A STUDY ON PRODUCTION OF LIQUID SMOKE FROM CHERRY WOOD AND ITS CHEMICAL COMPOSITION AND FUNCTIONAL EFFICACY

by

Suman Bisunke Sarki

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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A Study on Production of Liquid Smoke from Cherry Wood and its Chemical Composition and Functional Efficacy

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfilment of the requirements for the degree of B. Tech. in Food Technology

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Suman Bisunke Sarki

Department of Food Technology

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Institute of Science and Technology

Tribhuvan University, Nepal

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Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***Dissertation*** entitled ***A Study on Production of Liquid Smoke from Cherry Wood and its Chemical Composition and Functional Efficacy*** presented by **Suman Bisunke Sarki** has been accepted as the partial fulfilment of the requirement for the **B. Tech. degree in Food Technology.**

Dissertation committee

1. **Head of Department** _____
   (Mr. Kabindra Bhattarai, Asst. Prof.)
2. **External Examiner** _____
   (Mr. Bijaya Khanal, Senior food inspector)
3. **Supervisor** _____
   (Mr. Kunjal Shrestha, Teaching Asst.)
4. **Internal Examiner** _____
   (Mr. Sabin Bahadur Khatri, Teaching Asst.)

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(Suman Bisunke Sarki)

Abstract

Liquid smoke was generated from cherry wood chips utilizing a simplified assembly design, with specific procedural modifications implemented to minimize the formation of polycyclic aromatic hydrocarbons. The chemical composition of the resulting liquid smoke was systematically analyzed to assess its properties. Liquid smoke of different percentages is applied to the lean uncured pork meat and cured pork meat by injecting it with syringe. To evaluate its antimicrobial efficacy, liquid smoke solutions at concentration of 10%, 15%, and 20% were applied to lean uncured pork meat and cured meat and microbiological assessment was carried out. Sensory properties and changes in antioxidant activity and total phenolic count were determined by applying the same concentration of liquid smoke.

Reddish yellow liquid smoke having acidity of 0.2% was produced with the yield of 41 ml per kg per hour. Total solids of liquid smoke were found negligible. pH, Antioxidant and total phenolic count of the liquid smoke were found to be 3.24, 46% and 49.23 mg GAE/ml respectively. The storage life of meat smoked with 10%, 15% and 20% liquid smoke was found to be 20, 20, and 25 days and for cured meat smoked with same amount of liquid smoke was 20, 25, and 30 days respectively. Sensory analysis identified cured meat treated with 20% liquid smoke as the most favorable. Antioxidant activity of cured meat treated with liquid smoke was higher as compared to uncured meat with same concentration and also decreasing rate was lesser for cured meat during 10-day storage period. Although, total phenolic content was initially similar in both meat types, its degradation rate was lower in cured samples.

Contents

List of Tables

List of Figures

List of Plates

List of Abbreviation

Abbreviation	Full form
ANOVA	Analysis of variance
LSD	Least significant difference
PP	Primary smoke flavoring
VOC	Volatile organic compound
wb	Wet basis
HPLC	High performance liquid chromatography
CFU	Colony forming unit
gm	Gram
HDPE	High density polyethylene
GAE	Gallic acid equivalent
PAH	Polycyclic aromatic hydrocarbon
TPC	Total plate count
ml	Milliliter
kg	Kilogram
mg	Milligram
ºC	Degree Celsius
WHO	World health organization
FAO	Food and agriculture organization

Part I

Introduction

1.1 General introduction

Smoking have been used for centuries to preserve food, particularly meats. Perhaps, to defend against canines, a man hung a catch over a fire. As time passed, it was found that drying meat in smoke-filled caverns increased its shelf-life and flavor. Since then, smoking has become a popular method for producing smoked food with unique flavors and inactivating enzymes and bacteria (Mcdonald, 2015; Simko, 2005). Wood smoke has been used for generations to treat a wide range of foods, including meats, poultry, fish, scallops, cheese, prunes, paprika and malt for whiskey and beer production. Typically, the technique involves salting, partial drying, and may be heating. Because of its antioxidant and antimicrobial property, the goal of extending shelf-life of the food items, prevention of food illness, imparting desirable color, aroma and enhancement of the smokey flavor can be achieved. Nowadays, the preservation aspect of smoking is usually not a consideration because conventional preservation is no longer necessary due to the development of different current food storage practices. As a result, smoking is mostly employed to improve the flavor and color of food and attain the characteristic smoked flavor and color of the food (Lingbeck *et al.*, 2014; Sikorski and Sinkiewicz, 2014).

Though, smoking has different advantages, it is also hazardous to our health due to the presence of PAHs. European Commission (2008) has mentioned that, PAH8; benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene are the markers of the carcinogenic potential of PAHs in food, whether alone or in combination. Particularly, Parliament (2003) has mentioned the specific amounts of benzo[a]pyrene (BaP) and benzo[a]anthracene (BaA) that can be present in the main product.

Traditionally, foods were smoked with direct smoking that involves placing the meat (food products) and wood smoke in the same chamber. Direct smoking can be hot and cold. Temperatures can rise to 30°C during cold smoking, and the fire is not kept maintained. Temperatures can rise to 130°C during hot smoking, which involves

maintaining the fire throughout the process (Ledesma *et al.*, 2016). As time passed, the way of smoking has evolved. At present, the meat can be smoked directly using different kind of smoke generators, or liquid smoke can be applied for flavoring (Meier, 2008).

According to Burdock (2016), liquid smoke is a water soluble yellow to red liquid used for flavoring. Liquid smoke can be considered as, any or all of formulations made from its primary products (PP) also known as primary liquid smoke. Liquid smoke primary products, are the interim products formed from condensation of wood smoke during production of liquid smoke (Parliament, 2003; Simon *et al.*, 2005).

1.2 Statement of the problem

Despite the fact that traditional smoking contains antibacterial and antioxidative substances like pyrocatechol that enhance food quality, these food products also include toxic ingredients like polycyclic aromatic hydrocarbons. These compounds are regarded as potential genotoxic and carcinogenic to humans (European Commission, 2008; WHO, 2006). Meier (2008) along with Simon *et al.* (2005) have explained the liquid smoke flavorings can be utilized in meat and food items due to the minimized level of polycyclic aromatic hydrocarbons and other additional benefits. Though European countries have been producing liquid smoke for several decades, there have been scant (almost no) studies on liquid smoke flavorings made from wood species that are available locally in Dharan. This means that liquid smoke flavoring preparations based on local limits cease to exist.

This study intends to produce LSF PP free of tarry fractions, which reduces the occurrence of PAHs by a significant proportion. This work aims to boost commercial liquid smoke production in Dharan with locally available woods like cherry, *Shal, Bhogate.*

1.3 Objectives of the study

1.3.1 General objectives

The general objective of this study is to study the production of liquid smoke from cherry wood and its chemical composition and functional efficacy.

1.3.2 Specific objectives

The Specific objectives of this study are as follows:

1. To design simple assembly for liquid smoke production.
2. To produce liquid smoke flavoring from cherry wood chips.
3. To evaluate chemical and functional constituents of liquid smoke.
4. To check the effectiveness of liquid smoke in meat preservation.
5. To carry out sensory analysis of smoked and smoked cured meat.
6. To study changes in antioxidant and total phenolic count during 10-day storage period.

1.4 Significance of the study

This research has been done to create a tar-free liquid smoke flavoring product that may be used either on its own or after additional processing in food. These flavors of liquid smoke have lower PAH levels. The chances of carcinogenicity and mutagenicity caused by PAHs present in food products; smoked with wood smoke is reduced in liquid smoke (Sannino, 2008). Liquid smoke is simple to use, concentration can be regulated and is amenable to analysis. It shows a marked contribution on product safety by controlling the growth of microbial pathogens (Suñen *et al.*, 2001; Wendorff *et al.*, 1993). Cherry wood which is a hardwood species, produces cleaner, mild, sweet and fruity smoke which is more stable, during pyrolysis. Furthermore, these flavorings are cost-effective since they don't require a smoke generator, don't pollute the environment, are quicker to apply, produce more throughput per unit, and are repeatable and reproducible because the liquid smoke flavoring's concentration is more stable (Meier, 2009). Through this research, locally made liquid smoke flavorings can be introduced in Nepalese market. Additionally, Nepali locals engaged in the smoked meat industry lack access to labs for carcinogen quantification. Therefore, it is essential to have a smoke flavor production assembly that can generate an appropriate smoke. This study is also important since it may serve as the foundation for harnessing the potential of Nepal's native wood species to make commercial liquid smoke flavoring goods. Finally, because liquid smoke can now be created locally, this discovery opens the door for additional research on the topic.

1.5 Limitations of the study

1. Concentration of liquid smoke could not be done that requires membrane filtration.
2. Comprehensive characterization of the prepared liquid smoke could not be done because it requires highly sophisticated methods like GC-MS or HPLC.
3. Only one type of wood species is used for the liquid smoke production.
4. The change in antioxidant and total phenolic count was studied for only 10 days because of time constraints.

Part II

Literature review

2.1 General introduction to liquid smoke

Liquid smoke is a water-based colloidal mixture created by capturing and condensing smoke and steam produced during the pyrolysis of wood (Saloko *et al.*, 2013). Simply, liquid smoke is the aqueous condensate of natural wood smoke (Moeller, 1997). Liquid smoke is a renewable energy source that is the result of combustion or pyrolysis of raw materials containing hemicellulose, cellulose, and lignin that can be used for various purposes; both food and non-food applications. According to European Parliament and Counsil (2008), under regulation (EC) No. 1334/2008, liquid smoke is classified as a smoke flavoring, which is defined as: "*A product obtained by fractionation and purification of a condensed smoke yielding primary smoke condensates, primary tar fractions and/ or derived smoke flavourings."*

According to FAO/WHO (2001), "Liquid smoke flavorings are complex mixtures of components of smoke obtained by subjecting untreated hardwoods to (a) pyrolysis in a limited and controlled amount of air, (b) dry distillation between 200 and 800ºC, or (c) superheated steam between 300 and 500ºC. The source materials must not contain detectable amounts of pesticides, wood preservatives, or other extraneous matter that may result in hazardous constituents in the wood smoke. The major flavoring principles of liquid smoke flavorings are carboxylic acids, compounds with carbonyl groups and phenolic compounds."

Different terms like; wood vinegar, bio-oil, pyrolysis liquid, pyrolysis oil, bio-crude oil, biofuel oil, pyroligneous acid, wood liquid, and wood oil are used in place of liquid smoke. Nowadays, liquid smoke is already widely used in the food industry. They can be applied to meat products by various ways such as dipping, spraying, or aerosol treatments similar to the treatment in traditional smokehouse. Liquid smoke can be used as flavoring because of its specific aroma and taste, also suitable to use as preservative because of its antimicrobial property (Meier, 2009; Milly *et al.*, 2005; Suñen *et al.*, 2001). It has ability

to preserve food as a result of its antimicrobial compounds and antioxidants, such as aldehydes, carboxylic acids, and phenols (Rorvik, 2000).

Not only in food industry, liquid smoke is also used in agriculture. Risfaheri *et al.* (2018) has found that, liquid smoke can be used to improve soil quality and neutralize soil acids, kill plant pests, control plant growth, repel insects, accelerate growth on roots, stems, tubers, leaves, flowers and fruit. Thus, liquid smoke is believed to be able to replace the function of chemical pesticides which are very harmful to the health as well as to environment. Liquid smoke made from palm kernel shells inhibits black pod disease in cacao fruit (Faisal and gani, 2018). Hendra *et al.* (2014) has mentioned that liquid smoke can be used as a latex of rubber latex lump. It is also used often to preserve wood so that it lasts longer to decompose and avoid termites.

Similarly, the primary products (PP) of liquid smoke refer to the aqueous extract made from condensation of smoke generated by pyrolysis of wood. At point no. 7 in Parliament (2003) following regulation has been mentioned.

"This Regulation covers liquid smoke flavorings as defined in Directive 88/388/EEC. The production of these liquid smoke flavorings starts with the condensation of smoke. The condensed smoke is normally separated by physical processes into a water-based primary smoke condensate, a water-insoluble high-density tar phase and a water-insoluble oily phase. The water-insoluble oily phase is a by-product and unsuitable for the production of liquid smoke flavorings. The primary smoke condensates and fractions of the water-insoluble high-density tar phase, the "primary tar fractions", are purified to remove components of smoke which are most harmful to human health. They may then be suitable for use as such in or on foods or for the production of derived liquid smoke flavorings made by further appropriate physical processing such as extraction procedures, distillation, concentration by evaporation, absorption or membrane separation and the addition of food ingredients, other flavorings, food additives or solvents, without prejudice to more specific Community legislation."

2.2 Historical Background

The use of liquid smoke flavors (LSFs) began gaining attention in the early 1970s (Meier, 2009) although, the application of LSFs to meat dates as far back as 1811. The concept of

liquid smoke as a water-based condensate of natural wood smoke was officially developed, described, and patented in the United States in 1930 (Patent No. 1753358) by Wright (Moeller, 1997). In his patent, Wright (1930) stated that the innovation was intended to produce a superior condensate from gases released by hardwood. He had already introduced a product to the market with promising commercial success. Based on this claim, it is reasonable to assume that liquid smoke flavoring may have been used informally even before its formal documentation. Around the same time, other similar products containing ingredients such as common salt also emerged (Wright, 1930). Furthermore, Sedacca (2016) noted that Wright who was a pharmacist at Kansas city, had initially developed a liquid smoke flavoring ingredient as early as 1895. He also mentioned that, Wright was inspired by the memory of "A drop of liquid trickling down the stove-pipe" in the print shop he worked at as a teenager.

