# EFFECTS OF DIFFERENT OAK VARIETIES *(QUERCUS Spp.)* ON CHEMICAL AND SENSORY QUALITY OF *RAKSHI* (FINGER MILLET WHISKY).

by

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# Effects of Different Oak Varieties (Quercus Spp.) on Chemical and Sensory Quality of Rakshi (finger millet whisky)

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## Approval Letter

**This *dissertation* entitled "*Effects of different oak varieties (Quercus Spp.) on chemical and sensory quality of rakshi (finger millet whisky)* " presented by Ritesh Khadka has been accepted as the partial fulfillment of the requirements for the B.Tech. degree in Food Technology.**

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# Abstract

The aim of this research was to evaluate the effects of Nepalese origin oak cubes on rakshi maturation, comparing it with American white oak effects. *Rakshi*, made from finger millet, was distilled twice and its alcohol content was adjusted to approximately 73% v/v. Along with a control, five types of oak cubes and one non-oak variety were used for a one-year maturation period. A little amount of the whisky samples were taken every three months and analyzed qualitatively and semi-quantitatively for their sensory characteristics to evaluate maturation progression. The whisky base spirit for this research was produced from finger millet grain using traditional distilling methods practiced in Nepal.

Proximate analysis of the raw millet confirmed its suitability as a fermentation substrate. Following a 40-day fermentation, the mash had stable pH and alcohol levels proportional to sampling depth after 40 days of fermentation. The matured whisky was analyzed for pH, total dissolved solids (TDS), conductivity, color development, and chemical composition using spectrophotometry and gas chromatography-mass spectrometry (GC-MS**).** The findings support that chemical composition, including maturation, resulted in significant increase of certain constituents like ethyl acetate, acetic acid, some higher alcohols (isoamyl alcohol) with reported incerease of about 41%, 56%, 75% respectively, while strongly reduced concentrations of methanol (from 50.29 to 11.5ppm) reached safe levels in treated samples. Sensory evaluations covering appearance, aroma, mouthfeel, body, aftertaste, and overall acceptability revealed that all treated samples outperformed the control, with Sample D (*Quercus lamellosa, Phalat)* showing strong similarity to the reference (Sample B, American oak) in both chemical and sensory attributes. Radar chart analyses further illustrated the progressive enhancement of desirable flavor characteristics such as vanilla, smoky, and fruity notes, particularly in Nepalese oak-treated samples. The findings underscore the potential of Nepalese oak and other varieties as a viable alternative to imported oak species in whisky maturation, with recommendations for further studies on oak seasoning and potential for barrel aging.

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## List of abbreviations

| Abbreviations | Full Form |
|---|---|
| ABV | Alcohol by volume |
| ANOVA | Analysis of variance |
| AOAC | Association of official analytical chemists |
| CDFT | Central Department of Food Technology |
| DFTQC | Department of Food Technology and Quality Control |
| EBC | European brewery commission |
| FSSAI | Food Standard and Safety Authority of India |
| GCMS | Gas Chromatography Mass Spectroscopy |
| HSD | Honestly significance difference |
| LSD | Least significance difference |
| GCMS | Gas chromatography Mass Spectroscopy |
| HPLC | High performance liquid chromatography |
| SA/V | Surface area per volume |
| SD | Standard Deviation |
| TDS | Total Dissolved Solids |

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# Part I

# Introduction

## 1.1 General introduction

Whisky (whiskey) is one of the most popular spirit-based drinks. Depending on the region, whisky is defined differently. According to (EU Regulations Whisky) whisky is a spirit drink produced via distillation of mash made from malted grains or from grains, distillation is conducted at less than 94.8% by volume. Whisky should mature for at least 3 years in wooden barrels of a capacity not exceeding 700L. Only water and plain caramel (for coloring) can be added to the distillate; no other colorings and flavorings can be added. Scotch whisky is the most popular whisky type. It is produced entirely from malted barley via double distillation in large copper pot stills. Volatile phenolic compounds such as cresol and guaiacol which form in roasted barley are responsible for the specific taste of whisky. Whisky originating from Northen part of the USA is made from a mixture of corn, rye, wheat, barley and other grains and is aged in oak barrels for at least 2 years (Rhodes et al., 2009, Bathgate & Taylor, 1977, Christoph et al., 2007, Lea et al., 2003).

Ageing is among the most important and the costliest factors influencing the quality of distilled beverages. A wide range of distilled alcoholic drinks are now matured in oak casks, most notably whiskies and brandies but also many rums and liquors. While probably originally serving only as a means of storage and transportation, the time spent in the oak cask is now seen as fundamentally contributing to the finished taste and aroma. The process of maturation and ageing is characterized by changes in color and favor of the maturing spirit and a decline in both the volume and the alcoholic content. The time required for satisfactory maturation varies according to the characteristics of the raw distillate, the size, wood origins and treatment of the cask and the environment in which the spirit is matured (Mosedale et al. 1998, Nishimura and Matsuyama 1989).

Due to the limited sources and availability of American white oak (*Quercus. alba)* and European oak (*Quercus. robur)* has prompted extensive research within the alcoholic beverage industry into alternative wood species and maturation techniques. This pursuit has led to the emergence of diverse oak varieties, including Japanese oak, Slovenian oak, Spanish oak, and Indian oak. Among these, Japanese oak, specifically Mizunara (*Quercus mongolica*), has become particularly notable for barrel construction, especially in whisky production. A prominent example showcasing the efficacy of Mizunara is the Yamazaki 12-Year-Old Single Malt Japanese Whisky, frequently recognized as a benchmark for high-quality Japanese whisky (Noguchi, 2017).

Nepal possesses a rich diversity of oak (*Quercus*) species, particularly prevalent in its Himalayan and hilly regions. Despite their abundance, the full potential of these natural resources remains largely unrealized. Currently, these extensive oak forests are primarily exploited for timber in furniture production and as a source of fuel wood. To maximize the benefits from these plentiful arboreal assets, it is imperative to initiate comprehensive scientific investigations. Such research endeavors would facilitate a deeper understanding of the various oak species present in Nepal, enabling their optimal utilization for diverse applications beyond their current scope (Vetaas et al., 1998).

*Rakhi* (also spelt *raksi, raushi)* is a generic term that refer to strong alcoholic Nepalese drink. It is made by fermenting grains like finger millet *(Eleusine coracana)*, rice (*Oryzasativa)*, wheat *(Triticum spp.)* and maize (Zea mays) etc. but mainly finger millet using *murcha* (Rai, 2016). It is highly prevalent in the cultures of the Limbu and Rai peoples, as well as in other ethnic groups generally. The tradition of offering *jand* to show a hospitality is a unique way. It is also used in several joyful events, ritual rites, settling disputes and appeasing deities (Karki et al., 2019,D. Karki et al., 2012)

## 1.2 Statement of the Problem

Freshly distilled spirits are inherently clear, colorless, and typically possess a harsh, pungent flavor profile, which is particularly pronounced in unaged finger millet whiskey. To mitigate these undesirable characteristics and enhance overall palatability, maturation (aging) is a crucial process (Reazin, 1981). However, it is noteworthy that currently, there are no widespread or standardized aging practices employed for such spirits in Nepal, or any specifically designated or studied local wood types utilized for this purpose. Consequently, there exists a significant opportunity to explore and implement scientifically informed maturation techniques to refine the quality of indigenous Nepali alcoholic beverages.

Finger millet whiskey, locally known as *rakshi*, has a long-standing history of production in Nepal. The conversion of underutilized millet crops into high-quality *Jand* and *rakshi* presents a significant opportunity to enhance the economic well-being of local populations. Beyond its economic benefits, this practice also serves to promote and preserve traditional cultural heritage. Furthermore, in contemporary developing nations, there is an increasing demand for gluten-free food and beverage options. This demand is driven by individuals with celiac disease and other gluten intolerances who require alternatives to products derived from wheat, barley, or rye (D. B. Karki & Kharel, 2012; R. Karki et al., n.d.; Rai, 2016).

Since the home-made alcohol is not aged so it contains strong and thick flavor along with long and warming finish which is deficient of more flavors, aroma and color and contain more unwanted congeners. So, it must be aged for more flavor, aroma and appearance. American distillers like Koval distilleries in Chicago, USA produces pure millet malt whisky aged in American oak barrels at a good price and has been awarded for its distinct quality attributes. Whereas in a country like ours the production of finger millet fermented beverages are high in number but have quiet small market value compared to foreign whiskies.

The Himalayan belt of Nepal is home to a diverse array of oak (*Quercus*) species, thriving at elevations ranging from approximately 1000 m to 2500 m above sea level. Prominent among these are *Quercus lanta* (Ban oak also known as "Bajrath"), *Quercus lamellosa* (layered acorn oak, locally known as "*phalant*"), *Q. semicarpifolia* (*khesru* or brown oak), *Q. lanata* (woolly-leaved oak), *Q. leucotricophora* (banj or Himalayan white oak), and *Q. glauca* (ring-cup oak). These oak populations flourish within Nepal's temperate forest ecosystems, which provide the requisite climatic conditions for their growth. In several regions, such as the *Tinjure* area of the Eastern Himalayas, local communities extensively utilize these oak species as Non-Timber Forest Products (NTFPs). Beyond oaks, beech family trees (Fagaceae), including Castanopsis indica (Indian chestnut, locally known as "dhale katus"), are also found in abundance, particularly in the forests of the Chuira and Mahabharat ranges (Negi & Naithani, 1995; Negi & Negi, 1994; Paudel et al., 2012; K. B. Shrestha et al., 2013).

## 1.3 Objectives

### 1.3.1 General Objective

To study effects of different oak varieties (*Quercus SPP*.) on chemical and sensory quality of *Rakshi* (Finger millet whisky).

### 1.3.2 Specific Objectives

1. To prepare finger millet distillate.
2. To season and char the cubes and ageing of distillate with treated oaks cubes for one year.
3. To conduct qualitative/semi-quantitative analysis.
4. To evaluate and compare various chemical and sensory parameters of aged *rakshi*.

## 1.4 Significance of the study

If ingredients with higher levels of beneficial compounds are used, the antioxidant activity of the jelly and its content of vitamins and minerals can be enhanced. Fortification with natural components offers opportunities to develop products with distinctive and desirable flavour characteristics. Numerous studies have emphasized the health-promoting properties of phenolic compounds, including gallic acid, quercetin, and resveratrol, which are associated with the prevention of lifestyle-related diseases and the promotion of overall health (Sotolář *et al.*, 2025)

The production of a jelly using dragon fruit juice and stinging nettle extract holds multiple advantages both to the consumer as well as the industry. Dragon fruit contributes attractive natural colour, flavour, and antioxidant compounds such as betalains and vitamin C, which can serve as natural alternatives to synthetic colorants and preservatives. Stinging nettle extract, on the other hand, provides additional nutrients such as iron, calcium, and polyphenols, which are known to improve antioxidant capacity and health benefits. The combination of these two ingredients may yield a novel confectionery product with improved health-promoting potential while maintaining consumer appeal. These fruits are still underutilized in Nepal despite of having high health benefits and the favourable climate.

Additionally, the research addresses the perishability of dragon fruit by developing a shelf-stable product, which can reduce post-harvest waste while offering economic benefits to growers and local producers. Overall, this research will contribute to the diversification of functional food products by utilizing underexplored natural resources such as dragon fruit and stinging nettle. It aligns with global trends in sustainable food production and functional product development, promoting the utilization of natural, health-promoting, and locally available ingredients in confectionery formulations.

## 1.5 Limitations of the study

1. The selection of wood samples was limited to only five varieties among numerous varieties based on their availability, potentiality and botanical similarities.
2. Only chemical groups like carbo-oxalic acid group, phenols, esters, aldehydes, ketone and fusel oils were evaluated from GCMS rather than the specific flavoring compound.

# Part II

# Literature Review

## 2.1 Alcohol

Recent research on humankind's ability to metabolize alcohol, by an enzyme called *alcohol dehydrogenase*, suggests that this ability was present 10 million years ago in gorillas, chimpanzees, and humans when they first identified alcoholic fruit as a safe and consumable food (Dudley, 2004).

The origins of alcoholic beverages are generally traced to ancient Egypt and Mesopotamia 6000 years ago. Evidence suggests that virtually all civilizations and cultures have independently developed some variety of alcoholic beverages. Early fermentation processes, utilizing diverse agricultural products such as wheat, rye, millet, rice, oats, barley, potatoes, and grapes, laid the foundation for modern alcohol production technologies (Jones, 2001). Almost all distilled alcoholic beverages share common generic origins based on their primary raw material, whether it is fruit, grain, or sugar. These distilled products were commonly referred to by the Latin name aqua vitae, which means "water of life" (Daiches et al., 1969).

Archaeological evidence suggests that the production and consumption of beer can be traced back to the period between 4000 and 3500 BCE. Concurrently, the development of distilled alcoholic beverages also occurred. The Sumerians and Egyptians are frequently recognized as the earliest known brewers of beer (Wolf and Bray 2008, 2007). Distillation first appeared in Mesopotamia around 4000 BCE. The process was used primarily in the manufacture of perfumes, but it represented a major step in alcohol production. Until distillation, the sugar in the ingredients produced the alcohol in the final product but still made it possible to concentrate alcohol further (Standage T. 2017).

Although perfume helped create the still, alchemy became the driving force behind its use in Europe. Sometime between 1000 and 1500 A.D., alchemists developed brandy while searching for the 'essence' of wine. Sometime between 800 and 1300 A.D, the practice of distillation was introduced in China and Europe (Kiple & Ornelas, 2000). As the tides of history have embed and flowed, different drinks have come to prominence in different times, places, and cultures, from stone age villages to ancient Greek dining rooms or Enlightenment coffee houses. Each one became popular when it met a particular need or aligned with a historical trend; in some cases, it then went on to influence the course of history in unexpected ways  (Dudley, 2004; Piggott et al., 1995; Wolf et al., 2007).

## 2.2 Whisky

Whisky is produced through the distillation of a cereal mash which may or may not include malted cereal grains. The saccharification process could be facilitated by the diastatic enzymes in the malt, along with other natural enzymes, and the mash is subsequently fermented by yeast (EU regulations whisky,Piggott, 2017a). The major grains used in whisky production are maize (*Zea mays*), barley (*Hordeum polysticum*), wheat (*Triticum vulgare*) and rye (*Secale montanum*). These grains have historically been used to produce whisky because of their high fermentable starch content, providing a good yield of alcohol. Cereal grains do not undergo natural fermentation and thus need to be converted into a fermentable substrate. This process involves the modification of their structural components (by malting or by milling and cooking) to release the starch, which is then converted into sugar by enzymes under controlled temperature (Jack, 2012, Piggott, 2003).

In 1494, the earliest documented commercial transaction of whiskey (then referred to as aqua vitae) took place between the Benedictine monastery at Lindores Abbey in Fife and the Court of King James IV in Holyrood, Edinburgh. The governments often impose excise duties, or taxes, on distillers, which played the major role in the growth of the whisky distilling industry. Since then, various remarkable similarities can be found for the development of international brands and markets all around the globe (Stewart, 2014).

## 2.3 Maturation

Freshly distilled whiskies and other distilled products generally have unacceptable sensory characteristics (undesirable levels of sulphury, feinty, cereal and sour aromas) and are traditionally matured in oak casks for several years to produce a premium product (Philp, J .1989). The spirit in oak casks, mellows these immature characters and results in the development of desirable flavors, such as sweet, dried fruit, woody, spicy and nutty notes. The appearance also changes, with the clear newly distilled spirit developing a golden, amber color over time. Moreover, the clear freshly distilled spirit's appearance changes over time, developing into an intense golden, amber hue overtime. (Poisson & Schieberle, 2008).

The effect of maturation is determined by the cask used and the cask is determined by the type of cask used (American or Spanish), the treatment employed on the cask surface (charred or wine treated) and number of times cask has been used, and even the climatic condition. (Philp, J. 1989).

The mechanisms underlying maturation in oak casks remain incompletely understood. Research has been conducted to identify compounds, known as flavor congeners, that contribute to the flavor and aroma profile of whisky. Correlations between descriptive flavors and chemical analyses of mature whiskies, have identified over 400 flavor congeners (Philp, J. 1989). The principal flavor congeners include esters, carbonyl compounds, sulfur compounds, lactones, phenols, and nitrogenous bases, encompassing both desirable and undesirable components. In certain cases, the origin and synthesis methods of these compounds have been further studied, with the involvement of the maturation stage being subsequently confirmed. Changes in taste or aroma will be due to changes in these flavor congeners(Nishimura and Matsuyama, 1989)

Oak contains numerous volatile compounds which are in the range of 100 peaks as detected by gas chromatography-mass spectrometry. (Masson & Puech, 2000). Recent research has shown that ethanol and water do not form a completely homogeneous mixture across the entire compositional range (Delahunty et al., 1993). At certain concentration, ethanol exits as a micro-emulsion within water. In whisky, which primarily consists of ethanol and water, flavor components are retained within the aqueous emulsion through the stabilizing effect of ethanol (Conner, 1998). The aggregation of ethanol is influenced by the presence of wood extractives, which enhance the solubility of aroma compounds and subsequently reduces their release into the headspace (Conner 1999). This effect, along with the related physical and chemical changes, suggests that larger ethanol polymer hydrates form in wood-matured spirits, allowing them to better dissolve and retain aroma compounds. The wood extractives, particularly water-soluble components such as gallic acid, sugars and ionic components, compete with ethanol for water for hydration. This competition reduces ethanol's solubility in water, thereby increasing the amount of ethanol available to dissolve other congeners, such as esters. This reduces ethanol solubility and consequently increases the amount of ethanol available to solubilize other congeners such as esters (Piggott, 2017).

### 2.3.1 Chemical reactions

The major chemical reaction and changes that effect the final quality of the sprit are described according to Nishimura and Matsuyama (1989) are as follows.

1) Direct extraction of wood components.

2) Decomposition of the macromolecules forming the framework of wood, such as lignin, cellulose and hemicelluloses, followed by elution into the spirits.

3) Reactions of wood components with components of the unaged distillates.

4) Reactions involving only the extracted wood materials.

5) Reactions involving only the distillate components.

6) Evaporation of the low-boiling compounds through the wood of the cask.

7) Formation of stable molecular clusters of ethanol and water.

### 2.3.2 Maturation reactions

The reactions that occur during maturation can be distinguished under the two headings; additive and subtractive reactions as described in (Russell et al., 2014)

- Additive reactions include the reactions that introduce or form new aroma compounds.
- Subtractive reactions include the reactions that remove and alter the constituents of new formed spirit.

#### 2.3.2.1 Additive reactions

Several compounds found in mature whisky derive from oak lignin. Mechanisms for their formation have been proposed by many authors (Baldwin et al, 1967, Puech et al, 1977, Nishimura et al, 1983, Nishimura and Matsuyama, 1989, Sarni et al., 1990)

The heat applied during charring break down the structure of lignin and hemicelluloses polymers in the wood. The combined effect of oxidation and hydrolysis generates a higher level of color and extractives from the cask wood (Mosedale, 1995). Several compounds found in mature whisky derive from oak lignin. Mechanisms for their development have been postulated by many authors and the following pathway have been verified (Nishimura and Matsuyama, 1989) for the origin of lignin degraded products in matured distillate.

1. Degradation of lignin to aromatic compounds due to toasting or charring of casks
2. Extraction of lignin and monomeric compounds from the wood.
3. Ethanolysis of lignin results in formation of aromatics.
4. Further conversion of compounds in the spirit.

The ethanol-lignin complex extracted during maturation breaks down to form coniferyl and sinapyl alcohols. With further oxidation these compounds result into vanillin and syringaldehyde respectively (Reazin, 1995, Puech, 1984). Vanillin is naturally present in oak wood, but the concentrations are greatly increased by heat treatment, during which it is formed by the thermal degradation of lignin. In addition to vanillin, lignin degradation also produces coniferaldehyde, sinapaldehyde, syringaldehyde, and vanillic and syringic acids as described above ( Nishimura K., 1983).

Russell et al., (2014) studied that the hemicellulose usually breakdown to give sugars such as glucose or xylose but the formed products are too little in amount to affect any sugar level. One of the most important groups of phenolic compounds are the tannins, which is being loosely defined by group of water-soluble plant polyphenols (Haslam, 1989). The major constituents extracted from oak during maturation which include gallic and ellagic acids and the various complex combinations of these acids with sugars, which are known as gallo- and ellagitannins (e.g. vescalagin and castalagin). These compounds are generally non-volatile and have no aroma but may play a role in modifying the mouthfeel and taste of mature whisky (Glabasnia & Hofmann, 2007).

Hemicelluloses generally degrade to release sugars such as glucose and xylose. However, the quantities produced are generally insufficient to contribute significantly to the overall sugar content (Cutzach et al., 1997).

All the aroma components are not formed only by the degradation of structural polymers, some of them are already present in oak wood e.g. oak lactone. (Waterhouse & Towey, 1994).Oak lactones, a group of compounds exist as two distinct isomers: cis and trans oak lactones. The ratio of these two isomers varies among species and even within species, depending on the source forest. The *cis* isomer predominates in whiskies matured in American oak. Waterhouse & Towey (1994), whereas the trans isomer predominates in whiskies matured in Japanese oak (Noguchi et al., 2010).

Another potential source of aroma and flavor compounds in refill casks arises from their prior use in aging other spirits, such as bourbon, red wine, or brandy (Bryson, 2014). The residual compounds retained within the wood from these earlier maturations can interact with the new spirit, enriching its aromatic profile and contributing to greater complexity during subsequent aging processes. The various combinations of depletion and migration from deeper in the stave wood slow the rate of extraction and consequently maturation. Eventually, a point is reached where the cask fails to produce any sensory improvement, and it is then termed "exhausted" (J.R. Piggott et.al. 1989, Conner, J et, al, 2003, Philp, J ,1989).

