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Microbes and Microbial Technology

Vol-2

JV'n Ms. Anshika Kushwaha

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CHAPTER 1

Mushroom Cultivation.

Mushroom cultivation:

It is a technical process. As mushroom professionals often talk in a technical language, a few of these terms will first be explained.

In the early years of mushroom culture in the Netherlands, compost was scooped into the mushroom trays and then inoculated with spores. A nine week wait followed, until the mycelium spawned sufficiently, flushing started and the grown mushrooms could be harvested by hand.

The cultivation process hasn't changed that much, but the way the successive steps are performed differs immensely. Hardly anything is done by hand anymore in modern mushroom farming. These changes started to take place when three young mushroom growers from Mook set up the 'Coöperatieve Nederlandse Champignonkwekersvereniging' (Cooperative Dutch Mushroom Growers Association: CNC) in 1953. One of their activities was to organize the preparation of compost, resulting in the delivery of ready prepared compost permeated with spawn to most mushroom growers' doors. Mushroom cultivation can be divided into five phases:

Phase 1: Composting

The growing cycle of mushrooms starts with compost. Compost preparation starts with horse manure. The compost factories get the horse manure from large horse breeding companies that pay the compost factories to collect the manure. Straw, gypsum, chicken manure and water are added to the horse manure. The straw improves the structure, gypsum ensures the proper acidity and the two manures are the nutrients. The compost is produced in tunnels in order to prevent the smell from becoming a nuisance. As manure emits ammonia, compost factories purify the air with ammonia wash to prevent the emission of gases. The indoor fresh compost looks like earth from a forest. Dark brown, full of trampled bits of straw. The compost is steaming, due to the composting process: heat is generated which digests the components. What's left is a very fertile, nutritious source for mushrooms. On one batch of compost, two to three flushes of mushrooms can be grown. A square metre of compost (which is equal to 90 kilos) yields a maximum of 35 kilos of mushrooms. After that it's no longer lucrative to reuse the compost. The leftover compost can still be used as a soil conditioner in other agricultural companies.

Phase 2: Spawning

In a tunnel, the indoor fresh compost is pasteurized at 57-60 degrees Celsius. This kills all possible bacteria. The compost stays in the tunnel to mature for six days, after which the compost is mixed with spawn that will produce the mushrooms: the mycelium. The compost is then moved to another tunnel where the mycelium can spread through the compost. The mycelium grows quickly; after two weeks it has completely permeated the compost, which means that is has reached the point that it is ready for the growers. At this time the compost looks like light brown peat.

Most mushroom growers do not produce their own spawn, as it is a very sophisticated process. Specialized companies produce the spawn by inoculating grain with spores. The grain is sterilized first to prevent infection and it's kept moist, exactly the way mushrooms like it. Ten kilo of spores (22 pounds) provides about five hundred kilos of inoculated grain (1100 pounds). The grain is incubated in a bag for two weeks at 25 degrees Celsius (75 degrees Fahrenheit), then transferred to a refrigerator at 2 degrees Celsius (35 degrees Fahrenheit) to harden it. In this way, the spawn gets a shelf life of 6 months without the mycelium losing its vitality.

Phase 3: Casing

The matured compost is spread onto long stainless steel boxes, the mushroom beds. The beds are inside special dark rooms called cells. The temperature in the cells is kept nice and warm, at about 23 degrees Celsius. A layer of peat casing material is added on top of the compost to keep the compost moist. Over a period of six days, 20 to 25 litres of water is sprinkled on each m2 in each cell because more moisture is needed. After this, the fungus has two days to grow through the covering layer of casing soil.

Phase 4: Pinning

Mushrooms only grow in the wild in autumn. However, they can be cultivated year round by recreating autumn conditions. Therefore, the temperature in the cell is gradually lowered from 23 to 17 degrees Celsius over four days. The mushroom grower starts to lower the temperature once he sees that the mycelium has grown to its full extent. The temperature shock is a sign for the mycelium to start sprouting the mushrooms. The same thing happens in nature. Mycelium grows well in mild autumn weather, and after an October storm, the mushrooms will start appearing. The mycelium starts to form little buds, which will develop into mushrooms. Those little white buds are called pins. In this phase, air temperature and humidity can influence growth. Low air temperature and low humidity produce more buds, which yield smaller mushrooms. Higher air temperature and humidity produce fewer but larger mushrooms.

Phase 5: Harvesting

After this, the temperature is kept steady at 18 degrees Celsius. Mushrooms grow best at this temperature; they' will grow 3 cm (1 inch) in a week, which is the normal size for harvesting. In week 3 the first flush is harvested. Mushrooms destined for selling fresh are still harvested by hand; mushrooms destined for preserving are being picked and sorted mechanically. Although hand-picking is a lot of work, it offers the best guarantee that the mushrooms will be removed from the beds undamaged. On average, a picker can harvest between 18 and 30 kilos of mushrooms an hour. The mushrooms are picked from the beds with a rotating motion and sorted by the pickers based on quality, size and weight. Nine days after the first flush, the second flush will be harvested. The second flush often consists of larger, but fewer mushrooms than the first flush.

After the second flush of mushrooms has been picked, the cells need to be cleaned. First the cell is pasteurized with steam to kill any remaining fungus to ensure that there is no transfer from cycle to cycle. During steam-cleaning, the temperature in the cells reaches 70 degrees Celsius for eight hours. After steam-pasteurization, the compost is removed from the beds. The empty cell is thoroughly cleaned one more time and then it is ready to be filled again.

Mushroom farms receive the spawn in the exact composition they requested. The same goes for the casing. However, this doesn't mean that mushroom growers of today have an easy life. Mushroom cultivation is not just a matter of setting the climate control dials and waiting for the mushrooms to grow. Compost is and will always be a natural product, and no matter how hard compost factories try to deliver a constant quality, there will always be differences in each delivered truckload of compost. The structure might a little coarser or finer, the humidity a little higher or lower. The grower has to determine the exact conditions of the compost (by smelling, feeling and looking) and adjust the growing process accordingly. Mushroom cultivation is a true profession, and the knowledge of the grower determines his success.

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- **Competitive questions from today topic (2 questions Minimum)-**

The first smallpox vaccine is an example of

A. Heat killed vaccine
B. Chemically attenuated vaccine
C. Live vaccine
D. Vaccine with adjuvant

Exam NameDBT JRF 2015

Fertility factor is related to conjugation in the same way as bacteriophage P1 to

- Transformation
- Transduction
- Efflux
- Transposition

Exam NameDBT JRF 2015

- **Suggestions to secure good marks to answer in exam-**
  - Give answer with complete labeled diagrams.
  - Explain answer with key point answers

- **Questions to check understanding level of students-**
  - What are the steps in mushroom cultivation?
  - What are the nutritional benefits of mushroom.

