ALAGAPPA UNIVERSITY

[Accredited with 'A+' Grade by NAAC (CGPA:3.64) in the Third Cycle
and Graded as Category–I University by MHRD-UGC]

KARAIKUDI – 630 003

DIRECTORATE OF DISTANCE EDUCATION

M.Sc. [Home Science – Nutrition and Dietetics]

365 24

LAB II - NUTRITIONAL BIOCHEMISTRY, FUNCTIONAL FOODS AND NUTRACEUTICALS AND FOOD SERVICE MANAGEMENT

II - Semester

ALAGAPPA UNIVERSITY

[Accredited with 'A+' Grade by NAAC (CGPA:3.64) in the Third Cycle
and Graded as Category–I University by MHRD-UGC]

(A State University Established by the Government of Tamil Nadu)

KARAIKUDI – 630 003

Directorate of Distance Education

M.Sc. [Home Science – Nutrition and Dietetics]

II - Semester

365 24

LAB II - NUTRITIONAL BIOCHEMISTRY, FUNCTIONAL FOODS AND NUTRACEUTICALS AND FOOD SERVICE MANAGEMENT

Author

Dr Sona Jain, *Clinical Nutritionist And Dietician, Lucknow*

"The copyright shall be vested with Alagappa University"

All rights reserved. No part of this publication which is material protected by this copyright notice may be reproduced or transmitted or utilized or stored in any form or by any means now known or hereinafter invented, electronic, digital or mechanical, including photocopying, scanning, recording or by any information storage or retrieval system, without prior written permission from the Alagappa University, Karaikudi, Tamil Nadu.

Information contained in this book has been published by VIKAS® Publishing House Pvt. Ltd. and has been obtained by its Authors from sources believed to be reliable and are correct to the best of their knowledge. However, the Alagappa University, Publisher and its Authors shall in no event be liable for any errors, omissions or damages arising out of use of this information and specifically disclaim any implied warranties or merchantability or fitness for any particular use.

Vikas® is the registered trademark of Vikas® Publishing House Pvt. Ltd.

VIKAS® PUBLISHING HOUSE PVT. LTD.
E-28, Sector-8, Noida - 201301 (UP)
Phone: 0120-4078900 • Fax: 0120-4078999
Regd. Office: 7361, Ravindra Mansion, Ram Nagar, New Delhi 110 055
• Website: www.vikaspublishing.com • Email: helpline@vikaspublishing.com

Work Order No. AU/DDE/DE1-291/Preparation and Printing of Course Materials/2018 Dated 19.11.2018 Copies - 500

LAB II - NUTRITIONAL BIOCHEMISTRY, FUNCTIONAL FOODS AND NUTRACEUTICALS AND FOOD SERVICE MANAGEMENT

SYLLABI

NUTRITIONAL BIOCHEMISTRY

1. Determination of Moisture Content in Food Sample.
2. Determination of Carbohydrates, Proteins and Fats in Food Sample.
3. Determination of Gluten Content in Wheat.
4. Estimation of Acidity in Wheat Flour.
5. Estimation of Fiber, Phosphorous and Iron Content in anyone Food.
6. Determination of Calcium Content in Milk.

FUNCTIONAL FOODS AND NUTRACEUTICALS

1. Manufacturing Aspects of Selected Nutraceuticals (Demonstration)
   (a) Lycopene,
   (b) Isoflavonoids
   (c) Prebiotics
   (d) Probiotics
   (e) Glucosamine
   (f) Phytosterols
2. Spirulina Cultivation (Industrial Visit)

FOOD SERVICE MANAGEMENT

1. Causes and Prevention of Food-Borne Illnesses in Food Service Operations.
2. The Levels of Management and the Various Production and Service Positions in a Food Operation (Field Visits)

INTRODUCTION

NOTES

Nutrition is the nourishment of an organism to support its functions, with substances called *nutrients*. In humans, nutrition more specifically refers to the consumption, absorption, utilization and excretion of essential chemical compounds found in foods and drinks that are required by the body to produce energy as well as to assist the body to grow and develop. Nutrients also help the body prevent or fight diseases more effectively. There are six major classes of nutrients which include carbohydrates, fats, proteins, vitamins, minerals and water. Nutrients cannot be created by the body and thus must be obtained through diet. Nutritional science is the study of nutrients, their function and how they are involved in health and disease. Nutritional scientists employ many of the techniques used in biochemistry, although nutritionists are more health oriented and concerned particularly with nutrients. In fact, the wealth of knowledge of nutrition is greatly attributed to biochemists.

Nutritional Biochemistry is a discipline that encompasses the knowledge of nutrients and other food components with emphasis on their range of function and influence on mammalian physiology, health, and behaviour. Fundamentally, the nutritional biochemistry is an integrative science whose foundation is derived from knowledge of other biological, chemical, and physical sciences, but it is distinguished in its application of this knowledge to understanding the interactive relationships among diet, health, and disease susceptibility. For example, nutritional biochemistry is rooted in analytical methodology that permits the purification of individual nutrients and the determination of their structures, as well as in classical biochemical approaches that identify metabolic pathways and elucidate the role of dietary components in regulating metabolism and gene expression. Nutrition research has resulted in an increased awareness world-wide that disease prevention and improved health can be accomplished by means of dietary change. This has led to the development of '*Nutraceuticals*', also called '*Functional Foods*' which can be defined as, 'Foods which provide enhancement to health or performance through the addition of ingredients that they would not otherwise contain, or the fortification of ingredients already present.' The purpose of Nutraceuticals is to maintain or improve key functional aspects of the human body, such as digestive systems, immune system, cardiovascular system, dental health, bone strength, and so on.

Foodborne illnesses are usually infectious or toxic in nature and caused by bacteria, viruses, parasites or chemical substances entering the body through contaminated food or water. Foodborne pathogens can cause severe diarrhoea or debilitating infections including meningitis. Hence, the food management is very significant for good health.

This book, *Lab. II: Nutritional Biochemistry, Functional Foods and Nutraceuticals & Food Service Management*, focuses on the various significant aspects of Nutritional Biochemistry, Functional Foods and Nutraceuticals, and Food Service Management.

NUTRITIONAL BIOCHEMISTRY

1. DETERMINATION OF MOISTURE CONTENT IN FOOD SAMPLE

Aim: To determine Water Moisture content in Food Sample.

Theory

Food moisture analysis involves the whole coverage of the food items in the world because food are comprising a considerable amount of water rather than other ingredients foods are vital components which are consumed by the people at each and every moment for the surviving in the world. Basically there are several kinds of foods are available for the consumption as raw foods, processed foods and modified foods in the market. Moisture content of the food material is important to consider the food to suitable before the consumption, because moisture content affects the physical, chemical aspects of food which relates with the freshness and stability for the storage of the food for a long period of time and the moisture content determine the actual quality of the food before consumption and to the subsequent processing in the food sector by the food produces.

Moisture is an important factor in food quality, preservation and resistance to deterioration. Determination of moisture content also is necessary to calculate the content of other food constituents on a uniform basis (i.e., dry weight basis). The dry matter that remains after moisture analysis is commonly referred to as total solids.

While moisture content of foods is not given on a nutrition label, it must be determine to calculate total carbohydrate content. Moisture content of foods can be determined by a variety of methods. Moisture content influences the taste, texture, weight, appearance and shelf life of food stuff. Even a slight deviation of from a defined standard can adversely impact the physical properties of a food material. For example, substances which are too dry could affect the consistency of the end product, conversely, excess moisture may cause food material to agglomerate or become trapped in the piping systems during production. Also the rate of microbial growth increases with total water content, possible resulting in spoiled batches that need to be disposed of.

Determination of moisture is important economically to the processor and the consumer moisture content of a food product will affect its stability and quality. Moisture is inversely proportional to the amount of dry matter in the food.

The importance of determination of moisture content in food stuff includes the following.

Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of different reasons.

Legal and Labelling Requirements

There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.

Economic: The cost of many foods depends on the amount of water they contain. Water is an inexpensive ingredient and manufactures often try to incorporate as much as possible in a food, without exceeding some maximum legal requirements.

Microbial Stability: The prosperity of micro-organisms to grow in foods depend on their water content. For this reason many foods are dried below some critical moisture content.

Food Quality: The texture, taste, appearances and stability of foods depends on the amount of water they contain.

It is therefore important for food scientists to be able to reliably measure moisture contents. A number of analytical techniques have been developed for this purpose, which vary in their accuracy, cost, speed, sensitivity, specificity, ease of operation, etc. The choice of an analytical procedure for a particular application depends in the nature of the food being analysed and the reason the information is needed.

The Types of Water in a Food Stuff

- **Bulk Water:** Bulk water is free from any other constituent so that each water molecule is surrounded only by other water molecules. It therefore, has physico-chemical properties that are the same as those of pure-water, e.g., melting point, boiling point, density, compressibility, heat of vaporization electron magnetic absorption spectra.
- **Capillary or Trapped Water:** Capillary water is held in narrow channels capillary forces. Trapped water is held within spaces within a food that are surrounded by a physical barrier that prevent the water molecules from easily escaping, e.g., an emulsion droplet or a biological cell. The majority of this type of water is involved in normal water, water bonding and so it has physico-chemical properties similar to that of bulk water.
- **Physically Bonded Water:** A significant fraction of the water molecule in many foods is not completely surrounded by other water molecules, but is in molecular contact with other food constituent, e.g., protein, carbohydrate or minerals. The bonds between water molecules and these constituents are often significantly different from normal water, water bond and so this type of water has different physico-chemical properties than bulk water, e.g., melting point, boiling point, density, compressibility, heat of vaporization, electro-magnetic absorption spectra.
- **Chemically Bonded Water:** Some of the water molecule present in a food may be chemically bonded to other molecules as water of crystallization is as hydrate, e.g., $NaSO_4.10H_2O$. These bonds are much stronger than

the normal water, water bond and therefore, chemically bonded water has very different physico-chemical properties as compared to bulk water, such as lower melting point, higher boiling point.

The method used for the determination will depend on the food that is being analysed. The methods applied can be classified into two groups: direct or indirect methods. The existing official (reference) methods for food analysis are all direct methods and include Oven Drying, Vacuum Oven Drying, Azeotropic Distillation and Karl Fischer Titration.

In principle, the moisture content of a food can therefore be determined accurately by measuring the number or mass of water molecules present in a known mass of sample. It is not possible to directly measure the number of water molecules present in a sample because of the huge number of molecules involved. A number of analytical techniques commonly used to determine the moisture content of foods are based on determinations of the mass of water present in a known mass of sample.

But there are a number of practical problems associated with these techniques that make highly accurate determinations of moisture content difficult or that limit their use for certain applications. For these reasons, a number of other analytical methods have been developed to measure the moisture content of foods that do not rely on direct measurement of the mass of water in a food. Instead, these techniques are based on the fact that the water in a food can be distinguished from the other components in some measurable way.

Methods of Determining Moisture Content in Foods

Oven Drying Methods: With oven drying, the sample is heated under specified conditions, and the loss of weight is used to calculate the moisture content of the sample.

- **Forced Oven Draft:** Sample is rapidly weighed into a moisture pan and placed in the oven for an arbitrarily selected time if no standard method exists. Drying time periods for this method are 0.75–24 hours, depending on the food sample.
- **Vacuum Oven:** Drying under reduced pressure (25-100 mm Hg) allows a more complete removal of water and volatiles without decomposition within 3-6 hours drying time.
- **Microwave Oven:** A precise and rapid technique that allows some segments of the food industry to make in process adjustments of moisture content before final packaging. In vacuum microwaves, a drying time of 10 minutes can yield results equivalent to those of five hours in a standard vacuum oven.

Advantages of the oven drying and vacuum oven drying methods are their easy handling and possibility for simultaneous determinations. Their limitations are based on the dependence on relative humidity conditions during weighing and

desiccating and on the possible loss of other volatiles being eventually present in food samples.

In addition, the oven drying method applying temperatures of about 100°C can lead to decomposition of the food (e.g., Maillard Reaction) and the release of water.

Infrared Drying

Employs penetration of heat into the sample being dried, as compared to heat conductivity and convection as with conventional ovens. Required drying time can be as little as 10-25 minutes.

Distillation Methods

Distillation techniques involve co-distilling the moisture in a food sample with a high boiling point solvent that is immiscible in water, collecting the mixture that distills off, and then measuring the volume of water. Includes direct and reflux distillation, e.g., Dean and Stark Distillation Method (Zoetrope Distillation Method).

The Azeotropic Distillation method is very little applied for food analysis. The advantages include the relatively short analysis times and the relatively easy handling. However, the accuracy of the method is limited due to reading problems, not sufficient glassware cleaning and the possibility that other volatiles can distil over.

Chemical Methods – Karl Fischer Titration

This technique is particularly appropriate to food products that show erratic results when heated or submitted to a vacuum. It is the method of choice for low-moisture foods, such as dried fruits and vegetables, candles, chocolate, roasted coffee, oils and fats, and low-moisture foods high in sugar or protein.

Physical Methods

A number of analytical methods have been developed to determine the moisture content of foods that are based on the fact that water has appreciably different bulk physical characteristics that the food matrix, e.g., density, electrical conductivity or refractive index. These methods are usually only suitable for analysis of foods in which the composition of the food matrix does not change significantly, but the ratio of Water-to-Food Matrix changes. For example, the water content of Oil-In-Water Emulsions can be determined by measuring their density or electrical conductivity because the density and electrical conductivity of water are significantly higher than those of oil.

If the composition of the food matrix changes as well as the water content, then it may not be possible to accurately determine the moisture content of the food because more than one food composition may give the same value for the physical property being measured. In these cases, it may be possible to use a combination of two or more physical methods to determine the composition of the

food, e.g., density measurements in combination with electrical conductivity measurements.

Electric (Dielectric or Conductivity) - Moisture content is determined by measuring the change in capacitance or resistance to an electric current passed through a sample.

Hydrometry Used to determine moisture/solid content of beverages and sugar solutions. Measuring the specific gravity or density of the sample via one of the following instruments:

- **Pycnometer:** Used to compare the weights of equal volumes of a liquid and water. Yields density of the liquid compared to water.
- **Hydrometer:** A standard weight on the end of a spindle which displaces a weight of liquid equal to its own weight. In a low-density liquid, weight will sink to a greater depth.
- **Westphal Balance:** Functions on the principle that the plummet on the balance will be buoyed by the weight of liquid equal to the volume displaced.

Refractometry: Measures moisture content of oils and syrups as a function of the degree of refraction of a light beam as it passes through the sample.

Infrared Analysis: Measures the energy that is reflected or transmitted by the sample when exposed to infrared light.

Freezing Point: Measures the solutes present by determining the freezing point of the sample. Used principally to measure for added water content.

1. Oven Drying Method for the Thermally Stable Products

Principle

$$Moisture\% = \frac{weight\ loss \times 100}{weight\ of\ the\ sample}$$

In oven drying methods, the sample is heated under specified conditions, and the loss of weight is used to calculate the moisture content of the sample. The amount of moisture determined is highly dependent on the,

1. Type of Oven Used
2. Conditions within the Oven
3. Time and Temperature of Drying

Forced Draft Oven: The sample is rapidly weighed into a pre-dried moisture pan covered and placed in the oven for an arbitrarily selected time, if non-standardized method exists. Drying time is about 0.75–24 hours. Some liquid samples are dried initially on a steam bath at 100°C to minimize spattering. In these cases, drying times are shortened to 0.75–3 hours. Samples high in carbohydrates should not be dried in a forced draft oven but rather in a vacuum

oven at a temperature no higher than 70°C. The user of this method must be aware of sample transformation, such as,

- Browning which suggests moisture loss of the wrong form.
- Lipid oxidation and a resulting sample weight gain can occur at high temperatures in a forced draft oven.

Vacuum Oven: By drying under reduced pressure (25-100 mm Hg), one is able to obtain a more complete removal of water and volatiles without decomposition within a 3–6 hours drying time. The following are important points in the use of a vacuum drying oven:

1. Temperature used depends on the product, such as 70°C for fruits and other high sugar products. Even with reduced temperature, there can be some decomposition.

2. If the product to be assayed has a high concentration of volatiles, you should consider the use of a correction factor to compensate for the loss.
3. Analysts should remember that in a vacuum heat is not conducted well. Thus pans must be placed directly on the metal shelves to conduct heat.
4. Evaporation is an endothermic process; thus a pronounced cooling is observed. Because of the cooling effect of evaporation, when several samples are placed in an oven of this type, you will note that the temperature will drop. Do not attempt to compensate for the cooling effect by increasing the temperature, otherwise samples during the last stages of drying will be overheated.

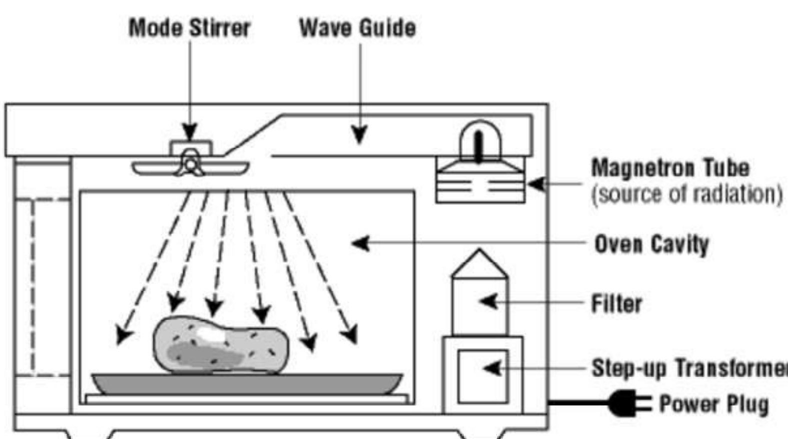
5. The drying time is a function of the total moisture present, nature of the food, surface area per unit weight of sample, whether sand is used as a dispersant and the relative concentration of sugars and other substances capable of retaining moisture or decomposing.

Forced Draft Ovens: These have the least temperature differential across the interior of all ovens, usually not greater than 1°C. Air is circulated by a fan that forces air movement throughout the oven cavity. Forced draft ovens with air distribution manifolds appear to have added benefit where air movement is horizontal across helving. Thus, no matter whether the oven shelves are filled completely with moisture pans or only half filled, the result would be the same for a particular sample.

Two features of some vacuum ovens contribute to a wider temperature spread across the oven. One feature is a glass panel in the door. Although from an educational point of view, it may be fascinating too observe some samples in the drying mode; the glass is a heat sink. The second feature is the way by which air is bled into the oven. If the air inlet and discharge are on opposite sides, conduct of air is virtually straight across the oven.

Microwave Oven: This is rapid technique that allowed some segments of the food industry to make in-process adjustment of the moisture content in food products before final packaging. Power settings are dependent upon the type of sample and the recommendations of the manufacturer of the microwave moisture analyser. Next, the internal balance is trade with two sample pads on the balance. As rapidly as possible, a sample is placed between the two pads and then pads are centered on the pedestal, and weighed against the tare weight. Time for the drying operation is set by the operator and 'Start' is activated. The following are some considerations when using a microwave analyser for moisture determination:

1. The sample must be of a uniform, appropriate size to provide for complete drying under the conditions specified.
2. The sample must be centrally located and evenly distributed, so some portions are not burned and other areas are under processed.



3. The amount of time used to place an appropriate sample weight between the pads must be minimized to prevent moisture loss or gain before weight determination. Any oven method used to evaporate moisture has as its foundation the fact that the boiling point of water is 100°C. Free water is the easiest of the three forms of water to remove. Moisture removal is sometimes best achieved in a two-stage process. Liquid products (e.g., juices, milk) are commonly pre-dried over a steam bath before drying in an oven. Products, such as bread and field-dried grain are often air dried, then ground and oven dried, with the moisture content calculated from moisture loss at both air and oven drying steps. Particle size, particle size distribution, sample sizes, and surface area during influence the rate and efficiency of moisture removal.

Moisture loss from a sample during analysis is a function of time and temperature.

Decomposition happens when time is extended too much or temperature is too high. Thus, most methods for food moisture analysis involve a compromise between time and a particular temperature at which limited decomposition might be a factor. For example, carbohydrates decompose at 100°C according to the following reaction:

$$C_6H_{12}O_6 \rightarrow 6C + 6H_2O$$

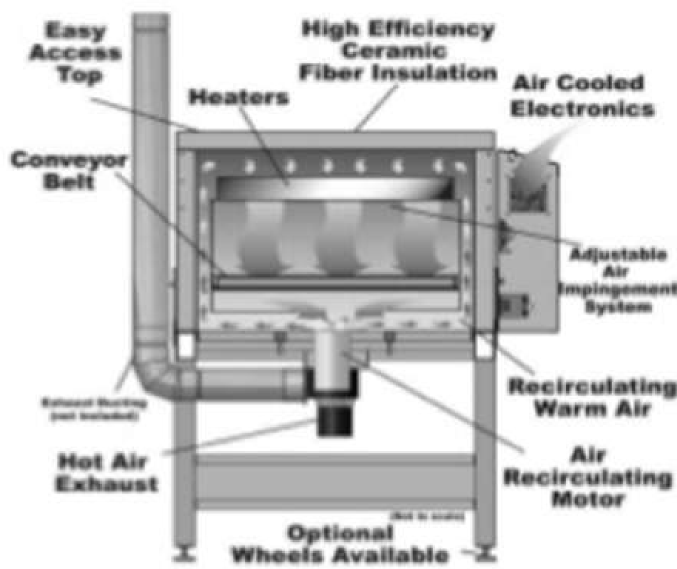
The moisture generated in carbohydrate decomposition is not the moisture that we want to measure. Certain other chemical reactions (e.g., Sucrose Hydrolysis) can result in utilization of moisture, which would reduce the moisture for measurement. A less serious problem is the loss of volatile constituents, such as acetic, propionic, and butyric acids; and alcohols, esters, and aldehydes among flavour compounds.

While weight changes in oven drying methods are assumed to be due to moisture loss, weight gains also can occur due to oxidation of unsaturated fatty acids and certain other compounds. Consider the temperature variation in three types of ovens: Convection (Atmospheric), Forced Draft, and Vacuum.

The greatest temperature variation exists in convection oven. This is because hot air slowly circulates without the aid of a fan. Air movement is obstructed further by pans placed in the oven. When the oven door is closed, the rate of temperature recovery is generally slow. This is dependent also upon the load placed in the oven and upon the ambient temperature.

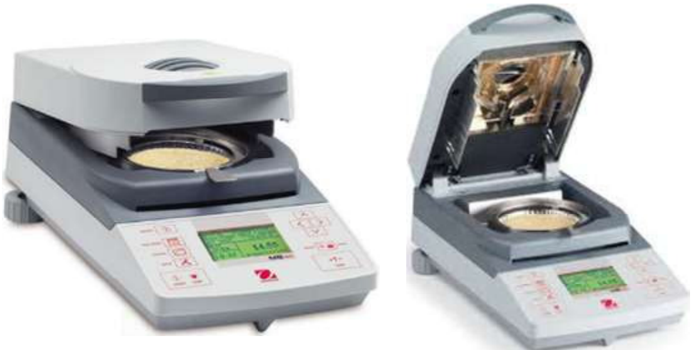
Infrared Drying

Infrared drying involves penetration of heat into the sample being dried, as compared with heat conductivity and convection with conventional ovens. Such heat penetration to evaporate moisture from the sample can significantly shorten the required drying time to 10-25 minutes. The infrared lamp used to supply heat to the sample results in a filament temperature of 2000-2500 K.



Rapid Moisture Analyser

Many rapid moisture/solids analysers are available to the food industry. In addition to those based on infrared and microwave drying as described previously, compact instruments that depend on high heat are available, such as analyzsers that detect moisture levels from 50 ppm to 100% using sample weights of 150 mg to 40 g.



Materials Required

Moisture Oven, Moisture dishes, Motar and Pestle, Oven maintained at 105°C, Weight Balance, Carrot

Procedure

1. Firstly three moisture dishes were washed with water and dried thoroughly. Weight of each dishes were taken by using weight balance.
2. Then carrot sample was peeled and cut into small pieces and put into moisture dishes until the weight to nearest about 5 g of carrot sample.
3. These three samples were covered with lid and placed in a moisture oven for 3 hours until it become to a constant weight.

4. After that dishes were transferred to a desiccator and measured the weight of each samples as soon as dishes were cool.
5. Weight losses were calculated in each samples and get the average moisture content of carrot sample.

Observations

Analysing the moisture content of carrot sample.

Moisture % of carrot sample from moisture balance = 87.06%

Results from Oven Drying Method

Dish No.	Weight of Empty Dish	Weight of Initial Sample	Weight of Final Sample	Weight Loss
1.	45.48g	5.03g	0.494g	4.536g
2.	12.74g	5.04g	0.548g	4.492g
3.	22.05g	5.09g	0.534g	4.556g

Calculations

$$\text{Moisture Percentage of Carrot Sample} = \frac{\text{Weight loss} \times 100}{\text{Weight of the sample}}$$

$$\text{Moisture Percentage of Sample 1} = \frac{4.536g \times 100}{5.03g}$$

$$= 90.18$$

$$\text{Moisture Percentage of Sample 2} = \frac{4.492g \times 100}{5.04g}$$

$$= 89.13\%$$

$$\text{Moisture Percentage of Sample 3} = \frac{4.556g \times 100}{5.09g}$$

$$= 89.51\ \%$$

$$\text{Average Moisture Content of Carrot} = \frac{90.18 + 89.13 + 89.51\%}{3}$$

$$= 89.61\%$$

2. Determination of the Moisture Content of the Products with Volatile Compounds.

Theory

The terms 'Moisture Content' and 'Water Content' have been used interchangeably to designate the quantity of water contained in food. The total water content of food involves the concepts of 'Free' and 'Bound' water, equilibrium moisture content, moisture adsorption, moisture desorption, etc.

The most important term is bound water. It is the bound water and not frees water on which the ultimate accuracy of a method for moisture content measurement is related. Bound water was defined by many researchers as the water which remains in an unchanged from when the food is subjected to a particular treatment, e.g., water which does not freeze at low temperatures or which is not available as solvent water. However, all water, other than surface water should be regarded as bound to a greater or lesser extent. Therefore, a corresponding energy input is necessary to remove such bound water from a food when moisture is being determined.

The different methods are available for moisture analyses in food. Oven Dry Method and Reflux Method (Dean and Stark Method) were used for the proximate analysis of moisture in given food stuffs.

Generally thermally stable foods are more suitable for oven drying methods since more than 10% sugary food of fatty foods can be decompose by the heat generated by the oven and it can give higher reading for the moisture content. (False Positive Answer).