Ledford (1981) introduced an automated system designed to tightly regulate raw materials, temperature, and other processing conditions, resulting in a consistently high-quality product. In an earlier development, Dainius *et al.* (1979) created a liquid smoke flavoring free from detectable levels of 3,4-benzopyrene. Their formulation included propylene glycol and propylene glycol-soluble wood tar, which were subsequently removed through co-distillation. Likewise, Underwood and Grameat (1991) developed an aqueous wood smoke solution using rapid pyrolysis combined with precise temperature control. Moeller (1997) described a technique for producing tar-reduced liquid smoke flavoring by treating the initial liquid smoke with activated carbon. This process removes tar content effectively, resulting in a final product that is fully water-soluble.

Simon *et al.* (2005) referencing Meier and Guillen, described a general method for producing water-soluble primary products from smoke condensate, which structurally aligns with the process outlined by Hollenbeck (1963). In terms of usage, liquid smoke flavorings (LSFs) were initially utilized in the United States and Eastern Europe. Currently, the quality standards for LSFs products in Europe are overseen by Parliament (2003). The development of aerosol technology, first introduced by Hickory Specialties in 1969, marked a significant advancement in the production of LSFs (Meier, 2009).

Liquid smokes are derived from the condensation of wood smoke generated by the burning of wood chips or sawdust in an oxygen-restricted environment. Commercially available full-strength liquid smokes are commonly fractionated, purified and concentrated to produce aqueous, oil or dry powder products. In food systems, liquid smokes have been widely employed to add flavor attributes identical to those of smoked food items(Montazeri *et al.*, 2013; Nollet and Toldrá, 2010). The flavor of wood smoke is produced through the regulated pyrolysis of wood's primary constituents: cellulose, hemicellulose, and lignin. There is quick degradation of hemicellulose followed by cellulose and finally lignin, during pyrolysis (Cadwallader, 2007).

2.3 Wood and its components

Wood consists of 40-45% of cellulose, 20-30% of hemicellulose, 20-33% lignin and other components those vary depending on species of wood and especially on hardwood and softwood (Sjostrom and Alen, 1999). The composition of softwood and hardwood has been reported by Doelle and Bajrami (2018), which is shown in table 2.1 below.

Table 2.1 Composition of hardwood and softwood

Composition	Hardwood	Softwood
Cellulose	40-44%	40-44%
Hemicellulose	15-35%	30-32%
Lignin	18-25%	25-32%
Extractives	2%	5%

Source: Doelle and Bajrami (2018)

2.4 Wood smoke, its nature and composition

Cellulose, hemicellulose, and lignin are the main components of wood that pyrolyze under controlled conditions to produce wood smoke. The most easily breaking down material during pyrolysis is hemicellulose, which is followed by cellulose and lignin. Polysaccharides (cellulose and hemicellulose) during pyrolysis give mainly furans, acids,

alcohols, anhydro sugars, easters, and aldehydes and are predominantly responsible for the staining and bactericidal effects of smoke. The most significant family of smoke taste chemicals, known as phenols, are produced when lignin is thermally degraded (Cadwallader, 2007). According to Toth and Potthast (1984) following thermal decompositions occur during the course of pyrolysis:

1. Drying - up to about 170ºC.
2. Pyrolysis of hemicellulose – between 200 and 260ºC
3. Pyrolysis of cellulose – between 260 and 310ºC
4. Pyrolysis of lignin – between 310 and 500 ºC

Smoke is thought to consist of two phases; a dispersing gas phase, also known as the vapor phase and a dispersed liquid phase, also known as the particulate phase that contains smoke particles (Lawrie and Ledward, 2006). Wood smoke has been shown to include over 300 different chemicals spread across these two phases. As a whole, wood smoke is most frequently composed of phenols, organic acids, alcohols, carbonyls, hydrocarbons, and certain gaseous substances like carbon dioxide (CO_2), carbon monoxide (CO), oxygen (O_2), nitrogen (N_2), and nitrous oxide (N_2O)n (Pearson and Gillet, 1996). Chemical composition of wood smoke is shown in table 2.2 and 2.3.

Table 2.2 Major components of wood smoke

S.N.	Class of compounds	Major compounds present
1	Organic acids	The major acid is acetic acid
2	Alcohols	Simplest alcohol is methanol
3	Gaseous Compounds	Carbon dioxide (CO_2), carbon monoxide (CO), oxygen (O_2), nitrogen (N_2), and nitrous oxide (N_2O)
4	Hydrocarbons	Benz (a) anthracene, dibenz (a, h) anthracene, benz (a) pyrene, benzopyrene, benzo (g, h, i) pyrelene, pyrene, and 4-methyl pyrene
5	Phenols	Guaiacol, 4-methylguaiacol, phenol, 4-ethlguaiacol, o-eucresol, m-cresol, p-cresol, 4-propyl guaiacol, eugenol, 4-vinylguaiacol, vanillin, 2,6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol, 2,6 dimethoxy-4-propylphenol, and 2,6 dimethoxy-4-ethylphenol
6	carbonyls	2-pentanone, valeraldehyde, 2-butanone, butanal, acetone, propenal, croto-naldehyde, ethanal, isovaleraldehyde, acrolein, isobutyraldehyde, diacetyl, 3-methyl-2-butanone, pinacolene, 4-methyl-3 pentatone, 5-methly furfural, methyl vinyl ketone, furfural, methacrylaldehyde, methyl glyoxal, etc.

Source: Pearson and Gillet (1996)

Table 2.3 Chemical composition of smoke produced per kg of wood

Chemical	g/kg wood
Carbon monoxide	80-370
Methane	14-25
VOCs (C2-C7)	7-27
Aldehydes	0.6-5.4
Substituted furans	0.15-1.7
Benzene	0.6-4
Alkyl benzenes	1-6
Acetic acid	1.8-2.4
Formic acid	0.06-0.08
Nitrogen oxides	0.16-0.24
Methyl chloride	0.01-0.04
Naphthalene	0.24-1.6
Substituted naphthalene	0.3-2.1
Oxygenated monoaromatics	1-7
Total particle mass	7-30
Particulate organic carbon	2-20
Oxygenated PAHs	0.15-1
Chlorinated dioxins	1×10-5-4×10-5
Normal alkanes (C24-C30)	1×10-3-6×10-3
Sodium	3×10-3-2.8×10-2
Magnesium	2×10-4-3×10-3
Aluminum	1×10-4-2.4×10-2

Silicon	3×10-4-3.1×10-2
Sulfur	1×10-3-2.9×10-2
Chlorine	7×10-4-2.1×10-2
Potassium	3×10-3-8.6×10-2
Calcium	9×10-4-1.8×10-2
Titanium	4×10-5-3×10-3
Vanadium	2×10-5-4×10-3
Chromium	2×10-5-3×10-3
Manganese	7×10-5-4×10-3
Iron	3×10-4-5×10-3
Nickel	1×10-6-1×10-3
Copper	2×10-4-9×10-4
Zinc	7×10-4-8×10-3
Bromine	7×10-5-9×10-4
Lead	1×10-4-3×10-3

Source: Larson and Koenig (1993)

2.5 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are large group of organic compounds with two or more fused aromatic (benzene) rings (WHO, 2000). Low molecular weights PAHs (2 or 3 rings) occur in the atmosphere predominantly in the vapor phase, whereas multi-ring PAHs (5 or more) are largely bound to particles. 4 rings PAHs are partitioned between the vapor and particulate phases depending upon the atmospheric temperature (Srogi, 2007). They are released during incomplete burning of organic matters and are also found in wood smoke. PAHs are primarily produced during the breakdown of lignin, and numerous types have been detected in wood smoke (Pearson and Gillet, 1996). Among them, benzopyrene is commonly used as a marker for PAH presence (Gullen *et al.*, 2000).

PAHs are referred to be carcinogens. Due to its mutagenic qualities, this class of chemicals has been placed to the list of contaminants by the US Environmental Protection Agency and the European Union. A small number of these substances have synergistic effects but are not carcinogenic. In most cases, PHAs are solids at room temperature. These substances have low vapor pressures, high melting and boiling temperatures, low water solubility, and high lipid solubility. Generally speaking, molecules become less soluble in water as their molecular mass increases (Anyakora, 2013). The toxic effects of PAHs depend on the duration and mode of exposure. Short-term effects include skin and eye irritation, nausea, vomiting, and inflammation, while long-term effects are associated with skin, lung, bladder, and gastrointestinal cancers: kidney, and liver damage, and cataracts, as well as genetic mutation, cell damage, and cardiopulmonary related mortality (Agarcia *et al.*, 2014; ATSDR, 1995).

2.6 Functionalities of wood smoke

Utilizing wood smoke is beneficial since its diverse components serve a variety of purposes. In addition to serving as antioxidants, phenols also add flavor and color to smoked goods, have a bacteriostatic effect that aids in preservation, and aid in color development. The distinct flavor of smoked meat is also attributed to phenols. Maga has demonstrated a strong smoke flavor in neutral, carbonyl, and basic fractions as well, despite the fact that phenolics have historically been believed to be the class of chemicals that possess essential flavor. Alcohols similarly act as carriers for other organic compounds found in wood smoke, rather than serving as effective bactericides. Methanol is the simplest example of such an alcohol (Underwood and Shoop, 1998).

Organic acids contribute modestly to preservation, with carbon chains ranging from 1 to 10. The lighter acids are typically found in the vapor phase of smoke. Despite their limited preservative effect, they play a key role in coagulating surface proteins on smoked meat. Likewise, wood smoke contains more than 20 carbonyl compounds, with the short-chain varieties being especially important for developing its aroma, flavor, and color (Pearson and Gillet, 1996).

Wood smoke also contains a significant group of compounds known as hydrocarbons, including benz (a) anthracene, dibenz (a, h) anthracene, benz (a) pyrene, benz (e) pyrene,

benzo (g, h, i) pyrelene, pyrene, and 4-methyl pyrene. Among these, benz (a) pyrene and dibenz (a, h) anthracene are identified as carcinogenic. These hydrocarbons contribute little or nothing to the preservative effects or sensory qualities such as taste and aroma of smoked meats. Since, most of these compounds exist in the particulate phase of smoke, they can be removed from the vapor phase without compromising the smoke's preservative or flavor enhancing properties. The use of liquid smoke flavoring has been shown to reduce or eliminate these harmful substances (Pearson and Gillet, 1996). Benzopyrenes are water-insoluble, which is one of the reasons liquid smoke flavoring formulations are effective in minimizing their presence (Lawrie and Ledward, 2006). The functions of smoke have been shown in table 2.4 below.

Table 2.4 Functions of major chemical compounds in wood smoke

S.N.	Class of chemical compounds	Functions
1	Phenols	Antioxidants, color and flavor, bacteriostatic effects, color development
2	Organic acids	Coagulation of surface proteins of smoked meat and minor preservative actions
3	Alcohols	Carriers of other organic compounds and bactericides (little role)
4	Carbonyls	Aroma, flavor, color
5	Hydrocarbons	No or negligible role in preservative or organoleptic properties.

Source: Pearson and Gillet (1996)

2.7 Smoking with curing smoke

The discovery of fire marked a major turning point in human history, serving a wide range of purposes, from providing energy for cooking to offering protection. One of the earliest methods of food preservation developed was the use of smoke to extend the shelf-life of meat and fish (Pausas and Keeley, 2009; Simon *et al.*, 2005). Some studies have defined smoking as the process of penetration of volatiles resulting from thermal destruction of wood into the surface of meat or fish products. Smoking is a preservation technique that involves exposing meat, fish, and occasionally other foods to curing smoke. It is the a method of treating these products with smoke (Toth and Potthast, 1984).

The fundamental principle of smoking, then, is to either directly or indirectly expose wood smoke to the food item being smoked. However, due to dependence in a number of internal and external parameters for their release and deposition during wood pyrolysis, not all of the chemicals found in wood smoke are found in smoked products (Pearson and Gillet, 1996). The significance of smoking lies primarily in the absorption of the vapor phase by the food's surface and its interstitial moisture, rather than in the direct settling of smoke particles. This vapor phase is rich in compound such as phenols, carbonyls, alcohols, and polycyclic hydrocarbons (Lawrie and Ledward, 2006).

Smoke gives meat and other smoked foods their distinctive color, flavor, and taste. It also offers antimicrobial properties and acts as a preservative. However, there are potential health risks due to the formation of compounds such as polycyclic aromatic hydrocarbons, N- nitroso compounds, and possible heterocyclic aromatic amines, which are known to be potentially carcinogenic (Meier, 2009). Direct smoking of meat has been linked to cancer in humans due to presence of carcinogenic and mutagenic polycyclic aromatic hydrocarbons (PAHs), such as benzo (a) pyrene, found in wood smoke (Meatidi *et al.*, 2016).

Smoking often combined with drying and salting is considered one of the earliest methods used to preserve food. It has even been referred to as humanity's original seasoning (Meier, 2009). Curing and smoking meat are closely connected practices, frequently carried out together i.e. cured meats are often smoked, and smoked meats are typically cured. Additionally, smoking is often intertwined with cooking, as heat has

traditionally been used alongside smoke. However, smoke and heat don’t always go hand in hand. They can be applied simultaneously or independently. As a result, meat can be either hot smoked or cold smoked, though even cold smoking generally involves a slight rise in temperature (Pearson and Gillet, 1996).

