STUDIES ON DEVELOPMENT OF *MASMIN* AND *MASMIN* BASED PRODUCTS USING LIQUID SMOKE TECHNOLOGY

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Ph.D. Thesis under the Faculty of Marine Sciences

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Dedication

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This is to certify that this thesis entitled ***"Studies on Development of Masmin and Masmin Based Products Using Liquid Smoke Technology"*** *embodies the original work done by Mr. Nithin. C. T., Reg No: 4195, under my guidance and supervision in the Fish Processing Division of Central Institute of Fisheries Technology, Cochin. I further certify that no part of this thesis has previously been formed the basis of award of any degree, diploma, associateship, fellowship or any other similar titles of this or in any other University or Institution.*

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Declaration

I, ***Nithin. C. T.****, do hereby declare that the thesis entitled* ***"Studies on Development of Masmin and Masmin Based Products Using Liquid Smoke Technology"*** *is a genuine record of bona fide research carried out by me under the supervision of* ***Dr. T. K. Srinivasa Gopal****, Former Director (CIFT), Emeritus Scientist (KSCSTE),Central Institute of Fisheries Technology, Cochin and has not previously formed the basis of award of any degree, diploma, associateship, fellowship or any other similar titles of this or any other University or Institution.*

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Nithin. C. T

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List of Abbreviations

°C	-	Degree Celsius
µg	-	Microgram
µl	-	Microlitre
µm	-	Micrometre
2, 4-DNPH	-	2, 4-dinitrophenylhydrazine
AA	-	Amino acid
AC	-	Alternating current
ACE	-	Acenaphtene
ACY	-	Acenaphthylene
$AgNO_3$	-	Silver nitrate
ANT	-	Anthracene
atm.	-	Atmospheric pressure
BaA	-	Benzo(a)anthracene
BaP	-	Benzo(a)pyrene
BbF	-	Benzo(b)fluoranthene
BF3-methanol	-	Boron trifluoride-methanol
BgP	-	Benzo(g,h,i)perylene
BkF	-	Benzo(k)fluoranthene
CD	-	Cross direction
CF	-	Liquid smoke produced from coconut fibre
CH	-	Liquid smoke produced from coconut husk
CHR	-	Chrysene
CL	-	Commercial liquid smoke

CMLS	-	Commercial liquid smoked
Conc.	-	Concentration
CP	-	Liquid smoke produced from coconut fibre powder
$CuSO_4$	-	Copper sulphate
Da	-	Dalton
DHA	-	Docosahexaenoic acid
DhA	-	Dibenzo(a,h)anthracene
EMC	-	Equilibrium moisture content
EPA	-	Eicosapentaenoic acid
Ex/Em	-	Excitation/emission
FFA	-	Free fatty acid
FID	-	Flurescence insitu detector
FLR	-	Fluorene
FLT	-	Fluoranthene
g	-	Gram
H_2SO_4	-	Sulphuric acid
Hcl	-	Hydrochloric acid
HNO_3	-	Nitric acid
HPLC	-	High-performance liquid chromatograph
hr	-	Hour
Hz	-	Hertz
ID	-	Internal diameter
IMP	-	Improved
IcP	-	Indeno(1,2,3-cd)pyrene-
INDLS	-	Indigenous liquid smoked

K_2SO_4	-	Potassium sulphate
Kg	-	Kilogram
KOH	-	Potassium hydroxide
LDPE	-	Low density polyethylene
L	-	Litre
L/hr	-	Litre per hour
MD	-	Machine direction
CD	-	Cross direction
mg	-	Milligram
milli.eq	-	Milli equivalent
min	-	Minute
ml	-	Millilitre
mm	-	Millimetre
MUFA	-	Monounsaturated fatty acids
N	-	Normal/Normality
Nacl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NAP	-	Naphthalene
nm	-	Nano meter
N	-	Newton
OPA	-	O-phthalaldehyde
OTR	-	Oxygen transmission rate
PAH	-	Polycyclic Aromatic Hydrocarbon
PE	-	Polythene
PEST/PE	-	Polyester/polyethylene laminate

PHE	-	Phenanthrene
ppm	-	Parts per million
PUFA	-	Polyunsaturated fatty acids
PYR	-	Pyrene
RH	-	Relative humidity
RPM	-	Revolution per minute
Sec	-	Second
SFA	-	Saturated fatty acids
SPSS	-	Statistical package for the social sciences
SS	-	Stainless steel
Std	-	Standard
TBA	-	Thiobarbituric acid value
TCA	-	Trichloroacetic acid
TMA-N	-	Tri-methyl amine-Nitrogen
TPC	-	Total phenolic content
TVBN	-	Total volatile base nitrogen
Vol.	-	Volume
V	-	Volt
%w/v	-	Percent Weight/Volume
Wt	-	Weight
WVTR	-	Water vapour transmission rate

INTRODUCTION

Smoking is an age old food preservation method and is still widely used in most of the countries. It gives a characteristic flavour and colour to the product and increases its shelf life as a consequence of combined effects of preliminary salting/cooking and antimicrobial activity of smoke components. Traditional smoking involved hanging meat on the ceiling of a dwelling and permitting smoke resulting from fire to pass around and through the product. In due course of time, the technology has evolved in various dimensions to meet the safety, quality, consumer and industrial requirements.

Smoked foods are still in demand due to the fondness for their typical flavour; 15% of fish for human consumption in European market is offered in the form of cold or hot smoked products (Stolyhwo & Sikorski, 2005). Smoked and salted fish in France accounts for 17% of the market share of aquatic products (Girard & Paquotte, 2003). Smoke-cured bacon is a traditional Chinese delicacy used as the main meat food in Hubei, Sichuan, Hunan, Guizhou and Yunnan provinces of China (Yu & Sun, 2005). "*Katsuobushi*" is a traditional and popular smoked, dried, fermented product in Taiwan and Japan (Mitou, Shigemori, Aoshima & Yokoyama, 2008).