According to the experiment observation given by the dry oven method carrot contains 89.61% of moisture and the reading given by the moisture balance was 87.06%, dry oven method answer was higher than moisture balance answer since oven drying method can be caused the decomposition of other compounds, such as sugar, etc., other than evaporation of moisture. Furthermore, heating causes calculation of all substances that evaporate or form a volatile azeotropes below 100°C. They include many alcohols, flavoring agents, or acetic acid.

The important practical considerations in this experiment are;

- The dried sample should be cooled in a dedicator before getting the final weight. Thus it will not give a chance to absorb atmospheric moist during cooling. Since dried foods have high possibility to absorb water from atmosphere. Cooling in to room temp is important since the differences in temperature will create convection currents around the balance pan, which will severely disrupt method accuracy.
- To get a more accurate result this experiment should be carried out for 3 samples and the average of moisture must be calculated (the method of doing it has mentioned under calculation).

The oven drying method was a time consuming method (at least it will take 4 hours to give constant weight) to detect moist and when compare to moisture balance. It will give the final reading within few minutes (15 - 20 minutes). Hence this newest method is more popular in industrial usage and it is considered as more accurate method when compare to oven dry methods. Another negative point of this method was case hardening and charring of the food due to high heat. Applying sand wash method can be used to prevent case hardening in selected food items like milk and ice cream. By this method can increase the evaporation efficiency due to more space area and it dispersed the fat globules well.

Dean and Stark Distillation Method or Reflux Distillation Method

Reflux is a distillation technique involving the condensation of vapors and the return of this condensate to the system from which it originated. It is used in industrial and laboratory distillations.

Principle

Distillation methods are practically useful for the foods of low moisture content and product containing volatile oils, such as herbs and spices since the oils remain dissolved in the organic solvent.

Distillation methods are based on direct measurement of the amount of water removed from a food sample by evaporation. In contrast, evaporation methods are based on indirect measurement of the amount of water removed from a food sample by evaporation. Basically, distillation methods involve heating a weighed food sample in the presence of an organic solvent that is immiscible with water. The water in the sample evaporates and is collected in a graduated glass tube where its volume is determined.

Distillation methods are best illustrated by examining a specific example, namely the Dean and Stark method. It also known as entertainment method. A known weight of food is placed in a flask with an organic solvent, such as xylene or toluene. The organic solvent must be,

- Insoluble with Water.
- Have a higher boiling point than water.
- Be less dense than water.
- Be safe to use.

The flask containing the sample and the organic solvent is attached to a condenser by a side arm and the mixture is heated. The water in the sample evaporates and moves up into the condenser where it is cooled and converted back into liquid water, which then trickles into the graduated tube. When no more water is collected in the graduated tube, distillation is stopped and the volume of water is read from the tube.

There are three potential sources of error with distillation should be eliminated if observed:

1. Formation of emulsions that will not break. Usually this can be controlled by allowing the apparatus to cool after distillation is completed and before reading the amount of moisture in the trap.
2. Clinging of water droplets to dirty apparatus. Clean glassware is essential, but water seems to cling even with the best cleaning effort. A burette brush, with the handle end flattened so it will pass down the condenser, is needed to dislodge moisture droplets.

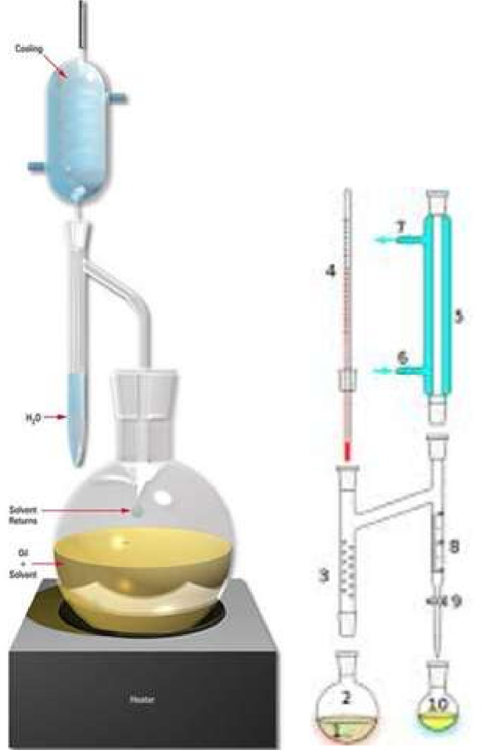
3. Decomposition of the sample with production of water. This is principally due to carbohydrate decomposition to generate water ($C_6H_{12}O_6 \rightarrow 6H_2O + 6C$). If this is measurable problem, discontinue method use and find an alternative procedure.

Advantages

- Suitable for application to foods with low moisture contents.
- Suitable for application to foods containing volatile oils, such as herbs or spices, since the oils remain dissolved in the organic solvent, and therefore do not interfere with the measurement of the water.
- Equipment is relatively cheap, easy to setup and operate.
- Distillation methods have been officially sanctioned for a number of food applications.
- Minimize the oxidation and decomposition, while reduce chemical reaction by heat.

Disadvantage

- Destructive.
- Relatively time consuming.
- Involves the use of flammable solvents.
- Not applicable to some types of foods.



Dean-Stark apparatus set up

1: Stirrer bar/anti-bumping granules
2: Still pot
3: Fractionating column
4: Thermometer/Boiling point temperature
5: Condenser
6: Cooling water in
7: Cooling water out
8: Burette
9: Tap
10: Collection vessel

Practical Considerations

There are a number of practical factors that can lead to erroneous results:

1. Emulsions can sometimes form between the water and the solvent which are difficult to separate.
2. Water droplets can adhere to the inside of the glassware.
3. Decomposition of thermally labile samples can occur at the elevated temperatures used.

Materials Required

Dean and Stark Distillation Unit, Drying Oven, Measuring cylinder 100 ml, Beaker 250 ml, Pipette 2 ml, Long Glass Rod, Toluene

Procedure

1. All the glassware were thoroughly cleaned and dried by heating an oven.
2. A 50 ml of toluene and pumice stones were transferred into the boiling flask.
3. The calibrated arm was fixed on the flask and boiled until the graduated tubes get a constant volume of water.
4. A volume of 2 ml of water was added to the flask and boiled until a constant volume is obtained.
5. An accurately weighed sample (about 2 g) was added and was boiled until the graduated tube indicates a constant volume for the extracted water layer. This is equal to the same value of water in grams.

Observations

Step	Volume of Collected Water
After adding 50 ml Toluene	1.8 ml
After adding 2 ml Water - For calibrating	3.7 ml
After adding Clove Powder	3.9 ml

Sample	Weight of Sample	Volume of Collected Water	Moisture % from Moisture Balance
Cinnamon	2 g		7.8%
Clove	2 g	0.2 cm^3	22.17%

Calculation

Error of the Apparatus = 2.0-(3.7-1.8) cm3

= 0.1 cm^3

Error Percentage = (0.1/2)×100 = 5%

Moisture Percentage

$$= \frac{\text{Volume of water collected in the graduated tube + error} \times 100}{\text{Weight of the sample}}$$

$$= \frac{0.2 + (0.2 \times 5\%) * 100}{2}$$

= 10.5%

According to the observations and the calculation the moisture % in cloves was 10.5%. Generally it is moist content should in between 8% to 10%. Therefore the experimental result can be considered as an accurate finding. Though moisture balance reading was higher than normal value. It gave 22.17% moisture %. The weight loss was the measurement used moisture balance method. Hence it gave higher result because of the evaporation of volatiles along with the moisture evaporation. So it had been reduced the weight more than the expected from that method.

In this practical, the moisture in the cinnamon sample did not evaporate successfully. So the moisture level could not be obtained by the distillation method. Moisture balance reading for cinnamon was 7.8%.

The important practical considerations of the distillation method were:

- The apparatus must be in dry, very clean and it should not trapped the water droplets inside the wall. Therefore to remove trapped water it should boiled with toluene. To get a significant reading known amount of water can be added to toluene, since the Dean and Stark apparatus may not have a scale from its bottom. But practically removing all the inside trapped water was impossible. Therefore there was an error which we could not detect.
- The calibration of the apparatus is important before the doing the experiment. By this step can find the error of this apparatus which occur normally. According to the calculation the error was 5%. This should be added to the amount of water obtained at last (3.9-3.7 = 0.2 cm^3). This error occurred due to improper condensation of water.

Practical Considerations

1. **Sample Dimensions:** The rate and extent of moisture removal depends on the size and shape of the sample, and how finely it is ground. The greater

the surface area of material exposed to the environment, the faster the rate of moisture removal.

2. **Clumping and Surface Crust Formation:** Some samples tend to clump together or form a semi-permeable surface crust during the drying procedure. This can lead to erroneous and irreproducible results because the loss of moisture is restricted by the clumps or crust. For this reason samples are often mixed with dried sand to prevent clumping and surface crust formation.
3. **Elevation of Boiling Point:** Under normal laboratory conditions pure water boils at 100°C. Nevertheless, if solutes are present in a sample the boiling point of water is elevated. This is because the partial vapour pressure of water is decreased and therefore a higher temperature has to be reached before the vapour pressure of the system equals the atmospheric pressure. Consequently, the rate of moisture loss from the sample is slower than expected. The boiling point of water containing solutes (T_b) is given by the expression, $T_b = T_0 + 0.51$ m, where T_0 is the boiling point of pure water and m is the molality of solute in solution (mol/kg of solvent).
4. **Water Type:** The ease at which water is removed from a food by evaporation depends on its interaction with the other components present. Free water is most easily removed from foods by evaporation, whereas more severe conditions are needed to remove chemically or physically bound water. Nevertheless, these more extreme conditions can cause problems due to degradation of other ingredients which interfere with the analysis.
5. **Decomposition of Other Food Components:** If the temperature of drying is too high, or the drying is carried out for too long, there may be decomposition of some of the heat-sensitive components in the food. This will cause a change in the mass of the food matrix and lead to errors in the moisture content determination. It is therefore normally necessary to use a compromise time and temperature, which are sufficient to remove most of the moisture, but not too long to cause significant thermal decomposition of the food matrix. One example of decomposition that interferes with moisture content determinations is that of carbohydrates.

   $$C_6H_{12}O_6 \xrightarrow{\text{Heat}} 6C + 6$$

   The water that is released by this reaction is not the water we are trying to measure and would lead to an over estimation of the true moisture content. On the other hand, a number of chemical reactions that occur at elevated temperatures lead to water absorption, e.g., Sucrose Hydrolysis (Sucrose + $H_2O \xrightarrow{\text{Heat}}$ Fructose + Glucose), and therefore lead to an underestimation of the true moisture content. Foods that are particularly susceptible to thermal decomposition should be analysed using alternative methods, e.g., chemical or physical.

6. **Volatilization of Other Food Components:** It is often assumed that the weight loss of a food upon heating is entirely due to evaporation of the water. In practice, foods often contain other volatile constituents that can also be lost during heating, e.g., flavours or odours. For most foods, these volatiles only make up a very small proportion and can therefore be ignored. For foods that do contain significant amounts of volatile components (e.g., spices and herbs). It is necessary to use alternative methods to determine their moisture content, e.g., distillation, chemical or physical methods.
7. **High Moisture Samples:** Food samples that have high moisture contents are usually dried in two stages to prevent 'Spattering' of the sample, and accumulation of moisture in the oven. Spattering is the process whereby some of the water jumps out of the food sample during drying, carrying other food constituents with it. For example, most of the moisture in milk is removed by heating on a steam bath prior to completing the drying in an oven.
8. **Temperature and Power Level Variations:** Most evaporation methods stipulate a definite temperature or power level to dry the sample so as to standardize the procedure and obtain reproducible results. In practice, there are often significant variations in temperatures or power levels within an evaporation instrument, and so the efficiency of the drying procedure depends on the precise location of the sample within the instrument. It is therefore important to carefully design and operate analytical instruments so as to minimize these temperature or power level variations.
9. **Sample Pans:** It is important to use appropriate pans to contain samples, and to handle them correctly, when carrying out a moisture content analysis. Typically aluminum pans are used because they are relatively cheap and have a high thermal conductivity. These pans usually have lids to prevent spattering of the sample, which would lead to weight loss and therefore erroneous results. Pans should be handled with tongs because finger prints can contribute to the mass of a sample. Pans should be dried in an oven and stored in desiccators prior to use to ensure that no residual moisture is attached to them.

2. DETERMINATION OF CARBOHYDRATE, PROTEINS AND FATS IN FOOD SAMPLE

Aim: To test foods for the presence of Carbohydrates, Lipids and Proteins in Food Sample.

Theory

Carbohydrates, proteins and lipids are essential components of a healthy diet. Scientists have designed tests to determine the presence of these nutrients in food. Such information may help to maintain a balance intake of the macromolecules. In this lab you will use chemical tests to verify the presence of these nutrients in

known samples. You will then use these tests to determine the presence of carbohydrates (simple sugars and starches), lipids (fats and oils) and protein in food samples that are provided.

1. Analytical Methods for Proteins in Foods

What are Proteins?

Proteins are large biological molecules made up of large number of amino acid units, Amino acids are molecules consisting of both the amino (–NH_2) group and carboxylic group (–COOH). In proteins, the amino acid units are linked together by specific linkages called peptide linkages. Because of the complex nature of protein, our body takes a longer time to break down protein molecules. Compared to carbohydrates, proteins are a much slower and longer-lasting source of energy. Most proteins are soluble in acidic or alkaline solutions, but insoluble in water.

Proteins give colour reactions due to one or more radicals or groups present in the complex protein molecules. All proteins do not give all colour reactions because all of them do not contain the same amino acid units, This property of protein can be used for the detection of protein in a given sample.

Biuret test is a method used for the detection of peptide bond in a protein molecule. In the Biuret test, the nitrogen atoms in the peptide chain react with copper Ions in the reagent to form a violet coloured complex.

$$+ \ Cu^{2+} + OH^- \rightleftharpoons$$

Biuret Complex
Pink or Violet

Xanthoproteic test is used for the identification of protein containing aromatic amino acid units. By heating with nitric acid, the benzene ring in the amino acid unit is nitrated and forms a yellow coloured nitro-compound which turns to orange colour with alkali.

For many years, the protein content of foods has been determined on the basis of total nitrogen content, while the Kjeldahl (or similar) method has been almost universally applied to determine nitrogen content (AOAC, 2000). Nitrogen content is then multiplied by a factor to arrive at protein content. This approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen, and that nearly all of the nitrogen in the diet is present as amino acids in proteins. On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N × 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content.

This use of a single factor, 6.25, is confounded by two considerations. First, not all nitrogen in foods is found in proteins: it is also contained in variable quantities of other compounds, such as free amino acids, nucleotides, creatine and choline, where it is referred to as Non-Protein Nitrogen (NPN). Only a small part of NPN is available for the synthesis of (non-essential) amino acids. Second, the nitrogen content of specific amino acids (as a percentage of weight) varies according to the molecular weight of the amino acid and the number of nitrogen atoms it contains (from one to four, depending on the amino acid in question). Based on these facts, and the different amino acid compositions of various proteins, the nitrogen content of proteins actually varies from about 13 to 19 percent. This would equate to nitrogen conversion factors ranging from 5.26 (1/0.19) to 7.69 (1/0.13).

In response to these considerations, Jones (1941) suggested that N x 6.25 be abandoned and replaced by N x a factor specific for the food in question. These specific factors, now referred to as 'Jones Factors', have been widely adopted. Jones factors for the most commonly eaten foods range from 5.18 (nuts, seeds) to 6.38 (milk). It turns out, however, that most foods with a high proportion of nitrogen as NPN contain relatively small amounts of total N (Merrill and Watt, 1955; and 1973). As a result, the range of Jones factors for major sources of protein in the diet is narrower. Jones factors for animal proteins, such as meat, milk and eggs are between 6.25 and 6.38; those for the vegetable proteins that supply substantial quantities of protein in cereal-/legume-based diets are generally in the range of 5.7 to 6.25. Use of the high-end factor (6.38) relative to 6.25 increases apparent protein content by 2 percent. Use of a specific factor of 5.7 (Sosulski and Imafidon, 1990) rather than the general factor of 6.25 decreases the apparent protein content by 9 percent for specific foods. In practical terms, the range of differences between the general factor of 6.25 and Jones factors is narrower than it at first appears (about 1 percent), especially for mixed diets. Table 1 gives examples of the Jones factors for a selection of foods.

Because proteins are made up of chains of amino acids joined by peptide bonds, they can be hydrolysed to their component amino acids, which can then be measured by ion-exchange, gas-liquid or high-performance liquid chromatography. The sum of the amino acids then represents the protein content (by weight) of the food. This is sometimes referred to as a 'True Protein'. The advantage of this approach is that it requires no assumptions about, or knowledge of, either the NPN content of the food or the relative proportions of specific amino acids - thus removing the two problems with the use of total N x a conversion factor. Its disadvantage is that it requires more sophisticated equipment than the Kjeldahl method, and thus may be beyond the capacity of many laboratories, especially those that carry out only intermittent analyses. In addition, experience with the method is important; some amino acids (e.g., the sulphur-containing amino acids and tryptophan) are more difficult to determine than others. Despite the complexities of amino acid analysis, in general there has been reasonably good agreement among laboratories and methods (King-Brink and Sebranek, 1993).

NOTES

Table 1 Specific (Jones) Factors for the Conversion of Nitrogen Content to Protein Content (Selected Foods)

Food	Factor
Animal Origin	
Eggs	6.25
Meat	6.25
Milk	6.38
Vegetable Origin	
Barley	5.83
Corn (Maize)	6.25
Millets	5.83
Oats	5.83
Rice	5.95
Rye	5.83
Sorghums	6.25
Wheat: Whole Kernel	5.83
Bran	6.31
Endosperm	5.70
Beans: Castor	5.30
Jack, Lima, Navy, Mung	6.25
Soybean	5.71
Velvet Beans	6.25
Peanuts	5.46

Source: Adapted and modified from Merrill and Watt (1973).

Practical Considerations

1. It is recommended that protein in foods be measured as the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water) plus free amino acids, whenever possible. This recommendation is made with the knowledge that there is no official Association Of Analytical Communities (AOAC) method for amino acid determination in foods. Clearly, a standardized method, support for collaborative research and scientific consensus are needed in order to bring this about.
2. Related to the previous recommendation, food composition tables should reflect protein by sum of amino acids, whenever possible. Increasingly, amino acid determinations can be expected to become more widely available owing to greater capabilities within government laboratories and larger businesses in developed countries, and to the availability of external contract laboratories that are able to carry out amino acid analysis of foods at a reasonable cost for developing countries and smaller businesses.

3. To facilitate the broader use of amino acid-based values for protein by developing countries and small businesses that may lack resources, FAO and other agencies are urged to support food analysis and to disseminate updated food tables whose values for protein are based on amino acid analyses.
4. When data on amino acids analyses are not available, determination of protein based on total N content by Kjeldahl (AOAC, 2000) or similar method x a factor is considered acceptable.
5. A specific Jones factor for nitrogen content of the food being analysed should be used to convert nitrogen to protein when the specific factor is known. When the specific factor is not known, N x the general factor 6.25 should be used. Use of the general factor for individual foods that are major sources of protein in the diet introduces an error in protein content that is relative to the specific factors and ranges from -2 percent to +9 percent. Because protein contributes an average of about 15 percent of energy in most diets, the use of N × 6.25 should introduce errors of no more than about 1 percent in estimations of energy content from protein in most diets ([-2 to + 9 percent] × 15).
6. It is recommended that only amino acid analysis be used to determine protein in the following:
   - Foods used as the sole source of nourishment, such as infant formula.
   - Foods/formulas designed specifically for special dietary conditions.
   - Novel foods.

Test for Proteins: Using a Known Protein

1. Place 2 ml of uncooked egg white in labelled test tube.
2. In another test tube put 2ml of water.
3. Add 2ml of fresh Biuret's solution to each test tube, and shake the test tubes gently.
4. Record the colors of the test and the control in the table.

Positive Test - Test Biuret solution changes from blue to a pink or purple violet colour.

2. Analytical Methods for Fats in Food

There is perhaps more agreement on standardized methods of analysis for fat than for protein and carbohydrate. Most fat in the diet is in the form of triglyceride, three fatty acids esterified to a glycerol molecule backbone. There are also non-glyceride components, such as sterols, e.g., cholesterol. While there is considerable interest in the roles that these non-glyceride components may play in metabolism, they are not important sources of energy in the diet (FAO, 1994).

There are accepted AOAC gravimetric methods for crude fat, which includes phospholipids and wax esters, as well as minor amounts of non-fatty material (AOAC, 2000). Total fat can be expressed as triglyceride equivalents determined as the sum of individual fatty acids and expressed as triglycerides (FAO, 1994). This method is satisfactory for the determination of fat in a wide variety of foods.

Practical Considerations

1. For energy purposes, it is recommended that fats be analysed as fatty acids and expressed as triglyceride equivalents, as this approach excludes waxes and the phosphate content of phospholipids, neither of which can be used for energy (James, Body and Smith, 1986).
2. A gravimetric method, although less desirable, is acceptable for energy evaluation purposes (AOAC, 2000).

Test for Lipids: Using a Known Lipid

1. Place a drop of cooking oil on a piece of unglazed paper spread it thinly over a small area.
2. Repeat another piece of unglazed paper but use water.
3. Allow drying time (you can speed this up by waving the paper in the air).
4. Compare the piece of paper to see which is translucent (allows light to pass through).
5. Record your finding in the table.

Positive Test

Light passes through the paper with lipids on it.

3. Analytical Methods for Carbohydrate in Food

Theory

The food we eat is one of the necessary factors in our daily life that provides nutritional support for the human body. Food consists of both organic and inorganic substances. Carbohydrates, Fats and Proteins are the main organic substances present in the food, which provide energy.

What are Carbohydrates?

One of the main components of our daily diet is carbohydrates. This type of foods includes sugars, starch and fibres. They are composed of sugar molecules that contain carbon, hydrogen and oxygen. Carbohydrates are classified into simple carbohydrates and complex carbohydrates.

Simple Carbohydrates

Simple carbohydrates are composed of one or two sugar units. Because of their smaller size, simple carbohydrates can be broken down and can be absorbed by the body easily. So they act as the quickest source of energy. They are found in fruits in the form of fructose, milk in the form of lactose and table sugar in the form of sucrose.

There are two types of simple carbohydrates: Monosaccharides and Disaccharides.

Monosaccharides are the simplest carbohydrates, consisting of only one sugar unit. Glucose, fructose and galactose are examples of monosaccharides. They have the capability of reducing cupric (Cu^{2+}) ions into cuprous (Cu^{+}) ions due to the presence of free Aldehydic and Ketonic groups and are called reducing sugars. These reducing sugars give positive results in Benedict's test and Fehling's test because they reduce the cupric ion present in the Benedict's Fehling's solution and form a precipitate of cuprous oxide. Depending upon the concentration of sugar, green, orange or brick red precipitates are obtained.

$$CH_2OH(CHOH)_4\ CHO + 2Cu^{++}\ SO_4^{-} + 2H_2O \xrightarrow{\text{heating}}$$

Glucose (Reducing Agent) — Cupric sulphate (Blue solution)

$$CH_2OH(CHOH)_4\ COOH + Cu_2O + 4H^{+} + SO4$$

Gluconic acid (Oxidised sugar) — Cuprous Oxide (Red Precipitate)

Disaccharides are composed of two chemically-linked monosaccharide units. Sucrose, lactose and maltose are examples of disaccharides. Sucrose is a non-reducing disaccharide. When it is boiled with HCl, sucrose undergoes hydrolysis to form glucose and fructose, which gives positive result with Benedict's and Fehling's solutions.

Complex Carbohydrates

Complex carbohydrates are composed of long chains of simple carbohydrate units. Because of their larger size, they can be broken down into simple carbohydrates.

Complex carbohydrates can be classified as Oligosaccharides and Polysaccharides.

Oligosaccharides consist of less than 10 monosaccharide units. Disaccharides are also a class of oligosaccharides. Raffinose and Stachyose are examples of Oligosaccharides.

Carbohydrates, made up of large number of monosaccharide units, are called polysaccharides. Starch, glycogen and cellulose are example of polysaccharides. Starch gives a blue-black complex with iodine.

Carbohydrate content of food has for many years, been calculated by differences, rather analysed directly. Under this approach the other constituents in the food protein, fat.

Total carbohydrate content of foods has, for many years, been calculated by difference, rather than analysed directly. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as **total carbohydrate by difference** and is calculated by the following formula:

100 - (Weight in grams [Protein + Fat + Water + Ash + Alcohol] in 100 g of Food)

It should be clear that carbohydrate estimated in this fashion includes fibre, as well as some components that are not strictly speaking carbohydrate, e.g., organic acids (Merrill and Watt, 1973). Total carbohydrate can also be calculated from the sum of the weights of individual carbohydrates and fibre after each has been directly analysed.

Available carbohydrate represents that fraction of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism. It does not include dietary fibre, which can be a source of energy only after fermentation. Available carbohydrate can be arrived at in two different ways: it can be estimated by difference, or analysed directly. To calculate available carbohydrate by difference, the amount of dietary fibre is analysed and subtracted from total carbohydrate, thus:

100 - (Weight in grams [Protein + Fat + Water + Ash + Alcohol + Dietary Fibre] in 100 g of Food)

This yields the estimated weight of available carbohydrate, but gives no indication of the composition of the various saccharides comprising available carbohydrate. Alternatively, available carbohydrate can be derived by summing the analysed weights of individual available carbohydrates. In either case, available carbohydrate can be expressed as the weight of the carbohydrate or as monosaccharide equivalents. For a summary of all these methods, see Table 2.

Dietary fibre is a physiological and nutritional concept relating to those carbohydrate components of foods that are not digested in the small intestine. Dietary fibre passes undigested from the small intestine into the colon, where it may be fermented by bacteria (the microflora), the end result being variable quantities of short-chain fatty acids and several gases such as carbon dioxide, hydrogen and methane. Short-chain fatty acids are an important direct source of energy for the colonic mucosa; they are also absorbed and enter into intermediary metabolism (Cummings, 1981).

Table 1 Total and Available Carbohydrate

Total Carbohydrate
By Difference: 100 - (Weight in grams [Protein + Fat + Water + Ash + Alcohol] in 100 g of Food) By Direct Analysis: Weight in grams (Monosaccharides + Disaccharides + Oligosaccharides + Polysaccharides, including Fibre)
Available Carbohydrate
By Difference: 100 - (weight in grams [Protein + Fat + Water + Ash + Alcohol + Fibre] in 100 g of Food) By Direct Analysis: Weight in grams (Monosaccharides + Disaccharides + Oligosaccharides + Polysaccharides, excluding Fibre)*

* May be expressed as weight (anhydrous form) or as the monosaccharide equivalents (hydrous form including water).