2.8 Smokig with liquid smoke

Some manufacturers employ liquid smoke flavoring, which offers a number of benefits over real wood smoke. First of all, it eliminates the need for smoke generator installation, which typically necessitates a significant financial investment. Second, because the liquid smoke flavoring's composition is more consistent, the procedure is easier to repeat. Third, potential carcinogen-related issues can be mitigated by preparing liquid smoke flavoring without the particle phase. Fourth, even in plants situated in heavily populated regions, the application of liquid smoke flavoring is simple and produces minimal air pollution. Fifth, the flavoring of liquid smoke is applied more quickly than in traditional smoking, which increases throughput per unit (Muratore *et al.*, 2007; Pearson and Gillet, 1996; Varlet *et al.*, 2009). There are more drawbacks of directly smoking food. The primary consequence of merely the food's surface coming into contact with the smoke is the loss of flavor control. Therefore, using a well-characterized and regulated smoke flavor-bearing product to add wood smoke flavor to food is highly desirable from the perspective of flavor control (Hollenbeck, 1963). "Liquid smoke flavoring" is one such product that bears flavors.

2.8.1 Properties of liquid smoke flavoring

Chemical composition as well as other physical properties of liquid smoked depends primarily on the wood type and moisture content of wood, the latter influences the pyrolysis temperature and the duration of smoke generation (Cadwallader, 2007). Commercial full-strength liquid smoke is primarily made up of water (11–92%), tar (1–17%), acids (2.8–9.5%), carbonyl compounds (2.6–4.6%), and phenol derivatives (0.2–2.9%). Additionally, a variety of ingredients may be incorporated during its production. adjusting the levels of phenol derivatives, carbonyl compounds, and organic acids allows manufacturers to fine-tune the aroma and color of the product. However, the exact

formulation of commercial liquid smoke is typically kept confidential, so only broad, general information is available regarding its chemical composition (Baltes *et al.*, 1981).

A study conducted by Montazeri *et al.* (2013) on various commercial liquid smoke flavors revealed several key characteristics. Freshly prepared samples were bright yellow, though their color gradually changed over time, likely due to the settling and condensation of inherent compounds. Despite these changes, no visible turbidity or precipitate was observed during two months of storage, and color varied among samples. The pH levels showed a broad range from 1.5 to 7.7, indicating the presence of both acidic and alkaline types depending on production methods. Titratable acidity also varied significantly, ranging from 0.7% to 10%. Regarding phenol content, some refined samples contained no detectable phenols, while others had up to 3.22 ± 0.03 mg/ml. Overall, refined liquid smoke samples tended to have reduced levels of acidity, phenols, and carbonyls, which suggests they are less likely to alter the original flavor and appearance of food products.

The physical and chemical properties outlined below are compiled from multiple sources such as FAO/WHO (2001); Hollenbeck (1963); Moeller (1997); Montazeri *et al.* (2013); Simon *et al.* (2005); Underwood and Shoop (1998); Wright (1930)

State: Liquid

Color: Amber, yellow to red, reddish brown

Solubility: Aqueous extract is water soluble

Total solids: Variable (can also be zero)

Titratable acidity: 0.7 – 4 % (w/v)

Lead content: not more than 2 mg/kg

Benzo (a) pyrene: not more than 2 µg/kg

Carbonyls: 2-25% (as heptaldehyde)

Phenols: 0.1 – 16% (as 2,6-dimethoxyphenol)

2.8.2 Properties of primary products

Despite the extensive research on smoke production, comparatively fewer studies have addressed the chemical characterization of primary smoke condensates. Simon et al. (2005) conducted a detailed analysis of the composition of liquid smoke's primary products, revealing that hardwoods tend to yield higher levels of acidic compounds than softwoods. This difference is primarily due to the greater abundance of pentosans in hardwoods, as opposed to the hexosans predominant in softwoods. The presence of glucuronic acids in hardwoods further contributes to increased acidity, as these compounds decompose into various carboxylic acids during pyrolysis. Moreover, hardwoods exhibit a higher concentration of syringol relative to guaiacol. Simon noted that flavor compounds other than phenols reach their peak concentration at pyrolysis temperatures around 500°C, whereas phenolic compounds peak at approximately 650°C. He also emphasized that maintaining a moisture content between 20–30% (wet basis) is optimal for minimizing particulate matter in smoke, and that production temperatures should ideally remain below 650°C to ensure favorable product characteristics (Simon *et al.*, 2005).

The chemical composition of primary smoke products is strongly influenced by production parameters. Referencing additional literature, Simon et al. (2005) reported substantial variability in the composition of certain components, even under identical production conditions with deviations ranging from 10% to 66%. This finding underscores the inherent variability in primary smoke condensates across batches. Nevertheless, controlling critical factors such as temperature, wood species, and moisture content within defined limits is essential for achieving consistent and desirable chemical profiles (Simon *et al.*, 2005).

2.8.3 Application of liquid smoke

2.8.3.1 Scope of application

Despite of being so many uses of liquid smoke most important are listed below:

1. As liquid smoke flavorings
2. As preservatives

3. As colorants

Source: Varlet *et al.* (2009)

2.8.3.2 Way of application

Liquid smoke can be applied to meat in several ways including dipping, brushing, spraying, injecting or incorporating it into marinades, brines or even the meat itself during processing like in sausage or salami or as aerosols (Schneck, 1981). According to Pearson and Gillet (1996) various methods exist for integrating liquid smoke flavoring into food formulations. Those methods are listed below:

1. Direct addition to meat emulsion
2. Direct dipping of the food item into the solution
3. Spraying of product with liquid smoke flavoring
4. Atomization into a fog and releasing this fog into the smokehouse
5. Vaporization by putting on a hot surface
6. Injecting into the meat

The methods and timing of liquid smoke application are influenced by its chemical properties in cured meats with low pH levels, particularly those below 5.5, the increased reaction rate can lead to the formation of nitrogen dioxide; a toxic, reddish brown gas through the conversion of nitric oxides. To mitigate this risk, highly acidic smoke formulations should be avoided. Therefore, it is generally recommended to apply liquid smoke after curing process is complete. Additionally, specialized low acid smoke flavorings are available that are compatible with curing agents such as nitrite and nitrate, making them suitable for use in pickle or brine solutions. If acidic smoke flavorings are added to brines or pickles with limited buffering capacity, nitrite levels may diminish before the solution is injected into the product (Schneck, 1981; Underwood and Shoop, 1998). Although, liquid smoke flavoring imparts smoky characteristics, it does not eliminate the need for cooking meat products. To achieve desirable smoke coloration, cooking remains a crucial step following the application of liquid smoke. Consequently, such flavoring agents are typically introduced prior to or during the cooking process of meat and other food items.

2.8.4 Principle of use

Being water-based condensate derived from natural smoke, the fundamental concept underlying the use of liquid smoke flavoring is straightforward. Liquid smoke flavoring is based on following principles:

1. Carcinogenic polycyclic aromatic hydrocarbons which are found in smoke are lipophilic in nature. It makes them insoluble in water (Lawrie and Ledward, 2006; Underwood and Shoop, 1998)
2. Traditional smoking offer limited control over the smoking process, whereas liquid smoke flavoring enables consistent, reproducible, and precisely regulated flavor development (Hollenbeck, 1963).

2.8.5 Production of liquid smoke

The production methods for liquid smoke flavoring have undergone substantial transformation over the past 85 years. The earliest technique, involving the creation of an aqueous condensate, was first documented by Wright (1930). Since then, the processes used to produce liquid smoke flavoring have continuously evolved, reflecting advancements in both technology and industrial practices. According to Abdullah *et al.* (2017), schematic diagram for production of liquid smoke is shown in fig. 2.1 below.

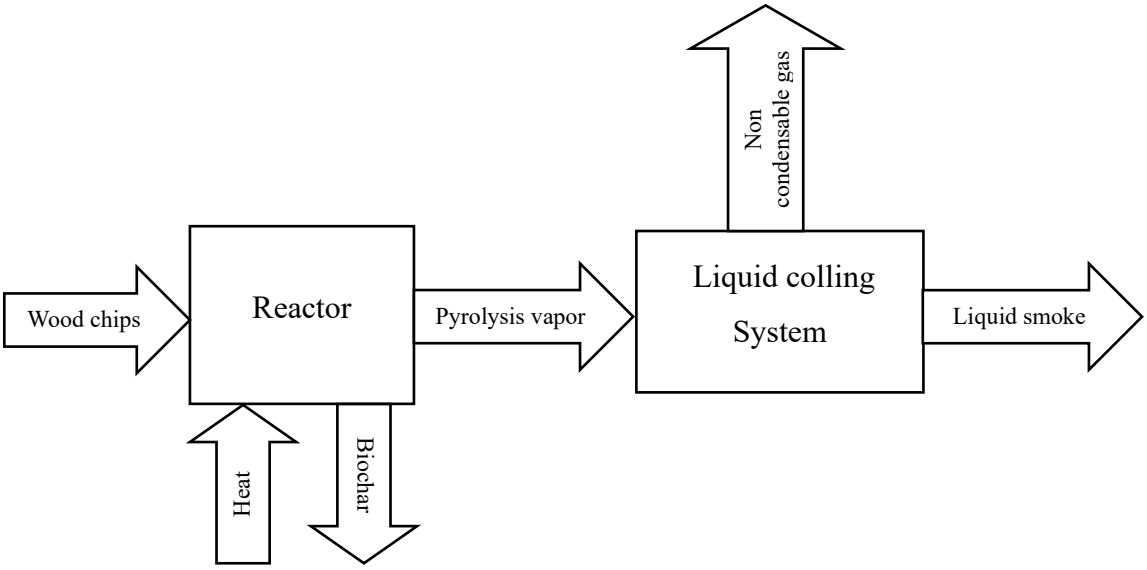

Fig. 2.1 Schematic diagram for liquid smoke production

Above fig. 2.1 shows that the process begins with the preparation of raw materials, followed by their introduction to a reactor where pyrolysis is carried out. In reactor pyrolysis vapors are generated those are subjected to liquid colling system and get condensed. As result liquid smoke as primary product is obtained.

Basic principle for liquid smoke production has been explained by Meier (2009), which is presented as flowchart in fig. 2.2 below.

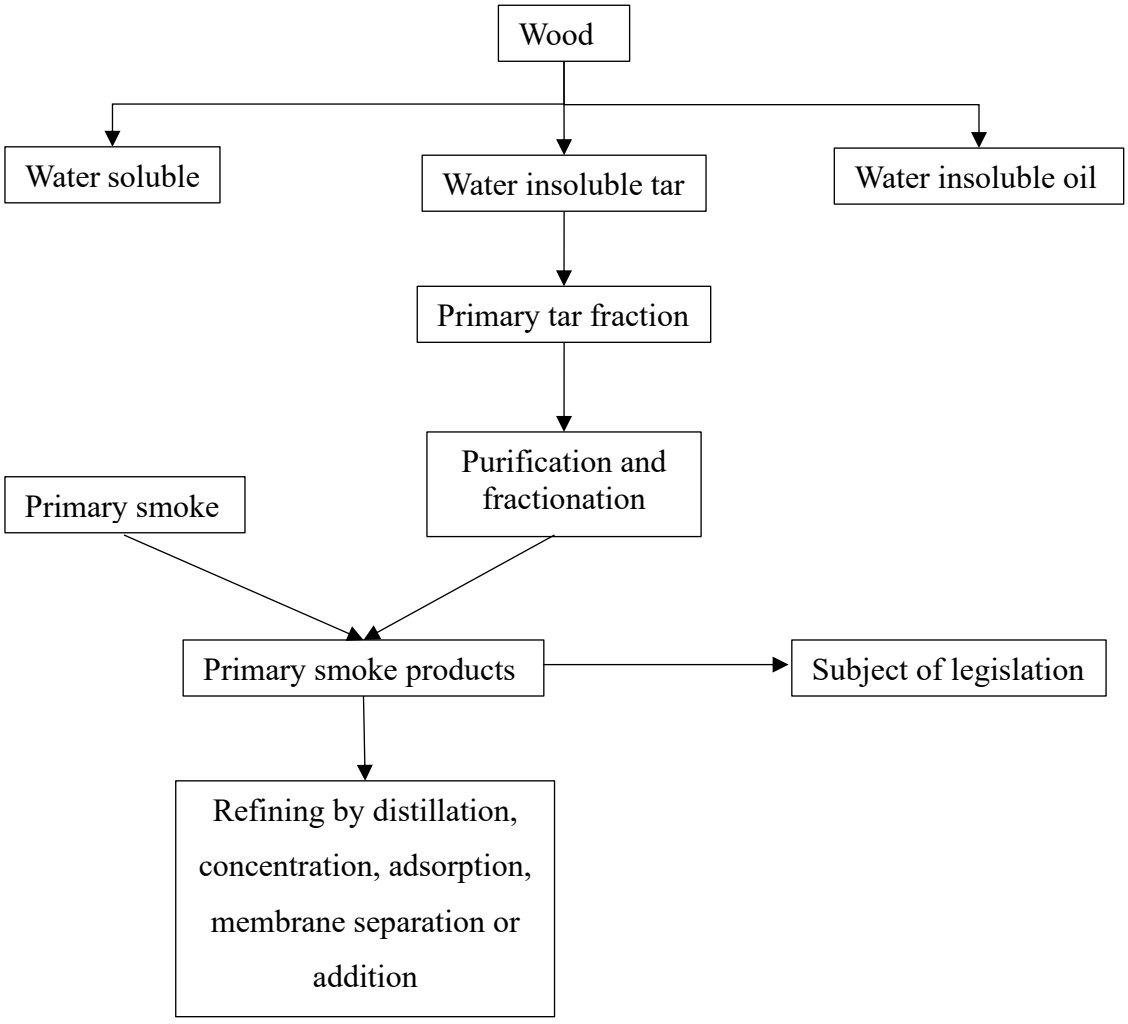

Fig. 2.2 Principle of liquid smoke flavoring production

Wood undergoes thermal decomposition in an oxygen-free environment, producing vapors that are subsequently condensed using either water or vegetable oils. These volatile compounds are continuously extracted from the high-temperature reaction zone and collected using specialized condensation systems. The resulting raw condensates are

categorized based on their solubility in water as shown in fig. 2.2, those that dissolve in water are termed as primary smoke condensates, while the water insoluble, tar like fraction is referred to as the main primary tar fraction, typically purified through solvent extraction. Both fractions are subjected to further refinement processes, including distillation, solvent extraction, evaporation, absorption, or membrane filtration. Additionally, the condensation process yields other water insoluble oily by products, which are generally not utilized in liquid smoke production (Meier, 2009; Simon *et al.*, 2005).