Masmin or *mas* is a traditional smoked and dried product from skipjack tuna (*Katsuwonus pelamis*) produced in Union Territory of Lakshadweep, India. *Masmin* production contributes a major share to the economy of islands through domestic trade and export to countries like Sri Lanka, Singapore and Malaysia. For the production of *masmin*, skipjack tuna is beheaded, eviscerated, cleaned, cooked in sea water and allowed to cool in the same

water for about 6 hrs. After cooling, the loins are separated (Alternatively, in some other practices, raw fish is loined first and then wrapped in coconut leaves to prevent the disintegration during cooking). These cooked and cooled loins are then smoked for 4 to 5 hrs using dried coconut husk as the major smoke source (coconut leaf wrapping if used is removed after this first stage smoking), followed by sun drying for about 10 days. This smoking and drying cycle is repeated several times until the final product is formed. *Masmin* is very hard in texture and resembles a dark piece of wood and can be stored up to one year with proper packaging. A fall in overseas market demand for *masmin* has been observed, which is majorly attributed to the inconsistent quality and inconvenient size of the product (Antony, Muraleedharan, & Mukundan, 2003). Due to the heavy smoking and unhygienic handling practised during its preparation, traditional *masmin* also possess the threat of contamination with carcinogenic Polycyclic Aromatic Hydrocarbons (PAH).

Commendable efforts have been made from the scientific community for salvaging the *masmin* industry from the present bottle necks. Improved methods for the production of *masmin* by resorting to controlled smoking and drying conditions have been suggested by many researchers (Muraleedharan & Valsan, 1980; David, Rajagopalaswami, & Sugumar, 1990; Nair, Nair, Joseph, & Cyriac, 1994; Yathavamoorthi, Mumthaz, Jinu, Bindu, Suseela, & Srinivasa Gopal, 2010). Several convenient products such as *mas* fingers, *mas* granules (Antony, Muraleedharan, & Mukundan, 2003), smoked *masmin* flakes (Srinivasa Gopal, Yathavamoorthi, Mumthaz, Bindu, & Suseela, 2010), *masmin* powder (Bindu, Yathavamoorthi, Mumthaz, & Srinivasa Gopal, 2010) etc. have also been developed. However the threat of PAH contamination in *masmin* could not be completely resolved yet.

PAH are ubiquitous environmental contaminants formed during incomplete combustion of organic materials. They originate from environmental sources (natural and anthropogenic), industrial food processing (heating, drying, smoking etc.), packaging materials and from certain cooking practices (grilling, roasting, frying etc.) (EFSA, 2008). Inhalations, dermal contact and consumption of contaminated foods are the three major ways of human exposure to PAH (Silva, Adetunde, Oluseyi, Olayinka, & Alo, 2011). Among them, diet is considered to be the most potential source as it accounts for 88 to 98% of such exposures (Farhadian, Jinap, Hanifah, & Zaidul, 2011).

Most important factor affecting the formation of PAH during traditional smoking is the temperature of smoke generation. PAH content in smoke can be minimised by limiting the temperature of the smouldering to 300– 400°C (Stolyhwo & Sikorski, 2005). The contamination can also be reduced by resorting to indirect smoking or by switching to use of liquid smokes (Visciano, Perugini, Conte, & Amorena, 2008).

Use of liquid smoke or smoke flavourings in food industry is gaining importance due to their convenience of use, combined with low content of PAH without compromising the flavour and preservative properties of smoke. Most of the traditional smoking practices are nowadays being largely replaced by use of smoke flavourings.

With a prime objective of reducing the PAH contamination in *masmin*, the present study is aimed at development of *masmin* and *masmin* based convenience product using liquid smoke technology. Success of such an attempt will have multifaceted advantages viz; it provides an opportunity for the *masmin* industry to regain its charm in international markets, longer smoking time required for traditional *masmin* production can be reduced to lesser duration of dipping or spraying with liquid smoke (increase in production rate), reduction in environmental pollution by limiting the

liberation of carcinogenic PAH in to atmosphere, promote replication of the technology for production of other smoked foods and thus help reducing the dietary PAH consumption of the masses.

One of the problems which could arise during commercial adoption of such a technology will be dearth of liquid smoke in the islands due to reduced access to mainland and high price of the commodity. To ensure consistent supply of liquid smoke and there by leading to self sufficiency of the industry, development of a small scale facility for production of liquid smoke is also envisaged under the present study.

Present study is proposed with the following objectives:

- To develop a low cost and effective method for production of an indigenous liquid smoke.
- To evaluate the quality and efficiency of the indigenously developed liquid smoke and comparison of the same with a commercial liquid smoke
- To standardise the production of *masmin* and *masmin* based products by using liquid smoke.
- To evaluate the effectiveness of liquid smoking in reducing the PAH content of *masmin* and *masmin* based products.
- To study the influence of process modifications on the nutritional value of *masmin* and *masmin* based products.
- Identification of appropriate packaging materials and storage conditions for the products.
- Shelf life evaluation of the developed products.

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Chapter 1

Review of the Literature

Contents

1. Smoking

Smoking as a food preservation method has been used from time immemorial. Traditional smoking methods developed in the 19th century are still used for imparting flavour, colour and aroma to foods. Smoke is considered to be an aerosol (solid and liquid particle dispersed in gaseous medium) generated by controlled smoldering of wood in the absence of or at reduced oxygen levels (Holley & Patel, 2005).

1.1 Smoke components

Smoke is a mixture of air, water vapour, CO_2, CO and at least several hundred organic compounds such as phenols, aldehydes, ketones, alcohols, acids, hydrocarbons, esters and ethers (Guillen & Errecalde, 2002). During smoking they get deposited on the surface of the food and later penetrate into the interior layers. They are generated as a result of thermal degradation of wood, followed by the oxidation of some of the products of pyrolysis under limited oxygen supply.

1.1.1 Phenols

Suitability of smoke for treating food depends primarily on its phenolic content, since they are mainly responsible for imparting the desirable sensory properties and are also valuable as antioxidants and antimicrobial agents (Stołyhwo & Sikorski, 2005). Phenols and phenolic esters are produced during the pyrolysis of lignin present in smoke source. They mainly include guaiacol (2-methoxyphenol) and syringol (2, 6-dimethoxyphenol). Along with them, a variety of associated compounds with methyl, ethyl, propyl, vinyl, allyl and propenyl side chains are also produced during such pyrolysis (Maga, 1988). Major phenolic compounds identified in wood smoke includes; phenol, p-cresol, o-cresol, guaiacol, 4-methyl guaiacol, 4-ethyl guaiacol, syringol, eugenol, 4-propyl guaiacol and isoeugenol (Serot & Lafficher, 2003).

Exact mechanism of inactivation of microbes due to phenolic compounds is still unknown. Some of the studies suggest that these compounds penetrate the microbial cell wall and interfere with the cellular metabolism (Davidson & Branden, 1981). Other pathways include inhibition of active sites of enzymes (Guynot, Ramos, Seto, Purroy, Sanchis, & Marın, 2003).

