

**LIQUID SMOKE: PRODUCTION ASSEMBLY DESIGN
MODIFICATION, PREPARATION AND EVALUATION**

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**Liquid Smoke: Production Assembly Design Modification, Preparation
and Evaluation**

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Approval Letter

This *dissertation* entitled *Liquid Smoke: Production Assembly Design Modification, Production and Evaluation* presented by Sajal Bhattarai has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

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Research starts with a gap in knowledge and ends with fulfillment of that gap. But what makes it interesting are not just the answers we arrive at during the quest for knowledge, but also the journey that lies within. There have been certain marvelous people in that journey I have come across. Without acknowledging these people, I believe my dissertation work can never be a success. So, I have tried to thank all these people in order as mentioned hereafter.

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Abstract

Liquid smoke from *Shorea*, *Pinus*, *Prunus* and *Citrus* were prepared and their physicochemical properties were studied. They were then applied to pork ham by dipping. Preliminary sensory was carried which gave the range of optimum dipping volume. RSM was then used to generate optimized dipping volumes based on descriptive sensory evaluation carried out on two parameters, aroma and taste. Then, vinegar (5% v/v) was added to the optimized product in concentrations of 5% (v/v) of primary liquid smoke to prepare a formulated liquid smoke. Formulated flavors were then compared with each other as well as the market sample based on descriptive sensory evaluation to obtain best results.

So produced primary liquid smoke had maximum possible acidity of 0.2% (w/v) with optimum production time of 10 h. Primary liquid smokes prepared from *Shorea*, *Pinus*, *Prunus* and *Citrus* under optimized time had no significant differences among their physicochemical properties. The optimized dipping volumes (ml/g) of these liquid smokes were found to be 89.75%, 111.69%, 130.25% and 130.25% respectively. Comparison of market liquid smoke with formulated liquid smoke resulted that lab prepared liquid smoke was superior to market sample in terms of aroma and taste. Liquid smoke from *Shorea* received highest sensory scores. The cost incurred for production of primary liquid smoke was Rs. 50.36 per liter. It was concluded that primary liquid smoke from *Shorea* can be prepared in pilot plant scale with acidity of 0.2% (w/v) and optimum production time of 10 h incurring a cost of Rs. 50.36 per liter.

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List of Abbreviations

| Abbreviations | Full forms |
|----------------------|---------------------------------|
| ANOVA | Analysis of variance |
| LSD | Least significant difference |
| LSF | Liquid smoke flavoring |
| PP | Primary product of liquid smoke |
| VOC | Volatile organic compound |
| w/v | Weight by volume |
| wb | Wet basis |

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PART I

Introduction

1.1 General introduction

Fire was a revolutionary finding. It served as a multipurpose tool from being used as a source of energy for cooking food, to as a tool for security (Pausas and Keeley, 2009). But, where there is fire, there is bound to be smoke. So, smoke can be considered as an inseparable accessory when we talk about cooking food over fire. Therefore, practices of smoking food can undoubtedly be assumed to have dated back to the times when our ancestors used to gather food and cook them over fire.

Over the course of time, way of smoking has evolved. Referring to various sources, Meier (2009) has mentioned that at present, we can use various smoke generators to smoke the meat directly, or we could also apply liquid smoke flavorings to them.

According to Moeller (1997), liquid smoke is the aqueous condensate of natural wood smoke. Liquid smoke has been explained as a water-soluble yellow to red liquid used for flavoring by Burdock (2010). It has also been defined as a type of artificial flavoring used to give food a smoky taste (Philip, 2005). To be more specific, 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). These have been subjected to regulations (Parliament, 2013) and under acceptance by law, can be used to create derived formulations as well (Meier, 2009). Parliament (2003) allows the use of PP as such in foods or after processing into derived flavorings if these solutions are prepared without any prejudice to legislations.

These solutions are not only used to impart flavor, color and texture, but in certain cases are also used to provide enhanced shelf life for food products (Ramakrishnan and Moeller, 2002). Other advantages include ease of application, speed, uniformity of the product, reproducibility of physical and chemical properties, and cleanliness of application (Meier, 2009). Other benefits include reduction in certain carcinogenic components such as benzo (a) pyrene, etc. which are relatively insoluble in water. (Soares *et al.*, 2016) has studied

antimicrobial effects of liquid smoke flavoring and thus liquid smoke flavoring can be said to also have antimicrobial effects. They can be applied to meat products by various ways such as dipping, spraying, or aerosol treatments like the treatment in traditional smokehouses (Meier, 2009). According to Meier (2009), liquid smoke flavor has potential to replace traditional smoking and can also be added to foodstuffs such as soups, sauces, savories, cheese, and spices.

1.2 Statement of the problems

Direct smoking of meat is associated with cancer in humans owing to carcinogenic and mutagenic polycyclic aromatic hydrocarbons (PAHs) such as benzo (a) pyrene present in the produced wood smoke (Tamakawa, 2008; Narayan, 2014; Parada et al., 2017). Referring to Pzczola, Meier (2009) has mentioned that liquid smoke flavorings have shown to have reduced levels of polycyclic aromatic hydrocarbons; and owing to other benefits as well, therefore, are used as liquid smoke flavorings for meat and food products.

Regarding its use in Dharan, no documented production of liquid smoke flavoring was found. A preliminary survey was carried out in 20 places (shops) in Dharan where smoke meat products are sold, to know whether they had any knowledge on the use of smoke flavoring. The questions and the results of the survey has been put in in Appendix C and D respectively. It was found that there is minimum knowledge in people regarding the use of liquid smoke in meat and other food items. This could certainly mean that there is high risk of consumers of smoked meat to get exposed to the carcinogens present in the wood smoke. Moreover, lack of production of liquid smoke flavoring in Dharan may be one of the reasons why there is minimum knowledge on liquid smoke flavoring and its use. It is also to be noted here that local men involved in smoked meat businesses have minimal (in fact almost no access) access to laboratories that have smoke carcinogen testing.

This research has, therefore aspired to reintroduce a method of production of a tar free liquid smoke primary product based on what has been explained by (Parliament, 2003; Simon *et al.*, 2005). Liquid smoke flavorings made by Hollenbeck (2013) has also been referred to, which over the course of time seems to have been generalized as primary products (PP) of smoke condensation (Parliament, 2003; Simon *et al.*, 2005; Meier 2009). Such primary product can be used as it is, or further refined and modified according to

commercial requirements and be sold as liquid smoke flavorings (Parliament, 2013). Moreover, none of researches have been done in liquid smoke flavorings prepared from wood varieties within the local reach in Dharan. This means liquid smoke flavoring preparations based on local constraints seize to exist. This project aims to prepare LSF PP free of tarry fractions that (theoretically) reduces the chances of presence of PAHs by large amounts. This work, therefore, will help to promote commercial liquid smoke production in Dharan. It will also help us gain knowledge about preference of locally available woods viz. *Shorea robusta* (local name = *sakhuwa*), *Pinus roxburghii* (local name = *sallo*), *Prunus* (local name = *aarubakhada*) and *Citrus maxima* (local name = *bhogate*) in production of liquid smoke.

1.3 Objectives of the research

1.3.1 General objective

The general objective of this research is, liquid smoke production assembly design modification, preparation and evaluation of liquid smoke produced from different wood varieties.

1.3.2 Specific objectives

The specific objectives of this research are as follows:

1. To modify liquid smoke production assembly design by Hollenbeck (1963).
2. To produce primary liquid smoke using locally available wood varieties.
3. To evaluate aroma and taste of pork ham using prepared liquid smoke.
4. To compare sensory and physiochemical properties of laboratory prepared and commercially available liquid smoke.

1.4 Significance of the study

This research has been done with an aim to produce liquid smoke flavoring (tar-free) primary product which can be used as such, or after further processing in foods. Such liquid smoke flavorings have reduced levels of PAHs (Underwood and Shoop, 1998; Pearson and Gillet, 1996). So, use of liquid smoke flavoring primarily reduces the chances of carcinogenicity

and mutagenicity caused due to PAHs (Tamakawa, 2008) present in wood smoke. To add, such flavorings are economic as they do not require a smoke generator; they do not create environment pollution, they are faster to apply, result in more through-put per unit and are also repeatable and reproducible as the concentration of the liquid smoke flavoring is more constant (Meier, 2009). So, through this research, locally made liquid smoke flavorings can be introduced in Nepalese market. Moreover, local people of Nepal involved in business related to smoked meat have no access to laboratories for quantization of carcinogens. So, a smoke flavor production assembly that can produce suitable smoke flavor is necessary. Another significance of this research is that it could form the basis for exploitation of the potential of local wood varieties of Nepal, in preparing commercial liquid smoke flavoring products. Last but not the least, this work finally paves way for further researches regarding liquid smoke as well, since now it can be locally produced.

1.5 Limitations and delimitations

The major limitations and delimitations in this research are:

1. For analysis of a full profile chemical characterization of the prepared liquid smoke flavoring, process of either gas chromatography-mass spectroscopy (GC-MS), or High-Performance Liquid Chromatography (HPLC) has to be applied which seems rather inaccessible inside the campus premises. Moreover, the chemicals required for analysis of flavor compounds are considered toxic and are not permitted to be imported by the government. So, these analyses could not be done.
2. Another limitation is that concentration of the prepared smoke flavoring could not be done as it requires membrane filtration.
3. Temperature of pyrolysis could not be measured.
4. A delimitation of this work is that the locally available wood varieties taken for the liquid smoke flavoring preparation were few owing to time and resource limitation.
5. Another delimitation is that the production apparatus was constructed for a pilot plant. Its industrial feasibility has not been studied.
6. Likewise, only aroma and taste were evaluated for sensory evaluation.

PART II

Literature review

2.1 Introduction to liquid smoke and its primary products (PP)

Liquid smoke is not completely liquefied smoke under pressure, as the name suggests. It has instead been explained as a water-soluble yellow to red liquid used for flavoring by Burdock (2010). This suggests that the liquid smoke is water soluble. There are literatures which have mentioned oil-based liquid smokes as well (Underwood and Shoop, 1998; Meier, 2009). It is also defined as a type of artificial flavoring used to give food a smoky taste (Philip, 2005). These solutions are obtained from pyrolysis of wood smoke and are not only used to impart flavor, color and texture, but in certain cases are also used to provide enhanced shelf life for food products (Ramakrishnan and Moeller, 2002). They can be applied to meat products by various ways such as dipping, spraying, or aerosol treatments similar to the treatment in traditional smokehouses (Meier, 2009). It can, therefore, be concluded that liquid smoke extracts may or may not be further processed into forming derived smoke solutions.

The terms “liquid smoke flavoring” and “liquid smoke” have been used synonymously by various scientists such as Wright (1930), Hollenbeck (1963), Moeller (1997), Meier (2009). Though these scientists have not used the terms interchangeably in the same documents, it can be easily inferred that “liquid smoke” is a general term for the products and “liquid smoke flavoring” is a functional term for the product owing to the product being used as a flavoring substance.

There are other terms such as “wood vinegar” (ACG, 2019) or “pyroligeneous acid” (Mathew and Zakaria, 2015) that have been used in an unclear but nearly synonymous manner to liquid smoke or smoke flavoring. But these products have wider use such as application in wounds to heal them, etc. Thus, considering the flavoring property of liquid smoke we are concerned in this research, those terms have not been considered synonymous to the term “liquid smoke flavoring”

Similarly, the primary products (PP) of liquid smoke refer to the aqueous extract made from condensation of smoke generated by pyrolysis of wood. Simon *et al.* (2005) mentions in detail being based on Parliament (2003) guidelines, that primary products are interim

products obtained on the way to production of liquid smoke. Parliament (2003) mentions following lines in point no 7 as definitions of primary products.

“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.” (Parliament, 2003)

It is to be strictly noted that the directions have not given idea on mixing of the tar phase and primary smoke condensates. It has instead been mentioned that, these products should be purified to remove harmful components and may be used as such on foods. Production of derived flavorings remains beyond the scope of this paper.

Parliament (2003) also mentions in point no 8 that these primary smoke condensates and fractionated tar fraction are known by the name of “primary products” and derived products are those that are made from these PP (s). These primary products are subjected to regulations by Parliament (2013), where the producer must clearly explain the way, equipment and materials used to produce these solutions along with the analyses performed to check the levels of unwanted hazards such as benzo (a) pyrene in them. If the levels of benzopyrene exceed 10 µg/kg, these solutions are ineligible to be further used as they are or processed in production of commercial liquid smoke flavorings. This suggests that one may or may not use these primary products as liquid smokes based on the level of benzo (a) pyrene present in them.

Thus, liquid smoke can be considered as either of primary products or the derived products that can be used in and on food items with an intention of providing smoky flavor to foods. FAO (2001) gives following definition of liquid smoke flavorings.

“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 800°C, or (c) superheated steam between 300 and 500°C. 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.”

Thus, concluding from all these, a broader definition can be derived.

Liquid smokes are therefore products of operations in following order (a) pyrolysis in limited and controlled amount of air (b) dry distillation between 200 to 800°C or superheated steam between 300 and 500°C; to give primary products (c) which may be used as such, or after processing to give derived products without prejudice to required specific community standards.

2.2 Primary product as a liquid smoke

Although the primary products are known to be interim products; and are generally used to produce derived flavorings; no restrictions have been found in literatures regarding their use as liquid smoke.

2.3 History and use of liquid smoke

According to Meier (2009), history of use of liquid smoke flavors (LSFs) started in early 1970s, although early treatments of meat with LSFs can be dated back to 1811. Liquid smoke flavoring, however, as the aqueous condensate of natural wood smoke, was developed, described and documented in 1930 in U.S. Patent no. 1753358 issued to Wright (Moeller, 1997). In the patent, Wright (1930) has mentioned that the development was actually an invention to produce an improved condensate of the gases driven off from hardwood, which he had previously placed in the markets and had found a good potential in trade. So, referring

to his statement, the undocumented use of smoke flavor can be assumed to have been practiced even before the documentation of the development. Other products like the condensate also appeared around the development which comprised of common salt in it (Wright, 1930). According to Sedacca (2016), Wright created liquid smoke flavoring ingredient for the first time in 1895.

Since then, various modifications and improvements have been brought in production of liquid smoke flavoring. Moeller (1997) mentions the improved liquid smoke flavoring compositions and techniques for manufacture of the same to have been described in U.S. Pat. No. 3106473 issued to *Hollenbeck*, U.S. Pat. No. 3873741 issued to *Melcer et al.*, U.S. Pat. No. 4298435 issued to *Ledford*, U.S. Pat. No. 4154866 issued to *Dainius et al.*, and U.S. Pat. No. 4,994,297 issued to *Underwood*.

Hollenbeck (1963) discovered that aqueous wood smoke flavored solution of superior flavor and reproducible properties free of undesirable materials could be produced by repetitive or single countercurrent extraction of wood smoke with cool to warm water and advisably until the titrable acidity of the acidic and phenolic substances in the liquid smoke extract is at least 3% by weight of solution. Melcer *et al.* (1975) described the controlled carbonization combustion wood in the presence of regulated critical concentration of air.

Ledford (1981) introduced the automated process that controlled the raw materials, temperatures and other process variables within narrow limits to yield a uniformly high-quality product. Dainius *et al.* (1979) produced liquid smoke flavoring that contained no detectable amount of 3,4-benzopyrene; and consisted of propylene glycol together with propylene glycol-soluble wood tar which were later co-distilled out. Similarly, Underwood and Graham (1991) used the method of fast pyrolysis and temperature control to produce aqueous wood smoke solution. Moeller (1997) has described a method to produce tar-depleted liquid smoke flavoring comprising the step of contacting a starting material liquid smoke flavoring, with an activated carbon with enough active sites such that the tar-depleted liquid smoke flavoring is totally water-miscible.

Simon *et al.* (2005), referring to Meier and Guillen, has explained a general method for production of water-soluble primary products of smoke condensate. This method schematically resembles the process given by Hollenbeck (1963).

Regarding the application of LSFs, they were first applied in the United States and Eastern Europe. Today, Parliament (2013) regulates the quality of the liquid smoke flavoring or the primary products of smoke condensate produced in Europe. The aerosol technology was first applied by Hickory Specialties in 1969, and was the breakthrough for producing LSFs (Meier, 2009). Today, several liquid smoke flavorings are available in the market. Costello (2017) has mentioned some top liquid smoke flavoring manufacturers on a global level as follows:

- Kerry
- Besmoke
- FRUTAROM Savory Solutions
- Azelis
- Redbrook Ingredient Services
- MSK Specialist Ingredients
- Red Arrow International

According to Meier (2009), liquid smoke flavorings can replace traditional smoking processes.

2.4 Wood and its components (softwood vs. hardwood)

Wood consists of 40-60% cellulose, 20-30% hemicellulose, and 20-30% lignin (Pearson and Gillet, 1996). However, there are two broad types of woods found, viz. softwood and hardwood. Referring to Smook, Robertson (2013) has mentioned the variations in various components of softwood and hardwood. Softwood has been shown to contain more lignin than hardwood (Table 2.1).

Table 2.1 Variations in various components of wood

| Components | Softwoods | Hardwoods |
|----------------|-----------|-----------|
| Celluloses | 42% ± 2% | 45% ± 2% |
| Hemicelluloses | 27% ± 2% | 30% ± 5% |
| Lignin | 28% ± 3% | 20% ± 4% |
| Extractives | 3% ± 2% | 5% ± 3% |

Source: Robertson (2013)

2.5 Wood smoke, its nature and composition

Regarding to the term “curing smoke”, it is produced by pyrolytic changes of wood (Toth and Potthast, 1984). According to Meier (2009), this smoke comprises of the products mainly derived from the pyrolytic changes of two major polymers present in wood - polysaccharides (cellulose and hemicelluloses) and lignin. A characteristic spectrum of pyrolytic products has been obtained from each of these two polymers. The polysaccharides pyrolyze to give mainly furans, acids, alcohols, anhydrosugars, esters, and aldehydes, and are predominantly responsible for the staining and bactericidal effects of smoke. The lignin on the other hand degrades to release compounds such as guaiacol, syringol, and their derivatives and are generally responsible for the typical smoky flavor. The course of thermal decomposition of the wood during pyrolysis is as follows (Toth and Potthast, 1984):

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

Smoke can be assumed to have two phases - a dispersed liquid phase or the particulate phase containing smoke particles, and a dispersing gas phase or vapor phase (Lawrie and Ledward, 2006). More than 300 compounds have been found to be distributed in these two phases in wood smoke.

The chemical components most commonly found in wood smoke as a whole include phenols, organic acids, alcohols, carbonyls, hydrocarbons, and some gaseous components, such as carbon dioxide (CO₂), carbon monoxide (CO), oxygen (O₂), nitrogen (N₂), and nitrous oxide (N₂O) (Pearson and Gillet, 1996). Chemical composition of wood smoke is given in Table 2.2 and 2.3

2.6 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are class of organic compounds that have fused aromatic rings (hence, the name polynuclear). They are released during incomplete burning of organic matters and are also found in wood smoke. They are mainly released from lignin degradation (Pearson and Gillet, 1996). There are many PAHs identified in wood smoke. Out of these, benzopyrene is known as an indicator compound for PAHs (Guillén *et al.*, 2000)

PAHs are known as cancer-causing agents. European Union and United States Environmental Protection Agency have added this class of compounds in the list of pollutants because they have mutagenic and carcinogenic properties. Few of these compounds are not carcinogenic but play synergistic roles. PAHs are generally solids at room temperature. These compounds have high melting and boiling points, low vapor pressures, and high lipid solubility and low water solubility. Increment in the molecular mass generally reduce their solubility in water (Anyakora, 2013). The solubility of various PAHs as well as the structure of benzo [a] pyrene, has been given in Appendix B

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 ₂ O) |
| 4 | Hydrocarbons | Benz (a) anthracene, dibenz (a, h) anthracene, benz (a) pyrene, benzopyrene, benzo (g, h, i) pyrene, pyrene, and 4-methyl pyrene. |
| 5 | Phenols | Guaiacol, 4-methylguaiacol, phenol, 4-ethylguaiacol, o-eucresol, m-cresol, p-cresol, 4-propyl guaiacol, eugenol (4-allylguaiacol), 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, propanal, crotonaldehyde, ethanal, isovaleraldehyde, acrolein, isobutyraldehyde, diacetyl, 3-methyl-2-butanone, pinacolene, 4-methyl-3-pentanone, α-methyl-valeraldehyde, tiglic aldehyde, 3-hexanone, 2-hexanone, 5-methyl furfural, methyl vinyl ketone, furfural, methacrylaldehyde, methyl glyoxal, etc. |

Source: Pearson and Gillet (1996)

Table 2.3 Chemical composition (with concentration) 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 | 0.6-4.0 |
| Alkyl benzenes | 1-6 |
| Acetic acid | 1.8-2.4 |
| Formic acid | 0.06-0.08 |
| Nitrogen oxides | 0.2-0.9 |
| Sulfur dioxide | 0.16-0.24 |
| Methyl chloride | 0.01-0.04 |
| Naphthalene | 0.24-1.6 |
| Substituted naphthalenes | 0.3-2.1 |
| Oxygenated monoaromatics | 1-7 |
| Total particle mass | 7-30 |
| Particulate organic carbon | 2-20 |
| Oxygenated PAHs | 0.15-1 |
| Individual PAHs | 10 ⁻⁵ -10 ⁻² |
| 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 |

| Chemical | g/kg Wood |
|-----------|---|
| 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)

2.7 Functionalities of wood smoke

Various components of wood smoke have various functions to play, which make the use of it advantageous.