CHAPTER 2

Fermentative production of alcoholic beverages.

Fermentation involved in alcohol production:

Fermentation naturally occurs in yeast and some bacteria. Alcohol is commercially produced by using yeast. During fermentation, sugar is anaerobically converted into ethanol, water, and carbon dioxide. Louis Pasteur first worked on the living identity of yeast and its capability to convert fruit sugar into alcohol (alcoholic fermentation). He also worked lactic acid production by bacteria (lactic acid fermentation). In today's time the science that deals with fermentation is called zymology.

Chemically alcoholic fermentation is divided into two phases: in the first phase the glucose (sugar) is converted into pyruvate (glycolysis) and the second phase involves the conversion of pyruvate in alcohol. So basically, the second phase is the fermenting step of the reaction. Alcoholic fermentation produces all different types of alcoholic beverages, where the type of beverage depends on the source of sugar and final alcohol content. For making beers and lagers barley is frequently used and for making wines the sugar source is crushed grapes. To increase the alcoholic contents of the beverage, distillation is done because yeast cannot tolerate high levels of alcohol and die. In the process of distillation, fermented solution is heated to vaporize ethanol at the temperature around 78.5 ° C and after that ethanol vapors are collected and condensed in another flask to get concentrated ethanol. The alcoholic beverages that are produced by distillation are vodka, rum, and other spirits.

Alcoholic fermentation has another application in the bread making industry, where yeast is mixed with dough for the fermentation process. The only difference here is unlike beverages, the carbon dioxide produced during reaction makes the dough rise and ethanol evaporates from it.

Alcoholic Fermentation

Alcoholic fermentation is a complex biochemical process during which yeasts convert sugars to ethanol, carbon dioxide, and other metabolic byproducts that contribute to the chemical composition and sensorial properties of the fermented foodstuffs. Alcoholic fermentation is the basis for the manufacturing of alcoholic beverages such as wine and beer. Control of fermentation is generally considered as a prerequisite to determine the quality of the final product. In this context, fermentation monitoring is a growing need, which calls for fast, low-cost, and nondestructive methods providing real-time or online information in order to assure an effective control at all stages of the process.

Role of Yeast in Production of Alcoholic Beverages:

Introduction

Although there is a distinction between beer, wine and liquor as well as other lesser known alcoholic beverages, they share one thing in common. They are the fermentation products of **yeasts**, mostly *Saccharomyces cerevisiae* or in the case of beers, usually *S. carlsburgiensis*. Yeasts, as you recall, are not mycelial. They are unicellular fungi that reproduce asexually by budding or fission. The reaction by which alcoholic beverages are produced is generally referred to as **fermentation** and may be summarized as:

Yeast + Glucose è Alcohol (Ethanol) + CO2

This reaction is also important in baking bread, but the desired product is then the carbon dioxide rather than alcohol. The production of alcohol occurs best in the absence of oxygen. However, from the yeast's point of view, alcohol and carbon dioxide are waste products, and as the yeast continues to grow and metabolize in the sugar solution, the accumulation of alcohol will become toxic when it reaches a concentration between 14-18%, thereby killing the yeast cells. This is the reason why the percentage of alcohol in wine and beer can only be approximately 16%. In order to produce beverages (liquor) with higher concentrations of alcohol, the fermented products must be distilled.

What is Alcohol?

Distilled and Undistilled Alcohol

There are two categories of alcoholic beverages: distilled and undistilled. Undistilled drinks are also called fermented drinks. Fermentation is the process by which bacteria or yeast chemically converts sugar into ethanol. Wine and beer are both fermented, undistilled alcoholic beverages. Wineries ferment grapes to make wine and breweries ferment barley, wheat, and other grains to make beer.

Distillation is a process which follows fermentation. The process converts a fermented substance into one with an even higher concentration of alcohol. Distillation concentrates alcohol by separating it from the water and other components of a fermented substance. Liquors and spirits are distilled alcoholic beverages. They contain more alcohol by volume than undistilled drinks. In general, a distilled alcoholic beverage will have a higher alcohol proof.

What is an alcohol beverage?

An alcohol beverage is simply any drink that contains ethanol/ ethyl alcohol.

Beer, wine, and spirits all start with a process called fermentation, which is the natural result of yeast digestion of the sugars found in ingredients like fruit, cereal grains, or other starches. Fermentation results in two substances: ethanol and carbon dioxide.

Ethanol is the alcohol people drink, but there are other types of alcohol that may be harmful or even fatal to drink. Throughout this website, we use “alcohol” to refer only to ethanol.

Almost any yeast and source of sugar can produce alcohol. Trace amounts of ethanol may occur in non-alcohol beverages, including juices. Some beer, wine, and spirits drinks are designed to look and taste like alcohol beverages but contain very low levels of alcohol. These beverages are often classed under legislation as “non-alcohol” beverages.

Although recipes vary, alcohol beverages are generally divided into three broad categories:

- Beers, typically made with barley that is sprouted and roasted into malt (sometimes other grains are used or added), then cooked with water, fermented with yeast, and flavored with the flowers of the hops plant.
- Wines, made from grapes and sometimes other fruits that are juiced and fermented.
- Distilled spirits, made from grain, fruit, or other sugar sources that are fermented and then distilled in a heating and cooling process that concentrates the alcohol.

These categories are far from complete. It is important to note that there are alcohol beverage products (locally homemade, regionally traditional, etc.) that may fall outside of commonly accepted beer, wine, or spirits. For example, liqueurs are a specialty including a diverse group of alcohol beverages obtained by addition of sugar and water to fruit distillates or herbs, seeds, and essences. Other alcohol beverages are made from rice, apples, cassava, and many other sugar sources, even including the milk of horses.

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- **Competitive questions from today topic (2 questions Minimum)-**

Marine organisms that require oxygen levels typically in the range of 2-10 % for growth would be classed under

E. facultative anaerobes
F. aerotolerant anaerobes
G. obligate aerobes
H. microaerophiles

Exam NameDBT JRF 2015

H1N1, H1N2, H2N1, H3N1 and H3N2 are subtypes of which influenza virus?

- influenza A
- influenza B
- influenza C
- influenza D

Exam NameDBT JRF 2015

- **Suggestions to secure good marks to answer in exam-**
  - Give answer with complete labeled diagrams.
  - Explain answer with key point answers

- **Questions to check understanding level of students-**
  - What are the steps in Alcoholic beverages?
  - Give name of some alcoholic beverages.