#### 2.3.2.2 Subtractive Activity

The sensory character of the new-make spirit which is matured is cask may be modified by several interactions that have been shown to take place during maturation. The different activities that take place may be:

- **Evaporation of low boiling point and high volatile compounds through the cask**

While evaporation occurs from all casks, the extent to which factors such as wood porosity and stave thickness influence the rate of evaporation, and consequently the quality of the matured spirit, remains inadequately understood (Maga, 1989). Warehouse conditions, including temperature, humidity, and airflow dynamics around the cask, are critical factors that influence the rate of evaporation and, subsequently, the overall quality of the matured spirit(Reid, 1994).

- **Adsorption/degradation by charred surface of the cask**

The charring of the inner surface of the cask induces pyrolysis of the oak, resulting in the thermal degradation of its organic compounds. The 'active' carbon layer plays a vital role in the removal of immature character from the maturing spirit like sulphur containing compounds. During maturation slow adsorption and oxidation may be the possibilities for these reductions (Conner et al., 1993).

- **Masking of immature characters**

The immature character of a spirit is masked by the direct sensory interaction, where the presence of strong wood aromas like esters, carbonyls, sulphur compounds, lactones, phenols, and nitrogenous bases. All these imparted compounds have both desirable and undesirable character (Mosedale, 1995). Masking effects may also be present due to alterations in the whisky matrix that diminish the volatility of distillate components. The decrease in pH that occurs during maturation, which is influenced by cask-specific factors, influences the ionization state of weak bases and subsequently impacts their volatility (Conner et al., 1993)

#### 2.3.2.3 Interactive Reactions

The original distillate components may undergo chemical reactions with each other or with the wood constituents, leading to the formation of new compounds. Such reactions are typified by esterification, though they could theoretically include oxidation and acetal formation. During maturation, the concentration of esters increases because of esterification of free acids by ethanol. A large fraction of this is the formation of ethyl acetate from acetic acid, either extracted from the cask wood or the product of ethanol oxidation (Piggott et al., 1995; Reazin, 1981) .The changes in pH during maturation, which may be cask dependent, affect the ionization state of weak bases and, consequently, their volatility (Conner et al., 1993). The solubility parameters of many amphiphilic compounds present in malt distillates are changed due to the wood extractives (Conner et al., 1994).

## 2.4 Oak Barrels

In ancient times, when metals were scarce and costly, wood was the primary material used for vessels and containers for storage and transportation. As people began to experiment with storing alcohol in different containers, they noticed that certain types of wood imparted distinct flavors to the beverage. Among these, oak wood emerged as a particularly suitable material due to its strength, impermeability, and ability to enhance the taste of stored alcohol (J. Michael, 2017).

Oak barrels are used not only for whisky maturation but other alcoholic beverages like wine, rum, brandy, beer are also matured in it (J. Mosedale & Andrew Ford, 1996).

Nowadays oak barrels are considering not only the vessel for storage but also the active reactor vessel. Alamo-Sanza et al., (2017) Mid-20th century saw a decline in the use of wood, as materials such as cement and stainless steel gained popularity However, beginning in the 1990s, wooden barrels enjoyed a significant revival and became a worldwide fashion (JF Gautier, 2003,VL Singleton, 2000).

The culture of oak maturation has flourished in every part of world among them America and Europe are the largest producer of oak barrels. Oak wood for casks primarily comes from two sources: American oak and European oak. American oak, the more common choice, is mainly derived from Quercus alba but can also include other white oak species. Other European oaks are *Quercus robur, Quercus petrae, Quercus sessiflora* etc (Garde-Cerdán & C Ancín-Azpilicueta, 2006,Cadahía et. al, 2003).

Japan's use of native oak, like Mongolian Oak (Q. mongolica) commonly known as mizunara oak, in making whiskey has helped it become one of the world's top five whiskey producers (Noguchi, 2017). According to U.S. regulations to be called Bourbon, the whiskey must be aged in new, charred American oak barrels for at least 2 years (Booth et al., 1989).

And it is common practice for Scotch whisky distillers to use casks which have previously held Bourbon whiskey. These barrels are reused multiple times to age both Scotch malt and grain whiskies, until no flavor can be extracted from these casks (exhausted). To be called scotch whisky the spirit must be aged for at least 3 years (Philp, J .1989).

The other features that are important in the consideration of woods for cooperage include flexibility, permeability, porosity. Oak wood is suitable for barrel making because it is bendable for the shaping of the barrel bilge (the bulging center of the barrel shape) (Nishimura and Matsuyama, 1989).

### 2.4.1 Oak varieties in Nepal

The Himalayan landscape is a tapestry of diverse forest ecosystems, ranging from the arid, deciduous woodlands of the subtropical foothills to the verdant, coniferous forests of the subalpine zone (Vetaas et al., 1998). Oak Various species of oak are found in Himalayan belt of Nepal from above 1000 m from sea level to 2700 m (S. S. Negi, 1994).

Major oak varieties that are scattered in Nepal mostly in eastern hilly areas are: *Q. semicarpofolia (khesru), Q.lamellosa (falant, layered acorn oak), Q. leucotricophora (banj oak, Himalayan white oak), Q. lanata , Q. glauca* etc (Ohsawa et. al., 1986,B. Pradhan & GPS. Ghimire, 1994, Vetaas, 1997,Vetaas & Chaudhary, 1998b).

### 2.4.2 Taxonomical classification of oak.

| | |
|---|---|
| Kingdom | Plantae |
| Phylum | Magnoliophyta |
| Class | Magnoliopsida |
| Order | Fagales |
| Family | Fagaceae |
| Genus | Quercus |

### 2.4.3 Other maturation Variables

There are numerous factors that are responsible for the change in flavor like type of barrel used, fill strength, warehouse condition, duration of maturation, environment of maturation. Among them the type of cask used is regarded as the most dominant factor in maturation (Nishimura and Matsuyama., 1989).

### 2.4.4 Maturation time

The quality of distilled beverages is significantly affected by the ageing process, which is both crucial and expensive. To achieve the production of high-quality mature spirits, the product undergoing maturation should be stored in barrels for an extended period (J. R. Mosedale & Puech, 1998).

At the beginning of the ageing process, there's a quick extraction of substances from the cask wood which is driven by the rapid diffusion of readily available compounds. The gradual increase in extraction over time is caused by the release of additional hydrolysable tannins and lignin breakdown products. This occurs through a combination of spirit-induced hydrolysis and oxidation by air. The evaporation of spirit, which increases the concentration of all non-volatile congeners, may also contribute to this steady increase (Piggott et al., 1995, Piggott, 2003, Reazin, 1983,Caldeira et al., 2007, Canas et.al., 2007).

On prolonged maturation the spirit's alcohol content can decrease significantly (up to 50% v/v), which can impact the solubility of both wood and distillate components (Russell et al., 2014).

### 2.4.5 Fill strength and warehouse condition

The concentration of alcohol in a spirit significantly impacts the extraction and development of flavor compounds during the maturation process. Malt and grain whiskies are basically filled into barrels at a specific alcohol concentration. Malt whiskies are usually diluted to between 57% and 70% alcohol by volume before aging, while grain whiskies are often filled at a higher strength, but generally less than 80% alcohol by volume (Piggott, 2017)

Lower alcohol levels promote the extraction of water-soluble components from the wood, including hydrolysable tannins, glycerol, and sugars. Higher alcohol concentrations in distillates lead to the extraction of more ethanol-soluble compounds, such as lactones. In general, increasing the initial alcohol strength of the spirit results in lower levels of color, solids, and volatile acids during the maturation process (Piggott et al., 1995a, ,Fujii et. al., 1992, Nishimura and Matsuyama,1989).

Temperature and humidity are the two most important environmental factors that affect the aging process, these can affect both evaporative losses and the rate and progress of maturation (Mosedale, 1995, Philp, J .1989). Higher temperature increases the evaporation loss of both ethanol and water. In high humid condition more ethanol is lost than water which results in decrease in strength of alcohol percentage. Whereas at low humid condition more water is lost compared to alcohol and the strength increases. Application of these results to the ware-house environment is not straightforward, as conditions can vary on a seasonal, monthly and even daily basis (Philp, J. 1989,Russell et al., 2014).

## 2.5 Oak alternatives

The construction and maintenance of oak barrels is an expensive process. Cost may vary from the wood source, process, market demand and reputation of the barrel maker.

Wooden barrels are both expensive and high maintenance. Furthermore, they experience greater ethanol loss due to evaporation. If the quality of the wood and the workmanship are poor, the quality of the spirits will be affected and evaporation losses will increase. Furthermore, barrels lose their extractable substances after a few years and must be replaced. Costs rise in proportion to the length of the ageing period, because of tied up capital (Singleton et al, 1986).

Recently, there have been advancements in the aging process of alcoholic beverages. One such method involves introducing fresh wood pieces (oak chips or staves) into inert containers. This technique offers distinctive flavors that were not possible before, and new approaches to managing distilled beverages. Since wood is being put into distillate and not distillate into wood, the entire surface area is usable and not just 40% of it. The result is a compelling application that has been adopted by many (Stutz et al., 1999).

Oak alternatives also reduce the cost by reducing the cost associated with topping up and lost due to evaporation and work well in long lasting steel or glass tanks (Jackson, 2000). From different research and studies alternative, it has been proven that oak alternatives can have proven to be comparable in quality to oak barrels. The flavors oak contribute to wines and spirits are a result of seasoning (claimed) and heat treatments (confirmed), which modify the oak's key constituents (Canas et. al., 2009, González-Centeno et. al., 2021).

Oak chips have the ability to impart oak flavors in a matter of weeks compared to the years-long barreling methods showing a similar intensity. However, there are conflicting theories about the comparisons of oak barrels and oak chips. It is claimed that some of the oak alternatives are likely to bring a monochromatic, harsh profile; missing delicate mixture of aromatic compounds like that a mature rink usually can deliver. Nevertheless, it is unknown how true this can be or if it is just propaganda by the vested spirit and barrel interests (Arapitsas et al., 2004).

The rising demand for American and French oak barrels has resulted in substantial price hikes and limited availability. In response, cooperages have explored alternative oak sources, particularly in Eastern Europe. These regions, including Romania, Hungary, and Russia, provide oak species with properties like French oak. In recent years, researchers have extensively studied a variety of oak species like Slovenian oak (Chira & Teissedre, 2015), Spanish oaks (Cadahía et al., 2001, Cadahía et al., 2009), Hungarian oaks (Guchu et al., 2006,Díaz-Maroto et al., 2008), Russian oaks (J. Mosedale, 1996), Romanian)ian oaks (Prida & Puech, 2006). These studies of the same species but different origin showed similar characteristics to American and French oaks, suggesting that they are suitable for barrel production for quality wines. Some authors even state that these origins have intermediate characteristics between French and American oaks. Although there are variety of wood species only oak and chestnut are the only ones approved today by the Organization of Vine and Wine (Cadahía et al., 2001b ,Fernández de Simón et al., 2003, Prida & Puech, 2006)

## 2.6 Origins of color in matured whisky.

The natural color of matured whisky is known to originate during maturation in oak casks, where the virtually colorless distillate changes into golden brown associated with the final product. Color related components are introduced to the spirit by their extraction from the cask wood, the three major constituents of which are lignin, cellulose and hemicellulose (Conner, 2003, Sharp, 1983).

Heat treatment of casks during manufacture promotes thermal degradation of these wood polymers and it is the breakdown of hemicellulose in particular that is thought to contribute to whisky color during maturation. Certain tannic substances derived from lignin are also believed to contribute to whisky color; however, the degradation of this wood polymer predominantly influence flavor (Mosedale, 1998).

The reactions involved in the formation of colors during maturation are very complex and consequently very little is known about the exact chemical structure of natural color arising during maturation. The different color intensities and hues associated with different whiskies, however, are known to depend on several cask variables such as cask history, fill strength and maturation age ( Piggott & Conner, 1995 , Nishimura and Matsuyama 1989).

## 2.7 Important chemical compound in whisky

### 2.7.1 Ethyl Alcohol

Alcoholic strength measurement is the key quantitative measurement in whisky production, as it helps measure the efficiency of the overall carbohydrate conversion process. Ethyl alcohol determines the excise tax paid to the government and ensures that consumers receive the alcohol declared on the product's label. Alcoholic strength is normally defined as the concentration of ethyl alcohol in a solution by volume at a specific temperature. Alcohol concentration is normally expressed as a percentage or as degrees proof (Russell et al., 2014).

### 2.7.2 Aldehyde Group

Phenolic aldehydes such as vanillin contribute to olfactory characteristics of wine with notes of vanilla, coffee, dark chocolate and smoke, with a synergistic effect of whiskey lactone (Spillman et al., 1997). Vanillin (4-hydroxy-3-methoxybenzaldehyde) and Syringaldehyde (hydroxy-3,4-dimethoxybenz-aldehyde) is formed, as a lignin degradation product, mainly during coopering and is subsequently extracted into wines and spirits during barrel maturation (Dubois, 1989).

If aromatic aldehydes form a major proportion of oak wood volatile compounds, their sensory role is still largely a matter for conjecture. Grassy notes have been considered synonymous with aldehydic, green and leafy characters in the whisky flavor wheel of (Lee et al., 2001). However, the technical term - aldehydic - is better understood as fresh or dried grassy characters (Lee at el, 2000). Grassy seems a more useful term than green, since the term is widely used to imply a lack of maturity in wines (young) and associated with use of green malt in whisky (Wishart, 2009).

### 2.7.3 Phenol Groups

Volatile phenols, such as eugenol and iso-eugenol, guaiacol and some of its derivatives, showed an increasing tendency with increasing maceration time, Toasted wood chips resulted in higher levels of these phenols compared to non-toasted chips. This is because the heat treatment breaks down phenolic aldehydes, leading to the formation of more volatile phenols. (Chatonnet, 1999, Singleton, 1995).

Eugenol (2-methoxy-4-(2-propenyl) phenol), a volatile phenol, is produced by the lignins breakdown during wood toasting and contributes the character (Aiken & Noble, 1984,and smoke (Aiken & Noble, 1984, Feuillat et al., 1999). Phenolic aldehydes such as vanillin contribute to olfactory characteristics of distillate with notes of vanilla, coffee, dark chocolate and smoke, with a synergistic effect of whiskey lactone. If aromatic aldehydes form a major proportion of oak wood volatile compounds (Spillman et al., 1997, Boidron et al., 1988).

### 2.7.4 Furfuranic compounds

(furfural, 5-hydroxymethylfurfural, and 5-methylfurfural) are supplied by the wood and by distillation. Furfural (2-furancarboxaldehyde) originates from degradation of monosaccharides produced by partial hydrolysis of hemicellulose. It contributes to the character of dried fruits, and particularly that of burned almonds. (Sauvageot & Feuillat, 1999, Spillman et al., 1997,Chatonnet et al., 1999).

The aroma note associated with furfural at 90% recognition threshold (839 mg/L) (Lee *et al.*, 2000b) was only described by a minority of distillers (<10%) as grainy, greater numbers used marzipan (coconut, cake mix, almond, nutty, walnut oil and coumarin-like - 54%), sweet (26%) and oily (15%) as descriptors (Lee *et al.*, 2000b, K. Lee et al., 2000).

Pentose sugars from the breakdown of cell walls in cereal husks yield furfural during pyrolysis processes, both malting and distillation (Bathgate, 1977, Singleton, 1995).Furfural yield appears to be a function of pH: when wash contained a high number of lactic acid bacteria, furfural concentration increased. (Jack, 2014)

### 2.7.5 Ethyl Esters

Esters are an essential family among the aroma components of whiskeys. Even present in small amounts, they have very intense odor characterized by pleasant aromas, such as *fruity* and *floral* aromas that generally contribute positively to the global quality of whiskeys (Lee et al., 2001).

Ester formation occurs during maturation through esterification of spirit fatty acids(Reazin, 1981) (Reazin, 1981), primarily as ethyl acetate (Nishimura and Matsuyama,

1989) (Nishimura & Matsuyama, 1989). Acetic acid is generated through hydrolysis of side chain acetyl groups in oak cell wall hemicelluloses (Nishimura *et al*., 1983) and through oxidation of ethanol.

In distillations, solveinty and fruity characters are related to the heads (foreshot) and the characters pass to floral and feints (tails) at the late distillation stages (Jounela-Eriksson, 2019) Conner, 1992). Esters, such as ethyl acetate, are often perceived as solvent-like. Of these esters, ethyl acetate is the most abundant at (typically) 175 mg/L in but has little overall flavor impact with a high threshold (33 mg/L).

Although individual esters are not abundant whisky congeners, the total esters form a key flavor component Salo, (1972), contributing particularly to roundness in whisky (Charalambous, 2014).

### 2.7.6 Higher alcohol

Higher alcohol acetates are correlated with freshness and fruitiness character, while fatty acids can contribute with *fruity*, *cheese*, *fatty* and *rancid* notes to the whiskeys sensory, causing a positive contribution to the overall sensory properties when present at levels lower than 300 mg L−1 (Reazin, 1983).

### 2.7.7 Nitrogen compounds

There are 5 classes of nitrogen which are much important for whisky flavor: aliphatic amines, quinolines, thiazoles, pyrazines and pyridines. Of these the heterocyclic compounds, pyridines, pyrazines and thiazoles, are believed to make a significant contribution to the whisky's flavor character. Although present in very low concentration, of the order of ugl-1 or mg 1-, due to low odor threshold levels and distinctive odor properties their importance is most likely. In general, the odors of pyrazines are associated with pleasant roasted foods. The terms burnt, roasted and nutty, and nutty, roasted, earthy, fruity and woody", have been used as descriptors for these most often.(Conner et al., 1993).

### 2.7.8 Oak Lactones

The two oak lactones present in matured whiskies are β-Methyl-ϒ-octalactones (whisky lactones) and ϒ-nonalactone which was discovered in bourbon (Arapitsas et al., 2004) .

There are four stereoisomers but only the cis (3S, 4S) and trans (3S, 4R) ismers are found in wood extracts. The sensory character of the lactones is described as a weak coconut note, faint musty, earthy and hay note and that of the latter is described as a spicy note, weak coconut and green walnut note (Koppenhoefer et al.,1994, Maga, 1996, Nykänen, 1986).

These lactones, the so called oak or whisky lactones, derive solely from oak and may be formed from the oxidation of lipids, (Maga, 1989, Reazin, 1981) claimed that their flavor was modified by the presence of furfural.

## 2.8 Distillation

Distillation is more than just a simple separation process: formation of new flavor compounds will occur, while others are eliminated. Most of the flavor formation is due to heat-mediated reactions, such as the Maillard reactions described previously. In terms of flavor removal, one of the most important reactions during distillation is a reduction in the level (Poisson & Schieberle, 2008, Poisson & Schieberle, 2008).

The process of distillation uses the concepts of relative volatility and boiling points to separate two or more liquid components in a combination. The greater the difference in relative volatility the greater the nonlinearity and the easier it is to separate the mixture using distillation. In this procedure the liquid mixture is boiled in a still to produce vapor, which is then removed from the still by combination. Due to differences in relative volatility or boiling points, the vapor is rich in light components, and the liquid is rich in heavy components (Mujtaba, 2003).

Two distinct distillation systems have been used for production of whiskies; the batch or pot still, normally providing a double distillation (occasionally triple), to produce highly flavored spirit, and the continuous column still to produce lighter flavored spirits normally used as the base for blending.

### 2.8.1 Batch Distillation

Batch distillation, perhaps the oldest operation used for separation of liquid mixtures. For centuries and today, batch distillation is widely used for the production of fine chemicals and specialized products such as alcoholic beverages, essential oils perfume, pharmaceutical and petroleum products. It is the most frequent separation method in batch processes (Lucet et al., 1996). Pot Distillation is the main distillation type that has been used for whisky production from the starting time. A still consists of three major parts, the pot containing the liquid to be distilled, the swan neck and Lyne arm, and the condenser. There are many minor variations on this basic pattern. The pot can assume many shapes conical, onion, cylindro-conical, inverted cone, ball and lantern – provided that sufficient volume and surface area is maintained. Spirit is usually double distilled but there are some distilleries which practices triple distillation to produce lighter spirit at natural higher strength (Adams, 1910)

Wash typically contains about 8 % v/v ethanol, and efficient distillation should result in the collection of virtually all the ethanol, at about 21-23 % v/v, as low wines. The second, spirit, distillation proceeds in essentially the same way, except that a portion of the distillate (middle cut) must be selected as new spirit (Whitby, 1992)

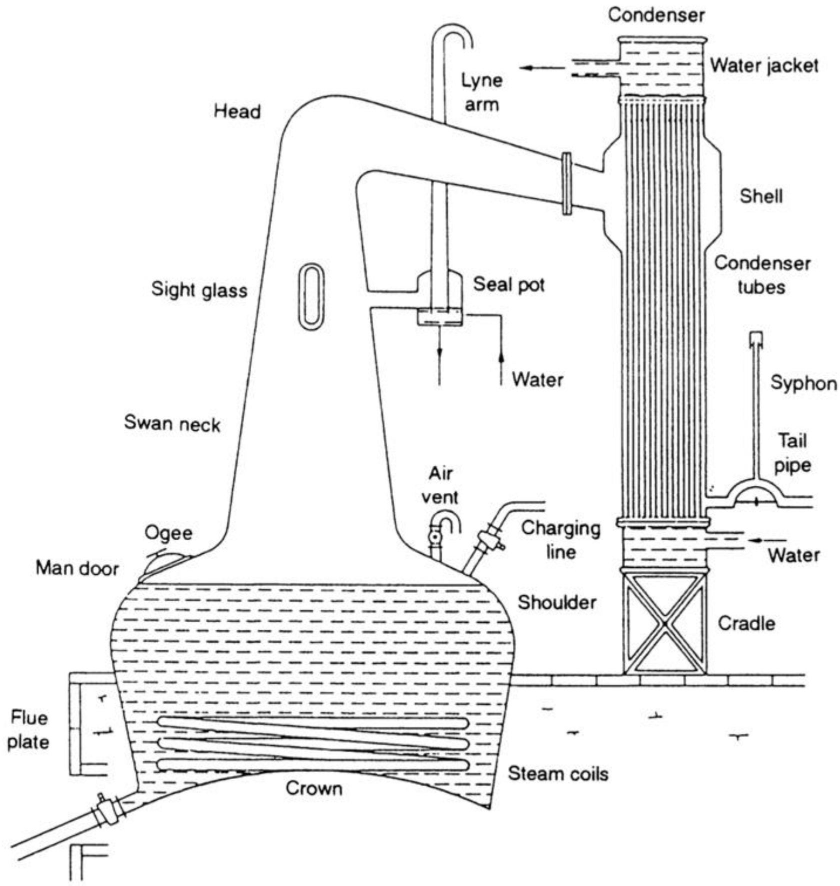


**Figure 2.1**: Batch Distillation

### 2.8.2 Continuous Distillation.

In whisky production, column stills serve to distill lighter-bodied grain spirits, which are primarily intended for blending purposes, though occasionally bottled as single grain expressions. The introduction of continuous distillation technology occurred in 1827, with subsequent refinements to the continuous still apparatus patented by Aeneas Coffey in 1830 (Moss and Hume, 1981). This type of apparatus is still known as the Coffey or patent still (Whitby, 1992).