Chemically, dietary fibre can comprise: cellulose, hemicellulose, lignin and pectins from the walls of cells; resistant starch; and several other compounds. As more has been learned about fibre, a variety of methods for analysis have been developed. Many of these measure different components of fibre, and thus yield different definitions of, and values for, it. Three methods have had sufficient collaborative testing to be generally accepted by such bodies as AOAC International and the Bureau Communautaire de Reference (BCR) of the European Community (EC) (FAO, 1998): the AOAC (2000) enzymatic, gravimetric method - Prosky (985.29); the enzymatic, chemical method of Englyst and Cummings (1988); and the enzymatic, chemical method of Theander and Aman (1982). Monro and Burlingame (1996) have pointed out, however, that at least 15 different methods are applied for determining the dietary fibre values used in food composition tables. Their publication, and the FAO/WHO report on carbohydrates in human nutrition (FAO, 1998), discuss these issues in more detail. The effect of having such a variety of methods for dietary fibre, each giving a somewhat different value, affects not only the values in food composition tables for dietary fibre per se, but also those for available carbohydrate by difference.

Practical Considerations

1. Available carbohydrate is a useful concept in energy evaluation and should be retained. This recommendation is at odds with the view of the expert consultation in 1997, which endorsed the use of the term 'Glycaemic Carbohydrate' to mean 'Providing Carbohydrate for Metabolism' (FAO, 1998). The current group expressed concerns that 'Glycaemic Carbohydrate' might be confused or even equated with the concept of 'Glycaemic Index', which is an index that describes the relative blood glucose response to different 'Available Carbohydrates'. The term 'Available' seems to convey adequately the concept of 'Providing Carbohydrate for Metabolism', while avoiding this confusion.
2. Carbohydrate should be analysed by a method that allows determination of both available carbohydrate and dietary fibre. For energy evaluation purposes, standardized, direct analysis of available carbohydrate by summation of individual carbohydrates (Southgate, 1976; Hicks, 1988) is

preferred to assessment of available carbohydrate by difference, i.e., total carbohydrate by difference minus dietary fibre. This allows the separation of mono- and disaccharides from starches, which is useful in determination of energy content.

3. Determination of available carbohydrate by difference is considered acceptable for purposes of energy evaluation for most foods, but not for novel foods or food for which a reduced energy content claim is to be made. In these cases, a standardized, direct analysis of available carbohydrate should be carried out.
4. 'Dietary Fibre' is a useful concept that is familiar to consumers and should be retained on food labelling and in food tables. Because the physical characteristic of solubility/insolubility does not strictly correlate with fermentability/non-fermentability, the distinction between soluble and insoluble fibre is not of value in energy evaluation, nor is it of value to the consumer.
5. The AOAC (2000) analysis - Prosky (985.29) or similar method should be used for dietary fibre analysis.
6. Because dietary fibre can be determined by a number of methods that yield different results, when the Prosky method is not used the method used should be stated and the value should be identified by INFOODS tag names (Klensin *et al*., 1989). In addition, the method should be identified with the tag name in food composition tables.
7. Further research and scientific consensus are needed in order to develop standardized methods of analysis of resistant starch.

Materials Required

Hot plate, Test tube holder, Test tube rack, Test tubes, Stoppers, 250 mL beaker, Thermometer, 10 mL graduated cylinder, 1 mL droppers, Spot plate, 3% glucose solution, 1% starch solution, Cooking oil, Uncooked egg white, Benedict's solution, Biuret solution, Iodine solution, Unglazed paper, Distilled water, Food samples

Procedure

1. Tests for Carbohydrates

Test for Simple Sugar: Using a Known Sugar

1. Place 2 mL of 3% glucose solution in a labelled test tube.
2. In another test tube put 2 mL of water (the control).
3. Add 5 drops of Benedict's solution to each test tube.
4. Place the test tube in a hot water bath (about 75°C) for 3 minutes.
5. Record the colours of the test tube and control in the table.

Positive Test: A light green to red-brown precipitate forms. The colour depends on the amount of sugar present.

Test for Starch: Using a Known Starch

1. Place 3 drops of 1% starch solution on a spot plate.
2. In another depression on the spot plate place 3 drops of water (the control).
3. Add 3 drops of iodine solution to each sample.
4. Record the colours of the test and the control in the table.

Positive Test: The iodine solution changes from a brown colour to a blue/black colour.

3. DETERMINATION OF GLUTEN CONTENT IN WHEAT

Theory

Gluten is a protein composite found in food processed from wheat and related grain species. Gluten is the composite of Gliadin and Glutenin which are water insoluble protein which is conjoined with starch in the endosperm.

Gluten is a source of protein, additives and use in cosmetic other dermatological preparation.

Gluten is known to cause adverse health issue ranging from progressive malnutrition, diarrhea and vomiting, abdominals pain in those suffering from gluten sensitivity, primarily as a result of coeliac disease.

Materials Required: For Testing Gluten Apparatus/Reagent

Beaker, Analytical balance, Strives or spatula, Sieve, Brine solution, Water

Principle

It is a reaction that involve Salt Solution (Brine) and the flour sample in order to obtain a Water Insoluble Protein (Gluten).

Test Sample

Line Flour

Bagging Flour

Procedure

1. 10 g of flour is weighed in a small plastic container using analytical balance.
2. 5 ml of 2.1% salt solution is added.
3. The flour is their mixed with a stirrer until it forms dough.
4. The salt solution is then added to the dough and allow stay for 10 minutes.

5. The dough is then washed manually with NaCl solution until all the starch and soluble proteins are washed away.
6. The residue (gluten) is rinsed with water.
7. The gluten is then rubbed between palms and finally weighed to determine the weight of the wet gluten.

Result

Gluten Content (g)	Result	Remark
30,000	Standard	Sufficient
Less Than 30,000	Absent	Unsufficient
Higher Than 30,000	Present	Sufficient

4. ESTIMATION OF ACIDITY IN WHEAT FLOUR

Aim: Estimation of Acidity in Wheat Flour.

Theory

India produced 72.8 million tonnes of wheat during 2003. The kernel of wheat is usually 1/8-1/4 inch long. In some kinds of wheat the tip of each kernel is covered by stiff hairs called the brush. Wheat grains are avoid in shape, rounded in both ends. Along one side of the grain there is a grease, a folding of the aleurone and all covering layers.

Percentage Distribution of Carbohydrate in Wheat

Carbohydrate	Endosperm	Green	Poor
Starch	95.8	31.5	14.1
Sugar	1.5	36.4	7.6
Cellulose	0.3	16.8	35.2
Hemicellulose	2.4	15.3	43.1

Protein: Its content depends on the variety grown, climate and soil conditions. Proportion of different proteins in wheat grain at percentage of total protein are:

Albumin 5-10

Globulin 5-10

Prolamine 40-50

Glutelin 40-50

Wheat proteins are rich in glulamic acid and iron tryptophan glulamic acid and aspartic acid in the amide form as glutamine and asparagine. The high concentration of amide is important in determining the characteristics of gluten. The bran and germ proteins have a higher content of essential amino acids that the inner endosperm proteins. Thus, the biological value of endosperm protein is much less than that of the whole wheat protein.

Wheat is consumed mostly in the form of flour obtained by milling the grain whole a small quantity is converted into breakfast foods, such as wheat flakes and puffed wheat. Indian wheats are hard and the moisture content is usually 8-10 percent.

Various steps are involved in making the flour. The traditional procedure for millions wheat in India has been store grinding to obtain whole wheat flour. In modern milling, the wheat is subjected to creaming to remove various types of impurities together with damaged kernels.

1. **Vibrating Screen:** This remove bits of strains and other course received and second screen remove foreign materials like seeds.
2. **Aspirator:** It lifts off lighter impurities in the wheat. The stream of grain is directed across screens while air suits off the dust and lighter particles.
3. **Disc Separator:** After the aspirator it moves into a disc separator consisting of discs revolving on a horizontal axis. The surface of the discs intended to catch individual grains of wheat but reject larger or smaller material.
4. **Scourer:** The wheat then moves into the scourer, a machine in which beaters attached to a central shaft throw a wheat violently against the surrounding drum, puffins each kernel and breaking off the kernel hairs.
5. **Magnetic Separator:** The stream of wheat next passes over a magnetic separator that pulls out iron and steel particles contaminating during harvesting.
6. **Washer Stoner:** High speed motor spins the wheat in the water both. Excess water is thrown out by centrifugal force. Stones drop to the bottom are removed, lighter material float off leaving only the clean wheat.
7. **Tempering:** Wheat is tempered, before the start of grinding the process in which moisture is added. Tempering aids in separation of the bran from the endosperm and helps to provide constant controlled, amount of moisture and temperature throughout milling. The percentage of moisture, length of soaking, time and temperature are three important factors in tempering with different requirement in soft, medium and hard wheat. Dampened wheat is held in a bin for 8-24 hours. The outer layers of wheat tend to be brittle and tempering together the bran coat to permit more complete separation of endosperm within the kernel tempering also mellows or conditions the endosperm so the floury particles break move freely in milling.
8. **Entoleter:** Discs revolving at high speed in the scourer aspirator hurl the wheat against finger like pins. The impact cracks down any unsolved kernel which are rejected.
9. **Grinding Bin:** The 'First Break' rolls of a mill and are corrugated rather than smooth, break into coarse particles.

10. **Sifter:** The broken particles of wheat and bran go into a box like sifter where they are shaken through a series of cloth or screens to separate larger from the smaller particles. Larger particles are shaken off from the top by leaving the final flour to shift towards the bottom.
11. **Purifier:** The top fractions and particles of endosperm graded by size are carted to separate purifiers. In a purifier a controlled flow of air lifts off bran particles while cloth a screen separate and grade coarse fractions by size and qualities.
12. **The Down Purifier:** Four or five additional break rolls with successively final corrugations and each followed by a sifter are usually used to remove the coarse stock from the sifter and reduce the wheat particles granular middlings as free from bran as possible. Germ particles being somewhat plastic will be flattened by a later passage through the smooth reduction role and tend to be easily separated.

The process is repeated over and over again. Sifters and purifiers reducing roll until the maximum amount of flour is separated consisting of at least 72 percent of wheat.

Method of Estimation of Acidity

Materials Required

Conical flask, Stirrer, Titration stand, Filter paper (Dry), Whatman Filter Paper No.1

Reagents Required

1. Neutral Ethyl Alcohol-90% (V/V)
2. Standard Sodium Hydroxide Solution - Approx. 0.05 N
3. Phenolphthalein Indicator - Dissolve 0.1 gm in 100 ml of 60% Ethyl Alcohol.

Procedure

1. Weigh 5 gm of sample in a stoppered conical flask and add 50 ml of neutral ethyl alcohol.
2. Stopper the flask, swirl gently and allow to stand for 24 hours with occasional swirling.
3. Filter the alcoholic extract through a dry filter paper.
4. Titrate 10 ml of the alcoholic extract with standard sodium hydroxide solution to a pink and point using phenolphthalein as indicator.
5. Subtract titre value of blank alcohol.
6. Whatman Filter Paper No.1 or equivalent is to be used for filtration process.

Calculation

Alcoholic acidity with 90 % alcohol calculated as H_2SO_4 on dry basis.

$$= \frac{\text{Titre Value} \times 24.52 \times \text{Normality of NaOH}}{\text{Weight of sample (dry weight)}}$$

Result: Calculate the Result and write the findings.

5. ESTIMATION OF FIBER, PHOSPHOROUS AND IRON CONTENT IN ANY ONE FOOD.

Aim: Estimation of Fiber, Phosphorous and Iron Content in Any One Food.

1. Estimation of Iron in Spinach

There has always been good myth about how high the iron content in spinach and how we should eat regularly to achieve our required supplements of iron. Spinach also contains high levels of oxalates which bind iron to form ferrous oxalate, making iron in spinach unavailable leads to decrease in iron absorption. The purpose of this investigation is to see an effect on in iron content of spinach through boiling the spinach for different length of time in water. This will determine how much iron has been lost from the spinach and also give a good indication on which is the best way to cook spinach to preserve as much of the iron as possible.

The United States Department of agriculture states that 180 g of boiled spinach contains 6.43 mg of iron. Although there is still high iron content compared to other types of food but it has approximately the same amount of iron as other vegetables and even less than other, such as Cooked Broccoli and Cauliflower.

The non-heme iron in spinach is not properly absorbed unless eaten with sufficient quantity of vitamin. Spinach also contains high levels of oxalates which bind iron to form ferrous oxalate, making iron in spinach unavailable leads to decrease in iron absorption.

To determine the iron content of spinach, the different sample of water left from boiling the spinach have been titrated with standard solution of 'Potassium Permanganate'. Potassium permanganate is an oxidizing agent so it will oxidize Iron (II) in the spinach to Iron (III), it will need to be working in an acidic medium in order to be reduced into Mn^{2+}.

Materials Required

Spinach, Flask, Burette, Pipette, Conical flask, Titration stand

Reagents Required

1. Standardized Potassium Permanganate
2. Zinc powder
3. Sulphuric acid

Procedure

1. 100 grams sample of spinach was boiled with 500 ml of water for 10,15,20,25 and 30 minutes.
2. Volume of remaining water after boiling was measured then bottled and stored in fridge overnight, keeping solution at constant temperature before used in the experiment.
3. Burette was filled with a Standardized Potassium Permanganate at 0.0138 mol and value read, 25 ml of solution (water from spinach after 10 minutes of boiling) was pipette out in to conical flask.
4. A rice grain amount of zinc powder was placed into the conical flask.
5. Approximately 25 ml of sulphuric acid was added into the conical flask and titrated against the potassium permanganate until a bright pink colour was observed in the flask.
6. The value on the burette was recorded to the nearest 0.05 ml. The same procedure was used for all the solution.

Observation

The Table 1 below shows that as the boiling time of the spinach increased, the amount of iron lost through boiling also had increased. However the percentage mass of the iron lost was not that significant because it was less than 1% of the total weight of the spinach in all the different samples. According to these results only small amounts of iron were present in the water after boiling and although there seems to be an apparent increase in the amount of iron lost through boiling, the percentage different shows that this is not a significant amount even through there was change present. This suggests that spinach is able to retain majority of its iron content during boiling in its leaves.

The graph in Figure 1 below shows the relationship between the boiling time of spinach and the percentage of Iron (II) in the 100 gm of spinach boiled. The relationship between these five averages seems to be linear and strong. In theory as the spinach boils for a longer time, it will become more susceptible to the heat and therefore lose its nutrients more readily then for a shorter amount of time. From the results it is hard to judge whether it is linear or exponential trend as the percentage are small and could have a gradual exponential curve forming.

Table 1 *Data Regarding Iron Content Variations with Boiling Time*

Boiling Times	Average Titre Value(ml)	Iron (II) Concentration (mol^{-1})	Mass of Iron (Mg)	Iron (II) Percentage
10	14.78	0.0408	57.0	0.0570
15	15.15	0.0418	58.7	0.0587
20	16.55	0.0458	63.7	0.0637
25	17.79	0.0491	68.8	0.0688
30	18.65	0.0515	72.1	0.0721

Boiling Time (In Minutes)

Fig. 1.1 *Graphical Representation of Iron Content Variations with Boiling Time*

Result

From the data obtained and graph drawn, we can conclude that boiling the spinach for longer length of time have an effect on the iron content in spinach. There is an obvious trend shown that as the boiling time increases for the spinach there is an increased percentage of Iron (II) in water. This shows that in order to retain maximum iron in the spinach, it will only need to be boiled for a short time before consuming.

2. Determination of Dietary Fibre Determination in Grain Products

Theory

The dietary fibre is edible parts of plants' carbohydrates that are resistant to digestion in human small intestine. Diets naturally rich in dietary fibre support to prevent constipation, improve gastrointestinal health, glucose tolerance and the insulin response, and reduce the risk of colon cancer, hyperlipidemia, hypertension and other coronary heart disease risk factors. About 45% of the dietary fibre intake comes from grains and grain mixtures. In Latvia, there are neither data, nor investigations of the dietary fibre content in grain products during processing and in the end products in which manufacturers and consumers are interested. The aim

of this paper was an approval of dietary fibre determination methodology by using analytical equipment the Full Option Science System (FOSS) analytical fiber. The Total Dietary Fibre (TDF) content was analysed in the samples of rye whole grain flour, rye whole grain bread, and wheat biscuit experiments showed that the fibre content in rye whole grain flour is 13.8 g 100 g^{-1}, in rye whole grain bread –11.6 g 100 g^{-1}, and in the wheat biscuit –1.9 g 100 g^{-1}.

It is generally believed that E.H. Hipsley in 1953 was the first to use 'Dietary Fiber' as a term for the non-digestible constituents that make up the plant cell wall. These constituents were known to include cellulose, hemicellulose, and lignin (DeVries *et al.*, 1999).

By 1976, the dietary fibre definition had been broadened to include all indigestible polysaccharides, such as gums, modified celluloses, mucilages, oligosaccharides, and pectins (Trowell *et al*., 1976). It remained primarily a physiological definition, identifying dietary fibre on the basis of edibility and resistance to digestion. The definition was broadened to reflect chemical research findings obtained in the interim years. In later years scientists began to seek consensus on a dietary fibre definition with the aim to quantify it in foods for nutrition improvement and labeling purposes. By the 1981 workshop of the Association of Official Analytical Chemists (AOAC) in Canada, general consensus had been achieved on methodology that would quantify dietary fibre, as defined by H. C. Trowell in 1976, and adopted in collaborative study by 43 laboratories in 29 countries (Prosky, 1990). The method was adopted by AOAC as the first official Enzymatic-Gravimetric Method of analysis for total dietary fibre, in foods-AOAC official Method 985.29 (Andrews, 1998). After that American Association of Cereal Chemists (AACC) adopted the method as AACC approved Method 32-05. Also adopted as official methods were: AOAC 992.16, total Dietary Fiber, Enzymatic Method and AOAC 994.13, total Dietary Fiber (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin)–Gas Chromatographic–Colometric–Gravimetric Method (Uppsala Method), (Andrews, 1998).

At the end of 90th last century before scientists had realized the international survey of 147 scientists for dietary fibre methodology (DeVries *et al*., 1999), 65% of the scientists supported the physiological definition; while an additional 5% favored using it in combination with the chemical definition. Fifty nine per cents supported the inclusion of digestion-resistant oligosaccharides in that definition. Workshop participants acknowledged that AOAC 985.29/AACC 32-05 did not quantify non-digestible oligosaccharides. After that, methods were developed to specifically measure oligosaccharides–methods 997.08, 999.03, 2001.02, 2000.11, 2001.03, 2002.02, and (IFST, 2007). Codex committee on nutrition and foods for special Dietary Uses (CCNFSDU) and International Life Sciences Institute (ILSI) took part in dietary fibre methodology development (ILSI, 2007). The issue of a definition for dietary fibre has been discussed and debated in the scientific community for many years (ILSI, 2007). A consensus has been

developed basing on clear scientific evidence that the definition of dietary fibre should be based on the physiological properties of food constituents, not merely on their physiochemical characteristics. This consensus is reflected in the original definition developed in CCNFSDU, AACC and numerous other definitions, including US national academy of institute of Medicine and Health council of the Netherlands. Each of these definitions is based on the physiological property of non-digestion and non-absorption in the small intestine, with one or more desirable health effects (ILSI, 2007).

Dietary fibre definition adopted at the 28th session of CCNFSDU in November 2006 is as follows: 'Dietary fibre means carbohydrate polymers with a Degree of Polymerization (DP) not lower than 3, which are neither digested nor absorbed in the small intestine. A degree of polymerization not lower than 3 is intended to exclude monosaccharides and disaccharides. It is not intended to reflect the average DP of a mixture. Dietary fibre consists of one or more:

- Edible carbohydrate polymers naturally occurring in the food as consumed.
- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means.
- Synthetic carbohydrate polymers' (AACC, 2007).

Grain foods provide complex carbohydrates important not only as a source of energy, but also a source of fibre in human diet and therefore a benefit to human health (Poutanen, 2006). consumers are interested in higher content of dietary fibre in grain products, which improves health because dietary fibres are an extremely beneficial component. there is strong and expanding epidemiological evidence linking high intake of cereal fibre to reduced risk of chronic and cardiovascular disease (Poutanen, 2006). A diet naturally high in fibre helps prevent constipation, reduce the risk of colon cancer, improves gastrointestinal health, effect of satiety, and impacts weight loss by reducing food intake at meals (Leeds, 1982). Such dietary fibre component as â-glucan lowers cholesterol level in blood and effected diabetes disease (Shinnick *et al*., 1991), but the World Health Organization has recommended that total fibre intake be 25 g per day (Viscione, 2007).

Materials Required

Rye whole grain flour, Rye whole grain bread, Wheat biscuit

Procedure

1. The samples used in experiments were obtained from:
   - Rye whole grain flour.
   - Rye whole grain bread (baked from whole meal grain flour with scalding and sourdough).
   - Wheat biscuit (baked from wheat flour type 405 at the Department of Food Technology).

2. The total dietary fibre in these samples was determined according to the AOAC approved method no. 985.29 by FOSS.
3. The samples of every type were used in duplicates.
4. The samples were defatted and dried with a particle size less than 0.5 mm.
5. After weighing, each sample was enzymatically digested with á amylase and incubated at 100°C, and then the samples were digested with protease and amylo-glucosidase and were incubated at 60°C.
6. The determination procedure of TDF is shown in Figure 1.
7. After digestion, the total fibre content was precipitated by adding 95% ethanol. Then the solution was filtered and fibre was collected, dried and weighed.
8. The protein and ash content were determined to correct any of these substances which might remain in the fibre.

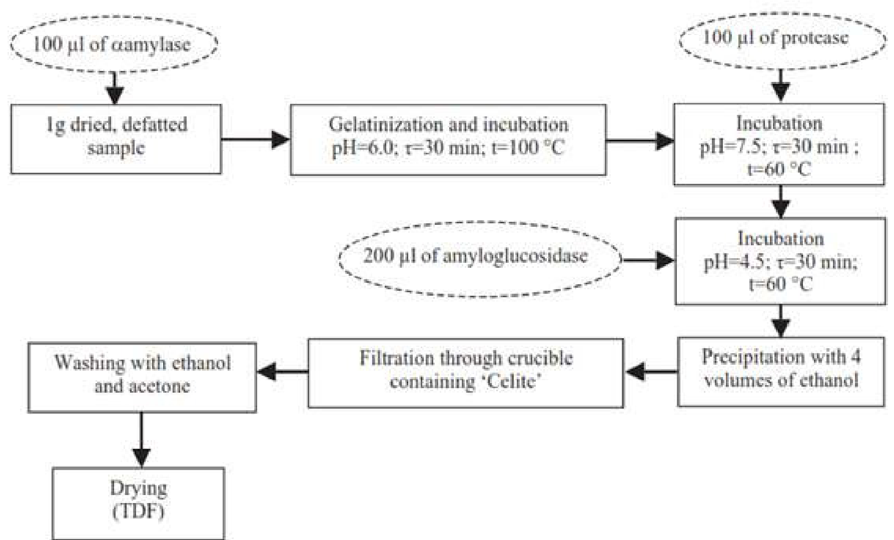


Fig. 1 *TDF Determination Procedure*

9. The experiments were carried out by using enzymatic processing with incubation in a thermostatic shaking water bath.
10. The TDF residues were filtrated by using the Filtration Module.
11. The protein content was determined by using Kjeldahl (AACC, 1995) nitrogen equipment. Each determination process took 2 days.

Calculation

For calculations, basic equation applied is as follows:

$$\text{Content of Fibre-Residue Weight} - \text{Weight of (Protein} + \text{Ash)}$$

Results

The content of total dietary fibre was determined in Rye Whole Grain Flour, Rye Whole Grain Bread, and Wheat Biscuit. The results of total dietary fibre were obtained in three independent repetitions by using FOSS Analyticals, as shown below in Table 1.

Table 1 *Results of Experiments Compared to the Literature Data*

Sample	Experimental Data of TDF, g 100 g^{-1}	Literature Data of TDF, g 100 g^{-1}		
		Kujala, 1999	Lindhauer, 2005	Fineli, 2008
Rye Whole Grain Flour	13.8 ± 0.7	13.6	–	13.9
Rye Whole Grain Bread	11.6 ± 0.6	9.9	9.0	11.0
Wheat Biscuit	1.9 ± 0.1	–	–	1.3

Conclusion

Fibre content in grain depends on corn sort and climatic, growing and harvesting conditions. In the milling process, the kernel can be ground and fractionated into different types of flour. Whole grain rye flour contains all parts of the grain. In the literature it was found that the average dietary fibre content usually is between 12% and 15% of dry matter (Kujala, 1999). This research showed that the amount of total dietary fibre in whole grain rye flour is 13.8 ± 0.7 g $100g^{-1}$.

The fibre content in rye bread is about three times higher (about 8.5 –10 g 100 g^{-1}) than in wheat bread. The latest research data demonstrate that in the rye whole grain bread total dietary fibre content is 11.6 ± 0.6 g $100g^{-1}$. This amount is higher than that in the literature and should be taken into account corn sort, and that bread was baked from whole grain flour.

Wheat biscuit baked from wheat flour 550. Type contains very limited amount of fibre-according Fineli Food Composition Database – 1.3 g $100g^{-1}$. New experimental data 1.9 ± 0.1 g $100g^{-1}$ and the literature data demonstrate high comparability.

6. DETERMINATION OF CALCIUM CONTENT IN MILK

Aim: Determination of Calcium Content in Milk.

Theory

Calcium an important mineral for the body; Calcium is an important component of a healthy diet and a mineral necessary for life. It is a mineral that people need to build and maintain strong bones and teeth. It is also very important for other physical functions such as muscle control and blood circulation. It contains dissolved carbohydrates, proteins, vitamins, fats, minerals like calcium, magnesium and sodium.

Components of Milk

Milk contains Water, Carbohydrates, Fats, Proteins, Enzyme, Vitamins, Organic Acids and Minerals like Phosphorus, Potassium and Calcium. Milk Fat is secreted in the form of Fat Glucose. Fat soluble Vitamins A, D, E, K are present along with essential fatty acid in milk. Milk contains Vitamin D which promoter calcium absorption in the gut and maintains adequate serum calcium and phosphate concentration to enable normal mineralization of bone, without sufficient vitamin D bone can become thin. Vitamin D prevents Rickets in children and Osteomalacia in adult. Important milk protein is casein, present in large amount. Milk also contains other proteins including enzymes. Milk proteins apart from casein are more water soluble. Milk also contains different carbohydrates like lactose, glucose and galactose. Lactose gives sweet taste to milk. Milk contains various minerals like calcium, magnesium, sodium and potassium. Mineral contribute to important physiological processes.

Milk is an excellent source of dietary calcium for those whose bodies tolerate it because it has a high concentration of calcium and the calcium in milk is excellently absorbed.