Ali and Fiqri (2020) has also mentioned about the simple design for the production of liquid smoke from coconut shells and rubber seeds as a food preservative. A schematic diagram for liquid smoke production is shown in fig. 2.3 below.

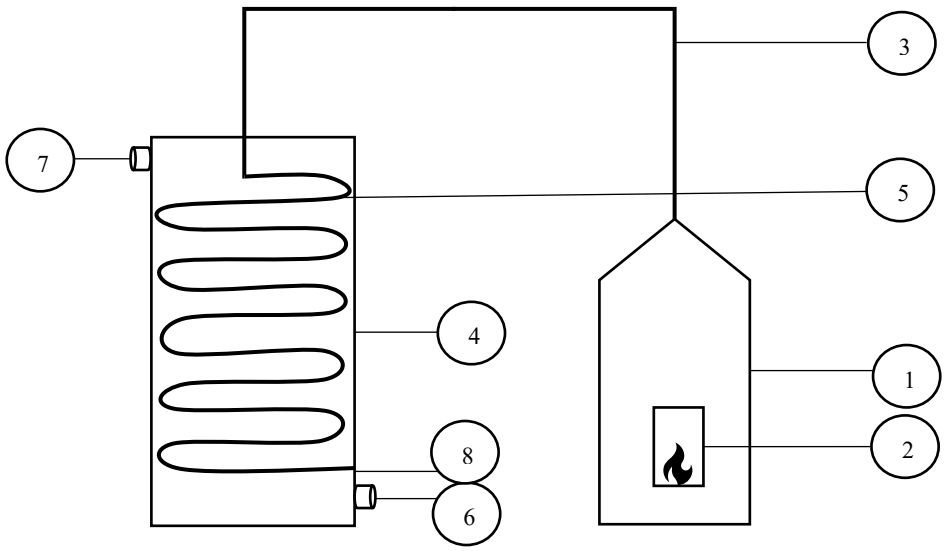

Fig. 2.3 Design for liquid smoke production

Where,

1 = Furnace
2 = Source of heat
3 = Smoke pipe
4 = Condenser
5 = Spiral copper tube
6 = Water in
7 = Water out
8 = Liquid smoke

Hardwood chips or other selected biomass are cleaned and dried to reduce moisture. The organic biomass is placed in a sealed pyrolysis chamber and heated to approximately 400ºC for hours. The organic matters undergo thermal decomposition in absence of oxygen. This high temperature environment breaks down the lignocellulosic components into volatile compounds. The resulting smoke is then directed to a cooling system, typically a water-cooled condenser coil, where water is flowing in counter current direction. It condenses into a liquid form. This raw liquid smoke is subsequently filtered to remove tar and particulates. Purification can be achieved using activated charcoal. The charcoal filtration improves antioxidant properties and reduces harmful components like PHAs present in liquid smoke. The final product is diluted to appropriate concentrations and can be applied to food items such as fish to extend shelf life and enhance flavor (Ali and Fiqri, 2020; Susanto *et al.*, 2024).

According to Wang *et al.* (2024), for the enhancement of smoke particle entrapment, fine water mist or water sprays are used. It helps to entrap smoke components by enhancing the condensation and trapping process. Application of water mist increases the contact of soluble or condensed phases in liquid form.

2.8.6 Controlling PHAs during production

The concentration of polycyclic aromatic hydrocarbons in liquid smoke depends on several key factors related to the production process, raw materials, and post-processing. Those factors include pyrolysis temperature, type of biomass, smoke condensation and collection, purification techniques, storage and packaging, environmental factors like light and oxygen (Simko, 2005).

According to various researchers: Nithin *et al.* (2018); Simko (2005); Simon *et al.* (2005); Underwood and Grameat (1991), following strategies can be applied to control the level of PHAs in liquid smoke.

1. By lowering the pyrolysis temperature : Pyrolysis is a crucial process which involves the thermal decomposition of biomass in the absence of oxygen, resulting in the formation of gas, liquid, and solid products. Temperature around 400ºC

reduces the PAH formation. Pyrolytic temperature between 500 ºC to 900 ºC leads to the formation of PAHs, those have potential health risk.

2. Distillation and adsorption purification : Fractional distillation separates volatile compounds from heavier PAHs and activated carbon traps PAHs effectively.
3. Usage of cleaner biomass : Selection of low-resin woods, avoidance of soft woods and avoidance of contaminated biomass significantly reduce PAHs.
4. Smoke condensation and collection : Rapid cooling and separation of heavy fractions can reduce PAHs levels.
5. Storage and packaging : Light exposure and oxygen can degrade PAHs and also selection of LDPE as packaging material can also reduce their concentration over time.
6. Moisture content : Moisture content of about 20-30 ºC can reduce the PAHs in liquid smoke.
7. Elimination of tarry fraction : Tarry fractions along with other particulate matters are the section which contains most of PAHs. So, tarry fractions have to be strictly avoided during the preparation of primary product.

2.8.7 Advantages of liquid smoke

Liquid smoke is a natural preservative; it acts as a natural preservative, as it contains antimicrobial compounds that inhibit spoilage organism, making it a viable alternative to synthetic preservatives. It also has therapeutic properties as it exhibits antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral activities, anti-diabetic, wound healing, and ulcer healing effects (Surboyo *et al.*, 2024). As compared to traditional smoking, liquid smoke significantly reduces the formation of harmful compounds like PAHs, and tar which reduces the risk of carcinogen (Xin *et al.*, 2022). The application of liquid smoke is easier, efficient and uniform as it shortens processing time, ensures consistent flavor, and allows for reproducibility in food manufacturing. Liquid smoke minimizes environmental pollution compared to conventional smoking methods, making it a more sustainable choice. Application of liquid smoke to food products improve texture and nutritional profile; addition to products like fish balls increases hardness, gel strength, protein, and fat content while reducing water content. It is versatile across different food products like meat, fish, dairy and plant based (Permanasari *et al.*, 2020). Sawdust from

wood industries and other waste biomass like husk, coconut shell can be utilized for the production of liquid smoke (Risfaheri *et al.*, 2018; Surboyo *et al.*, 2024).

2.8.9 Disadvantages of liquid smoke

Anotable limitaion of liquid smoke flavoring lies in its reduced preservative efficacy, as it incorporates only a fraction of the compounds present in traditional smoke. This diminiished concentration results in less antimicrobial and antioxidant activity compared to conventional smoking techniques. Furthermore, during prolonged storage, heavier particulates tend to settle at the bottom of the container, which can lead to a gradual decline in flavor intensity and overall effectiveness. The water-based nature of most liquid smoke formulations also restricts their application to food products that are compatible with aqueous solutions. Nevertheless, oil-based variants have been developed to broaden the scope of use across a more diverse range of food items (Simon *et al.*, 2005). Commercial production of liquid smoke requires industrial facilities and advanced laboratories, making it costly and impractical for small-scale production.

2.8.10 Application on meat

Liquid smoke acts as a natural preservative. Because of its antimicrobial compounds that inhibit spoilage organisms, make it an effective alternative to synthetic additives, also its antioxidant activity makes it suitable for application on meat products (Surboyo *et al.*, 2024). Permanasari *et al.* (2020) has reported that, application of liquid smoke on foods can significantly increase the shelf life and also enhance the flavor and texture of food products. Indiarto *et al.* (2019) has explained that, the total microbial growth in liquid smoked meat ball is lower as compared to the control sample i.e. without the addition of liquid smoke. Siskos *et al.* (2007) and Xin *et al.* (2021) have also mentioned that with liquid smoke meat can be stored for longer period i.e. till microbial load becomes 1.0×10^6 as mentioned by Thatcher *et al.* (1968).

Using liquid smoke in the production of cured meats is a widely adopted technique. Acidic compounds in smoke enhance the nitrite curing reaction, speeding up pink color development and helping form a skin on wieners that aid peeling. Phenolic substances are key to the smoky flavor, through their intensity can sometimes taste medical. They also interact with carbonyls to create resinous compounds that add surface sheen. Carbonyls

lend sharpness to flavor but mainly contribute to the smoky brown color through a browning reaction with meat proteins (Schneck, 1981). Curing generally incorporates nitrites and salts, which contribute antioxidant properties that inhibit oxidative reactions. Although phenolic compounds derived from wood pyrolysis exhibit antioxidant and bacteriostatic effects, they remain vulnerable to degradation overtime. While the integration of curing and smoking enhances the shelf life and sensory attributes of meat products, their influence on preserving phenolic compounds appears to be additive rather than synergistic (Kumar *et al.*, 2004).

Part III

Materials and methods

3.1 Materials

The materials used for this work has been explained in this section.

3.1.1 Materials for construction

Materials that are used for the construction of liquid smoke production assembly are shown in table 3.1 below.

Table 3.1 Construction materials for construction of the assembly

Construction materials	Location	Source
Furnace (stainless steel)	Smoke generator	Hanuman store, hardware, Dharan
Copper tube	Smoke transfer pipe	Hanuman store, hardware, Dharan
Stainless steel	Condenser	Shyam store, hardware, Dharan
Rubber tube	Connected to copper tube's end	CCT lab

3.1.2 Wood chips

Cherry (*Prunus cerasoides*) wood chips having dimension of 5 mm × 4 mm were collected from local area of Dharan, during autumn season.

3.1.3 Lean pork meat

Lean pork meat was brought from the local meat shop at Dharan-16.

3.1.3 Chemicals and apparatus

All the chemicals, laboratory glassware and equipment used for study were analytical grade quality and obtained from Centra Campus of Technology, laboratory.

3.2 Methodology

3.2.1 Working procedure

The working procedure is presented as flowchart in fig. 3.1 below.

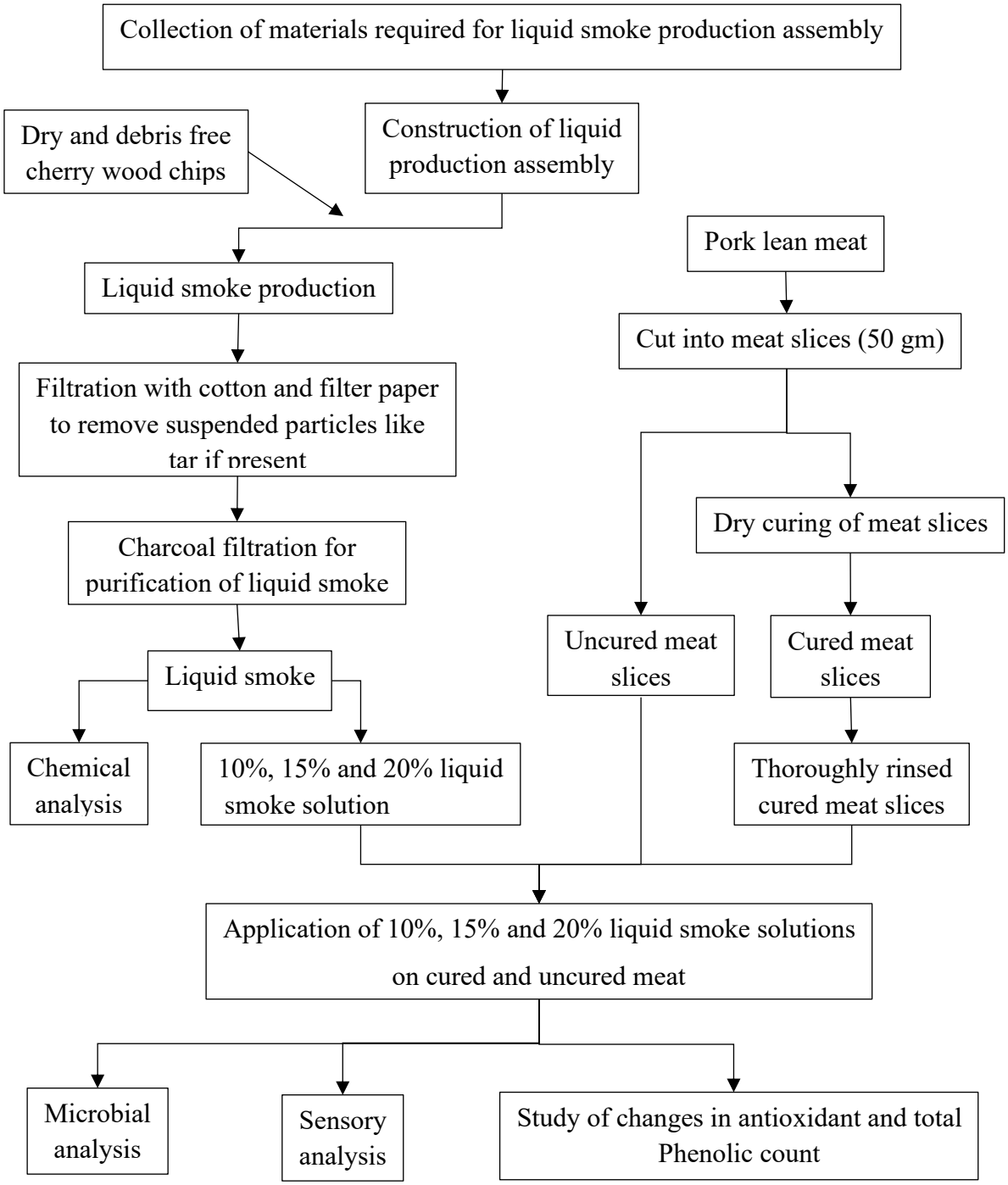

Fig. 3.1 Working procedure

3.2.2 Design of the assembly

The design of the assembly was developed by integrating foundational principles outlined by Meier (2008) and Simon *et al.* (2005), and further structured according to the practical framework proposed by Ali and Fiqri (2020). This design ensured that the design was both theoritically grounded and aligned with proven methodologies relevant to the intended application.