This continuous still comprises two adjacent columns constructed side-by-side. The wash or beer is preheated by passing it through a tube winding through the second column (rectifier) and is then fed into the first column (analyzer) toward the top. Steam is sparged into the base of the column, and as the wash falls, the volatiles are stripped out and removed from the top of the column. The vapor then passes to the base of the rectifier, and the separation into alcohol and water occurs. The spirit product is removed at a level toward the top of the column (Piggott et al. 2003).

## 2.9 Heat treatment of wood.

Heat treatment constitutes a crucial stage in the production of maturation casks for distilled spirits. Two primary heating methodologies are employed: toasting is a milder but more prolonged form of heat treatment, while charring is more rapid and involves heating the inner face of the cask with a gas burner until the inner face catches fire and becomes carbonized. The fundamental aims of both treatments converge on achieving similar outcomes (J. R. Mosedale, 1995).

- Degradation of wood polymers to yield flavor compounds
- Destruction of resinous or unpleasant aroma compounds present in the wood
- Production of a layer of 'active' carbon on the inner surface of casks (charring only).

This controlled burn creates physical changes that transform the oak barrel from a mere container to a chemical reaction chamber, a filter, and an infusion vessel (Bathgate & Taylor, 1977). Not only physical changes but other chemical changes also occur during charring i.e pyrolysis and autolysis (Clyde at al. 1993).

Regarded cis- and trans-lactone, eugenol, vanillin and syringaldehyde as the most important volatiles present in oak woods from a sensory standpoint. The optimum temperature for extraction of vanillin and syringaldehyde was proposed as 165 to 215 °C (Pérez-Coello et al. 1999).

### 2.10 Seasoning

The period between oak tree felling and coopering involves a drying process which is referred to as seasoning. This drying may be a natural process, involving open-air storage for a period commonly between one and three years, and/or an artificial process, involving controlled temperature and humidity conditions. The drying process is important to the resultant structural integrity of the barrels. It prevents the wood from shrinking after construction, thus ensuring a tight container. This aspect is, obviously, not important to non-barrel oak wood (e.g. chips). Any aroma and flavor effects of seasoning, however, should be equally important to all oak wood used in winemaking. The concentration of oak-derived flavor volatiles can change considerably because of the drying process and/or other factors during this period (Sefton et al. 1993, Cown et al. 1996, Chatonnet et al. 1994). Francis et al., (1992) found that seasoning oak wood for 12 months resulted in aroma changes from 'spicy' to those that are 'more distinct and intense. Air seasoning results in an increased mycoflora attacking cell wall lignin and polysaccharide.

### 2.11 Millet

The word "millet" refers to a group of small-seeded annual grasses that are of minor importance in the West but a staple in the diets of African and Asiatic people (Audu et al., 2008). Millet can be summarized as small, cultivated grass with kernels that are grouped as members of the Poaceae family, but they can belong to various tribes or even subfamilies. The word itself comes from the French word "mille," which means "thousand." This means that there are one thousand grains in a handful of millet. It is one of the oldest crops and is referred to as 'Artha-Kandaka' in ancient Sanskrit Literature, meaning 'Dancing grain'(Hiremath & Geetha, 2019). Millet was first mentioned in written records 10,300 years ago in Northern China and Korea. Paleoethnobotanists have concluded from observing the growing methods and remnants of the storage vessels that the ancient grain was foxtail millet. Additionally, millet is described as priyangava (foxtail millet), aanava (barnyard millet), and shycamka (black finger) in Yajurveda scripts(Arendt & Dal Bello, 2008). It is one of the oldest crops and is referred to as ‘Artha-Kandaka’ in ancient Sanskrit Literature, meaning ‘Dancing grain’(Hiremath & Geetha, 2019).

According to Arendt & Dal Bello, (2008), there can be confusion when studying different millet-related literature because different common names and even different proper names are widely used for the same species. For example, millet is commonly referred to as kodo in Nepal, which is *Eleucine coracana*, whereas kodo millet which is common in India is *Paspalum scrobiculatum*.

Common millet (Panium miliaceum), finger millet (Eleusine coracana), pearl millet (Pennisetum typhoideum), barnyard millet (Eichinochloa frumentacea), and foxtail millet (Setaria italica) are the five common types of millets. Finger millet is well-known in many parts of Asia as an antidiabetic meal. Compared to other typical cereals, it's thought to have higher fiber and less carbohydrates (Ravindran, 1991). The general English name for some more commonly cultivated millet has been discussed in Table 2.1 with its vernacular name and taxonomy (Arapitsas et al., 2004).

### 2.11.1 Introduction to Finger Millet

Finger millet was First domesticated in Africa probably originating in the highlands of Uganda and Ethiopia some 3000 years ago and after some period it was introduced into India(‘Lost Crops of Africa’, 1996). Finger millet, also known as ragi in India, is another staple food in Eastern Africa and Asia e.g. India, Nepal (Léder, 2004). Africa is the primary origin and production region for several grain crops, including sorghum and other types of millet. Some people have referred to these ancient crops as "lost crops" or even "orphan crops". Finger millet gets its name from the way the plant's head resembles a hand's fingers. It has a few possibly extremely helpful brewing-related qualities. Research has found that certain Tannin-containing malts have a high diastatic power; in particular, the β-amylase activity is significantly higher than that of sorghum.(Léder, 2004).

Of all major cereals, like wheat, rice, maize, etc. This crop is one of the most nutritious. Indeed, some varieties appear to have high levels of methionine, an amino acid lacking in the diets of hundreds of millions of the poor who live on starchy foods such as cassava and plantain(‘Lost Crops of Africa’, 1996). Although a gluten-free grain with a low-glycemic index with nutritional and nutraceutical advantages, Finger millet is neglected and underutilized (Amadou et al., 2013).

Finger millet (Eleusine coracana) is the fourth most pivotal food crop in Nepal, thriving in hectares 265,401 of land. With a robust yield of 326,443 MT and a productivity of 1.23 T/h (‘STATISTICAL INFORMATION ON NEPALESE AGRICULTURE’, 2079).Finger millet is the most significant crop among the millets in Nepal in terms of both area and yield, with proso and foxtail millet following closely behind. Among millet groups, finger millet is the most important crop and the fourth important cereal crop after rice maize, wheat in Nepal. Finger millet plays a crucial role in both relay cropping systems and mono-cultivation, mid and high hills. While not only a staple for direct human consumption, but finger millet also emerges as a key player in alcoholic beverage production

The dark brown seed coat of the little millet grain is rich in polyphenols, including tannins, flavonoids, and phenolic acids and their derivatives. Phytic acid, an antinutrient that binds minerals, is also abundant in it (Sripriya et al. 1996). According to Namiki, (1990), each of these compounds can scavenge radicals, making them potential antioxidants. The significance of antioxidant compounds is increasing because of their dual applications as lipid stabilizers in the food industry and as antioxidants that suppress excessive oxidation, which causes aging and cancer, in preventative medicine. Other bioactive compounds like ferulic acid-rich arabinoxylans or feraxans, ferulic acid, caffeic acid, and quercetin have been associated with certain health-promoting properties and have been found bio accessible in the grain. Finger millet has demonstrated possible nutraceutical effects considering the growing interest in natural remedies over synthetic alternatives for the treatment of food-dependent illnesses. Some important health effects such as anti-diabetic, antioxidative, anti-inflammatory, and antimicrobial properties have been reported in recent trials with the grain (Udeh et al. 2017). Numerous phenolic compounds and flavonoids found in finger millet have been identified by several recent research (Chandrasekara & Shahidi, 2012; Chethan & Malleshi, 2007; Hithamani & Srinivasan, 2014).

### 2.11.2 Traditional finger millet-based alcoholic beverages in Nepal

The history of alcoholic beverage production in Nepal extends to antiquity. These traditional technologies were indigenously developed by diverse ethnic groups and are intrinsically linked to socio-cultural practices, including the celebration of festivals, feasts, and marriage ceremonies. This suggests that these beverages hold significant cultural value and are not merely consumables, but rather integral components of ritualistic and communal events (Ethnic Fermented Foods and Alcoholic Beverages of Asia, 2001).

Although generations of people have learnt how to brew at home, they are mostly unaware of the intricate workings of microbiological biochemistry or its enormous scope. In fact, the exact nature of fermentation is still not fully known to them.

Traditional alcoholic beverages in Nepal possess profound ritual significance and are deeply embedded within the cultural heritage of its diverse ethnic groups. Among the *matwali* communities (non-Brahmin Nepali groups, including, but not limited to, the Limbu, Rai, Gurung, Tamang, Sunwar, Newar, and Sherpa), the provision and consumption of substantial quantities of these beverages are often integral to social activities. Specifically, *jand* and *raksi* are essential components in the solemnization of marriage ceremonies among non-Brahmin Hindu Nepalese and Buddhist tribes, highlighting their importance in key life-cycle events. Among the various fermented finger millet alcoholic beverages, jand, toongba, nigar and rakshi are the major alcoholic fermented liquor traditionally consumed in various parts of Nepal (Kharel et al. 2009). There is different alcoholic beverages made from fermented mash of millet some of them are Jand, Nigar, Tongba, Rakshi etc. Among them Rakshi is described below

### 2.11.3 Rakshi

An unaged congeneric spirit obtained by pot distillation of the slurry of fermented cereal (*Jand*) is known as *Rakshi*. The product like whiskey and has alcohol contents varying, generally between 15 to 40% (v/v). *Jand* and *Rakshi* finds a prominent place in Limbu and Rai culture and other ethnic groups in general. The tradition of offering *jand/rakshi* to guests is a unique way of showing hospitality. It has also been used in several festive occasions, ritual rites, settling disputes and appeasing deities (Tamang, 2020)

After the completion of fermentation, the mash is mixed with some portion of water, poured into the *phoshi* (a flat bottom copper, brass or aluminium pot) to about 1/3 of its volume and *paini* (earthen pot having holes at the bottom) is placed over it. *Nani* (earthen pot for collecting distillate) is kept inside the *paini* and *bata* (condenser) is placed on top of the *paini*. The *bata* is filled with cold water and firing is made. During distillation, water and other volatiles are evaporated, passed through the small holes of *paini* and condensed on the cold surface of *bata*, which in turn is collected in the *nani*. The water in the *bata* is chaged from time to time when its temperature exceeds 45 $_{\circ}$C. As the number of water changes increases, the distillate becomes weaker in alcohol content. Thapa et al., (2004) A conventional distillation assembly is shown in

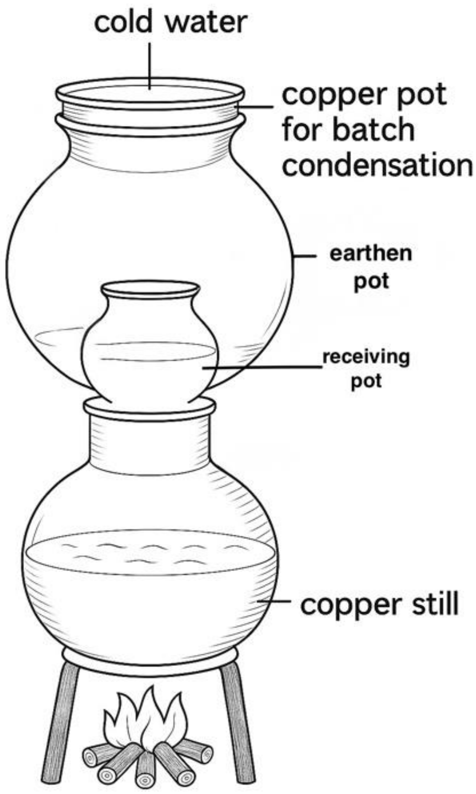


**Figure 2.2**: Distillation process of fermented Jand.

## 2.12 Traditional starter culture used in the context of Nepal

### 2.12.1 Murcha

*Murcha* (also spelt *marchaa, marcha*) is a traditional ingredient used to make alcoholic beverages like (such as jand (undistilled) and raksi (distilled) in the Himalayan region, It is an amylolytic starter that convert starches into sugars during the fermentation process (Tsuyoshi et al. 2005).

Murcha preparation involves use of over 40 wild plants. Microbiologically, *murcha* is a mixed culture containing saccharifying molds, fermenting yeasts, and acidifying bacteria. *Saccharomyces cerevisiae* is the dominant yeast in these *murcha* plants. KC et al., (2001) Other yeast types identified include *Saccharomyces bayanus*, *Saccharomycopsis fibuligera*, *Pichia anomala*, *Candida glabrata*, *Candida versatilis*, *Saccharomyces capsularis*, and *Pichia burtonii* (H. Shrestha et al., 2002) .

Some examples of notable amylolytic (starch hydrolyzing) molds are strains of *Aspergillus oryzae*, and species of *Mucor* and *Rhizopuss* (KC et al., 2004).

# Part III

## Materials and methods

### 3.1 Materials

#### 3.1.1 Finger millet

Finger millet (*Eleucine coracana*) for the preparation of whiskey was purchased from the local market of Dharan, and it was verified by Regional Agriculture Research Center, Tarahara-Sunsari Nepal. (P74H+3H)

#### 3.1.2 Murcha

*Murcha* was collected from native makers of district Terhathum, Basantapur. (4CM4+WX)

#### 3.1.3 Wood variety and sources

Six different species of wood were collected. All 6 of them are from the Fagaceae family of the plantae kingdom as shown in Table. Among them five are oak species which are American oak (*Quercus alba*), layered acorn oak (*Q. lamellosa*), *Quercus lanta* (Bajrath), *Quercus semecarpifolia* (Kharsu), *Quercus leucotrichophora* (Banj) and beech wood from Indian chestnut tree (*Castanopsis indica*) which is found commonly in Nepal mostly along the Churia and Mahabharat range. Apart from American white oak, all wood specimens were sourced from the Tinjure ridge, located at the juncture of two eastern Nepali districts Tehrathum and Sankhuwasava with help of locals. American oak was purchased online and collected. The heart wood (central part of trunk) of the matured trees was collected of the locally available wood.

The wood collected were heartwood and cut few years back. After they were collected, woods were left for sun drying.

### 3.1.4 Glassware and equipment

Small level stainless steel distillation pot designed and made by Central campus of technology Dharan was used for secondary distillation. Glassware required for analysis of fermented wash/mash was obtained from the Central campus of technology Dharan. Further analysis of matured whisky was done in Raj Brewery Quality Control lab, Ramgram-Parasi (GJC4+4F) and Jawalakhel Group of industries central lab Kathmandu (7MV7M8C7+HX9). All the necessary glassware and instruments were used from these two labs.

### 3.1.5 Lists of equipment.

**Table 3.1**: Lists of equipment.

| S.N. | Equipment | S.N. | Equipment |
|---|---|---|---|
| 1. | Stainless steel vessels | 9. | Anton paar alcolyzer model no DMA 4500 M |
| 2. | Weighing arrangement | 10. | pH Meter EUTECH INSTRUMENTS (pH 700) |
| 3. | Heating arrangement | 11. | Conductometer HANNA instruments |
| 4. | Distillation set | 12. | Digital Weighing balance |
| 5. | Pycnometer | 13. | Spectrophotometer Hach |
| 6. | Titration apparatus | 14. | Gas Chromatography |
| 7. | Other routine glasswares | 15. | Hydrometer |
| 8. | Food grade silicon tube rubber pipe | 16. | Sensory glasses (wine or tulip shaped) |

### 3.1.6 Glass jars and fermentation vessels

Fermentation vessels of about 50 liters and primary distillation pot was used of own home. Glass jars of 3 liters were purchased from Bhatbhateni Dharan. (R76H+RQ)

### 3.1.7 Chemicals

All chemicals used were of analytical grade and were obtained from the laboratory of Central Campus of Technology and Raj brewery.

### 3.1.8 Oak varieties

This project incorporates several oak varieties, in addition to a non-oak species selected based on its application in a prior study. Taxonomical name and sources shown in **Table 3.2.**

| S.N in this research | Common Name | Local Name | Scientific Name | Sources |
|---|---|---|---|---|
| 2 (B) | American white oak | White oak | *Quercus alba* | Imported |
| 3 (C) | Ban oak, Woolly oak | Bajrath | Quercus lanta | Tinjure range |
| 4 (D) | Layered acorn oak | *Phalant, gogane, salshi, Banga, pharat, bongset* | *Quercus lamellosa* | Tinjure range |
| 5 (E) | Brown Oak | *Kharsu* | *Quercus Semecarpifolia* | Tinjure range |
| 6 (F) | Banj Oak | *Banjh, Banj* | *Quercus Leucotrichophora* | Tinjure range |
| 7 (G) | Indian chestnut | *Dhalne Katus, Banjh katus, chyakpal* | *Castanopsis indica* | Tinjure range |

**Table 3.2**: Oak varieties with their taxonomical name and sources.

## 3.2 Methods

### 3.2.1 Preparation of fermented mash of millet

The preparation was done at home scale level since all the indigenous tools required were easily available. About 150 kg of millet was brought and cleaned with water for multiple times. Then the cleaned millet batch of 15 kg along with water was cooked on open fire at temperature of 92°C in. Continuous stirring was also done with big wooden stirrer to avoid burning at the bottom. Bursting of the millet grains indicate the completion of cooking. Time taken for cooking was about 2.5 hours. The hot sticky mash was spread in bamboo mat as soon as possible for rapid cooling and breaking of big lump of mash with wooded stirrer. Following a cooling period, approximately 1g/100g of *murcha* powder was incorporated into the substrate and thoroughly mixed. The mixture was subsequently divided into two sacks and placed on a wooden flank for a duration of two days for biomass development. The sacks were then sealed, allowing for minimal air exchange. Incubation resulted in a distinctive sweat-like aroma permeating the surrounding room.

Due to its ready availability, 50-liter PVC plastic drums were selected as fermentation vessels for the incubated mash. The drums were completely sealed and placed on a wooden bed to facilitate fermentation, avoiding direct contact with the concrete floor. Fermentation occurred from late October to mid-December 2023, coinciding with the winter season in Nepal. The cool and humid winter conditions facilitated a slower fermentation process, with temperatures ranging from 18 to 25°C.

Finger millet (15 kg batch)

↓

Cleaning several times / pounding / winnowing

↓

Washing with squeezing in mesh bag

↓

Cooking and steaming (until few grains start bursting indicating the completion of cooking)

↓

Spreading in bamboo mat

↓

Inoculation with murcha (~1g/100 g)

↓

Placing in two sacks for biomass development

↓

Putting the mash into PVP container

↓

Fermentation at cool temperature (~40 days)

↓

*Jand*

**Figure 3.1:** Preparation of finger millet jand**.**

### 3.2.2 Distillation of Fermented mash

After a fermentation period of 40 days, the mash was determined to be fully fermented, then the mash is taken for distillation. The primary distillation was done in the wash still by traditional method that is commonly practiced in the indigenous households of Nepal. The traditionally formed finger millet spirit commonly known as *Rakshi* has 20±1.07~22 v/v alcohol content. Before taking the mash for distillation, 1/3 part of water was added in the mash and the distillation set-up is made which is shown in fig from literature. So produced *rakshi* was *paanch pane* (meaning the water for condensation was changed 5 times). Total of 7 batch distillation was done for the complete distillation and about 60 liters of 22% abv distillate was obtained. The alcohol percent of the distillate couldn't be increased efficiently by traditional method, so secondary distillation was done, the spirit still for second distillation was set up in the laboratory. For second distillation about 60 liters of primary distillate was taken. Following distillation, the resulting distillate underwent fractional separation based on alcohol content. Only fractions exceeding 60% ABV were selected for subsequent blending and maturation processes. The final spirit was then adjusted to an approximate alcohol by volume abv of 73%.

Fermented mash

↓

Addition of 1/3 part of water and mixing

↓

Distillation in traditional distillation set as shown in **Figure 2.2**

↓

Distillate of about 22% abv collected (~60 ltr)

↓

Distillate taken for secondary distillation in lab pilot label distillation

↓

Distillate exceeding above 60% abv collected and maintained 73% abv.

**Figure 3.2**: Primary and secondary distillation process.

### 3.2.3 Oak cubes preparation

Oak cubes of Nepalese origin were collected form himalyan area of Terathum and Sankhuwasava which is also known as Tinjure hills. These oak trees were felled one to three years ago. Residents reported that their girths were so large that multiple individuals could not encircle them with their arms combined, indicating an age exceeding fifty years. For research purposes, only the heartwood of these trees, which had undergone natural seasoning in their natural environment was extracted. The wood pieces were washed with hot water and dried in sun for over a week to further accelerate cleaning and seasoning. These woods were sectioned into (2*2*2) $cm^3$ cubes**.**

The consistency in size helps maintaining the surface area per volume (SA/V) ratio to be constant. The dimensions of the purchased oak variety were studied and noted. The common physical properties like moisture content and mass of the wood chips were also calculated.

Subsequently, the cubes were subjected to a toasting process using a liquefied petroleum gas (LPG) burner. Each side was exposed to the flame for 30 seconds. This duration was experimentally determined to achieve a charring depth of approximately 2-4 millimeters. Excess charr and dust was removed and washed with the same alcohol used for maturation.

### 3.2.4 Cleaning of glass jars

Three-liter glass jars equipped with screw-on caps were procured. These jars were thoroughly cleaned with hot water to eliminate any dust and residual odors. Prior to maturation, they were rinsed with the same alcohol as described in the previous section.