Phenols act as antioxidants, contribute to the color and flavor of smoked products, have a bacteriostatic effect that contributes to preservation; and contribute to color development as well. Phenols are also responsible for the characteristic flavor of the smoked meat. Underwood and Shoop (1998) mention “*While the phenolics historically have been thought, to be the essential flavor containing class of substances, Mga has shown substantial smoke flavor in neutral, carbonyl and basic fractions as well.*” Similarly, alcohols play the role of carrier of other organic compounds in wood smoke and have little role as bactericides. The simplest of one of such alcohols is methanol.

Organic acids have minor preservative actions and the number of carbons in the chain range from 1 to 10, with lighter ones in the vapor phase of smoke. They however have an

important role in coagulation of surface proteins of smoked meat. Similarly, over 20 carbonyl compounds are present in wood smoke. Among these, short chained species play major roles in smoke aroma, flavor and color (Pearson and Gillet, 1996).

Another important class of compounds which is present in the wood smoke is hydrocarbons, some of which are benz (a) anthracene, dibenz (a, h) anthracene, benz(a)pyrene, benz (e) pyrene, benzo (g, h, i) pyrene, pyrene, and 4-methyl pyrene. Among these, benz (a) pyrene and dibenz (a, h) anthracene, are recognized as being carcinogens (Pearson and Gillet, 1996). These hydrocarbons have no or negligible role in imparting preservative and organoleptic properties to the smoked meats (Pearson and Gillet, 1996). Most of these compounds since are present in the particulate phase of smoke, can be separated from the vapor phase without affecting the preservative and organoleptic functionality of the smoke. Use of liquid smoke flavoring has shown elimination or reduction in these carcinogenic compounds (Pearson and Gillet, 1996). The benzopyrenes are insoluble in water (Lawrie and Ledward, 2006) and this forms one of the basis for liquid smoke flavoring preparations. The functions of smoke have been shown in Table 2.4.

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)

2.8 Smoking with curing smoke

Fire was a revolutionary finding. It served as a multipurpose tool from being used as a source of energy for cooking food, to as a tool for security (Pausas and Keeley, 2009). Use of smoke to preserve meat and fish products is one of the oldest technologies in food preservation (Miculis *et al.*, 2011). Toth and Potthast (1984) defined smoking as the treatment of meats or meat products, fish, and sometimes other foods with curing smoke. Regarding its use in meat and fish preservation; Miculis *et al.* (2011) has defined smoking as the process of penetration of volatiles resulting from thermal destruction of wood into the surface of meat or fish products. It is to be noted that the second definition does not necessarily mention the direct role of fire and its heat in meat preservation as mentioned by Toth and Potthast (1984); but rather mentions penetration of the product (i.e. the volatiles) of the thermal decomposition of wood due to the heat of the fire.

Thus, in one or the other way, the core idea of smoking lies in bringing wood smoke in contact with the food item to be smoked, either directly or indirectly. Out of all the compounds present in wood smoke, however, not all of them occur in smoked product because of dependencies in various external and internal parameters for their release and deposition during wood pyrolysis (Pearson and Gillet, 1996). According to Lawrie and Ledward (2006), it is the absorption of the vapor phase by interstitial water and the surface of the food that makes smoking significant rather than the direct deposition of particulate phase on the surface. This vapor phase contains mainly phenols, carbonyls, alcohols and polycyclic hydrocarbons as well.

Smoke imparts its characteristic color, flavor and taste to the meat or other food items which have undergone smoking process. Smoke also has antimicrobial and preservative effects (Meier, 2009). However, risks lie in introduction of compounds called polycyclic aromatic hydrocarbons, N - nitroso compounds, and possibly also heterocyclic aromatic amines which are potentially carcinogenic (Meier, 2009). Direct smoking of meat is associated with cancer in humans owing to carcinogenic and mutagenic polycyclic aromatic hydrocarbons (PAHs) such as benzo (a) pyrene present in the produced wood smoke (Tamakawa, 2008; Narayan, 2014; Parada *et al.*, 2017).

Smoking, together with drying and salting, is perhaps the oldest process to preserve foodstuffs. It has also been called man's first spice (Meier, 2009). Curing and smoking of meat are closely interrelated and are often practiced together, that is, cured meat is commonly smoked, and vice versa. Smoking of meat is also difficult to separate from cooking, since heat has traditionally been applied at the same time as smoke. However, the application of smoke and heat together are not necessarily closely allied, as smoke and heat can be applied either together or separately. Thus, there are both hot and cold smoking of meat; however, even cold smoking usually requires some increase in temperature (Pearson and Gillet, 1996).

2.9 Smoking methods

According to Pearson and Gillet (1996), liquid smoke flavoring is used by some processors and has several advantages over natural wood smoke. First, it does not require the installation of a smoke generator, which usually requires a major financial outlay. Second, the process is more repeatable, as the composition of liquid smoke flavoring is more constant. Third, liquid smoke flavoring can be prepared with the particle phase removed, and thereby possible problems from carcinogens can be alleviated. Fourth, liquid smoke flavoring application creates little atmospheric pollution and can be applied easily, even in plants located in densely populated areas. And fifth, liquid smoke flavoring application is faster than conventional smoking, resulting in more through-put per unit. In addition to that, according to Hollenbeck (1963), direct smoking of foods, also, has additional shortcomings. Chief among these is the lack of flavor control due to only the surface of the food coming in contact with the smoke. Thus, from the standpoint of flavor control, it is greatly desirable to add wood smoke flavor to a food by means of a well characterized and controlled smoke flavor bearing product (Hollenbeck, 1963). One of such flavors bearing products is the "liquid smoke flavoring".

2.10 Properties of liquid smoke flavoring

The following physical properties have been summarized being based on various literatures (Wright, 1930; Hollenbeck, 1963; Moeller, 1997; Underwood and Shoop, 1998; FAO, 2001; Parliament, 2003; Simon *et al.*, 2005; Meier, 2009; Montazeri *et al.*, 2013)

State: Liquid

Colour: Amber, yellow to red, reddish brown

Solubility: Aqueous extract is water soluble

Total Solids: Variable (can also be zero)

Titration acidity: 0.7 - 4% (w/v).

Guillén and Ibargoitia (1996) have studied change in acidity of smoke products with increasing temperatures and incorporate a wide range from 0.0557 to 0.670% (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)

2.10.1 Variation in properties

Chemical properties of liquid smoke vary according to the wood type used, temperature of pyrolysis and various other factors. Montazeri *et al.* (2013) has mentioned that *Baltes* had found major proportion of commercial full-strength liquid smoke to be composed of water (11 – 92%), tar (1 – 17%), acids (2.8 – 9.5%), carbonyl containing compounds (2.6 – 4.6%) and phenol derivatives (0.2 – 2.9%). He further mentions that various ingredients may be used during manufacture of liquid smoke. He also mentions referring to various sources that by controlling the phenol derivatives, carbonyl-containing compounds and organic acids content of liquid smoke, desired aroma and color characteristics can be achieved. The composition and constituents of commercial liquid smoke are corporate secrets and are not generally disclosed to buyers. So, only general information exists on the chemical properties of liquid smoke produced.

According to study done by Montazeri *et al.* (2013) on various commercial liquid smoke flavors, following characteristics were observed:

Color: Freshly prepared liquid smokes were bright yellow, but the color changed slowly possibly due to deposition and further condensation of intrinsic components. Different

samples showed variations in color. These solutions showed no visible turbidity or precipitate formation in 2 months of storage time.

pH and titrable acidity: wide variations in pH and titrable acidity have been reported. pH may range from 1.5 - 7.7. Therefore, both acidic and alkaline liquid smokes are present, depending on production variables. Similarly, titrable acidity (TA) ranged from 0.7 - 10%.

Total phenol content: It was seen that certain refined samples had no detectable phenols in them whereas one had about 3.22 ± 0.03 mg/ml of total phenols in them.

It was seen that, the refined liquid smoke samples had lower amount of acidity, phenols and carbonyls in them. Lower amount of phenols and carbonyls would mean lesser interference in the original flavor and color of food items respectively.

2.10.2 Properties of primary products

Comparatively, less studies have been dedicated to chemical characterization of primary smoke condensates. Simon *et al.* (2005) has studied composition of liquid smoke primary products. According to him, hardwood yields more acids than softwoods owing to more pentosans compared to hexosans in softwood. Hardwoods are rich in Glucuronic acids that decompose to give various carboxylic acids and thus increased acidity as compared to softwoods. Similarly, hardwoods have syringol content more than guaiacol content. He also mentions that the flavor compounds besides phenols reach a maximum at pyrolysis temperatures of 500°C and phenols reach maximum at 650°C. He mentions that a moisture content of 20-30% (wb) is ideal for minimizing particulate matters in smoke. Similarly, ideal temperature for production is below 650°C. Composition of primary products are strongly dependent on the parameters of smoke production. Referring to other sources, Simon *et al.* (2005) mention that variations with deviation from 10% to 66% in composition of certain components in batches of same production conditions have also been seen. From this, we can conclude that composition of primary smoke condensates of same batches is highly variable, even when produced in same conditions. However, temperature, wood variety and moisture content are to be kept within specified ranges for achieving desired properties.

To conclude, temperature of pyrolysis should be below 650°C, moisture content should be around 20 - 30% (wb) and oxygen should be limited to prevent excessive emission of

polycyclic aromatic hydrocarbons. Variations in composition of primary smoke condensates are integral part of production process.

Underwood and Shoop (1998) has mentioned that these primary smoke condensates can also be used as such in foods, but for various reasons, mixing of PSC with tar fractions are done. But he also mentions that this tar fraction is where most of the hydrocarbons and PAHs are located.

2.11 Application of liquid smoke

2.11.1 Scope of application

Liquid smoke flavoring has various uses (Ramakrishnan and Moeller, 2002). Out of those, these are the most prominent ones.

- a. As liquid smoke flavorings
- b. As preservatives
- c. As colorants

According to Underwood and Shoop (1998), today, liquid smoke flavors are mainly used for organoleptic purposes.

2.11.2 Way of application

They can be applied to meat products by various ways such as dipping, spraying, or aerosol treatments similar to the treatment in traditional smokehouses (Meier, 2009). Pearson and Gillet (1996) mention various ways of application of liquid smoke flavoring to food items. These include:

- i. direct addition to meat emulsion
- ii. direct dipping of the food item into the solution
- iii. spraying of product with liquid smoke flavoring
- iv. atomization into a fog and releasing this fog into the smokehouse
- v. vaporization by putting on a hot surface

Out of these methods, spraying seems to be most promising. Concentrated liquid smoke flavoring flavorings are generally diluted before their application. Pearson and Gillet (1996)

have written that a typical smoke solution preparation made by various meat processors contain 20% liquid smoke flavoring, 5% citric acid or acetic acid (vinegar), and 65 - 75% water.

Application of liquid smoke flavoring, however, does not make it unnecessary to cook the meat items (Pearson and Gillet, 1996). Pearson and Gillet (1996) also mention that for good smoke color formation, cooking after the application of liquid smoke flavoring is also essential. Therefore, these solutions are applied before or during cooking of meat and food items.

Underwood and Shoop (1998) have mentioned that application methods and timings are dependent on properties of liquid smoke as well. In acidic meats, there are chances of formation of nitrogen dioxide from nitric oxides in cured meat due to increment in rate of reaction. Nitrogen dioxide is a toxic reddish-brown gas and may form during curing if the pH of meat is too low (< 5.5). Highly acidic liquid smokes should be avoided in such cases. Thus, in general, liquid smoke should be applied once the curing process has completed. We can also find special low acid smoke flavorings that are compatible with curing agents such as nitrite and nitrate to be used in items such as pickle solutions. When brines or pickles with little buffering capacity are added with acidic smoke flavorings, nitrites could deplete before the pickle is pumped into the product.

2.12 Principle of use

The principle behind use of liquid smoke flavoring is simple. According to Wright (1930), it is an aqueous condensate of smoke. As mentioned earlier, Liquid smoke flavoring is based on following principles:

1. First, referring from what has been mentioned earlier in this literature (Section 2.4 - 2.9), carcinogens from hydrocarbon fraction of the smoke, called PAHs and benzopyrenes, are insoluble in water (Lawrie and Ledward, 2006; Underwood and Shoop, 1998).
2. Direct smoking, as pointed out by Hollenbeck (1963), lacks control over smoking process. Liquid smoke flavoring, produces reproducible, controlled and constant flavor.

The principle for production of liquid smoke flavoring has been given in Section 2.13.

Besides other advantages (Section 2.15), these two reasons form the basis for the production and use of liquid smoke flavoring.

2.13 Liquid smoke production

Liquid smoke flavoring production process has evolved over past 85 years. The first liquid smoke flavoring as an aqueous condensate was production method was documented by Wright in 1930 as mentioned earlier in the text. Since then, production processes have evolved through the history. Among the improved processes mentioned by (Moeller, 1997); besides Hollenbeck (1963) and Moeller (1997), all other methods require heavy expenses in construction and is beyond the scope of the author of this paper. In addition, because there is limited practice in use of liquid smoke flavoring in Nepal, starting material required for the tar-depleted liquid smoke flavoring (Moeller, 1997) remains unavailable, or very rare on the market. Thus, the method introduced by Hollenbeck (1963) remains within the scope of the author and has been described in detail, in the literature. Toth and Potthast (1984) has hinted an idea of the use of the same in an example for industrial equipment for smoke condensation.

The production method also resembles the schematic method referred by Simon *et al.* (2005) in his review paper referring to *Guillen and Meyer*, but by the identity of production of water-soluble primary smoke condensate. This scheme has also been generalized in parliament (2013) as a method to produce the primary product of smoke condensate. Since the scheme of production of the water-soluble primary product resembles to the scheme given by Hollenbeck (1963), we can conclude that the processes to produce the primary product of liquid smoke flavoring is merely the representation of Hollenbeck's original design. Hence, both the scheme and Hollenbeck's original design prove to be acceptable processes have been presented for a clear understanding of the process of liquid smoke flavoring production.

The method has also been described clearly by Meier (2009) and Simon *et al.* (2005) and have been presented for understanding the principle of liquid smoke flavoring flavor production.

2.13.1 Meier's and Simon's review

The method highlighted by Meier (2009) basically involves smoke generation from wood and its condensation. Wood is thermally degraded in the absence of oxygen, and the vapors are condensed either in water or vegetable oils. The volatile smoke constituents are continuously removed from the hot reaction zone and condensed in special equipment. The raw products are divided into different classes according to their solubility in water (Fig. 2.1); water-soluble condensates are called “primary smoke condensates.” The water insoluble tarry phase is cleaned mostly by extraction and called “primary tar fraction.” Both fractions are refined through further process steps such as extraction, distillation, and concentration by evaporation, absorption, or membrane filtration. During condensation other water insoluble oily products are formed that are not utilized. It is schematically shown in Fig. 2.1 and the production concept drawn from the review done by Simon *et al.* (2005) has been shown in Fig. 2.2.

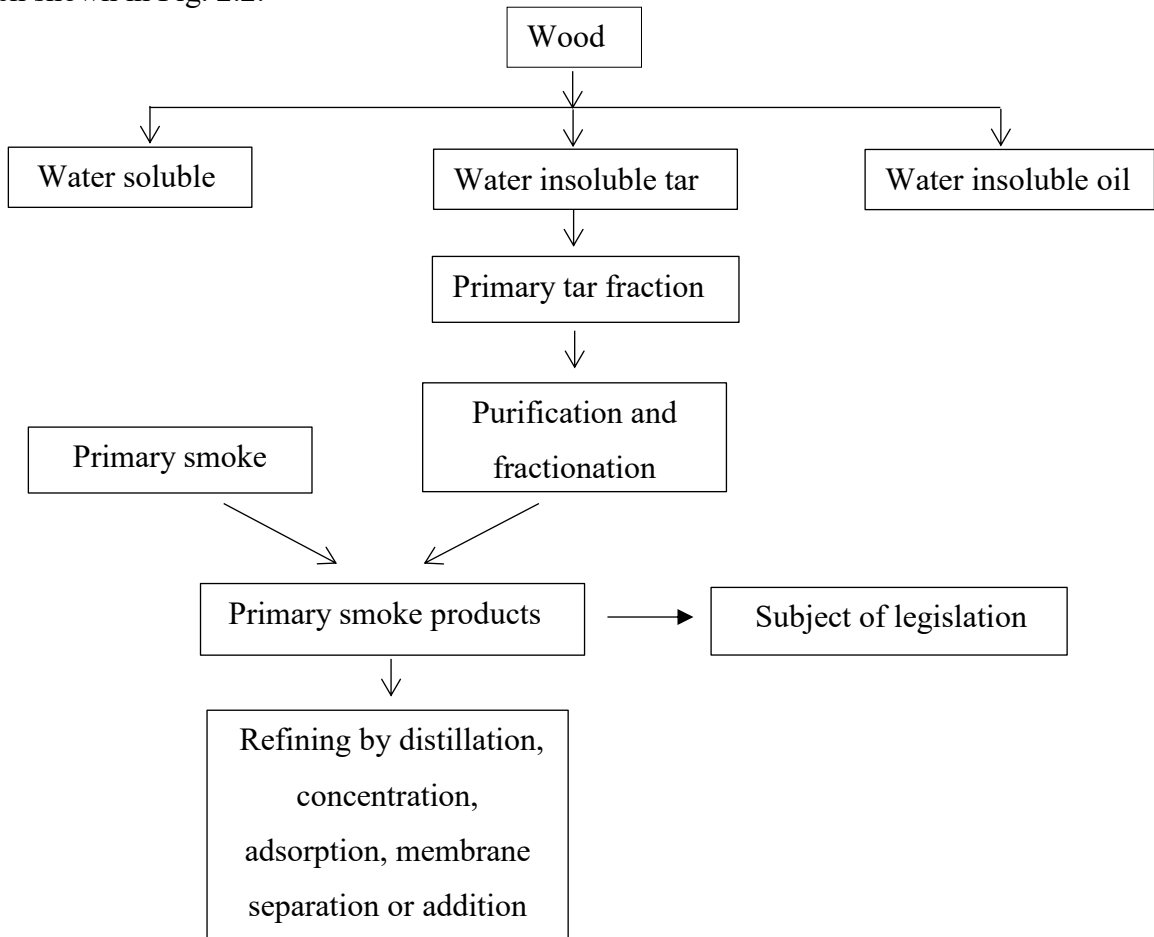


Fig. 2.1 Principle of Liquid smoke flavoring production

Source: Meier (2009)

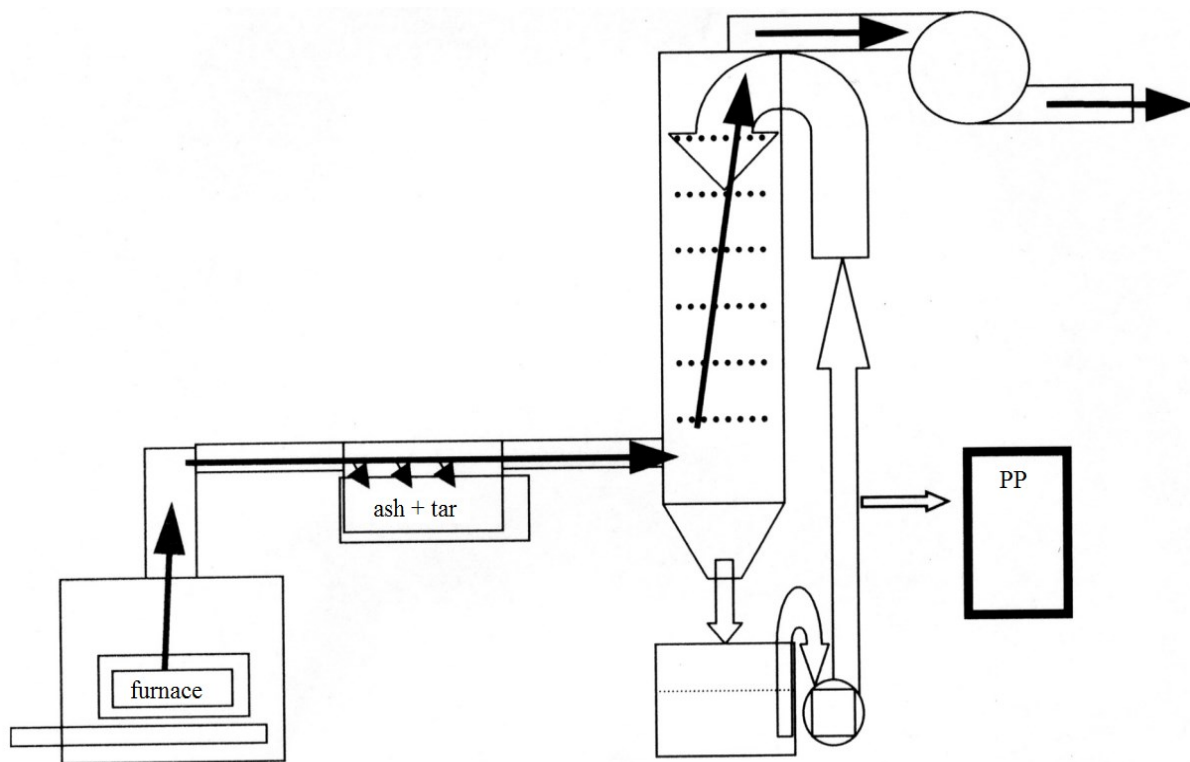


Fig. 2.2 Smoke primary product (PP) production

Source: Simon *et al.* (2005)

2.13.2 Hollenbeck's method

The method given by Hollenbeck, (1963) is relatively simple to understand and operate and involves countercurrent extraction of water soluble components of smoke by cool to warm water in an absorption tower after the smoke has been separated from the particulate matter, until the titrable acidity in terms of acetic acid and other phenolic substances reaches 3% by weight of the solution. The particulate matter is separated in a settling chamber before it is passed through the tower where the soluble components get solubilized in cool to warm water. He mentions the limit of the concentration to be kept from 3% to 8% with best one being 6% so that excessive acidity does dissolve tars and other unwanted compounds of the smoke.