3.2.3 Construction of the assembly

The construction of the assembly was carried out according to the design mentioned in figure 3.2.

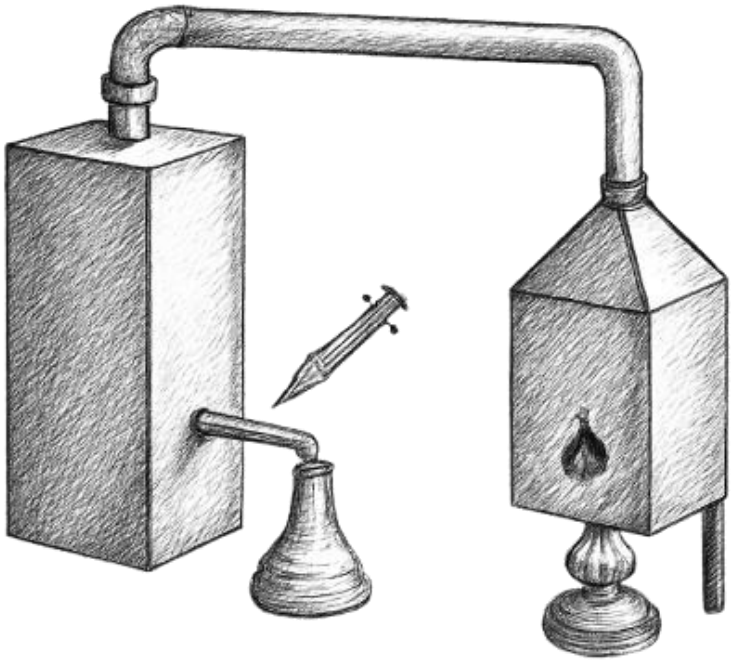

Fig. 3.2 Design assembly for liquid smoke production

3.2.4 Operation of the assembly

The assembly was operated in manual mode; wherein wood chips were introduced into the smoke generation chamber by placing them on a pan and applying direct heat from an open flame. As the biomass underwent thermal decomposition, the resulting smoke was

channeled into condenser through a smoke pipe positioned at a higher elevation than the combustion zone. This elevation facilitated the gravitational settling of particulate matter, thereby improving the purity of the smoke. The smoke then entered the condensation unit, where it passed through a spiral copper coil submerged in ice water maintained below 10ºC. The spiral configuration of the coil was intentionally designed to maximize surface area, thereby enhancing the efficiency of condensation. To further aid in the entrapment of smoke compounds, 50 ml distilled water was injected into the system via syringe. End product was collected in conical flask and again injected into the system using syringe for the purpose of attaining maximum possible concentration of liquid smoke. With referencing Wang *et al.* (2024) it can be concluded that, the water injected after the condensation section helps to entrap smoke components by enhancing the condensation and trapping process. Injecting water into the cooled smoke vapor path increases the contact between water and smoke particles or vapors, aiding further condensation or capture of soluble or condensed phases in liquid form. The condensed liquid smoke was subsequently collected in a liquid smoke collector for further analysis and application.

3.2.5 Testing the assembly

The assembly was tested for its successful operation using sawdust of mix wood that was collected from furniture manufacturers inside the CCT periphery.

3.2.5.1 Leakage test

Smoke leakage of the assembly was tested by visual assessment.

3.2.5.2 Test for smoke transfer

To confirm the transfer of smoke, the assembly was operated while conducting a visual inspection, utilizing the visible nature of smokes as a means of assessment.

3.2.6 Yield of liquid smoke

The yield was then measured in volume of primary liquid smoke produced per gram of sawdust burnt in an interval of one hour, using the formula given below.

$$\text{Yield} = \frac{L}{W \times t}$$

Where,

L = lirers of liquid smoke pruduced in one batch production

W = Weight of sawdust burnt during production of one batch of liquid smoke.

t = liquid smoke production time.

3.2.7 Heating temperature

There was no direct exposure of sawdust to open flames, indicating that the temperature within the smoke generator remained below 400 ºC . To regulate the heat, water was intermittently sprinkled over the heating area. The assembly's design and operation ensured that the sawdust was consistently heated at a temperature lower than its ignition threshold.

3.2.8 Air supply

There was no direct exposure of the smoke generation section to the outer air. However, the design permitted reaching of air inside through the net present below the burner.

3.2.9 Analysis of liquid smoke

3.2.9.1 Color

Color of the produced liquid smoke was determined with visual assessment.

3.2.9.2 pH

pH of the liquid smoke was measured using digital pH meter.

3.2.9.3 Acidity

Total titratable acidity was measured by following the method mentioned by Sadler and Murphy (2010).

3.2.9.4 Total solids

Total solid was measured as explained by Simon *et al.* (2005)

3.2.9.5 Antioxidant

Antioxidant of the liquid smoke was calculated by DPPH assay at 517 nm as described by Budaraga (2019). Liquid smoke samples were prepared at different concentrations ranging from 50 ppm to 250 ppm and reacted with a 50 ppm DPPH solution in methanol, incubated for 30 minutes at 27ºC in the dark and absorbance was measured. Antioxidant activity was calculated by using formula given below.

$$\text{Antioxidant} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{control}} \times 100$$

where,

$A_{control}$= Absorbance of control

A_{sample}=Absorbance of sample

3.2.9.6 Total phenol count

Total phenolic content was determined with FC reagent based on the procedure mentioned by Stankovic (2011). For the analysis, the methanolic solution of the extract in the concentration of 1 mg/ml was prepared. After that 0.5 ml of the methanolic extract, 2.5 ml of the Folin-Ciocalteu reagent (10%) dissolved in water, and 2.5 ml of 7.5% of $NaHCO_3$ were mixed to prepare the reaction mixture. Blank was prepared using 0.5 ml methanol, 2.5 ml of 7% of $NaHCO_3$, and 2.5 ml of the 10% Folin-Ciocalteu reagent. The samples were kept in incubation in thermostat at 45ºC for 45 minutes and the absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was noted. The same method was repeated for the standard solution of gallic acid and the calibration line was constructed. The concentration of phenolics (mg/ml) was obtained from the calibration line and then the content of phenols in samples was expressed in terms of gallic acid equivalent (mg of GAE/gm of extract).

3.2.10 Microbial analysis

Total plate count of raw lean meat, smoked meat and cured smoked meat was determined for microbial analysis. TPC of different samples was carried out using spread plate technique. For this 1 gm of the sample was aseptically collected, crushed, and thoroughly

mixed with 9ml of diluent using a vortex mixer. Serial dilutions were prepared by transferring 1 ml of the homogenized mixture into 9 ml of sterile diluent, achieving a final dilution of 10^{-5}. Form this dilution, 0.1 ml was pipetted onto the surface of nutrient agar and evenly spread using sterile glass spreader. The plates were then incubated at 30ºC for 24 hours, after which colonies were counted to determine the microbial load. TPC was performed for every 5 days, until total count didn't exceed 1.0×10^6 CFU/gm after the value meat is considered unacceptable.

For microbial analysis of liquid smoked sample, 50 ml of 10%, 15% and 20% of liquid smoke was injected to 3 pieces of pork lean meant 50 gm each, having dimension of 3cm×3cm×1cm using syringe. After 2 hours, the sample was air dried at room temperature and stored at refrigerated temperature of approximately 4ºC keeping inside HDPE and required sample for microbial analysis was taken aseptically.

Similarly, for cured smoked meat, three pieces of pork lean meat weighing 50 gm each (dimension 3cm×3cm×1cm) was subjected to dry curing for curing purpose. After completion of curing process, the samples were rinsed thoroughly under cold running water to remove excess surface salt and curing mix which helps to prevent over salty smoke flavoring on the surface. After that 50 ml of 10%, 15% and 20% liquid smoke solution was applied through injection process to each cured sample and left rest for 2 hours. After that the samples were air dried at room temperature and stored at refrigerated temperature approximately of 4ºC keeping inside HDPE for microbial analysis.

Thus, total 7 samples (A, B, C, D, E, F, and G) were prepared for microbial analysis. Table 3.2 shows the samples prepared for the analysis below.

Table 3.2 Samples and their respective title

Sample	Title
A	Control (untread lean pork meat)
B	Uncured lean pork meat treated with 10% liquid smoke solution
C	Uncured lean pork meat treated with 15% liquid smoke solution
D	Uncured lean pork meat treated with 20% liquid smoke solution
E	Cured lean pork meat treated with 10% liquid smoke solution
F	Cured lean pork meat treated with 15% liquid smoke solution
G	Cured lean pork meat treated with 20% liquid smoke solution

3.2.11 Sensory analysis

For sensory analysis 9 points hedonic rating (1= dislike extremely, 9= like extremely) method as mentioned by Ranganna (1986) was used. The semi-trained panelist members consisted of research students and faculties of Central Campus of Technology, Dharan who had some previous experience in sensory analysis.

The prepared 10%, 15% and 20% liquid smoke solutions were applied to pork lean meat and cured pork lean meat with injection method and left overnight at refrigerated temperature of approximately 4ºC. The lean meat used for sensory evaluation was cut into different pieces weighing approximately 50 gm having the dimension of 3cm×3cm×1cm. In the morning those samples were taken out and wiped with blotting paper. Then they were baked in gas oven. After baking, these samples were subjected to descriptive sensory evaluation. Different parameters like color, taste, flavor, texture, overall acceptance were used to evaluate the sensory characteristics of liquid smoke.

3.2.12 Statistical analysis

The data were examined using a two-way ANOVA with no blocking at the level of significance of 5%. The LSD method was to compare the treatment means (Genstat 5 Version 12.1, Lawes Agricultural Trust, Totmeatsted Experimental 35 Station, 2009).

Part IV

Results and discussions

This study aimed to produce a liquid smoke flavor devoid of harmful compounds such as polycyclic aromatic hydrocarbons (PHAs), utilizing a straightforward assembly and locally sourced cherry wood chips. Comprehensive analyses were conducted to evaluate parameters including color, acidity, pH, and antioxidant activity. The results and corresponding discussion are presented in the subsequent section.

4.1 Construction of liquid smoke production assembly

A basic setup for liquid smoke production was developed in accordance with the procedures outlined in the methodology section. The system was thoroughly inspected to ensure it was free from any leaks and functioning properly. There were no leaks except smoke generation section which was negligible.

4.2 Yield

The smoke yield was found to be 41 ml per kg per hour.

4.3 Physical characteristics

4.3.1 Color

The color of produced liquid smoke was reddish yellow. Burdock (2016) and Xin *et al.* (2021) have also mentioned smoke as a yellow to red liquid used for imparting smoke flavor.

4.3.2 Odor

The liquid smoke emitted a distinct smoky aroma. When used on meat, the product has consistently delivered a convincing smoky flavor as mentioned by Montazeri *et al.* (2013).

4.4 Chemical characteristics

Chemical constituents of obtained liquid smoke are tabulated below in table 4.1.

Table 4.1 Chemical constituents of liquid smoke

Parameters	Values
Acidity (%)	0.2±0.01
pH	3.24±0.03
Total solids	Negligible
Antioxidant (%)	46±0.23
Total phenolic count (mg GAE/ml)	49.23±0.52

Values are the means of triplicates ± standard deviations.

Percentage of acidity of the liquid smoke was found to be 0.2±0.01. Xin *et al.* (2021) has also mentioned the same level of acidity in liquid smoke, but is lower as compared to the Montazeri *et al.* (2013) i.e. 0.4 – 7%. The variation in acidity of liquid smoke stems from the diverse types of wood used and the distinct makeup of each. Additionally, differences in processing methods and refining techniques contribute to fluctuations in acidity levels. The lower value of acidity also helpful when there are simultaneous liquid smoke application and curing. Smoke flavors with high acidity can interfere with the curing process of meat by disrupting the function of nitrites. When the pH of meat drops below 5.5, nitrite rapidly converts to nitric oxide. However, this accelerated reaction may lead to the further transformation of nitric oxide into nitrogen dioxide, that is a harmful, reddish-brown gas. To avoid this, smoke flavors with lower acidity are generally preferred in meat curing. In cases where highly acidic liquid smoke is used, it is typically applied after the curing process, allowing sufficient time for nitric oxide to bind effectively with myoglobin before any further chemical changes occur Underwood and Shoop (1998).

The pH value of liquid smoke was 3.24±0.03 as measured. This pH value is in accord with the pH value of commercial smoke liquids (2.3-5.7) mentioned by Montazeri *et al.* (2013) but it is slightly higher as compare to report reported by Xin *et al.* (2021) that is due to the variation in acidic compounds.

Total solids for the sample were found to be negligible. Underwood and Shoop (1998) has reported that, total solids encompass both soluble and insoluble components, the insoluble portion may include tar like substances that may contain polycyclic aromatic hydrocarbons (PAHs). So, in this context, maintaining the minimal total solids is beneficial.

The percentage of antioxidant of produced liquid smoke was found to be 46±0.23, which is higher as compare to antioxidant mentioned by Maulina (2020)and Xin *et al.* (2021). The variation in antioxidant is due to the difference in source of production i.e. biomass, pyrolysis condition, purification process and also the concentration of liquid smoke. The overall chemical composition, including organic acids and volatiles, further contributes to the variation in antioxidant.

