Appearance of brown ring Appearance of bluish ring	Indicates the presence of steroids Indicate the presence of phytosterols
0.5ml of plant extract was added with 2 ml of DPPH in ethanol solution	Violet colour of DPPH turned into pale yellow colour	Indicates presence of antioxidant

Result:

Presence of nutraceuticals compounds in green tea and spinach extract

Name of nutraceutical compounds	Ethanol extract of Green tea	Ethanol extract of Spinach
	Presence/ Absence	Presence/ Absence
Saponin	++	+
Flavonoid	++	+
Tannins	+++	-
Steroids	-	+
Phenols	++	+
Phytosterol	+	++
Antioxidant	++	++

Interpretation:

According to phytochemical screening, it was observed that most of the nutraceuticals present in both green tea and spinach. Saponin, phytosterols, phenol present in high amount in green tea and spinach. Tanins is present in green tea but absent in spinach.

4. Antioxidative sensor of foods: Estimation of antioxidative compounds in foods:

Estimation of total antioxidant by DPPH radical scavenging activity

Antioxidants are substances that neutralize free radicals and their action. There are natural antioxidant enzymes in our body like superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase, glutathione reductase etc. Antioxidants acta s free radical scavengers and prevent or slow the damage done by free radicals. When a balance between ROS production and antioxidant defense is less, oxidative stress results which ultimately dysregulate the cellular function and leads to many chronic diseases.

Fruits and vegetables are known as good source of antioxidants such as retinol, ascorbic acid, alpha tocopherol, carotenoids, phenolic compounds etc.

Principle:

DPPH (2,2 - diphenyl-1-picryl hydrazyl) is a stable free radical widely used to test the ability of compounds acts as free radicals scavenger and hydrogen doner to evaluate antioxidant activity. When antioxidant react with DPPH the violet colour of DPPH is reduced to light yellow colour. The absorbance at 517nm is taken and the reduced OD value indicate the more discolouration due to presence of strong antioxidant compounds.

This test is more acceptable model for evaluating the free radical scavenging activities.

Requirements:

- Food extract or fruit juice,
- DPPH (1mm in ethanol/methanol): 1M= 1000MM, 1 Milimolar= 0.001 Mole
  Molecular weight of DPPH is 394g/mol
  1mm= 0.001 mole = 1/1000, 394/1000 = 0.394gX 100 – 394 mg.
  To make 1 mm solution of DPPH, 394 mg of DPPH is to be dissolved in 1 litre of ethanol.
- Test tubes
- 1 ml Micropipette with tips
- Colorimeter or spectrophotometer
- Conical flask

Procedure:

Preparation of standard: Different concentration of ascorbic acid was taken as a standard (1mg/ml, 2mg/ml, 5mg/ml, 10mg/ml),

- 1 milimolar solution of DPPH was prepared in ethanol
- Different concentration of lemon juice was taken in separate test tubes (Concentration – 100µl, 200µl, 500µl, 1ml) also different concentration of ascorbic acid was also taken.

- Total volume was made upto 1.5 ml by using ethanol.
- 1.5ml of DPPH solution was added to all the sample and standards.
- The mixture was shaken and allowed to stand at room temperature for 15 minutes.
- 3 ml of ethanol was used as blank and set as 0.
- The absorbance of all the test tubes was measured at 517 nm in a spectrophotometer/ colorimeter against DPPH solution (1mm) as a control.

Result:

OD of DPPH solution (Control) was 2.0. Different concentration of sample and its OD value are given below.

DPPH radical scavenging percentage = $\frac{\text{OD of DPPH solution (Control)} - \text{OD of sample}}{\text{OD of DPPH solution (Control)}} \times 100$

Table 1 DPPH radical scavenging percentage of standard ascorbic acid at different concentrations.

Concentration of standard	**OD value**	**DPPH radical scavenging percentage**
1mg/ml	1.28	36%
2mg/ml	1.05	47.5%
5mg/ml	0.75	62.5%
10mg/ml	0.25	87.5%

Table 2 DPPH radical scavenging percentage of sample at different concentrations

Concentration of sample	**OD value**	**DPPH radical scavenging percentage**
100 µl	1.69	15.5%
200 µl	1.25	37.5%
500 µl	0.88	56%
1000 µl	0.50	75%

Interpretation:

The antioxidant activity is elevated with increase of the concentration of lemon juice. Lemon juice has free radical scavenging activity. For this reason the antioxidant activity increases with increasing concentration of lemon juice. The rate of discoloration also increases die to increase in antioxidant concentration. The purple colour of DPPH become lighter and turns yellow due to DPPH radical scavenging properties of lemon juice. Standard ascorbic acid showed increase in the DPPH radical scavenging percentage as concentration of ascorbic acid increases as it is a known antioxidant.