Concluding from Sections 2.1, 2.2 and Fig. 2.1 and 2.2; the product given by Hollenbeck can be concluded as a Primary Smoke Product which further needs to be refined. The design of the apparatus given by Hollenbeck is shown in Fig. 2.3.

In the Fig. 2.3, smoke is generated by burning sawdust (12) held on a grate (11) by a burner (19) from below in the smoke generator (12) in limited air supply. The smoke then rises and moves through the outlet (14) into a settling chamber (15). The diameter of the settling chamber is eight times to that of the outlet channel. After settlement of heavy particles, the relatively light and volatile components move through the outlet again to release into the absorption tower (16). The smoke is drawn upwards by an exhaust (21). The tower is packed with ceramic saddles (26) for providing enough area for the smoke to mix with water supplied in counter current direction of smoke through the pipe at the top (20). The water and smoke solution (22) get collected at a beaker downwards (16) which is kept cool by circulating cold water (17) continuously. The liquid smoke flavoring thus collected is then again recirculated through a pump to reach the pipe (20) above. Additional water is supplied from a reservoir (24) through a controlling valve (23). The recycling is done until the titrable acidity of the collected liquid smoke flavoring reaches 3% by weight of the solution. It is then left for settlement of heavy particles and finally filtered through cellulose pulp filter to get liquid smoke flavoring. (Hollenbeck, 1963)

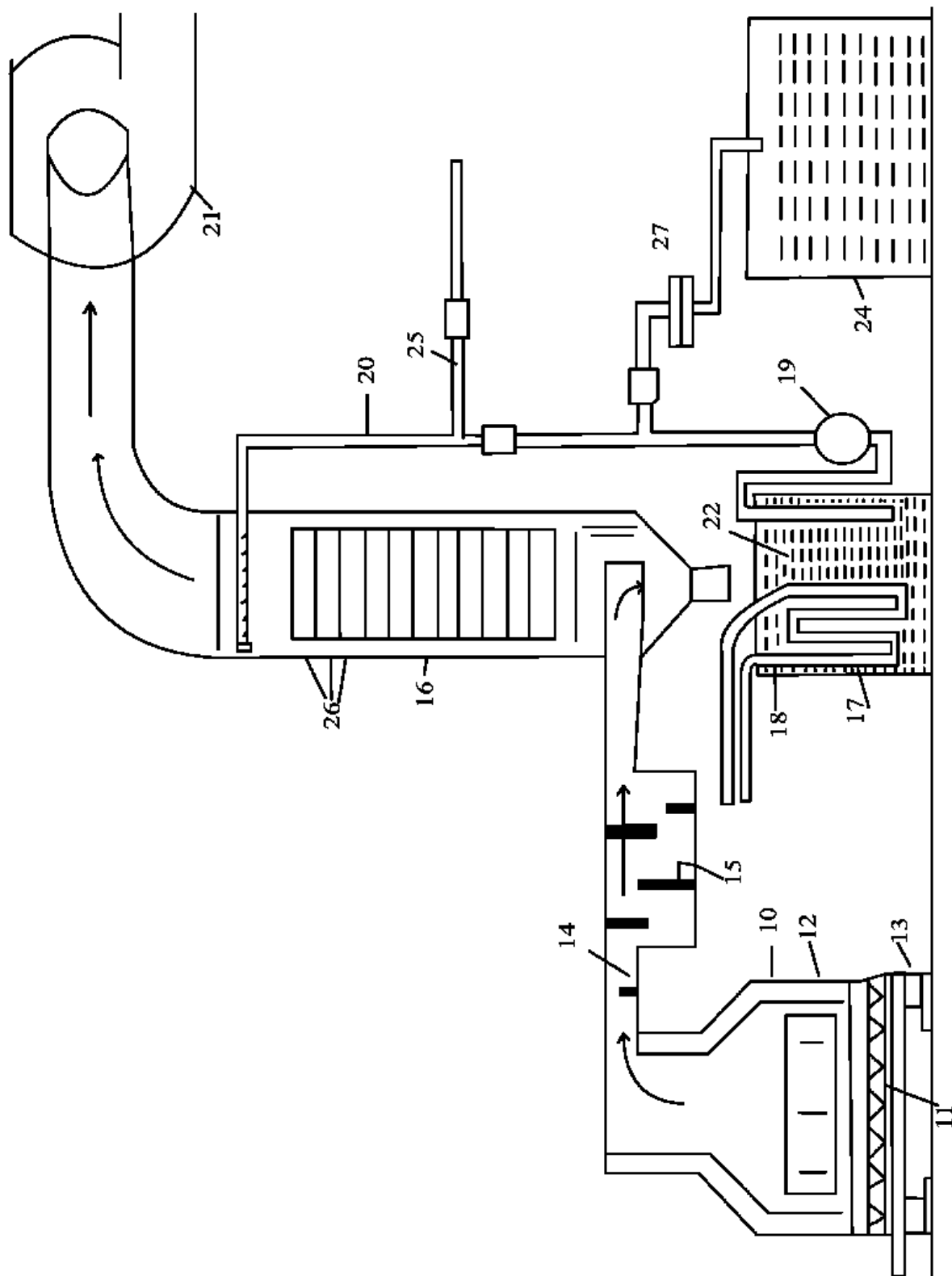


Fig. 2.3 Design for producing liquid smoke flavoring

Source: Hollenbeck (1963)

2.14 Controlling PAHs during production

The levels of PAHs have been found to be dependent upon various factors such as temperature of pyrolysis, wood type, oxygen availability, etc. (Ledesma *et al.*, 2016). Tamakawa (2008) has mentioned that CODEX requires a total of ten parameters to be controlled in order to control level of PAHs contamination during smoking process. Out of all these, temperature control and control of oxygen concentration seem to be the most promising factors. It has been shown by García and Simal (2005) that heating the sawdust below 400°C of temperature gives lower levels of PAHs as compared to ignition and flaming. According to Simon *et al.* (2005), the parameters that need to be maintained for minimum emission of PAHs are

- a) Temperature of pyrolysis below 650°C
- b) Limited Oxygen Supply in the region of pyrolysis
- c) Moisture content of about 20 - 30 % (wb)

Babic *et al.* (2018) have studied the potential of use of gravels in minimizing PAHs content of smoke during smoking process. They have suggested that gravels have a good potential in reduction of PAHs while smoking of foods. Hence, gravels have also been used in this research for minimization of PAHs.

Underwood and Shoop (1998) clearly mention that tarry fractions along with other particulate matters is the section which contains most of PAHs. Therefore, tarry fractions have to be strictly avoided while preparing the primary product with an aim to minimize the content of carcinogens. It is also important to note here, that benzo (a) pyrene and PAHs have very low solubility in water (Underwood and Shoop, 1998). They also mention that an organic content of 30% (w/v) is sufficient to limit the solubilization of PAH (benzopyrene) to less than 1 ppb.

2.15 Advantages and disadvantages of liquid smoke

Liquid smoke flavoring has several advantages over traditional direct smoking processes. According to Hollenbeck (1963), liquid smoke flavoring has reproducible flavor. Similarly, other advantages include ease of application, speed, uniformity of the product,

reproducibility of physical and chemical properties, and cleanliness of application (Meier, 2009). Another benefit includes reduction in certain carcinogenic components such as benzo(a)pyrene, etc. which are relatively insoluble in water. Soares *et al.* (2016) has studied on the antimicrobial effects of liquid smoke flavoring and thus liquid smoke flavoring can be said to also have antimicrobial effects. The compounds in liquid smoke have been lumped together as three functional groups: carboxylic acids, phenols, and carbonyls by Ramakrishnan and Moeller (2002), which suggests the preservative effects and importance of liquid smoke flavoring as a flavoring additive to be used in food. In addition, since liquid smoke flavoring is packed and can be retrieved in bottles, it eliminates the need for burning wood that could otherwise cause environmental pollution and respiratory problems.

The disadvantage of liquid smoke flavoring is in that since only a fraction of smoke is incorporated in it, it has lesser preservative effects than the actual smoke itself. Demerits also lie in settlement of heavy particles at the bottom of bottle used for storage over the course of time, thereby bringing decrement in the intensity of the flavor and other effects. Another disadvantage is that it can be used only with food items with which water can be compatibly used. There are, however, other liquid smoke flavorings made in oil rather than water for diverse use as well (Meier, 2009).

Advantages of liquid smoke

1. Reproducible flavor
2. Ease of application
3. Speed, uniformity of the product,
4. Reproducibility of physical and chemical properties
5. Cleanliness of application
6. Reduction in certain carcinogenic components such as benzo(a)pyrene
7. Minimization of respiratory problems.
8. Less environmental problems
9. Utilization of waste sawdust from wood industries.

Disadvantages of liquid smoke

1. Lower concentration than smoke itself
2. Reduced preservative actions as compared to original smoke
3. Reduced flavor intensity as compared to original smoke
4. Settlement of particles over long storage time decreasing its intensity
5. Industrial production and sophisticated lab required for commercialization
6. Construction costs may be expensive for non-regular consumers.

2.16 Descriptive sensory analysis

‘Sensory analysis can be considered to be an interdisciplinary science that uses human panelists sensory perception related to thresholds of determination of attributes, the variance in individual sensory response experimental design to measure the sensory characteristics and the acceptability of food products, as well as many other materials’. (Singh and Maharaj, 2014). Descriptive sensory analysis uses several techniques that seek to discriminate between a range of products based on their sensory characteristics and also to determine a quantitative description of the sensory differences that can be identified, not just the defects (Singh and Maharaj, 2014)

2.17 Analysis of liquid smoke

Simon *et al.* (2005) has given various methods for analysis of liquid smoke. Likewise, titrable acidity can be easily calculated by using the method given by Sadler and Murphy (2010).

PART III

Materials and methods

3.1 Materials

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

3.1.1 Materials for construction.

For construction of liquid smoke production assembly, following construction materials were collected from different sources which is shown in Table 3.1

Table 3.1 Construction materials for the construction of the assembly

| Construction materials | Location | Source |
|------------------------|------------------------|--------------------------|
| Concrete | Smoke generator | Campus Premises |
| Iron | Smoke transfer Pipe | Lalit Hardware, Dharan |
| Tin | Settling tank | Suresh Tin Works, Dharan |
| Motor (18 Watts) | Liquid smoke collector | Balaji Hardware, Ktm. |
| Aquarium Pipes (16 mm) | Solubilization tower. | Pet House, Dharan |

3.1.2 Local woods for production

The Liquid smoke flavoring was prepared from 4 locally available wood varieties. Their name and sources have been mentioned in Table 3.2

Table 3.2 Name and Source of local wood varieties

| S.N. | Wood varieties | English name | Scientific name | Source/location |
|------|----------------|--------------|-----------------------------|---------------------------|
| 1 | Sal | Sal | (<i>Shorea robusta</i>) | CCT, Dharan |
| 2 | Sallo | Pine | (<i>Pinus roxburghii</i>) | Sai Kastha Udhyog, Dharan |
| 4 | Bhogate | Pomelo | (<i>Citrus maxima</i>) | Vijaypur, Dharan |
| 6. | Arubakhada | Plum | (<i>Prunus</i>) | Sirutar, Bhaktapur |

3.1.3 Commercial liquid smoke

Market liquid smoke sample was obtained from local market. It was known as “Star Brand Smoke Flavor, Artificial Smoke Flavoring Agent” (Industrial Raw Material) and produced by International Flavors and Fragrances India Pvt. Ltd, Chennai, India.

3.2 Methodology

The research approach has been given in Appendix E.

3.2.1 Designing the assembly

The basis for design of the assembly was taken from Hollenbeck (1963). Hollenbeck’s design was chosen because of it was based on basic principle of production as explained by Parliament (2003). Comparing the principles given by Hollenbeck (1963) and Parliament (2003); it can be easily said that principle of liquid smoke production given by Parliament (2003) resembles Hollenbeck’s original design. These principles have been explained in Section 2.12 - 2.13. The assembly was designed such that liquid smoke flavor prepared was a water-based smoke flavoring owing to the fact that PAHs have limited solubility in water.

The basic principle of production involves extraction and solubilization of soluble fractions in water for a water based aqueous flavoring. However, there have been certain modifications done according to research aims without affecting the basic principle of production.

The noteworthy parts of the modified design include the following:

Modification 1: The heating Process in the smoke generator was made based on the principle of plate heating instead of direct heating of wood dust by burner.

Reason for modification 1: This was carried out being based on the research by Sadler and Murphy (2010) where plate heating showed control in heating temperatures, and thus lesser generation of Polycyclic Aromatic Hydrocarbons (PAHs).

Modification 2: The outlet channel from the smoke generator was modified into a long swan neck design

Reason for modification 2: Volatile substances rise up and particulate matters generally heavier than air, settle down. So, a swan neck design can facilitate preliminary settling of the particulate matter of smoke before entering the settling chamber.

Modification 3: The recycling of the produced liquid smoke flavoring by a pump will be slightly modified according to the design presented in the Fig. 3.1 - Fig. 3.7 where the pump will be directly soaked in the collected liquid smoke flavor below the absorption column.

Reason for modification 3: This was done according to convenience since only underwater pump of required capacity was available in local market.

Modification 4: The absorption column was packed with gravels of size 20 -30 mm.

Reason for modification 4: This was done because Babic *et al.* (2018) has found that gravel filters helped in reduction of PAHs during smoking.

The design used for liquid smoke production was as shown in Fig. 3.1 to 3.7. It is to be noted that in settling tank, the outlet pipe is at a higher altitude than the inlet pipe and of a smaller diameter. This was done so that only (as far as possible) the lighter portion of smoke representing the volatile portion would rise up instead of heavier section that would otherwise represent tars, particulate matters and heavier hydrocarbons.

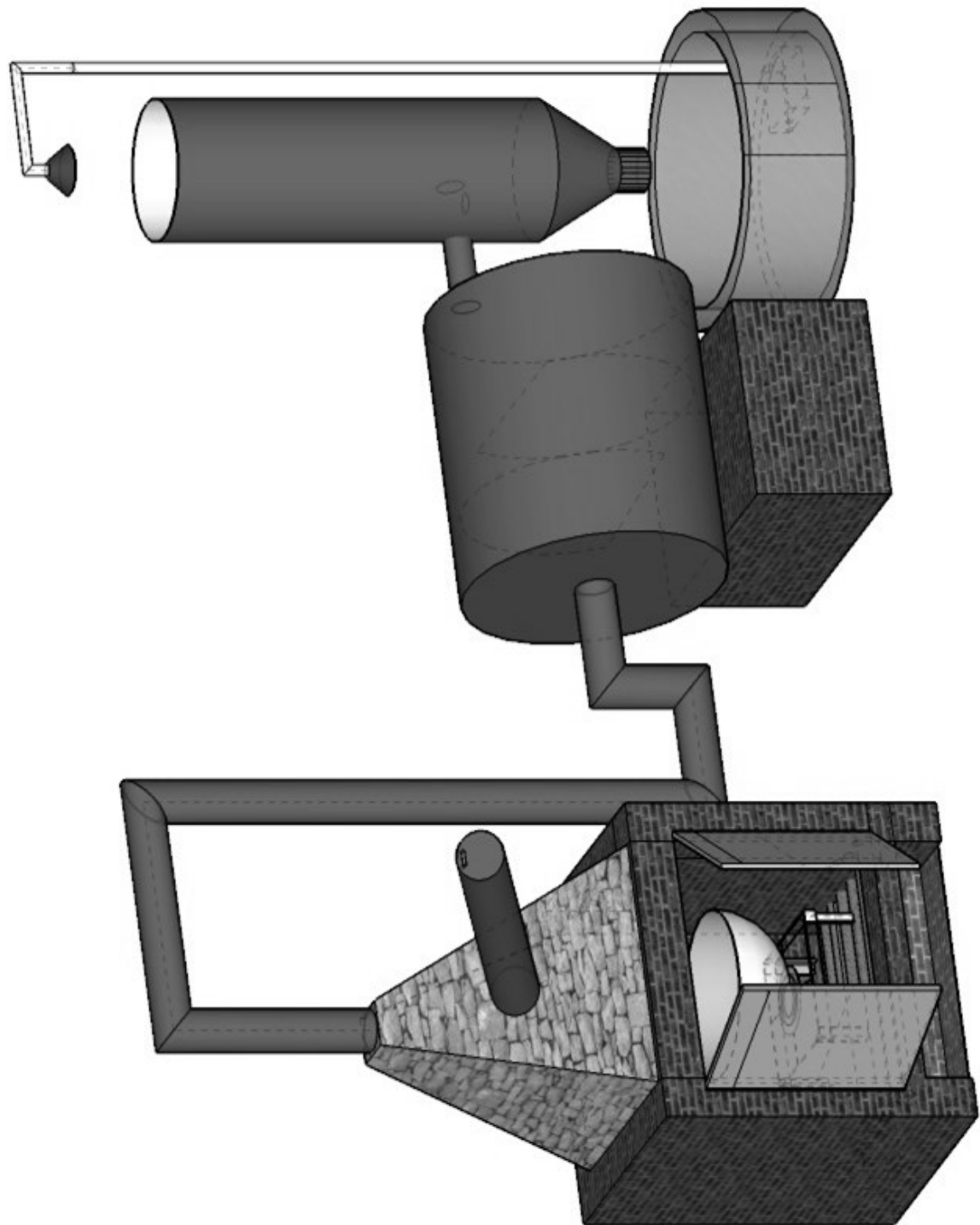


Fig. 3.1 Liquid smoke flavoring preparation assembly (general unlabeled view)