CHAPTER 3
Technology of purification for ethyl alcohol.

Purification:

Distillation

Ethylene hydration or brewing produces an ethanol–water mixture. For most industrial and fuel uses, the ethanol must be purified. Fractional distillation at atmospheric pressure can concentrate ethanol to 95.6% by weight (89.5 mole %). This mixture is an azeotrope with a boiling point of 78.1 °C (172.6 °F), and *cannot* be further purified by distillation. Addition of an entraining agent, such as benzene, cyclohexane, or heptane, allows a new ternary azeotrope comprising the ethanol, water, and the entraining agent to be formed. This lower-boiling ternary azeotrope is removed preferentially, leading to water-free ethanol.

At pressures less than atmospheric pressure, the composition of the ethanol-water azeotrope shifts to more ethanol-rich mixtures, and at pressures less than 70 torr (9.333 kPa), there is no azeotrope, and it is possible to distill absolute ethanol from an ethanol-water mixture. While vacuum distillation of ethanol is not presently economical, pressure-swing distillation is a topic of current research. In this technique, a reduced-pressure distillation first yields an ethanol-water mixture of more than 95.6% ethanol. Then, fractional distillation of this mixture at atmospheric pressure distills off the 95.6% azeotrope, leaving anhydrous ethanol at the bottom.

Molecular sieves and desiccants

Apart from distillation, ethanol may be dried by addition of a desiccant, such as molecular sieves, cellulose, and cornmeal. The desiccants can be dried and reused. Molecular sieves can be used to selectively absorb the water from the 95.6% ethanol solution. Synthetic zeolite in pellet form can be used, as well as a variety of plant-derived absorbents, including cornmeal, straw, and sawdust. The zeolite bed can be regenerated essentially an unlimited number of times by drying it with a blast of hot carbon dioxide. Cornmeal and other plant-derived absorbents cannot readily be regenerated, but where ethanol is made from grain, they are often available at low cost. Absolute ethanol produced this way has no residual benzene, and can be used to fortify port and sherry in traditional winery operations.

Membranes and reverse osmosis

Membranes can also be used to separate ethanol and water. Membrane-based separations are not subject to the limitations of the water-ethanol azeotrope because the separations are not based on vapor-liquid equilibria. Membranes are often used in the so-called hybrid membrane distillation process. This process uses a pre-concentration distillation column as first separating step. The further separation is then accomplished with a membrane operated either in vapor permeation or pervaporation mode. Vapor permeation uses a vapor membrane feed and pervaporation uses a liquid membrane feed.

Other techniques

A variety of other techniques have been discussed, including the following:

- Salting using exploit its insolubility will cause a phase separation with ethanol and water. This offers a very small potassium carbonate impurity to the alcohol that can be removed by distillation. This method is very useful in purification of ethanol by distillation, as ethanol forms and with water.
- Direct ethanol under ambient conditions using on a carbon nanospike film as the catalyst;

Grades of ethanol

Denatured alcohol

Pure ethanol and alcoholic beverages are heavily taxed as psychoactive drugs, but ethanol has many uses that do not involve its consumption. To relieve the tax burden on these uses, most jurisdictions waive the tax when an agent has been added to the ethanol to render it unfit to drink. These include bittering agents such as denatonium benzoate and toxins such as methanol, naphtha, and pyridine. Products of this kind are called *denatured alcohol.*

Absolute alcohol

Absolute or anhydrous alcohol refers to ethanol with a low water content. There are various grades with maximum water contents ranging from 1% to a few parts per million (ppm) levels. If azeotropic distillation is used to remove water, it will contain trace amounts of the material separation agent (e.g. benzene). Absolute alcohol is not intended for human consumption. Absolute ethanol is used as a solvent for laboratory and industrial applications, where water will react with other chemicals, and as fuel alcohol. Spectroscopic ethanol is an absolute ethanol with a low absorbance in ultraviolet and visible light, fit for use as a solvent in ultraviolet-visible spectroscopy.

Pure ethanol is classed as 200 proof in the U.S., equivalent to 175 degrees proof in the UK system.

Rectified spirits

Rectified spirit, an azeotropic composition of 96% ethanol containing 4% water, is used instead of anhydrous ethanol for various purposes. Spirits of wine are about 94% ethanol (188 proof). The impurities are different from those in 95% (190 proof) laboratory ethanol.

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- **Competitive questions from today topic (2 questions Minimum)-**

After infection of cattle, Dictyocaulus viviparus larvae reach the lungs via

I. Intestine, portal vein, liver, heart, lung
J. Intestine, abdominal cavity, liver, heart, lung
K. Intestine, lymphatics, mesenteric lymph nodes, thoracic duct, heart, lungs
L. Intestine, abdominal cavity, thoracic duct, heart, lungs

Exam NameDBT JRF 2015

Hjarres disease in poultry is caused by

- Mycoplasma gallisepticium
- Mucoid strain of E. coli
- Newcastle disease virus
- Mycobacterium avium

Exam NameDBT JRF 2015

- **Suggestions to secure good marks to answer in exam-**
    - Give answer with complete labeled diagrams.
    - Explain answer with key point answers

- **Questions to check understanding level of students-**
    - What is the purification process?
    - Explain purification process.

CHAPTER 4

Technology of production of beer.

Brewing:

Brewing is the production of beer by steeping a starch source (commonly cereal grains, the most popular of which is barley) in water and fermenting the resulting sweet liquid with yeast. It may be done in a brewery by a commercial brewer, at home by a homebrewer, or by a variety of traditional methods such as communally by the indigenous peoples in Brazil when making cauim. Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests that emerging civilizations, including ancient Egypt and Mesopotamia, brewed beer. Since the nineteenth century the brewing industry has been part of most western economies.

The basic ingredients of beer are water and a fermentable starch source such as malted barley. Most beer is fermented with a brewer's yeast and flavoured with hops. Less widely used starch sources include millet, sorghum and cassava. Secondary sources (adjuncts), such as maize (corn), rice, or sugar, may also be used, sometimes to reduce cost, or to add a feature, such as adding wheat to aid in retaining the foamy head of the beer. The most common starch source is ground cereal or "grist" - the proportion of the starch or cereal ingredients in a beer recipe may be called grist, grain bill, or simply mash ingredients.

Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. There are three main fermentation methods, warm, cool and spontaneous. Fermentation may take place in an open or closed fermenting vessel; a secondary fermentation may also occur in the cask or bottle. There are several additional brewing methods, such as Burtonisation, barrel-ageing, double dropping, and Yorkshire Square.