5. Proximate analysis of foods: Ash content, Moisture content, PH etc:

Principle:

Proximate Analysis stands for a method, which determines the values of the macronutrients in food samples. These food component measurements are essential in the food industry for product development, quality control (QC) or regulatory purposes. Analyses used are rapid methods but time consuming for QC. Moisture, ash, protein, fat, carbohydrates, fibre etc, are known as "proximates" and the process of determination of these contents is known as "Proximate analysis".

a. Moisture content of foods:

Moisture assays can be one of the most important analyses performed on a food product. One of the most fundamental and important analytical procedures that can be performed on a food product is an assay for the amount of moisture. The dry matter that remains after moisture removal is commonly referred to as total solids. This analytical value is of great economic importance to a food manufacturer because water is an inexpensive filler. The following listing gives some examples in which moisture content is important to the food processor.

1. Moisture is a quality factor in the preservation of some products and affects stability in
(a) Dehydrated vegetables and fruits
(b) Dried milks
(c) Powdered eggs
(d) Dehydrated potatoes
(e) Spices and herbs
2. Moisture is used as a quality factor for
(a) Jams and jellies to prevent sugar crystallization
(b) Sugar syrups
(c) Prepared cereals – conventional, 4–8%; puffed, 7–8%
3. Reduced moisture is used for convenience in packaging or shipping of
(a) Concentrated milks
(b) Liquid cane sugar (67% solids) and liquid corn sweetener (80% solids)
(c) Dehydrated products (these are difficult to package if too high in moisture)
(d) Concentrated fruit juices
4. Moisture (or solids) content is often specified in compositional standards (i.e., Standards of Identity) (a) Cheddar cheese must be ≤39% moisture.
(b) Enriched flour must be ≤15% moisture

Estimation of Moisture content in foods:

The food material was weighed and heated under carefully specified temperature and the loss of weight is taken as a measure of the moisture content of the sample. The value obtained for

moisture depends on type of oven, temperature and length of drying. Therefore, the methods provide same time approximate rather than accurate moisture values. The rate at which moisture can be removed from the surface of a solid phase is a function water vapour pressure and of the drying temperature.
The moisture content was measured by-

$$\frac{\text{Weight of wet sample(g)} - \text{Weight of dry sample}}{\text{Weight of wet sample(g)}} \times 100$$

b. Ash content of foods:

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. Determining the ash content of a food is part of proximate analysis for nutritional evaluation and it is an important quality attribute for some food ingredients. Also, ashing is the first step in the preparation of a sample for specific elemental analysis. It is measure to estimate the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl.

Determination of ash:

For the determination of ash, clean empty crucible was placed in a muffle furnace at 60^0C for an hour, cooled in desiccator and then weight o of empty crucible was noted (W1). One gram of each of 1 sample was taken in crucible (W2). The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550 C for 2-4 h. The appearances of gray white ash o indicate complete oxidation of all organic matter in the sample. After ashing furnace was switch off. The crucible was cooled and weighed (W3). Percent ash was calculated by following formula:

$$\% \text{ of Ash} = \frac{\text{Difference in Weight of Ash} \times 100}{\text{Weight of sample}}$$

Difference in weight of Ash- W3-W1

c. Measurement of pH of foods:

The term pH is a measure of acidity of basicity of an aqueous solution. The lower value indicated acidity and higher value indicated basic. The range of p H is 0- 14. pH 7 is considered neutral. Citrous have acidic pH.

Result:

Food sample used: Red apple

Weight of sliced apple: 81.6g

1. Percentage of moisture =

$$\frac{\text{Weight of wet sample(g)} - \text{Weight of dry sample}}{\text{Weight of wet sample(g)}} \text{X } 100$$

$$= \frac{81.6-10.74}{81.6} \text{x } 100$$

= 86.84 g%

2. Percentage of ASH=

$$\frac{\text{Difference in weight of ASH}}{\text{Weight of sample (g)}} \text{x } 100$$

Difference in weight of ASH- W3- W1

W3= Foil with ash weight = 2.78g

W1= Emply foil= 0.54g

So, $\frac{2.78-0.54}{81.6} \text{x } 100$

= 2.6g%

3. pH of apple- 5.28

Interpretation:

The moisture content of fresh apple was 86.84 % which contribute the bulky weight and high-water content. This high-water content makes it ideal for fruit juice making and used as a supplement to whole fruits.

The ash content was 2.6% which is used to determine the amount and type of minerals present in foods.

Fruit juices pH level normally ranges between 4-6 and this red apple have acidic pH of 5.28.