### 3.2.5 Maturation of the distilled spirit

The distillate, with a volume of 2.25 liters was matured in contact with the oak cubes (previously detailed). The glass containers holding the distillate and oak cubes were then transferred to a controlled environment where they were not interrupted from direct sunlight and significant diurnal temperature fluctuations. Maturation was conducted on the ground floor of own home from 2nd January 2024 to 27 December 2024. "The jars were positioned on wooden planks rather than being placed directly on the concrete floor."

### 3.2.6 Sampling method

This study focuses on the maturation kinetics of different varieties. Samples were collected at 90-day intervals and stored in a refrigerator. After one year, with a total of four sampling was done, the collected samples were subjected to different chemical and sensory analysis.

**Table 3.3**: Sampling method.

| Sample Name | Sample placed (January 2, 2024) ml | 90 days (April 1, 2024) ml | 180 days (June 30, 2024) ml | 270 days (September 28, 2024) ml | 360 days (December 27, 2024) ml |
|---|---|---|---|---|---|
| 1 | 2,250 | 125 | 180 | 180 | 200 |
| 2 | 2,250 | 125 | 180 | 180 | 200 |
| 3 | 2,250 | 125 | 180 | 180 | 200 |
| 4 | 2,250 | 125 | 180 | 180 | 200 |
| 5 | 2,250 | 125 | 180 | 180 | 200 |
| 6 | 2,250 | 125 | 180 | 180 | 200 |
| 7 | 2,250 | 125 | 180 | 180 | 200 |

### 3.2.7 Analytical Procedure.

Although different authors have described different methods and parameters to analyze raw materials, fermented mash, distillate and matured whisky only those parameters and related methods, which were feasible in the laboratory, were determined in this study.

For raw materials moisture content, Nitrogen and crude protein, crude fat, ash content, carbohydrate, and dry matter was analyzed. Acidity, pH and alcohol content were analyzed for fermented mash.

#### 3.2.7.1 Proximate analysis of raw material.

##### 3.2.7.1.1 Moisture

Moisture content was determined by oven drying method at 105±5 ºC (Ranganna, 1986).

#### 3.2.7.1.2 Nitrogen and crude protein

Total nitrogen and crude protein content were determined by micro-kjeldahal method according to (Ranganna, 1986) using 1 g of powdered sample and protein content was found out by multiplying the percent nitrogen by 6.25.

#### 3.2.7.1.3 Crude fat

Crude fat content was determined by petroleum ether extraction method as per (AOAC, 2005)

#### 3.2.7.1.4 Ash Content

Ash content was determined by direct method as per using 5 g of powdered sample and a furnace temperature of 550 ± 5º C.

Total carbohydrate content was determined by difference.

#### 3.2.7.1.5 Alcohol content.

**a. Alcohol content of fermented mash.**

The percentage of alcohol by volume from specific gravity was determined according to (AOAC, 2005). The relative specific gravity of the distillates was determined by taking 200 g of *jand* (mash) and equal volume of water using a 50 ml specific gravity bottle (pycnometer) and ethanol (%v/v) was found by referring to the reference table.

**b. Alcohol content of distillate during distillation.**

During secondary distillation, every 200 ml of distillate alcohol content was determined using the alcohol hydrometer as per (AOAC, 2005)

**c. Alcohol content of final blended distillate and matured whisky.**

Alcohol content was determined by specific gravity method (AOAC, 2005) and the values were expressed in % (v/v).Also, the alcohol content was determined using Anton paar alcolyzer model no DMA 4500 M In Raj brewery. The sample was diluted 10 times in volumetric flask of 100ml and was analyzed.

#### 3.2.7.1.6 Acidity.

For acidity determination of fermented mash, 25 g of well-mixed sample was ground in a mortar and pestle with $CO_2$-free distilled water, volume made up to 250 ml with distilled water and filtered through Whatman No. 40 filter paper. The filtrate was the titrate using 0.1*N* sodium hydroxide and the values were expressed in % (m/v) as lactic acid (Ranganna, 1986)

#### 3.2.7.1.7 pH.

For pH determination of fermented mash, ten grams of the sample was homogenized with 20 mL of carbon dioxide free distilled water for 1 min, and the pH of slurry was determined by a digital pH meter of EUTECH INSTRUMENTS (pH 700) which was calibrated and standardized with standard buffers at 20ºC.

#### 3.2.7.1.8 Conductivity.

Conductivity of matured whisky was determined by using HANNA instruments Made in Romania.

#### 3.2.7.1.9 Color

The color intensity of matured whisky samples was determined spectrophotometrically according to the method outlined in (AOAC, 2005). Specifically, the absorbance of each sample was measured at a wavelength of 525 nm using a 1 cm quartz cuvette.

### 3.2.8 Gas Chromatography Mass Spectoscopy

A gas chromatography-mass spectroscopy system was employed for the analysis. The instrumentation comprised an Agilent model 8890 gas chromatograph equipped with 50-meter column. Helium was used as the carrier gas. The GC oven temperature was programmed with a ramping format, and the system was couped with Triple Quadrupole Mass Spectroscopy for detection and identification. Some congeners that were analyzed by GCMS are listed by:

- Carboxylic acids:- acetic acid, propionic acid, isobutyric acid, butryic acid, isovaleric acid, valeric acid, hepatonic acid, octanoic acid, decanoic acid.
- Ketones:- Acetone
- Aldehydes:- Acetaldehyde, Furfural
- Esters:- ethyl acetate, methyl acetate, iso propyl acetate, isobutyl acetate, isoamyl acetate.
- Alcohols:- Methanol, 2-Butanol, 1-propanol, 2-methyl-propanol, 1- butynal, Isoamyl alcohol.

### 3.2.9 Sensory evaluation.

A sensory evaluation of matured whisky was conducted across four sessions utilizing a panel of semi-trained assessors. Panelists were selected based on their established familiarity with whisky and their regular engagement in sensory analysis. A nine-point hedonic scale (9 = like extremely, 1 = dislike extremely) was employed to assess overall acceptability. Concurrently, panelists were instructed to identify and record distinct flavor profiles perceived at different stages of the sensory evaluation using a separate table. Seven maturation variables, assessed at 90-day intervals over a one-year period, were subjected to sensory analysis. This resulted in a total of 28 samples, in addition to a base spirit control

A 5 mL aliquot of each sample, at 70% alcohol by volume, was presented to the sensory panel in covered sensory glasses. Distilled water and a dropper were provided to allow panelists to dilute the samples and record the emergence of new flavor profiles at varying alcohol concentrations. Sensory analysis was conducted under controlled conditions, maintaining a temperature range of 20-24° Celsius and appropriate ambient lighting as per standard. Specimen of sensory score card is given in Appendix A.

### 3.2.10 Data Analysis

All the data obtained in this work was analyzed by the statistical program known as R programming. MS- Excel 2025 was also employed for the general calculations, graph and diagram construction. Keynote MAC OS version was used for presentation.

# Part IV

## Results and Discussion

Finger millet whisky, produced using traditional Eastern Nepalese methods and subjected to double distillation, was adjusted to 73% alcohol by volume. Oak cubes of varying varieties were prepared and introduced into glass containers, each containing 2.25 L of the millet whisky. Samples were withdrawn at 90-day intervals over a period of one year for subsequent qualitative and quantitative analyzes.

### 4.1 Proximate analysis of raw materials

The proximate analysis of raw millet was performed in laboratory of Central Campus of Technology and results are tabulated in **Table 4.1**

**Table 4.1**: Proximate composition of raw millet.

| Parameters | Percentage |
|---|---|
| Crude fat (%, db) | 2.77±0.13 |
| Crude fiber (%, db) | 3.89±0.49 |
| Crude protein (%, db) | 8.87±0.18 |
| Dry matter (%, db) | 88.22±0.16 |
| Moisture (%, m/m) | 11.28±0.20 |
| Total ash (%, db) | 1.83±0.18 |
| Total carbohydrate (%, db) | 83.54±0.17 |

The values in table are arithmetic mean ± S.D. of triplicate samples.

The crude fat, crude fiber, crude protein, dry matter, moisture, total ash and total carbohydrate content of the germinated millet was found to be 2.77 %, 3.89%, 8.87 %, 88.22 %, 11.28 %, 1.83 % and 83.54 % respectively which is within the range as compared to (Ratnavathi et al., 2022, Kumar et al., 2021, Rani & Bhardwaj, 2021, Mohapatra et al., 2019).

## 4.2 Analysis of fermented mash.

After a 40-day fermentation period, fermented mash samples underwent quantitative analysis to determine pH, acidity, and alcohol content. Samples were collected from the top, middle, and bottom sections of three separate PVC containers to assess any stratification effects.

### 4.2.1 pH of fermented mash

pH of fermented mash as of three different regions of PVC container i.e. top, middle and bottom region are tabulated in **Table 4.2**.

**Table 4.2**: pH of fermented mash.

| SAMPLE | TOP | MIDDLE | BOTTOM | MEAN |
|---|---|---|---|---|
| **Vessel I** | 4.15 | 4.05 | 4.22 | 4.14 |
| **Vessel II** | 4.11 | 4.03 | 4.15 | 4.1 |
| **Vessel III** | 4.18 | 4.03 | 4.04 | 4.08 |

The average pH of the samples A, B, and C was found to be 4.14, 4.1 and 4.08 respectively.

(D. B. Karki, 2013)found that the pH of *jand* prepared from finger millet has pH value of 4.17. The pH of the *jand* was found between 4.27 to 5.09 and was in the range as described by (Lee et al., 2023).

### 4.2.2 Alcohol percent of fermented mash

Alcohol percent of fermented mash as of three different regions of PVC container i.e. top, middle and bottom region are tabulated in table below **Table 4.3**. The values are shown in v/v percentage.

**Table 4.3**: Alcohol percent of fermented mash.

| SAMPLE | TOP | MIDDLE | BELOW | MEAN |
|---|---|---|---|---|
| **Vessel I** | 13.2 | 13.2 | 13.8 | 13.4 |
| **Vessel II** | 13.3 | 13.2 | 13.9 | 13.46 |
| **Vessel III** | 13.1 | 13.2 | 13.7 | 13.33 |

## 4.3 Analysis of oak cube

Different stages of oak were analyzed and results showed in **Table 4.4**.

**Table 4.4**: Physical analysis of oak cubes.

| Sample | Moisture content of charred (%) | Surface area. ($cm^3$) | No of cubes | Weight (10 pcs) gm |
|---|---|---|---|---|
| B | 6.95 | 24 | 10 | 63.86 |
| C | 6.9 | 24 | 10 | 76 |
| D | 4.3 | 24 | 10 | 39.5 |
| E | 6.26 | 24 | 10 | 68.9 |
| F | 7.67 | 24 | 10 | 64.08 |
| G | 7.13 | 24 | 10 | 62.9 |

**Table 4.4** presents the physical analysis of charred oak cubes used for maturation. It shows that the moisture content (M.C) is relatively consistent across all samples, with the notable exception of Sample D, which exhibits a lower moisture content compared to the other samples. The data provided specifically represents to the cubes utilized in the maturation process.

## 4.4 Analysis of matured whisky

### 4.4.1 pH

pH of seven sample matured for 360 days was analyzed and shown in the **Table 4.5** whereas the pH of unmatured distillate at the zero day was 4.74.

**Table 4.5**: pH of matured whisky.

| Samples | pH reading |
|---|---|
| A | $4.71 \pm 0.25^{ae}$ |
| B | $4 \pm 0.10^{b}$ |
| C | $4.47 \pm 0.03^{c}$ |
| D | $4.74 \pm 0.07^{d}$ |
| E | $4.83 \pm 0.06^{e}$ |
| F | $4.66 \pm 0.5^{a}$ |
| G | $4.8 \pm 0.01^{ae}$ |

The values in **Table 4.5** are arithmetic mean ± S.D. of triplicate samples. Figures in the parentheses indicate standard deviation and similar alphabet as superscript indicates no significant differences ($p>0.05$) by Tukey HSD.

The pH values of both matured and unmatured samples ranged from 4.00 to 4.84, with a mean pH of 4.46, indicating that all samples fell within the specified acceptable range (Christoph et al., 2007, (Nishimura and R. Matsuyama., 1989). Various alcoholic beverages, including wine, whisky, brandy, and beer, exhibit pH values within this acidic range. Sensory evaluations suggest that samples with a pH closer to 4.0 possess more desirable flavor profiles compared to those with a pH above 4.5.

### 4.4.2 TDS and conductivity

Total dissolved solid (TDS) and conductivity of 360 days matured whisky was analyzed and shown in **Table 4.6**.

**Table 4.6**: TDS and conductivity of matured whisky.

| Samples | TDS | Conductivity |
|---|---|---|
| A | 5.77±0.02[a] | 8.97±0.05[a] |
| B | 11.53±0.25[b] | 18.22±0.10[b] |
| C | 14.55±0.10[c] | 22.38±0.06[c] |
| D | 11.23±0.08[b] | 17.23±0.10[d] |
| E | 19.61±0.04[d] | 30.13±0.25[e] |
| F | 16.6±0.10[e] | 25.49±0.15[f] |
| G | 21.07±0.16[f] | 32.53±0.60[g] |

The values in table are arithmetic mean ± S.D. of triplicate samples. Figures in the parentheses indicate standard deviation and similar alphabet as superscript indicates no significant differences ($p>0.05$) by Tukey HSD. TDS and conductivity determine the extraction the woods components.

### 4.4.3 Analysis of optical properties (color absorbance).

Samples of whisky at various maturation stages were subjected to spectrophotometric analysis to determine their color properties. The transmittance of visible light at a wavelength of 525 nm was measured. The resulting data is visually represented in the graph

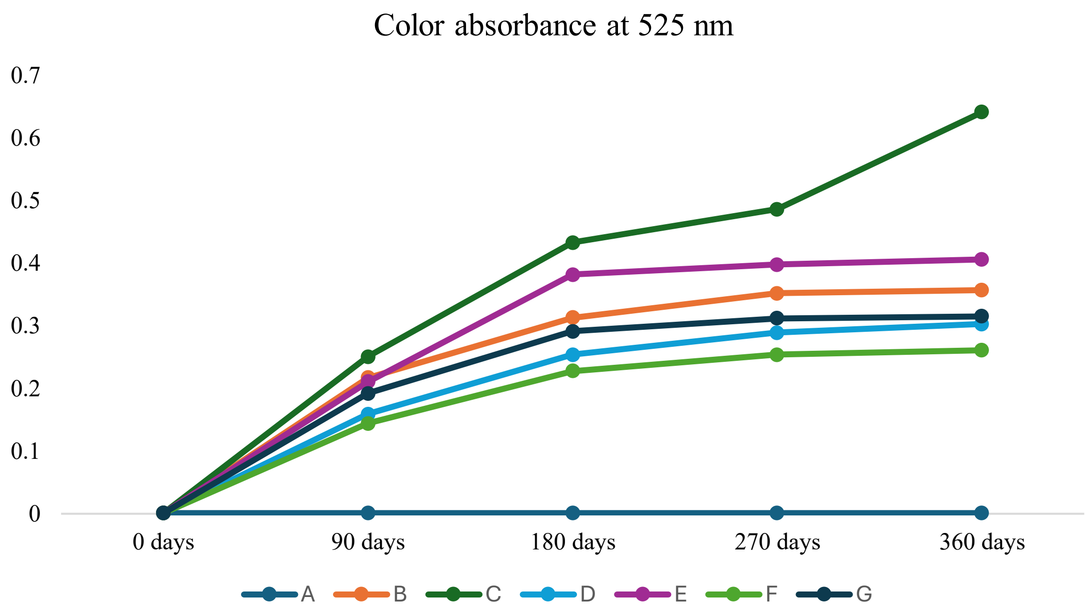


**Figure 4.1**: Absorbance at 525nm

Absorbance of samples at 90 days interval at 525nm.To further validate the colorimetric analysis, spectrophotometric measurements were conducted at a wavelength of 430 nm. This wavelength is also commonly employed in color assessment of whiskies and other spirits, as it corresponds to a region sensitive to yellow hues, which are characteristic of maturation color. This cross-verification step ensures the robustness and reliability of the color data obtained through spectrophotometric analysis. The consistency between the results obtained at 525 nm and 430 nm provides further confidence in the accuracy of the reported color intensities.

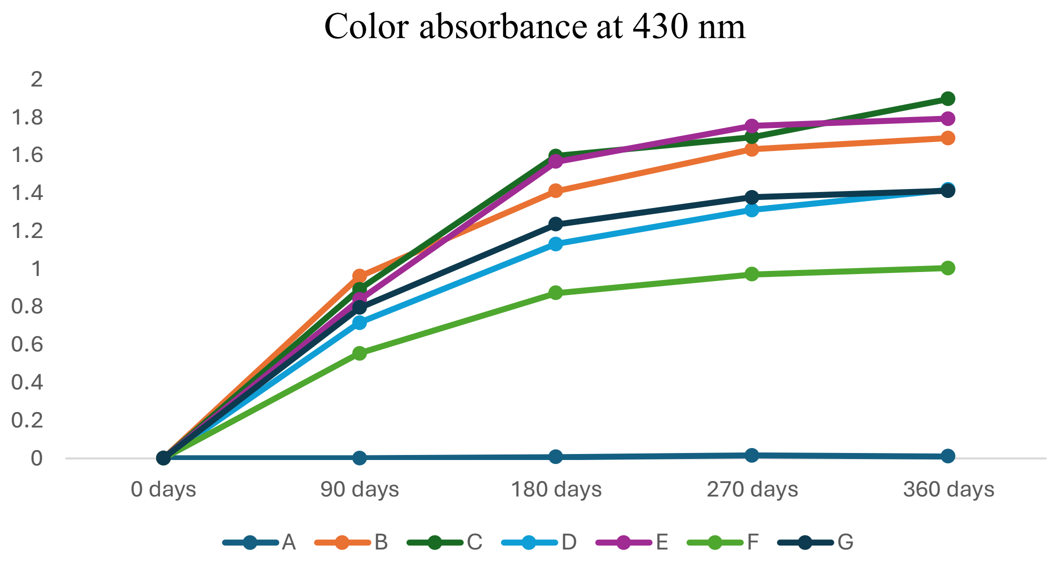


**Figure 4.2**: Absorbance at 430nm.

**Figure 4.1** and **Figure 4.2** clearly shows that the color units increase over the time which is one the truth of maturation science. All the sample are gradual increment order over the time. Sample G has high absorbance in both 525 and 430 over every time interval. In this research we have taken sample B as reference sample so form this graph, we could conclude that the sample E, D and G are close to sample B in every time interval. The color development is comparable to that of (Reazin, 1983).

Color is entirely due to substances extracted from the charred oak barrel. Newly distilled whisky is colorless. The color of the aging whisky changes gradually from colorless to light and deep yellow, then to amber, and finally to reddish brown. The rate of increase declines rapidly after the first six months of maturing (Mosedale, 1995).

### 4.5 Chemical analysis of matured whiskies.

Studies employing GCMS restrict the quantitative analysis to approximately five distinct chemical categories of congeners, specifically: carboxylic acids, ketones, aldehydes, esters, and alcohols. The availability of certified reference standards facilitated the comprehensive analysis of eleven distinct carboxylic acid congeners in the whisky samples. These included acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, 4-methylvaleric acid, hexanoic acid, heptanoic acid, octanoic acid, and decanoic acid. Quantitative assessment indicated that the concentrations of all these acids were below 10 parts per million (ppm), with the notable exception of acetic acid. Consequently, due to its significantly higher concentration relative to the other carboxylic acids identified, acetic acid was selected as the primary reference compound for further analytical considerations within this specific congener class.

Two aldehydes were analyzed for this research they are acetaldehyde and furfural. Only acetone was analyzed for ketone group for this research due to availability constraint. Five different esters were analyzed, and they are Ethyl Acetate, Methyl Acetate, Isopropyl Acetate, Isobutyl Acetate, Isoamyl Acetate. Among all these 5 different esters only ethyl acetate was taken for analysis. Six different alcohols were analyzed, and they are Methanol, 2-Butanol, 1-Propanol, 2-Methyl-Propanol, 1-Butanol and Isoamyl Alcohol. Only Methanol and Isoamyl alcohol are taken for further data analysis because of their health importance and flavor impact.

**Table 4.7**: Data presentation of chemical analysis.

| Sample name | Acetic acid | Acetaldehyde | Acetone | Ethyl acetate | Methanol | Active and isoamyl | Furfural |
|---|---|---|---|---|---|---|---|
| A | $19.02^{a}$ | $47.81^{a}$ | $1.02^{a}$ | $644.38^{a}$ | $50.29^{a}$ | $138.08^{a}$ | $0.18^{a}$ |
| B | $155.25^{b}$ | $31.84^{bde}$ | $1.97^{b}$ | $925.17^{b}$ | $12.55^{b}$ | $245.29^{b}$ | $7.56^{b}$ |
| C | $122.13^{c}$ | $29.15^{b}$ | $1.80^{c}$ | $822.63^{c}$ | $11.52^{b}$ | $224.53^{c}$ | $4.76^{c}$ |
| D | $182.37^{d}$ | $37.15^{c}$ | $1.81^{c}$ | $935.90^{b}$ | $12.70^{b}$ | $243.85^{bc}$ | $7.26^{b}$ |
| E | $121.05^{c}$ | $35.39^{cd}$ | $1.89^{d}$ | $932.06^{b}$ | $12.12^{b}$ | $257.79^{b}$ | $5.19^{c}$ |
| F | $94.95^{e}$ | $30.54^{be}$ | $1.97^{b}$ | $953.98^{d}$ | $12.33^{b}$ | $247.30^{b}$ | $3.03^{d}$ |
| G | $106.29^{f}$ | $33.43^{cde}$ | $1.91^{d}$ | $900.47^{e}$ | $11.60^{b}$ | $237.97^{bc}$ | $5.50^{c}$ |

The values are mean and are the results shown in parts per million (ml/L).

### 4.5.1 Acetic Acid

The acetic acidity of sample A, B, C, D, E, F and G was found to be 19.02, 155.25, 122.13, 182.37, 121.05, 94.95 and 106.29 ppm. The significance test shows us that samples are statistically different indicating that their difference among each sample.