Total phenolic count of produced liquid smoke was found to be 49.23±0.52 mg GAE/ml. This value is slightly higher as compared to the total phenolic count obtained by Maulina (2020) i.e. 42.23 mg GAE/ml, and the study done by Xin *et al.* (2021) i.e. 29.1±1.5 mg GAE/ml. The variation in total phenolic count in liquid smoke is due to the type of biomass used, pyrolysis temperature, and refining processes. Raw materials with more lignin yield higher phenolics. Refining and purification steps reduce phenol levels while removing impurities.

4.5 Microbial analysis

The total plate count obtained from microbial analysis of raw meat, meat smoked with 10%, 15% and 20% of liquid smoke and cured meat smoked with 10%, 15% and 20% liquid smoke solution is shown in table 4.2 below.

Table 4.2 TPC of different samples

Days	TPC (CFU/gm)						
	A	B	C	D	E	F	G
0	4.2×10^4 (a)	4.2×10^4 (a)	4.2×10^4 (a)	4.2×10^4 (a)	4.2×10^4 (a)	4.2×10^4 (a)	4.2×10^4 (a)
5	4.5×10^5 (b)	3.9×10^4 (a)	3.8×10^4 (a)	3.7×10^4 (a)	3.7×10^4 (a)	3.6×10^4 (a)	3.5×10^4 (a)
10	1.5×10^6 (c)	6.6×10^4 (a)	5.9×10^4 (a)	5.3×10^4 (a)	5.9×10^4 (a)	5.3×10^4 (ab)	4.8×10^4 (a)
15	-	4.6×10^5 (b)	2.5×10^5 (b)	9.6×10^4 (b)	2.2×10^5 (b)	9.8×10^4 (b)	8.9×10^4 (ab)
20	-	1.7×10^6 (c)	1.3×10^6 (c)	3.7×10^5 (b)	1.5×10^6 (c)	3.6×10^5 (c)	1.2×10^5 (b)
25	-	-	-	1.4×10^6 (c)	-	1.6×10^6 (d)	8.7×10^5 (c)
30	-	-	-	-	-	-	1.6×10^6 (d)

'-' TPC of samples, whose count exceeded threshold value of 1.0×10^6 CFU/gm was not measured and were discarded and small letters inside brackets are used to show if there is any significant difference in the change in TPC of different samples in 5 days, when they were stored at refrigerated temperature.

A is the control (untreated pork meat), B, C, and D are pork meats treated with 10%, 15%, and 20% liquid smoke respectively, and E, F, and G are cured pork meats treated with the same increasing liquid smoke concentrations of 10%, 15%, and 20%, respectively.

TPC count of different samples is shown in fig. 4.1 below in the form of line graph.

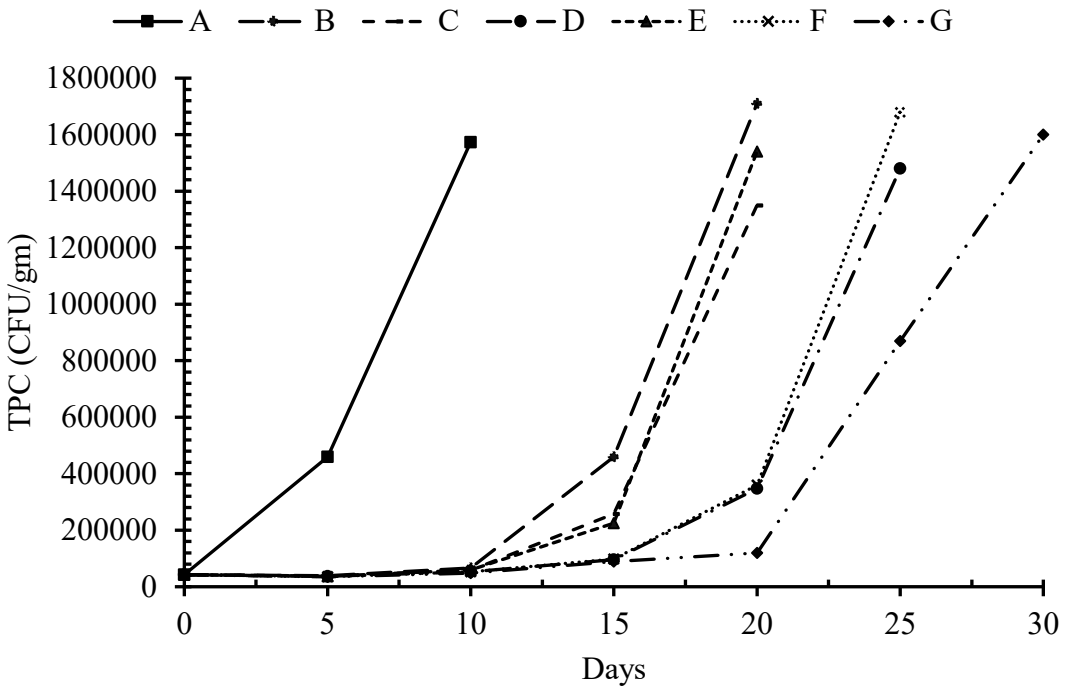

Fig. 4.1 Line graph showing TPC of different samples during storage

In fig. 4.1, the TPC of samples in different 5 days has been shown. Where, A represents raw lean meat, B represents meat smoked with 5% liquid smoke, C represents meat smoked with 10% liquid smoke, D represents meat smoked with 15% liquid smoke, E represents cured meat smoked with 5% liquid smoke, F represents cured meat smoked with 10% liquid smoke and G represents cured meat smoked with 15%.

The initial microbial load of untreated lean meat was found to be 4.24×10^4 CFU/gm, this value is in range with the TPC mentioned by Mangal *et al.* (2018). He has also mentioned that initial microbial load can vary with the condition of pig, sanitary condition of abattoir and hygiene practices followed during analysis. Fig 4.1 shows that the TPC of untreated pork meat increased significantly in following days and became 1.5×10^6 CFU/gm in 10^{th} day.

TPC of pork lean meat and cured pork lean meat treated with 10%, 15%, and 20% liquid smoke decreased during the first 5 days of storage. TPC of cured pork meat treated with 20% liquid smoke decreased to minimum 3.5×10^4 CFU/gm as compared to others. After five days of storage at refrigerated temperature, TPC of all treated samples

increased. After 10 days of storage, TPC of pork meat treated with 10% and 15% liquid and cured pork meat treated with 10% liquid smoke increased significantly and became 1.7×10^6 CFU/gm, 1.3×10^6 CFU/gm and 1.5×10^6 CFU/gm respectively in 20^{th} day.

Likewise, TPC of the pork meat treated with 20% liquid smoke and cured pork meat treated with 15% liquid smoke decreased in first 5 days. TPC of the sample was found to be increased slightly till 10^{th} day. After 10^{th} the TPC growth was found to be significant and ultimately became 1.4×10^6 CFU/gm and 1.6×10^6 CFU/gm respectively in 25^{th} day. In similar way, the TPC of cured meat treated with 20% liquid smoke, increased significantly from 15^{th} day and became 1.6×10^6 CFU/gm in 30^{th} day.

The value of TPC of sample treated with liquid smoke has decreased in first week as compared to the initial count due to the preservative action of liquid smoke and curing (Indiarto *et al.*, 2019; Siskos *et al.*, 2007). By observing above table 4.2 and fig. 4.1, it can be concluded that untreated pork meat can be stored for 10 days only at refrigerated temperature, but the smoked meat and cured smoked meat can be stored for longer period as compared to raw pork meat at similar conditions. which is also mentioned by Siskos *et al.* (2007) in his work. With increase in percentage of liquid smoke, TPC has been lowered so it can be concluded that, with increase in concentration of liquid smoke storage life meat can be increased.

By referencing Thatcher *et al.* (1968), when the microbial load of the samples exceeded 1.0×10^6, the sample was discarded because it is unacceptable.

4.6 Storage period of raw and smoked samples

Storage period based on microbial analysis of raw pork meat, pork meat smoked with 10%, 15% and 20% liquid smoke and cured pork meat smoked with 10%, 15% and 20% liquid smoke is shown in table 4.3 below.

Table 4.3 Storage period of raw and smoked samples

Sample	Storage period (days)
Pork meat (non-treated)	10
Pork meat smoked with 10% liquid smoke	20
Pork meat smoked with 15% liquid smoke	20
Pork meat smoked with 20% liquid smoke	25
Cured pork meat smoked with 10% liquid smoke	20
Cured pork meat smoked with 15% liquid smoke	25
Cured pork meat smoked with 20% liquid smoke	30

Table 4.3 shows how smoking and curing, combined with different concentrations of liquid smoke, can extend the storage period of pork meat. Generally, higher percentages of liquid smoke and curing increased shelf life, allowing storage up to 30 days in the best case here. Whereas, the storage life of non-treated pork meat was only 10 days.

4.7 Sensory Analysis

The prepared liquid smoke was applied to the pork lean meat and cured pork lean meat and it was evaluated by nine semi-trained panelists using a 9-point hedonic scale to assess its sensory attributes including color, flavor, taste, texture, and overall acceptance. The sensory scores obtained from the panelists for different samples were analyzed statistically using Analysis of Variance (ANOVA) without blocking, followed by the Least Significant Difference (LSD) test at a 5% significance level. This approach allowed determination of significant differences in sensory perceptions among the samples based on the panelists' ratings. The mean sensory scores of different parameters are shown in Appendix D. The ANOVA and LSD table for sensory evaluation are presented in Appendix C

4.7.1 Color

The mean sensory scores in term of color, obtained from different panelists are shown in fig. 4.1 in the form of bar diagram. Where sample A is the control (untreated pork meat), B, C, and D are pork meats treated with 10%, 15%, and 20% liquid smoke respectively, and E, F, and G are cured pork meats treated with the same increasing liquid smoke concentrations of 10%, 15%, and 20%, respectively.

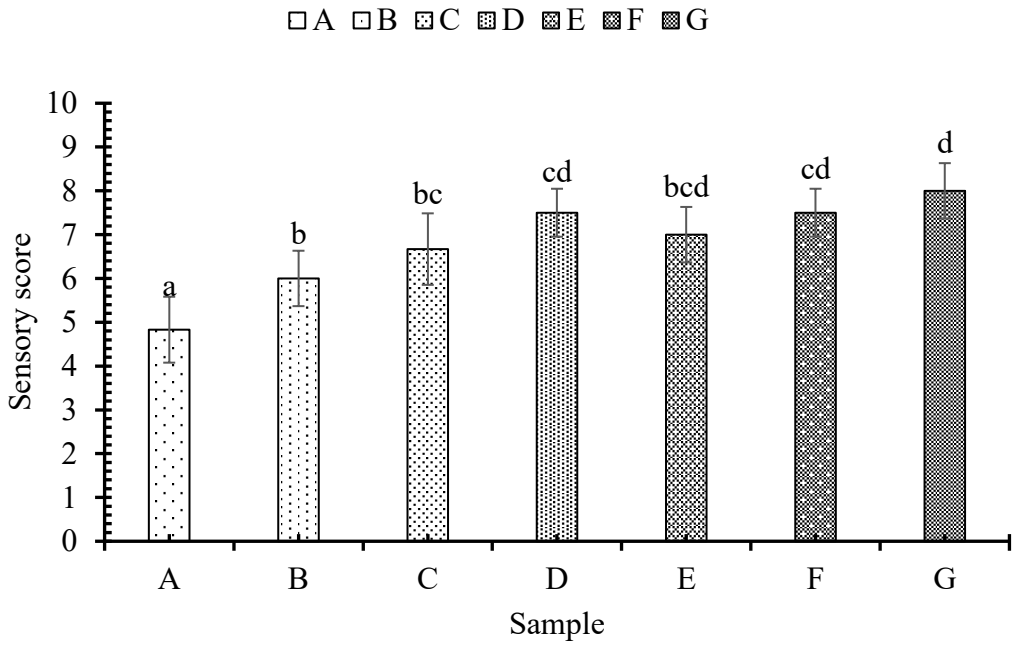

Fig. 4.2 Mean score of samples in terms of color

The same subscripts used on the top of bar diagram in fig. 4.1 indicate that, there is no significant difference between the samples, whereas different subscripts indicate, the samples are significantly different with respect to color at $p<0.05$.

The mean scores for color were found to be 4.83, 6, 6.67, 7.5, 7, 7.5 and 8 for the samples A, B, C, D, E, F and G respectively. Though, the mean score for sample G is the highest, there is no significant difference between sample D, E, F and G statistically in terms of color at $p<0.01$. Fig. 4.1 shows the mean score of sample A was found to be lowest and also there was significant difference between sample A and other samples in terms of color.

Liquid smoke darkens meat by reducing brightness and decreasing red and yellow color intensities, creating a golden-brown color through chemical reactions like the Maillard reaction. Phenolic compounds in liquid smoke contribute to the smoky color, flavor, and antioxidant effects. Curing helps stabilize and retain color, especially in red meats, with the final color influenced by liquid smoke concentration, application method, and curing conditions (Gurtler and Wason, 2024; Indiarto *et al.*, 2019).

4.7.2 Flavor

The mean sensory scores for flavor, collected from different panelists, are presented in Figure 4.3 as a bar diagram. Where, A is the control (untreated pork meat), B, C, and D are pork meats treated with 10%, 15%, and 20% liquid smoke respectively, and E, F, and G are cured pork meats treated with the same increasing liquid smoke concentrations of 10%, 15%, and 20%, respectively.