Step 1: Milling the grain

Beginning In the brew house, different types of malt are crushed together to break up the grain kernels in order to extract fermentable sugars to produce a milled product called grist.

Step 2: Mash Conversion

The grist is then transferred into a mash tun, where it is mixed with heated water in a process called mash conversion. The conversion process uses natural enzymes in the malt to break the malt’s starch down into sugars.

Step 3: Lautering

The mash is then pumped into the lauter tun, where a sweet liquid (known as wort) is separated from the grain husks.

Step 4: The boil

The wort is then collected in a vessel called a kettle, where it is brought to a controlled boil before the hops are added.

Step 5: Wort separation and cooling

After boiling, the wort is transferred into a whirlpool for the wort separation stage. During this stage, any malt or hop particles are removed to leave a liquid that is ready to be cooled and fermented.

Step 6: Fermentation

To start the fermentation, yeast is added during the filling of the vessel. Yeast converts the sugary wort into beer by producing alcohol, a wide range of flavors, and carbon dioxide (used later in the process to give the beer its sparkle).

Step 7: Maturation

After fermentation, the young “green” beer needs to be matured in order to allow both a full development of flavors and a smooth finish.

Step 8: Filtration, carbonation, and cellaring

After reaching its full potential, the beer is filtered, carbonated, and transferred to the bright beer tank, where it goes through a cellaring process that takes 3-4 weeks to complete. Once completed, the beer is ready to be packaged.

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- **Competitive questions from today topic (2 questions Minimum)-**

  Hygromycin B, generally used as a selection marker in plant transformation protocols is

  M. an aminocyclitol antibiotic produced by Streptomyces hygroscopicus
  N. an aminoglycoside bacteriocidal antibiotic isolated from the bacterium Streptomyces kanamyceticus
  O. a beta-lactam antibiotic that is part of the amino-penicillin family and is roughly equivalent to amoxicillin in terms of activity
  P. an ammonium butanoate antibody produced by Streptomyces hygroscopicus

  Exam NameDBT JRF 2016

  In order to extract Penicillin G from fermentation broth, the pH of the broth is adjusted to pH 2.5. This is done because:

  - Most of the Penicillin is in neutral uncharged form at this pH and hence extraction is better.
  - Most of the Penicillin is in ionic form and hence extraction is better.
  - Penicillin is highly stable at this pH
  - Most of the enzymes are precipitated at this pH, which increases the extraction efficiency of Penicillin.

  Exam NameDBT JRF 2015

- **Suggestions to secure good marks to answer in exam-**
  - ➢ Give answer with complete labeled diagrams.
  - ➢ Explain answer with key point answers
- **Questions to check understanding level of students-**
  - ➢ What is the brewing process?
  - ➢ Beer is made from?

CHAPTER 5

Technology of production of wine.

Winemaking:

Winemaking or vinification is the production of wine, starting with the selection of the fruit, its fermentation into alcohol, and the bottling of the finished liquid. The history of wine-making stretches over millennia. The science of wine and winemaking is known as oenology. A winemaker may also be called a vintner. The growing of grapes is viticulture and there are many varieties of grapes.

Winemaking can be divided into two general categories: still wine production (without carbonation) and sparkling wine production (with carbonation – natural or injected). Red wine, white wine, and rosé are the other main categories. Although most wine is made from grapes, it may also be made from other plants. (See fruit wine.) Other similar light alcoholic drinks (as opposed to beer or spirits) include mead, made by fermenting honey and water, and kumis, made of fermented mare's milk.

Process

There are five basic stages to the wine making process which begins with harvesting or picking. After the harvest, the grapes are taken into a winery and prepared for primary ferment. At this stage red wine making diverges from white wine making. Red wine is made from the must (pulp) of red or black grapes and fermentation occurs together with the grape skins, which give the wine its color. White wine is made by fermenting juice which is made by pressing crushed grapes to extract a juice; the skins are removed and play no further role. Occasionally white wine is made from red grapes; this is done by extracting their juice with minimal contact with the grapes' skins. Rosé wines are either made from red grapes where the juice is allowed to stay in contact with the dark skins long enough to pick up a pinkish color (maceration or saignée), or (less commonly) by blending red wine with white wine. White and rosé wines extract little of the tannins contained in the skins.

To start primary fermentation yeast may be added to the must for red wine or may occur naturally as ambient yeast on the grapes or in the air. Yeast may be added to the juice for white wine. During this fermentation, which often takes between one and two weeks, the yeast converts most of the sugars in the grape juice into ethanol (alcohol) and carbon dioxide. The carbon dioxide is lost to the atmosphere.

After the primary fermentation of red grapes the free run wine is pumped off into tanks and the skins are pressed to extract the remaining juice and wine. The press wine is blended with the free run wine at the winemaker's discretion. The wine is kept warm and the remaining sugars are converted into alcohol and carbon dioxide.

The next process in the making of red wine is malo-lactic conversion. This is a bacterial process which converts "crisp, green apple" malic acid to "soft, creamy" lactic acid softening the taste of the wine. Red wine is sometimes transferred to oak barrels to mature for a period of weeks or months; this practice imparts oak aromas and some tannin to the wine. The wine must be settled or clarified and adjustments made prior to bottling.

The time from harvest to drinking can vary from a few months for Beaujolais nouveau wines to over twenty years for wine of good structure with high levels of acid, tannin or sugar. However, only about 10% of all red and 5% of white wine will taste better after five years than it will after just one year. Depending on the quality of grape and the target wine style, some of these steps may be combined or omitted to achieve the particular goals of the winemaker. Many wines of comparable quality are produced using similar but distinctly different approaches to their production; quality is dictated by the attributes of the starting material and not necessarily the steps taken during vinification.

Variations on the above procedure exist. With sparkling wines such as Champagne, an additional, "secondary" fermentation takes place inside the bottle, dissolving trapped carbon dioxide in the wine and creating the characteristic bubbles. Sweet wines or off-dry wines are made by arresting fermentation before all sugar has been converted into ethanol and allowing some residual sugar to remain. This can be done by chilling the wine and adding sulphur and other allowable additives to inhibit yeast activity or sterile filtering the wine to remove all yeast and bacteria. In the case of sweet wines, initial sugar concentrations are increased by harvesting late (late harvest wine), freezing the grapes to concentrate the sugar (ice wine), allowing or encouraging botrytis cinerea fungus to dehydrate the grapes or allowing the grapes to raisin either on the vine or on racks or straw mats. Often in these high sugar wines, the fermentation stops naturally as the high concentration of sugar and rising concentration of ethanol retard the yeast activity. Similarly in fortified wines, such as port wine, high proof neutral grape spirit (brandy) is added to arrest the ferment and adjust the alcohol content when the desired sugar level has been reached. In other cases the winemaker may choose to hold back some of the sweet grape juice and add it to the wine after the fermentation is done, a technique known in Germany as süssreserve.