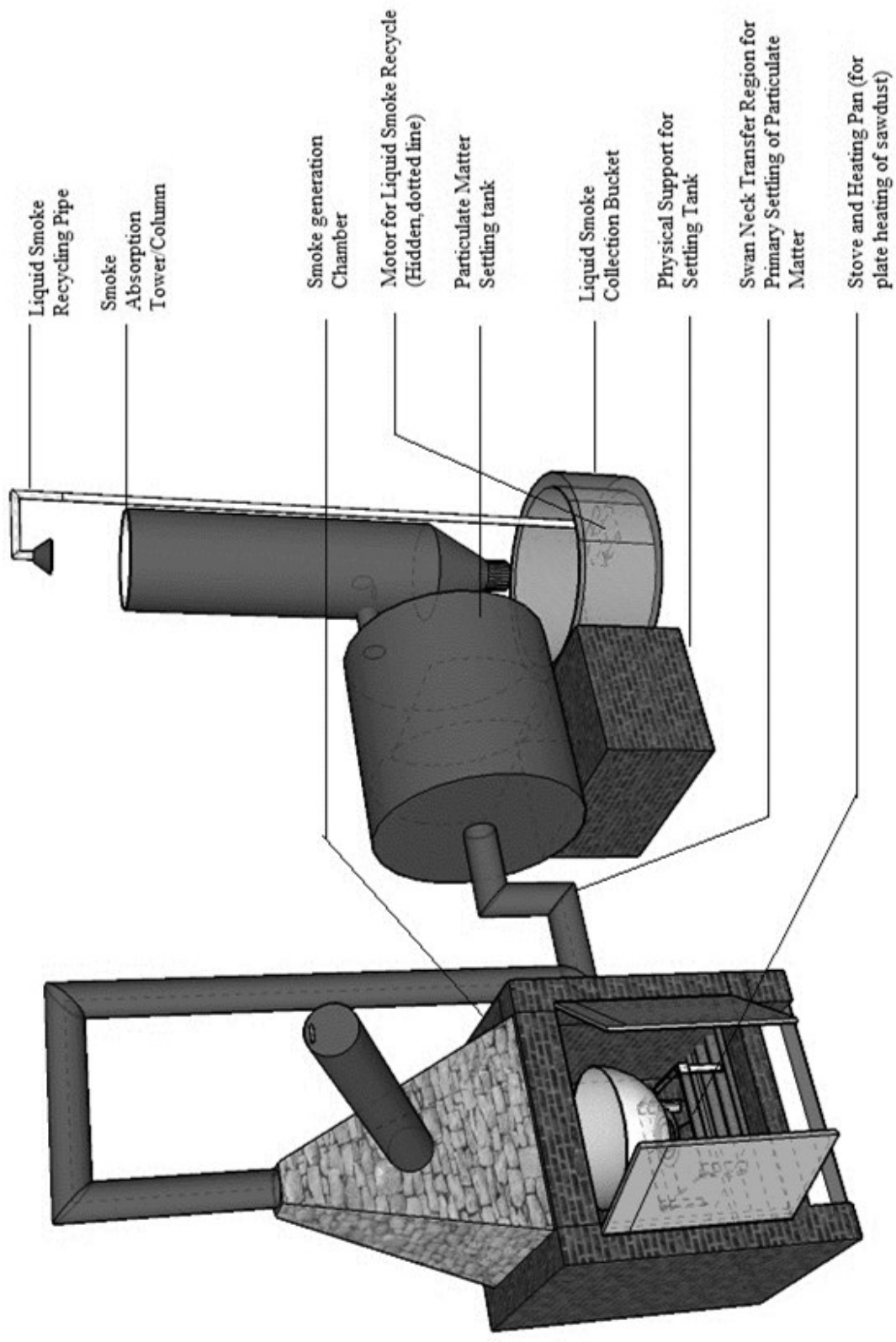


Fig. 3.2 Liquid smoke flavoring preparation assembly (labelled view)

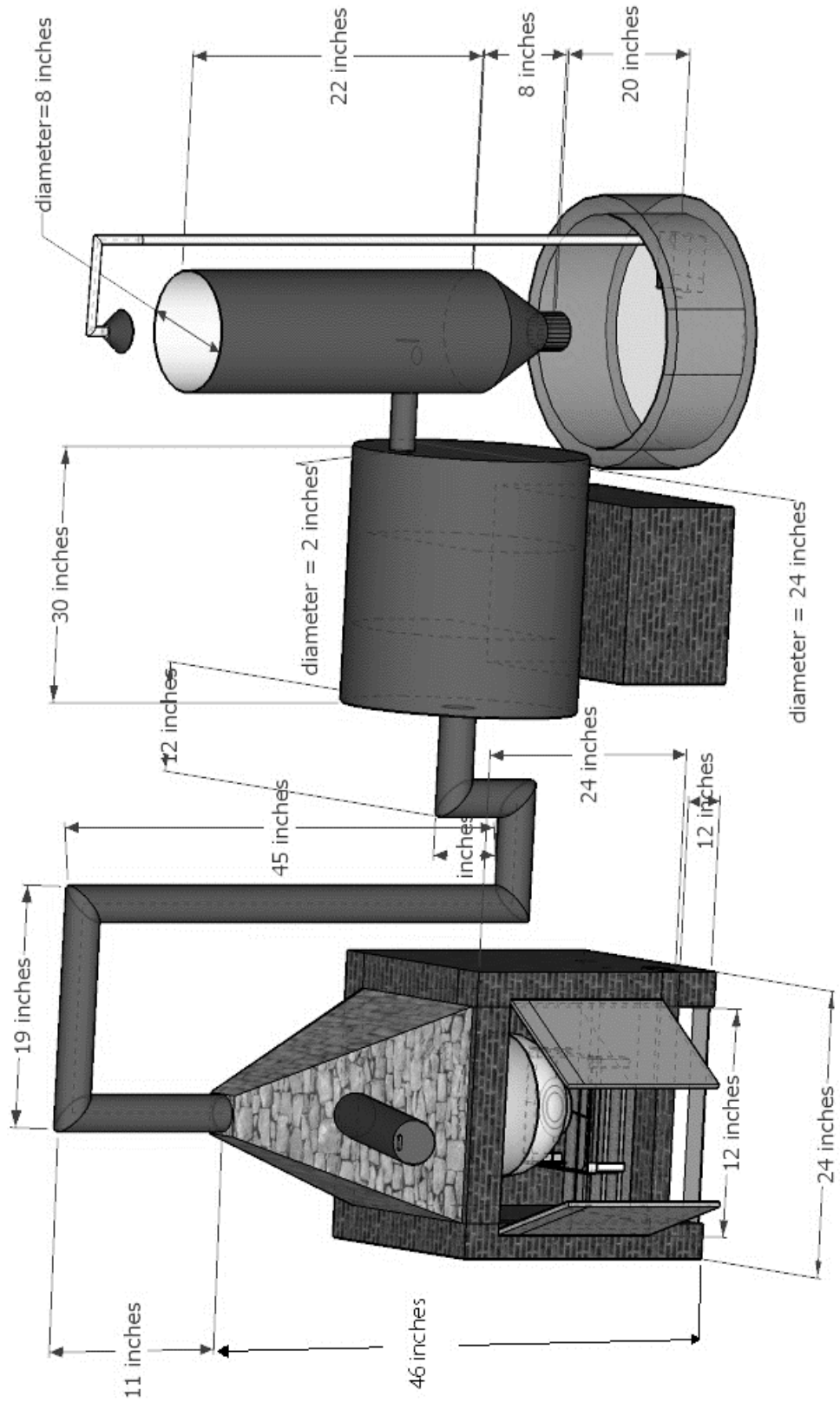


Fig. 3.3 Liquid smoke flavoring preparation assembly (with dimensions)

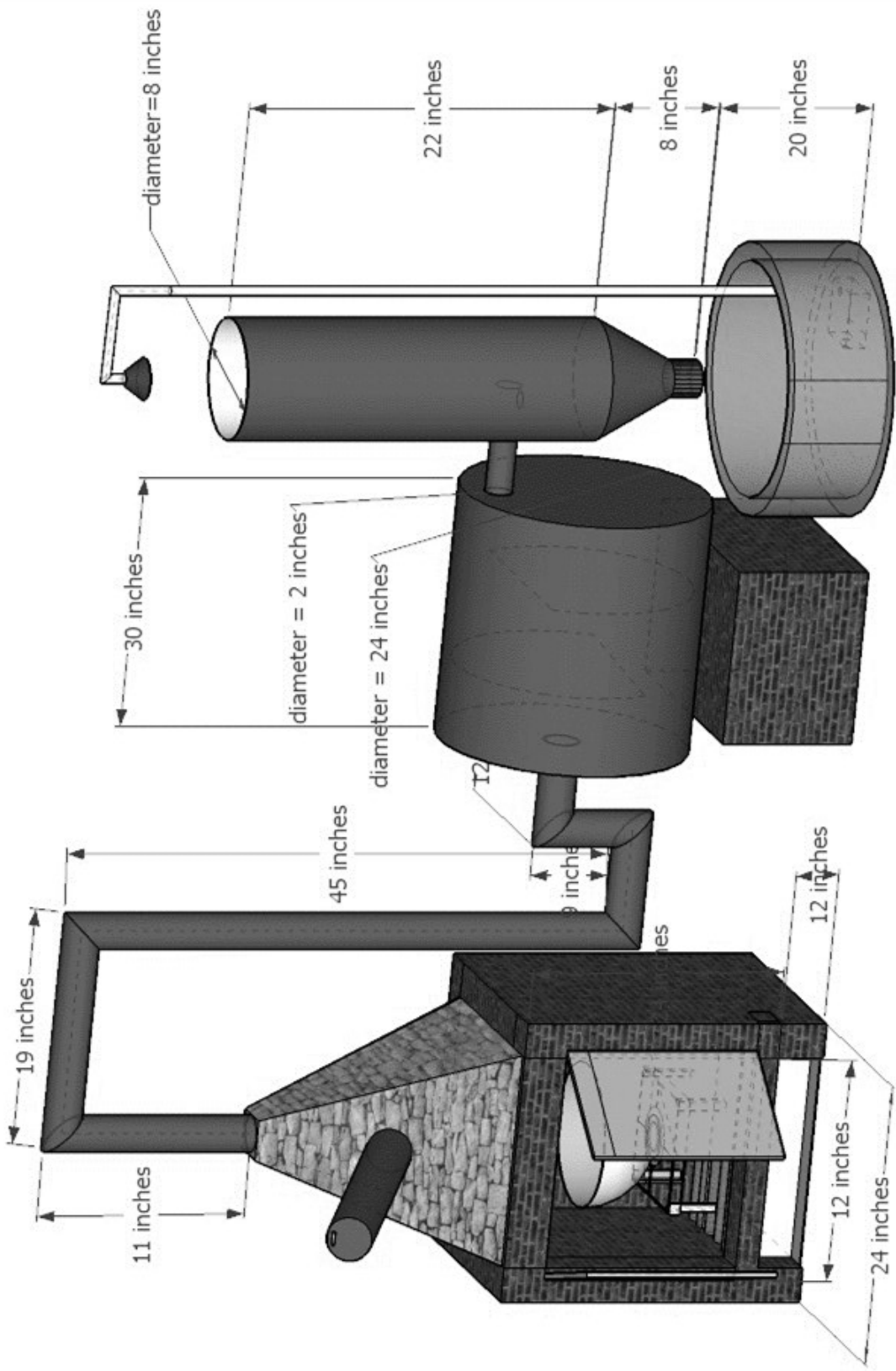


Fig. 3.4 Liquid smoke flavoring preparation assembly (with dimensions)

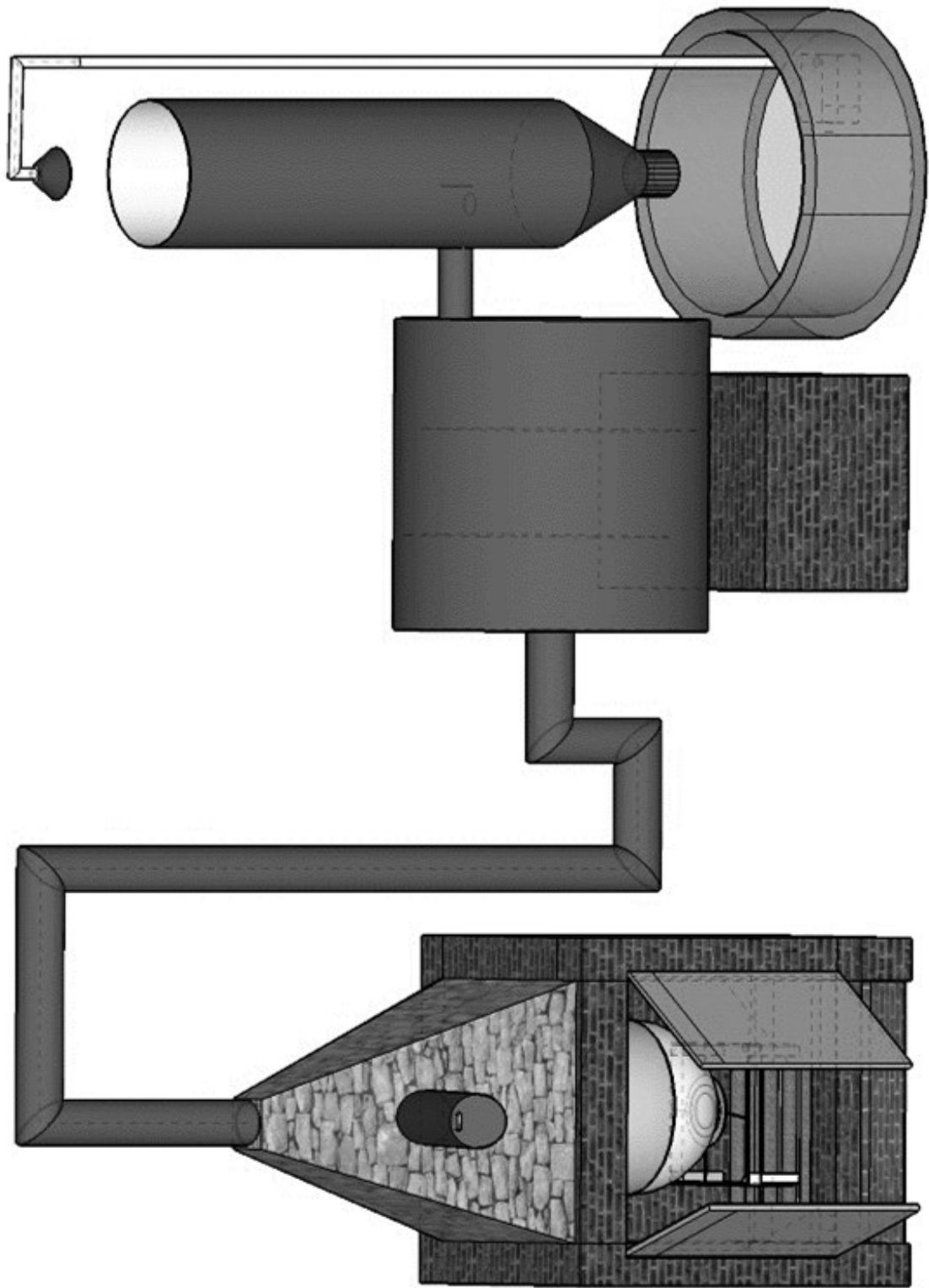


Fig. 3.5 Liquid smoke flavoring preparation assembly front view (a)

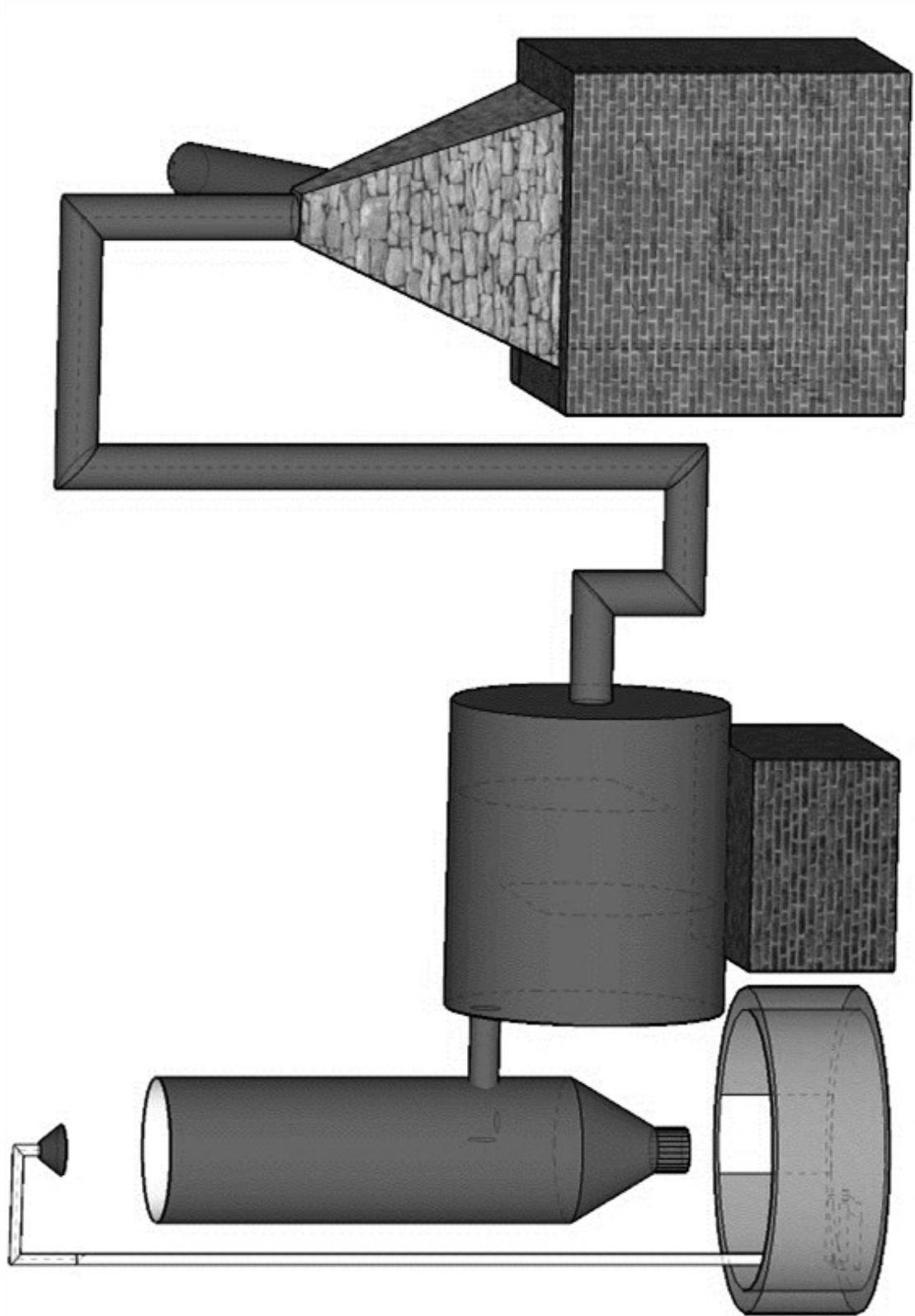


Fig. 3.6 Liquid smoke flavoring preparation assembly back view

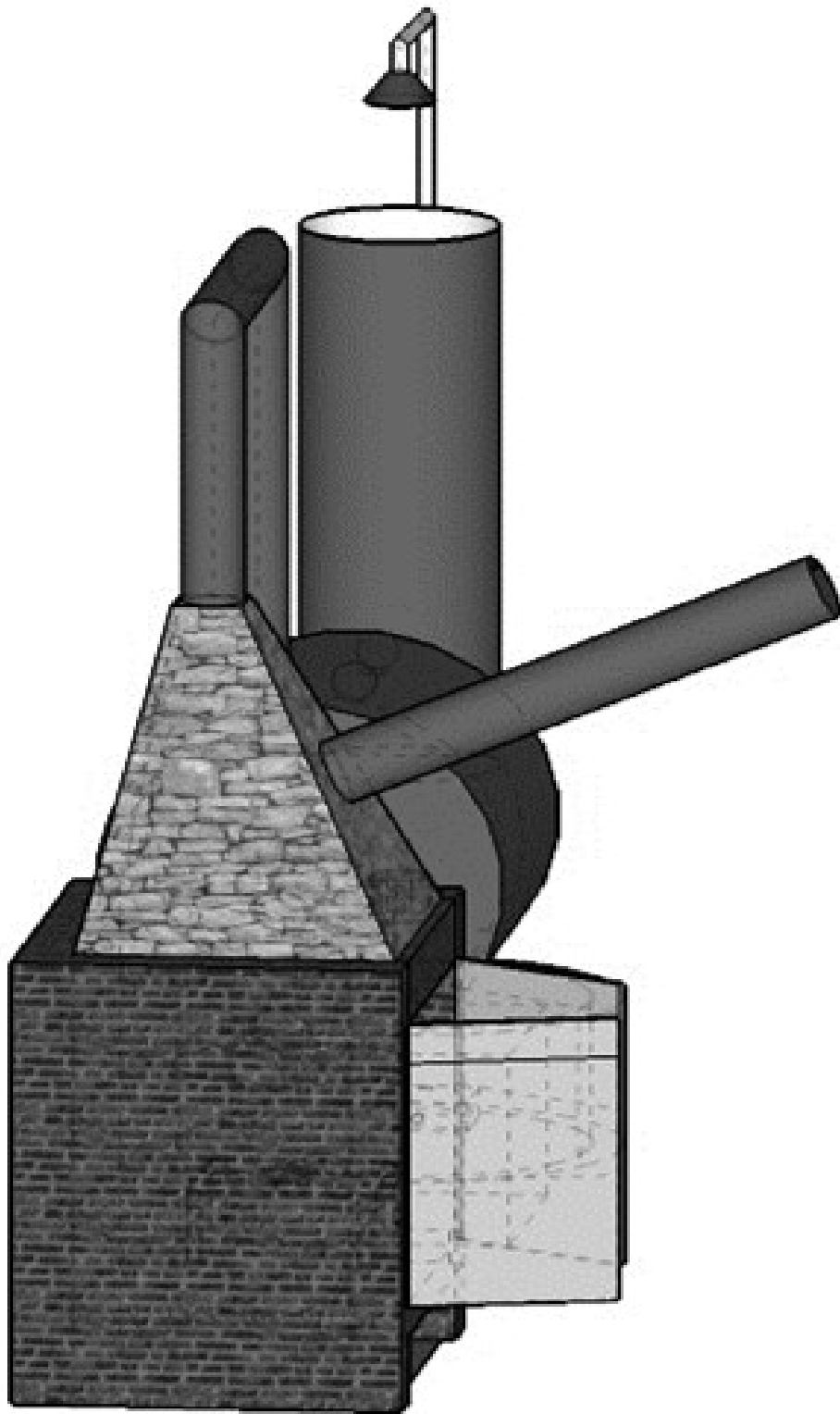


Fig. 3.7 Liquid smoke flavoring preparation assembly left view

3.2.2 Construction of the assembly

The construction of the assembly was carried out according to the design mentioned in Section 3.2.1.