Principle

In this experiment the determination of calcium in milk is based on a Complexometric Titration of calcium with an aqueous solution of the disodium salt of EDTA at high pH value (12).

Complexometric titration is a type of titration based on complex formation between the analyse and titrant.

Such compounds are capable of forming chelate complex with many cations in which the cation is bound in a ring structure.

The ring results from the formation of a salt like bond between the cation and the carboxyl groups together with a coordinate bond through the lone pair of electrons of the nitrogen atom.

The common form of the agent is Disodium Salt EDTA.

It is colourless and can be weighed and dissolved in water to form stable solution.

At high pH (>10) the remaining protons leave EDTA forming EDTA anion:

Reagent Required: Indicator Solochrome Dark Blue

The solochrome dark blue indicator is a suitable indicator in this case.

The dye itself has a blue colour.

This blue dye also forms a complex with the calcium ions changing colour form blue to pink red in the process, by the dye-metal ion complex is less stable than the EDTA-metal ion complex.

As a result, when the calcium ion-dye complex is titrated with EDTA the Ca^{2+} ions react to form a stronger complex with the EDTA changing the dye colour to blue.

Ca-Indicator + $EDTA4^{-} \rightarrow$ Ca-$EDTA^{2}$ + Indicator.

Materials Required

Distilled water, Sodium hydroxide solution, Erlenmeyer flask, Magnesium hydroxide, EDTA solution, Titration stand

Procedure

1. Combine 10 ml of sample, 40 ml distilled water and 4 ml of 8 ml sodium hydroxide solution into an Erlenmeyer flask and allow solution to stand for about 5 minutes with occasional swirling.
2. A small of magnesium hydroxide may precipitate during this time. Do not add the indicator until you have given the precipitate a chance to form.
3. Then add 6 drops of the Solochrome Dark Blue solution.
4. After that start to titrate with EDTA solution.
5. Repeat titration for three trials.
6. Observe and record the values in the following table.

Results

	EDTA (Volume L)
1	
2	
3	
Average	

Calculation

1. Calculate the moles of EDTA required to complex the Ca^{2+} ions in the sample.

   Number of Moles (for EDTA) = Molarity of EDTA + Volume of EDTA in L

   Note: Ratio = Ca^{2+} : EDTA = 1 : 1, i.e., Moles of EDTA = Moles of Ca^{2+}
2. Calculate Weight of Ca^{2+}:

   Weight of Ca^{2+} = Number of Moles × Molecular Weight (40.78)

   % of Ca^{2+} = (Weight of Ca^{2+} / Weight of Sample) × 100

$$\frac{\text{Amount of Calcium} = (\text{Molarity of EDTA (in litre)} \times 40.78}{\text{Weight of Sampl}} \times 100$$

FUNCTIONAL FOODS AND NUTRACEUTICALS

1. MANUFACTURING ASPECTS OF SELECTED NUTRACEUTICALS (DEMONSTRATION)

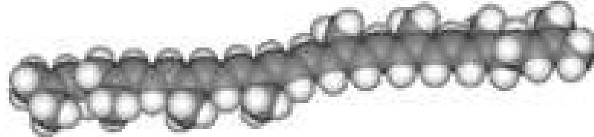
Aim: Demonstration of manufacturing aspects of selected Nutraceuticals.

A. Lycopene

Theory

Lycopene (from the neo-Latin *Lycopersicum*, the tomato species) is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables, such as red carrots, watermelons, gac, and papayas, but it is not in strawberries or cherries. Although lycopene is chemically a carotene, it has no vitamin A activity. Foods that are not red may also contain lycopene, such as asparagus and parsley.

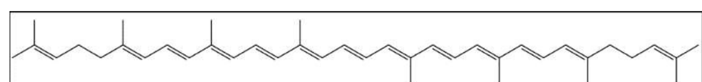
In plants, algae, and other photosynthetic organisms, lycopene is an intermediate in the biosynthesis of many carotenoids, including beta-carotene, which is responsible for yellow, orange, or red pigmentation, photosynthesis, and photoprotection. Like all carotenoids, lycopene is a tetraterpene. It is insoluble in water. Eleven conjugated double bonds give lycopene its deep red color. Owing to the strong color, lycopene is useful as a food colouring (registered as E160d) and is approved for use in the USA, Australia and New Zealand (registered as 160d) and the European Union.



Names	
IUPAC Name (6*E*,8*E*,10*E*,12*E*,14*E*,16*E*,18*E*,20*E*,22*E*,24*E*,26*E*)- 2,6,10,14,19,23,27,31-Octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene	
Other Names ψ,ψ-Carotene	
Identifiers	
CAS Number	502-65-8
3D Model (JSmol)	Interactive Image
ChEBI	CHEBI:15948
ChEMBL	ChEMBL501174
ChemSpider	394156
ECHA InfoCard	100.007.227 394156
EC Number	207-949-1
E number	E160d (Colours)
PubChem CID	446925
UNII	SB0N2N0WV6

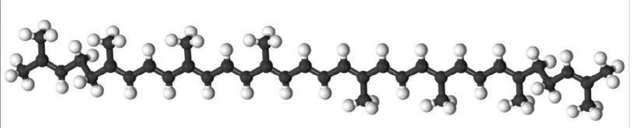
Properties	
Chemical Formula	$C_{40}H_{56}$
Molar Mass	536.888 g·mol^{-1}
Appearance	Deep Red Solid
Density	0.889 g/cm^3
Melting Point	177 °C (351 °F; 450 K)
Boiling Point	660.9 °C (1,221.6 °F; 934.0 K) at 760 mmHg
Solubility in Water	Insoluble
Solubility	Soluble in CS_2, $CHCl_3$, THF, Ether, C_6H_{14}, Vegetable Oil Insoluble in CH_3OH, C_2H_5OH
Solubility in Hexane	1 g/L (14 °C)
Vapor Pressure	1.33·10^{-16} mmHg (25 °C)

Structure of Lycopene



Skeletal Formula of All-Trans Lycopene

NOTES



Ball-And-Stick Model of All-Trans Lycopene

Lycopene is a symmetrical tetraterpene assembled from eight isoprene units. It is a member of the carotenoid family of compounds, and because it consists entirely of carbon and hydrogen, is also a carotene. Isolation procedures for lycopene were first reported in 1910, and the structure of the molecule was determined by 1931. In its natural, all-trans form, the molecule is long and straight, constrained by its system of 11 conjugated double bonds. Each extension in this conjugated system reduces the energy required for electrons to transition to higher energy states, allowing the molecule to absorb visible light of progressively longer wavelengths. Lycopene absorbs all but the longest wavelengths of visible light, so it appears red.

Plants and photosynthetic bacteria naturally produce all-trans lycopene. When exposed to light or heat, lycopene can undergo isomerization to any of a number of cis-isomers, which have a bent rather than linear shape. Different isomers were shown to have different stabilities due to their molecular energy (highest stability: 5-cis ≥ all-trans ≥ 9-cis ≥ 13-cis > 15-cis > 7-cis > 11-cis: lowest). In human blood, various cis-isomers constitute more than 60% of the total lycopene concentration, but the biological effects of individual isomers have not been investigated.

Carotenoids like lycopene are found in photosynthetic pigment-protein complexes in plants, photosynthetic bacteria, fungi, and algae. They are responsible for the bright orange–red colors of fruits and vegetables, perform various functions in photosynthesis, and protect photosynthetic organisms from excessive light damage. Lycopene is a key intermediate in the biosynthesis of carotenoids, such as beta-carotene, and xanthophylls.

Dispersed lycopene molecules can be encapsulated into carbon nanotubes enhancing their optical properties. Efficient energy transfer occurs between the encapsulated dye and nanotube, light is absorbed by the dye and without significant loss is transferred to the nanotube. Encapsulation increases chemical and thermal stability of lycopene molecules; it also allows their isolation and individual characterization.

Biosynthesis

The unconditioned biosynthesis of lycopene in eukaryotic plants and in prokaryotic cyanobacteria is similar, as are the enzymes involved. Synthesis begins with mevalonic acid, which is converted into dimethylallyl pyrophosphate. This is then condensed with three molecules of isopentenyl pyrophosphate (an isomer of dimethylallyl pyrophosphate), to give the 20-carbon geranylgeranyl pyrophosphate.

Two molecules of this product are then condensed in a tail-to-tail configuration to give the 40-carbon phytoene, the first committed step in carotenoid biosynthesis. Through several desaturation steps, phytoene is converted into lycopene. The two terminal isoprene groups of lycopene can be cyclized to produce beta-carotene, which can then be transformed into a wide variety of xanthophylls.

Staining and Removal

Lycopene is the pigment in tomato-containing sauces, turning plastic cookware orange, and is insoluble in water. It can be dissolved only in organic solvents and oils. Because of its non-polarity, lycopene in food preparations will stain any sufficiently porous material, including most plastics. To remove this staining, the plastics can be soaked in a solution containing a small amount of household bleach.

Diet

Consumption by Humans

Absorption of lycopene requires that it be combined with bile salts and fat to form micelles. Intestinal absorption of lycopene is enhanced by the presence of fat and by cooking. Lycopene dietary supplements (in oil) may be more efficiently absorbed than lycopene from food.

Lycopene is not an essential nutrient for humans, but is commonly found in the diet mainly from dishes prepared from tomatoes. The median and 99th percentile of dietary lycopene intake have been estimated to be 5.2 and 123 mg/d, respectively.

Sources

Dietary sources of lycopene	
Source	**mg Wet Weight**
Gac Aril	2~6 per gram
Raw Tomato	4.6 per cup
Tomato Juice	22 per cup
Tomato Paste	75 per cup
Tomato Ketchup	2.5 per tablespoon
Watermelon	13 per Wedge
Pink Grapefruit	2 per half Grapefruit

Fruits and vegetables that are high in lycopene include autumn olive, gac, tomatoes, watermelon, pink grapefruit, pink guava, papaya, seabuckthorn, wolfberry, and rosehip. Ketchup is a common dietary source of lycopene. Although gac (*Momordica cochinchinensis* Spreng) has the highest content of lycopene of any known fruit or vegetable (multiple times more than tomatoes), tomatoes and tomato-based sauces, juices, and ketchup account for

more than 85% of the dietary intake of lycopene for most people. The lycopene content of tomatoes depends on variety and increases as the fruit ripens.

Unlike other fruits and vegetables, where nutritional content, such as vitamin C is diminished upon cooking, processing of tomatoes increases the concentration of bioavailable lycopene. Lycopene in tomato paste is up to four times more bioavailable than in fresh tomatoes. Processed tomato products, such as pasteurized tomato juice, soup, sauce, and ketchup contain a higher concentration of bioavailable lycopene compared to raw tomatoes.

Cooking and crushing tomatoes (as in the canning process) and serving in oil-rich dishes, such as spaghetti sauce or pizza greatly increases assimilation from the digestive tract into the bloodstream. Lycopene is fat-soluble, so the oil is said to help absorption. Gac has high lycopene content derived mainly from its seed coats. Cara cara navel, and other citrus fruit, such as pink grapefruit, also contain lycopene. Some foods that do not appear red also contain lycopene, for example, asparagus, which contains about 30μg of lycopene per 100-g serving (0.3 μg/g) and dried parsley and basil, which contain around 3.5–7.0 μg/g of lycopene.

Safety Value

In humans, the Observed Safe Level for lycopene is 75 mg/day, According to study.

Adverse Effects

Lycopene is non-toxic and commonly found in the diet, mainly from tomato products. There are cases of intolerance or allergic reaction to dietary lycopene, which may cause diarrhea, nausea, stomach pain or cramps, gas, and loss of appetite. Lycopene may increase the risk of bleeding when taken with anticoagulant drugs. Because lycopene may cause low blood pressure, interactions with drugs that affect blood pressure may occur. Lycopene may affect the immune system, the nervous system, sensitivity to sunlight, or drugs used for stomach ailments.

Lycopenemia is an orange discoloration of the skin that is observed with high intakes of lycopene. The discoloration is expected to fade after discontinuing excessive lycopene intake.

Lycopene Extract from Tomato

Lycopene extract from tomato is a lycopene-rich extract prepared from the ripe fruits of tomato (*Lycopersicon esculentum* L.). The product is manufactured by crushing tomatoes, to produce crude tomato juice that is then separated into serum and pulp. The pulp is subsequently extracted using ethyl acetate as a solvent. The final extract consists of tomato oil in which lycopene together with a number of other constituents that occur naturally in tomato, are dissolved and dispersed. These constituents include fatty acids and acylglycerols, unsaponifiable matter, water soluble matter, phosphorous compounds, and phospholipids.

The major colouring principle in tomato extract is all-trans-lycopene, however, minor amounts of cis-isomers and other carotenoids and related substances including β-carotene, phytofluene, phytoene and tocopherols are also present. The intended use of lycopene extract from tomato is as a food colour in dairy products, non-alcoholic flavoured drinks, cereal and cereal products, bread and baked goods and spreads, to provide colour shades from yellow to red. Lycopene extract from tomato may also be used in food supplements. The use levels of the extract, expressed as lycopene added to food, may vary from 2 mg/l in bottled water to 130 mg/kg in ready-to-eat cereals. Lycopene in the extract was shown to be stable when stored at room temperature and at 4°C for up to 37 months. When used as a food colour, lycopene remained stable in the food matrix under appropriate storage conditions. Lycopene stability depends on the particular food to which it is added, as well as on the production process.

Principle

The major colouring principle of lycopene extract from tomato is all-trans-lycopene. Lycopene in tomatoes and tomato products consists predominantly of all-trans-lycopene (35-96% of the total lycopene content) and low levels of cis-lycopenes (1-22% of the total lycopene content) (Schierle *et al.,* 1997). Lycopene for food use is also manufactured by chemical synthesis or produced by fermentation of *Blakeslea trispora*.

The lycopene content in tomato typically ranges from 70 to 130 mg/kg and depends on the variety, geographic location, technique of cultivation, climatic conditions and degree of ripeness of tomato fruits. The tomato extract described in this application is the ethyl acetate extract of ripe tomato fruits with lycopene content ranging from 150 to 250 mg/kg. The lycopene content of tomato extract ranges from 5% to 15%, depending on the nature of the fruit from which it was extracted, and the amount of tomato seed oil that is included in the extract.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) had previously evaluated lycopene (both natural and synthetic) to be used as a food colour at its eighth, eighteenth, and twenty-first meetings (FAO/WHO, 1965, 1975, 1978), but was not able to establish an Acceptable Daily Intake (ADI) due to the limited information available. At its sixty-seventh meeting JECFA agreed that both synthetic lycopene and lycopene extracted from *Blakeslea trispora* were acceptable as food colours and established a group ADI of 0-0.5 mg/kg bw/day for both preparations.

Lycopene extract from tomato is a dark-red viscous liquid. It is freely soluble in ethyl acetate and *n*-hexane, partially soluble in ethanol and acetone, and insoluble in water. A solution in *n*-hexane shows an absorption maximum at approximately 472 nm.

Method of Manufacture

Lycopene extract from tomato is produced from a tomato variety with high lycopene content, within the range of 150 to 250 mg/kg. This particular variety is not generally marketed for direct consumption, but is used primarily in the production of this lycopene extract. The extract is produced by crushing tomatoes into crude tomato juice that is then separated into serum and pulp. The tomato pulp is then extracted with ethyl acetate. The final product is obtained after solvent removal by evaporation under vacuum at 40-60°C.

Composition

Lycopene extract from tomato contains carotenoids (5-15% w/w) as well as non-carotenoid components. The carotenoid fraction of the tomato extract consists mainly of lycopenes, of which ~86 % is all-trans-lycopene, ~6% is 5-cis-lycopene, ~2% is 9-cis-lycopene and ~2% is 13-cis-lycopene, and ~4% are other carotenoids. The major non-carotenoid components of tomato extract include fatty acids and acylglycerols (69-74%), phospholipids (8.9-14%), and waxes (5-8.4%).

The chemical composition of tomato extract as provided to the Committee is detailed in Table 1. The reported values were determined using the analytical methods described in the report of a study that aimed at a full qualitative and quantitative characterization of the extract.

Table 1. Chemical Composition of Lycopene Extract from Tomato

Compound	Content [%]	
	Min	Max.
Unsaponifiable matter	13.4	31.4
Lycopene	4.9	15
Phytoene	0.5	1.1
Phytofluene	0.4	0.9
β-Carotene	0.1	0.5
Tocopherols	1.0	3.0
Sterols	1.5	2.5
Others (i.e. waxes)	5.0	8.4
Fatty acids and acylglycerols of which*	69	74
Myristic acid (14:0)	0.5	0.6
Palmitic acid (16:0)	22.5	23.0
Stearic acid (18:0)	5.1	5.4
Oleic acid (18:1)	12.4	13.5
Linoleic acid (18:2)	46.7	48.7
Linolenic acid (18:3)	8.8	10.9
Arachidic acid (20:0)	0.9	1.1
Behenic acid (22:0)	0.5	—
Free fatty acids	5	
Water	0.5	0.9

NOTES

Compound	Content [%] Min	Max.
Water and soluble matter	2.7	4.8
Lactic acid	0.5	0.7
Other organic acids		0.1
Others	2.2	4.0
Total Phosphorus	0.4	0.5
Organic phosphorus	0.3	0.5
Phospholipids (estimated from phosphorus determined by ICP)	8.9	14
Nitrogen	0.16	0.31
Ash	0.7	0.8

* % of total peak area

All-trans-lycopene is an unsaturated acyclic hydrocarbon with chemical formula C40H56 and molecular weight of 536.85. Its Chemical Abstract Service (CAS) Number is 502-65-8.

The chemical name of all-trans-lycopene is (all-E)-2, 6, 10, 14, 19, 23, 27, 31-octamethyl- 2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30-dotriacontatridecaene. Common names include ψ, ψ-carotene, all-trans-lycopene, and (all-E)-lycopene. The structural formula of all-trans-lycopene.

All-trans-lycopene

Carotenoids from tomato or tomato extract can be analysed using HPLC (Ishida *et al.,* 2001).

Impurities

According to the sponsor, tomato extract may contain residues of ethyl acetate, which is used as solvent in the production process. The Committee established a specification limit for ethyl acetate of not more than of 50 mg/kg. The sponsor also provided information on other potential contaminants including heavy metals and arsenic. Based on this information, the Committee established the specification limits for lead and arsenic

Stability

Lycopene is susceptible to chemical changes, such as oxidation followed by degradation or isomerization when exposed to light, heat and oxygen. Lycopene present in tomato extract was shown to be stable under storage at 4°C and room temperature when tested over a time period ranging from 18 to 37 months. Lycopene stability was assessed for nine batches of tomato extract using spectrophotometry and HPLC.

Analytical Methods

Analytical methods used to support the specifications for tomato extract are based on general tests in the Combined Compendium of Food Additive Specifications for identity and purity. The specifications monograph cites specific tests for limits on lead (not more than 1 mg/kg), arsenic (not more than 3 mg/kg), sulfated ash (not more than 0.1%) and loss-on-drying (not more than 2%). The headspace gas chromatography method for residual solvent (ethyl acetate) is described in the new specifications monograph.

The assay is intended to define both the content of total lycopenes and total carotenoids in the extract. The HPLC method of assay provided to the Committee was designed to determine total lycopenes (all-trans-lycopene and cis-lycopene isomers), while total carotenoids are determined spectrophotometrically using a method compatible with that published in the Combined Compendium of Food Additive.

Rationale for Proposed Specifications

The specifications for tomato extract were developed from considerations proposed by the sponsor, based on the Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on the safety of lycopene oleoresin from tomatoes (EFSA, 2008), as well as on the existing JECFA specifications for synthetic lycopene (FAO/WHO, 2006) and lycopene isolated from *Blakeslea trispora.*

Functional Uses

Lycopene extract from tomato is intended for use as a food colour. It provides the similar colour shades, ranging from yellow to red, as do the natural and synthetic lycopenes. Lycopene extract from tomato is also used as a food/dietary supplement in products where the presence of lycopene provides a specific value (for example, antioxidant or other claimed health benefits). The product may also be used as an antioxidant in food supplements.

Food Categories and Use Levels

Lycopene extract from tomato is intended for use in the following food categories: baked goods, breakfast cereals, dairy products including frozen dairy desserts, dairy product analogues, spreads, bottled water, carbonated beverages, fruit and

vegetable juices, soybean beverages, candy, soups, salad dressings, and other foods and beverages.

According to the sponsor, the use levels of tomato extract, expressed as lycopene levels added to food, depend on its intended function and may vary from 2 mg/l in bottled water to 130 mg/kg in ready- to-eat cereals. Food and beverage products will be formulated in such a way that they will provide about 2 mg lycopene per serving.

Reactions in Foods

The chemical structure of lycopene, particularly the long chain of conjugated carbon-carbon double bonds, predisposes lycopene to isomerization and degradation upon exposure to light, heat, and oxygen (Lee and Chen, 2002) and the subsequent loss of its colouring properties (Xianquan *et al.*; Yang *et al*. 2006); this would render tomato extract ineffective as a food colour.

The Committee received data on lycopene stability in representative foods based on monitoring of the lycopene content in food and the colour of food during 5 days storage under fluorescent light and storage conditions appropriate for each food (room temperature, 4°C, or frozen). The concentration of lycopene in different food products, to which the commercial product Lyc-O-Mato Oleoresin containing 6% lycopene was added, was in the range of 0.5 to 60 mg/kg (Table 2). Equivalent commercial food products, which were either not coloured or coloured with control colorants, such as â-carotene, were used as control samples. Both the test and control samples were analyzed for colour using a Hunter Colorimeter and for lycopene content using HPLC.

Table 2 *Lycopene Stability in Foods Prepared with Lyc-O-Mato Oleoresin Containing 6% Lycopene*

Food	Lycopene level in food (mg/kg)	Control colorant level in food (mg/kg)
Orange gelatine	10-30	Yellow 6/Red (40)
Yellow cake	20-30	β-Carotene (80)
Lemon beverage	3-60	Not coloured
Orange hard candy	5-20	Not coloured
Ice cream	10-20	Not coloured
Salad dressing	20-50	Not coloured
Margarine	0.5-1.0	β-Carotene (2)

Visual inspections and Hunter Colorimetry showed no significant changes in colour after 5 days of storage. The HPLC data showed that ninety-five percent of the added lycopene was recovered at the time of formulation and ninety percent 5 days after formulation. These results demonstrate that tomato extract is stable in a variety of foods under appropriate storage conditions.

Lycopene stability was also assessed in a fruit preparation containing apple and Aloe vera formulated with tomato extract. The level of lycopene in the product decreased from approximately 83 mg/kg to 77 mg/kg after four months of storage.

Table 3 *Proposed Uses and Use Levels of Tomato Extract*

Food Category*	GSFA Food Category and Food-Use	Use level (mg/kg)
Milk Products	0.1.1.2 Flavoured milk and milk drinks	30
	01.2.1 Fermented milk beverages	30
	13.4 Milk-based meal replacements	9-40
Dairy Product Analogues	01.3.3 Imitation milks	30
	01.5 Dry milk	30
	01.5.2 Soy milks	30
	01.7 Yoghurt	20-40
	01.7 Frozen Yoghurt	20-40
Fats and Oils	02.2.1.2 Margarine-like spreads	20
Soft Candy	05.2 Chewy and nougat candy	15
	05.2 Fruit Snacks	15
Hard Candy	05.2 Hard candy	20-70
Chewing Gum	05.3 Chewing gum	15
Breakfast Cereals	06.3 Ready-to-eat cereals	30-130
	06.5 Instant and regular hot cereals	9-20
	07.1.2 Crackers and crisp breads	60
	07.2.1 Cakes, cookies	30
Egg Products	10.4 Egg-based desserts	20
Soups and Soup Mixes	12.5.1 Soups	30
	12.6.1 Salad dressings	30
Gravies and Sauces	12.6.2 Tomato-based sauces	30
	12.9.1.1 Soybean beverage	20-40
Beverages and Beverage Bases	14.1.1.1 Bottled water	2-15
Processed Fruits and Fruit Juices	14.1.2.1 Fruit juice	4-20
	14.1.2.2 Vegetable juice	4-20
	14.1.3 Nectars	4-20
	14.1.4 Energy, sport, and isotonic drinks	4-15
	14.1.4.1 Carbonated beverages	4-20
	14.1.4.2 Fruit-flavoured drinks	9-15
	14.1.5 Tea, ready-to-drink	3-15
Baked Goods and Baking Mixes	15.1 Cereal and energy bars	40-80

B. Isoflavonoids

Theory

Isoflavones are 4-benzopyrone derivatives formed in the shikimic acid pathway. The following are the best-known representatives of isoflavones: genistein, with its precursor biochanin A, daidzein, with its precursor formononetin and glycitein, prunetin and purearin, occurring mostly as glycosides, for example, genistin, daidzin and ononin.

NOTES

the general formula biochanin A genistein

daidzein formonetin glycitein

prunetin puerarin

The Structural Formulas of Some of the Isoflavonoids

Isoflavonoids have demonstrated usefulness in the treatment of diabetes, some allergies, inflammation, bacterial and viral infections and high cholesterol and triglyceride levels. Moreover, they may be useful for the treatment of hormone-dependent diseases, as they bind to the estrogen receptor and behave as selective estrogen receptor agonists or antagonists. The effects of these compounds on cells are determined by many factors, including concentration, receptor status, presence or absence of endogenous estrogens, and the identity of the target tissue. Conjugated isoflavones are inactive compounds but become active when the glucose residue is removed. Isoflavonoids bioavailability depends, to a large extent, on the intestinal microflora, since bacterial enzymes in the intestines convert isoflavonoids into various metabolites. Of all known phytoestrogens, this group has been studied most extensively.

Isoflavonoids occur in large quantities in plants belonging to the families *Fabaceae* and *Iridaceae*. They are considered a semi-specific chemotaxonomic marker of *Fabaceae* and *Iridaceae*, but they are also found in representatives of *Cupressaceae*, *Liliaceae*, *Gramineae*, *Polygonaceae*, *Ranunculaceae*, *Lamiaceae*, *Rosaceae* and *Apiaceae* families.

The extraction of active compounds from plant material is a key step in the development of analytical methods for phytochemistry. The optimal extraction method should be simple, safe, reproducible, inexpensive, and suitable for industrial applications. Moreover, the extract should have the same isoflavone composition and profile as the plant sample. The efficient isolation of analytes requires the optimization of many parameters, including temperature, sample amount, time, and type of extraction solvent.

Extraction of isoflavones is performed using similar principles as for other polyphenols. The process is usually performed with methanol, ethanol, acetonitrile, acetone or their mixtures with water. Isoflavonoids are present in plant material in free forms, called aglycones, but they are present mostly as glycosides.