Structurally, phenols are aromatic hydrocarbons composed of benzene to which varying number of hydroxyl groups are attached. In addition to this, they can have other functional groups such as aldehydes, ketones, acids and esters. Increasing the number of hydroxy groups present on the benzene ring causes the resulting phenol to be quiet reactive with metal and sensitive to light and oxygen. Simple phenols, when dissolved in water, display acidic properties while in alkaline conditions, they are solubilized by dissociation to form phenolates. Ether forms of phenols are insoluble in water and can be

converted to phenol ketones at low temperature or can form condensation products by reacting with aldehydes (Maga, 1988).

Fat content of the food can significantly influence the amount of individual phenols in smoked foods. Some of them show lower recovery in water compared to lipid systems, whereas for some compounds (eg: Guaiacol), percentage recovery is essentially the same in both systems (Issenberg, Kornreich, & Lustre, 1971). Thus, depending upon the levels of individual phenols in smoke and amount of fat in food, the amount of total phenols in the food can vary. Method of smoke production also has a significant bearing on the phenolic concentration in food and thus affects the product flavour. Maga (1988) found that electrostatically filtered smoke was lower in phenols, carbonyls and acids when compared to smoke resulting from normal pyrolysis. Nature of the wood used in the smoking process can also influence the concentration of phenolic compounds (Guillen & Ibargoitia, 1998; Guillen & Marzanos, 1999).

Issenberg and Lustre (1970) has reported that all phenolic compounds produced in wood smoke do not end up as such in smoked foods. Especially, coniferaldyhyde and sinapladehyde are present in wood smoke at fairly significant levels. However, they are prone to addition reactions and react with meat proteins to form other compounds. Apparently some of these compounds can be associated with certain smoked foods in unreacted state also (Radecki & Grzybowski, 1981). Another factor to be considered relative to phenols in food is their degree of penetration into smoked foods, as a rule of the thumb, there is a higher deposition on the outer surfaces. It should also be noted that with storage, Amount of individual free phenols in a food can dramatically decrease during storage due to the covalent binding of quinones, resulting from the oxidation of phenols (Maga, 1988).

1.1.2 Carbonyls

Carbonyl class represents the largest number of compounds identified to date from wood smoke. Most carbonyls are thought to originate through thermal decomposition and rearrangement of cellulose and hemicellulose via classical carbohydrate degradation schemes (Kim, Kurata, & Fujimaki, 1974). Despite of their contribution to aroma of food matrices, carbonyl compounds, especially aldehydes contribute to colour and the texture in smoked foods (Varlet *et al*., 2007). Development of the characteristic colour of smoked foods is a result of maillard reaction, in which the carbonyl groups present in the smoke react with the amino groups present on the food surface to produce a brown colouration (Maga, 1988). Ruiter (1979) identified formaldehyde, glycolic aldehyde, glyoxal, acetone, hydroxyacetone, methylglyoxal, diacetyl, furfural etc. to be the major carbonyls associated with the formation of colour in smoked foods. Guillen & Ibargoitia (1998) reported that reactions between carbonyls and proteins can impart greater firmness to meat products thereby affecting the texture of the products. Carbonyls are also thought to contribute to the overall sensory properties of wood smoke.

Formaldehyde is a carbonyl of potential concern due to its toxicity. In smoke, it has been found at levels of up to 200 mg/100g of wood and at a concentration of up to 50 mg/kg in smoked meats (Toth & Potthast, 1984). However, formaldehyde and acrolein in smoke are known to exhibit antibacterial properties by penetrating the cell wall and subsequent inactivation of enzymes on the cytoplasmic membrane and cytoplasm. Another mechanism of bacterial inactivation due to carbonyl compounds is through interfering nutrient uptake by cells (Painter, 1998). Major carbonyl compounds identified in smoked fish are Ethanal (acetaldehyde), butanal, 3-Methylbutanal, 2-Methylbutanal, 2-Butenal, 2-Methyl-2-butenal, Pentanal,

(E)-2-Pentenal, 2-Furancarboxaldehyde (furfural), 5-Methyl-2-furancarboxaldehyde (5-methyl-furfural), Benzaldehyde, 4-Methylbenzaldehyde, Benzeneacetaldehyde, Hexanal, 4-Hydroxy-2-(E)-hexenal, Heptanal, 2-Heptenal, Octanal, Nonanal, Decanal, 2,4-Hexadienal, 2,4-Heptadienal, 2,4-Hecadienal, 2-Decenal, 2-Undecenal, Dodecanal, Tetradecanal, Hexadecanal, (1H)-Pyrrole-2-carboxaldehyde (Varlet *et al*., 2007).

1.1.3 Furans

Furans represent a class of five-membered oxygen containing heterocyclic compounds that most probably result from the dehydration of glucose, which in turn is an intermediate of the thermal degradation of cellulose. Other furans such as furfuraldehyde can be formed from pentosans which are degradation products of hemicellulose. Furfuraldehyde in turn can condense to form numerous analogues that possess sweet, fruity and grassy aromas (Toth & Potthast, 1984). The major furan found in smoked foods is maltol. Kim, Kurata, and Fujimaki (1974) found that furans also can contribute to the overall sensory properties of wood smoke and tend to soften the heavy smoky aromas associated with phenolic compound. Radecki, Grzybowski, Halkiewicz, and Lamparczyk (1977) have also reported that a furan fraction isolated by the steam distillation of a commercial liquid smoke possess a sweet, fragrant and floral aroma, which also was felt to soften heavy smoky aromas.

1.1.4 Other smoke components

Two other groups of flavour compounds that have received relatively little attention as to their potential role in wood smoke flavour chemistry are the lactones and pyrazines. Lactones in wood smoke are primarily derived from the thermal degradation of cellulose and hemicellulose via the

intermediates butyrolactone and 2-butenolide. These compounds in turn can form hydroxyacids (Maga, 1988). A rather extensive group of lactones has been identified by Kim, Kurata, and Fujimaki (1974) and all possessed aroma properties that can be characteristic and beneficial to wood smoke aroma (Maga & Katz, 1976). Pyrazines are heterocyclic nitrogen containing compounds that are thermally produced and possess potent and unique sensory properties (Maga, 1988). Since wood is a nitrogen source, a large number of these compounds are present in wood smoke. Kim, Kurata, and Fujimaki (1974) found the compound pyrazines, methylpyrazine and 2, 5-(or 2, 6) dimethylpyrazine at a combined level of 1 mg% in the basic fraction of wood smoke. A series of eight pyrazine compounds including pyrazine, 2-methoxy-3-ethylpyrazine, 2-ethoxy-3-ethylpyrazine, 2-propyl-3, 6-dimethylpyrazine, 2-butyl-3, 5-dimethylpyrazine, 2-butyl-3, 6-dimethylpyrazine, 2-acetyl-3-methylpyrazine and 2-acetyl-3, 5-dimethylpyrazine has been identified from smoke generated by different wood sources (Maga, 1985). In addition to this, numerous compounds belonging to chemical groups such as hydrocarbons, alcohols and acids are present in smoke and smoked foods. Methanol is a compound of special interest in view of its toxicology. It is primarily derived from the methoxy groups of lignin and upon oxidation forms formaldehyde, which also presents toxicological concerns (Toth & Potthast, 1984).