3.2.3 Operation of the assembly

The assembly was operated on manual mode whereby, sawdust was fed at the smoking generation section by putting it in a pan and heating over fire. The smoke generated from the smoke generation chamber transferred to the settling tank through a primary smoke transfer pipe and then swan-neck pipe for settlement of particulate matters. The settling chamber was designed in such a way that the outlet pipe was in a higher altitude and of a smaller diameter. The other important feature of this tank was that the path of smoke coming from the inlet of tank was continuously blocked by tin blockades making then functionally similar to baffles and thereby, facilitating the settlement of particulate matter and the heavier section of the smoke. Therefore, only the most volatile part of smoke came out of the settling tank and reach the absorption column. The smoke on reaching the column came in contact in numerous stones used for loading the column. This increased the surface area for the contact of the smoke with water coming in countercurrent direction in the same column. Smoke rose from lower altitude to higher altitude, and continuously recirculated water came from above, thereby dissolving the soluble portion of the smoke with it. The solution was collected in a bucket below, known as the primary smoke condensate. This aqueous extract was continuously recirculated using a motor until highest possible concentration was reached. This solution was taken for further analysis in lab and to be used as liquid smoke flavoring.

Precautions taken during operation

1. Since stones are used in the absorption column, there could be a high chance of sand particles and stone debris to be present in the prepared smoke flavor. So, stones were properly washed before operation. Washing of stones involved following procedures.

Gravels (20-25 mm) → Soaking in water overnight → Repeated washing with scrubbing by warm water until no visible particles appeared in water → Dipping in HCl solution (0.1N) overnight → Washing with warm distilled water until no acid residue was observed in Litmus test → Dipping in NaOH solution (0.1N) overnight → Washing with warm distilled water

until no acid residue was observed in litmus test → Packing into the absorption column
Ready for operation

2. The door of the smoke generator was tightly closed to prevent unnecessary leakage of smoke into the surrounding.
3. The fire in the burner was kept at minimum. This had two advantages; first that it saved the LPG gas being used; and second, it controlled the heating temperature.
4. Cool Distilled water (Temperature not greater than 10°C) was used for production to prevent miscalculations due to buffering potential of non-distilled water. Warm water was not used because solubility of PAHs increase at higher temperatures (Anyakora, 2013)
5. Frequent sprinkling of water at an interval of 15 min was done in the heating zone to control the temperature rise.

3.2.4 Testing the assembly

The assembly was tested for its successful operation using sawdust of hardwood from *Sal (Shorea robusta)*.

3.2.4.1 Leakage test

The assembly was tested for smoke leakage by visual assessment.

3.2.4.2 Test for smoke transfer

The assembly was tested whether smoke transferred to the absorption column. Since smoke can be visibly seen, visual assessment was done while operating the assembly.

3.2.4.3 Yield of liquid smoke

The final concentration of the liquid smoke flavoring was accessed based on titrable acidity (% w/v in terms of acetic acid) of the solution. Once the constant level of titrable acidity was detected, the production was stopped. The yield was then measured in volume of primary liquid smoke produced per gram of sawdust burnt in an interval of one hour; this can be represented by following formula

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

where,

L = liters of liquid smoke produced in one batch of production

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

t = time taken by liquid smoke to reach maximum possible acidity.

3.2.5 Heating temperature

No direct contact of sawdust with fire was observed. So, it can be inferred that the heating temperature of the sawdust in smoke generator was lower than ignition temperature because sawdust was heated on a plate. Further temperature was controlled by sprinkling water from time to time in the region where saw dust was being heated.

The design and working of the assembly were such that the temperature of sawdust being heated was lower than ignition temperature.

3.2.6 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.

3.2.7 Production from local woods

Four of local wood varieties were selected for preparation of liquid smoke flavoring. These were Pine (*Pinus*), Sal (*Shorea robusta*), Pomelo (*Citrus maxima*), and Plum (*Prunus*). Pine belongs to softwood variety, Sal belongs to hardwood variety, Pomelo belongs to *Citrus* fruit variety, and Plum belongs to sweet fruit variety. These woods represented the types of woods majorly used for producing smoke flavorings around the world.

3.2.8 Liquid Smoke formulation

Liquid smoke formulation was prepared from the primary smoke condensate by mixing it with 5% (v/v liquid smoke) vinegar as mentioned by Pearson and Gillet (1996).

3.2.9 Analysis of liquid smoke

Smoke flavorings were analyzed for total acidity, pH, total solids and TSS.

- pH was measured using a hand pH Meter
- Total Solids was measured as explained by the method of Simon *et al.* (2005).
- Total Titrable acidity was measured as explained by Sadler and Murphy (2010).

3.2.10 Sensory evaluation

The prepared liquid smoke flavors were subjected to sensory evaluation for their acceptance as a flavoring additive. These liquid smokes were applied to pork ham by dipping method. They were dipped overnight in refrigerator in temperatures of approximately 4°C. The pork ham used for sensory evaluation was cut into uniform thickness of about 1 cm and a surface area of 9 cm² such that each piece weighed approximately 50 g. The sensory was carried out in three stages where first two stages involved optimization of dipping volume per gram of pork ham (% , ml/g) and the final one involved the comparison of prepared smoke flavors with commercial liquid smoke currently used in a local meat processing industry. Each of these stages were carried out for four wood varieties independently. After optimization of dipping volume concerned with liquid smoke of every wood variety, the optimized samples were formulated by adding vinegar solution in concentrations of 5 ml vinegar per 100 ml of liquid smoke. In third sensory, these optimized liquid smokes prepared from wood varieties were compared with each other and with the market sample.

Preliminary sensory evaluation: Preliminary sensory evaluation was carried out for finding out the range of application of the product. Repeated trials were done to find out the dipping volumes that imparted weakest and strongest flavor in pork ham. This gave the minimum volume and the maximum volume in which the ham needed to be dipped for best sensory results. Accordingly, the range of dipping volume in ml per gram of meat was identified.

Sensory evaluation of liquid smoke applied to Pork ham (dipping volume optimization): This sensory evaluation was carried out being based on the details obtained from preliminary sensory evaluation. Once the range of dipping volume was identified, experimental design was set up using software Stat-ease Design Expert (trial) version 11.1.2.0. Response Surface

methodology Optimal (custom) design was followed in which value of numeric factors was set to 1. The factor name being Dipping volume; its units being % (ml/g); low level being 50% and high level being 200%. Quadratic design model was selected with design space as 'best', and optimality as 'Integrated Variance (IV)' optimal design. Replicate points were set to 6. Blocks were set to '3', since runs were distributed to three sets, 7:00 am, 5:30 pm and 7:01 am next morning. The application generated 16 runs/variations out of which non-repeating variations were subjected to sensory evaluation. This stage was carried out independently for all four wood varieties and best among each of them were selected by ANOVA of received mean sensory scores.

Cured pork hams each of 50 g (volume = 9 cm³) were dipped overnight in prepared smoke flavor in dipping volumes generated by the experimental design. In the morning, these samples were taken out from the flavor solution and wiped with blotting paper. Then they were baked at 325°F for 20 min in gas oven. After baking these samples were subjected to descriptive sensory evaluation (taste and aroma) being based on directions by Singh and Maharaj (2014). Responses were recorded accordingly and analyzed (one-way ANOVA) for best sample based on sensory scores.

During the evaluation procedure, the variables X, A, B, C, D, E, F, G, H and I were used to represent dipping volumes of 0% (ml/g), 50% (ml/g), 69.53% (ml/g), 111.69% (ml/g), 130.25% (ml/g), 149%, 165.5% (ml/g), 182% (ml/g) and 200% (ml/g) respectively.

Comparison of market and laboratory prepared liquid smoke applied to pork ham by sensory evaluation: This sensory evaluation involved comparison on aroma and taste of optimized smoke flavors with that of commercial flavor [Star Brand Smoke Flavor, Artificial Smoke Flavoring Agent"]. The market flavor was used according to instructions given in bottle. Total solids and acidity were made equivalent to the laboratory prepared primary liquid smoke. Meat of 50 g was then dipped in the prepared market sample. Similarly, lab prepared solutions (in best one as given by sensory stage 2) of all four wood varieties were subjected to formulation based on Pearson and Gillet (1996) and applied in meat by dipping meat samples in them. These samples were subjected to descriptive sensory analysis and analyzed for taste and aroma. The comparison was done on taste and aroma by performing ANOVA of sensory scores obtained for all four wood varieties and market sample. The sample that received the best scores was recommended for use.

3.2.11 Statistical analysis

The generation of optimized dipping volumes, using Response Surface Methodology (RSM) for was done by using Design Expert v.11.1.2.0 (trial). Similarly, statistical analyses (ANOVA and multiple comparisons) for analyzing the obtained sensory scores, as well as the physicochemical properties of prepared liquid smokes were carried out using GenStat v.12.

3.2.12 Graphical designs

The graphical designs were made using Sketchup, v. 2017.

3.2.13 Construction and Production costs

The construction and production were carried out from financial grant obtained from Central Campus of Technology, Dharan-14.

PART IV

Results and discussions

A survey was carried out to know about the knowledge of liquid smoke in major places of Dharan, where smoked (*sekuwa/barbeque*) meats are sold and traded in local market. Accordingly, four primary liquid smoke were prepared from four local varieties of wood viz. *Shorea*, *Pinus*, *Citrus* and *Prunus*. They were applied to pork ham by dipping process and baked. Then they were subjected to descriptive sensory evaluation based on aroma and taste. Later on, once the optimum dipping volume for each of the wood variety was identified, they were formulated and compared with market sample by applying in pork ham by similar dipping process.

4.1 Results of survey

The survey involved a total of 12 questions and the results of survey has been shown graphically in Appendix D. The corresponding questions of answers have been presented in Appendix C. Results of survey indicated that majority of people had no idea on use of liquid smoke. Answers received in question on whether people consumed smoked meat items or not, suggested that that 95% of people answering the survey consumed or sold smoked meat items. Similarly, answers received in questions on whether people involved in local smoked meat businesses had any knowledge on liquid smoke suggested that among the people who undertook survey, none of them had knowledge on use of liquid smoke. Moreover, answers received also suggest that one of the reasons why people undertaking survey had no any knowledge on use of liquid smoke, could also be the lack of production of liquid smoke locally.

It was thus concluded that there was minimum knowledge of people regarding use of liquid smoke. Moreover, lack of production of liquid smoke on a local level was also a cause of very low knowledge of liquid smoke amongst local people involved in meat businesses.

Therefore, it was reasonable to construct an assembly that produced liquid smoke. Hence, liquid smoke production assembly was successfully designed and tested in meat pilot plant of Central Campus of Technology.

4.2 Modified liquid smoke production assembly

The assembly was constructed in terrace of Meat Pilot Plant of Central Campus of Technology. The constructed assembly was according to the design explained in Section 3.2.1. It has been shown in plates P5, P6 and P13. The plates P1 - P4 show the various snaps taken during construction works.

4.3 Operation and testing of assembly

4.3.1 Smoke leakage test

No leakage was visibly seen in the assembly except the smoke generation section. Negligible amount smoke seemed to leak from the door of smoke generator. This can be neglected since smoke is volatile and is prone to escape when it finds a way.

4.3.2 Test for smoke transfer

Smoke successfully transferred to the absorption column and was visibly seen coming out of the upper region of the absorption column.

4.3.3 Yield

Acidity as % (w/v) acetic acid was taken as the standard for production. Hollenbeck (1963) mentions that acidity of liquid smoke produced should be between 3 - 8% (w/w). It has also been mentioned in by Simon *et al.* (2005) that acidity of some commercial liquid smoke ranged from 0.7 - 20% (w/v). A much lower value of acidity has been observed during the study by Guillén and Ibargoitia (1996) where they have studied the change in acidity of smoke products with increasing temperatures and incorporate a range of 0.0557 to 0.670% (w/v) as well.

However, it was observed that acidity of primary liquid smoke produced showed no significant increment between the interval on 9 and 10 h of production time. At this time, the acidity reached 0.2% (w/v). Therefore, liquid smoke was produced until this maximum possible concentration was reached.

Hence, this concentration was considered as the maximum concentration possible; and yield was calculated accordingly. The increment pattern of titrable acidity is given in Fig.

4.1. The corresponding statistical analysis (ANOVA) has been shown in Table A.21 and Table A.22.

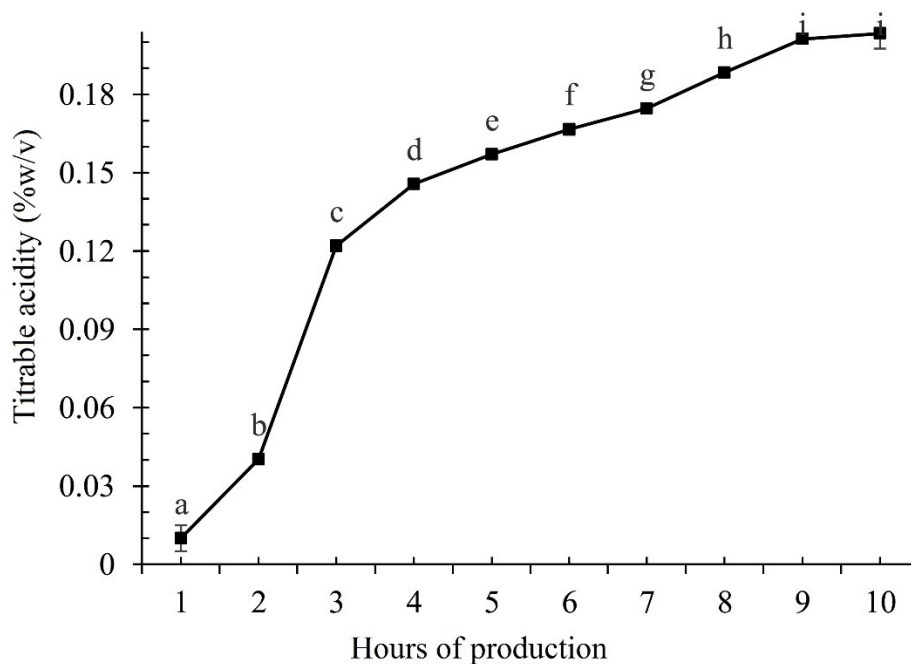


Fig. 4.1 Change in titrable acidity with time of production

In the Fig. 4.1, two different alphabets represent significantly different ($p < 0.05$) values. The vertical bars on each of the values represent error bars (standard deviations)

The yield of the constructed liquid smoke flavoring production assembly was thus calculated to be 2 L per 10 h per 3 kg of sawdust consumed.

4.4 Production of liquid smoke

Liquid smoke was prepared from four local wood varieties. Their physicochemical properties were determined before and after formulation.

4.4.1 Sensory and physicochemical properties

Color: The color of produced flavors was amber color to reddish yellow. Burdock (2010) has also mentioned liquid smoke as a yellow to red liquid used for imparting smoke flavor.

Odour: The odour of the flavor was characteristic smoky odor. Each of different wood varieties had their characteristic smell. This could be because liquid smoke produced from

different woods have different compositions (Montazeri *et al.*, 2013). This product has shown convincing results when applied to meat to give a smoky taste which should justify for the definition given by Philip (2005).

Chemical properties of the liquid smoke flavoring prepared has been tabulated in Table 4.1.

It can be seen in Table 4.1 that acidity has increased after formulation. The formulation involved addition of vinegar solution (5% acetic acid solution) in concentrations of 5 ml vinegar per 95 ml of liquid smoke solution. Only vinegar was added since the original solution was very dilute. This addition showed significant increment in the acidity. Total solids were negligible in both cases. The values of pH decreased after addition of vinegar in all these solutions.

Table 4.1 Chemical Properties of Liquid Smoke

| S.N | Wood variety (scientific name) | Parameters under study | Values before formulation | Values after formulation |
|-----|-----------------------------------|---------------------------|------------------------------|-----------------------------|
| 1 | <i>Shorea robusta</i> | Total Solids | Negligible | Negligible |
| | | Acidity (%) | 0.22 ^a ± 0.0219 | 0.41 ^c ± 0.018 |
| | | pH | 5.5 ^{a'} ± 0.057 | 4.8 ^{a''} ± 0.057 |
| 2 | <i>Pinus roxburghii</i> | Total Solids | Negligible | Negligible |
| | | Acidity (%) | 0.18 ^a ± 0.011 | 0.39 ^c ± 0.018 |
| | | pH | 5.5 ^{a'} ± 0.1 | 4.9 ^{a''} ± 0.057 |
| 3 | <i>Prunus</i> | Total Solids | Negligible | Negligible |
| | | Acidity (%) | 0.2 ^a ± 0.014 | 0.4 ^c ± 0.012 |
| | | pH | 5.4 ^{a'} ± 0.115 | 4.8 ^{a''} ± 0.057 |
| 4 | <i>Citrus maxima</i> | Total Solids | Negligible | Negligible |
| | | Acidity (%) | 0.19 ^a ± 0.047 | 0.4 ^c ± 0.009 |
| | | pH | 5.4 ^{a'} ± 0.115 | 4.8 ^{a''} ± 0.057 |
| 5. | Commercial sample | Total Solids | 1.5% | Negligible |
| | | Acidity (%) | 3.6 ^b ± 0.047 | 0.42 ^c ± 0.007 |
| | | pH | 2.9 ^{b'} ± 0.012 | 4.8 ^{a''} ± 0.045 |

In Table 4.1, acidity is in terms of % acetic acid (w/v). The values given are means of triplicates. Similar alphabets in superscripts represent that the values are significantly similar to each other ($p < 0.05$). Statistical analysis (ANOVA) of the properties of various liquid smokes have been shown in Table A.23 - A.26.