The process produces wastewater, pomace, and lees that require collection, treatment, and disposal or beneficial use.

Synthetic wines, engineered wines or fake wines, are a product that do not use grapes at all and start with water and ethanol and then adds acids, amino acids, sugars, and organic compounds.

The grapes

The quality of the grapes determines the quality of the wine more than any other factor. Grape quality is affected by variety as well as weather during the growing season, soil minerals and acidity, time of harvest, and pruning method. The combination of these effects is often referred to as the grape's *terroir*.

Grapes are usually harvested from the vineyard from early September until early November in the northern hemisphere, and mid February until early March in the southern hemisphere. In some cool areas in the southern hemisphere, for example Tasmania, harvesting extends into May.

The most common species of wine grape is Vitis vinifera, which includes nearly all varieties of European origin.

Harvesting and destemming:

Harvest is the picking of the grapes and in many ways the first step in wine production. Grapes are either harvested mechanically or by hand. The decision to harvest grapes is typically made by the winemaker and informed by the level of sugar (called °Brix), acid (TA or Titratable Acidity as expressed by tartaric acid equivalents) and pH of the grapes. Other considerations include phenological ripeness, berry flavor, tannin development (seed color and taste). Overall disposition of the grapevine and weather forecasts are taken into account.

Mechanical harvesters are large tractors that straddle grapevine trellises and, using firm plastic or rubber rods, strike the fruiting zone of the grapevine to dislodge the grapes from the rachis. Mechanical harvesters have the advantage of being able to cover a large area of vineyard land in a relatively short period of time, and with a minimum investment of manpower per harvested ton. A disadvantage of mechanical harvesting is the indiscriminate inclusion of foreign non-grape material in the product, especially leaf stems and leaves, but also, depending on the trellis system and grapevine canopy management, may include moldy grapes, canes, metal debris, rocks and even small animals and bird nests. Some winemakers remove leaves and loose debris from the grapevine before mechanical harvesting to avoid such material being included in the harvested fruit. In the United States mechanical harvesting is seldom used for premium winemaking because of the indiscriminate picking and increased oxidation of the grape juice. In other countries (such as Australia and New Zealand), mechanical harvesting of premium winegrapes is more common because of general labor shortages.

Crushing and primary (alcoholic) fermentation

Crushing is the process when gently squeezing the berries and breaking the skins to start to liberate the contents of the berries. Destemming is the process of removing the grapes from the rachis (the stem which holds the grapes). In traditional and smaller-scale wine making, the harvested grapes are sometimes crushed by trampling them barefoot or by the use of inexpensive small scale crushers. These can also destem at the same time. However, in larger wineries, a mechanical crusher/destemmer is used. The decision about destemming is different for red and white wine making. Generally when making white wine the fruit is only crushed, the stems are then placed in the press with the berries. The presence of stems in the mix facilitates pressing by allowing juice to flow past flattened skins. These accumulate at the edge of the press. For red winemaking, stems of the grapes are usually removed before fermentation since the stems have a relatively high tannin content; in addition to tannin they can also give the wine a vegetal aroma (due to extraction of 3-isobutyl-2-methoxypyrazine which has an aroma reminiscent of green bell peppers.) On occasion, the winemaker may decide to leave them in if the grapes themselves contain less tannin than desired. This is more acceptable if the stems have 'ripened' and started to turn brown. If increased skin extraction is desired, a winemaker might choose to crush the grapes after destemming. Removal of stems first means no stem tannin can be extracted. In these cases the grapes pass between two rollers which squeeze the grapes enough to separate the skin and pulp, but not so much as to cause excessive shearing or tearing of the skin tissues. In some cases, notably with "delicate" red varietals such as Pinot noir or Syrah, all or part of the grapes might be left uncrushed (called "whole berry") to encourage the retention of fruity aromas through partial carbonic maceration.

Pressing

Pressing is the act of applying pressure to grapes or pomace in order to separate juice or wine from grapes and grape skins. Pressing is not always a necessary act in winemaking; if grapes are crushed there is a considerable amount of juice immediately liberated (called free-run juice) that can be used for vinification. Typically this free-run juice is of a higher quality than the press juice. Pressed juice is typically lesser in quality due to the release and increase of total phenolic compounds, as well as browning index and the C6-alcohol levels. These compounds are responsible for the herb-like taste perceived in wine with pressed grapes. However, most wineries do use presses in order to increase their production (gallons) per ton, as pressed juice can represent between 15%-30% of the total juice volume from the grape.

Presses act by positioning the grape skins or whole grape clusters between a rigid surface and a movable surface and slowly decrease the volume between the two surfaces. Modern presses dictate the duration and pressure at each press cycle, usually ramping from 0 Bar to 2.0 Bar.

Sometimes winemakers choose pressures which separate the streams of pressed juice, called making "press cuts." As the pressure increases the amount of tannin extracted from the skins into the juice increases, often rendering the pressed juice excessively tannic or harsh. Because of the location of grape juice constituents in the berry (water and acid are found primarily in the mesocarp or pulp, whereas tannins are found primarily in the exocarp, or skin, and seeds), pressed juice or wine tends to be lower in acidity with a higher pH than the free-run juice.

Cold stabilization

Cold stabilization is a process used in winemaking to reduce tartrate crystals (generally potassium bitartrate) in wine. These tartrate crystals look like grains of clear sand, and are also known as "wine crystals" or "wine diamonds". They are formed by the union of tartaric acid and potassium, and may appear to be [sediment] in the wine, though they are not. During the cold stabilizing process after fermentation, the temperature of the wine is dropped to close to freezing for 1–2 weeks. This will cause the crystals to separate from the wine and stick to the sides of the holding vessel. When the wine is drained from the vessels, the tartrates are left behind. They may also form in wine bottles that have been stored under very cold conditions.

Secondary (malolactic) fermentation and bulk aging

During the secondary fermentation and aging process, which takes three to six months, the fermentation continues very slowly. The wine is kept under an airlock to protect the wine from oxidation. Proteins from the grape are broken down and the remaining yeast cells and other fine particles from the grapes are allowed to settle. Potassium bitartrate will also precipitate, a process which can be enhanced by cold stabilization to prevent the appearance of (harmless) tartrate crystals after bottling. The result of these processes is that the originally cloudy wine becomes clear. The wine can be racked during this process to remove the lees.