1.2 Factors influencing smoke composition

1.2.1 Wood source

Type of wood used as smoke source can significantly influence the organoleptic properties of smoke. In general, it has been reported that, hard woods such as beech and oak, produce smoke that is high in acids, while soft woods such as pine and spurce, have high levels of carbonyls in their smoke

(Maga, 1988). Ziemba (1957) reported that, smoke from oak wood contained more organic acids than beach wood. In comparing the carbonyl composition of smoke derived from different wood sources, Borys (1978) found that fir contained more aliphatic ketones, cyclic ketones and aromatic carbonyl compounds than alder. In the case of fir, 39 compounds were identified, while 28 compounds were found in alder wood smoke. Khvan, Shaposhnikov, Radakova, Alsufev, and Kondakova (1975) also found difference in major volatile compounds in smoke produced from different woods. Smoke from oak was found to contain more volatile acids, neutral and basic compounds to pine derived smoke. Fujimaki, Kim, and Kurata (1974) investigated the influence of wood source on resulting aroma acceptability and composition of smoke condensate from different wood species. Wood source used were two oak species (*Queretis acuta* and *Q. Seriata*), cherry, bamboo, pine and cedar. Results indicated that, total and individual amount of volatile materials varied significantly amongst wood sources. Baltes, Wittkowski, Söchtig, Block, and Toth (1981) reported a major finding that, in terms of phenols, the major difference between hardwood and softwood is the higher proportion of 4-methylguaiacol and the lower proportion of syringol, 4-methylsyringol and 4-propenylsyringol in smoke produced from soft wood.

1.2.2 Smoke generation conditions

1.2.2.1 Combustion temperature

Thermal degradation of hemicelluloses, cellulose and lignin of wood take place at 180–300, 260–350 and 300–500°C, respectively. Oxidation of the decomposition products can occur at temperatures reaching up to 900-1200°C. Smoke produced at 650–700°C is considered to be the richest in components responsible for imparting the desirable sensory properties to the products.

Fenner and Lephardt (1981) while examining lignin decomposition over a wide range of temperatures reported that, major decomposition of lignin occurred at 300 to 480°C and was maximum at 385°C. Maximum phenolic compound formation was observed at approximately 320°C, while at higher temperatures secondary degradation products began to appear. Toth (1980) also reported a general trend of an increase in phenolic content of smoke with a gradual decline at the higher temperatures. Simon, Rypinski, and Tauber (1966) reported that, actual weight percentage of acids and phenols decreased with increasing combustion temperature, while carbonyl content showed an increasing trend. However, the total number of volatiles increased by a factor of 4.5, over the temperature range evaluated (300°C- 415°C). Maximum phenolic content was obtained around a temperature of 300°C. However, Baltes, Wittkowski, Söchtig, Block, and Toth (1981) reported maximum phenol, carbonyl and acid production at 600°C.

1.2.2.2 Presence of air

Amount of air present in the combustion chamber during smoke generation is an important factor influencing the smoke composition. Wasserman and Fiddler (1969) investigated the effect of varied oxygen composition (0-59%) of the gas mixture used during smoke generation and has reported major differences in the composition of smoke. In general, they found that as oxygen percentage increased, furans, typified by the compound furfuryl alcohol, decreased. Phenolic compounds (guaiacol) and carbonyls (cuclotene) increased significantly at 10% oxygen, but then decreased as oxygen content increased. Maga (1985) reported that presence of air significantly influenced the amount of pyrazines formed from hickory smoke as the lack of air minimized formation of certain classes of unstable compounds and resulted in larger amounts of more stable pyrazines.

1.2.2.3 Wood moisture content

Gorbatov *et al*. (1971) have reported that a moisture content of 22-24% in smoke sources yielded high amount of phenols, acids and carbonyls. whereas moisture of 1.8 and 30% produced lower volatiles. Maga and Chen (1985) analysed the rate of pyrazine formation as influenced by hickory wood moisture content (4, 20 or 30%) at 290°C. and reported that the concentration was double when the moisture content was at 4%.

1.2.2.4 Wood particle size

The actual size of wood particle being used as a smoke source can indirectly influence resulting smoke flavour. The larger the particle, the higher the air flow required to obtain the same combustion rate as a smaller particle. Thus, if air flow is not altered according to particle size, combustion rates will differ, which in turn will influence final smoke composition (Borys, 1978).

1.3 Types of smoking

Traditional smoking involved hanging meat on the ceiling of a dwelling and permitting smoke resulting from fire that was used solely as a source of warmth to pass around and through the product. Today, smoke generation in many commercial operations have become quiet sophisticated to match the requirement of consumers. Now days, most of the countries stipulate adherence to techniques that minimize environmental pollution.

1.3.1 Hot and cold smoking

Major contrast between hot and cold smoking is the difference in temperature of smoke and smoking chamber. Cold smoking usually occurs at temperature of 15 to 25°C (Corretti, 1975), while for hot smoking, temperature

in the range of 55 to 80°C are employed (Draudt, 1963). Some processors also utilize warm smoking, where the smoke and chamber temperature is between 24 and 40°C (Butanski, 1979). Moist smoking at a temperature of 24-48°C and relative humidity in excess of 30% is also practiced for dry sausage and salamis (Reich, 1977). Dark smoking is another process whereby a dense smoke is permitted to blacken the surface of the food (Shaposhnikov, Khavan, Stepanova, & Kondakova, 1972). Raw ham and bacon are usually cold smoked, while cooked ham and frankfurters are typical hot smoked products. During hot smoking, the food will undergo some degree of cooking as well as absorb and react with components in smoke. In cold smoking, smoky characters are obtained without undergoing cooking. Cold smoking of a food may take up to several weeks for obtaining the desirable characteristics, where as hot smoking only requires several hours (Toth, 1982). Cold smoking seems to be adaptable to most fish species (Maharova, Goncharova, Pasternak, & Kosinova, 1985) and shows better yield characteristics (Simmonds and Avery, 1984). Hot smoke becomes associated with food approximately seven times faster than cold smoke (Draudt, 1963). Hot smoking can significantly increase the phenol composition in food with more variation in the fat portion than in lean portion (Toth, 1982). However, no variation in carbonyl content was observed between hot or cold smoked foods (Khlamova, Soloveva, & Petrakova, 1980).