The liquid smoke primary product prepared in the pilot plant was dilute when compared to what has been written in literature. It is lighter in color, has lower acidity, higher pH, and almost negligible total solids when compared to the market sample. This can be attributed to the use of gravels in packing of the absorption/solubilization column. Although dilute, this prepared flavor developed pleasant aroma and taste and had characteristic smoke flavor in it, which when applied to meat by dipping process generated very satisfactory results as obtained in Section 4.4.

Total solids

As it can be seen in Table 4.1, total solids for all of the samples is negligible except for the market sample. Simon *et al.* (2005) has mentioned total solids as a parameter to be studied in liquid smokes. However, no specifications can be found. Total solids represent both soluble and insoluble solids. Insoluble solids in liquid smoke can represent tarry fraction (which may further contain PAHs) in liquid smoke according to Underwood and Shoop (1998). Underwood and Shoop (1998) also mention that a typical liquid smoke may contain a soluble tar ranging from 0 - 2% (w/v). If that be the case, then negligible total solids could be advantageous.

pH and acidity

As it can be seen in Table 4.1 and Tables A.23 - A.26; titrable acidity of liquid smokes made from Sal, Pine, Pomelo and Plum are 0.22^a %, 0.18^a %, 0.19^a % and 0.2^a % respectively. These values are lower compared to commercial flavors mentioned by Montazeri *et al.* (2013) and Simon *et al.* (2005). However, these values are still larger than values seen during the study by Guillén and Ibargoitia (1996). So, acidity as such should not pose a problem in recognition of the prepared primary liquid smoke.

Moreover, it has been mentioned by Underwood and Shoop (1998) that high acid smoke flavor may hinder roles of nitrites in curing of meat because on reduction of pH of meat

below 5.5, conversion of nitrite to nitric oxide happens so fast that nitric oxide may further change to nitrogen dioxide, which is a toxic reddish-brown gas. So, for cured meats, smoke flavors with low acidity are preferred. If used liquid smokes of higher acidity, they are applied after curing with sufficient time in between so that nitric oxide binds with myoglobin. The prepared samples had a pH range of 5.5 - 5.6. After addition of vinegar though, the pH decreased to 4.8 - 4.9. Hence, for precautionary reasons, they were applied to meat after curing and holding for 2 h in chilled storage.

Similarly, it has been mentioned by Pearson and Gillet (1996) that organic acids play the function of coagulating the surface proteins during smoking and have minor preservative actions. Since no experiments were carried out on preservative actions of liquid smoke; this means that, it could not be confirmed that the prepared liquid smoke had no major role in coagulation of surface protein and preservative actions. This poses no threat to its potential use as preservative and coagulator as well; but only after related experiments.

In addition to this, the commercial production of liquid smoke involves further processes such as distillation, etc. (Meier, 2009) that serve to make a concentrate liquid smoke solution which has not been done during this production. Underwood and Shoop (1998) mentions that there are many other different ways to manufacture liquid smoke for commercial purposes but does not clearly mention what these are because these are corporate secrets and have patented information. This provides us a good leverage on developing our own formulations for commercialization. So, further processing certainly could pave way for producing more concentrated and improvised products.

4.5 Results of sensory evaluation

Liquid smoke prepared from various wood were subjected to descriptive sensory evaluation for aroma and taste. Every wood was analyzed independently for its optimum dipping concentration in first two sages of evaluation. Once the best dipping concentration was identified, they were applied and compared with a market sample. The best among these was accordingly recommended for use.

4.5.1 Preliminary sensory

Preliminary sensory evaluation for *Shorea*, *Pinus*, *Citrus* and *Prunus* revealed that dipping volume of 50% (ml/g) showed no remarkable flavor development whereas dipping volume of 200% showed excessive flavor development. So, it was concluded that optimum range of dipping volume was 50 - 200% (ml/g). Thus, liquid smoke prepared were accordingly subjected to second stage sensory evaluation which optimized the dipping volume.

4.5.2 Dipping volume optimization

This sensory was carried out after applying RSM for a single factor (dipping volume) to get responses on aroma and taste. The table generated by Design Expert v.11 has been given in Appendix F, according to which sensory was carried out.

The sensory data received for optimization of dipping volume for primary liquid smoke prepared from *Shorea* has been given in Fig. 4.2; that for *Pinus* in Fig. 4.3; that for *Citrus* in Fig. 4.4 and that for *Prunus* in Fig. 4.5. Results of statistical analyses have been given in Table A.1 - A.16

Shorea robusta

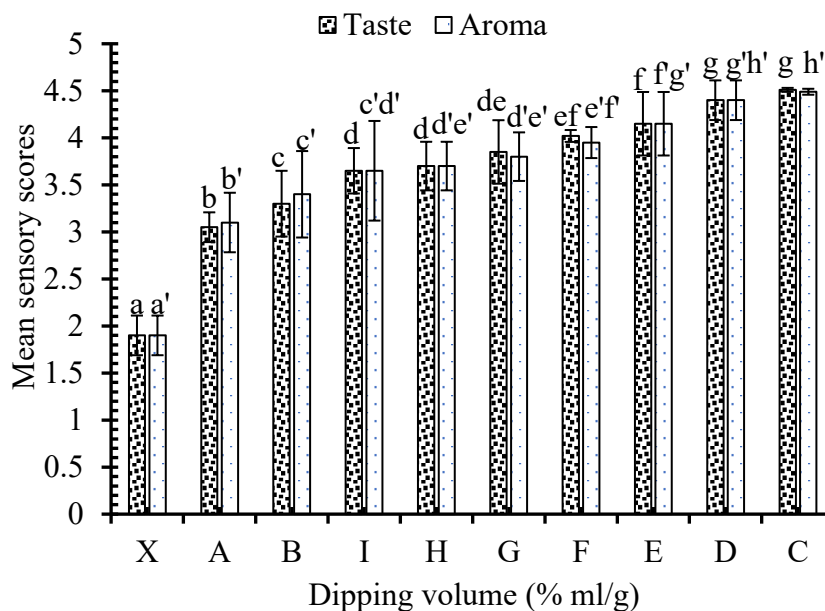


Fig. 4.2 Sensory scores received for optimization of dipping volume of primary liquid smoke made from *Shorea*

Similar alphabets in superscripts in Fig. 4.2 represent that the values are significantly similar to each other ($p < 0.05$). The statistical analysis (ANOVA ad multiple comparisons) have been shown in Tables A.13 - A.16.

The results in Fig. 4.2 show that highest score for aroma, and taste, both were given to dipping volume of 89.75% (ml/g). Although, scores for dipping volumes 111.69% (ml/g) and 89.75% (ml/g) are insignificant from each other, dipping volume of 89.75% (ml/g) was considered best for economic reasons. The mean sensory scores for aroma and taste given to dipping 89% (ml/g) are 4.4^{g'h'} and 4.51^g respectively.

Pinus roxburghii

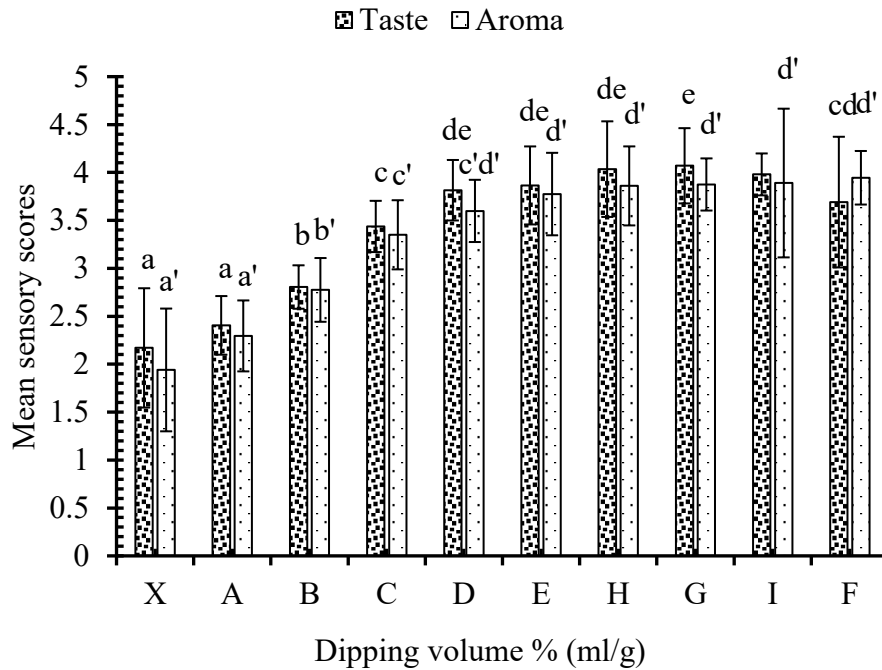


Fig. 4.3 Sensory scores received for optimization of dipping volume of primary liquid smoke made from *Pinus*

Similar alphabets in superscripts in Fig. 4.3 represent that the values are significantly similar to each other ($p < 0.05$). The statistical analysis (ANOVA ad multiple comparisons) have been shown in Tables A.9 - A.12

It can be seen in Fig. 4.3 that highest score for aroma as well as taste, was received by dipping volume of 111.69% after which the volumes had no significant differences between

them. The score received for aroma and taste were 3.59^{c'd'} and 3.81^{de}. Hence, dipping volume of 111.69% was taken as the best dipping volume.

Citrus maxima

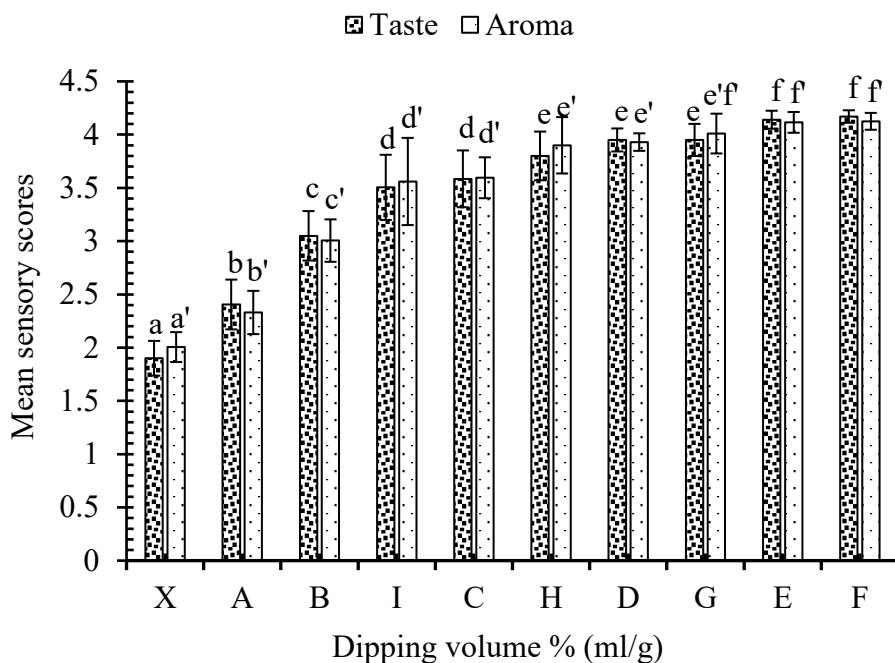


Fig. 4.4 Sensory scores received for optimization of dipping volume of primary liquid smoke made from *Citrus*

Similar alphabets in superscripts in Fig. 4.4 represent that the values are significantly similar to each other ($p < 0.05$). The statistical analysis (ANOVA and multiple comparisons) have been shown in Tables A.5 - A.8

It can be seen in Fig. 4.4 The mean sensory scores received for aroma and taste had no significant increment after dipping volume of 130.25% (ml/g). These scores received for aroma and taste were 4.12^f. and 4.14^f respectively. Hence, dipping volume of 130.25% (ml/g) was considered the best.

Prunus

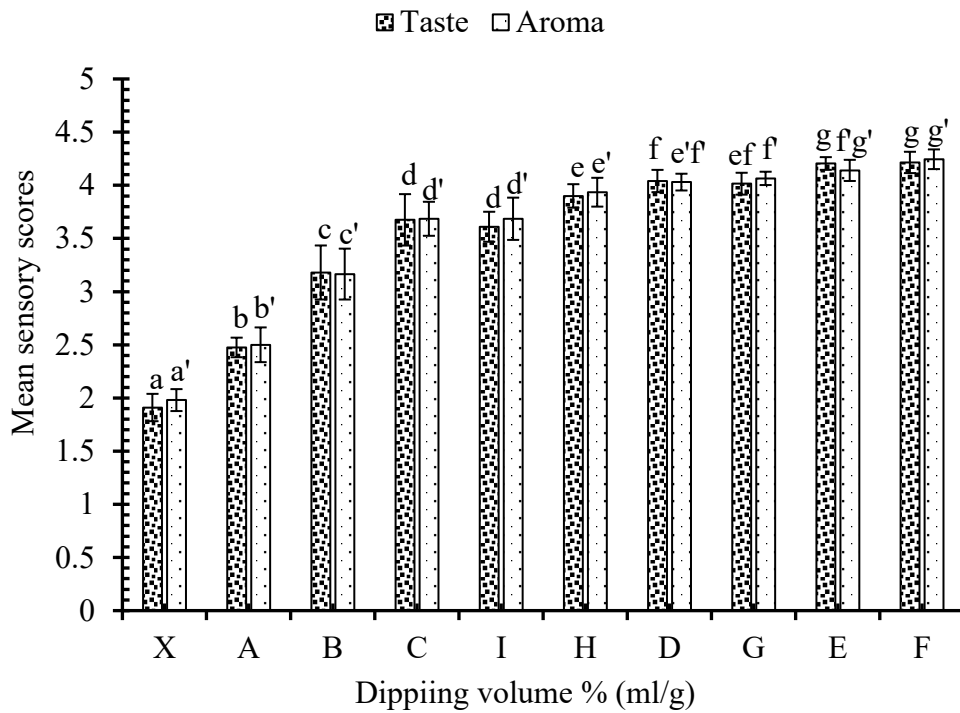


Fig. 4.5 Sensory scores received for optimization of dipping volume of liquid smoke made from *Prunus*

Similar alphabets in superscripts in Fig. 4.5 represent that the values are significantly similar to each other ($p < 0.05$). The statistical analysis (ANOVA and multiple comparisons) have been shown in Tables A.1 - A.4.

The mean sensory scores given for aroma and taste had no significant increment after dipping volume 130.25% (ml/g). The score for aroma and taste were found to be 4.14^{f'g'} and 4.21^g respectively. Hence, dipping volume of 130.25 % (ml/g) was selected as the best.

4.5.3 Comparisons with market (sensory)

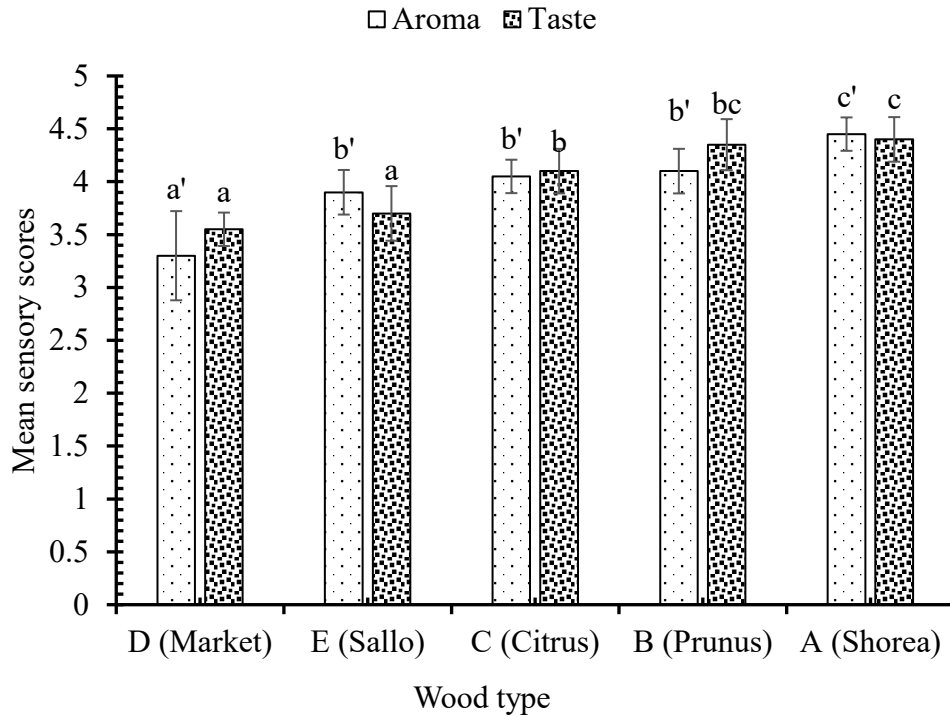


Fig. 4.6 Sensory scores received for market and laboratory prepared liquid smokes applied to pork ham

Similar alphabets in superscripts in Fig. 4.6 represent that the values are significantly similar to each other ($p < 0.05$). The statistical analysis (ANOVA and multiple comparisons) have been shown in Tables A.17 - A.20.

From Fig. 4.6 we can infer that the best mean sensory score for aroma of ham treated with liquid smoke was received by *Shorea*. The score was $4.45 \pm 0.1581^{c'}$. However, in case of taste, both *Prunus* and *Shorea* received values that were insignificant from each other. The scores were 4.35 ± 0.2415^{bc} and 4.4 ± 0.2108^c respectively.

But the score received by *Shorea* is still greater than *Prunus*. Hence considering the significant score received for aroma as well, we can conclude that the smoke flavor prepared from *Shorea robusta* has the best sensory appeal and is accordingly recommended for use at a dipping volume of 89.75% (ml/g).

4.6 Construction and Production cost

The construction costs incurred was Rs. 22,500. Excluding the construction costs, the overall cost of production for primary liquid smoke was estimated to be Rs. 50.36 per liter. The calculation of this production cost has been presented in Appendix G.

PART V

Conclusions and recommendations

Hence, following conclusions can be inferred from this work.

1. Liquid smoke production assembly was successfully designed and tested being based on original design by Hollenbeck (1963), after few modifications on a pilot plant scale in meat pilot plant of Central Campus of Technology. The liquid smoke so produced had a maximum acidity of 0.2% (w/v). Production time was optimized based on this acidity and was found to be 10 h. The cost of production was Rs. 50.36 per liter of primary liquid smoke produced.
2. Liquid smoke was prepared from four local wood varieties, viz, *Shorea*, *Pinus*, *Citrus* and *Prunus* under optimized production time of 10 h. Their physicochemical properties were analyzed. It was found that the physicochemical properties (total solids, pH and titrable acidity) had no significant differences between them. However, every wood variety gave characteristic odour.
3. Prepared liquid smokes were applied to pork ham by dipping. Preliminary sensory on taste and aroma provided the range of volume to be used for dipping. Then, dipping volume was optimized using RSM and descriptive sensory evaluation was carried out for taste and aroma. Optimized dipping volumes (based on taste and aroma) for liquid smokes of *Shorea*, *Pinus*, *Citrus* and *Prunus* were found to be 89.75% (ml/g), 111.69% (ml/g), 130.25% (ml/g) and 130.25% (ml/g) respectively. Prepared liquid smokes were then formulated by adding 5% vinegar solution (5 ml vinegar in 95 ml primary liquid smoke).
4. Pork ham were dipped in these formulated liquid smokes as well as market liquid smoke and were subjected to descriptive sensory evaluation on taste and aroma. It resulted that wood variety *Shorea robusta* received best sensory scores of 4.45 ± 0.1581^c and 4.4 ± 0.2108^c for aroma and taste respectively. Hence, *Shorea* was recommended for primary liquid smoke production.

Thus, it can be concluded that liquid smoke production assembly can be successfully constructed and primary liquid smokes with acidity 0.2% (w/v) can be prepared in a pilot plant scale being based on Hollenbeck's design with few modifications.

Likewise, following things are recommended for betterment and improvement of the produced product.