The secondary fermentation usually takes place in large stainless steel vessels with a volume of several cubic meters, oak barrels or glass demijohns (also referred to as carboys), depending on the goals of the winemakers. Unoaked wine is fermented in a barrel made of stainless steel or other material having no influence in the final taste of the wine. Depending on the desired taste, it could be fermented mainly in stainless steel to be briefly put in oak, or have the complete fermentation done in stainless steel. Oak could be added as chips used with a non-wooden barrel instead of a fully wooden barrel. This process is mainly used in cheaper wine.

Amateur winemakers often use glass carboys in the production of their wine; these vessels (sometimes called *demijohns*) have a capacity of 4.5–54 litres (0.99–11.88 imp gal; 1.2–

14.3 US gal). The kind of vessel used depends on the amount of wine that is being made, the grapes being used, and the intentions of the winemaker.

Malolactic fermentation

Malolactic fermentation occurs when lactic acid bacteria metabolize malic acid and produce lactic acid and carbon dioxide. This is carried out either as an intentional procedure in which specially cultivated strains of such bacteria are introduced into the maturing wine, or it can happen by chance if uncultivated lactic acid bacteria are present.

Malolactic fermentation can improve the taste of wine that has high levels of malic acid, because malic acid, in higher concentration, generally causes an unpleasant harsh and bitter taste sensation, whereas lactic acid is more gentle and less sour. Lactic acid is an acid found in dairy products. Malolactic fermentation usually results in a reduction in the amount of total acidity of the wine. This is because malic acid has two acid radicals (-COOH) while lactic acid has only one. However, the pH should be monitored and not allowed to rise above a pH of 3.55 for whites or a pH of 3.80 for reds. pH can be reduced roughly at a rate of 0.1 units per 1 gram/litre of tartaric acid addition.

The use of lactic acid bacteria is the reason why some chardonnays can taste "buttery" due to the production of diacetyl by the bacteria. Most red wines go through complete malolactic fermentation, both to lessen the acid of the wine and to remove the possibility that malolactic fermentation will occur in the bottle. White wines vary in the use of malolactic fermentation during their making. Lighter aromatic wines such as Riesling, generally do not go through malolactic fermentation. The fuller white wines such as barrel fermented chardonnay, are more commonly put through malolactic fermentation. Sometimes a partial fermentation, for example, somewhere less than 50% might be employed.

Blending and fining

Different batches of wine can be mixed before bottling in order to achieve the desired taste. The winemaker can correct perceived inadequacies by mixing wines from different grapes and batches that were produced under different conditions. These adjustments can be as simple as adjusting acid or tannin levels, to as complex as blending different varieties or vintages to achieve a consistent taste.

Fining agents are used during winemaking to remove tannins, reduce astringency and remove microscopic particles that could cloud the wines. The winemakers decide on which fining agents are used and these may vary from product to product and even batch to batch (usually depending on the grapes of that particular year).

Gelatin [gelatine] has been used in winemaking for centuries and is recognized as a traditional method for wine fining, or clarifying. It is also the most commonly used agent to reduce the tannin content. Generally no gelatin remains in the wine because it reacts with the wine components, as it clarifies, and forms a sediment which is removed by filtration prior to bottling.

Besides gelatin, other fining agents for wine are often derived from animal products, such as micronized potassium caseinate (casein is milk protein), egg whites, egg albumin, bone char, bull's blood, isinglass (Sturgeon bladder), PVPP (a synthetic compound), lysozyme, and skim milk powder.

Some aromatized wines contain honey or egg-yolk extract.

Non-animal-based filtering agents are also often used, such as bentonite (a volcanic clay-based filter), diatomaceous earth, cellulose pads, paper filters and membrane filters (thin films of plastic polymer material having uniformly sized holes).

Preservatives

The most common preservative used in winemaking is sulfur dioxide (SO_2), normally added in one of the following forms: liquid sulfur dioxide, sodium or potassium metabisulphite. Another useful preservative is potassium sorbate.

Sulfur dioxide has two primary actions, firstly it is an anti microbial agent and secondly an anti oxidant. In the making of white wine it can be added prior to fermentation and immediately after alcoholic fermentation is complete. If added after alcoholic fermentation it will have the effect of preventing or stopping malolactic fermentation, bacterial spoilage and help protect against the damaging effects of oxygen. Additions of up to 100 mg per liter (of sulfur dioxide) can be added, but the available or free sulfur dioxide should be measured by the aspiration method and adjusted to 30 mg per liter. Available sulfur dioxide should be maintained at this level until bottling. For rose wines smaller additions should be made and the available level should be no more than 30 mg per liter.

In the making of red wine, sulfur dioxide may be used at high levels (100 mg per liter) prior to ferment to assist in color stabilization. Otherwise, it is used at the end of malolactic ferment and performs the same functions as in white wine. However, small additions (say, 20 milligrams per litre (7.2×10^{-7} lb/cu in)) should be used to avoid bleaching red pigments and the maintenance level should be about 20 mg/L. Furthermore, small additions (say 20 mg per liter) may be made to red wine after alcoholic ferment and before malolactic ferment to overcome minor oxidation and prevent the growth of acetic acid bacteria.

Without the use of sulfur dioxide, wines can readily suffer bacterial spoilage no matter how hygienic the winemaking practice.

Potassium sorbate is effective for the control of fungal growth, including yeast, especially for sweet wines in bottle. However, one potential hazard is the metabolism of sorbate to geraniol which is a potent and unpleasant by-product. The production of geraniol occurs only if sorbic acid is present during malo-lactic fermentation. To avoid this, either the wine must be sterile bottled or contain enough sulfur dioxide to inhibit the growth of bacteria. Sterile bottling includes the use of filtration.

Some winemakers practice natural wine making where no preservative is added. Once the wine is bottled and corked, the bottles are put into refrigeration with temperatures near 5 °C (41 °F).

Filtration

Filtration in winemaking is used to accomplish two objectives, clarification and microbial stabilization. In clarification, large particles that affect the visual appearance of the wine are removed. In microbial stabilization, organisms that affect the stability of the wine are removed therefore reducing the likelihood of re-fermentation or spoilage.

Bottling

A final dose of sulfite is added to help preserve the wine and prevent unwanted fermentation in the bottle. The wine bottles then are traditionally sealed with a cork, although alternative wine closures such as synthetic corks and screwcaps, which are less subject to cork taint, are becoming increasingly popular. The final step is adding a capsule to the top of the bottle which is then heated for a tight seal.