The amount of acetic acid in the matured samples is within in the range as comparison with acetic acid (Nykänen et al, 1968). A rapid increase in total acidity takes place during the early stages of maturing. Salo et al., (1972) also found that the acetic acid was within the range of 130-200 ppm in the matured whisky.

### 4.5.2 Ethyl acetate

The ethyl acetate of sample A, B, C, D, E, F and G was found to be 644.38, 925.17, 822.63, 935.90, 932.06, 953.98 and 900.47 ppm respectively. The same letters in the superscript shows that sample there is no statistical difference between sample B, D and E while different letters show difference ($p \leq 0.05$).

Ethyl acetate comes under the esters group which character is already described in literature above. The values are in lower sides as compared to (Wiśniewska et al. 2015, Diéguez et al. 2002). Among the different component's esters have the greatest influence on the aroma of whisky. These compounds mainly form during the fermentation and aging processes. Esters are characterized by an intense aroma and a low odor detection threshold. Depending on the concentration of esters, they may have a positive or negative effect on the aroma of an alcoholic beverage. For example, ethyl acetate at high concentrations gives a solvent-like or vinegar note to the final product, while at low concentrations it softens the sharp aroma of other beverages (Campo et al. 2007).

The increase in esters with age is probably the most regular and consistent of all the characteristics and therefore may be regarded as the most reliable index for determination of age.

### 4.5.3 Acetaldehyde

The Acetaldehyde of sample A, B, C, D, E, F and G was found to be 47.81, 31.84, 29.15, 37.15, 35.39, 30.54 and 33.43 ppm respectively. The letters in the superscript shows that sample is significant difference or not ($p \leq 0.05$).

Acetaldehyde comes under the aldehydic group which is already described in literature above and related with fresh grassy flavor. The rate of increase in aldehydes is greatest during the first 3 years and proceeds at an average for this period. Similar findings were observed by (Piggott et al., 1979).

### 4.5.4 Acetone

The Acetone of sample A, B, C, D, E, F and G was found to be 1.02, 1.97, 1.80, 1.81, 1.89 and 1.91 ppm respectively. The letters in the superscript shows that sample is significant difference or not ($p \leq 0.05$). Acetone comes under the ketone group. The values were similar as described by (Fazzalari, 1978).

### 4.5.5 Methanol

The methanol of sample A, B, C, D, E, F and G was found to be 50.29, 12.55, 11.52, 12.70, 12.12, 12.33 and 11.60 ppm respectively. The letters in the superscript shows that sample is significant difference or not ($p \leq 0.05$).

From the data the untreated sample has quite high value of methanol content as compared to treated sample which suggests that the oak cubes have some scavenging properties also. According to WHO (2014), methanol concentration of 6-27 ppm in beer and 10-220 ppm in spirits are not harmful. The methanol concentration of the samples was within safety level. Aylott et al, (2010) also analyzed 56 different malt whiskies, and the values of methanol content was within the range of 4.7 to 16.4 which is comparable to the methanol of the project samples. The results of treated samples were comparable to the findings of (Aylott, 2022). Karki, (2013) found that undistilled beverage of finger millet was 182.69 g/100L alc which is quite high than the distilled whisky.

### 4.5.6 Active and Isoamyl

The isoamyl alcohol of sample A, B, C, D, E, F and G was found to be 138.08, 245.29, 224.53, 243.85, 257.79, 247.30 and 237.97 ppm respectively. The letters in the superscript shows that sample is significant difference or not ($p \leq 0.05$). Sample B, D, E, F have significantly no difference.

The fusel oil (higher alcohols) constitutes an important component of whisky so far as character and quality are concerned. Its content is largely determined by the method of distilling used. The high evaporation point, and chemical sluggishness of this constituent are responsible for the fact that the original content at age zero remains practically unchanged, although a slight loss has been found to occur in most cases (Reazin, 1981). The variation of results is very small with comparison to findings of (Aylott et al., 1994; Garcia et al., 2013).

### 4.5.7 Furfural.

The furfural of sample A, B, C, D, E, F and G was found to be 0.18, 7.56, 4.76, 7.26, 5.19, 3.03 and 5.50 ppm respectively. The letters in the superscript shows that sample is significant difference or not ($p \leq 0.05$). Sample B and D have No significant difference.

From various studies newly made doesn't contain furfural, as the sample A also have 0.18 ppm that is almost 0. Furfural is formed in the process of charring the barrel, extracts of uncharred oak wood show only trace of furfural (Liebmann et al, 1949).

## 4.6 Sensory analysis

Seven different samples of six oak varieties and a control were subjected for sensory analysis which were matured for one year. The samples were judged for color, aroma, mouthfeel, body, aftertaste, and overall acceptability. The data obtained from sensory analysis were statistically examined using one-way ANOVA followed by Tukey HSD using R-programming. The radar chart was also made by using R-programming.

### 4.6.1 Appearences

Sensory evaluation for Appearance yielded mean scores ranging from 5.13 to 8.13 across samples A through G, as presented in **Figure 4.3**. The mean scores were 5.13, 8.13, 7.38, 7.88, 7.38, 7.13, and 7.25 respectively for sample A, B, C, D, E, and F.

These mean scores are graphically depicted in **Figure 4.3** Statistical analysis using ANOVA, detailed in (Appendix C) revealed significant differences in aroma scores among the samples. Post-hoc analysis using Tukey's Honestly Significant Difference (HSD) test at a 95% confidence level ($\alpha$=0.05) identified specific groupings, as indicated by the letters above each bar in **Figure 4.3**.

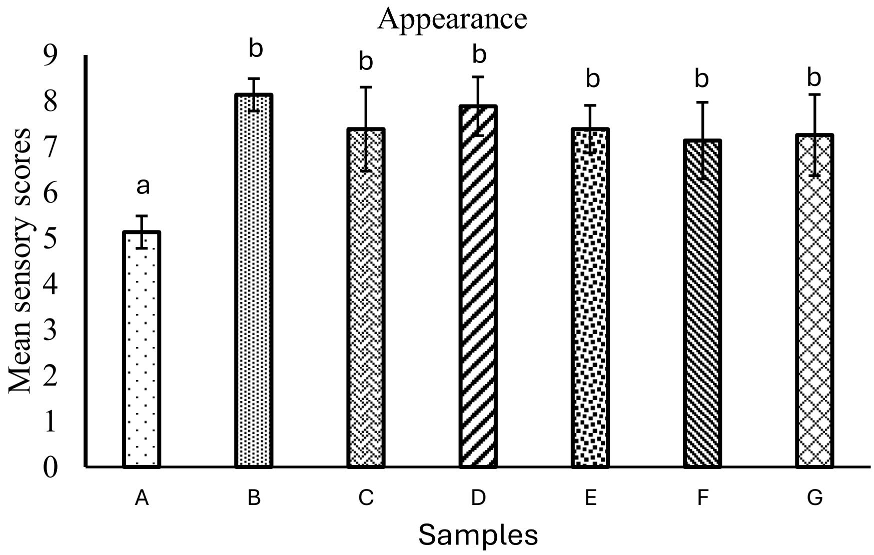


**Figure 4.3**: Sensory evaluation on attribute "Appearance".

Similar alphabet above the bar graph indicates no significant difference with only difference with control sample and error bars shows standard deviation of scores given by 8 panelists. Since there were not any significant difference in appearance between treated sample and may be due to same level of charring to oak cubes. Based on mean score with respect to appearance given in Appendix., the superiority/inferiority ($p \le 0.05$) of the *matured whisky* was found to rank as follows:

[B] > [D] > [C, E] > [G] > [F] > [A]

From the mean score it can be concluded that Sample D is comparable to the reference sample B and other samples as series.

### 4.6.2 Aroma

Sensory evaluation for aroma yielded mean scores ranging from 5.25 to 8.38 across samples A through G, as presented in **Figure 4.4** the mean scores were 5.25, 8.38, 7.25, 8.25, 6.5, 7.13, and 7.63 respectively for sample A, B, C, D, E, and F. The error bars show the standard deviation between the sensory panelist scores These mean scores are graphically depicted in **Figure 4.4**.

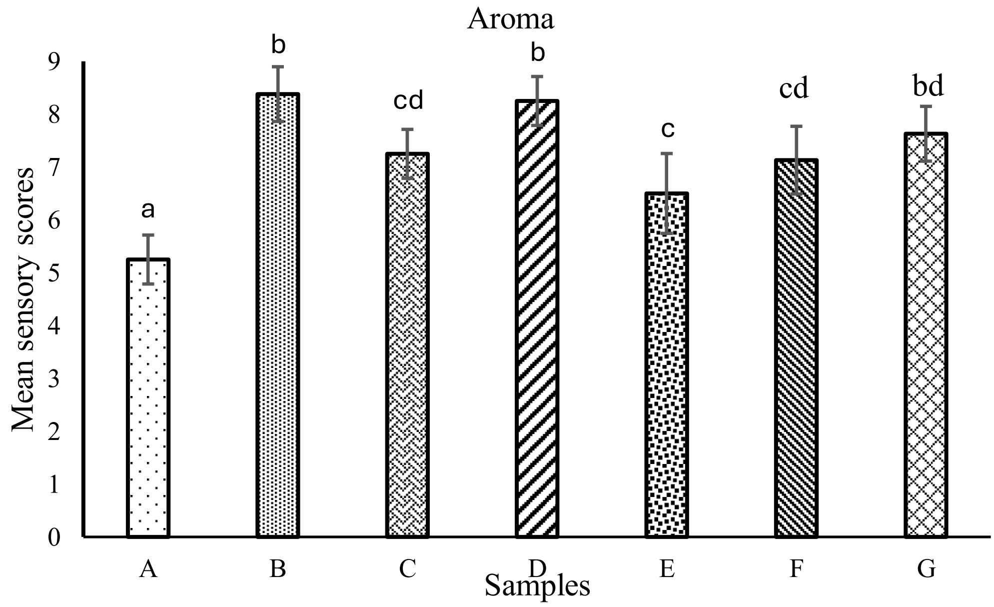


**Figure 4.4**:Sensory evaluation on attribute "Aroma".

The sensory evaluation demonstrated significant variations in aroma across the different samples. Samples B and D exhibited the highest mean aroma scores, significantly exceeding those of samples A and E. This suggests that the sample D and G is comparable to reference sample B among the treated group. Based on mean score with respect to Aroma given in Appendix., the superiority/inferiority (p≤0.05) of the *matured whisky* was found to rank as follows:

[B] > [D] > [C, G] > [F] > [E] > [A]

Samples B and D exhibited the highest mean aroma scores, significantly exceeding those of samples A and E. This suggests that the sample D and G is comparable to reference sample B among the treated group.

### 4.6.3 Body

Sensory evaluation for body yielded mean scores ranging from 5.25 to 8.38 across samples A through G, as presented in **Figure 4.5**. The mean scores were 5.25, 8.38, 7.13, 8, 6.75, 7, and 7.13 respectively for sample A, B, C, D, E, and F.

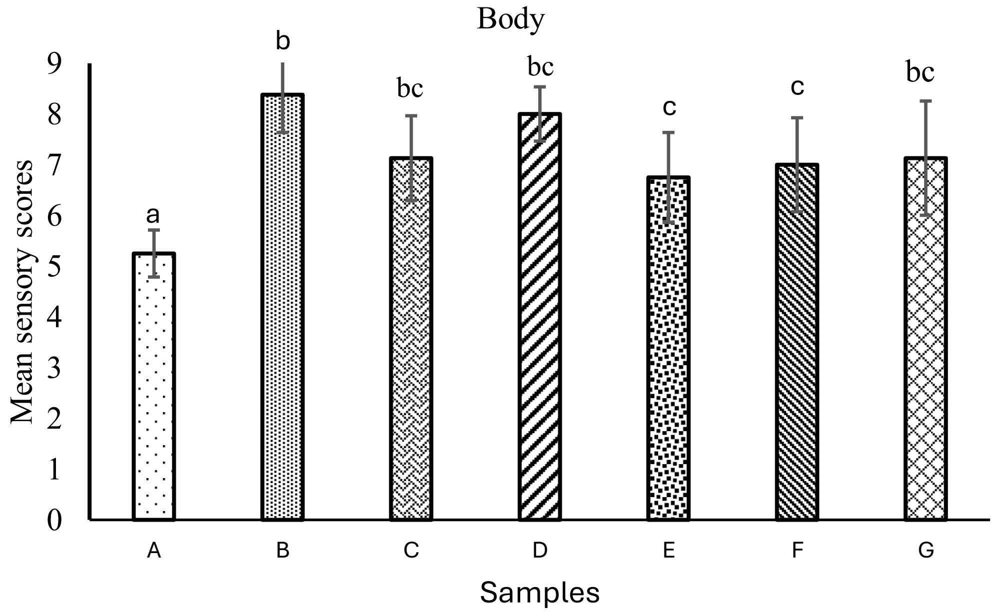


**Figure 4.5**: Sensory evaluation on attribute "Body".

Based on mean score with respect to Body given in Appendix C , the superiority/inferiority (p≤0.05) of the matured whisky was found to rank as follows:

[B] > [D] > [C, G] > [F] > [E] > [A]

Significant differences in body were observed across the samples during sensory evaluation. Sample B and D exhibited the highest mean body scores, significantly exceeding sample A. The mean and Tukey HSD suggest that the sample C, D, and G is comparable to reference sample B among the treated group. Whereas Sample E and F are significantly different and is not comparable to reference sample B.

### 4.6.4 Mouthfeel

Sensory evaluation for mean yielded mean scores ranging from 5.63 to 8.13 across samples A through G, as presented in **Figure 4.6** the mean scores were 5.63, 8, 6.88, 8.13, 6.5, 8.88, and 7.38 respectively for sample A, B, C, D, E, and F.

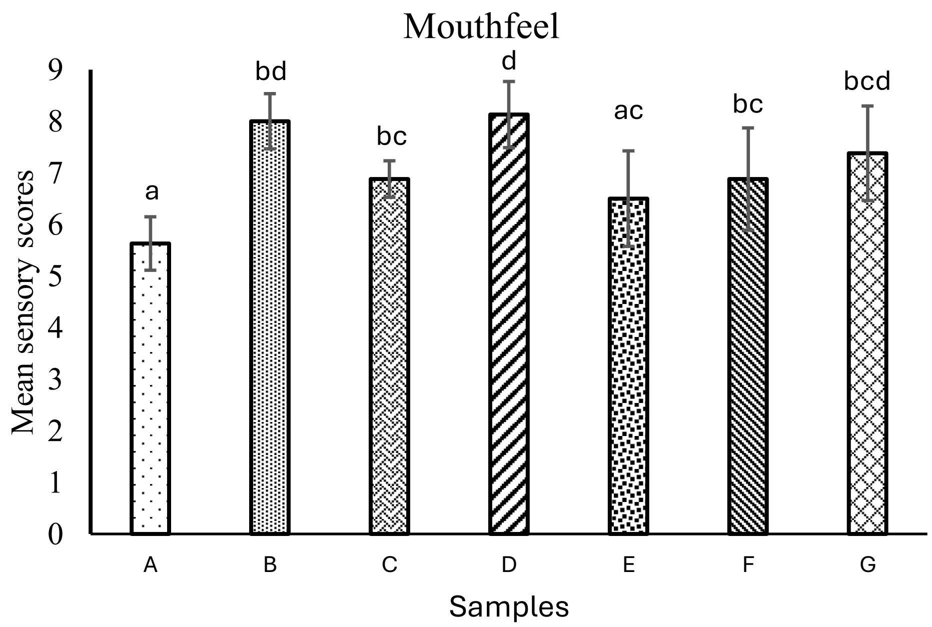


**Figure 4.6**: Sensory evaluation on attribute "Mouthfeel".

Based on mean score with respect to mouthfeel given in Appendix C ., the superiority/inferiority ($p \leq 0.05$) of the *matured whisky* was found to rank as follows:

[B] > [D] > [G] > [C, F] > [E] > [A]

The sensory evaluation demonstrated significant variations in mouthfeel across the different samples. Samples D and B exhibited the highest mean mouthfeel scores, significantly exceeding samples A and C. Among all the other attributes sample D score exceeded the score of reference sample D. The mean and Tukey HSD suggests that the sample C, F and G is comparable to reference sample B among the treated group. Whereas Sample E and A are significantly different and is not comparable to reference sample B.

### 4.6.5 Aftertaste

Sensory evaluation for aftertaste yielded mean scores ranging from 5.25 to 7.88 across samples A through G, as presented in **Figure 4.7** the mean scores were 5.25, 7.88, 6.75, 7.75, 6.25, 6.75 and 7.38 respectively for sample A, B, C, D, E, and F.

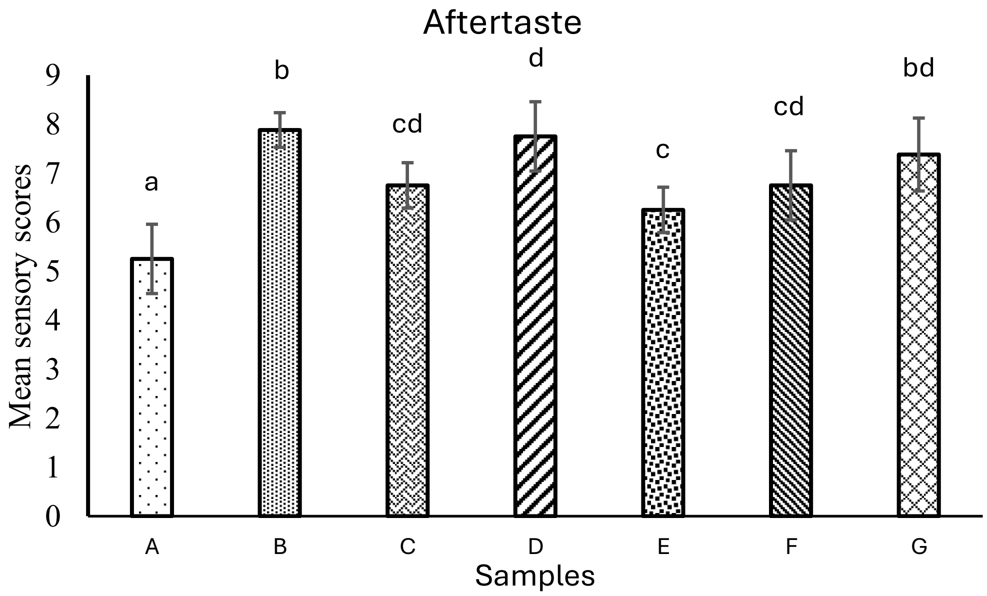


**Figure 4.7**: Sensory evaluation on attribute "Aftertaste".

Based on mean score with respect to mouthfeel given in Appendix C ., the superiority/inferiority (p≤0.05) of the matured whisky was found to rank as follows:

[B] > [D] > [G] > [C, F] > [E] > [A]

The sensory evaluation demonstrated significant variations in aftertaste across the different samples. Samples B and D followed by sample G exhibited the highest mean aftertaste scores, significantly exceeding samples A and C.

### 4.6.6 Overall

Sensory evaluation for aftertaste yielded mean scores ranging from 5.25 to 8.25 across samples A through G, as presented in **Figure 4.8** the mean scores were 5.25, 8.13, 6.75, 8.25, 6.25, 6.88 and 7.38 respectively for sample A, B, C, D, E, and F.

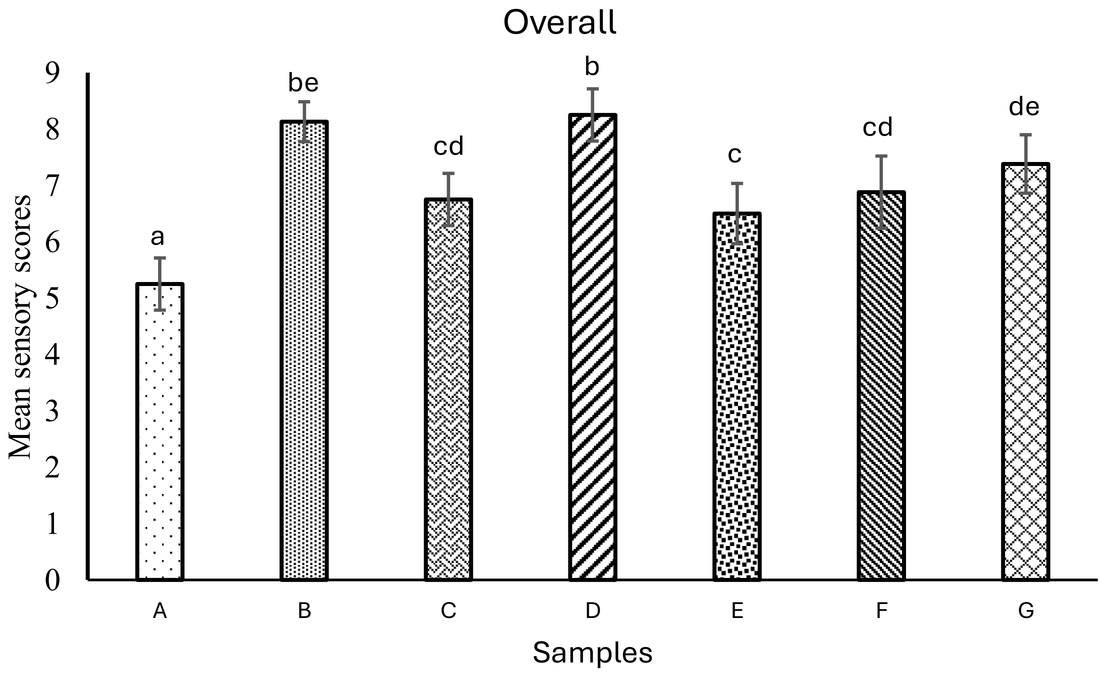


**Figure 4.8**: Sensory evaluation on attribute "Overall".

Based on mean score with respect to mouthfeel given in Appendix C ., the superiority/inferiority ($p \leq 0.05$) of the matured whisky was found to rank as follows:

[B] > [D] > [G] > [F] > [C] > [E] > [A]

The sensory evaluation demonstrated significant variations in overall across the different samples. Samples D and B exhibited the highest mean overall scores, significantly exceeding samples A and C. Although the reference sample was taken as B but the score for overall was highest for sample D.