1.3.2 Smoldering

Smoldering is a kind of indirect smoking in which smoke is generated in an enclosed chamber by exposing the smoke source (usually saw dust) to heating coil or gas flame and the generated smoke is allowed to interact with the food which is kept in a separate chamber. The amount of tar and other solid particle entering the food chamber can be controlled by the distance, the

smoldering unit is from the food chamber; longer the distance, greater the opportunity for tar to settle out. In some cases, baffles and filters are fixed in the passage of smoke to eliminate such toxic compounds. Smoke generation temperature during smoldering shows wide variations. Normally when a large quantity of air is present, the temperature can go above 800°C. A moisture content of 30 % in the smoke source can lower the smouldering temperature by 100-300°C, when compared to a completely dry wood sample (Maga, 1988).

1.3.3 Friction smoking

Friction smoking is a flameless method of smoking, wherein an intact wood piece is pressed against a rapidly rotating metal wheel and heat produced by friction cause the wood to undergo pyrolysis. Air is passed through the centre of the friction wheel to give a cooling effect. Friction smoke generator can be operated either in continuous and discontinuous manner. Continuous operation produces a smoke temperature of 140-160°C. During discontinuous operation, the friction wheel is in operation for 10 seconds and then rested for several minutes; smoke temperatures just above ambient temperature can be obtained by this method. Other factors which affect the smoke temperature during friction smoking are; force applied to the wood block, wheel speed and shape of friction edges (Klettner, 1975; Khavan, 1971; Maga, 1988). In contrast with smoldering, during friction smoking, amount of air in the chamber is inversely proportional to the smoke temperature. The actual temperature of wood pyrolysis using friction ranges from 450-560°C, but due to the rapid cooling smoke temperature can be relatively low (Toth, 1980). The rapid cooling associated with friction smoke generation does not permit sufficient time and temperature for the primary

products of wood pyrolysis to undergo extensive secondary reactions, thus limiting the number of compounds formed (Maga, 1988).

1.3.4 Wet smoking or condensate smoking

This is another method of flameless smoke generation. In this method sawdust and superheated steam containing varying amount of heated air is blended to cause sawdust pyrolysis. Pyrolysis usually occurs at a temperature of 300-400°C and the resulting smoke is moist, dense & low in oxygen, with a temperature of approximately 80°C. The method requires short treatment time, but has improved smoke yield (Schuldt, 1979; Fessman & Fessman, 1979).

1.3.5 Fluidization

During fluidization, air is heated to 300-400°C by means of an electric heater and mixed with sawdust under high velocity. Because of the resulting turbulence, the sawdust is suspended or fluidized and undergo pyrolysis under high temperature. A cyclonic separator is used to separate smoke and charred sawdust (Maga, 1988). Temperature of smoke produced by this method does not go much beyond ambient temperatures.

1.3.6 Two stage Smoking

Two stage smoking is a modification and extension of the fluidization technique. As the name indicates, this method of smoke production is carried out in two stages. First stage involves initial wood pyrolysis of sawdust by the action of nitrogen or carbon dioxide at a temperature of 300 to 400°C. During second stage, the reactants of the first stage are mixed with oxygen or air at 200°C, and further pyrolysis occurs. The second stage promotes oxidation, condensation and polymerization reactions thus resulting in a more complex mixture of potential flavour compounds in the smoke. Factors influencing

smoke composition and yield are temperature and amount of gases (Maga, 1988). Klettner (1979) found that maximum smoke production was obtained with a first stage temperature of 400°C, with a nitrogen level of 1500 L/hr and a second oxidation temperature of 200°C. Both fluidization and two-stage smoking results in dry, dense smoke containing oxygen.

1.3.7 Carbonization

Production of smoke by carbonization requires specialized equipment in which, sawdust is pressed together in a tubular casing by means of a tapered screw. As a result most of the air associated with sawdust is eliminated. At the end of the compression device is a variable temperature electrical heating element which, because of lack of air in the compressed sawdust causes the product to give off smoke during carbonization. Generally during carbonization, pyrolysis occurs at a temperature of 300-400°C which result in dry and dense smoke with low oxygen content (Maga, 1988).

1.3.8 Electrostatic smoking

During electrostatic smoking, food is sent into a tunnel where an electrostatic field is created. Smoke particles are given a positive charge and get deposited onto the surface of the food which is negatively charged. Distance between wires and treatment time are the two factors significantly influencing the amount of flavour compounds deposited during electrostatic smoking. Other factors found to be important were skin density, presence of scales and the amount of subcutaneous fat (Kurko & Mezenova, 1985). Although this procedure will change the composition of smoke, the efficiency of smoking is still higher than that of the traditional smoking. Ruiter (1979) indicated that the electrostatic field modifies the smoke compound ratio in the vapour phase, mainly by increasing the level of carbonyl compounds to the

detriment of phenolic compounds. Maga (1988) reported that electrostatically filtered smoke was found to be lower in acids, phenols and carbonyls. A variety of electrostatically smoked sausages gave less smoky aroma and colour than normal smoked ones, but sensory score was found to be similar (Rusz, 1977). In contrast, Girard, Talon, and Sirami, (1982) reported, electrostatic smoking, as compared to the traditional smoke house process, increased the content of phenolic compounds in bacon.

1.3.9 Liquid smoking

Liquid smoke or smoke flavouring is a smoke condensate that is dissolved in water, oil or smoke extracts in organic solvents. Smoke condensate can also be absorbed on the solids such as spices, salt, sugars, starch or protein, thus resulting in dry or powdered forms (Toth & Potthast, 1984). Use of liquid smoke is a more controllable, consistent process alternative to burning hardwood sawdust.

American Food and Drug Administration (FDA) and Canadian Department of National Health and Welfare accepted liquid smoke flavourings in 1960 and 1966, respectively. Japanese started using smoke flavourings in the year 1975. Towards mid 80s the technology was widely accepted in Europe (Underwood, 2005). Smoke flavourings are popular in US and about 90% of the hot dogs marketed there are flavoured using smoke flavourings. According to Varlet, Serot, and Prost (2010), 75% of smoked foods produced in the United States are treated with liquid smoke, while in Europe between 20% and 30% of smoked foods are produced using this method. Annual consumption of smoke flavourings around the globe amounts to about 100,000 tonnes (Yu, 2000).