Because glycosides are relatively unstable, the extraction method must be carefully considered in order to preserve the original isoflavone profile. Several studies have shown that isolation at high temperatures causes changes in isoflavone composition due to glycoside decomposition. The most common conversions of glycosides occurring during the extraction process are decarboxylation of malonyl glucosides to acetyl glucosides and ester hydrolysis of malonyl and acetyl glucosides to underivatized glucosides. It is also possible for any conjugated isoflavone to generate the aglycone form by cleavage of the glucosidic bond. Some glycosides, including malonyl and acetyl isoflavones, are particularly unstable. Due to these potential chemical alterations, the use of drastic temperature and pressure conditions available amount of aglycones to be extracted. Therefore, in order to extract the conjugated forms of isoflavonoids intact, mild extraction techniques, such as maceration or negative pressure cavitation extraction, are often favored. In the case of the extraction of aglycones, however, more drastic methods, such as microwave-assisted extraction or accelerated solvent extraction, may be performed.

The Most Common Occurred Isoflavonoid Glycosides Decomposition

The principles of Green Chemistry, formulated in 1998 by Anastas and Warner, involve friendly products and processes. Green Analytical Chemistry (GAC), which emerged from green chemistry, involves 12 principles designed to make analytical practices more environmentally friendly. The key challenge of GAC is to reach a compromise between the quality of the results and the environmental friendliness of analytical methods.

The most important areas of GAC associated with the extraction of plant isoflavones are:

- Automation and simplification of the process.
- Increasing operator safety.
- Reduction of sample size, solvent volume and waste production.
- Elimination of toxic reagents
- Minimization of energy and time.

Generally, a significant aspect of greening laboratory practices is the need to compromise between the performance parameters, for example, accuracy, precision, sensitivity) and GAC requirements. This experiment provide descriptions and comparisons of traditional and modern extraction methods, applied in the isolation of isoflavonoids from plant material, taking into account the major achievements and important areas of discussion in the reviewed field.

Traditional Methods of Extraction of Isoflavonoids

Conventional methods, such as infusion, decoction, percolation or maceration, i.e., direct simple solvent extraction, not supported by any additional source of energy, are still frequently used in phytochemistry laboratories. These techniques, as well as extraction under reflux and Soxhlet extraction, had been the most commonly used methods for the extraction of active compounds from plant material until new extraction methods were developed. As a matter of fact, the Soxhlet method is still the most highly cited extraction technique. This method was used for the extraction of isoflavonoids from *Dalbergia oliveri* (*Fabaceae*) to examine a growth disruption of *Aedes aegypti* caused by extracts and isolated isoflavonoids. For this purpose, the authors extracted air-dried and powdered *D. oliveri* heartwood using organic solvents in the order hexane, dichloromethane, ethyl acetate, and methanol. As the dichloromethane extract containing isoflavonoids was most active against *Aedes aegypti,* it was fractionated by column chromatography. In the separated fractions, (+)-medicarpin, (±)-violanone, and formononetin were found.

Another very popular traditional technique is extraction under reflux, Hot Reflux Extraction (HRE). This technique was employed in the isolation of six major isoflavonoids from *Radix Astragalii.* The dried roots were heated with methanol and then analyzed by High-Performance Liquid Chromatography (HPLC). HRE and methanol containing 0.1% butylated hydroxytoluene and hydrochloric acid (4:1) were used by Lutz et al. for exhaustive extraction of daidzein and genistein from quinoa seeds. Hot methanol was used for the isolation of isoflavonoids from *Pueraria lobata.* Ohwi root (kudzu, Gegen), an essential plant used in traditional herbal medicines in the treatment of diabetes, cardio vascular diseases and osteonecrosis. Then the plant extract was fractionated using column chromatography. Puerarin, daidzein, genistein, and daidzin were detected by HPLC analysis of the separated fractions.

Several experiments have shown that extraction at high temperatures, for example, Soxhlet technique, HRE causes changes in the isoflavone composition

due to conversion of the malonyl forms to acetyl forms, glucosides or even to free aglycones. Extraction at room temperature is a preferable technique to avoid degradation of thermolabile compounds. This type of extraction process was performed to evaluate the concentration of isoflavones in chickpea (*Cicer arietinum* L.). In this experiment, solid samples were suspended in 80% aqueous methanol with an addition of hydrochloric acid and shaken at room temperature. The extracts obtained by centrifugation were treated with ethyl ether and afterward with ethyl acetate, dried, re-suspended in methanol/water, hydrolyzed, and analyzed by HPLC. Fotso *et al*. applied extraction at room temperature (using dichloromethane with methanol followed by extraction with methanol) to isolate a new isoflavone, seputheisoflavone, from the root of *Ptycholobium contortum.* Brummitt. A crude extract was partitioned between n-hexane, chloroform, ethyl acetate, and n-butanol. Extensive column chromatography of ethyl acetate and n-butanol fractions yielded three new isoflavonoids. Moreover, four known compounds, genistein, isoliquiritigenin, thonningiol and medicarpin, were identified. A simple and efficient isolation of biochanin A and genistein from the leaves of *Dalbergia odorifera* T. Chen was tested by Ma *et al*. Extraction of target compounds was carried out at room temperature with the use of 80% ethanol, while isoflavanones from *Amorpha fruticosa* L. roots were isolated using acetone.

In another study, a new process was designed to obtain puerarin, daidzein and a total isoflavones fraction from the stem of *Pueraria lobata.* Ohwi by means of a butanol/water two-phase solvent system, alumina column purification, and recrystallization. The findings indicated that this solvent system produced the maximum yield of total isoflavones. Moreover, with the two-phase solvent procedure outlined above, the combination of solid–liquid extraction and liquid–liquid purification is achieved for the separation of daidzein and puerarin. This process, which can be viewed as relatively economically viable, may be adjusted for the production of the analyzed isoflavones on a larger scale.

Elimination of toxic reagents during the extraction procedure was achieved in experiments conducted by Gao *et al*., and Wang et al. in which kudzu root was extracted with 70% ethanol at room temperature. To remove impurities and further enrich the active ingredients, the extract was diluted with water and purified on a resin column. Compounds present in aqueous solution were adsorbed onto the resin and then desorbed using 70% ethanol. The eluates were collected in fractions, concentrated and analyzed for the content of puerarin, daidzin and daidzein using UV spectroscopy. Another green alternative used for the isolation of isoflavonoids from *P. lobata* roots was water extraction at room temperature with a subsequent purification and concentration of active compounds by a macroporous resin column.

One of the principles of GAC is automation and simplification of analytical procedures. Soxtec extraction meets these requirements. Soxtec is an automated and safe solvent extraction unit based on Soxhlet technique. It utilizes the following processes: boiling, rinsing, solvent recovery and auto-shut down. This equipment (Soxtec HT6) was applied in the isolation of isoflavones (genistein, daidzein, and

biochanin A) from *Arachis hypogaea* L. (peanut). The compounds of interest were extracted from the samples with ethanol. HPLC analysis confirmed that the Soxtec technique isolated significantly higher amounts of Isoflavonoids compared to other methods used in the experiment (ultrasound-assisted extraction and stirring methods). Microwave-assisted extraction was the only technique that produced comparable results. The higher yield of isoflavones obtained with the use of Soxtec may be associated with the conversion of malonyl conjugates to their corresponding â-glucosides or aglycones at higher temperature. However, the total amount of isoflavones extracted was constant.

Modern Isoflavonoid Extraction Techniques

Even though they can be adapted to suit multiple purposes, traditional extraction techniques do not solve every problem in the field of extraction because they consume a lot of energy and time and require large amounts of solvents (often toxic) and relatively high amounts of starting material. Moreover, traditional extraction methods are difficult to automate, produce considerable amount of waste and pose a risk of degradation of thermolabile compounds. Modern extraction methods can be considered a remedy for these problems, as they are easy to automate, require shorter extraction times and smaller amounts of solvents, and enable simultaneous processing of several samples. These benefits and the greater effectiveness of these methods result from the use of an additional source of energy, apart from heating.

The mechanism of modern techniques is different from the mechanism of simple solvent extraction. For example, during the microwave-assisted extraction of isoflavones, microwave energy is rapidly delivered to the solvent and the plant matrix. The energy is absorbed by polar substances (e.g. water) inside the matrix. Consequently, the internal temperature of the plant cells increases. The superheating causes water vaporization within the cells, which may rupture the cell walls and plasma membranes. Since isoflavones are accumulated primarily in the vacuole and less frequently in the cell wall, the cell disruption can facilitate the mass transfer of extractant into the plant matrix and target compounds into solvent, thus allowing effective extraction.

Microwave-Assisted Extraction (MAE)

MAE was first carried out in 1986 by Ganzler et al who extracted fats from food and pesticides from soil. The major advantages of MAE over conventional methods are its high efficiency, shorter time, and reduced use of solvents as well as a high precision and reproducibility. This technique was applied, for example, in the simultaneous extraction of isoflavonoids (puerarin-4-O-glucoside, puerarin-3'-methyoxy-4'-O-glucoside, daidzein-4',7-O-glucoside, puerarin, mirificin, daidzin, 6"-O-xylosylpuerarin, 3'-methoxypuerarin, genistin, sophoraside A, ononin, daidzein, genistein and formononetin) from the root of *Pueraria lobata.* Ohwi and Pueraria *thomsonii* Benth. The findings showed that the yield of target

compounds, extracted by MAE with 65% ethanol at 100 °C, was higher than those obtained with the use of0 other methods (accelerated solvent extraction (ASE), HRE and sonication).

Other researchers optimized MAE with the response surface methodology for HPLC-fluorescence determination of puerarin and daidzein in *P. thomsonii* roots. The optimized extraction procedure was carried out by soaking the sample in 70% methanol for 30 minutes. Next, it was subjected to microwave energy (11 min, 600 W). The proposed method demonstrated good recovery, satisfactory precision, and a good linear relation.

Ecofriendly and economical variants of MAE are Microwave-Assisted Aqueous Two-Phase Extraction (MA-ATPE) and Deep Eutectic Solvent-Based Microwave- Assisted Extraction (DES-MAE). MA-ATPE was applied in the isolation of genistein and biochanin A from *Dalbergia odorifera* T. Chen leaves. Ethanol, three salts (dipotassium hydrogen phosphate/ ammonium sulfate/citrate) and deionized water were chosen to construct an Aqueous Two-Phase System (ATPS). A salt was dissolved in deionized water, then ethanol was added and all components were mixed and held until two phases were formed. The formation of an aqueous two-phase system is due to the mutual exclusion of the ions and ethanol and their high affinity for the water molecules. Dipotassium hydrogen phosphate was selected from investigated salts to form the aqueous two-phase system. Compared with traditional MAE and the extraction under reflux, the content of genistein and biochanin A in the extracts increased when the new procedure was applied. Moreover, the phase-forming salt can be recyclable. The new 'green approaches' were investigated for the extraction of genistin, genistein, and apigenin from pigeon pea (*Cajanus cajan* (L.) Millsp.) Roots. The optimum conditions for DES-MAE, proposed by the single factor and Box-Behnken design tests, were as follows: extraction with 30% of water in 1, 6-hexanediol/choline chloride, temperature of 80 °C, time 11 minutes and microwave power of 600 W. The experiments proved the superiority of DES-MAE over other extraction methods, showing higher extraction efficiency in case of the new approach. Studies have shown the potential of MA-ATPE and DES-MAE as ecological methods for fast and efficient extraction of isoflavonoids from plant samples.

Conclusions

The choice of technique for the extraction of a desired metabolite from a specific plant needs to balance the efficiency and reproducibility of extraction, the simplicity of the procedure with the cost, time, safety, and the degree of automation. Isoflavonoids are present in plant material in free forms, as aglycones, but mostly as glycosides. Consideration of the ability of extraction methods to preserve the original isoflavone profile is particularly important since some glycosides have a relatively unstable character.

Traditional extraction techniques are not sufficient to perform every job in the extraction field because they consume a lot of energy and time and require large amounts of solvents (often toxic) and a relatively high amount of sample material.

Modern extraction methods bring numerous advantages, including improved yield and selectivity, optimized extraction time, and increased quality and safety of extracts, ease of translation to an industrial scale, and environmental friendliness. We hope that this review will facilitate the laboratory and commercial applications of the most suitable isolation techniques for the isolation of isoflavonoids from plant samples.

C. Prebiotics

Theory

P**rebiotics** are compounds in food that induce the growth or activity of beneficial microorganisms such as bacteria and fungi. The most common example is in the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

Dietary prebiotics are typically non-digestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them. They were first identified and named by Marcel Roberfroid in 1995. As a functional food component, prebiotics, like probiotics, are a conceptual intermediary between foods and drugs. Depending on the jurisdiction, they typically receive an intermediate level of regulatory scrutiny, in particular of the health claims made concerning them for marketing purposes.

Definition

The definition of prebiotics and the food ingredients that can fall under this classification, has evolved since its first definition in 1995. In its earliest definition, the term prebiotics was used to refer to non-digestible food ingredients that were beneficial to the host through their selective stimulation of specific bacteria within the colon. As a result of research suggesting that prebiotics could impact microorganisms outside of the colon, in 2016 the International Scientific Association for Probiotics and Prebiotics (ISAPP) produced the following definition of prebiotics: a substrate that is selectively used by a host microorganism to produce a health benefit.

Compounds that can be classified as prebiotics must also meet the following criteria:

- Non-digestible and resistant to breakdown by stomach acid and enzymes in the human gastrointestinal tract.

- Selectively fermented by intestinal microorganisms.
- Selectively target and stimulate the growth and activity of beneficial bacteria.

Thus, consumption of prebiotics may facilitate the health of the host. Based on the previous classifications, plant-derived carbohydrate compounds called oligosaccharides are the main source of prebiotics that have been identified. Specifically, fructans and galactans are two oligosaccharide sources which have been found to stimulate the activity and growth of beneficial bacterial colonies in the gut. Fructans are a category of carbohydrate consisting of FructoOligoSaccharides (FOS) and inulins, while galactans consist of GalactoOligoSaccharides (GOS). Other dietary fibers also fit the definition of prebiotics, such as resistant starch, pectin, beta glucans, and xylooligosaccharides.

The European Food Safety Authority (EFSA), the regulatory agency for product labeling, differentiates between 'prebiotic' and 'dietary fiber', stating that 'a cause and effect relationship has not been established between the consumption of the food constituents which are the subject of the health claims and a beneficial physiological effect related to increasing numbers of gastrointestinal microbiota'. Consequently, under EFSA rules individual ingredients cannot be labeled as prebiotics, but only as dietary fiber and with no implication of health benefits.

Function

Most prebiotic research has focused on the effects that prebiotics confer on *Bifidobacteria* and *Lactobacillus.* These bacteria have been highlighted as key probiotics and beneficial gut bacteria as they may have several beneficial effects on the host in terms of improving digestion (including but not limited to enhancing mineral absorption) and the effectiveness and intrinsic strength of the immune system. Both *Bifidobacteria* and *Lactobacillus* have been shown to have differing prebiotic specificity and selectively to ferment prebiotic fiber based on the enzymes characteristic of the bacterial population.

Thus, *Lactobacilli* prefers inulin and fructooligosaccharides, while *Bifidobacteria* displays specificity for inulin, fructooligosaccharides, xylooligosaccahrides and galactooligosaccharides. A product that stimulates bifidobacteria is described as a bifidogenic factor, a concept that overlaps, but is not identical with, being prebiotic. Studies have also shown that prebiotics, besides stimulating the growth of beneficial gut bacteria, can also inhibit the growth of detrimental and potentially pathogenic microbes in the gut, such as clostridia.

Mechanism of Action

Fermentation is the main mechanism of action by which prebiotics are used by beneficial bacteria in the colon. Both *Bifidobacteria* and *Lactobacillus* are bacterial populations which use saccharolytic metabolism to break down

substrates. The bifidobacterial genome contains many genes that encode for carbohydrate-modifying enzymes as well as genes that encode for carbohydrate uptake proteins. The presence of these genes indicates that *Bifidobacteria* contain specific metabolic pathways specialized for the fermentation and metabolism of plant-derived oligosaccharides, or prebiotics. These pathways in *Bifidobacteria* ultimately produce short chain fatty acids, which have diverse physiological roles in body functions.

Sources

Prebiotic sources must be proven to confer a benefit to the host in order to be classified as a prebiotic. Fermentable carbohydrates derived from fructans and xylans are the most well documented example of prebiotics, and galactooligosaccharides are enzymatically synthesized from lactose. However, there are additional endogenous prebiotics and exogenous food sources that can be classified as prebiotic sources. Additionally, functional foods containing prebiotic food ingredients serve as an additional prebiotic food source. However, the FOS and inulin content in food sources is very low, meaning it is difficult to consume sufficient prebiotics from food alone.

Endogenous

An endogenous source of prebiotics in humans is human breast milk, which contains oligosaccharides structurally similar to GOS, referred to as human milk oligosaccharides (HMOs). These HMOs were found to increase the *Bifidobacteria* bacterial population in breastfed infants, and to strengthen the infant immune system. Furthermore, HMOs play a role in the establishment of a healthy intestinal microbiota composition of newborns.

Exogenous

Indigestible carbohydrate compounds classified as prebiotics are a type of fermentable fiber, and thus can be classified as dietary fiber. However, not all dietary fiber can be classified as a prebiotic source. In addition to the food sources highlighted in the following table, raw oats, unrefined barley, yacon, and whole grain breakfast cereals are also classified as prebiotic fiber sources. The predominant type of prebiotic fiber may vary according to the food. For instance, oats and barley have high amounts of beta-glucans, fruit and berries contain pectins, seeds contain gums, onions and Jerusalem artichokes are rich in inulin and oligofructose, and bananas and legumes contain resistant starch.

Top 10 Foods Containing Prebiotics	
Food	**Prebiotic Fiber Content by Weight**
Raw, Dry Chicory Root	64.6%
Raw, Dry Jerusalem Artichoke	31.5%
Raw, Dry Dandelion Greens	24.3%
Raw, Dry Garlic	17.5%
Raw, Dry Leek	11.7%
Raw, Dry Onion	8.6%
Raw Asparagus	5%
Raw Wheat bran	5%
Whole Wheat flour, Cooked	4.8%
Raw Banana	1%

While there is no broad consensus on an ideal daily serving of prebiotics, recommendations typically range from 4 to 8 grams (0.14–0.28 oz) for general digestive health support, to 15 grams (0.53 oz) or more for those with active digestive disorders. Given an average 6 grams (0.21 oz) serving, below are the amounts of prebiotic foods required to achieve a daily serving of prebiotic fiber.

Food	Amount of Food to Achieve 6 g Serving of Fructans
Raw Chicory Root	9.3 g (0.33 oz)
Raw Jerusalem Artichoke	19 g (0.67 oz)
Raw Dandelion Greens	24.7 g (0.87 oz)
Raw Garlic	34.3 g (1.21 oz)
Raw Leek	51.3 g (1.81 oz)
Raw Onion	69.8 g (2.46 oz)
Cooked Onion	120 g (4.2 oz)
Raw Asparagus	120 g (4.2 oz)
Raw Wheat Bran	120 g (4.2 oz)
Whole Wheat Flour, Cooked	125 g (4.4 oz)
Raw Banana	600 g (1.3 lb)

Functional Food Applications

The use of prebiotics, specifically GOS, as a fundamental ingredient in the creation of functional foods has been seen in the following food sources:

- Fermented Milks/Yogurts
- Sports/Health Drinks
- Energy Bars
- Baby Foods
- Sugar-Free Candy/Chewing Gum
- Breakfast Cereals
- Bread/Baked Goods
- Meat Products
- Pet Foods

Research

Preliminary research has demonstrated potential effects on calcium and other mineral absorption, immune system effectiveness, bowel acidity, reduction of colorectal cancer risk, inflammatory bowel disease (Crohn's disease or ulcerative colitis) hypertension and defecation frequency. Prebiotics may be effective in decreasing the number of infectious episodes needing antibiotics and the total number of infections in children aged 0–24 months.

While research demonstrates that prebiotics lead to increased production of Short-Chain Fatty Acids (SCFA), more research is required to establish a direct causal connection. Prebiotics may be beneficial to inflammatory bowel disease or Crohn's disease through production of SCFA as nourishment for colonic walls, and mitigation of ulcerative colitis symptoms.

The immediate addition of substantial quantities of prebiotics to the diet may result in an increase in fermentation, leading to increased gas production, bloating or bowel movement. Production of SCFA and fermentation quality are reduced during long-term diets of low fiber intake. Until bacterial flora are gradually established to rehabilitate or restore intestinal bacteria, nutrient absorption may be impaired and colonic transit time temporarily increased with an immediate addition of higher prebiotic intake.

D. Probiotics

Theory

Probiotics are live microorganisms intended to provide health benefits when consumed, generally by improving or restoring the gut flora. Probiotics are considered generally safe to consume, but may cause bacteria-host interactions and unwanted side effects in rare cases.

A Bottle of Yakult, a Probiotic Drink Containing *Lactobacillus paracasei*

The original theory, similar to the modern concept, but not the term, is generally attributed to Nobel laureate Élie Metchnikoff, who postulated that yoghurt-consuming Bulgarian peasants lived longer lives because of that custom. In 1907, he wrote: 'The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the microbiota in our bodies and to replace the harmful microbes by useful microbes.'

A growing probiotics market has led to the need for stricter requirements for scientific substantiation of putative benefits conferred by microorganisms claimed to be probiotic. Although there are numerous claimed benefits marketed towards using consumer probiotic products, such as reducing gastrointestinal discomfort, improving immune health, relieving constipation, or avoiding the common cold, such claims are not supported by scientific evidence, and are prohibited as deceptive advertising in the United States by the Federal Trade Commission.

In a clinical setting however, some probiotics have been found to be useful in treating specific medical conditions, such as antibiotic-associated diarrhea in children and *Clostridium difficile* infection in adults. One concern is that probiotics taken by mouth can be destroyed by the acidic conditions of the stomach. As of 2010, a number of microencapsulation techniques were being developed to address this problem.

An October 2001 report by the World Health Organization (WHO) defines probiotics as live microorganisms that, 'when administered in adequate amounts, confer a health benefit on the host'. Following this definition, a working group convened by the Food and Agriculture Organization (FAO)/WHO in May 2002 issued the *Guidelines for the Evaluation of Probiotics in Food*. A consensus definition of the term *probiotics*, based on available information and scientific evidence, was adopted after the aforementioned joint expert consultation between the FAO of the United Nations and the WHO. This effort was accompanied by local governmental and supra-governmental regulatory bodies' requirements to better characterize health claims substantiations.

That first global effort was further developed in 2010; two expert groups of academic scientists and industry representatives made recommendations for the evaluation and validation of probiotic health claims. The same principles emerged from those two groups as were expressed in the 'Guidelines' of FAO/WHO in

2002. This definition, though widely adopted, is not acceptable to the European Food Safety Authority (EFSA) because it embeds a health claim that is not measurable.

A group of scientific experts assembled in London on October 23, 2013, to discuss the scope and appropriate use of the term probiotic. That meeting was motivated by developments in the field that followed the formation of the 2001 definition, and the panel's conclusions were published in June 2014.

In Food

Live probiotic cultures are part of fermented dairy products, other fermented foods, and probiotic-fortified foods.

Some fermented products that contain Lactic Acid Bacteria (LAB) include: vegetables such as pickled vegetables, kimchi, pao cai, and sauerkraut; soy products such as tempeh, miso, and soy sauce; and dairy products such as yogurt, kefir, and buttermilk.

More precisely, sauerkraut contains the bacteria Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus pentosaceus, and Lactobacillus brevis, Leuconostoc citreum, Leuconostoc argentinum, Lactobacillus paraplantarum, Lactobacillus coryniformis, and Weissella sp. Kimchi contains the bacteria Leuconostoc spp, Weissella spp, and Lactobacillus spp. Pao cai contains L. pentosus, L. plantarum, Leuconostoc mesenteroides, L. brevis, L. lactis , L. fermentum. A list of many other bacteria found in several Asian fermented fruits and vegetables is also available. Kefir contains *Lactobacillus acidophilus, Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. bulgaricus, *Lactobacillus helveticus*, *Lactobacillus kefiranofaciens*, *Lactococcus lactis,* and Leuconostoc species. Buttermilk contains either *Lactococcus lactis* or *Lactobacillus bulgaricus*.

Probiotic Manufacturing Process

Compiling a Library of Strains

It all starts with a few bacteria from the cell bank. Vials are kept at -80°C to guarantee genetic stability during long periods. Cell bank vials are thawed and several quality controls processes are conducted in order to ensure that the strains are free of any contaminants and their genetic.

Inoculum Preparation

A strain specific culture medium rich in nutrients is prepared under sterile conditions.

Industrial Growth

The inoculum is gradually introduced in the adapted culture medium: the bacteria becomes activated and start to multiply.

Pre-Fermentation and Fermentation

Once the quality and purity of the inoculum is checked, it is transferred under sterile conditions to a pre-fermentor, where growth conditions are continuously monitored (pH temperature, pressure) the culture is then transferred to industrial fermentor.

Centrifugation or Ultra-Filtration

Live bacteria are then separated from the culture medium by centriguation or ultra-filtration: around 75% of water is removed at this stage, resulting in 50-100 times concentration of the live bacteria. The bacteria are then mixed with an adapted cryprotective formula, to help them survive the freeze drying process.

Freeze-Drying

During freeze-drying, bacteria are exposed to a very low temperature and the remaining water is eliminated by sublimation (passage from solid to gaseous phase without liquid intermediary) under low pressure. Bacteria from a solid 'cake', containing 2-4% water.

Milling

The solid cake is milled to obtain a fine, homogeneous powder. Each grain of powder may contain 1 billion of bacteria. This powder can be either delivered as bulk bacteria culture or further blended. In order to obtain the desired formulation available Kefir contains *Lactobacillus acidophilus*, *Bifidobacterium bifidum, Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus, Lactobacillus kefiranofaciens, Lactococcus lactis,* and Leuconostoc species. Buttermilk contains either *Lactococcus lactis* or *Lactobacillus bulgaricus*.

Other acidic bacteria, said to be probiotic, can also be found in Kombucha. This drink contains Gluconacetobacter xylinus. It also contains Zygosaccharomyces sp., Acetobacter pasteurianus, A. aceti, and Gluconobacter oxydans. Manufacturing process to be add after this.

Side Effects

The manipulation of the gut microbiota is complex and may cause bacteria-host interactions. Though probiotics are considered safe, some have concerns about their safety in certain cases. Some people, such as those with immunodeficiency, short bowel syndrome, central venous catheters, cardiac valve disease and premature infants, may be at higher risk for adverse events. In severely ill people

with inflammatory bowel disease there is a risk of the passage of viable bacteria from the gastrointestinal tract to the internal organs (bacterial translocation) as a consequence of bacteremia, which can cause adverse health consequences. Rarely, consumption of probiotics by children with lowered immune system function or who are already critically ill may result in bacteremia or fungemia, i.e., bacteria or fungi in the blood, which can lead to sepsis, a potentially fatal disease.