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- **Competitive questions from today topic (2 questions Minimum)-**

  Cells are broken to release the contents by using various enzymes. Which of the following combination is FALSE?

  Q. Lysozyme ? bacteria
  R. Cellulase ? plant cell
  S. Chitinase ? fungus
  T. Cellulase ? bacteria

  Exam NameDBT JRF 2015

  Archea is considered as a separate group from bacteria and eukaryotes, based on

  - genome sequence.
  - 16S rRNA gene sequence.
  - 23S rRNA gene sequence.
  - EFTu sequence.

  Exam NameDBT JRF 2015

- **Suggestions to secure good marks to answer in exam-**
  - Give answer with complete labeled diagrams.
  - Explain answer with key point answers
- **Questions to check understanding level of students-**
  - What is the wine making process?
  - Wine is made from?

CHAPTER 6

Distilled beverage (whisky).

History of distilling:

The first distilled spirits were made from sugar-based materials, primarily grapes and honey to make grape brandy and distilled mead, respectively. The earliest use of starchy grains to produce distilled spirits is not known, but their use certainly dates from the Middle Ages. Some government control dates from the 17th century. As production methods improved and volume increased, the distilled spirits industry became an important source of revenue. Rigid controls were often imposed on both production and sale of the liquor.

The earliest stills were composed simply of a heated closed container, a condenser, and a receptacle to receive the condensate. These evolved into the pot still, which is still in use, particularly for making malt whiskeys and some gins. The next refinement was heating the alcohol-containing liquid in a column made up of a series of vaporization chambers stacked on top of one another. By the early 19th century large-scale continuous stills, very similar to those used in the industry today, were operating in France and England. In 1831 the Irishman Aeneas Coffey designed such a still, which consisted of two columns in series.

Since distillation requires that the liquid portion of a fermentation mixture be vaporized, considerable heat must be applied to the process. The fuel used in distilling spirits has always been that which has been most readily available at the particular time and place. Peat, coal, and wood were the fuels used historically, while the fuels of choice today are coal, natural gas, and oil. The high steam requirement for continuous-still operation inhibited the development of rectifying columns for production of spirits until after the Industrial Revolution.

Whisky

Whisky or **whiskey** is a type of distilled alcoholic beverage made from fermented grain mash. Various grains (which may be malted) are used for different varieties, including barley, corn, rye, and wheat. Whisky is typically aged in wooden casks, generally made of charred white oak.

Whisky is a strictly regulated spirit worldwide with many classes and types. The typical unifying characteristics of the different classes and types are the fermentation of grains, distillation, and aging in wooden barrels.

Etymology

The word *whisky* (or *whiskey*) is an anglicisation of the Classical Gaelic word *uisce* (or *uisge*) meaning "water" (now written as *uisce* in Modern Irish, and *uisge* in Scottish Gaelic). Distilled alcohol was known in Latin as *aqua vitae* ("water of life"). This was translated into Old Irish as *uisce beatha* ("water of life"), which became *uisce beatha* in Irish and *uisge beatha* Scottish Gaelic. Early forms of the word in English included *uskebeaghe* (1581), *usquebaugh* (1610), *usquebath* (1621), and *usquebae*

History

It is possible that distillation was practised by the Babylonians in Mesopotamia in the 2nd millennium BC, with perfumes and aromatics being distilled, but this is subject to uncertain and disputed interpretations of evidence.

The earliest certain chemical distillations were by Greeks in Alexandria in the 1st century AD, but these were not distillations of alcohol. The medieval Arabs adopted the distillation technique of the Alexandrian Greeks, and written records in Arabic begin in the 9th century, but again these were not distillations of alcohol. Distilling technology passed from the medieval Arabs to the medieval Latins, with the earliest records in Latin in the early 12th century.

The earliest records of the distillation of alcohol are in Italy in the 13th century, where alcohol was distilled from wine. An early description of the technique was given by Ramon Llull (1232–1315). Its use spread through medieval monasteries, largely for medicinal purposes, such as the treatment of colic and smallpox.

The art of distillation spread to Scotland and Ireland no later than the 15th century, as did the common European practice of distilling "aqua vitae", spirit alcohol, primarily for medicinal purposes. The practice of medicinal distillation eventually passed from a monastic setting to the secular via professional medical practitioners of the time, The Guild of Barber Surgeons. The earliest mention of whisky in Ireland comes from the seventeenth-century *Annals of Clonmacnoise*, which attributes the death of a chieftain in 1405 to "taking a surfeit of aqua vitae" at Christmas. In Scotland, the first evidence of whisky production comes from an entry in the *Exchequer Rolls* for 1494 where malt is sent "To Friar John Cor, by order of the king, to make aquavitae", enough to make about 500 bottles.

James IV of Scotland (r. 1488–1513) reportedly had a great liking for Scotch whisky, and in 1506 the town of Dundee purchased a large amount of whisky from the Guild of Barber Surgeons, which held the monopoly on production at the time. Between 1536 and 1541, King Henry VIII of

England dissolved the monasteries, sending their monks out into the general public. Whisky production moved out of a monastic setting and into personal homes and farms as newly independent monks needed to find a way to earn money for themselves.

Production

Distillation

A still for making whisky is usually made of copper, since it removes sulfur-based compounds from the alcohol that would make it unpleasant to drink. Modern stills are made of stainless steel with copper innards (piping, for example, will be lined with copper along with copper plate inlays along still walls). The simplest standard distillation apparatus is commonly known as a pot still, consisting of a single heated chamber and a vessel to collect purified alcohol.

Column stills are frequently used in the production of grain whisky and are the most commonly used type of still in the production of bourbon and other American whiskeys. Column stills behave like a series of single pot stills, formed in a long vertical tube. Whereas a single pot still charged with wine might yield a vapour enriched to 40–60% alcohol, a column still can achieve a vapour alcohol content of 95.6%; an azeotropic mixture of alcohol and water.

Aging

Whiskies do not mature in the bottle, only in the cask, so the "age" of a whisky is only the time between distillation and bottling. This reflects how much the cask has interacted with the whisky, changing its chemical makeup and taste. Whiskies that have been bottled for many years may have a rarity value, but are not "older" and not necessarily "better" than a more recent whisky that matured in wood for a similar time. After a decade or two, additional aging in a barrel does not necessarily improve a whisky.

While aging in wooden casks, especially American oak and French oak casks, whisky undergoes six processes that contribute to its final flavor: extraction, evaporation, oxidation, concentration, filtration, and colouration. Extraction in particular results in whisky acquiring a number of compounds, including aldehydes and acids such as vanillin, vanillic acid, and syringaldehyde. Distillers will sometimes age their whiskey in barrels previously used to age other spirits, such as rum or sherry, to impart particular flavours.