1.3.9.1 Advantages of Liquid smoking

Liquid smoking has been reported to possess the following advantages (1) it can be fractionated to intensify smoke flavour and to remove potentially harmful compounds before it is used with food, (2) it has application to a wide variety of foods that are not traditionally smoked, (3) can be used to intensify the flavour of traditionally smoked foods, (4) there is closer control over the amount of smoke flavour the product receives, (5) it can be used at consumer level as well as commercial processing levels, (6) it represents cost savings since wood and smoking equipment are not required as part of food smoking plant, (7) there is less environmental pollution associated with its use and (8) it can be applied in various ways such as spraying on the surface, dipping and actual mixing in with the food (Maga,1988). The major interest on application of liquid smoke in food industry is their lower PAH content. Swastawati, Agustini, Darmanto, and Dewi (2007) found that the level of carcinogenic PAH in liquid smoke produced from lamtoro wood and corn cob was below detectable limit. Da Porto, Moret, and Soldera (2006) reported that the level of benzo(a)pyrene (BaP), a carcinogenic PAH in the distillates obtained from smoked marc was lower than 0.03 µg/kg.

1.3.9.2 Production of Liquid smoke

European Union regulation No. 2065/2003 (EC, 2003) has set a formal production process of smoke flavourings for approval in member countries. The process starts with the condensation of smoke and the condensed smoke is 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 is unsuitable for the production of smoke flavourings. The primary smoke condensates and fractions of the

water-insoluble high-density tar phase are purified to remove components of smoke which are most harmful to human health. They are then suitable for use as such on foods or for the production of derived smoke flavourings which can be made by further processing such as extraction, distillation, concentration by evaporation, absorption and addition of food ingredients, other flavourings, food additives, solvents etc.

Freshly generated liquid smoke that is condensed in water usually has a bright yellow colour and with time darkens due to the formation of brown coloured condensation or polymerization products. These compounds along with carcinogenic Polycyclic Aromatic Hydrocarbons, settle out during stabilization and storage. The resulting liquid phase can then be decanted, resulting in a liquid preparation that is low in PAH. There is another kind of smoke flavouring called artificial smoke flavouring. The term 'artificial flavouring' means a flavouring containing any sapid or aromatic constituent which was manufactured by a process of synthesis or other similar artifice (CFR, 2005).

Considerable number of patents has been filed on methods for producing liquid smokes. A method by burning saw dust and then condensing the smoke to yield liquid smoke was described in U.S. Pat. No. 4,883,676 (Sophianopoulos, Darley, & Sair, 1989). U.S. Pat. No. 3,106,473 (Clifford, 1963) describes a method of burning wood and mixing the smoke with counter streaming water or steam. U.S.Pat. No 4,359,481 (Johannes & Franciscus, 1982) describes a process of producing liquid smoke by destructive distillation and final separation of the tar fractions by cooling. Guillen and Manzanos (1999) produced an aqueous smoke flavouring from thyme by pyrolysing dried ground thyme plant in a round-bottom flask smoke generator and filtering the condensate obtained to remove the tar phase.

1.4 Smoked products

Smoked foods enjoy a huge market demand due to the fondness for their typical flavour. In France, the market share for smoked and salted fish accounts 17% of the total aquatic products (Girard & Paquotte, 2003). Introduction of smoked salmon in 1990s was a mile stone in the field and the product enjoys the largest market for smoked fish. Each year 45,000 tonnes of salmon are used in France to produce 18,000 tonnes of smoked salmon, 15% of which is exported to Italy, Belgium and Germany (Cardinal, Cornet, Serot, & Baron 2006). Trout and herrings are the next preferred species in smoked food industry. Global production of smoked herring and salmon is about 38,000 and 86,000 tonnes, respectively. In addition to these principal fish species, buckling, eel, halibut, mackerel and sprats in smoked form are also relished, particularly in Germany, Poland and UK (FAO, 2001).

Smoked and dry-cured ham (*prosciutto*) from Dalmatia (southern part of Croatia) is a delicacy with excellent international demand. It is made by salting, pressuring, smoking and dry-curing ham for 12–24 months without any additives such as nitrites or ascorbic acid (Dirinck, Van, & Vandendriessche, 1997). Smoke-cured bacon is a traditional Chinese food used as the main meat food in Hubei, Sichuan, Hunan, Guizhou and Yunnan provinces of China. It is made by salting, smoking and dry-curing of pork. The product has a distinctive flavour considered as tangy, delicate, meaty and salty (Yu & Sun, 2005).

1.4.1 Kipper

Kipper is traditional smoked product obtained from herring (*Clupea harengus*). In United Kingdom, Japan and in some North American regions it is often preferred as breakfast. For production of Kipper, whole herring is split

in butterfly fashion from tail to head along the dorsal ridge, gutted and washed. They are then soaked in brine for approximately 10 min. During the next step, the fishes are oak smoked above fires for 6-12 hrs. The same method is being used for over 120 years which gives them their unique flavour (FAO, 2001).

1.4.2 *Katsoubushi*

Katsuobushi is a traditional and popular seasoned product in both Taiwan and Japan. The product is made specifically from five species of tuna viz; Skipjack tuna (*Katsuwonus pelamis*), Eastern little tuna (*Eythynnus affinis*), Bullet tuna (*Auxis rochei)*, Frigate tuna (*Auxis thazard*) and Oriental bonito (*Sarda orientalis*) (Collette & Nauen, 1983). These species are collectively called as "bonito fishes" The production process includes boiling, smoking, drying and fermenting. Fishes are beheaded, gutted, filleted and the belly area is trimmed off. The fillets are then arranged in a basket and cooked just below boiling for an hour or two. The rib bones are removed after cooking. The fillets are then smoked using oak, pasania, or castanopsis wood; this process can take up to a month. They are smoked for 5–6 hrs in one session, left to rest for one day, then fired and smoked again the next day, repeating this smoking and resting cycle 12–15 times. The built up tar is cleaned from the surface using a grinder. Next step in the production is drying the fillets with the assistance of a mould. The fillets are sprayed with a culture of *Aspergillus glaucus* and left for 2 weeks in a closed cultivation room. The mould ferments the fillets and also siphons out any residual moisture. Mould from surface is continually scraped off, with further sun drying. *Katsuobushi* is usually made into slices and consumed as a soup stuff. Due to the heavy smoking during its production, *Katsuobushi* contains high level of PAH. The layer of tar on the surface, forms up to about 3% of the product's weight and

contains 20–40 times more Benzo(a)pyrene than the meat of the deeper layers (Kikugawa, Kato, & Hayatsu, 1986).