It has been suggested that *Lactobacillus* contributes to obesity in humans, but no evidence of this relationship has been found.

Global Consumption

In 2015, the global retail market value for probiotics was US$41 billion, including sales of probiotic supplements, fermented milk products, and yogurt, which alone accounted for 75% of total consumption. Innovation in probiotic products in 2015 was mainly from supplements, which produced US$4 billion and was projected to grow 37% globally by 2020. Consumption of yogurt products in China has increased by 20% per year since 2014.

Regulation

The European Food Safety Authority has rejected all petitions by commercial manufacturers for health claims on probiotic products in Europe due to insufficient research, and thus inconclusive proof of effectiveness. Occurring over many years, the scientific reviews established that a cause-and-effect relationship had not been sufficiently proven in the products submitted. The European Commission placed a ban on putting the word ‘probiotic’ on the packaging of products because such labeling misleads consumers to believe a health benefit is provided by the product when no scientific proof exists to demonstrate that health effect.

In the United States, the FDA and Federal Trade Commission have issued warning letters and imposed punishment on various manufacturers of probiotic products whose labels claim to treat a disease or condition. Food product labeling requires language approval by the Food and Drug Administration, so probiotic manufacturers have received warning letters for making disease or treatment claims. The Federal Trade Commission has taken punitive actions, including a US$21 million fine coordinated by 39 different state governments against a major probiotic manufacturer for deceptive advertising and exaggerated claims of health benefits for a yogurt and probiotic dairy drink.

Yogurt Labeling

The National Yogurt Association (NYA) of the United States gives a *Live & Active Cultures Seal* to refrigerated yogurt products that contain 100 million cultures per gram, or frozen yogurt products that contain 10 million cultures per gram at the time of manufacture. In 2002, the US Food and Drug Administration (FDA) and World Health Organization recommended that ‘the minimum viable numbers of each probiotic strain at the end of the shelf-life’ be reported on labeling,

but most companies that give a number report the viable cell count at the date of manufacture, a number that could be much higher than what exists at consumption. Because of the variability in storage conditions and time before eating, it is difficult to tell exactly how many or how much active culture remains at the time of consumption.

Allergies

There is no good evidence that probiotics are effective in preventing or treating allergies.

Antibiotic-Associated Diarrhea

Antibiotics are a common treatment for children, with 11% to 40% of antibiotic-treated children developing diarrhea. Antibiotic-Associated Diarrhea (AAD) results from an imbalance in the colonic microbiota caused by antibiotic therapy. These microbial community alterations result in changes in carbohydrate metabolism, with decreased short-chain fatty acid absorption and osmotic diarrhea as a result. A 2015 Cochrane review concluded that a protective effect of some probiotics existed for AAD in children. In adults, some probiotics showed a beneficial role in reducing the occurrence of AAD and treating *Clostridium difficile* disease.

Probiotic treatment might reduce the incidence and severity of AAD as indicated in several meta-analyses. For example, treatment with probiotic formulations including *L. rhamnosus* may reduce the risk of AAD, improve stool consistency during antibiotic therapy, and enhance the immune response after vaccination.

The potential efficacy of probiotics to treat AAD depends on the probiotic strains and dosage. One review recommended for children *L. rhamnosus* or *Saccharomyces boulardii* at 5 to 40 billion colony forming units/day, given the modest number needed to treat and the likelihood that adverse events are very rare. The same review stated that probiotic use should be avoided in pediatric populations at risk for adverse events, such as severely debilitated or immune-compromised children.

Bacterial Vaginosis

Probiotic treatment of bacterial vaginosis is the application or ingestion of bacterial species found in the healthy vagina to cure the infection of bacteria causing bacterial vaginosis. This treatment is based on the observation that 70% of healthy females have a group of bacteria in the genus *Lactobacillus* that dominate the population of organisms in the vagina. Currently, the success of such treatment has been mixed since the use of probiotics to restore healthy populations of *Lactobacillus* has not been standardized. Often, standard antibiotic treatment is used at the same time that probiotics are being tested. In addition, some groups of women respond to treatment based upon ethnicity, age, and number of sexual partners, pregnancy, and the pathogens causing bacterial vaginosis. In 2013 researchers found that

administration of hydrogen peroxide producing strains, such as *L. acidophilus* and *L. rhamnosus*, were able to normalize vaginal pH and rebalance the vaginal microbiota, preventing and alleviating bacterial vaginosis.

Blood Pressure

The consumption of probiotics may modestly help to control high blood pressure.

Cholesterol

Preliminary human and animal studies have demonstrated the efficacy of some strains of Lactic Acid Bacteria (LAB) for reducing serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its re-absorption (where it enters the blood as cholesterol).

A meta-analysis that included five double-blind trials examining the short-term (2–8 weeks) effects of a yogurt with probiotic strains on serum cholesterol levels found a minor change of 8.5 mg/dL (0.22 mmol/L) (4% decrease) in total cholesterol concentration, and a decrease of 7.7 mg/dL (0.2 mmol/L) (5% decrease) in serum LDL concentration.

Diarrhea

Some probiotics are suggested as a possible treatment for various forms of gastroenteritis, and a Cochrane Collaboration meta-analysis on the use of probiotics to treat acute infectious diarrhea based on a comprehensive review of medical literature through 2010 (35 relevant studies, >4500 participants) reported that use of any of the various tested probiotic formulations appeared to reduce the duration of diarrhea by a mean of 25 hours (vs. control groups, 95% confidence interval, 16–34 hours), also noting, however, that "the differences between the studies may be related to other unmeasured and unexplored environmental and host factors" and that further research was needed to confirm reported benefits.

Eczema

Probiotics are commonly given to breast-feeding mothers and their young children to prevent eczema, but there is no good evidence they are effective for this purpose.

Helicobacter Pylori

Some strains of Lactic Acid Bacteria may affect *Helicobacter pylori* infections (which may cause peptic ulcers) in adults when used in combination with standard medical treatments, but no standard in medical practice or regulatory approval exists for such treatment.

Immune Function and Infections

Some strains of (LAB) may affect pathogens by means of competitive inhibition, i.e., by competing for growth and some evidence suggests they may improve immune function by increasing the number of IgA-producing plasma cells and increasing or improving phagocytosis, as well as increasing the proportion of T

lymphocytes and natural killer cells. LAB products might aid in the treatment of acute diarrhea, and possibly affect rotavirus infections in children and travelers' diarrhea in adults, but no products are approved for such indications. A large study demonstrated that probiotics may decrease dental caries in children. Two reviews reported reduction of the incidence of respiratory-tract infections in adults. Probiotics do not appear to change the risk of infection in older people.

Inflammatory Bowel Disease

Probiotics are being studied for their potential to influence inflammatory bowel disease. There is some evidence to support their use in conjunction with standard medications in treating ulcerative colitis and no evidence of their efficacy in treating Crohn's disease.

A live formulation of lyophilized *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* (VSL#3) has shown effectiveness in the small clinical trials, some of which were not randomized nor double-blinded, that had been done as of 2015; more high-quality clinical trials are needed to determine safety and effectiveness.

Irritable Bowel Syndrome

Probiotics are under study for their potential to affect irritable bowel syndrome, although uncertainty remains around which type of probiotic works best, and around the size of possible effect.

Lactose Intolerance

Ingestion of certain active strains may help lactose-intolerant individuals tolerate more lactose than they would otherwise have tolerated.

Necrotizing Enterocolitis

Several clinical studies provide evidence for the potential of probiotics to lower the risk of necrotizing enterocolitis and mortality in premature infants. One meta-analysis indicated that probiotics reduce these risks by more than 50% compared with controls.

Recurrent Abdominal Pain

A 2017 review based on moderate to low-quality evidences suggests that probiotics may be helpful in relieving pain in the short term in children with recurrent abdominal pain, but the proper strain and dosage are not known.

Urinary Tract

There is no good evidence that probiotics are of benefit in the management of infection or inflammation of the urinary tract.

General Research

Formulations

Supplements such as tablets, capsules, powders, and sachets containing the bacteria have been studied. However, probiotics taken orally can be destroyed by the acidic conditions of the stomach. As of 2010, a number of microencapsulation techniques were being developed to address this problem.

Multiple Probiotics

Preliminary research is evaluating the potential physiological effects of multiple probiotic strains, as opposed to a single strain. As the human gut may contain several hundred microbial species, one theory indicates that this diverse environment may benefit from consuming multiple probiotic strains, an effect that remains scientifically unconfirmed.

Strains

There is only preliminary evidence for most probiotic health claims. Even for the most studied probiotic strains, few have been sufficiently developed in basic and clinical research to warrant approval for health claim status by a regulatory agency such as the Food and Drug Administration or European Food Safety Authority, and, as of 2010, no claims had been approved by those two agencies. Some experts are skeptical about the efficacy of different probiotic strains and believe that not all subjects benefit from probiotics.

Scientific Guidelines for Testing

First, probiotics must be alive when administered. One of the concerns throughout the scientific literature resides in the viability and reproducibility on a large scale of observed results for specific studies, as well as the viability and stability during use and storage, and finally the ability to survive in stomach acids and then in the intestinal ecosystem.

Secondly, probiotics must have undergone controlled evaluation to document health benefits in the target host. Only products that contain live organisms shown in reproducible human studies to confer a health benefit can actually claim to be probiotic. The correct definition of health benefit, backed with solid scientific evidence, is a strong element for the proper identification and assessment of the effect of a probiotic. This aspect represents a major challenge for scientific and industrial investigations because several difficulties arise, such as variability in the site for probiotic use (oral, vaginal, intestinal) and mode of application.

Thirdly, the probiotic candidate must be a taxonomically defined microbe or combination of microbes (genus, species, and strain level). It is commonly admitted that most effects of probiotics are strain-specific and cannot be extended to other probiotics of the same genus or species. This calls for a precise identification

of the strain, i.e., genotypic and phenotypic characterization of the tested microorganism.

Fourthly, probiotics must be safe for their intended use. The 2002 FAO/WHO guidelines recommend that, though bacteria may be Generally Recognized As Safe (GRAS), the safety of the potential probiotic should be assessed by the *minimum* required tests:

- Determination of antibiotic resistance patterns.
- Assessment of certain metabolic activities, for example, D-lactate production, bile salt deconjugation.
- Assessment of side effects during human studies.
- Epidemiological surveillance of adverse incidents in consumers (after market).
- If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition.
- If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required.

In Europe, the EFSA has adopted a premarket system for safety assessment of microbial species used in food and feed productions to set priorities for the need of risk assessment. The assessment is made for a selected group of microorganisms, which if favorable, leads to a 'Qualified Presumption of Safety' status.

Fifthly and finally, probiotics must be supplied in adequate numbers, which may be defined as the number able to trigger the targeted effect on the host. It depends on strain specificity, process, and matrix, as well as the targeted effect. Most of the reported benefits demonstrated with the traditional probiotics have been observed after ingestion of a concentration around 10^7 to 10^8 probiotic cells per gram, with a serving size around 100 to 200 mg per day.

Glucosamine ($C_6H_{13}NO_5$) is an amino sugar and a prominent processor in the biochemical synthesis of glycosylated proteins & lipids. Glucosamine is part of the structure of the polysacccharides, chitosan and chitin. Glucosamine in one of the most abundant mono saccharides. It is produced communically by the hydrolysis of crustacean ekosteletons or, less commonly by fomentation of a grain such as corn or wheat manufacturing.

Most glucosamine is manufactured by processing chitin from the shells of shellfish including shrimp, lobster and crabs. To meet the demands of vegetarians and others with objections to shellfish, manufacturers have Glucosamine products to market made using fungus and from fermenting corn.

E. Glucosamine

Theory

Glucosamine is one of the most popular dietary supplements sold in the United States. Most clinical trials have focused on its use in osteoarthritis of the knee. The reported adverse effects have been relatively well studied and are generally uncommon and minor. No significant supplement drug interactions involving glucosamine have been reported. The National Institutes of Health sponsored Glucosamine/chondroitin Arthritis Intervention Trial, the largest randomized, double-blind, placebo-controlled study involving the supplement, still has not confirmed whether glucosamine is effective in the treatment of osteoarthritis. Despite conflicting results in studies, there is no clear evidence to recommend against its use. If physicians have patients who wish to try glucosamine, it would be reasonable to support a 60-day trial of glucosamine sulfate, especially in those at high risk of secondary effects from other accepted treatments. The decision to continue therapy can then be left to patients on an individual basis, while the physician monitors for possible adverse effects. Glucosamine should be used with caution in patients who have shellfish allergies or asthma, and in those taking diabetes medications or warfarin.

Glucosamine and chondroitin sulfate are among the most popular dietary supplements sold in the United States. The U.S. consumer market for glucosamine and chondroitin was estimated at $810 million in 2005. Glucosamine is also one of the most studied supplements, with more than 20 randomized controlled trials involving over 2,500 patients. Glucosamine sulfate attracted the attention of the scientific community after two long-term clinical trials showed that it could slow the progression of anatomic joint structure changes in knee osteoarthritis and control the progression of symptoms. Subsequent trials have had conflicting results, including the largest study, the National Institutes of Health–funded Glucosamine/chondroitin Arthritis Intervention Trial (GAIT). Although most studies are of glucosamine alone, it is often sold in combination with chondroitin. It is not known if this combination is better than glucosamine alone, but animal studies suggest that this may be the case. This article focuses on a literature review of glucosamine and its use in osteoarthritis.

Pharmacology

Glucosamine (2-amino-2-deoxy-β-d-glucopyranose) is an endogenous aminomonosaccharide synthesized from glucose and utilized for biosynthesis of glycoproteins and glycosaminoglycans. Glucosamine is present in almost all human tissues, highly concentrated in connective tissues of the human body, and found at highest concentrations in the cartilage. In humans, about 90 percent of glucosamine is absorbed when administered as an oral dose of glucosamine sulfate, and is rapidly incorporated into articular cartilage. Glucosamine can be found in many forms, including sulfate, hydrochloride, *N*-acetyl-glucosamine, or chlorohydrate

salt, and as a dextrorotatory isomer. There is some dispute over which form is most effective. Pooled findings from studies using a specific commercial glucosamine sulfate product called Dona suggest that this formulation reduces osteoarthritis pain, whereas other formulations do not. Another study performed in China provides some evidence that glucosamine hydrochloride and glucosamine sulfate are equally effective.

The sulfate salt of glucosamine forms one half of the disaccharide subunit of keratan sulfate, which decreases in patients with osteoarthritis. Hyaluronic acid (found in articular cartilage and synovial fluid) is composed of repeating dimeric units of glucuronic acid and *N*-acetylglucosamine. Possible mechanisms of action for the chondroprotective effect of glucosamine include direct stimulation of chondrocytes, incorporation of sulfur into cartilage, and protection against degradative processes within the body through altered gene expression. The exact mechanism of action for the possible effect of glucosamine is unknown.

Uses and Effectiveness

Glucosamine has been studied for many uses, including treatment of temporomandibular joint disorder and rheumatoid arthritis, but most trials have focused on its use in osteoarthritis. Clinical trials have yielded conflicting results. Double-blind studies enrolling more than 400 persons found glucosamine and ibuprofen (Motrin) to be equally effective in reducing symptoms of knee and temporomandibular joint osteoarthritis. In four studies involving more than 500 persons, glucosamine failed to provide any meaningful improvement in symptoms. A recent study concluded that most of the trials with positive outcomes were funded by manufacturers of glucosamine products, whereas most trials performed by neutral researchers failed to find benefit.

Two of the largest placebo-controlled trials conducted before 2007 were in Europe and used the glucosamine sulfate formulation. In the 2001 Belgian study, 212 persons with osteoarthritis of the knee were followed for three years, received either placebo or oral glucosamine sulfate in a dosage of 1,500 mg daily, and were evaluated using the Western Ontario and McMaster Universities (WOMAC) osteoarthritis index. The WOMAC index is the most commonly employed questionnaire in clinical research to assess degree of pain and stiffness, as well as functional impairment caused by osteoarthritis. The study showed that patients taking glucosamine sulfate had modest pain reduction (average of 11.7 percent relative reduction in the WOMAC index compared with baseline) and reduced joint-space narrowing compared with placebo, as measured by weight-bearing anteroposterior view radiography (0.06 mm versus 0.31 mm). Both differences were statistically significant; however, there was no correlation between improvement in symptoms and radiographic findings.

In a similar 2002 trial conducted in Prague, Czech Republic, 202 patients with osteoarthritis of the knee were given placebo or 1,500 mg of Dona and were followed for three years. Those receiving Dona showed statistically significant

improvement in symptoms of pain and stiffness compared with placebo (26 percent versus 16 percent mean reduction in the WOMAC index) and radiographic evidence of decreased narrowing in the medial joint compartment (mean gain of 0.04 mm versus 0.19 mm of joint-space narrowing).

The most recent meta-analysis of glucosamine was conducted in 2005 and included 20 randomized controlled trials with a total of 2,570 patients. The investigators found that current evidence:

- Does not analyze the long-term effectiveness and toxicity of glucosamine.
- Does not differentiate which joints and which levels of severity of osteoarthritis warrant this therapy.
- Does not differentiate which dosage and route of administration are best.
- Does not demonstrate whether glucosamine modifies the long-term progression of osteoarthritis.

When restricting the analysis to eight studies with the highest-quality design, no overall improvement in pain or function was found. The investigators concluded that there was high-quality evidence that glucosamine was not as useful for symptom improvement as had been previously thought.

The authors of the 2006 GAIT also were unable to conclude whether glucosamine is useful in the treatment of osteoarthritis. GAIT was the first major clinical trial to directly compare glucosamine alone, chondroitin alone, combination glucosamine/chondroitin, a cyclooxygenase inhibitor, and placebo. It included more than 1,500 patients who were followed for six months. The WOMAC index was the primary outcome measure. Although radiographic data have yet to be published, the authors concluded that, compared with placebo, glucosamine alone or in combination with chondroitin did not reduce pain significantly after six months in patients with osteoarthritis of the knee. They did suggest that a combination of the two may be effective in a subgroup of patients with moderate to severe knee pain. An important finding in this study was a placebo effect of around 60 percent, suggesting that the sample size used was possibly inadequate. Additional concerns about the study have been raised, including the attrition rate, limitations in data analysis, and the use of glucosamine hydrochloride preparation rather than the glucosamine sulfate preparation. A 2008 study of glucosamine sulfate in more than 200 patients with hip osteoarthritis showed no reduction in symptoms or progression of arthritis compared with placebo.

Glucosamine combined with chondroitin has been used in a topical form in a few small, randomized, double-blind, placebo-controlled trials with favorable results. One study showed statistically significant improvement in pain reduction after eight weeks with a glucosamine/chondroitin preparation compared with placebo (visual analog scale measurements). There have also been some initial studies suggesting that the addition of glucosamine to Non-Steroidal Anti-

Inflammatory Drugs (NSAIDs) could decrease NSAID use in those patients already taking them. Because the anti-inflammatory ability of glucosamine is different from that of NSAIDs, it is possible the two might have a synergistic effect in alleviating some types of inflammation.

Finally, a 2007 double-blind, placebocontrolled study of 51 Japanese patients with rheumatoid arthritis showed that glucosamine hydrochloride in a dosage of 1,500 mg daily significantly improved symptoms according to patients' self-evaluation and physician global evaluation. It did not, however, alter measures of inflammation as determined through blood tests.

Contraindications, Adverse Effects, and Interactions

The reported adverse effects have been generally uncommon and minor. Glucosamine is produced from the shells of lobster, crab, and shrimp. However, the antigen proteins associated with seafood allergies are not found in the shell, and there have been no reports of reactions in persons with shell-fish allergies who take glucosamine. There also have been no significant supplement–drug interactions involving glucosamine. In one case report, the addition of glucosamine sulfate to a stable-dose regimen of warfarin (Coumadin) appeared to magnify the anticoagulant effects of warfarin in a 69-year-old man. Only one person has been reported to have had an allergic reaction to oral glucosamine.

In a large open trial (n = 1,208), the most common adverse effects of oral glucosamine sulfate (1.5 g daily) were epigastric pain or tenderness (3.5 percent), heartburn (2.7 percent), diarrhea (2.5 percent), and nausea (1 percent). There was a single case report of a glucosamine-chondroitin sulfate compound triggering difficulty walking and climbing steps because of shortness of breath in a 52-year-old woman with longstanding intermittent asthma. Finally, it has been hypothesized that glucosamine is associated with reducing the effectiveness of diabetes medications. To date, this has been refuted, and the use of glucosamine in patients with diabetes has not been shown to affect insulin sensitivity or induce insulin resistance. Nonetheless, the Arthritis Foundation recommends that patients with diabetes monitor their blood glucose levels more often when taking glucosamine. Scientific evidence for the safe use of glucosamine during pregnancy is not available.

Bottom Line

The use of glucosamine is widespread. Physicians should be encouraged to have open discussions with patients, as well as inform them about the controversy regarding the supplement's effectiveness. Because of glucosamine's potential for benefit, there is no reason to recommend against its use, especially in persons at high risk of secondary effects from other accepted treatments.

In trials that have found benefit with glucosamine, most focused on the glucosamine sulfate preparation, and most showed improvements after 30 to 90

days of therapy. Therefore, it would be reasonable to support a 60-day trial of glucosamine sulfate. The decision to continue therapy can then be left to patients on an individual basis, while the physician monitors for possible adverse effects. Caution is advised in patients with shellfish allergies or asthma, and in those taking diabetes medications or warfarin. If a patient chooses to try glucosamine therapy, the physician should recommend glucosamine sulfate (from a reputable source) in a dosage of 500 mg orally three times daily. Key points about glucosamine are summarized in *Table 1*.

Table 1 *Key Points about Glucosamine*

Effectiveness Osteoarthritis of the knee: controversial, probably effective. Pain in rheumatoid arthritis: limited data, possibly effective.
Adverse Effects Common: epigastric pain or tenderness, heartburn, diarrhea, nausea. Severe or rare: potential hypersensitivity (theoretical).
Interactions Diabetes medications: reduced effectiveness (theoretical). Warfarin (Coumadin): increased anticoagulation effect.
Contraindications Allergy to shellfish. Asthma. Use of warfarin or diabetes medications.
Dosage 500 mg orally three times daily.
Bottom line Already widely in use. No clear clinical data for or against use in the indicated conditions, but reasonable to discuss or support a 60-day trial of glucosamine sulfate, especially in patients at high risk of secondary effects from other accepted treatments.

F. Phytosterols

Theory

Phytosterols, which encompass plant sterols and stanols, are phytosteroids, similar to cholesterol, which occur in plants and vary only in carbon side chains and/or

presence or absence of a double bond. Stanols are saturated sterols, having no double bonds in the sterol ring structure. More than 200 sterols and related compounds have been identified. Free phytosterols extracted from oils are insoluble in water, relatively insoluble in oil, and soluble in alcohols.

Phytosterol-enriched foods and dietary supplements have been marketed for decades. Despite well documented LDL cholesterol lowering effects, no scientifically proven evidence of any beneficial effect on CardioVascular Disease (CVD) or overall mortality exists.

Structure

β-sitosterol	**campesterol**	**cholesterol**
stigmasterol	**Stigmastanol**	
campestanol	**brassicasterol**	**ergosterol**
lupeol	**cycloartenol**	

Nomenclature for Steroid Skeleton

The molecule on the left is β-sitosterol. Nomenclature for steroid skeleton is on the right.

- By removing carbon 24^2, campesterol is obtained.
- By removing carbons 24^1 and 24^2, cholesterol is obtained.
- Removing a hydrogen from carbons 22 and 23 yields stigmasterol (stigmasta-5, 22-dien-3β-ol).
- By hydrogenating the double bond between carbons 5 and 6, β-sitostanol (Stigmastanol) is obtained.
- By hydrogenating the double bond between carbons 5 and 6 and removing carbon 24^2, campestanol is obtained.
- Removing carbon 24^2 and hydrogens from carbons 22 and 23, and inverting the stereochemistry at C-24 yields brassicasterol ergosta-5, 22-dien-3 β-ol).
- Further removal of hydrogens from carbons 7 and 8 from brassicasterol yields ergosterol (ergosta-5, 7, 22-trien-3β-ol). Important: Ergosterol is not a plant sterol. Ergosterol is a component of fungal cell membranes, serving the same function in fungi that cholesterol serves in animal cells.
- Esterification of the hydroxyl group at carbon 3 with fatty/organic acids or carbohydrates results in plant sterol esters, i.e., oleates, ferulates and (acyl) glycosides.
- Actually, Lupeol is a triterpenoid, not strictly a sterol; it is not a gonane.

Dietary Phytosterols

The richest naturally occurring sources of phytosterols are vegetable oils and products made from them. Sterols can be present in the free form and as fatty acid esters and glycolipids. The bound form is usually hydrolyzed in the small intestines by pancreatic enzymes. Some of the sterols are removed during the deodorization step of refining oils and fats, without, however, changing their relative composition. Sterols are therefore a useful tool in checking authenticity.

As common sources of phytosterols, vegetable oils have been developed as margarine products highlighting phytosterol content. Cereal products, vegetables, fruit and berries, which are not as rich in phytosterols, may also be significant sources of phytosterols due to their higher intakes.

The intake of naturally occurring phytosterols ranges between ~200–300 mg/day depending on eating habits. Specially designed vegetarian experimental diets have been produced yielding upwards of 700 mg/day. The most commonly occurring phytosterols in the human diet are β-sitosterol, campesterol and stigmasterol, which account for about 65%, 30% and 3% of diet contents, respectively. The most common plant *stanols* in the human diet are sitostanol and campestanol, which combined make up about 5% of dietary phytosterol.