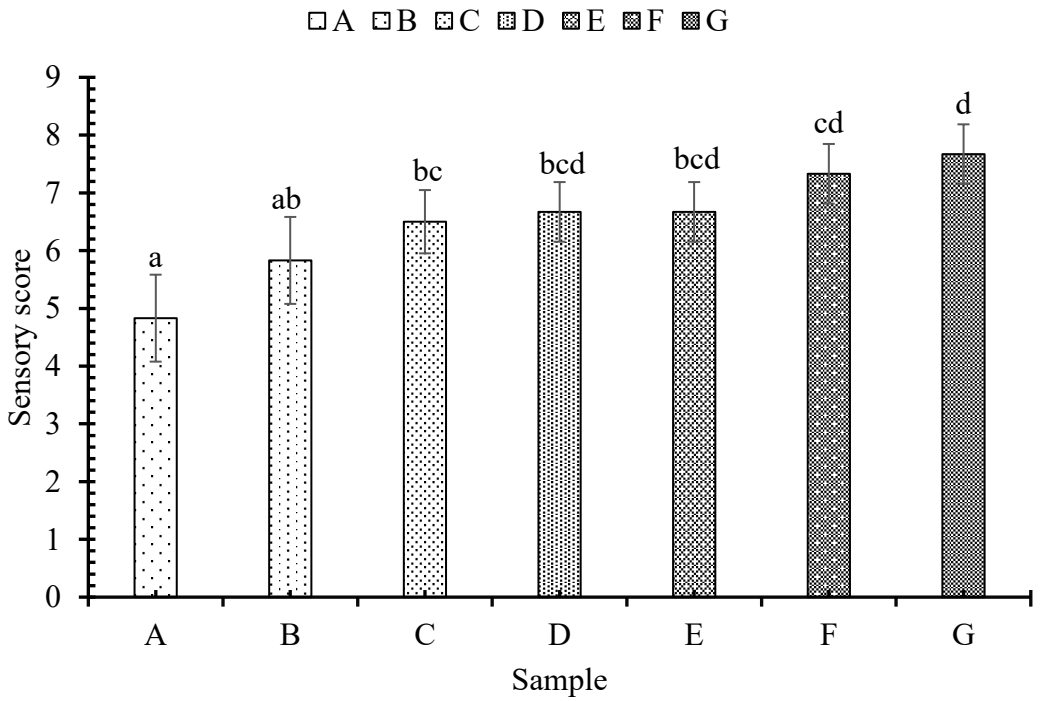

Fig. 4.3 Mean score of samples in terms of flavor

As illustrated in Figure 4.2, identical subscripts placed above the bars indicate no statistically significant difference in flavor among the corresponding samples, while differing subscripts denote significant variation at the $p < 0.05$ level.

The average flavor scores for samples A through G were 4.83, 5.83, 6.5, 6.67, 6.67, 7.33 and 7.67 respectively. Although sample G received the highest mean score, statistical analysis at $p < 0.01$ revealed no significant differences in flavor among samples D, E, F, and G. Conversely, sample A exhibited the lowest mean score and was found to be significantly different from samples C, D, E, F and G.

Liquid smoke enhances meat flavor by adding smoky, barbequed, and meaty notes, contributing to a more complex and appealing taste. Curing works with liquid smoke by developing deeper flavors and improving overall flavor stability in the meat. Together, they create characteristic smoke profiles with improved sensory acceptance (Cadwallader, 2007; Indiarto *et al.*, 2019).

4.7.3 Taste

The mean sensory scores in term of taste, obtained from different panelists are shown in fig. 4.3 in the form of bar diagram. Where A is the control (untreated pork meat), B, C, and D are pork meats treated with 10%, 15%, and 20% liquid smoke respectively, and E, F, and G are cured pork meats treated with the same increasing liquid smoke concentrations of 10%, 15%, and 20%, respectively.

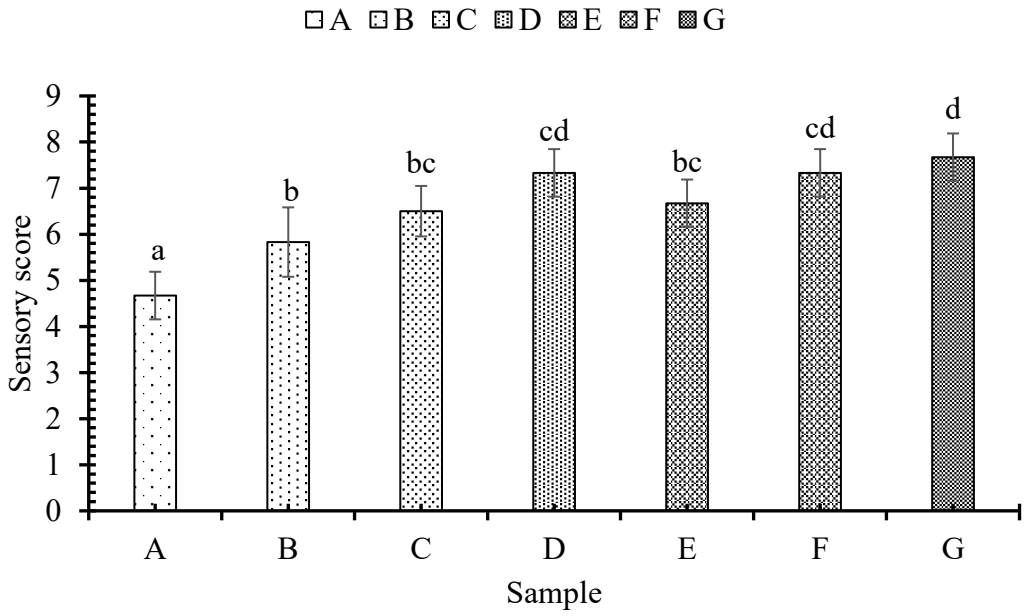

Fig. 4.4 Mean score of samples in terms of taste

As illustrated in Figure 4.3, identical subscripts placed above the bars indicate no statistically significant difference in taste among the corresponding samples, while differing subscripts denote significant variation at the $p < 0.05$ level.

The average taste scores of samples A, B, C, D, E, F and G were found to be 4.67, 5.83, 6.5, 7.33, 6.67, 7.33 and 7.67 respectively. Above fig. 4.3 shows that there is no significant difference between samples C, D, E and F, between B, C and E and between D, F and G. But A is significantly different from other samples at $p<0.01$.

Liquid smoke enhances the taste of pork meat by imparting rich smoky and savory flavors, making the meat more flavorful and appealing. Curing contributes to taste development by intensifying the flavors through salt and seasoning, while helping preserve the meat. Together, liquid smoke and curing create a balanced, improved taste profile.

4.7.4 Texture

Figure 4.4 displays the average texture scores from various panelists using a bar graph. Samples A through G represent different treatments: A is the control (untreated pork meat), B, C, and D are pork meats treated with 10%, 15%, and 20% liquid smoke respectively, and E, F, and G are cured pork meats treated with the same increasing liquid smoke concentrations of 10%, 15%, and 20%, respectively.

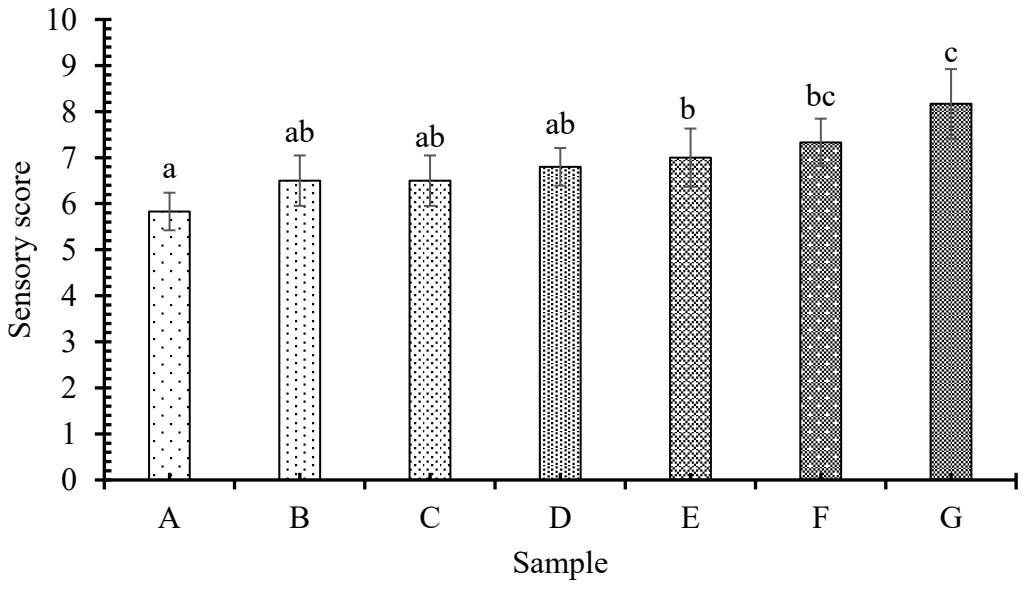

Fig. 4.5 Mean score of samples in terms of texture

As shown in Figure 4.3, identical subscripts placed above the bars indicate no statistically significant difference in texture among the corresponding samples, while differing subscripts denote significant variation at the $p < 0.05$ level.

The average texture scores of samples A, B, C, D, E, F and G were found to be 5.83, 6.5, 6.5, 6.8, 7, 7.33 and 8.17 respectively. From fig. 4.4 it can be concluded that there is no significant difference between samples A, B, C, and D, in between samples B, C, D, E, and F as well as in between F and G at $p<0.05$. Sample G had the largest and sample A had the lowest mean value, also there is significant difference between them at $p<0.05$.

Liquid smoke modifies meat texture by affecting protein interactions, water retention, and phenolic compound binding, resulting in varied effects like increased firmness or softness depending on product type and smoke concentration. Curing synergizes with these effects by preserving texture through microbial and oxidative control during storage (Gurtler and Wason, 2024; Indiarto *et al.*, 2019).

4.7.5 Overall acceptance

Figure 4.5 displays the average overall acceptance scores from various panelists using a bar graph. Samples A through G represent different treatments: A is the control (untreated pork meat), B, C, and D are pork meats treated with 10%, 15%, and 20% liquid smoke respectively, and E, F, and G are cured pork meats treated with the same increasing liquid smoke concentrations of 10%, 15%, and 20%, respectively.

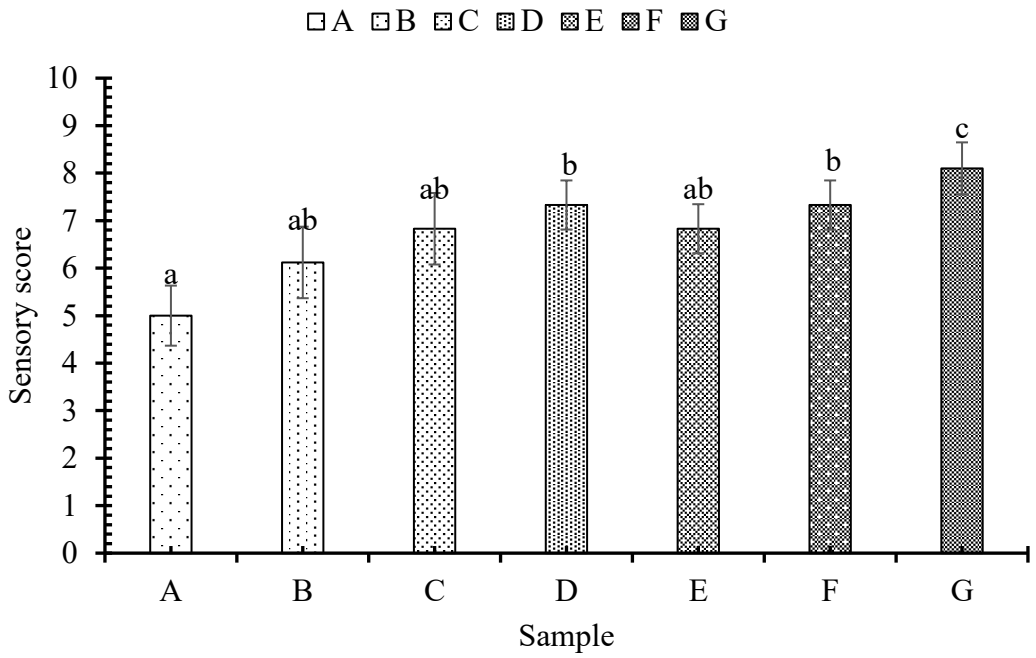

Fig. 4.6 Mean score of samples in terms of overall acceptance

The same subscripts used on the top of bar diagram in fig. 4.1 indicate that, there is no significant difference between the samples, whereas different subscripts indicate, the samples are significantly different with respect to overall acceptance at $p<0.05$.

The mean values for samples A, B, C, D, E, F and G were found to be 5, 6.12, 6.83, 7.33, 6.83, 7.33 and 8.1 respectively. The average overall acceptance value for sample G was found to be highest and also it is significantly different from other samples at $p<0.05$.

The combined used of liquid smoke and curing positively influences the overall acceptance of meat by enhancing flavor, color, texture and shelf-life. Liquid smoke adds desirable smoky and savory notes, while curing intensifies taste and helps preserve the meat, resulting in improved sensory appeal. Based on sensory analysis and microbial analysis the sample G which was cured and smoked with 20% liquid smoke solution, was found to be the best.