1.4.3 *Masmin*

Masmin or *mas* is a traditional smoked and dried product from skipjack tuna (*Katsuwonus pelamis*). It is a traditional fishery product of Lakshadweep islands. The method of preparation of this product has passed down through generations and the process is followed even today with very little modifications. Processing employed is almost similar to *Katsuobushi*. However, the fermentation step is not followed for production of *masmin*. Skipjack tuna loins are cooked in sea water and allowed to cool in the same water for about 6 hrs. After cooling, the loins are smoked for 4 to 5 hrs followed by sun drying for about 10 days. This smoking and drying cycle is repeated several times until the final product is formed. *Masmin* is very hard in texture and resembles a dark piece of wood. It can be stored for a period of one year with proper packaging.

Conversion of surplus landing of skipjack tuna to *masmin* provides a cheap and efficient way of preserving the catches for future consumption. *Masmin* production contributes a major share to the economy of islands through domestic trade and export to countries like Sri Lanka, Singapore and Malaysia. However a fall in the overseas market demand for *masmin* has been reported (Antony, Muraleedharan, & Mukundan, 2003). The major reasons behind such trends are the inconsistent quality and inconvenient size of the material. Being a heavily smoked product *masmin* also possess the threat of contamination with carcinogenic Polycyclic Aromatic Hydrocarbons.

1.5 Major problems associated with smoked products

Major problems encountered with production and distribution of smoked seafood are the chances of contamination with spoilage and pathogenic microorganisms (Jeevanandam, Kakatkar, Doke, Bongirwar, & Venugopal, 2001), histamine formation (Lehane & Olley, 2000) and the content of Polycyclic Aromatic Hydrocarbons (Lawrence & Weber 1984; Farhadian, Jinap, Hanifah, & Zaidul, 2011).

1.5.1 *Listeria monocytogenes*

L. monocytogenes is an emerging safety concern in the field of smoked fish and fishery products (Lin, Jiang, & Li, 2008). It is a facultative anaerobic bacterium which occurs widely in nature and may enter fish production plants by way of seawater, utensils, personnel or raw material, thus contaminating the finished products. Once ingested to the body, the organism penetrates the intestinal tract to cause systemic infections resulting in a condition called "listeriosis". Cold smoked fishery products carry a particular risk of listeriosis (FAO/WHO, 2004). Many cases of listeriosis associated with cold smoked rainbow trout have been reported (Heinitz & Johnson, 1998; Miettinen, Siitonen, Heiskanen, Haajanen, Björkroth, & Korkeala, 1999). Vacuum packaged products possess higher chances of listeriosis as they allow enough time for the bacterium to multiply to hazardous levels if the storage temperature is not maintained below 4℃ (Rørvik, Yndestad, & Skjerve, 1991).

1.5.2 *Clostridium botulinum*

C. botulinum in fishery products has attracted particular attention due to their heat resistance and spore forming characteristics. Chances of proliferation of *C. botulinum* in fishery products are mainly associated with

products stored in vacuum or modified atmospheres. It shows that hot smoked and cold smoked fishes are good substrates for *C. botulinum* that could produce spores and toxin. The spores of *C. botulinum* are very common in nature and are also found in the gills & viscera of finfish, crabs and shellfish. *C. botulinum* type E is the most common form found in marine environments and in fishes (Hielm, Hyytiä, Andersin, & Korkeala, 1998). *C. botulinum* type E outbreaks associated with vacuum packaged hot smoked rainbow trout and white fish have been recorded (Öberg, 1994; Korkeala *et al*., 1998). Hot smoked fish seem to have higher risk of *C. botulinum* E than cold smoked fish, possibly due to severe under processing and the heating procedure as a spore activating step (Southcott & Razzell, 1973; Eklund, 1992). According to Hyytiä, Hielm, and Korkeala (1998), 3% of 64 samples of cold smoked rainbow trout were positive with *C. botulinum* type E spores between 40 and 290 per kg. Moreover 5% hot smoked fish products contained spores, indicating that *C. botulinum* can survive the heat treatments employed in fish smoking.

1.5.3 Histamine

Biogenic amines have been used as chemical indicators of seafood quality, especially for vacuum packed cold smoked salmon (Jorgensen, Huss, & Dalgaard, 2000). Levels of the same between 3 and 240 mg/kg have been detected in cold smoked salmon (Jorgensen, Dalgaard, & Huss, 2000). Histamine is one among the biogenic amine, formed by bacterial decarboxylation of the amino acid, histidine. Histamine at high concentration (500 ppm) causes ailments which typically lasts up to 24 hrs, producing allergy like symptoms such as facial flushing, nausea and headache (Naila, Flint, Fletcher, Bremer, & Meerdink, 2012). Mishandling coupled with high temperature abuse is common practice in handling fish in the tropics and

subtropics, which significantly enhance histamine formation (Guizani, Al-Busaidy, Al-Belushi, Mothershaw, & Rahman, 2005). Proper cold chain management is the most effective method for curtailing the chances of histamine formation. Smoked fishery products have a particular threat of histamine and other biogenic amine formation, as they are exposed to elevated temperatures during various stages of processing. Scombroid fishes such as tuna, mackerel, bonito and saury, that contain high levels of free histidine in their muscle are often implicated in histamine poisoning incidents (Taylor, 1986). Fishes from other families, such as salmon (Salmonidae), have also been regularly reported to have high content of histamine. A histamine level of 50 ppm is an indicator of decomposition of fish (Food and Drug Administration, 1995) and several countries have set legal limits of histamine concentrations that are regarded as safe for human consumption: Australia, 200 ppm (Australian Food Standards Code, 2001), Europe, 100 ppm (EC, 2003), USA, 50 ppm (FDA, 1998) and South Africa, 100 ppm (South African Bureau of Standards, 2001). FSSAI (2017) has recommended a maximum level of 200 ppm histamine in smoked fishery products marketed in India; for dried and salted fish the maximum tolerable limit is fixed as 400 ppm.

1.5.4 Polycyclic Aromatic Hydrocarbon (PAH)

Polycyclic Aromatic Hydrocarbons comprise the largest class of chemical compounds consisting of two or more condensed aromatic rings (Simko, 2002). About 660 different compounds belonging to the PAH group have been identified so far (Sanders & Wise, 1997). The main health concern on PAHs is due to the fact that some of them have proven to be highly carcinogenic in laboratory animals, having been also implicated in different types of human cancers due to a metabolic activation in mammalian cell to "dihydrodiol epoxides" causing errors in DNA replication. PAHs show wide

variations in their molecular mass ranging from Indene (116 Da) to Dibenzopyrenes (302 Da) (Toth & Potthast, 1984). Light PAHs, of molecular mass below 216 Da, are not regarded as carcinogenic as they are relatively volatile, soluble and more degradable than the higher molecular weight compounds.