Table 1 *Sterol Composition in Crude Oils (As Percentage of Total Sterol Fraction)*

	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Δ7-Stigmasterol
Coconut oil	0.6 – 2	0 – 0.9	7 – 10	12 – 18	50 – 70	5 – 16	0.6 – 2	2 – 8
Corn oil	0.2 – 0.6	0 – 0.2	18 – 24	4 – 8	55 – 67	4 – 8	1 – 3	1 – 4
Cottonseed oil	0.7 – 2.3	0.1 – 0.9	7.2 – 8.4	1.2 – 1.8	80 – 90	1.9 – 3.8	1.4 – 3.3	0.7 – 1.4
Olive oil	0 – 0.5		2.3 – 3.6	0.6 – 2	75.6 – 90	3.1 – 14		0 – 4
Palm oil	2.2 – 6.7		18.7 – 29.1	8.9 – 13.9	50.2 – 62.1	0 – 2.8	0 – 5.1	0.2 – 2.4
Palm kernel oil	1 – 3.7	0 – 0.3	8.4 – 12.7	12.3 – 16.1	62.6 – 70.4	4 – 9	0 – 1.4	0 – 2.1
Peanut oil	0.6 – 3.8	0 – 0.2	12 – 20	5 – 13	48 – 65	7 – 9	0 – 5	0 – 5
Rapeseed oil	0.4 – 2	5 – 13	18 – 39	0 – 0.7	45 – 58	0 – 6.6	0 – 0.8	0 – 5
Soybean oil	0.6 – 1.4	0 – 0.3	16 – 24	16 – 19	52 – 58	2 – 4	1 – 4.5	1.5 – 5
Sunflower oil	0.2 – 1.3	0 – 0.2	7 – 13	8 – 11	56 – 63	2 – 7	7 – 13	3 – 6

Health Claims

EFSA

The European Foods Safety Authority (EFSA) concluded that blood cholesterol can be reduced on average by 7 to 10.5% if a person consumes 1.5 to 2.4 grams of plant sterols and stanols per day, an effect usually established within 2–3 weeks. Longer-term studies extending up to 85 weeks showed that the cholesterol-lowering effect could be sustained. Based on this and other efficacy data, the EFSA scientific panel provided the following health advisory: 'Plant sterols have been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of coronary heart disease'.

FDA

The FDA has approved the following claim for phytosterols: For plant sterol esters: (i) Foods containing at least 0.65 g per serving of plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of supplies grams of vegetable oil sterol esters. For plant stanol esters: (ii) Foods containing at least 1.7 g per serving of plant stanol esters, eaten twice a day with meals for a total daily intake of at least 3.4 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the

food] supplies grams of plant stanol esters. Reviewing clinical trials involving phytosterol supplementation, the FDA concluded that when consumed in the range of 1 to 3 grams in enriched foods, phytosterols resulted in statistically significant (5-15%) reductions in blood LDL cholesterol levels relative to placebo. The FDA also concluded that a daily dietary intake of 2 grams a day of phytosterols (expressed as non-esterified phytosterols) is required to demonstrate a relationship between phytosterol consumption and cholesterol lowering for reduced CVD risk.

Cholesterol Lowering

The ability of phytosterols to reduce cholesterol levels was first demonstrated in humans in 1953. From 1954-82, phytosterols were subsequently marketed as a pharmaceutical under the name Cytellin as a treatment for elevated cholesterol.

Unlike the statins, where cholesterol lowering has been proven to reduce risk of Cardiovascular Diseases (CVD) and overall mortality under well-defined circumstances, the evidence has been inconsistent for phytosterol-enriched foods or supplements to lower risk of CVD, with two reviews indicating no or marginal effect, and another review showing evidence for use of dietary phytosterols to attain a cholesterol-lowering effect.

Coadministration of statins with phytosterol-enriched foods increases the cholesterol-lowering effect of phytosterols, again without any proof of clinical benefit and with anecdotal evidence of potential adverse effects. Statins work by reducing cholesterol synthesis via inhibition of the rate-limiting HMG-CoA reductase enzyme. Phytosterols reduce cholesterol levels by competing with cholesterol absorption in the gut via one or several possible mechanisms, an effect that complements statins. Phytosterols further reduce cholesterol levels by about 9% to 17% in statin users. The type or dose of statin does not appear to affect the cholesterol-lowering efficacy of phytosterols.

Because of their cholesterol reducing properties, some manufacturers are using sterols or stanols as a food additive.

Safety

Phytosterols have a long history of safe use, dating back to Cytellin, the pharmaceutical preparation of phytosterols marketed in the US from 1954-82. Phytosterol esters have Generally Recognized As Safe (GRAS) status in the US. Phytosterol-containing functional foods were subject to postlaunch monitoring after being introduced to the EU market in 2000, and no unpredicted side effects were reported.

A potential safety concern regarding phytosterol consumption is in patients with phytosterolaemia, a rare genetic disorder which results in a 50- to 100-fold increase in blood plant sterol levels and is associated with rapid development of coronary atherosclerosis. Phytosterolaemia has been linked to mutations in the ABCG5/G8 proteins which pump plant sterols out of enterocytes and hepatocytes into the lumen and bile ducts, respectively. Plant sterol levels in the blood have

been shown to be positively, negatively or not associated with CVD risk, depending on the study population investigated.

The link between plant sterols and CVD or CHD risk is complicated because phytosterol levels reflect cholesterol absorption.

Sterol vs Stanol

The equivalent ability and safety of plant sterols and plant stanols to lower cholesterol continues to be a hotly debated topic. Plant sterols and stanols, when compared head to head in clinical trials, have been shown to equally reduce cholesterol levels. A meta-analysis of 14 randomized, controlled trials comparing plant sterols to plant stanols directly at doses of 0.6 to 2.5 g/day showed no difference between the two forms on total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels. Trials looking at high doses (> 4 g/day) of plant sterols or stanols are very limited, and none have yet to be completed comparing the same high dose of plant sterol to plant stanol.

The debate regarding sterol vs. stanol safety is centered on their differing intestinal absorption and resulting plasma concentrations. Phytostanols have a lower estimated intestinal absorption rate (0.02 - 0.3%) than phytosterols (0.4 - 5%) and consequently blood phytostanol concentration is generally lower than phytosterol concentration.

Research

Phytosterols are under preliminary research for their potential to inhibit lung, stomach, ovarian and breast cancers, as well as colon and prostate cancers.

Functions in Plants

Sterols are essential for all eukaryotes. In contrast to animal and fungal cells, which contain only one major sterol, plant cells synthesize an array of sterol mixtures in which sitosterol and stigmasterol predominate. Sitosterol regulates membrane fluidity and permeability in a similar manner to cholesterol in mammalian cell membranes. Plant sterols can also modulate the activity of membrane-bound enzymes. Phytosterols are also linked to plant adaptation to temperature and plant immunity against pathogens.

Phytosterols, Phytostanols and Their Esters Chemical and Technical Assessment

Phytosterols and phytostanols, also referred to as plant sterols and stanols, are common plant and vegetable constituents and are therefore normal constituents of the human diet. They are structurally related to cholesterol, but differ from cholesterol in the structure of the side chain.

Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil, or from so-called ‘tall oil’, a by-product of the manufacture of wood pulp. These sterols can be hydrogenated

to obtain phytostanols. Both phytosterols- and stanols, which are high melting powders, can be esterified with fatty acids of vegetable (oil) origin. The resulting esters are liquid or semi-liquid materials, having comparable chemical and physical properties to edible fats and oils, enabling supplementation of various processed foods with phytosterol- and phytostanol esters.

Dietary intake of phytosterols ranges from 150-400 mg /day in a typical western diet. Phytosterols and phytostanols, in free or esterified form, are added to foods for their properties to reduce absorption of cholesterol in the gut and thereby lower blood cholesterol levels. It is now generally accepted that sterols and stanols have the same cholesterol lowering efficacy.

The daily doses, considered optimal for the purpose of lowering blood cholesterol levels, are 2-3 g of phytostanols and/or phytosterols, which translates to 3.4-5.2 g in esterified form. This recommended daily dose is typically divided in 1-3 portions of food providing 1.7-5.2 g ester, which equals 1-3 g phytostanol and/or phytosterol equivalents.

Phytosterols, phytostanols and their esters have not been evaluated previously by the Committee. However, these substances have been evaluated and approved for use in foods in a number of countries world-wide (the European Union, Australia, Switzerland, Norway, Iceland, Brazil, South Africa, Japan, Turkey and Israel). Furthermore, in the USA a 'self-GRAS' (GRAS = Generally Recognized As Safe) procedure has been followed for both phytosterols- and phytostanols, to which the US FDA raised no objections.

Principle

Phytosterols and phytostanols are a large group of compounds that are found exclusively in plants. They are structurally related to cholesterol but differ from cholesterol in the structure of the side chain. They consist of a steroid skeleton with a hydroxyl group attached to the C-3 atom of the A-ring and an aliphatic side chain attached to the C-17 atom of the D-ring. Sterols have a double bond, typically between C-5 and C-6 of the sterol moiety, whereas this bond is saturated in phytostanols.

Steroid Skeleton

Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil, or from so-called 'tall oil', a by-

product of the manufacture of wood pulp. Phytosterols can be hydrogenated to obtain phytostanols. Phytosterols and phytostanols are high melting powders. Phytostanol and phytosterol esters are chemically stable materials, having comparable chemical and physical properties to edible fats and oils. The substances are insoluble in water, but soluble in non-polar solvents, such as hexane, iso-octane and 2-propanol. The esters are also soluble in vegetable fats and oils.

Three separate dossiers on different commercially available materials were submitted to the committee for the assessment of the phytosterols, phytostanols and their esters.

- Phytosterols, phytostanols and ester mixtures thereof, derived from vegetable oil distillates
- Unesterified phytosterol and phytostanol mixtures derived from tall oil. Main constituents were sitosterol (40-65%), sitostanol (16-31%), campesterol (6-15%) and campestanol (2.5-11%).
- Phytostanol ester mixtures derived from either tall oil (stanol composition: about 94% sitostanol and about 6% campestanol), or vegetable oil (stanol composition: about 68% sitostanol and about 32% campestanol).

Manufacturig

Production of Sterols from Vegetable Oil Distillates

Edible vegetable oils, extracted from oil seeds, are typically refined to remove minor oil components like phosphatides, free fatty acids, pigments and odours, with the least possible damage to the glycerides and with minimal loss of oil. The conventional or caustic refining procedure comprises degumming, neutralization, bleaching and deodorization. In physical refining, the neutralization step is omitted and the residual free fatty acids are removed in the final deodorization step.

Deodorization is the last step in the edible oil refining process in which volatiles are removed, that can cause deterioration of the oil quality during use in products (flavour, odour, colour and taste stability). This process relies on the large volatility differences between the oil itself (triglycerides) and the volatile compounds to be removed and is carried out under reduced pressure, an elevated temperature in the presence of a stripping gas. The volatiles are recovered in a vapor condenser. With caustic refining the yield of volatiles distillate is approximately 0.3 - 0.4% on the processed oil volume. This distillate mainly contains free fatty acids, but also significant levels of tocopherols (5-15%) and phytosterols (8-20%).

In a transesterification (methanolysis) step, the glycerides are converted into fatty acid methyl esters and glycerol and the phytosterol-esters into free phytosterols and fatty acid methyl esters. After removal of the methanol/ glycerol phase, the methyl esters are removed and the free phytosterols and tocopherols removed by distillation. The phytosterols are separated from the tocopherols by solvent crystallization and filtration using food grade solvent. The phytosterols are further purified by re-crystallisation, mainly to remove wax-esters.

Production of Sterols from Wood Pulp/Tall Oil

Commercially grown coniferous trees (*Pinus sp*.) are the usual source of wood that is chemically digested in the so-called Kraft pulping process. In this alkaline process the wood chips are digested at pH 14 (hence the term 'soap') for about 18 hours at 50°C to free the wood fibers. The soapy material (black liquor pulping soap) is then separated from the cellulose pulp.

The soapy lipid phase which is obtained contains more than 2% phytosterols. One way of recovering these sterols is via solvent (methanol) extraction directly from the soap, after which the phytosterols are purified by precipitation from the solvent. More commonly the tall oil soap is acidified to produce an oily phase which is a mixture of free rosin and fatty acids and neutral components, most importantly consisting of sterols, fatty alcohols, squalene, waxes and other esters. This mixture is referred to as crude tall oil.

Crude tall oil is refined into different fractions, for example, rosin acids, fatty acids by distillation, where the phytosterols are concentrated, mostly as phytosterol esters, in the residue. This is known as tall oil pitch and serves as the raw material for the production of tall oil phytosterols. The concentration of phytosterols in tall oil pitch is in the range of 5-15%.

Pure phytosterols are obtained from the tall oil pitch, mainly containing high boiling fatty acid esters, resin acids and the phytosterols. The tall oil pitch is saponified with food-grade caustic soda to hydrolyze phytosterols esters and saponify the fatty acids. The mixture is then neutralized with a food- grade mineral acid (such as sulfuric acid, hydrochloric acid or phosphoric acid). Thereafter the aqueous phase is removed and any remaining water is removed by flash evaporation. The residual pitch is distilled in a number of steps to recover the phytosterol fraction. This fraction is finally purified via solvent re-crystallization using food-grade solvents.

Production of Phytostanols from Phytosterols

Starting with the unsaturated phytosterols from any of the processes described above, pure saturated phytostanols can be obtained by hydrogenation. In this process the double-bond in the sterol molecule is saturated by the addition of hydrogen. This reaction is carried out in a suitable solvent under high hydrogen pressure, generally using a noble-metal based catalyst.

Phytostanols thus produced mainly consist of sitostanol and campestanol. Phytostanols produced from tall oil sterols typically contain ~ 90% sitostanol and ~ 10% campestanol, whereas a blend of stanols obtained from vegetable oils, typically from soybean oil, contains 68 – 75% sitostanol and 25 - 32% campestanol.

It should be noted that stanols are also naturally-occurring. Especially in tall oil phytosterols, the level of phytostanols can be as high as 15%.

Production of Phytosterol and Phytostanol Esters

Phytostanol and phytosterol esters are produced via esterification of plant stanols or sterols with fatty acids from common vegetable oils. Thus, the fatty acid

composition of the esters is similar to the parent vegetable oil used as a source of the fatty acids.

Esterification of phytosterols or phytostanols modifies the physical properties from high-melting crystalline powders with low oil solubility into liquid or semi-liquid substances that can easily be incorporated into a variety of (fat containing) foods. The proportion of the phytosterol backbone is approximately 60% by weight of the ester and that of the fatty acid tail approximately 40 % by weight.

The phytosterols and phytostanols can be esterified with fatty acids from vegetable oils by two different routes:

- Direct esterification using free fatty acids.
- Trans-esterification using fatty acid methylesters.

Free Fatty Acid Route

This process consists of two consecutive steps:

- Preparation of free fatty acids.
- Esterification of free fatty acids and phytosterols/phytostanols.

The first step comprises hydrolysis of edible vegetable oil, e.g. sunflower oil triacylglycerides to form free fatty acids. After separation of the glycerol formed, the free fatty acids are purified from the unsaponifiable fraction and residual partial glycerides by distillation. In the second step the free fatty acids and phytosterols/phytostanols are esterified to form phytosterol or phytostanol esters in a process similar to the conventional manufacture of mono-acylglycerides. This reaction is carried out at elevated temperature (>180°C) using a food grade catalyst. The reaction is carefully controlled with respect to reaction temperature and time. After the esterification reaction the excess of free fatty acids is removed by distillation.

Methylester Route

In this process two similar steps are involved:

- Preparation of fatty acid methyl esters.
- Inter-esterification of the fatty acid methyl esters and the phytosterols/ phytostanols.

The first step comprises the methanolysis of edible vegetable oils to fatty acid methylesters and glycerol. In the second step these fatty methylesters are interesterified with the phytosterols and/or phytostanols by a similar process as used for the conventional chemical interesterification of fat blends. Also here the final purification involves deodorization to remove the excess methylesters and produce bland tasting and stable esters.

Commercial Suppliers

Depending on the manufacturer, the commercial product may be a mixture of the extracted sterols, a mixture of free sterols and stanols, sterol and stanol esters or stanol esters.

2. Spirulina Cultivation (Industrial Visit)

Aim: An industrial visit to study Spirulina cultivation.

Theory

In today's vicious lifecycle of hectic schedules and never sleeping generation, health is taking a hit and it has to be the highest priority for anyone living the same life schedule. Spirulina is substance the world is quite unaware of but has been on the increase of cultivation due to its usage in nutritional supplements and medicines.

It is actually an aquatic microorganism which is often referred to be of the fungus family but as a matter of fact belongs to the bacterium family. It has various benefits including cognitive development, physical growth and immunity. It was supposedly rediscovered in the 1960s and started finding its way in market supplements only by late 20th century.

Spirulina cultivation in India has been growing at a very fast pace especially in the areas of Tamil Nadu where they find it very economical and comfortable to cultivate and sell the produce here is a complete guide on how to grow Spirulina .

Spirulina are blue-green microorganisms that grow both in the ocean and fresh water. While often referred to as algae, they are not related to the algae species. However, they can engage in photosynthesis since they are autotrophs. Spirulina is very healthy, often serving as food source.

Spirulina Farm in India

NOTES

Spirulina Drink, Energy Bars, Chikki, and Pills

Spirulina is an aquatic microorganism often referred to as an algae, though it more closely resembles bacteria. It is used as a food supplement to combat malnutrition. 1 gram of Spirulina is said to be as nutritious as 100g of spinach or carrots, and is cheaper. It has an extremely high protein content, with 60-70% of its dry weight consisting of a balanced mix of various essential amino acids. Further it is very rich in beta carotene (to produce vitamin A), iron, vitamin B12, ganna-linolenic acid and other micronutrients. It has no cell wall and is therefore very easy to digest.

It improves physical growth as well as cognitive development. It also improves immunity, and therefore helps in fighting and preventing HIV/AIDS and anaemia. Studies show it is also effective against arsenic poisoning, a condition which is extremely hard to combat.

It can be consumed directly as the paste which is harvested or dried. This is highly useful for cheap, local distribution in rural areas. Further it is also suitable to be produced industrially for the middle class. This is in the form of pills sold in pharmacies or combined with various food products, such as rice, milk products, energy bars, candy, noodles, etc.

History

Spirulina was rediscovered during a European scientific mission in Chad, as a traditional food of the locals called dihé. It was a blue-green, dried cake made of a micro-organism which grew in the natural alkaline lagoons found in this region.

It was found that people had been consuming this for centuries not only in Chad, but also in Mexico and other areas. Even though people here had extremely poor diet, they did not suffer from malnutrition.

Nutrition

Malnutrition is a pathological condition caused by the lack of essential nutrients, such as amino acids, proteins, and vitamins. Hence it is caused not only by lack of food but also due to the poor quality of the food available in less economically developed areas. It can prevent physical growth as well as cognitive development and cause many other problems, such as immune dysfunction. UN figures show that over 250 million children suffer from malnutrition.

Spirulina has very high micronutrient content, is easy and cheap to produce locally. It is therefore a very realistic and also sustainable solution to the problem of malnutrition as opposed to food fortification or distribution programs. Fortification programs try to improve the quality of food by for example adding Vitamins A and D were to milk and margarine. These are not always effective since this food is usually not available to the people in rural areas who suffer most. Similarly food distribution programs are very effective during short term emergencies, however are not long term solutions to malnutrition. Spirulina production on the other hand can effectively combat malnutrition and simultaneously provides business opportunities for locals, particularly women.

Antenna Technologies has developed simple systems for the production of Spirulina, using simple tanks and specific culture medium recipes.

Suitable Conditions

Spirulina grows in solutions of specific minerals with the correct chemical balance and a pH of 8-11. There are various different recipes for this, depending on the budget available and the conditions. It needs a minimum of 20°C to grow substantially, though a temperature of 35-37°C is most effective. A good amount of sunlight is useful if the culture has a reasonable temperature and concentration. At very low temperature, low concentration of culture, or a culture that is struggling to grow.

Advantages	Disadvantages
- Can be produced locally and so has social as well as economic benefits. - Cheap to produce. - Uses simple technology and locally available materials. - Requires much less water to grow than vegetables. - Stimulates the education of local women about nutrition. - Can be combined with other products, for example, rice, etc., to be made into locally acceptable food products all around the world. - Very effective, contains most essential micronutrients in high concentrations so 1 gram per day can combat malnutrition within a month. - Very easy to digest. - Very safe, it is resistant to most contamination due to highly alkaline environment.	- Decentralized production means women need to be trained to produce, use technology, etc. - They also need to be aided in marketing their product and managing a business. - Initially smore expensive cumbersome to implement than food fortification programmes. - To cover initial investment, subsidies may still be required. - Cooking destroys the vitamins and nutrients in Spirulina so cannot be combined with all foods. - Does not combat iodine or folic acid deficiency.

Construction, Operations and Maintenance

A concentrated Spirulina culture is then used to seed the pond containing culture medium. This can be obtained from culture floating on an existing pond, or recently harvested. This is mixed into the culture medium and allowed to grow. It should be regularly agitated using an electric pump or by stirring manually. The temperature, pH and concentration of algae should be monitored. Once the concentration increases to about 0.5g/L (use a Secchi disk to measure) it must be harvested. This can be done by simply filtering it through a cloth to obtain a 'biomass' of about 10% dry matter per litre. The biomass obtained is then pressed in a cloth to produce a kind of cake. The culture medium can then be reused, by adding any of the ingredients which were used up by the Spirulina.

Harvesting Spirulina

Extruded Spirulina Laid Out to Dry

Empty Spirulina Tank

Spirulina is most nutritious in its wet form. However this lasts at most for a few days if refrigerated, and only a few hours at room temperature. Hence if it needs to be transported or stored it must be dried. If dried and packaged well it can be stored for at least a year without losing nutritional value. However if dried it acquires an unpleasant smell and taste, and is inconvenient to use. It can then also be combined with various other food products or simply packaged on its own.

The production of Spirulina requires manufacturing of a tank. The size of this depends on the scale of production, and the number of tanks. 1 tank of 18m2 produces approximately 150g of Spirulina per day.

Materials Required for Spirulina Culture

- 2 Polyethylene Sheets 2mm Thick.
- 4 Frames.
- 3 Bars (Metal/Bamboo, etc.).
- Bricks, Breeze Blocks, Packed Earth, or Planks for Walls.

Build up the walls, approximately 30cm high with packed earth, bricks or planks. The material to be used depends on the weather conditions, and presence of rodents, termites, etc. Cover the sides and the bottom with one polyethylene sheet. Create a temporary compartment (200L) to produce Spirulina needed to seed the whole tank. Reinforce the walls with metal/bamboo frames and cover with a second plastic sheet.

Spirulina is grown in large water tanks that are made of cement or plastic. Though it can be of any large size, the standard practice uses a tank of 10 × 5 × 1.5 feet. This is to ensure the ratio of other organic and inorganic products added to produce the best yield of Spirulina possible in the given area. A pump is also required to draw water and pump in water to the tank. It should be efficiently able to pump 1000 L of water as that's the amount required which will be filled up to a height of 2-3 feet in the tank.

Apart from these you require thermometer, pH sensors, air compressors, etc. for checking various growing conditional parameters. There is also a list of chemicals like urea, sodium chloride, sodium bi carbonate, sulphates of magnesium, potassium and ferrous, and phosphoric acid which is required for creating a suitable culture medium. The mixture after adding a kilogram Spirulina mother culture should be agitated for a week for about half an hour daily using a long stick.

Maintenance

This is a continuous process and can be carried out through the years. It is important to maintain the tank and repair if any damages incur during the process make sure to not overfill the tanks in any case as that will not gain you profits in any way rather make losses.

The culture medium and tanks need to be protected from contamination by foreign algae, insects and toxicity. Also the level of the pond and amount of nutrients needs to be maintained by regularly replacing the fertilizers and water. Further the temperature and pH need to be maintained. It is useful to replace a small amount of the solution with a completely fresh amount, to prevent deterioration of the culture medium.

Further, tanks usually need to be replaced or repaired after 3-4 years. Other equipment may also have to be replaced.

Required Conditional Parameters

Before deciding to start cultivating Spirulina, one should have all the resources and requirements ready. First and foremost, of that is the climatic conditions of the area. This is something which you cannot change and might end up spending a lot in trying to create a suitable artificial environment for the cultivation. The required temperature for good produce of Spirulina is between 25 °C and 35 °C.

Also, places that have good amount of sunlight present throughout the cultivation process is most suitable. The best temperature range is however around 35-37 °C. Cultivation during the rains or at places which are very wet should be avoided since that can contain the amount of sunlight which is necessary for a good yield. So, this is the first step of deciding whether your location is suitable for the cultivation of Spirulina and if it is move on to next steps.

Cultivating Procedure

Having a suitable tank is the first requirement and can be either made artificially in the form of tank or a pre-engineered tank can be used for the process. The culture medium has to be created after this and that can be done by basically mixing all the materials. All the chemicals are added to 1000 L of water and mixed thoroughly. Seeding is done with concentrated Spirulina mother culture which then multiplies in the medium over a period of time.

The pH, which is a level of basicity and acidity of the culture medium has to be monitored at regular intervals. Concentration of algae is the deciding factor for when Spirulina can be and should be harvested. Usually, the measure is about 0.5g of algae per litre of culture medium.

A Secchi disk can be used to measure this. Spirulina is a very nutritious substance and tends to lose its nutrition value very soon, almost within hours of harvest and hence it needs to be stored properly.

Harvesting of Spirulina

The algae concentration in the tank is the deciding factor for harvesting Spirulina. Like mentioned above, 0.5g/L is the ideal concentration. This is usually achieved 10 days after the seeding process is complete. The algae are collected and passed through a simple filter which drains out the water. The nutrient-laden water is then

returned to the ponds. Spirulina has a very high amount of protein comprising 55 to 77% of its weight when dried. Since protein is one of the most expensive nutrients to purchase, Spirulina production can help impoverished regions meet their protein intake demands. Manufactures also mix Spirulina in milk or juice to create energy drinks.

This is one of the most efficient **Spirulina cultivation methods**. After the filtration process, the algae are pressed upon by large weights to further reduce the moisture content. The product is now pretty dry and can be sent for further processing which is done in machines.

Next process involves processing the algae through machines which are used for making noodles and thin strips of algae is made for the ease of further processing. The noodle shaped algae are kept on a clean cloth and is dried under the sun.

It is allowed to dry foe a couple hour under the hot sun. It endures that the algae have completely dried now and is ready for the next process. The next step involves grounding of the algae in a similar way flour is produced.

The Spirulina is grounded and made into a powder which is then further tested. The testing of Spirulina is done in laboratories where they check the edibility of the product and mark it safe for consumption or not.

Leftover Water

Farmers can pump the leftover nutrient-rich water to minimize fertilizing costs. Spirulina replenish Oxygen in ponds. But the large number of nutrients can also encourage harmful algae growth.

Challenges in Spirulina Cultivation

There are certain challenges faced during the cultivation of Spirulina. Be careful with the temperature of the water and start cultivation with a clean tank to avoid any discolouration of the medium. Allow enough sunlight or the cultivation might turn pale and growth will stop.

Sometimes there could be foam formation in which case some ash water can be added to reduce foaming effect which will take a hit ton the produce. Urea is very important in the cultivation and if a gelatine type substance starts forming, it can make the mixing almost impossible. In that case, more urea should be added to the medium.

Do not allow the mixture to reach a stage where they might smell like ammonia. It is not good for the cultivation process and more water should be added. Keep the nutrient check on scale especially if the mixture starts turning lime green. There could be problems especially when you are starting out new and the harvesting should be done at the right time.