Packaging

Most whiskies are sold at or near an alcoholic strength of 40% abv, which is the statutory minimum in some countries although the strength can vary, and cask-strength whisky may have as much as twice that alcohol percentage.

Exports

Whisky is probably the best known of Scotland's manufactured products. Exports have increased by 87% in the decade to 2012 and it contributes over £4.25 billion to the UK economy, making up a quarter of all its food and drink revenues. In 2012, the US was the largest market for Scotch whisky (£655 million), followed by France (£535 million). It is also one of the UK's overall top five manufacturing export earners and it supports around 35,000 jobs. Principal whisky producing areas include Speyside and the Isle of Islay, where there are eight distilleries providing a major source of employment. In many places, the industry is closely linked to tourism, with many distilleries also functioning as attractions worth £30 million GVA each year.

In 2011, 70% of Canadian whisky was exported, with about 60% going to the US, and the rest mostly to Europe and Asia. 15 million cases of Canadian whisky were sold in the US in 2011.

Types

Whisky or whisky-like products are produced in most grain-growing areas. They differ in base product, alcoholic content, and quality.

- Malt whisky is made primarily from malted barley.
- Grain whisky is made from any type of grain.

Malts and grains are combined in various ways:

- *Single malt whisky* is whisky from a single distillery made from a mash that uses only one particular malted grain. Unless the whisky is described as *single-cask*, it contains whisky from many casks, and different years, so the blender can achieve a taste recognisable as typical of the distillery. In most cases, single malts bear the name of the distillery, with an age statement and perhaps some indication of some special treatments, such as maturation in a port wine cask.
- *Blended malt whisky* is a mixture of single malt whiskies from different distilleries. If a whisky is labelled "pure malt" or just "malt" it is almost certainly a blended malt whisky. This was formerly called a "vatted malt" whisky.

- *Blended whisky* is made from a mixture of different types of whisky. A blend may contain whisky from many distilleries so that the blender can produce a flavour consistent with the brand. The brand name may, therefore, omit the name of a distillery. Most Scotch, Irish and Canadian whisky is sold as part of a blend, even when the spirits are the product of one distillery, as is common in Canada. American blended whisky may contain neutral spirits.
- *Cask strength* (also known as *barrel proof*) whiskies are rare, and usually only the very best whiskies are bottled in this way. They are bottled from the cask undiluted or only lightly diluted.
- *Single cask* (also known as *single barrel*) whiskies are bottled from an individual cask, and often the bottles are labelled with specific barrel and bottle numbers. The taste of these whiskies may vary substantially from cask to cask within a brand.

Chemistry

Overview

Whiskies and other distilled beverages, such as cognac and rum, are complex beverages that contain a vast range of flavouring compounds, of which some 200 to 300 are easily detected by chemical analysis. The flavouring chemicals include "carbonyl compounds, alcohols, carboxylic acids and their esters, nitrogen- and sulphur-containing compounds, tannins, and other polyphenolic compounds, terpenes, and oxygen-containing, heterocyclic compounds" and esters of fatty acids. The nitrogen compounds include pyridines, picolines and pyrazines. The sulfur compounds include thiophenes and polysulfides which seem to contribute to whiskey's roasted character.

Flavours from treating the malt

The distinctive smoky flavour found in various types of whisky, especially Scotch, is due to the use of peat smoke to treat the malt.

Flavours from distillation

The flavouring of whisky is partially determined by the presence of congeners and fusel oils. Fusel oils are higher alcohols than ethanol, are mildly toxic, and have a strong, disagreeable smell and taste. An excess of fusel oils in whisky is considered a defect. A variety of methods are employed in the distillation process to remove unwanted fusel oils. Traditionally, American distillers focused on secondary filtration using charcoal, gravel, sand, or linen to remove undesired distillates.

Acetals are rapidly formed in distillates and a great many are found in distilled beverages, the most prominent being acetaldehyde diethyl acetal (1,1-diethoxyethane). Among whiskies the highest levels are associated with malt whisky. This acetal is a principal flavour compound in sherry, and contributes fruitiness to the aroma.

The diketone diacetyl (2,3-butanedione) has a buttery aroma and is present in almost all distilled beverages. Whiskies and cognacs typically contain more of this than vodkas, but significantly less than rums or brandies.

Polysulfides and thiophenes enter whiskey through the distillation process and contribute to its roasted flavor.

Flavours from oak

Whisky that has been aged in oak barrels absorbs substances from the wood. One of these is cis-3-methyl-4-octanolide, known as the "whisky lactone" or "quercus lactone", a compound with a strong coconut aroma.

Commercially charred oaks are rich in phenolic compounds. One study identified 40 different phenolic compounds. The coumarin scopoletin is present in whisky, with the highest level reported in Bourbon whiskey.

In an experiment, whiskey aged 3 years in orbit on the International Space Station tasted and measured significantly different from similar test subjects in gravity on Earth. Particularly, wood extractives were more present in the space samples.

Flavours and colouring from additives

Depending on the local regulations, additional flavourings and colouring compounds may be added to the whisky. Canadian whisky may contain caramel and flavouring in addition to the distilled mash spirits. Scotch whisky may contain added (E150A) caramel colouring, but no other additives. The addition of flavourings is not allowed in American "straight" whiskey, but is allowed in American blends.

Chill filtration

Whisky is often "chill filtered": chilled to precipitate out fatty acid esters and then filtered to remove them. Most whiskies are bottled this way, unless specified as *unchillfiltered* or *non-chill filtered*. This is done primarily for cosmetic reasons. Unchillfiltered whiskies often turn cloudy when stored at cool temperatures or when cool water is added to them, and this is perfectly normal.

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- **Competitive questions from today topic (2 questions Minimum)-**
  BCG vaccine
  U. is an attenuated M. tuberculosis strain
  V. reduces the incidence of tubercular meningitis
  W. induces protective CMI response against atypical mycobacteria
  X. protects against pulmonary tuberculosis
  Exam NameDBT JRF 2015

  Why catalase is induced in microbes during exposure to the pollutants?
  - Because it involve in biotransformation of that pollutant.
  - Because of oxidative stress produced due to exposure of pollutant.
  - Pollutants are general inducers of catalase
  - Because catalase in involved in the metabolism of metabolite generated from pollutants.

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- **Suggestions to secure good marks to answer in exam-**
  - Give answer with complete labeled diagrams.
  - Explain answer with key point answers
- **Questions to check understanding level of students-**
  - What is the whisky making process?
  - What is the duration of aging in whisky production process?

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