Sample B (American white oak) has high overall score in every aspect of sensory followed by sample D (*Quercus lamellosa*) and Sample G (*Castanopsis indica*).

## 4.7 Analysis of Flavor Attributes.

As in the Appendix A sensory specimen chart is divided into two primary sections. The first section records hedonic ratings for various constituents, assessing their preference. The second section focuses on flavor identification, where panelists indicate the perceived flavors by selecting from a provided list. Subsequently, these raw data are transformed into frequency data, which are then used to construct a radar chart. This graphical representation effectively visualizes changes in flavor profiles over time.

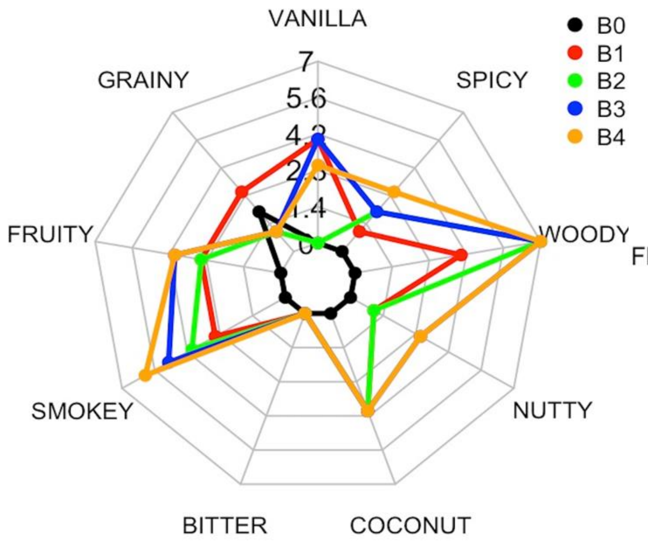


Fig: Sample B

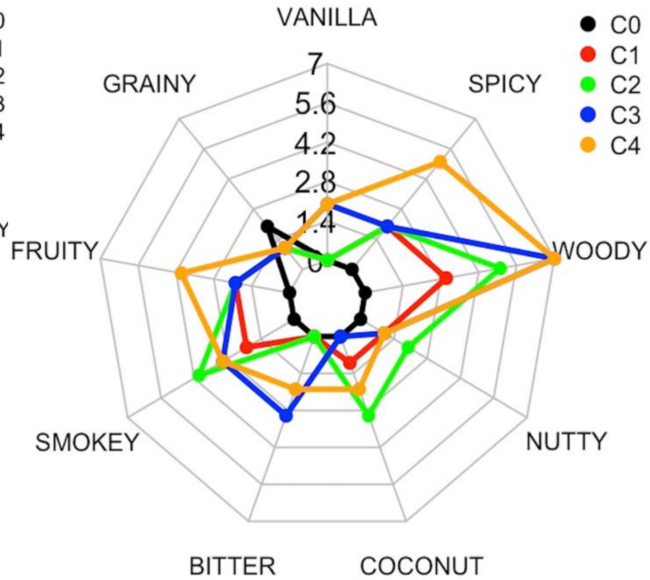


Fig: Sample C

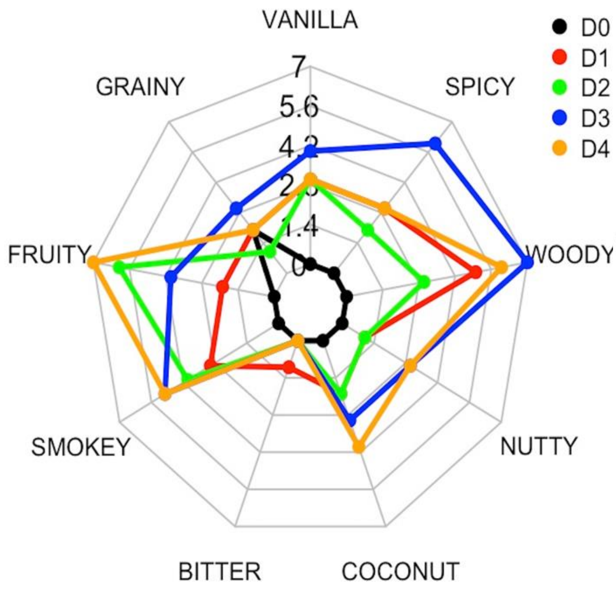


Fig: Sample D

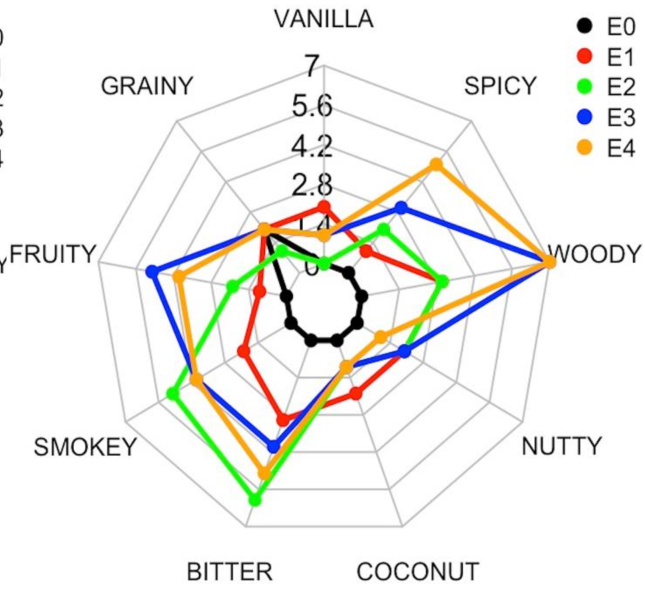


Fig: Sample E

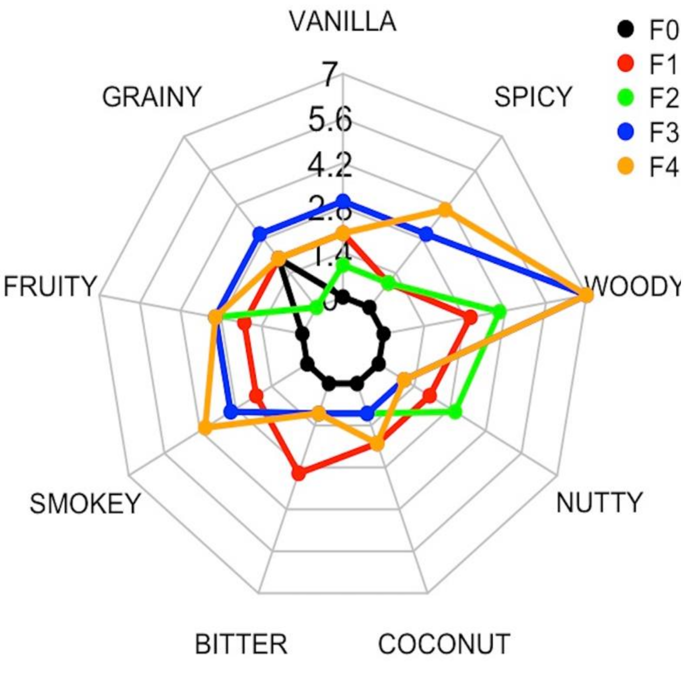


Fig: Sample F

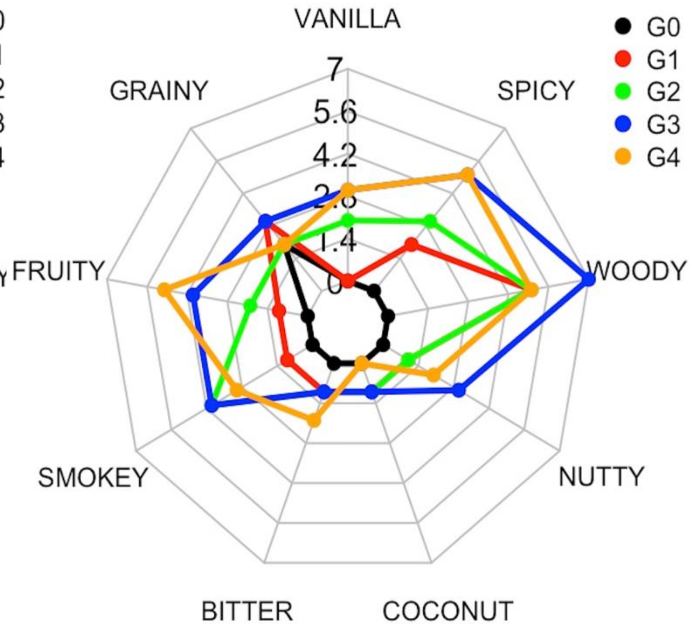


Fig: Sample G

**Figure 4.9**: Radar chart of Sample B, C, D, E, F and G.

From the presents experimental results, where panels B, C, D, E, F, and G represent various treated samples. The numerical suffix denoted by numerical increments (0, 1, 2, 3, 4), signifies the duration of maturation in months, specifically 0, 3, 6, 9 and 12 months.

A spider diagram, also known as a radar chart, web chart, or star chart, is a graphical method of displaying multivariate data in the form of a two-dimensional chart of three or more quantitative variables represented on axes starting from the same point.

Above figures visualize the temporal evolution of flavor profiles across six treated samples at different time period. A clear trend emerges, demonstrating the progressive emergence of desirable flavor attributes, such as fruity, vanilla and smokey notes, at various stages of the treatment period. Conversely, undesirable flavor characteristics show a reduction in intensity during later stages.

Sample "B" serves as the reference throughout this project. Several other samples exhibit resemblances to Sample "B" in specific flavor aspects. Notably, sample "D" *(Quercus lamellosa, Phalat)* shares comparable plot with sample "B" for flavor like vanilla, coconut and smoky notes. However, sample "D" distinguishes itself with a higher intensity of fruity flavor, a characteristic frequently associated with European oak varieties, such as French oak, as reported in the literature.

Other than oak species one non oak variety, Sample G *(Castanopsis indica, Phalat)* was also included which has similar flavor plot and overall hedonic score to that of sample B *(Quercus alba, American Oak)* and D *(Quercus lamellosa, Phalat)*.

# Part VI

## Conclusions and Recommendations

### 5.1 Conclusions

This study was conducted with the primary objective of investigating the maturation process of *rakshi* (finger millet whisky). However, due to the limited availability of distilled whisky, the comparative analysis was restricted to American white oak and a select few Nepalese oak varieties. Consequently, the conclusions derived from this research may not be universally applicable to all types of matured whiskies. Nevertheless, within the defined timeframe and scope of this work, several pertinent conclusions can be drawn regarding the specific conditions investigated.

In the light of the foregoing results and discussion, following conclusions can be drawn:

1. The consistent level of charring applied to all treated samples resulted in a nearly uniform final color across them.
2. The treated samples consistently outperformed the untreated control in every aspect of the sensory evaluation.
3. Sample D (*Quercus lamellosa, Phalat)* and was comparable to "Sample B" (American oak) in every aspect of sensory profile and chemical analysis.
4. Sensory attributes on hedonic scale demonstrated continuous improvement throughout the aging period.
5. Woody and smoky flavor notes were consistently identified in nearly all treated samples. A notable distinction was observed in Nepalese oak, which imparted a more pronounced fruity note compared to the imported American Oak.
6. Chemical analyses primarily focused on the identification and quantification of generic toxic compounds, rather than the characterization of flavoring agents. The results consistently indicated that concentrations of these toxic substances remained within established legal limits and below prescribed thresholds.
7. The addition of oak cubes led to a substantial reduction in methanol concentration from 50 to 12.13 ppm, suggesting that oak acts as an absorbent for this toxic compound.

8. Not only oak species but other non-oak variety found in Nepal (*Castanopsis indica, Katus)* hold significant potential for maturation.

## 5.2 Recommendation

From the present study, the following recommendations can be made:

1. Given the limited existing literature on the origin, traditional, functional, and other potential uses of Nepalese oak (Quercus species), further comprehensive research is strongly recommended. Specifically, the promising sensory attributes of Sample D and Sample G, characterized by their superior flavor and smoothness, indicate their significant potential**.**
2. Different seasoning, charring and toasting level is recommended for different oak species to know the hidden character of wood morphology.
3. Beyond oak, a variety of other tree species in Nepal possess comparable potential**.**
4. Other method of *rakshi* preparation can be studied for producing smooth and better taste.
5. For both personal consumption and research purposes, it's recommended to explore the use of various wood formats for maturation, including barrels and stakes.

# Part VI

## Summary

Oak species abundantly growing in the hilly regions were identified through a combination of literature review and ethnobotanical knowledge obtained from local inhabitants, who provided insights into the traditional uses and properties of these species. Based on morphological characteristics, including fruit (nut), tree structure, and leaf morphology and previous uses, five native oak species were selected for further study. In addition, *Quercus alba* (American white oak) was imported from the United States to serve as a reference species for comparative analysis.

All wood samples were thoroughly washed, air-seasoned under open environment, and uniformly cut into cubes measuring 2 × 2 × 2 $cm^3$. Prior to use in the maturation process, the wood cubes were toasted using an open flame burner. Finger millet (*Eleusine coracana*) was sourced from local markets and fermented using traditional *murcha* as the starter culture. The primary distillation was carried out using indigenous methods. The resulting distillate was then subjected to secondary distillation under controlled laboratory conditions to refine the product, and the final alcohol concentration was adjusted to approximately 70% v/v for subsequent maturation experiments. The amount of wood to be used was calculated in terms of surface area/volume ratio which were determined to be from 200 $cm^2$/ L.

A total of seven samples were prepared for maturation: six containing oak wood cubes and one control sample without oak, serving as a control. All samples were matured for a period of one year, with aliquots drawn at 90-day intervals for both chemical and sensory analyses. Chemical profiling was conducted using Gas Chromatography-Mass Spectrometry (GC-MS), which enabled the detection and characterization of key volatile compounds, including carboxylic acids, esters, aldehydes, alcohols, and ketones. Sensory evaluation was carried out through qualitative and semi-quantitative trials to identify and compare flavor attributes in the matured finger millet whiskey and the control.

The treated samples consistently surpassed the control sample in both chemical and sensory evaluations. Notably, Sample D (Quercus lamellosa, Phalat) proved to be comparable to the reference Sample B (American oak) across all sensory and chemical parameters. The sensory attributes of the treated samples showed continuous improvement

throughout the aging period. Chemical analyses, which primarily focused on identifying and quantifying generic toxic compounds, consistently demonstrated that the concentrations of these substances remained within established legal limits and below prescribed thresholds. Furthermore, the addition of oak cubes significantly reduced methanol concentration, suggesting that oak acts as an absorbent for this toxic compound. These findings indicate that not only various oak species, but also other wood varieties found in Nepal hold significant potential for maturation processes. Dominant flavor notes observed in the matured samples included caramel, grainy, fruity, smoky, bitter, coconut, nutty, woody, and spicy characteristics.

## References


Adams, A. B. (1910). The Distillation of Whiskey. ACS Publications, 2(2), 34–42. https://doi.org/10.1021/IE50014A003

Aiken, J. W., & Noble, A. C. (1984). Comparison of the Aromas of Oak- and Glass-Aged Wines. American Journal of Enology and Viticulture, 35(4), 196–199. https://doi.org/10.5344/AJEV.1984.35.4.196

Alamo-Sanza, M. del, & I, N. (2017). Oak wine barrel as an active vessel: A critical review of past and current knowledge. Taylor & FrancisM Del Alamo-Sanza, I NevaresCritical Reviews in Food Science and Nutrition, 2018•Taylor & Francis, 58(16), 2711–2726. https://doi.org/10.1080/10408398.2017.1330250

Amadou, I., Gounga, M. E., & Le, G. W. (2013). Millets: Nutritional composition, some health benefits and processing - A review. Emirates Journal of Food and Agriculture, 25(7), 501–508. https://doi.org/10.9755/ejfa.v25i7.12045

AOAC. (2005). Official Methods of Analysis of AOAC INTERNATIONAL 18th Edition. Washington, DC., d, 4–5.

Arapitsas, P., Antonopoulos, A., Stefanou, E., & Dourtoglou, V. G. (2004). Artificial aging of wines using oak chips. Food Chemistry, 86(4), 563–570. https://doi.org/10.1016/j.foodchem.2003.10.003

Arendt, Elke., & Dal Bello, Fabio. (2008). Gluten-free cereal products and beverages. Elsevier/Academic Press.

Audu, S. S., Ehanwo, A. A., Aremu, M. O., Tukura, B. W., Ambo, A. I., & Usman, A. (2408). Chemical composition of FInger Millet  (Eleusine coracana) Flour. FUW Trends in Science & Technology Journal, Www.Ftstjournal.Com e-ISSN, 3(2B), 905–908. www.ftstjournal.com

Aylott, R. (2022). Whisky analysis. Whisky and Other Spirits: Technology, Production and Marketing, 335–362. https://doi.org/10.1016/B978-0-12-822076-4.00009-7

Aylott, R., Clyne, A., Fox, A., & Walker, D. (1994). Analytical strategies to confirm Scotch whisky authenticity. Pubs.Rsc.Org. https://pubs.rsc.org/en/content/articlehtml/1994/an/an9941901741

Aylott, R., & MacKenzie, W. M. (2010). Analytical strategies to confirm the generic authenticity of Scotch whisky. Wiley Online Library, 116(3), 215–229. https://doi.org/10.1002/J.2050-0416.2010.TB00424.X

Bathgate, G. N., & Taylor, A. G. (1977). The qualitative and quantitative measurement of peat smoke on distiller's malt. Journal of the Institute of Brewing, 83(3), 163–168. https://doi.org/10.1002/J.2050-0416.1977.TB06812.X

Boidron, J., & Chatonnet, P. (1988). Influence du bois sur certaines substances odorantees des vins. 22, 275–294.

Booth, M., Shaw, W., & Morhalo, L. (1989). Blending and bottling. The science and technology of whiskies.

Bryson, Lew. (2014). Tasting whiskey : an insiders guide to the unique pleasures of the world's finest spirits. Storey Publishing.

Cadahía, E., Fernández de Simón, B., & Jalocha, J. (2003). Volatile compounds in Spanish, French, and American oak woods after natural seasoning and toasting. Journal of Agricultural and Food Chemistry, 51(20), 5923–5932. https://doi.org/10.1021/JF0302456

Cadahía, E., Muñoz, L., De Simón, B. F., & García-Vallejo, M. C. (2001a). Changes in low molecular weight phenolic compounds in Spanish, French, and American oak woods during natural seasoning and toasting. Journal of Agricultural and Food Chemistry, 49(4), 1790–1798. https://doi.org/10.1021/JF0006168

Cadahía, E., Muñoz, L., De Simón, B. F., & García-Vallejo, M. C. (2001b). Changes in low molecular weight phenolic compounds in Spanish, French, and American oak woods during natural seasoning and toasting. Journal of Agricultural and Food Chemistry, 49(4), 1790–1798. https://doi.org/10.1021/JF0006168

Cadahía, E., Simón, B. de, Sanz, M., & Poveda, P. (2009). Chemical and chromatic characteristics of Tempranillo, Cabernet Sauvignon and Merlot wines from DO Navarra aged in Spanish and French oak barrels. ElsevierE Cadahía, BF de Simón, M Sanz, P Poveda, J ColioFood Chemistry, 2009•Elsevier. https://www.sciencedirect.com/science/article/pii/S0308814608015343

Caldeira, M., Rodrigues, F., Perestrelo, R., Marques, J. C., & Câmara, J. S. (2007). Comparison of two extraction methods for evaluation of volatile constituents patterns in commercial whiskeys: Elucidation of the main odour-active compounds. Talanta, 74(1), 78–90. https://doi.org/10.1016/J.TALANTA.2007.05.029

Campo, E., Cacho, J., & Ferreira, V. (2007). Solid phase extraction, multidimensional gas chromatography mass spectrometry determination of four novel aroma powerful ethyl esters: Assessment of their occurrence and importance in wine and other alcoholic beverages. Journal of Chromatography A, 1140(1–2), 180–188. https://doi.org/10.1016/J.CHROMA.2006.11.036

Canas, S., Caldeira, I., & Belchior, A. P. (2009). Comparison of alternative systems for the ageing of wine bran\/dy. Oxygenation and wood shape effect. Ciencia e Tecnica Vitivinicola, 24(1).