Cost of Cultivation and Profits

Spirulina cultivation is a low investment and high-income farming. Those who have been in the business for a while know the economic benefits of it. The Spirulina farming cost is low and the profits that can be obtained are quite high. Finding buyers for the produce is also easy given there isn't much availability of it in the market and the produce can be sold for almost as high as Rs 1200/kg.

The cost of cultivation depends on the cost of raw materials, local labour, packaging and logistics and are dependent on the local market. It may vary from place to place.

Training on Cultivation

There are various programmes out in the market that provide Spirulina cultivation training to many people. Skill training in the field is very important to not make silly mistakes which might spoil the whole yield hence the training process is precise and doesn't take very long to learn either.

Once you have obtained the certification and the training you can either be your own boss and start cultivating in your own backyard or you can manage someone else's cultivation. The market is open and flexible to **Spirulina cultivation** and the scope is huge given it has a lot of benefits.

After this you are at a better position to cultivating and selling Spirulina in the market. It is one of the most efficient and simplest ways of earning money. The uses of Spirulina are immense and you are never going to run of buyers given you maintain quality and standard of the product.

Protection

Spirulina cultures change rapidly if not cared for properly cultures can grow quickly or perish in under a few hours. Spirulina ponds are easily contaminated by toxic microorganisms, and farmers must carefully control environmental conditions. Therefore Spirulina must grow in man mad pods. The water must be kept between 84 to 95 degrees Fahrenheit at all times.

FOOD SERVICE MANAGEMENT

1. CAUSES AND PREVENTION OF FOOD-BORNE ILLNESSES IN FOOD SERVICE OPERATIONS

Aim: To study the causes and relevant prevention related to food-borne illnesses.

Theory

Foodborne illness, also foodborne disease and colloquially referred to as food poisoning is any illness resulting from the spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as toxins such as poisonous mushrooms and various species of beans that have not been boiled for at least 10 minutes.

Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made, for example, the incubation period ranges from hours to days, depending on the cause and on how much was consumed. The incubation period tends to cause sufferers to not associate the symptoms with the item consumed, and so to cause sufferers to attribute the symptoms to gastroenteritis.

Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes, like bacteria, can pass through the stomach into the intestine and begin to multiply. Some types of microbes stay in the intestine, some produce a toxin that is absorbed into the bloodstream, and some can directly invade deeper body tissues.

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. There is a consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as food safety. Foodborne disease can also be caused by a large variety of toxins that affect the environment.

Furthermore, foodborne illness can be caused by pesticides or medicines in food and natural toxic substances such as poisonous mushrooms or reef fish.

Food safety is a scientific discipline describing handle, preparation, and storage of food in ways that prevent food-borne illness. The occurrence of two or more cases of a similar illnesses resulting from the ingestion of a common food is known as a food-borne disease outbreak. This includes a number of routines that should be followed to avoid potential health hazards. In this way food safety often overlaps with food defense to prevent harm to consumers. The tracks within this line of thought are safety between industry and the market and then between the market

and the consumer. In considering industry to market practices, food safety considerations include the origins of food including the practices relating to food labeling, food hygiene, food additives and pesticide residues, as well as policies on biotechnology and food and guidelines for the management of governmental import and export inspection and certification systems for foods. In considering market to consumer practices, the usual thought is that food ought to be safe in the market and the concern is safe delivery and preparation of the food for the consumer.

Food can transmit pathogens which can result in the illness or death of the person or other animals. The main mediums are bacteria, viruses, mold, and fungus (which is Latin for mushroom). It can also serve as a growth and reproductive medium for pathogens. In developed countries there are intricate standards for food preparation, whereas in lesser developed countries there are fewer standards and less enforcement of those standards. Another main issue is simply the availability of adequate safe water, which is usually a critical item in the spreading of diseases. In theory, food poisoning is 100% preventable. However this cannot be achieved due to the number of persons involved in the supply chain, as well as the fact that pathogens can be introduced into foods no matter how many precautions are taken. The five key principles of food hygiene, according to WHO, are:

- Prevent contaminating food with pathogens spreading from people, pets, and pests.
- Separate raw and cooked foods to prevent contaminating the cooked foods.
- Cook foods for the appropriate length of time and at the appropriate temperature to kill pathogens.
- Store food at the proper temperature.
- Use safe water and safe raw materials.

Food Safety Measures

- Access to sufficient amounts of safe and nutritious food is key to sustaining life and promoting good health.
- Unsafe food containing harmful bacteria, viruses, parasites or chemical substances, causes more than 200 diseases – ranging from diarrhoea to cancers.
- An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years (DALYs).
- Children under 5 years of age carry 40% of the foodborne disease burden, with 125 000 deaths every year.

- Diarrhoeal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230 000 deaths every year.
- Food safety, nutrition and food security are inextricably linked. Unsafe food creates a vicious cycle of disease and malnutrition, particularly affecting infants, young children, elderly and the sick.
- Foodborne diseases impede socioeconomic development by straining health care systems, and harming national economies, tourism and trade.
- Food supply chains now cross multiple national borders. Good collaboration between governments, producers and consumers helps ensure food safety.

Foodborne Illness Statistics

The Center for Disease Control and Prevention (CDC) estimates roughly 1 in 6 Americans (48 million people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases each year. Symptoms of foodborne illness include upset stomach, abdominal cramps, nausea, vomiting, diarrhea, fever, and dehydration; they can range from mild to severe and death.

Foodborne illness can affect anyone who eats contaminated food; however, certain populations are more susceptible to becoming ill with a greater severity of illness. These populations include infants and children, the elderly, pregnant women, people taking certain kinds of medications or immune suppressed (e.g., cancer patients, diabetics). Foodborne illness are usually infections or topic in nature and caused by bacteria, virus, parasites or chemical substances entering through contaminated for the water.

To prevent foodborne illness, it is necessary to understand how food becomes unsafe to eat and what proactive measures can be taken to keep food safe.

Causes of Foodborne Illness

The causes fall into the following 3 categories:

- **Biological hazards** include bacteria, viruses, and parasites. Bacteria and viruses are responsible for most foodborne illnesses. Biological hazards are the biggest threat to food safety. They can be inherent in the product or due to mishandling.
- **Chemical hazards** include natural toxins and chemical contaminants. Some natural toxins are associated with the food itself, i.e., certain mushrooms, PSP in molluscan shellfish, some are made by pathogens in the food when it is time/temperature abused, i.e., histamine development in certain seafood species. Some additives, such as sulfites, can be a hazard to some people. Chemical contamination can occur when products, i.e., cleaners are not used correctly.

- o **Food allergens** are a chemical hazard. Some people are sensitive to proteins in foods. Every food is different. Eight major food allergens include milk, eggs, fish, crustacean shellfish (lobster, crab, shrimp), wheat, soy, peanuts, tree nuts.

- **Physical hazards** can include metal shavings from cans and plastic pieces or broken glass.

Microbiology of Foodborne Illness

Bacteria are single-celled organisms which multiply by cell division, under appropriate environmental conditions. The conditions that influence bacterial growth are the food itself, acidity, time, temperature, oxygen, and moisture. Most bacteria need nutrients to survive. They obtain these nutrients from food. Bacteria grow best in food that is neutral to slightly acidic (acidity is measured by pH). Microorganisms have different acidity (pH), temperature, and oxygen requirements for optimal growth. Bacteria need time to grow and they grow rapidly between 41°F and 140°F. Bacterial growth is slowed at temperatures below 41°F and limited at temperatures above 140°F. Some bacteria require oxygen to grow (aerobic), some grow when there is no oxygen (anaerobic), and some can grow with or without oxygen (facultative). Bacteria will grow when food and water is available. If water is bound or tied up with, for example salts or sugars, it is not available to be used by bacteria. This concept of available water is referred to as water activity (Aw).

Some Bacteria can be Further Categorized

- Some bacteria are spore formers. The spore protects the organism during periods of environmental stress. When the conditions become suitable, the organism germinates from the spore and continues the growth cycle.
- Some bacteria produce toxins that cause illness.

Molds are a multi-cellular fungi that reproduce by fruiting bodies that break and release thousands of microscopic mold spores, each capable of growing under the right conditions. Molds can send ‘roots’ into the food to provide nourishment to the spore. Molds prefer damp, dark environments for optimal growth and they grow readily on almost any food, as well as walls, ceilings, and other areas of high moisture. Some molds produce toxins that can cause illness.

Viruses are the smallest known organisms. They cannot multiply in food they need a human host. Viruses are transmitted to food from infected people. Non-virus infections are characterised by nausea, vomiting, water, diarrhoea, Hepatitis. A virus can cause long lasting liver diseases & spreads typically through raw or under covered food.

Parasites include worms and protozoa. They cannot multiply in food; they multiply in a host cell. Some parasites such as fishborne trematodes, are only transmitted through food. Others, for example, tapeworms like Taenia Solium, may infect people through food or direct contract with animals.

Types of Foodborne Illness

Pathogens can cause different types of foodborne illness. Once a contaminated food is eaten, illness can be caused by the pathogens themselves (foodborne infection); caused by toxins produced in the food by pathogens (foodborne intoxication); and caused by toxins produced in the body by pathogens (foodborne toxin-mediated infection).

Prevention of Foodborne Illness

Following are some simple steps to keep food safe:

Cleanliness

Wash hands and food contact surfaces and utensils often, between tasks, and if they have become contaminated. Effective cleaning involves removing soil and debris, scrubbing with hot soapy water and rinsing, using potable/drinking water. Sanitizing involves the use of high heat or chemicals to reduce or eliminate the number of microorganisms to a safe level.

- Wash hands with warm water and soap for 20 seconds and dry with a disposable paper towel or clean hand cloth.
- Alcohol based hand sanitizers are not a replacement for handwashing. They are not effective if the hands are dirty, they are not effective against Norovirus, and they do not eliminate all types of microorganisms.
- Wash cutting boards, dishes, and utensils after preparing each food item and before you use it for the next food.
- Use hot, soapy water, rinse with hot water, and air dry or dry with a clean paper towel or clean dish cloth.
- Or wash in the dishwasher.
- Wash countertops after preparing each food item and before you use it for the next food.
- Use paper towels or clean dish cloths to wipe kitchen surfaces or spills.
- Wash countertops with hot soapy water, rinse with hot water and air dry or dry with a clean paper towel or clean dish cloth.

To sanitize for added protection for bacteria on surfaces, you can use the following:

- Dilute mixtures of chlorine bleach and water are a cost-effective method of sanitation. Chlorine bleach is a very effective sanitizer. It comes in several concentrations.

- If bleach is 8.25%: measure 1 teaspoon of bleach per 1 gallon of water or 1/8 teaspoon of bleach per 1 pint of water.
- Apply to the cleaned countertop and allow to sit for 1-2 minutes and air dry or dry with a clean paper towel.
- Alternatively, commercial products for sanitizing the home kitchen are available. Follow manufacturer instruction for use.
- Wash dish cloths often in a washing machine.
- Store sponge in a place so it can dry after use.
- To lower the risk of cross-contamination, sanitize the dish sponge often:
- Soak in a chlorine bleach solution for 1 min. See the following link on instructions for the bleach solution: Microwave heat a damp sponge for 1 min.
- Put sponge in dishwasher cycle.
- Replace the dish sponge often.

Preventing Cross Contamination

Separate to prevent cross contamination. Cross contamination is the transfer of harmful bacteria from uncooked food products, like raw meat, fish, and poultry or unclean people, countertops, and kitchen equipment to ready-to-eat foods.

Prevent cross contamination when grocery shopping.

- Physically separate raw meat, fish and poultry to prevent their juices from dripping onto other foods. This can be done by:
- Segregating raw meat, fish and poultry on one side of the shopping cart.
- Placing raw meat, fish and poultry in separate plastic bags (e.g. one bag for chicken, one bag for fish, etc.).

Designate reusable bags for grocery shopping only. Reusable bags for raw meat, fish, or poultry should never be used for ready-to-eat products.

- Frequently wash bags. Cloth bags should be washed in a machine and machine dried or air-dried. Plastic-lined bags should be scrubbed using hot water and soap and air-dried.
- Separate raw meat, fish and poultry in disposable plastic bags before putting them in a reusable bag
- Check that both cloth and plastic-lined reusable bags are completely dry before storing.

Prevent cross contamination when storing food in the refrigerator.

- In the refrigerator, store raw meats, fish, and poultry below ready-to-eat and cooked foods.

- When thawing frozen raw meat, fish and poultry, put the food in a plastic bag or on a plate on the lowest shelf to prevent juices from dripping onto other foods.

After thawing in the refrigerator, food should remain safe and of good quality for a few days before cooking. Food thawed in the refrigerator can be refrozen without cooking, although quality may be impacted. See *Chill* section for other methods for thawing.

Prevent cross contamination when handling, preparing, and serving food.

- Thoroughly wash your hands before and after handling different foods, after using the bathroom, and anytime they can become contaminated.
- Use separate cutting boards for Non veg.
- Wash and rinse cutting board, knives, and preparation area after cutting raw meat, fish or poultry. These items can be sanitized after cleaning.

Cook

Cook food thoroughly and use a thermometer to verify the proper temperature was reached.

To determine that the proper temperature was reached, place a food thermometer in the thickest part of the food and allow the it to equilibrate.

- Make sure it's not touching bone, fat, or gristle.
- For whole poultry, insert the thermometer into the innermost part of the thigh and wing and the thickest part of the breast.
- For combination dishes, place the thermometer in the center or thickest portion.
- Egg dishes and dishes containing ground meat or poultry should be checked in several places.

Clean your food thermometer with hot, soapy water before and after each use!

Food Thermometers – Why Use them?

Not only is it important to monitor the refrigerator temperature (chill foods); but using a thermometer is the only reliable way to ensure that a food is properly cooked.

Cooking

- **Color** is not a reliable indicator that the food has been cooked to the correct temperature to ensure that foodborne pathogens – bacteria, viruses – are destroyed. Determining "doneness" of hamburger cannot be safely done by looking at the brown color of the meat or of chicken by looking that the juices run clear.

- **Time** alone as an indicator that the food is cooked properly could result in a potential food safety hazard. Recipes may state 'x minutes/ pound'. However, different thicknesses of a food or ingredients that are used can alter the time needed at a specific temperature to make sure the food has reached the correct temperature to kill all pathogens.

  Food thermometers come in several types and styles and range in level of technology and price. There is a lot of good information on how to use a thermometer correctly, proper placement, and how to check to see if it is accurate. .

  Finally, pop-up temperature devices are commonly found in turkeys or oven roaster chickens. These devices have been around for a long time and indicate that the food has come to the correct temperature for safety. However, while these pop-up thermometers are reliable, it is often recommended that the temperature be checked in several places with a conventional thermometer to be sure.

Chill

Chill foods promptly. Cold temperatures slow the growth of harmful bacteria. Cold air must circulate to help keep food safe, so do not over fill the refrigerator. Maintain the refrigerator temperature at 41°F or below. Place an appliance thermometer in the rear portion of the refrigerator, and monitor regularly. Maintain the freezer temperature at 0°F or below:

- Refrigerate and/or freeze meat, poultry, eggs and other perishables as soon as possible after purchasing.
- Consider using a cooler with ice or gel packs to transport perishable food.
- Perishable foods, such as cut fresh fruits or vegetables and cooked food should not sit at room temperature more than two hours before putting them in the refrigerator or freezer (one hour when the temperature is above 90°F).
- There are three safe ways to thaw food: in the refrigerator (see Separate), in cold water, and in the microwave. Food thawed in cold water or in the microwave should be cooked immediately.
- Submerging the food in cold water. It is important to place the food in a bag that will prevent the water from entering. Check the water every 30 minutes to make sure it is cold. Cook food prior to refreezing.
- Microwave thawing. Cook food immediately once thawed because some areas of the food may become warm and begin to cook during the thawing process. Cook food prior to refreezing.
- Cool leftovers quickly by dividing large amounts into shallow containers for quicker cooling in the refrigerator.

Bacteria

- ***Salmonella, Campylobacter, and Enterohaemorrhagic Escherichia coli*** are among the most common foodborne pathogens that affect millions of people annually–sometimes with severe and fatal outcomes. Symptoms are fever, headache, nausea, vomiting, abdominal pain and diarrhoea. Examples of foods involved in outbreaks of salmonellosis are eggs, poultry and other products of animal origin. Foodborne cases with *Campylobacter* are mainly caused by raw milk, raw or undercooked poultry and drinking water. *Enterohaemorrhagic Escherichia coli* is associated with unpasteurized milk, undercooked meat and fresh fruits and vegetables.
- ***Listeria*** infection leads to unplanned abortions in pregnant women or death of newborn babies. Although disease occurrence is relatively low, listeria's severe and sometimes fatal health consequences, particularly among infants, children and the elderly, count them among the most serious foodborne infections. Listeria is found in unpasteurised dairy products and various ready-to-eat foods and can grow at refrigeration temperatures.
- ***Vibrio cholerae*** infects people through contaminated water or food. Symptoms include abdominal pain, vomiting and profuse watery diarrhoea, which may lead to severe dehydration and possibly death. Rice, vegetables, millet gruel and various types of seafood have been implicated in cholera outbreaks.

Antimicrobials, such as antibiotics, are essential to treat infections caused by bacteria. However, their overuse and misuse in veterinary and human medicine has been linked to the emergence and spread of resistant bacteria, rendering the treatment of infectious diseases ineffective in animals and humans. Resistant bacteria enter the food chain through the animals. Antimicrobial resistance is one of the main threats to modern medicine.

Chemicals

Of most concern for health are naturally occurring toxins and environmental pollutants:

- Naturally occurring toxins include mycotoxins, marine biotoxins, cyanogenic glycosides and toxins occurring in poisonous mushrooms. Staple foods like corn or cereals can contain high levels of mycotoxins, such as aflatoxin and ochratoxin, produced by mould on grain. A long-term exposure can affect the immune system and normal development, or cause cancer.
- Persistent Organic Pollutants (POPs) are compounds that accumulate in the environment and human body. Known examples are dioxins and Polychlorinated Biphenyls (PCBs), which are unwanted by-products of industrial processes and waste incineration. They are found worldwide in

the environment and accumulate in animal food chains. Dioxins are highly toxic and can cause reproductive and developmental problems, damage the immune system, interfere with hormones and cause cancer.

- Heavy metals such as lead, cadmium and mercury cause neurological and kidney damage. Contamination by heavy metal in food occurs mainly through pollution of air, water and soil.

2. THE LEVELS OF MANAGEMENT AND THE VARIOUS PRODUCTION AND SERVICE POSITIONS IN A FOOD OPERATION (FIELD VISITS)

Aim: To study various levels of management in a food operation.

The Levels of Management

Segmenting the management of an organization into levels is vital to maintaining the productivity and work performance of employees. Although when there is a change in the size of the business or the workforce, there would also be a change in the number of levels of the management.

Three Levels of Management

The three levels of management provide a separation between the managerial positions of the organization. The administrative rank of an organization worker determines the extent of authority, the status enjoyed and the chain of command that can be controlled by the worker. There are three levels of management found within an organization, where managers at these levels have different roles to perform for the organization to have a smooth performance, and the levels are:

- Top-Level Management/Administrative Level
- Middle-Level Management/Executory
- Low-level Management/Supervisory

The levels of Management and their Functions are discussed below:

Top Level Management

The Top-Level Management is also referred to as the administrative level. They coordinate services and are keen on planning. The top-level management is made up of the Board of Directors, the Chief Executive Officer (CEO), the Chief Financial Officer (CFO) and the Chief Operating Officer (COO) or the President and the Vice President.

The Top level management controls the management of goals and policies and the ultimate source of authority of the organization. They apply control and coordination of all the activities of the firm as they organize the several departments of the enterprise which would include their budge, techniques, and agendas.

The Top-level management is accountable to the shareholders for the performance of the organization. There are several functions performed by the top-level management, but three of them are the most important, and they are:

- To lay down the policies and objective of organization.
- Strategizing the plans of the enterprise and aligning competent managers to the departments or middle level to carry them out.
- Keeping the communication between the enterprise and the outside world.

Middle Level of Management

The Middle Level Management is also referred to as the executory level, they are subordinates of the top-level management and are responsible for the organization and direction of the low-level management. They account for the top-level management for the activities of their departments.

The middle-level managers are semi-executives and are made up of the departmental managers and branch manager. They could be divided into senior and junior middle-level management if the organization is big. They coordinate the responsibilities of the sub-unit of the firm and access the efficiency of lower-level managers.

The middle-level managers are in charge of the employment and training of the lower. They are also the communicators between the top level and the lower level as they transfer information, reports, and other data of the enterprise to the top-level. Apart from these, there are three primary functions of the middle-level management in the organization briefed below:

- To carry out the plans of the organization according to policies and directives laid down by the top level management.
- To organize the division or departmental activities.
- To be an inspiration or create motivation or junior managers to improve their efficiency.

Lower Level of Management

The lower level Management is also referred to as the supervisory of the operative level of managers. They oversee and direct the operative employees. They spend most of their time addressing the functions of the firm, as instructed by the managers above them.

The lower level managers are the first line of managers as they feature at the base of operations, so they are essential personnel that communicates the fundamental problems of the firm to the higher levels. This management level is made up of the foreman, the line boss, the shift boss, the section chief, the head

nurse, superintendents, and sergeants. They are the intermediary, the solve issues amidst the workers and are responsible for the maintenance of appropriate relationship within the organization. They are also responsible for training, supervising and directing the operative employees.

The lower managers represent the management to the operative workers as they ensure discipline and efficiency in the organization. The duty of inspiration and encouragement falls to them, as they strengthened the workforce. They also organize the essential machines, tools and other materials required by the employees to get their job done.

Briefed below are the primary function of the lower-level management:

- To allocate tasks and responsibilities to the operative employees.
- To ensure quality and be responsible for the production quantity.
- To communicate the goals and objective of the firm laid down by the higher level.
- Managers to the employees and also the suggestions, recommendations, appeals and information concerning employee problems to the higher level managers.
- To give instruction and guided direction to workers on their day to day jobs.
- To give periodic reports of the workers to the higher level managers.

Operations Management for Services

Operations management for services has the functional responsibility for producing the services of an organization and providing them directly to its customers. It specifically deals with decisions required by operations managers for simultaneous production and consumption of an intangible product. These decisions concern the process, people, information and the system that produces and delivers the service. It differs from operations management in general, since the processes of service organizations differ from those of manufacturing organizations.

In a post-industrial economy, service firms provide most of the GDP and employment. As a result, management of service operations within these service firms is essential for the economy.

The services sector treats services as intangible products, service as a customer experience and service as a package of facilitating goods and services. Significant aspects of service as a product are a basis for guiding decisions made by service operations managers. The extent and variety of services industries in which operations managers make decisions provides the context for decision making.

The six types of decisions made by operations managers in service organizations are: process, quality management, capacity and scheduling, inventory, service supply chain, and information technology.

Definition of Services

There have been many different definitions of service. Russell and Taylor (2011) state that one of the most pervasive, and earliest definitions is 'services are intangible products'. According to this definition, service is something that cannot be manufactured. It can be added after manufacturing, for example, product repair or it can stand alone as a service, for example, dentistry delivered directly to the customer. This definition has been expanded to include such ideas as 'service is a customer experience'. In this case the customer is brought into the definition as the experience the customer receives while 'consuming' the service.

A third definition of service concerns the perceived service as consisting of physical facilitating goods, explicit service and implicit service. In this case the facilitating goods are the buildings and inventory used to provide the service. For example, in a restaurant the facilitating goods are the building and the food. The explicit service is what is perceived as the observable part of the service (the sights, sounds and look of the service). In a restaurant the explicit service is the time spent waiting for service, the appearance of the facility and the employees, and the ambience of sounds and light and the decor. The implicit service is the feeling of safety, psychological well-being and happiness associated with the service.

Service is a Customer Experience

Comparison of Manufacturing and Services

According to Fitzsimmons, Fitzsimmons and Bordoloi (2014) differences between manufactured goods and services are as follows:

Simultaneous Production and Consumption: High contact services, for example, haircuts must be produced in the presence of the customer, since they are consumed as produced. As a result, services cannot be produced in one location and transported to another, like goods. Service operations are therefore highly dispersed geographically close to the customers. Furthermore, simultaneous production and consumption allows the possibility of self-service involving the customer at the point of consumption, for example, gas stations. Only low-contact services produced in the 'backroom', for example, check clearing can be provided away from the customer.

- **Perishable:** Since services are perishable, they cannot be stored for later use. In manufacturing companies, inventory can be used to buffer supply and demand. Since buffering is not possible in services, highly variable demand must be met by operations or demand modified to meet supply.
- **Ownership:** In manufacturing, ownership is transferred to the customer. Ownership is not transferred for service. As a result, services cannot be owned or resold.
- **Tangibility:** A service is intangible making it difficult for a customer to evaluate the service in advance. In the case of a good, customers can see it and evaluate it. Assurance of quality service is often done by licensing, government regulation, and branding to assure customers they will receive a quality service.

These four comparisons indicate how management of service operations are quite different from manufacturing regarding such issues as capacity requirements (highly variable), quality assurance (hard to quantify), location of facilities (dispersed), and interaction with the customer during delivery of the service (product and process design).

Service Industries

Industries have been defined by economists as consisting of four parts: Agriculture, Mining and Construction, Manufacturing, and Service. Services have existed for centuries. Early service was associated with servants. Servants were hired to do tasks that the wealthy did not want to do for themselves, for example, cleaning the house, cooking, and washing clothes. Later, services became more organized and were provided to the general public.

In 1900 the U.S. service industry, for example, consisting of banks, professional services, schools and general stores was fragmented, except for the railroads and communications. Services were largely local in nature and owned by entrepreneurs and families. The U.S. in 1900 had 31% employment in services, 31% in manufacturing and 38% in agriculture.

Services have now evolved to become the dominant form of employment in industrialized economies. Much of the world has progressed, or is progressing, from agricultural to industrial and now post-industrial economies. The U.S. Bureau of Labor Statistics provides a table of the employment of the 151 million people by industry in the U.S. for 2014.

M.Sc. [Home Science – Nutrition and Dietetics]

365 24

LAB II - NUTRITIONAL BIOCHEMISTRY, FUNCTIONAL FOODS AND NUTRACEUTICALS AND FOOD SERVICE MANAGEMENT

II - Semester

ALAGAPPA UNIVERSITY

[Accredited with 'A+' Grade by NAAC (CGPA:3.64) in the Third Cycle and Graded as Category–I University by MHRD-UGC]

KARAIKUDI – 630 003

DIRECTORATE OF DISTANCE EDUCATION

Vallal Dr.RM.Alagappa Chettiar

ISBN 978-93-5338-631-3