1. In the design, instead of plate heating, heating in an induction heater (where temperature can be controlled) can be performed. This will minimize PAHs content.
2. The design should be updated for installment of provisions for its cleaning (removal of deposited tars and particulate matters). Specially, the settling tank and the primary smoke transfer pipe should have a small cleaning hole at their bottom, which can be opened and the set can be cleaned to prevent path blockade by particulate matters in due course of time.
3. The best way for its concentration is membrane (osmosis) concentration. Therefore, the design should be expanded accordingly so that concentration of the produced primary product can be performed.
4. The prepared liquid smoke should be analyzed for its chemical properties., specially benzo (a) pyrenes and flavor compounds (carbonyls and phenols). For this, GC-MS or HPLC is required.

Summary

A survey suggested that no locally produced liquid smoke flavor was available in Dharan's meat market. Production assembly was redesigned and constructed according to local convenience and modifications without affecting the basic principle of productions, in meat pilot plant of CCT, Dharan being based on information by Parliament (2003) and Hollenbeck (1963). Certain modifications were made for potential improvisation. The major modifications in the design were the use of plate heating instead of direct ignition, introduction of swan neck primary smoke transfer pipe, placement of outlet pipe of settling chamber at a higher altitude than the inlet pipe. Packing of absorption/solubilization column with gravels of size 20 - 25 mm. The assembly was operated on a manual basis, by heating saw dust on a plate and passing of volatile section of the same so produced in a direction counter current to the direction of recirculating water thereby solubilizing the soluble components and formation of primary smoke condensate accordingly. Produced liquid smoke was dilute than the commercial counterpart. It had lower acidity, pH and total solids than the commercial counterpart. The pH of the prepared smoke flavors ranged from 5.5 to 5.6, its acidity being 0.2%. This product was then applied to meat by dipping overnight in varying concentrations (ml/g) and subjected to sensory analysis by semi trained panelists. Sensory was done in three stages and studied for two parameters (viz. aroma and taste) in which the first was a preliminary sensory and second was for optimization of dipping volume. The third stage sensory compared the prepared flavor and market flavor samples. The second stage sensory resulted that dipping volume of 89.75% (ml/g), 111.69% (ml/g), 130.25% (ml/g), and 130.25% (ml/g) were best for primary liquid smokes of *Shorea*, *Pinus*, *Citrus* and *Prunus* respectively. A liquid smoke formulation was also developed by adding 5% vinegar solution to it and applied to meat by dipping overnight. The market sample was also applied by same dipping process in concentrations as per the instructions. They were then subjected to ANOVA test with other four samples from four different woods. The wood variety *Shorea* received the best score of $4.450^{\circ} \pm 0.1581$ for aroma and for $4.4^{\circ} \pm 0.2108$ taste.

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Appendices

Appendix A

Statistical analyses of sensory scores received for optimization of dipping volumes of liquid smoke applied to pork ham.

Table A.1 ANOVA for aroma of *Prunus*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|----------|---------|--------|-------|
| Sample_liq_smoke_treatment | 9 | 52.26560 | 5.80729 | 281.07 | <.001 |
| Residual | 90 | 1.85950 | 0.02066 | | |
| Total | 99 | 54.12510 | | | |

Table A.2 Fisher's protected LSD test for sensory scores for aroma of *Prunus*

| Dip Volume | Mean Sensory Score |
|--------------------|------------------------------|
| X = 0% (ml/g) | 1.980 ± 0.1032 ^a |
| A = 50% (ml/g) | 2.500 ± 0.1632 ^b |
| B = 69.53% (ml/g) | 3.165 ± 0.2392 ^c |
| C = 89.75% (ml/g) | 3.685 ± 0.1599 ^d |
| I = 200% (ml/g) | 3.685 ± 0.1986 ^d |
| H = 182% (ml/g) | 3.935 ± 0.1355 ^e |
| D = 111.69% (ml/g) | 4.03 ± 0.0788 ^{e,f} |
| G = 165.5% (ml/g) | 4.065 ± 0.0625 ^f |
| E = 130.25% (ml/g) | 4.14 ± 0.0994 ^{f,g} |
| F = 149% (ml/g) | 4.245 ± 0.0926 ^g |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.3 ANOVA for taste of *Prunus*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|----------|---------|--------|-------|
| Sample_liq_smoke_treatment | 9 | 54.43863 | 6.04874 | 280.86 | <.001 |
| Residual | 90 | 1.93825 | 0.02154 | | |
| Total | 99 | 56.37687 | | | |

Table A.4 Fisher's protected LSD test for sensory scores for taste of *Prunus*

| Dip Volume | Mean Sensory Scores |
|--------------------|------------------------------|
| X = 0% (ml/g) | 1.910 ± 0.1286 ^a |
| A = 50% (ml/g) | 2.475 ± 0.0920 ^b |
| B = 69.53% (ml/g) | 3.180 ± 0.2540 ^c |
| I = 200% (ml/g) | 3.601 ± 0.1410 ^d |
| C = 89.75% (ml/g) | 3.675 ± 0.2418 ^d |
| H = 182% (ml/g) | 3.900 ± 0.1105 ^e |
| G = 165.5% (ml/g) | 4.010 ± 0.1028 ^{ef} |
| D = 111.69% (ml/g) | 4.040 ± 0.1048 ^f |
| E = 130.25% (ml/g) | 4.205 ± 0.0598 ^g |
| F = 149% (ml/g) | 4.215 ± 0.1001 ^g |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.5 ANOVA for aroma of *Citrus*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|----------|---------|--------|-------|
| Sample_liq_smoke_treatment | 9 | 52.17363 | 5.79707 | 134.01 | <.001 |
| Residual | 90 | 3.89325 | 0.04326 | | |
| Total | 99 | 56.06688 | | | |

Table A.6 Fisher's protected LSD test for sensory scores for aroma of *Citrus*

| Dip Volume | Mean Sensory Score |
|--------------------|-------------------------------|
| X = 0% (ml/g) | 2.005 ± 0.1403 ^a |
| A = 50% (ml/g) | 2.330 ± 0.2030 ^b |
| B = 69.53% (ml/g) | 3.005 ± 0.1992 ^c |
| I = 200% (ml/g) | 3.560 ± 0.4094 ^d |
| C = 89.75% (ml/g) | 3.595 ± 0.1921 ^d |
| H = 182% (ml/g) | 3.900 ± 0.2645 ^e |
| D = 111.69% (ml/g) | 3.930 ± 0.0823 ^e |
| G = 165.5% (ml/g) | 4.010 ± 0.1867 ^{e,f} |
| E = 130.25% (ml/g) | 4.115 ± 0.0973 ^f |
| F = 149% (ml/g) | 4.125 ± 0.0790 ^f |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.7 ANOVA for taste of *Citrus*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|----------|---------|--------|-------|
| Sample_liq_smoke_treatment | 9 | 52.92572 | 5.88064 | 148.05 | <.001 |
| Residual | 90 | 3.57475 | 0.03972 | | |
| Total | 99 | 56.50048 | | | |

Table A.8 Fisher's protected LSD test for sensory scores for taste of *Citrus*

| Dip Volume | Mean Sensory Score |
|--------------------|-----------------------------|
| X = 0% (ml/g) | 1.900 ± 0.1632 ^a |
| A = 50% (ml/g) | 2.400 ± 0.2338 ^b |
| B = 69.53% (ml/g) | 3.050 ± 0.2321 ^c |
| I = 200% (ml/g) | 3.505 ± 0.3059 ^d |
| C = 89.75% (ml/g) | 3.585 ± 0.2667 ^d |
| H = 182% (ml/g) | 3.800 ± 0.2285 ^e |
| D = 111.69% (ml/g) | 3.950 ± 0.1080 ^e |
| G = 165.5% (ml/g) | 3.950 ± 0.1509 ^e |
| E = 130.25% (ml/g) | 4.140 ± 0.0843 ^f |
| F = 149% (ml/g) | 4.170 ± 0.0586 ^f |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.9 ANOVA for aroma of *Pinus*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|---------|--------|-------|-------|
| Sample_liq_smoke_treatment | 9 | 48.5188 | 5.3910 | 26.94 | <.001 |
| Residual | 90 | 18.0125 | 0.2001 | | |
| Total | 99 | 66.5314 | | | |

Table A.10 Fisher's protected LSD test for sensory scores for aroma of *Pinus*

| Dip Volume | Dip Volume | Mean Sensory Score |
|--------------------|------------|-------------------------------|
| X = 0% (ml/g) | X | 1.940 ± 0.6402 ^a |
| A = 50% (ml/g) | A | 2.295 ± 0.3700 ^a |
| B = 69.53% (ml/g) | B | 2.775 ± 0.3327 ^b |
| C = 89.75% (ml/g) | C | 3.350 ± 0.3605 ^c |
| D = 111.69% (ml/g) | D | 3.599 ± 0.3244 ^{c,d} |
| E = 130.25% (ml/g) | E | 3.775 ± 0.4302 ^d |
| H = 182% (ml/g) | H | 3.860 ± 0.4121 ^d |
| G = 165.5% (ml/g) | G | 3.875 ± 0.2720 ^d |
| I = 200% (ml/g) | I | 3.890 ± 0.7756 ^d |
| F = 149% (ml/g) | F | 3.945 ± 0.2793 ^d |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.11 ANOVA for taste of *Pinus*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|---------|--------|-------|-------|
| Sample_liq_smoke_treatment | 9 | 45.1252 | 5.0139 | 28.10 | <.001 |
| Residual | 90 | 16.0580 | 0.1784 | | |
| Total | 99 | 61.1832 | | | |

Table A.12 Fisher's protected LSD test for sensory scores for taste of *Pinus*

| Dip Volume | Mean Sensory Score |
|--------------------|------------------------------|
| X = 0% (ml/g) | 2.170 ± 0.6223 ^a |
| A = 50% (ml/g) | 2.405 ± 0.3059 ^a |
| B = 69.53% (ml/g) | 2.805 ± 0.2266 ^b |
| C = 89.75% (ml/g) | 3.437 ± 0.2673 ^c |
| D = 111.69% (ml/g) | 3.815 ± 0.3156 ^{de} |
| E = 130.25% (ml/g) | 3.865 ± 0.4069 ^{de} |
| H = 182% (ml/g) | 4.035 ± 0.4989 ^{de} |
| G = 165.5% (ml/g) | 4.070 ± 0.3924 ^e |
| F = 149% (ml/g) | 3.980 ± 0.2188 ^{de} |
| I = 200(ml/g) | 3.691 ± 0.6815 ^{de} |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.13 ANOVA for aroma of *Shorea*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|----------|---------|-------|-------|
| Sample_liq_smoke_treatment | 9 | 50.60440 | 5.62271 | 58.81 | <.001 |
| Residual | 90 | 8.60400 | 0.09560 | | |
| Total | 99 | 59.20840 | | | |

Table A.14 Fisher's protected LSD test for sensory scores for aroma of *Shorea*

| Dip Volume | Mean sensory Scores |
|------------|--------------------------------|
| X | 1.900 ± 0.2108 ^a |
| A | 3.100 ± 0.3162 ^b |
| B | 3.400 ± 0.4594 ^c |
| I | 3.650 ± 0.5296 ^{c'd} |
| H | 3.700 ± 0.2581 ^{d'e'} |
| G | 3.800 ± 0.2581 ^{d'e'} |
| F | 3.950 ± 0.1649 ^{e'f} |
| E | 4.150 ± 0.3374 ^{f'g'} |
| D | 4.400 ± 0.2108 ^{g'h'} |
| C | 4.490 ± 0.0316 ^{h'} |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.15 ANOVA for taste of *Shorea*

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|------|----------|---------|-------|-------|
| Sample_liq_smoke_treatment | 9 | 52.76410 | 5.86268 | 98.81 | <.001 |
| Residual | 90 | 5.34000 | 0.05933 | | |
| Total | 99 | 58.10410 | | | |

Table A.16 Fisher's protected LSD test for sensory scores for taste of *Shorea*

| Dip Volume | Mean sensory Scores |
|--------------------|------------------------------|
| X = 0% (ml/g) | 1.900 ± 0.2108 ^a |
| A = 50% (ml/g) | 3.050 ± 0.1581 ^b |
| B = 69.53% (ml/g) | 3.300 ± 0.3496 ^c |
| I = 200% (ml/g) | 3.650 ± 0.2415 ^d |
| H = 182% (ml/g) | 3.700 ± 0.2581 ^d |
| G = 165.5% (ml/g) | 3.850 ± 0.3374 ^{de} |
| F = 149% (ml/g) | 4.020 ± 0.0632 ^{ef} |
| E = 130.25% (ml/g) | 4.150 ± 0.3374 ^f |
| D = 111.69% (ml/g) | 4.400 ± 0.2108 ^g |
| C = 89.75% (ml/g) | 4.510 ± 0.0210 ^g |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.17 ANOVA for stage 3 sensory on aroma

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------|-------------|-------------|-------------|-------------|--------------|
| Samples | 4 | 7.07000 | 1.76750 | 27.91 | <.001 |
| Residual | 45 | 2.85000 | 0.06333 | | |
| Total | 49 | 9.92000 | | | |

Table A.18 Fisher's protected LSD test for sensory 3 sensory scores for aroma

| Sample | Mean Sensory Score |
|---------------------|-----------------------------|
| D (Market) | 3.300 ± 0.4216 ^a |
| E (Sallo) | 3.900 ± 0.2108 ^b |
| C (<i>Citrus</i>) | 4.050 ± 0.1581 ^b |
| B (<i>Prunus</i>) | 4.100 ± 0.2108 ^b |
| A (<i>Shorea</i>) | 4.450 ± 0.1581 ^c |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.19 ANOVA for stage 3 sensory on taste

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---------------------|------|---------|---------|-------|-------|
| Samples | 4 | 5.83000 | 1.45750 | 30.51 | <.001 |
| Residual | 45 | 2.15000 | 0.04778 | | |
| Total | 49 | 7.98000 | | | |

Table A.20 Fisher's protected LSD test for sensory 3 sensory scores on taste

| Samples | Mean Sensory Score |
|---------------------|------------------------------|
| D (Market) | 3.550 ± 0.1581 ^a |
| E (<i>Sallo</i>) | 3.700 ± 0.2581 ^a |
| C (<i>Citrus</i>) | 4.100 ± 0.2108 ^b |
| B (<i>Prunus</i>) | 4.350 ± 0.2415 ^{bc} |
| A (<i>Shorea</i>) | 4.400 ± 0.2108 ^c |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.21 Analysis of variance for observed titrable acidity during production

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---------------------|------|-----------|-----------|---------|-------|
| Hour_of_Production | 9 | 1.186E-01 | 1.317E-02 | 1975.35 | <.001 |
| Residual | 20 | 1.334E-04 | 6.669E-06 | | |
| Total | 29 | 1.187E-01 | | | |

Table A.22 Fisher's LSD test for observed titrable acidity during production

| Hour of Production | Mean Titrable acidity |
|--------------------|------------------------------|
| 1 | 0.010 ± 0.0050 ^a |
| 2 | 0.040 ± 0.0001 ^b |
| 3 | 0.122 ± 0.0010 ^c |
| 4 | 0.145 ± 0.0001 ^d |
| 5 | 0.157 ± 0.0010 ^e |
| 6 | 0.166 ± 0.00208 ^f |
| 7 | 0.175 ± 0.0005 ^g |
| 8 | 0.188±0.0011 ^h |
| 9 | 0.201±0.0057 ⁱ |
| 10 | 0.203±0.0057 ⁱ |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference (p<0.05)

Table A.23 ANOVA for titrable acidity of various (wood type) liquid smoke before formulation

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---------------------|------|-----------|-----------|------|-------|
| Wood_Variety | 3 | 0.0023780 | 0.0007927 | 1.07 | 0.415 |
| Residual | 8 | 0.0059380 | 0.0007422 | | |
| Total | 11 | 0.0083160 | | | |

Table A.24 ANOVA for titrable acidity of various (wood type) liquid smoke after formulation

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---------------------|------|-----------|-----------|------|-------|
| Wood_Variety | 3 | 0.0012729 | 0.0004243 | 1.82 | 0.221 |
| Residual | 8 | 0.0018613 | 0.0002327 | | |
| Total | 11 | 0.0031343 | | | |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference ($p < 0.05$)

Table A.25 ANOVA for pH of various (wood type) liquid smoke before formulation

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---------------------|------|---------|---------|------|-------|
| Wood_Variety | 3 | 0.00917 | 0.00306 | 0.31 | 0.821 |
| Residual | 8 | 0.08000 | 0.01000 | | |
| Total | 11 | 0.08917 | | | |

Table A.26 ANOVA for pH of various (wood type) liquid smoke after formulation

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---------------------|------|----------|----------|------|-------|
| Wood_Variety | 3 | 0.002500 | 0.000833 | 0.25 | 0.859 |
| Residual | 8 | 0.026667 | 0.003333 | | |
| Total | 11 | 0.029167 | | | |

Values after the symbol '±' represent standard deviations and same alphabets on the superscripts represent no significant difference ($p < 0.05$)

Appendix B

Table A.27 Solubility of various PAHs

| IUPAC Name | Log of Octanol Water Partition | Water Solubility (mg/L) | Diffusivity in Air (cm ² /s) | Diffusivity in Water (cm ² /s) | Permeability (cm/h) |
|--------------------------|--------------------------------|-------------------------|---|---|---------------------|
| Acenaphthene | 3.92 | 3.90000 | 0.042100 | 0.000008 | 0.133000 |
| Acenaphthylene | 3.94 | 16.100000 | 0.043867 | 0.000000 | 0.141000 |
| Anthracene | 4.45 | 0.434000 | 324.0000 | 0.000008 | 0.225000 |
| Benz [a] anthracene | 5.76 | 0.009400 | 0.051000 | 0.000009 | 0.948000 |
| Benzo [b] fluornathene | 5.78 | 0.001500 | 0.022600 | 0.000006 | 0.699000 |
| Benzo [k]fluoranthene | 6.11 | 0.000800 | 0.022600 | 0.000006 | 1.200000 |
| Benzo [a]pyrene | 6.13 | 0.001620 | 0.043000 | 0.000009 | 1.240000 |
| Benzo[g, h, i]perylene | 6.63 | 0.000260 | NA | NA | 2.000000 |
| Chrysene | 5.81 | 0.002000 | 0.024800 | 0.000006 | 1.030000 |
| Dibenz [a, h] anthracene | 6.54 | 0.001030 | 0.020200 | 0.000005 | 1.680000 |
| Fluoranthene | 5.16 | 0.260000 | 0.030200 | 0.000006 | 0.513000 |
| Fluorene | 4.18 | 1.890000 | 0.036300 | 0.000008 | 0.171000 |
| Indeno [1, 2, 3-cd] | 6.70 | 0.000190 | 0.019000 | 0.000006 | 2.230000 |
| Naphthalene | 3.30 | 31.000000 | 0.059000 | 0.000008 | 0.069400 |
| Phenanthrene | 4.46 | 1.150000 | NA | NA | 0.229000 |
| Pyrene | 4.88 | 0.135000 | 0.027200 | 0.000007 | 0.324000 |

Source: Anyakora (2007)

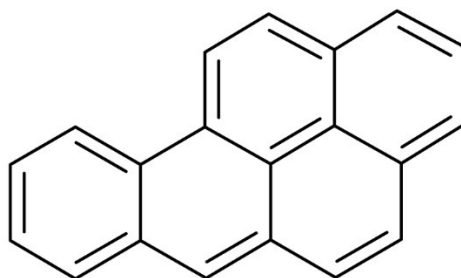


Fig A.1 Structure of Benzo [a] pyrene

Source: Anyakora (2007)

Appendix C

Please Read before answering

This questionnaire has been prepared for research work on liquid smoke, by Sajal Bhattarai, B. Tech Food (4th Year) Central Campus of Technology, Dharan-14

Questionnaire prepared by: *Sajal Bhattarai [B. Tech (Food) (batch 071-075)], CCT Dharan-14*

Purpose: *Questionnaire for Thesis work on "Liquid Smoke"*

Questionnaires related to knowledge of Liquid Smoke

Please answer the following questions by marking a tick (✓) in either of the boxes on the left-hand side.