Chandrasekara, A., & Shahidi, F. (2012). Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated in vitro digestion and microbial fermentation. Journal of Functional Foods, 4(1), 226–237. https://doi.org/10.1016/j.jff.2011.11.001

Charalambous, George. (2014). The Quality of foods and beverages V1 : Chemistry and technology. 462. https://books.google.com/books/about/The_Quality_of_Foods_and_Beverages_V1.html?id=dNvVSFdRLssC

Chatonnet, P. (1999). Discrimination and Control of Toasting Intensity and Quality of Oak Wood Barrels. American Journal of Enology and Viticulture, 50(4), 479–494. https://doi.org/10.5344/AJEV.1999.50.4.479

Chatonnet, P., Boidron, J., Dubourdieu, D., & One, M. P.-O. (1994). Evolution de certains composés volatils du bois de chêne au cours de son séchage premiers résultats. Oeno-One.Eu. https://oeno-one.eu/article/view/1720

Chatonnet, P., Cutzach, I., Pons, M., & Dubourdieu, D. (1999). Monitoring Toasting Intensity of Barrels by Chromatographic Analysis of Volatile Compounds from Toasted Oak Wood. Journal of Agricultural and Food Chemistry, 47(10), 4310–4318. https://doi.org/10.1021/JF981234T

Chethan, S., & Malleshi, N. G. (2007). Finger millet polyphenols: Optimization of extraction and the effect of pH on their stability. Food Chemistry, 105(2), 862–870. https://doi.org/10.1016/j.foodchem.2007.02.012

Chira, K., & Teissedre, P. L. (2015). Chemical and sensory evaluation of wine matured in oak barrel: effect of oak species involved and toasting process. European Food Research and Technology, 240(3), 533–547. https://doi.org/10.1007/S00217-014-2352-3

Christoph, N., chemistry, C. B.-C.-F. and fragrances:, & 2007, undefined. (2007). Flavour of spirit drinks: Raw materials, fermentation, distillation, and ageing. SpringerN Christoph, C Bauer-ChristophFlavours and Fragrances: Chemistry, Bioprocessing and Sustainability, 2007•Springer, 219–239. https://doi.org/10.1007/978-3-540-49339-6_10

Clyne, J., Conner, J. M., Paterson, A., & Piggott, J. R. (1993). The effect of cask charring on Scotch whisky maturation. Academic.Oup.ComJ CLYNE, JM Conner, A Paterson, JR PiggottInternational Journal of Food Science and Technology, 1993•academic.Oup.Com.https://academic.oup.com/ijfst/article abstract/28/1/69/7867576

Conner, J. M., Paterson, A., & Piggott, J. R. (1992). Analysis of lignin from oak casks used for the maturation of Scotch whisky. Journal of the Science of Food and Agriculture, 60(3), 349–353. https://doi.org/10.1002/JSFA.2740600312

Conner, J. M., Paterson, A., & Piggott, J. R. (1994). Agglomeration of ethyl esters in model spirit solutions and malt whiskies. Journal of the Science of Food and Agriculture, 66(1), 45–53. https://doi.org/10.1002/JSFA.2740660108

Conner, J. M., Paterson, A., & Piggott, J. R. (1999). Release of distillate flavour compounds in Scotch malt whisky. Wiley Online LibraryJM Conner, A Paterson, JR PiggottJournal of the Science of Food and Agriculture, 1999•Wiley Online Library. https://onlinelibrary.wiley.com/doi/pdf/10.1002/(SICI)1097-0010(19990515)79:7%3C1015::AID-JSFA321%3E3.0.CO;2-R

Conner, J., Reid, k., & Jack, F. (2003). Maturation and blending. In whisky.

Conner John M, Birkmyre, L., Paterson, A., & Piggott, J. R. (1998). Headspace concentrations of ethyl esters at different alcoholic strengths. Wiley Online Library. https://scijournals.onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1097-0010(199805)77:1%3C121::AID-JSFA14%3E3.0.CO;2-V

Cown, D. J., Haslett, A. N., Kimberley, M. O., & McConchie, D. L. (1996). The influence of wood quality on lumber drying distortion. Annales Des Sciences Forestières, 53(6), 1177–1188. https://doi.org/10.1051/FOREST:19960611

Cutzach, I., Chatonnet, P., Henry, R., & Dubourdieu, D. (1997). Identification of Volatile Compounds with a "Toasty" Aroma in Heated Oak Used in Barrelmaking. Journal of Agricultural and Food Chemistry, 45(6), 2217–2224. https://doi.org/10.1021/JF960947D

Daiches, D. (1978). Scotch whisky: its past and present. Cir.Nii.Ac.Jp. https://cir.nii.ac.jp/crid/1130000796052922496

Delahunty, C. M., Conner, J. M., Piggott, J. R., & Paterson, A. (1993). Perception of heterocyclic nitrogen compounds in mature whisky. Journal of the Institute of Brewing, 99(6), 479–482. https://doi.org/10.1002/J.2050-0416.1993.TB01187.X

Díaz-Maroto, M. C., Guchu, E., Castro-Vázquez, L., de Torres, C., & Pérez-Coello, M. S. (2008). Aroma-active compounds of American, French, Hungarian and Russian oak woods, studied by GC-MS and GC-O. Flavour and Fragrance Journal, 23(2), 93–98. https://doi.org/10.1002/FFJ.1859

Diéguez, S. C., Díaz, L. D., De La Peña, M. L. G., & Gómez, E. F. (2002). Variation of Volatile Organic Acids in Spirits during Storage at Low and Room Temperatures. LWT - Food Science and Technology, 35(5), 452–457. https://doi.org/10.1006/FSTL.2001.0882

Dubois, P. (1989). Influence of new oak cask on wine flavour. Agris.Fao.Org. https://agris.fao.org/search/en/providers/123819/records/647359c553aa8c896307bc99

Dudley, R. (2004). Ethanol, Fruit Ripening, and the Historical Origins of Human Alcoholism in Primate Frugivory 1 (Vol. 44). https://academic.oup.com/icb/article/44/4/315/800304

EU regulations whisky. (2019).

Fazzalari, F. A. (1978). Compilation of odor and taste threshold values data. In Published in 1978 in Philadelphia by The Society. Philadelphia : The Society, c1978. https://lib.ugent.be/catalog/rug01:000125672

Fermented Beverage Production - Google Books. (n.d.). Retrieved 19 April 2025, from https://books.google.com.np/books?hl=en&lr=&id=0aWR3fuA7QIC&oi=fnd&pg=PA1&dq=Fermented+Beverage+Production&ots=qFWoGbql5x&sig=68fbfpN5GyLl2PlT6t26HgChZQU&redir_esc=y#v=onepage&q=Fermented%20Beverage%20Production&f=false

Feuillat, F., Keller, R., Sauvageot, F., & Puech, J. L. (1999). Characterization of French Oak Cooperage (Quercus robur L., Quercus petraea Liebl.). Research of the Study Group on Barrel-Aging Burgundy Wines. American Journal of Enology and Viticulture, 50(4), 513–518. https://doi.org/10.5344/AJEV.1999.50.4.513

Forests and Forestry in Nepal - S. S. Negi, Sharad Singh Negi - Google Books. (n.d.). Retrieved 13 April 2025, from

Fujii, T., Kurokawa, M. and Saita, M. (1992) Studies... - Google Scholar. (n.d.). Retrieved 13 April 2025, from

Garcia, J., Vaz, B., & Corilo, Y. (2013). Whisky analysis by electrospray ionization-Fourier transform mass spectrometry. Elsevier. https://www.sciencedirect.com/science/article/pii/S0963996912004966

Garde-Cerdán, T., & C Ancín-Azpilicueta. (n.d.). Review of quality factors on wine ageing in oak barrels. ElsevierT Garde-Cerdán, C Ancín-AzpilicuetaTrends in Food Science & Technology, 2006•Elsevier. Retrieved 13 April 2025, from https://www.sciencedirect.com/science/article/pii/S092422440600015X

Gautier, J.-F. (2003). Le tonneau à travers Les âges. Dialnet.Unirioja.Es. https://dialnet.unirioja.es/servlet/articulo?codigo=3557395

Glabasnia, A., & Hofmann, T. (2007). Identification and Sensory Evaluation of Dehydro- and Deoxy-ellagitannins Formed upon Toasting of Oak Wood (Quercus alba L.). Journal of Agricultural and Food Chemistry, 55(10), 4109–4118. https://doi.org/10.1021/JF070151M

Guchu, E., Díaz-Maroto, M., M.S. Pérez-Coello, M.A. González-Viñas, & M.D. Cabezudo Ibáñez. (2006). Volatile composition and sensory characteristics of Chardonnay wines treated with American and Hungarian oak chips. ElsevierE Guchu, MC Díaz-Maroto, MS Pérez-Coello, MA González-Viñas, MDC IbáñezFood Chemistry, 2006•Elsevier. https://www.sciencedirect.com/science/article/pii/S0308814605006503

Haslam, E. (1989). Plant polyphenols: vegetable tannins revisited.

Hiremath, S. P., & Geetha, K. (2019). Nutritional Composition of Raw, Malted and Popped Finger Millet (Eleusine coracana) Varieties. International Journal of Current Microbiology and Applied Sciences, 8(02), 966–974. https://doi.org/10.20546/ijcmas.2019.802.112

Hithamani, G., & Srinivasan, K. (2014). Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum). Food Chemistry, 164, 55–62. https://doi.org/10.1016/j.foodchem.2014.04.107

Jack, F. R. (2014). Whisky. https://doi.org/10.1093/OXFORDHB/9780199742707.013.4

Jackson Micheal. (2017). Whiskey_ The Definitive World Guide.

Jackson, R. (2000). Wine science: principles, practice, perception.

Jounela-Eriksson, P. (2019). Predictive value of sensory and analytical data for distilled beverages. Degruyter.ComP Jounela-ErikssonFlavour, 1981•degruyter.Com, 145–164. https://doi.org/10.1515/9783110844061-013/PDF

Jyoti Prakash Tamang - Ethnic Fermented Foods and Beverages of India_ Science History and Culture-Springer Singapore (2020). (n.d.).

K Nishimura and R. Matsuyama. (1989). Reactions of wood components during maturation. Cir.Nii.Ac.Jp. https://cir.nii.ac.jp/crid/1574231875248834560

Karki, D. B. (2013). Improvement on Traditional Millet Fermentation Process and Its Brewing Quality Assessment. Central Department of Food Technology. Tribhuvan University, Dharan, Pp. 1À256.

Karki, D. B., & Kharel, G. P. (2012). Effect of finger millet varieties on chemical characteristics of their malts. Academicjournals.Org, 6(11), 308–316. https://doi.org/10.5897/AJFS12.029

Karki, R., Karki, D., Ojha, P., Shrestha, R., … P. B.-N., & 2023, undefined. (n.d.). Traditional Fermentation Practices and Brewing Dynamics of Nepalese Millet-Based Alcoholic Beverages. Researchgate.NetR Karki, DB Karki, P Ojha, R Shrestha, P Basnet, S Maharjan, U Manandhar, K KarkiNepal: Millets,

KC, J. B., Rai, B. K., Subba, D. K., & Ghimire, G. (2004). Practicals in Basic Biochemistry and Industrial Microbiology. Maya KC Kathmandu, Nepal.

KC, J. B., Subba, D. K., & Rai, B. K. (2001). Plants used in murcha preparation in eastern Nepal. Journal of Hill Research Sikkim Science Society, 14(2), 107–109.

Kharel, G. P., Aacharya, P. P., & Rai, B. K. (2011). Traditional foods of Nepal. Highlight Publication Pvt Ltd.

Kiple, K., & Ornelas, K. (2000). The Cambridge world history of food.

Koppenhoefer, B., Behnisch, R., Epperlein, U., Holzschuh, H., Bernreuther, A., Piras, P., & Roussel, C. (n.d.). Enantiomeric Odor Differences and Gas Chromatographic Properties of Flavors and Fragrances. A Selected Review.

Kumar, A., Tripathi, M., Joshi, D., & Kumar, V. (2021). Millets and millet technology. https://doi.org/10.1007/978-981-16-0676-2.PDF

Lea, A., & Piggott, J. (2003). Fermented beverage production.

Léder, I. (2004). Sorgum and millets.

Lee, K. Y. M., Paterson, A., Piggott, J. R., & Richardson, G. D. (2001). Sensory discrimination of blended Scotch whiskies of different product categories. Food Quality and Preference, 12(2), 109–117. https://doi.org/10.1016/S0950-3293(00)00037-9

Lee, K.-Y. M., Paterson, A., & Piggott, J. R. (2001). Origins of flavour in whiskies and a revised flavour wheel: A review. Wiley Online Library, 107(5), 287–313. https://doi.org/10.1002/j.2050-0416.2001.tb00099.x

Lee, K.-Y. M., Paterson, A., Piggott, J. R., & Richardson, G. D. (2000). Perception of whisky flavour reference compounds by Scottish distillers. Wiley Online LibraryKYM Lee, A Paterson, JR Piggott, GD RichardsonJournal of the Institute of Brewing, 2000•Wiley Online Library, 106(4), 203–208. https://doi.org/10.1002/j.2050-0416.2000.tb00058.x

Lee, S. M., Chen, Z. Y., Sheu, S. C., & Chen, C. W. (2023). Optimizing brewing beer production using Aspergillus oryzae solid-state fermentation of sorghum koji as an adjunct. Taylor & FrancisSM Lee, ZY Chen, SC Sheu, CW ChenInternational Journal of Food Properties, 2023•Taylor & Francis, 26(2), 3065–3081. https://doi.org/10.1080/10942912.2023.2270174

Liebmann, A., Chemistry, B. S.-I. & E., & 1949, undefined. (1949). Changes in whisky while maturing. ACS Publications, 41(3), 534–543. https://doi.org/10.1021/IE50471A021+

Lost Crops of Africa. (1996). In Lost Crops of Africa. National Academies Press. https://doi.org/10.17226/2305

Lucet, M., Charamel, A., Chapuis, A., Guido, G., & Loreau, J. (1996). Role of Batch Processing in the Chemical Process Industry. Batch Processing Systems Engineering, 43–48. https://doi.org/10.1007/978-3-642-60972-5_3

Maga, J. A. (1989). The contribution of wood to the flavor of alcoholic beverages. Food Reviews International, 5(1), 39–99. https://doi.org/10.1080/87559128909540844

Maga, J. A. (1996). Oak lactones in alcoholic beverages. Food Reviews International, 12(1), 105–130. https://doi.org/10.1080/87559129609541069

Masson, E., & Puech, J. (2000). The incidence of different modes of kiln drying on ellagitannin and volatile compound content of sessile oak (Quercus petraea (Matt.) Liebl.). https://www.cabidigitallibrary.org/doi/full/10.5555/20000614338

Mohapatra, D., Patel, A., & Kar, A. (2019). Effect of different processing conditions on proximate composition, anti-oxidants, anti-nutrients and amino acid profile of grain sorghum. Elsevier. https://www.sciencedirect.com/science/article/pii/S0308814618313566

Mosedale, J., and, A. F.-J. of the S. of F., & 1996, undefined. (n.d.). Variation of the flavour and extractives of European oak wood from two French forests. Wiley Online LibraryJR Mosedale, A FordJournal of the Science of Food and Agriculture, 1996•Wiley Online Library. Retrieved 16 April 2025, from https://scijournals.onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1097-0010(199603)70:3%3C273::AID-JSFA496%3E3.0.CO;2-L

Mosedale, J., & Andrew Ford. (1996). Variation of the flavour and extractives of European oak wood from two French forests. Wiley Online LibraryJR Mosedale, A FordJournal of the Science of Food and Agriculture, 1996•Wiley Online Library. https://scijournals.onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1097-0010(199603)70:3%3C273::AID-JSFA496%3E3.0.CO;2-L

Mosedale, J. R. (1995). Effects of oak wood on the maturation of alcoholic beverages with particular reference to whisky. Forestry, 68(3), 203–230. https://doi.org/10.1093/forestry/68.3.203

Mosedale, J. R., & Puech, J. L. (1998). Wood maturation of distilled beverages. Trends in Food Science & Technology, 9(3), 95–101. https://doi.org/10.1016/S0924-2244(98)00024-7

Mujtaba, I. M. (2003). Batch Distillation - Design and Operation.

Namiki, M. (1990). Antioxidants/antimutagens in food. Critical Reviews in Food Science and Nutrition, 29(4), 273–300. https://doi.org/10.1080/10408399009527528

Negi, S., & Naithani, H. (1995). Oaks of India, Nepal and Bhutan. https://doi.org/10.5555/19960600643

Negi, S., & Negi, S. (1994). Forests and forestry in Nepal.

Nishimura and Matsuyama (1989). (n.d.). Cask quality and warehouse conditions. Cir.Nii.Ac.Jp. Retrieved 11 April 2025, from https://cir.nii.ac.jp/crid/1573105975341990656

Nishimura K. (1983). Reactions of wood components during maturation. Cir.Nii.Ac.Jp. https://cir.nii.ac.jp/crid/1574231875248834560

Nishimura, K. (1989). Maturation and maturation chemistry. Cir.Nii.Ac.Jp. https://cir.nii.ac.jp/crid/1573387449970360320

Noguchi, Y. (2017). The influence of wood species of casks on matured whisky aroma : identification of unique character imparted to whisky by casks constructed of Japanese oak. https://www.ros.hw.ac.uk/handle/10399/3276

Noguchi, Y., Hughes, P., Priest, F., Conner, J., & Jack, F. (2010). The influence of wood species of cask on matured whisky aroma—identification of a unique character imparted by casks of Japanese oak. Distilled Spirits—New Horizons: Energy, Environment and Enlightenment, Nottingham University Press, Nottingham.

Nykänen, L. (1986). Formation and Occurrence of Flavor Compounds in Wine and Distilled Alcoholic Beverages. American Journal of Enology and Viticulture, 37(1), 84–96. https://doi.org/10.5344/AJEV.1986.37.1.84

Nykänen, L., Puputti, E., & Heikki, S. (1968). Volatile Fatty Acids in Some Brands of Whisky, Cognac and Rum. Journal of Food Science, 33(1), 88–92. https://doi.org/10.1111/J.1365-2621.1968.TB00890.X

Ohsawa, M., Shakya, P. R., & Numata, M. (1986). Distribution and succession of West Himalayan forest types in the eastern part of the Nepal Himalaya. Mountain Research & Development, 6(2), 143–157. https://doi.org/10.2307/3673268

Paudel, P. K., Bhattarai, B. P., & Kindlmann, P. (2012). An overview of the biodiversity in Nepal. Springer, 1–40. https://doi.org/10.1007/978-94-007-1802-9_1

Pérez-Coello, M. S., Sanz, J., & Cabezudo, M. D. (1999). Determination of Volatile Compounds in Hydroalcoholic Extracts of French and American Oak Wood. American Journal of Enology and Viticulture, 50(2), 162–165. https://doi.org/10.5344/AJEV.1999.50.2.162

Philip, J. M. (1989). Cask quality and warehouse conditions. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Philp%2C+J+.+%281989%29.+Cask+quality+and+warehouse+conditions.+In%3A+The+Science+and+Technology+of+Whiskies+%28edited+by+J.R.+Piggott%2C+R.+Sharp%2C+%26+R.E.B.+Duncan%29.+Pp.+264-294.+Harlow%3A+Longman.&btnG=

Piggott, J. R. (2017). Whisky. In Current Developments in Biotechnology and Bioengineering: Food and Beverages Industry (pp. 435–450). Elsevier Inc. https://doi.org/10.1016/B978-0-444-63666-9.00015-7

Piggott, J. R., & Conner, J. M. (1995). Whiskies. Alcohol and Temperance in Modern History: An International Encyclopedia, 2, 247–274. https://doi.org/10.1007/978-1-4757-5214-4_10

Piggott, J. R., & Conner, J. M. (2003). Whiskies. Fermented Beverage Production, 239–262. https://doi.org/10.1007/978-1-4615-0187-9_11

Piggott, J. R., Conner, J. M., & Paterson, A. (1995a). Flavour development in whisky maturation.

Piggott, J. R., Conner, J. M., & Paterson, A. (1995b). Flavour development in whisky maturation. Developments in Food Science, 37(C), 1731–1751. https://doi.org/10.1016/S0167-4501(06)80261-X

Piggott, J. R., & Jardine, S. P. (1979). Descriptive sensory analysis of whisky flavour. Wiley Online Library, 85(2), 82–85. https://doi.org/10.1002/J.2050-0416.1979.TB06830.X

Poisson, L., & Schieberle, P. (2008). Characterization of the most odor-active compounds in an American Bourbon whisky by application of the aroma extract dilution analysis. Journal of Agricultural and Food Chemistry, 56(14), 5813–5819. https://doi.org/10.1021/jf800382m

Pradhan, B., & Ghimire, G. P. S. (1994). The edapho-vegetation relation of Phulchowki Himalaya . Kathmandu Ecoprint, 1, 57-64.

Prida, A., & Puech, J. L. (2006a). Influence of geographical origin and botanical species on the content of extractives in American, French, and East European oak woods. Journal of Agricultural and Food Chemistry, 54(21), 8115–8126. https://doi.org/10.1021/JF0616098

Prida, A., & Puech, J. L. (2006b). Influence of geographical origin and botanical species on the content of extractives in American, French, and East European oak woods. Journal of Agricultural and Food Chemistry, 54(21), 8115–8126. https://doi.org/10.1021/JF0616098

PUECH, J., C, J., L, D., & G, A. (1977). Substances Phenoliques Des Eaux De Vie D'Armahnac ET DE Rhum. II. Substances Phenoliques Des Eaux De Vie D'Armahnac ET DE Rhum. II.

Puech, J.-L. (1984). Characteristics of oak wood and biochemical aspects of Armagnac aging. Ajevonline.OrgJL PuechAmerican Journal of Enology and Viticulture, 1984•ajevonline.Org. https://www.ajevonline.org/content/35/2/77.short

Rai, B. (2016). Basic Practical Manual on Industrial Microbiology. https://books.google.com/books?hl=en&lr=&id=ZdN1DwAAQBAJ&oi=fnd&pg=PR3&dq=Rai,+B.+K.+and+Subba,+D.+K.+(2016a).+%22Basic+Mannual+on+Industrial+Microbiology%22+(1st+ed.).&ots=gbcZq3Tbqq&sig=dVZJsEM943AdXJaEdc7ElhCrd9Y

Ranganna, S. (1986). Handbook of analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Education.

Rani, H., & Bhardwaj, R. D. (2021). Quality attributes for barley malt:"The backbone of beer". Wiley Online LibraryH Rani, RD BhardwajJournal of Food Science, 2021•Wiley Online Library, 86(8), 3322–3340. https://doi.org/10.1111/1750-3841.15858

Ratnavathi, C., & Tonapi, V. A. (2022). Nutritional properties of millets: nutricereals with health benefits to reduce lifestyle diseases and malnutrition. SpringerCV Ratnavathi, VA TonapiHandbook of Millets-Processing, Quality, and Nutrition Status, 2022•Springer, 129–149. https://doi.org/10.1007/978-981-16-7224-8_7

Ravindran, G. (1991). Studies on Millets: Proximate Composition, Mineral Composition, and Phytate and Oxalate Contents. In Food Chemistry (Vol. 39).

Reazin, G. H. (1981). Chemical Mechanisms of Whiskey Maturation. American Journal of Enology and Viticulture, 32(4), 283–289. https://doi.org/10.5344/AJEV.1981.32.4.283

Reazin, G. H. (1983). Chemical analysis of whisky.

Reid, K. J., & Ward, A. (1994). Evaporation losses from traditional and racked warehouses. In Proceedings of the Fourth Aviemore Conference on Malting, Brewing and Distilling (pp. 319-322). London: Institute of Brewing.