4.8 Change in antioxidant activity of liquid smoke applied uncured and cured pork lean meat during 10 days of storage

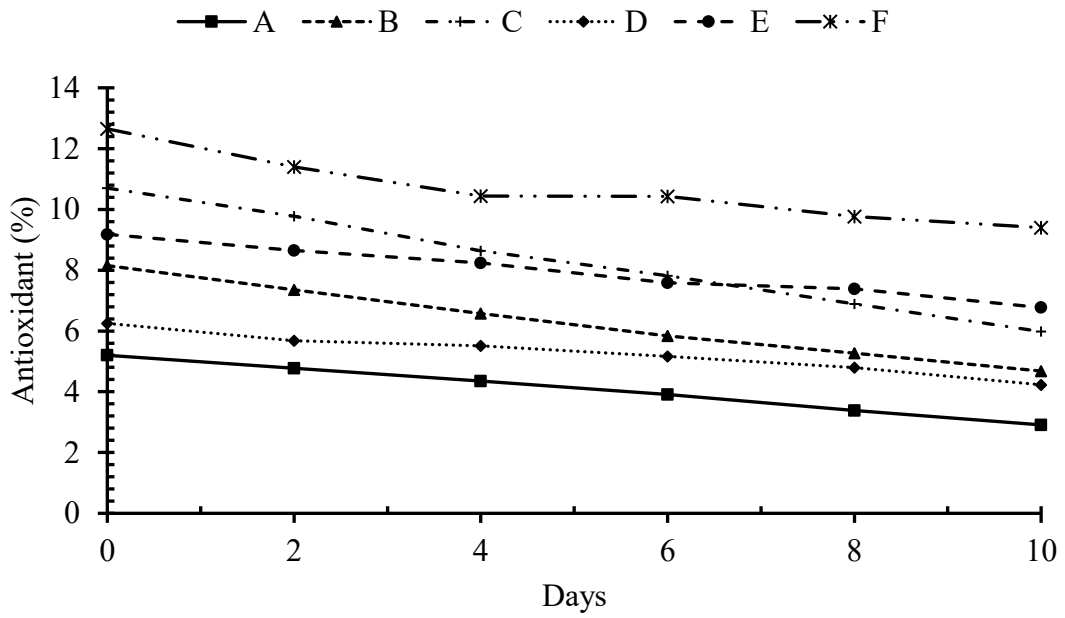

Fig. 4.7 Change in antioxidant activity of liquid smoke applied uncured and cured meat

Fig. 4.6 illustrates the change in antioxidant activity of uncured and cured pork lean meat treated with 10%, 15%, and 20% liquid smoke. Where, sample A, B and C represent pork lean meat treated with 10%, 15%, and 20% liquid smoke solutions and D, E and F represent cured pork lean meat treated with 10%, 15%, and 20% liquid smoke solutions respectively.

The percentage of antioxidant activity of samples A, B, C, D, E, and F was found to be 5.2, 8.12, 10.7, 6.25, 9.18 and 12.65 respectively in day 0, and it seemed to be decreased over storage period of 10 days and became 2.91, 4.68, 5.98, 4.23, 6.77, and 9.4 respectively. By observing above figure, it can be concluded that antioxidant increases with increase in percentage of liquid smoke concentration. Cured meats consistently exhibited higher initial and more sustained antioxidant activity compared to uncured meat.

4.9 Change in total phenolic count in liquid smoke applied uncured and cured meat during 10 days of storage

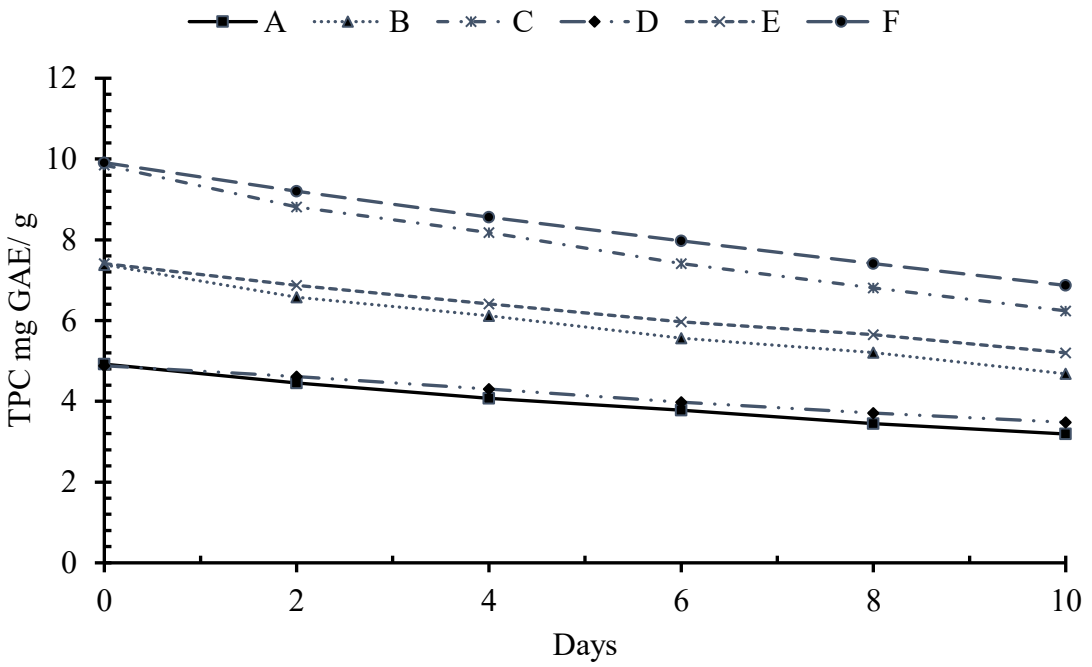

Fig. 4.8 Change in total phenolic count of liquid smoke applied uncured and cured meat

Fig. 4.7 shows the variation in antioxidant activity of both uncured and cured pork lean meat samples treated with smoke at concentration of 10%, 15%, and 20%. Samples A, B, and C correspond to uncured pork lean meat treated with 10%, 15%, and 20% liquid smoke solutions, respectively, while samples D, E, and F represent cured pork lean meat treated with the same respective concentrations of liquid smoke.

Over a 10 days storage period, both uncured and cured meat samples exhibited a decline in total phenolic content from 4.92, 7.38, 9.85, 4.88, 7.41, 9.91 mg GAE/ml to 3.19, 4.68, 6.24, 3.48, 5.2, 6.87 mg GAE/ml for sample A, B, C, D, E, and F respectively. Initially, the phenolic levels were nearly identical in samples treated with the same concentration of liquid smoke. However, unlike antioxidant activity, smoking and curing did not produce a synergistic effect in preserving phenolic compounds. Notably, the rate of phenolic degradation was slower in cured meat compared to uncured meat treated with same concentration of liquid smoke. Curing typically involves nitrites and salts those provide antioxidant protection that slows down oxidation reactions (Kumar *et al.*, 2004). The phenolic compounds from wood pyrolysis, which have antioxidant and bacteriostatic

properties, are still susceptible to degradation over time. The combined effect of curing and smoking improves shelf life and sensory characteristics, though their impact on phenolic retention appears additive rather than synergistic (Kumar *et al.*, 2004).

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of the study done, following conclusions can be drawn.

1. The basic setup which was free from any leakage, for liquid smoke production was developed
2. Liquid smoke was successfully produced and its chemical characteristics were evaluated after purifying through charcoal filtration.
3. Liquid smoke was applied to the meat through the injecting process, and it was found that, using liquid smoke, the storage ability of meat can be enhanced. 20% liquid smoke was found to be the most effective among other concentrations.
4. Smoking with 20% liquid smoke solution after curing gave the best result in terms of storage for the similar concentration of liquid smoke used.
5. The cured sample treated with 20% liquid smoke exhibited the highest antioxidant activity initially. Over a 10-day storage period, antioxidant levels declined across all samples; however, the rate of decline was slower in the cured meat compared to the uncured samples.
6. The total phenolic content in both cured and uncured samples treated with the same concentration of liquid smoke was nearly identical initially. However, over a 10-day storage period, the reduction in phenolic levels was less pronounced in the cured sample compared to the uncured one.

5.2 Recommendations

Based on this study following recommendations have been made.

1. In design, heating with induction heater can be carried out for better temperature control that reduces the PHAs
2. After condensation series of distillation column can be installed for efficient separation of undesirable compounds and absorption tower along with recycling provision can be installed for concentration of liquid smoke.

3. Carcinogenicity or PHAs content of produced liquid smoke can be determined using GC-MS or HPLC.
4. Membrane (osmosis) concentration is considered the most efficient method for the concentration so it can be done for concentration of liquid smoke.
5. Production of liquid smoke powder by spray drying using carriers.

Summary

This thesis explores the production, characterization, and application of liquid smoke derived from cherry wood chips, with a focus on its antimicrobial and antioxidant properties in meat preservation. A simplified assembly design was employed to generate liquid smoke while minimizing the formation of harmful polycyclic aromatic hydrocarbons. The resulting reddish-yellow liquid smoke exhibited an acidity of 0.2% and a yield of 41 ml/kg/h, antioxidant of 46%, total phenolic count of 49.23 mg GAE/ml with negligible total solids.

Liquid smoke solutions at concentrations of 10%, 15%, and 20% were prepared and injected into pork lean meat and cured meat to evaluate their preservative efficacy, sensory characteristics, change in antioxidant and total phenolic count in 10-day storage period. Microbiological assessments revealed that higher concentrations of liquid smoke extended the shelf life of both fresh and cured meat. Pork lean meat treated with 10%, 15%, and 20% was stored for 20, 20 and 25 days, microbial load was found to be 1.7×10^6 CFU/gm, 1.3×10^6 CFU/gm and 1.4×10^6 CFU/gm respectively. Similarly, cured meat treated with of 10%, 15%, and 20% liquid smoke could be stored for 20, 25, and 30 days respectively, the final microbial load for the sample was found to be 1.5×10^6 CFU/gm, 1.6×10^6 CFU/gm, and 1.6×10^6 CFU/gm respectively. Based on sensory analysis, cured meat treated with 20% liquid smoke solution was found the best. The antioxidant of cured meat was higher as compared to the uncured meat for the same concentration of applied liquid smoke but, total phenolic count was almost equal for both type of meats. The rate of decrease in antioxidant and total phenolic count was greater for uncured as compared to cured meat. These findings demonstrated that cherry wood derived liquid smoke is an effective natural preservative, enhancing both the antioxidant profile and microbial stability of meat products. The study supports its potential use in sustainable meat processing and storage practices.

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Appendices

Appendix A

Sensory evaluation card

Sensory evaluation of banana

Name…… Date: …./…/…

Observe the product by testing. Use appropriate scale to show your attitude by checking at the point that best describes feeling of the products. Write any of defects present described below. An honest expression of your personnel feeling will help me.

Sensory attributes	Sample A	Sample B	Sample C
Color			
Flavor			
Taste			
Texture			
Overall Acceptance			

Judge the above characteristics on the 1-9 scale described as follows:

Like extremely-9	Like slightly-6	Dislike moderately-3
Like very much-8	Neither like nor dislike-5	Dislike very much-2
Like moderately-7	Dislike slightly-4	Dislike extremely-1

Any comments:……

…….

Signature

Appendix B

Table B.1 Antioxidant for different samples during 10-day storage period

Sample / Day	Antioxidant (%)					
	A	B	C	D	E	F
0	5.2	8.15	10.7	6.25	9.18	12.65
2	4.77	7.35	9.78	5.68	8.65	11.4
4	4.35	6.57	8.64	5.51	8.24	10.44
6	3.91	5.84	7.82	5.16	7.58	10.43
8	3.38	5.27	6.89	4.79	7.38	9.77
10	2.91	4.68	5.98	4.23	6.77	9.4

Table B.2 Total phenolic count for different samples during 10-day storage period

Sample / Day	Total phenolic count (mg GAE/gm)					
	A	B	C	D	E	F
0	4.92	7.38	9.85	4.88	7.41	9.91
2	4.45	6.58	8.81	4.61	6.87	9.2
4	4.07	6.12	8.18	4.3	6.41	8.56
6	3.78	5.56	7.41	3.98	5.97	7.97
8	3.45	5.21	6.81	3.71	5.65	7.41
10	3.19	4.68	6.24	3.48	5.2	6.87

Appendix C

Table C.1 ANOVA (no interaction) for Color

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	6	41.9048	6.9841	19.64	<0.001
Panelist	5	4.5000	0.9000	2.53	0.050
Residual	30	10.6667	0.3556		
Total	41	57.0714			

Table C.2 ANOVA (no interaction) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	6	32.0000	5.3333	13.02	<0.001
Panelist	5	0.2143	0.0429	0.10	0.990
Residual	30	12.2857	0.4.95		
Total	41	44.5000			

Table C.3 ANOVA (no interaction) for overall acceptance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	6	27.9048	4.6508	9.90	<0.001
Panelist	5	0.5714	0.1143	20.24	0.940
Residual	30	14.0952	0.4698		
Total	41	42.5714			

Table C.4 ANOVA (no interaction) for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	6	39.2857	6.5476	24.12	<0.001
Panelist	5	2.8571	0.5714	2.11	0.092
Residual	30	8.1429	0.2714		
Total	41	50.2857			

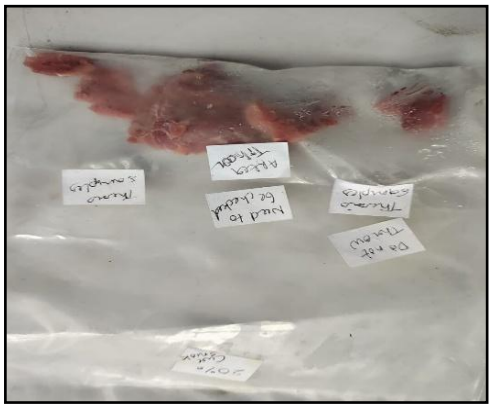
Table C.5 ANOVA (no interaction) for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	6	19.5714	3.2619	9.93	<0.001
Panelist	5	0.9762	0.1952	0.59	0.704
Residual	30	9.8571	0.3286		
Total	41	30.4048			

Color Plates

P1 : Liquid smoke production

P2 : Liquid smoke analysis



P3 : Storage of smoked sample inside HDPE

P4 : Plating

P5 : Colony count