1. Do you consume smoked meat items?
 Yes No

2. Do you sell smoked meat items?
 Yes No

3. How do you smoke meat and other food items you sell?
 Wood Smoke (direct) Liquid Smoke Others

4. How do you smoke meat and other food items you consume?
 Wood Smoke (direct) Liquid Smoke Others

5. Have you ever used liquid smoke?
 Yes No

6. Have you ever eaten items where liquid smoke has been used, to your knowledge?
 Yes No

7. Do you know what liquid smoke is ?
 Yes No
8. Do you know, that certain components of wood smoke (polycyclic aromatic hydrocarbons) are potential carcinogens?
 Yes No
9. Have you ever used liquid smoke in items you sell?
 Yes No
10. Do you know a place in Nepal, where liquid smoke is produced?
 Yes No
11. What do you think, is it because lack of production in Nepalese market, that you have less knowledge of liquid smoke ?
 Yes No
12. If there is production of liquid smoke in Nepal, what are the chances that you will be its regular customer?
 A lot Not Much Can't say

To be answered by the person who is answering this questionnaire

Name:

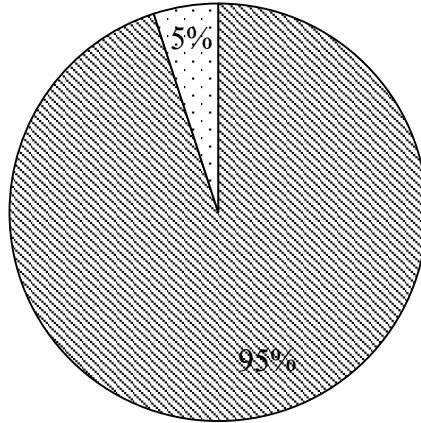
Address:

Contact number (at will):

Appendix D

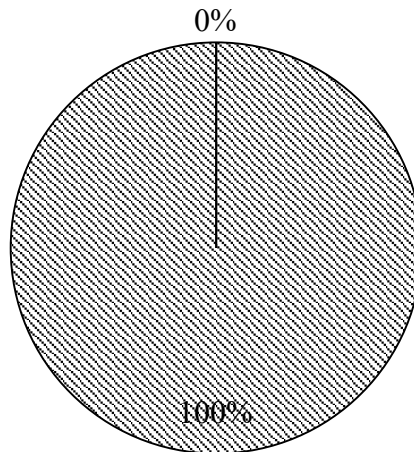
Results of Survey

▣ Yes □ No



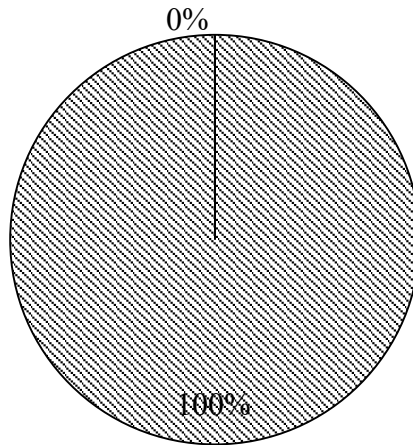
Question 1

▣ Yes □ No



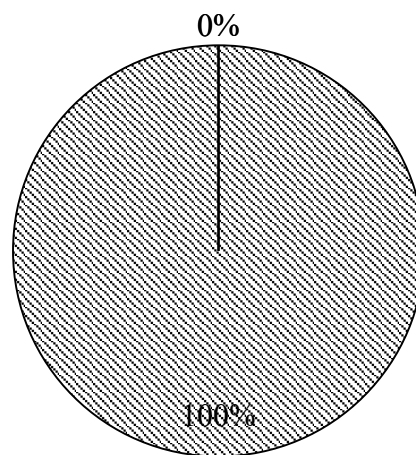
Question 2

☒ Wood Smoke ☐ Liquid Smoke



Question 3

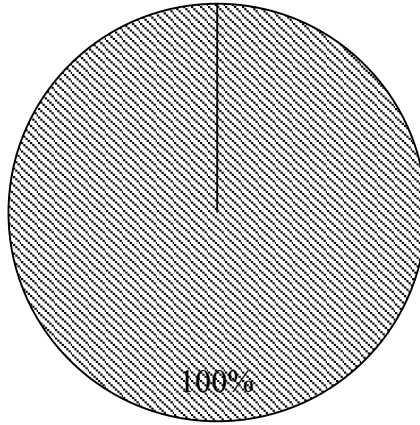
☒ Wood Smoke ☐ Liquid Smoke ☒ Others



Question 4

Yes No

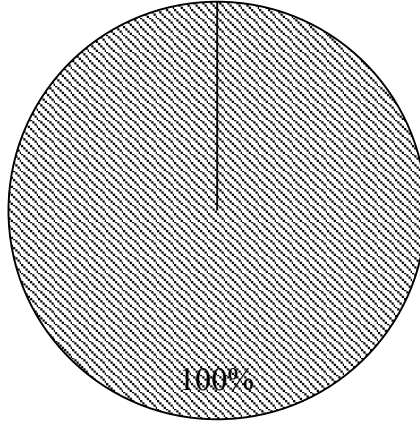
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Question 5

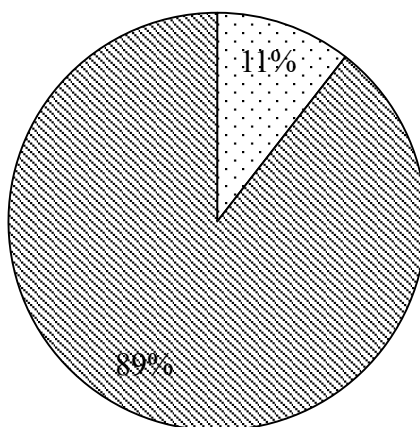
Yes No

0%



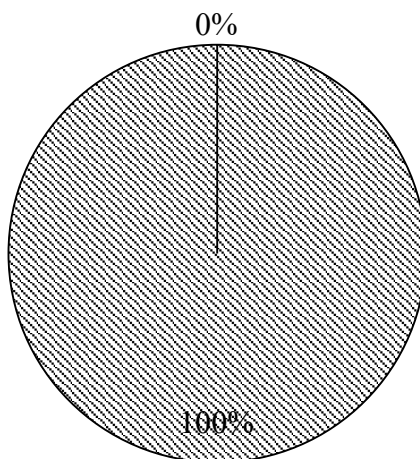
Question 6

Yes No



Question 7

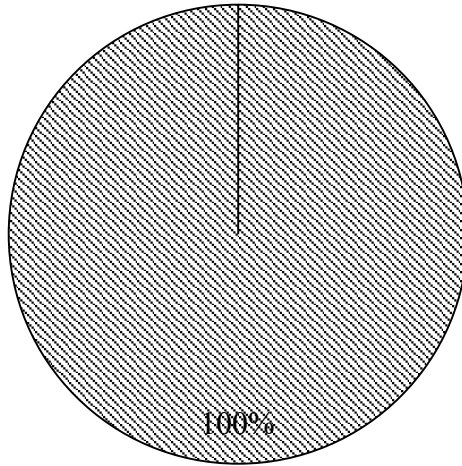
Yes No



Question 8

Yes No

0%

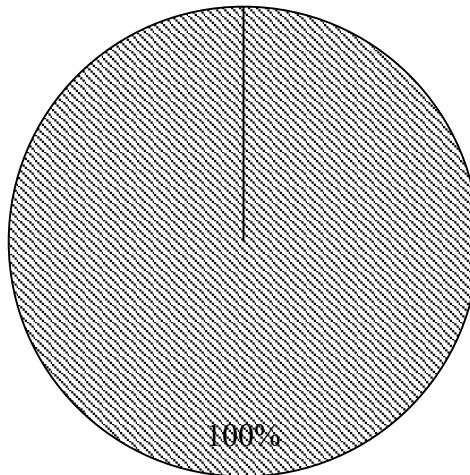


100%

Question 9

Yes No

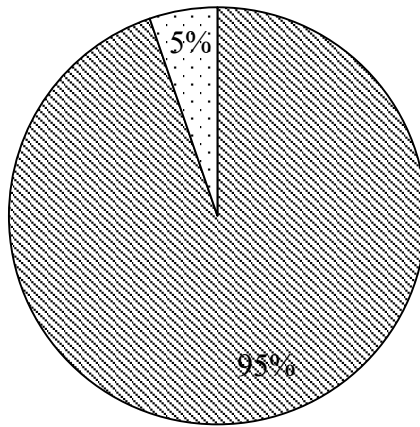
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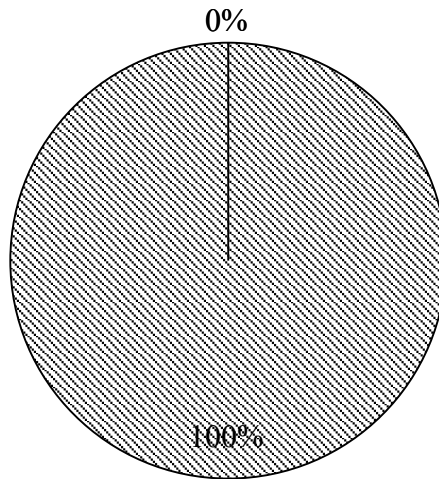
Question 10

Yes No



Question 11

A lot No much Can't say



Question 12

Appendix E

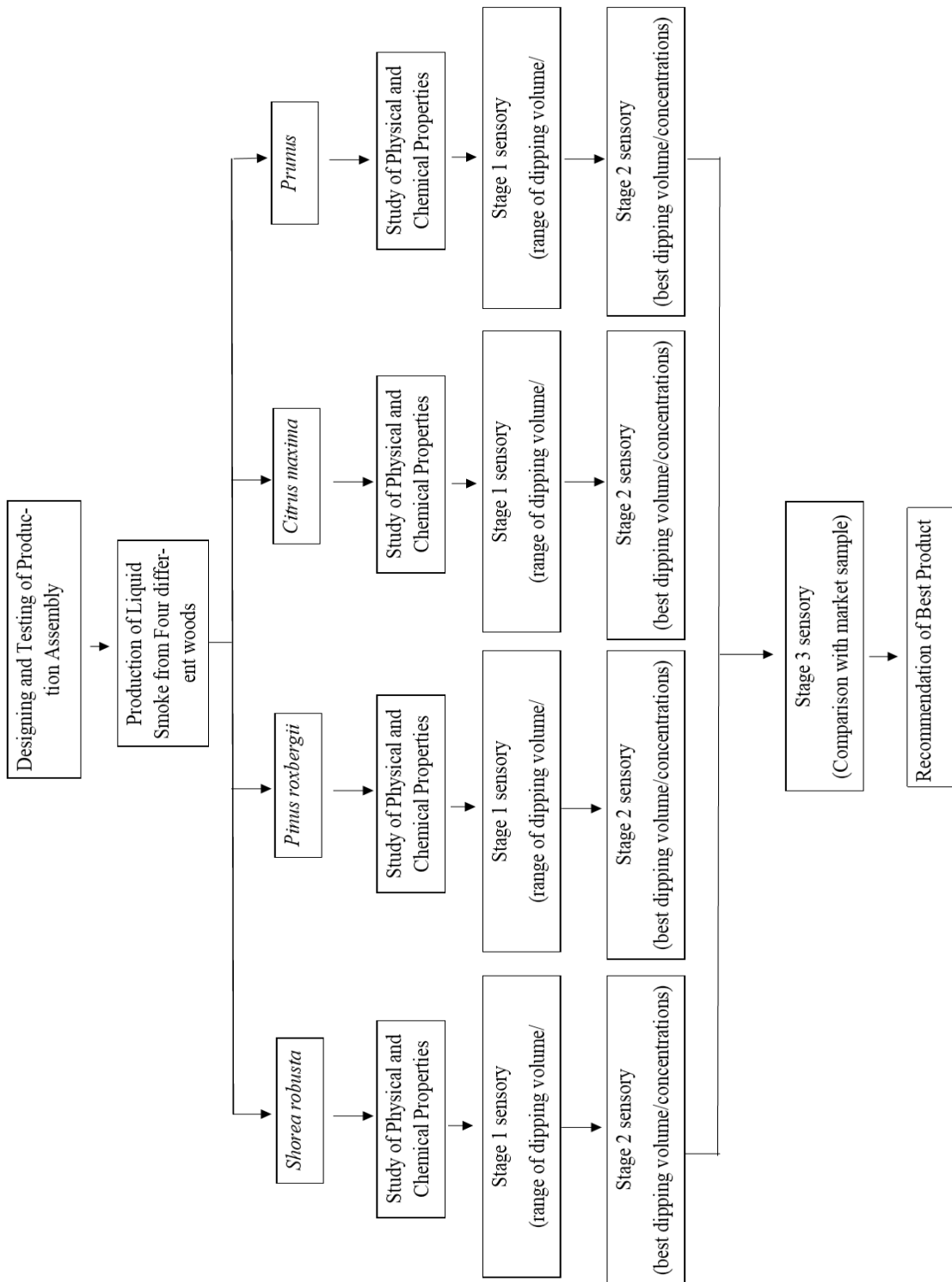


Fig. A.1 Research Approach

Appendix F

Table A.28 Single factor RSM Design by Design Expert v. 11

| Std | Blocks | Runs | Dipping Volume %(ml/g) |
|------------|----------------|-------------|-------------------------------|
| 1 | 7:00 am Sunday | 1 | 50 |
| 5 | 7:00 am Sunday | 2 | 111.69 |
| 10 | 7:00 am Sunday | 4 | 200 |
| 7 | 7:00 am Sunday | 6 | 165.50 |
| 11 | 5:30 pm Sunday | 11 | 89.75 |
| 13 | 5:30 pm Sunday | 12 | 182 |
| 12 | 5:30 pm Sunday | 13 | 130.25 |
| 16 | 7:00 am Monday | 14 | 149 |
| 14 | 7:00 am Monday | 16 | 69.53 |

Appendix G

Cost of Production-Calculation

The only electric appliance used was a motor of 18 Watts. Hence, converting this power to kWh, we get 0.018 kWh if used for 1 h. In a day, for a production time of 10 h, this becomes 0.18 kWh. In Nepalese context, the electricity cost for a single-phase electricity when 5A of current is used is Rs 4 per unit. Thus, for a production time of 10 h, it will be Rs. 0.72.

Hence, total cost for 1 liter of liquid smoke is estimated to be Rs. 50.36. It has been tabulated below in Table A.28

Table A.29 Cost of production

| Subject under study | Details |
|--|----------------|
| Liters of Liquid smoke produced in one batch | 2 liters |
| Working hours | 10 h |
| kWh consumed in 10 h | 0.18 kWh |
| Gas consumed in 10 h | 1 liter |
| Cost of 15 liters (1 cylinder) of LPG gas | Rs. 1500 |
| Cost of 1kWh of electricity | Rs. 4 |
| Cost of 1 liter of LPG gas | Rs. 100 |
| Cost of 0.18 kWh of electricity | Rs. 0.72 |
| Hence Total cost incurred during one batch of production | Rs.100.72 |
| Again, since 1 batch contains 2 liters of solution, cost of production for 1 liter of solution will be | Rs. 50.36 |

Appendix H

Plates and Photographs



Plate 1 Researcher with just-made and delivered settling tank and solubilization column



Plate 2 Welding and joining of smoke transfer pipe with settling tank



Plate 3 Plant under construction (metal works)



Plate 4 Smoke generation chamber under construction



Plate 5 Smoking chamber (close look)



Plate 6 Liquid smoke production assembly (top view)



Plate 7 Pork ham dipped in liquid smokes (a)



Plate 8 Pork hams dipped in liquid smokes (b)



Plate 9 Sawdust after plate heating (pyrolysis)

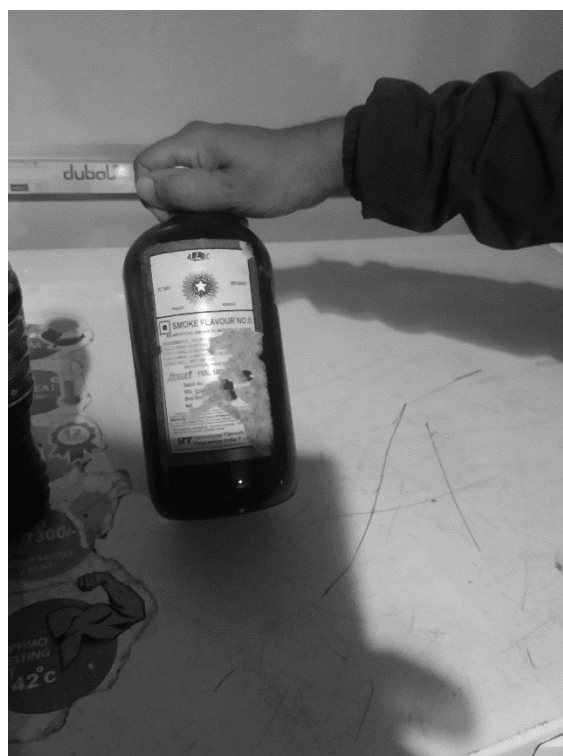


Plate 10 Commercial liquid smoke sample



Plate 11 Survey pic (a)

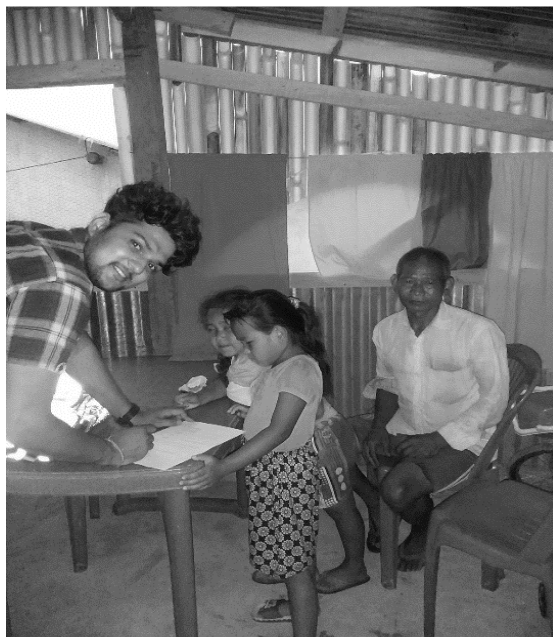


Plate 12 Survey pic (b)

Color Plates

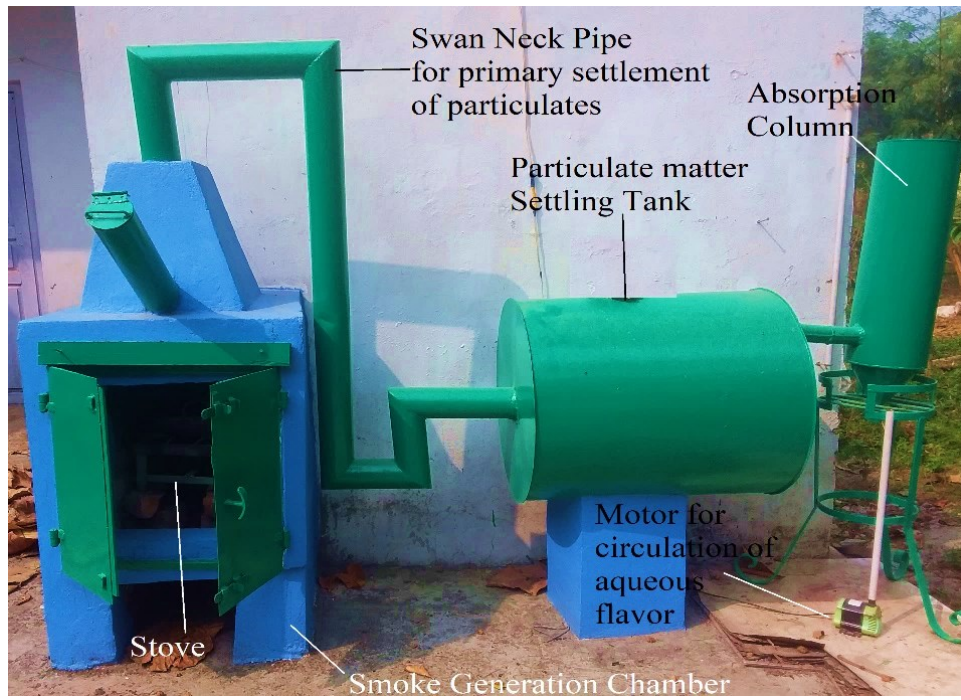


Plate 13 Liquid smoke flavor production assembly (front view)



Plate 14 Prepared liquid Smoke from *Pinus*, *Citrus*, *Prunus* and *Shorea* trees (from left to right)