Rhodes, C., Heaton, K., Goodall, I., & Brereton, P. (2009). Gas chromatography carbon isotope ratio mass spectrometry applied to the detection of neutral alcohol in Scotch whisky: an internal reference approach. ElsevierCN Rhodes, K Heaton, I Goodall, PA BreretonFood Chemistry, 2009•Elsevier. https://www.sciencedirect.com/science/article/pii/S0308814608011503

Russell, I., Stewart, G., New York, L., & Diego San Francisco, S. (2014). Whisky Technology, Production and Marketing. www.elsevierdirect.com/rights

Salo, P., Nykänen, L., & Suomalainen, H. (1972). Order thresholds and relative intensities of volatile aroma components in an artificial beverage imitating whisky. Journal of Food Science, 37(3), 394–398. https://doi.org/10.1111/J.1365-2621.1972.TB02647.X

Sarni, F., Moutounet, M., Puech, J. L., & Rabier, P. (1990). Effect of heat treatment of oak wood extractable compounds. Holzforschung, 44(6), 461–466. https://doi.org/10.1515/HFSG.1990.44.6.461/HTML

Sauvageot, F., & Feuillat, F. (1999). The Influence of Oak Wood (Quercus robur L., Q. petraea Liebl.) on the Flavor of Burgundy Pinot noir. An Examination of Variation Among Individual Trees. American Journal of Enology and Viticulture, 50(4), 447–455. https://doi.org/10.5344/AJEV.1999.50.4.447

Sefton, M., Francis, I., Pocock, K., & Williams, P. (1993). The influence of natural seasoning on the concentrations of eugenol, vanillin, and cis- and trans-β-methyl-γ-octalactone extracted from French and American. https://doi.org/10.5555/19940605730

Sharp, R. (1983). R. Sharp, Analytical techniques used in the study.

Shrestha, H., Nand, K., & Rati, E. R. (2002). Microbiological profile of Murcha starters and physio-chemical characteristics of POKO, a rice based traditional fermented food product of Nepal. Taylor & FrancisH Shrestha, K Nand, ER RatiFood Biotechnology, 2002•Taylor & Francis, 16(1), 1–15. https://doi.org/10.1081/FBT-120004198

Shrestha, K. B., Måren, I. E., Arneberg, E., Sah, J. P., & Vetaas, O. R. (2013). Effect of anthropogenic disturbance on plant species diversity in oak forests in Nepal, Central Himalaya. Taylor & Francis, 9(1), 21–29. https://doi.org/10.1080/21513732.2012.749303

Singleton, V., & Draper, D. E. (1961). Wood chips and wine treatment; the nature of aqueous alcohol extracts. Ajevonline.OrgVL Singleton, DE DraperAmerican Journal of Enology and Viticulture, 1961•ajevonline.Org. https://www.ajevonline.org/content/12/4/152.short

Singleton, V. L. (1995). Maturation of Wines and Spirits: Comparisons, Facts, and Hypotheses. American Journal of Enology and Viticulture, 46(1), 98–115. https://doi.org/10.5344/AJEV.1995.46.1.98

Singleton, V. L. (2000). Le stockage des vins en barriques: utilisation et variables significatives. Pascal-Francis.Inist.Fr. https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=1482101

Sousa, R. B., & Canes, S. (2007). Effect of heat treatment on the thermal and chemical modifications of oak and chestnut wood used in brandy ageing.

Spillman, P. J., Pollnitz, A. P., Liacopoulos, D., Skouroumounis, G. K., & Sefton, M. A. (1997). Accumulation of Vanillin during Barrel-Aging of White, Red, and Model Wines. Journal of Agricultural and Food Chemistry, 45(7), 2584–2589. https://doi.org/10.1021/JF970034Z

Sripriya, G., Chandrasekharan, " K, Murtyb, V. S., & Chandra, T. S. (1996). ESR spectroscopic studies on free radical quenching action of finger millet (Eleusine coracana). In Food Chemistry (Vol. 57, Issue 4).

Standage, T. (2017). Tom Standage - A History of the World in 6 Glasses-Bloomsbury Publishing PLC (2017).

Statistical information on Nepalese agriculture. (2079).

Stutz, T., Lin, S., & Herdman, I. (1999). Barrel Renewal Systems — A User's Perspective. American Journal of Enology and Viticulture, 50(4), 541–543. https://doi.org/10.5344/AJEV.1999.50.4.541

Tamang, J. P. (2019). Ethnic Fermented Foods and Alcoholic Beverages of Asia.

Thapa, S., & Tamang, J. P. (2004). Product characterization of kodo ko jaanr: Fermented finger millet beverage of the Himalayas. Food Microbiology, 21(5), 617–622. https://doi.org/10.1016/j.fm.2004.01.004

Udeh, H. O., Duodu, K. G., & Jideani, A. I. O. (2017). Finger millet bioactive compounds, bioaccessibility, and potential health effects - A review. In Czech Journal of Food Sciences (Vol. 35, Issue 1, pp. 7–17). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/206/2016-CJFS

Vetaas, O. R. (1997). The effect of canopy disturbance on species richness in a central Himalayan oak forest. Plant Ecology, 132(1), 29–38. https://doi.org/10.1023/A:1009751219823/METRICS

Vetaas, O. R., & Chaudhary, R. P. (1998a). Scale and species-environment relationships in a central Himalayan oak forest, Nepal. Plant Ecology 1998 134:1, 134(1), 67–76. https://doi.org/10.1023/A:1009768122692

Vetaas, O. R., & Chaudhary, R. P. (1998b). Scale and species-environment relationships in a central Himalayan oak forest, Nepal. Plant Ecology, 134(1), 67–76. https://doi.org/10.1023/A:1009768122692/METRICS

Ward, A., Hale, M. D., & Cardias-Williams, F. C. (1998). The Isolation of Fungi from Air and Kiln Drying Oak Wood Used for the Maturation of Alcoholic Beverages. Holzforschung, 52(4), 359–364. https://doi.org/10.1515/HFSG.1998.52.4.359/PDF/FIRSTPAGE

Waterhouse, A. L., & Towey, J. P. (1994). Oak Lactone Isomer Ratio Distinguishes between Wine Fermented in American and French Oak Barrels. Journal of Agricultural and Food Chemistry, 42(9), 1971–1974. https://doi.org/10.1021/JF00045A026/ASSET/JF00045A026.FP.PNG_V03

Whitby, B. R. (1992). Traditional distillation in the whisky industry. Ferment Institute of Brewing, 5(4), 261–267.

Wishart, D. (2009). The Flavour of Whisky. Significance, 6(1), 20–26. https://doi.org/10.1111/J.1740-9713.2009.00337.X

Wiśniewska, P., Dymerski, T., Wardencki, W., & Namieśnik, J. (2015). Chemical composition analysis and authentication of whisky. Journal of the Science of Food and Agriculture, 95(11), 2159–2166. https://doi.org/10.1002/JSFA.6960

Wolf, A., Bray, G., reviews, B. P.-O., & 2008, undefined. (2007). A short history of beverages and how our body treats them. Wiley Online LibraryA Wolf, GA Bray, BM PopkinObesity Reviews, 2008•Wiley Online Library, 9(2), 151–164. https://doi.org/10.1111/j.1467-789X.2007.00389.x

# APPENDICES

## Appendix A

## Specimen card of sensory evaluation by 9-point hedonic rating test

### Sensory evaluation

Name of panelist: ………………………………….……. Age: … Date………….

Name of Product: Traditionally made Local millet Rakshi matured in different oak varieties.

Details of product: This sample is matured for……. days in air sealed glass jar with 2cm^3 cube of different varieties of oak species.

| Sample | Color | Aroma | Mouthfeel | Body | Aftertaste | Overall |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |

Dislike extremely-1

Dislike very much-2

Dislike moderately-3

Dislike slightly-4

Neither like nor dislike-5

Like slightly-6

Like moderately-7

Like very much-8

Like extremely-9

Sensory participants are kindly requested to select (✓) the flavors you have noticed during the sensory evaluation.

| Sample | Caramel | Grainy | Fruity | Smokey | Bitter | Coconut | Nutty | Woody | Spicy | Others |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| | | | | | | | | | | |

Comments:

……………………………………………………………………………………………

List of panelists.

Table 5.1: Name of sensory panelist.

| S. N. | Names |
| --- | --- |
| 1 | Prof. Dr. Dhan Bahadur Karki, Central Campus of Technology |
| 2 | Asst. Prof. Kabindra Bhattarai, Central Campus of Technology |
| 3 | Amul Ghimire, Brew master, Raj Brewery |
| 4 | Roshan Parajuli, Quality control, Raj Brewery |
| 5 | Susham Shakhya, Brewer, Raj Brewery |
| 6 | Ritesh Khadka, Brewer, Raj Brewery |
| 7 | Kshitiz Bhusal, Brewer, Raj Brewery |
| 8 | Bikash Mishra, Brewer, AB InBev India |

## Appendix B

ANOVA Table and post hoc analysis of sensory hedonic scores.

Table 5.2: ANOVA table for hedonic sensory scores.

| | | Sum of squares | df | Mean squares | F | Sig. |
|---|---|---|---|---|---|---|
| Color | Between Groups | 45.464 | 6 | 7.577 | 16.321 | 0 |
| | Within Groups | 22.75 | 49 | 0.464 | | |
| | Total | 68.214 | 55 | | | |
| Aroma | Between Groups | 55.714 | 6 | 9.286 | 30.083 | 0 |
| | Within Groups | 15.125 | 49 | 0.309 | | |
| | Total | 70.839 | 55 | | | |
| Mouthfeel | Between Groups | 36.464 | 6 | 6.077 | 11.291 | 0 |
| | Within Groups | 26.375 | 49 | 0.538 | | |
| | Total | 62.839 | 55 | | | |
| Aftertaste | Between Groups | 40.607 | 6 | 6.768 | 18.171 | 0 |
| | Within Groups | 18.25 | 49 | 0.372 | | |
| | Total | 58.857 | 55 | | | |
| Overall | Between Groups | 50.857 | 6 | 8.476 | 34.254 | 0 |
| | Within Groups | 12.125 | 49 | 0.247 | | |
| | Total | 62.982 | 55 | | | |
| Body | Between Groups | 47.929 | 6 | 7.988 | 11.997 | 0 |
| | Within Groups | 32.625 | 49 | 0.666 | | |
| | Total | 80.554 | 55 | | | |

# Appendix C

Table C.1: Tukey table for Appearance

| SAMPLE | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| 1 | 8 | 5.13 | |
| 6 | 8 | | 7.13 |
| 7 | 8 | | 7.25 |
| 3 | 8 | | 7.38 |
| 5 | 8 | | 7.38 |
| 4 | 8 | | 7.88 |
| 2 | 8 | | 8.13 |
| Sig. | | 1 | 0.07 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 8.000

Table C.2: Tukey table for Aroma.

| SAMPLE | N | subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | a | c | d | b |
| 1 | 8 | 5.25 | | | |
| 5 | 8 | | 6.5 | | |
| 6 | 8 | | 7.13 | 7.13 | |
| 3 | 8 | | 7.25 | 7.25 | |
| 7 | 8 | | | 7.63 | 7.63 |
| 4 | 8 | | | | 8.25 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | 8 | | | | 8.38 |
| Sig. | | 1 | 0.12 | 0.554 | 0.12 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 8.000

Table C.3: Tukey table for Body.

| SAMPLE | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | a | c | b |
| 1 | 8 | 5.25 | | |
| 5 | 8 | | 6.75 | |
| 6 | 8 | | 7 | |
| 3 | 8 | | 7.13 | 7.13 |
| 7 | 8 | | 7.13 | 7.13 |
| 4 | 8 | | 8 | 8 |
| 2 | 8 | | | 8.38 |
| Sig. | | 1 | 0.051 | 0.051 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 8.000

Table C.4: Tukey table for Mouthfeel.

| SAMPLE | N | subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | a | c | d | b |
| 1 | 8 | 5.25 | | | |
| 5 | 8 | | 6.5 | | |
| 6 | 8 | | 7.13 | 7.13 | |
| 3 | 8 | | 7.25 | 7.25 | |
| 7 | 8 | | | 7.63 | 7.63 |
| 4 | 8 | | | | 8.25 |
| 2 | 8 | | | | 8.38 |
| Sig. | | 1 | 0.12 | 0.554 | 0.12 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 8.000

Table C.5: Tukey table for Aftertaste.

| SAMPLE | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | a | c | d | b |
| 1 | 8 | 5.25 | | | |
| 5 | 8 | | 6.25 | | |
| 3 | 8 | | 6.75 | 6.75 | |
| 6 | 8 | | 6.75 | 6.75 | |
| 7 | 8 | | | 7.38 | 7.38 |
| 4 | 8 | | | | 7.75 |
| 2 | 8 | | | | 7.88 |
| Sig. | | 1 | 0.659 | 0.399 | 0.659 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 8.000

Table C.6: Tukey table for Overall.

| SAMPLE | N | Subset for alpha = 0.05 | | | | |
|---|---|---|---|---|---|---|
| | | a | c | d | e | b |
| 1 | 8 | 5.25 | | | | |
| 5 | 8 | | 6.5 | | | |
| 3 | 8 | | 6.75 | 6.75 | | |
| 6 | 8 | | 6.88 | 6.88 | | |
| 7 | 8 | | | 7.38 | 7.38 | |
| 2 | 8 | | | | 8.13 | 8.13 |
| 4 | 8 | | | | | 8.25 |
| Sig. | | 1 | 0.739 | 0.177 | 0.058 | 0.999 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 8.000

# Appendix D

ANOVA Table and post hoc analysis of TDS, Conductivity and pH.

Table D.1: ANOVA table of TDS, Conductivity and pH.

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| TDS | Between Groups | 507.529 | 6 | 84.588 | 4968.814 | 0 |
| | Within Groups | 0.238 | 14 | 0.017 | | |
| | Total | 507.767 | 20 | | | |
| conductivity | Between Groups | 1188.732 | 6 | 198.122 | 2887.476 | 0 |
| | Within Groups | 0.961 | 14 | 0.069 | | |
| | Total | 1189.693 | 20 | | | |
| ph | Between Groups | 3.27 | 6 | 0.545 | 158.54 | 0 |
| | Within Groups | 0.048 | 14 | 0.003 | | |
| | Total | 3.319 | 20 | | | |

Table D.2: Tukey table for TDS

| Sample | N | Subset for alpha = 0.05 | | | | | |
|---|---|---|---|---|---|---|---|
| | | a | b | c | f | e | g |
| 1 | 3 | 5.7767 | | | | | |
| 4 | 3 | | 11.2 | | | | |
| 2 | 3 | | 11.5 | | | | |
| 3 | 3 | | | 14.5467 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | 3 | | | | 16.6 | | |
| 5 | 3 | | | | | 20 | |
| 7 | 3 | | | | | | 21.0733 |
| Sig. | | 1 | 0.13 | 1 | 1 | 1 | 1 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 3.000

Table D.3: Tukey table for Conductivity.

| Sample | N | Subset for alpha = 0.05 | | | | | |
|---|---|---|---|---|---|---|---|
| | | a | d | b | c | e | f |
| 1 | 3 | 8.9667 | | | | | |
| 4 | 3 | | 17.2333 | | | | |
| 2 | 3 | | | 18.2167 | | | |
| 3 | 3 | | | | 22.38 | | |
| 6 | 3 | | | | | 25.4967 | |
| 5 | 3 | | | | | | 30.1333 |
| 7 | 3 | | | | | | |
| Sig. | | 1 | 1 | 1 | 1 | 1 | 1 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 3.000

Table D.4: Tukey table for pH.

| Sample | N | Subset for alpha = 0.05 | | | | |
|---|---|---|---|---|---|---|
| | | c | a | b | d | e |
| 4 | 3 | 3.74 | | | | |
| 2 | 3 | | 4 | | | |
| 3 | 3 | | | 4.4667 | | |
| 6 | 3 | | | | 4.6567 | |
| 1 | 3 | | | | 4.7133 | 4.7133 |
| 7 | 3 | | | | 4.8033 | 4.8033 |
| 5 | 3 | | | | | 4.8333 |
| Sig. | | 1 | 1 | 1 | 0.092 | 0.228 |

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 3.000

# Appendix E

ANOVA Table and post hoc analysis of chemical analysis.

Table E.1: ANOVA table for chemical analysis.

| | | Sum of Squares | df | Mean Square | F |
|---|---|---|---|---|---|
| Acetic Acid | Between Groups | 47803.523 | 6 | 7967.254 | 359.416 |
| | Within Groups | 310.341 | 14 | 22.167 | |
| | Total | 48113.865 | 20 | | |
| Acetaldehyde | Between Groups | 706.239 | 6 | 117.706 | 49.534 |
| | Within Groups | 33.268 | 14 | 2.376 | |
| | Total | 739.506 | 20 | | |
| Acetone | Between Groups | 2.035 | 6 | 0.339 | 2739.821 |
| | Within Groups | 0.002 | 14 | 0 | |
| | Total | 2.037 | 20 | | |
| Ethyl Acetate | Between Groups | 216844.67 | 6 | 36140.778 | 748.162 |
| | Within Groups | 676.285 | 14 | 48.306 | |
| | Total | 217520.955 | 20 | | |
| Methanol | Between Groups | 124.272 | 6 | 624.33 | 1064.576 |
| | Within Groups | 8.21 | 14 | 0.586 | |
| | Total | 3754.211 | 20 | | |
| Active & Isoamyl | Between Groups | 3746.01 | 6 | 5003.292 | 79.28 |
| | Within Groups | 883.53 | 14 | 63.109 | |

| | | | | | |
|---|---|---|---|---|---|
| | Total | 30903.28 | 20 | | |
| Furfural | Between Groups | 96.617 | 6 | 16.103 | 78.395 |
| | Within Groups | 2.876 | 14 | 0.205 | |
| | Total | 99.492 | 20 | | |

## Color Plates

Fig: a

Fig: b

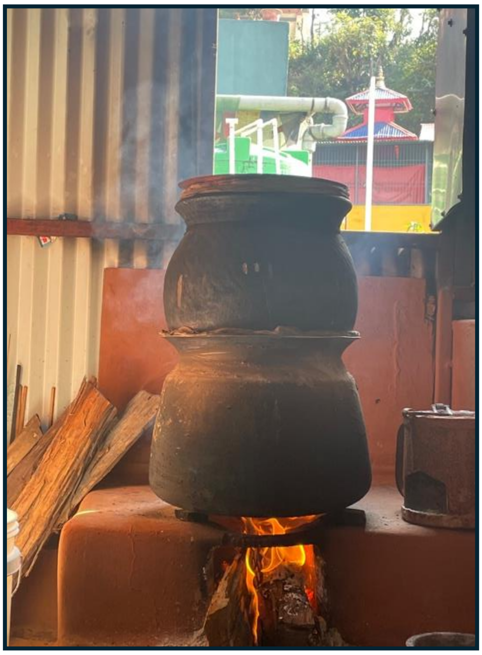

Fig: c

Fig: d

Fig: e

Fig: f

Fig: g

Fig: h

Fig: i

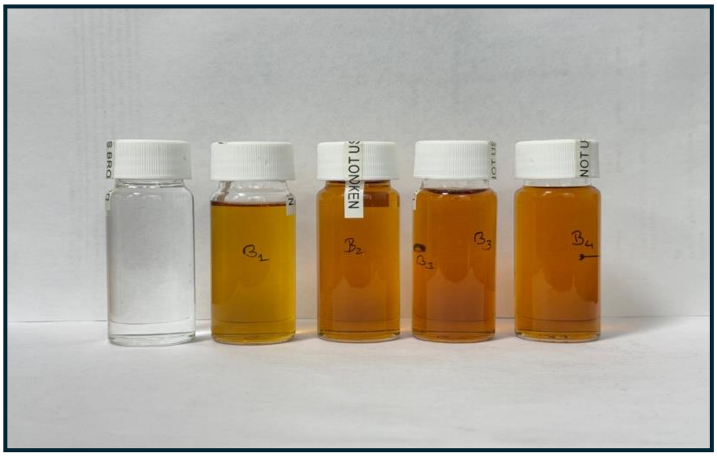

Fig: j

Fig: k

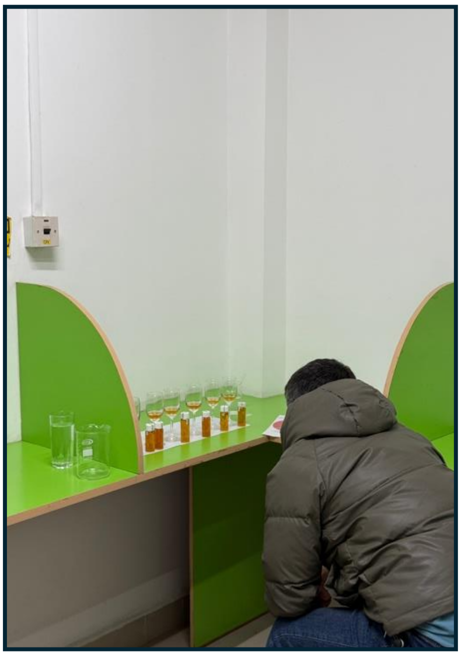

Fig: l

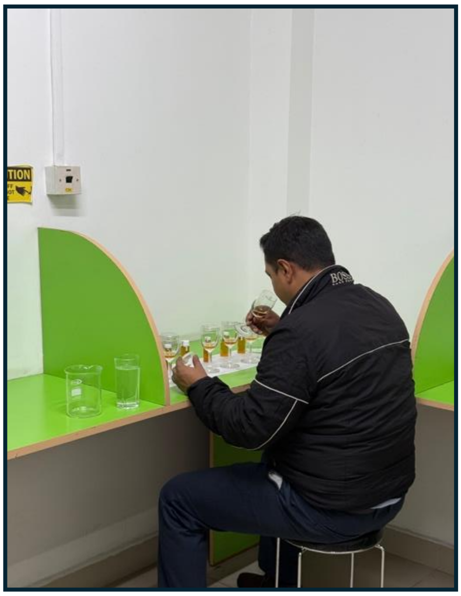

Fig: m