1.5.4.1 Sources of PAH

PAHs are ubiquitous environmental contaminants formed during incomplete combustion of organic materials. They originate from environmental sources (natural and anthropogenic), industrial food processing (heating, drying, smoking etc.), packaging materials and from certain cooking practices (grilling, roasting, frying etc.) (EFSA, 2008). Inhalations, dermal contact and consumption of contaminated foods are the three major ways of human exposure to PAHs (Silva, Adetunde, Oluseyi, Olayinka, & Alo, 2011). Among them, diet is considered to be the most potential source as it accounts for 88 to 98% of such exposures (Farhadian, Jinap, Hanifah, & Zaidul, 2011).

Exact mechanism of formation of PAHs in grilled/ smoked foods is not precisely known, it is generally considered that at least three possible mechanisms exist. The first mechanism is the pyrolysis of organic matter such as fat, protein and carbohydrates at temperatures above 200°C and PAH formation is augmented at a temperature of 500-900°C (Knize, Salmon, Pais, & Felton, 1999). The greatest concentrations of PAHs have been shown to arise from pyrolysis of fat (Bartle, 1991).

The second mechanism is due to the contact of lipids dripping at intense heat directly over the flame. This condition can generate volatile PAHs that in turn be adhered to the surface of the food as the smoke rises (Lijinsky, 1991; Wu, Wong, Lee, Shi, & Ong, 1997; Phillips, 1999; European

Commission, 2002; Farhadian, Jinap, Abas, & Sakar, 2010). The third mechanism is the incomplete combustion of charcoal which can generate PAH that are brought onto the surface of the food (Dyremark, Westerholm, Övervik, & Gustavsson, 1995; Wu, Wong, Lee, Shi, & Ong, 1997).

1.5.4.2 Metabolism of PAHs

PAHs enter the organism by inhalation, ingestion or penetration with a following distribution to the various organs, where they interact with aryl hydrocarbon hydroxylases, which are most abundant in the liver and hydrolysis them to dihydrodiols. These are the ultimate carcinogens and are also called as 'bay-region dihydrodiol epoxides". They form covalent adducts with proteins and nucleic acids and these DNA adducts are thought to initiate cell mutation and eventual malignancy (Stahl & Eisenbrand, 1988; Bartle, 1991).

1.5.4.3 Legislative limits for PAH

The US Environmental Protection Agency (EPA) has classified benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, chrysene, benzo(k)fluoranthene, dibenzo(a,h)anthracene and indeno(123-c,d), pyrene as probable human carcinogens (US EPA, 2002). Scientific Committee on Food (2002) stated that, benzo(a)pyrene (BaP) can be used as a marker for the occurrence and effect of carcinogenic PAHs in food. Maximum tolerable levels of benzo(a)pyrene in a variety of foodstuffs have been specified by European Union (EC, 2005), wherein a maximum of 5 µg/kg has been set for smoked fish and fishery products, excluding bivalve molluscs. For bivalve molluscs, a separate maximum limit of 10 µg/kg was specified. BaP content of 2 µg/kg was specified for non-smoked fish and a 5 µg/kg limit was fixed for non-smoked crustaceans & cephalopods. EU regulation No. 2065/2003 has

recommended that the level of BaP and benzo(a)anthracene (BaA) in a primary smoke condensate should not exceed 10 and 20 µg/L, respectively (EC, 2003).

During 2008, a scientific opinion adopted by the European Food Safety Authority (EFSA, 2008) concluded that BaP alone is not a suitable indicator for the occurrence and toxicity of PAHs in food and that eight specified PAHs (Known as "PAH8") viz: benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), benzo(g,h,i)perylene (BgP), dibenzo(a,h)anthracene (DhA) & indeno (1,2,3-cd)pyrene (IcP), for which oral carcinogenicity data are available, and/or a subgroup of these, Known as "PAH4" (sum content of BaP, CHR, BaA and BbF) are more suitable Indicators of PAH contamination in foods. It was further concluded that PAH8 would not provide much added value compared to PAH4. Based on these recommendations, European Union (2011) has set a new maximum level of 30 µg/kg for "PAH4" in smoked fish and fishery products, while maintaining a separate maximum level of 5 µg/kg for benzo(a)pyrene alone. FSSAI (2011) has also specified that the level of benzo(a)pyrene in smoked fishery products marketed in India should not be more than 5 µg/kg.

1.5.4.4 Factors affecting PAH concentrations in food

PAH levels in foods are mainly influenced by temperature and duration of cooking, type and fat content of the food, cooking process (fried, grilled, roasted, boiled and smoked), type of fuel used (electrical, gas, wood and charcoal) and proximity and direct contact with heat source (Akpambang, Purcaro, Lajide, Amoo, Conte, & Moret, 2009; Farhadian, Jinap, Abas, &

Sakar, 2010; Knize, Salmon, Pais, & Felton, 1999; Perelló, Martí-Cid, Castell, Llobet, & Domingo, 2009).

The most important factor affecting the formation of PAHs is the temperature of smoke generation. Amount of PAHs in smoke, formed during pyrolysis increases linearly with the smoking temperature within the interval 400–1000°C. According to Tilgner and Miler (1963), BaP is not formed if the temperature of wood pyrolysis in a two-stage smoke generator is below 425°C and that of oxidation of the volatile products of pyrolysis below 375°C. In oil sardines, smoked for 6 hrs at 45–70°C in a traditional kiln using smoke generated at 400–600°C, the concentration of BaP was about 12 µg/kg wet weights. When smoked at 45°C in filtered smoke generated at 300–400°C for 3.5 hrs and subsequent sun drying for 4–5 hrs, the fish contained only about 1.6 µg BaP/kg (Kaveriappa, 1985).

Hot smoking used for treating, a main part of meat production, brings about higher concentrations of PAH than cold smoking, (Potthast, 1978; Simko, Gombita, & Karovičová, 1991). Lean and fat trout fillets, hot smoked for 30 min by heating to an internal temperature of the fish to 82°C, contained, on average, 5.12 and 8.43 µg BaP/kg, respectively (Zabik, Booren , Zabik, Welch, & Humphrey, 1996).

Meat of fish from modern, automatic chambers, supplied with smoke produced under controlled conditions, contains generally about 0.1 µg/kg BaP, and products from traditional kilns up to several µg/kg (Stołyhwo & Sikorski, 2005). Petrun and Rubenchik (1966) found 4.2 to 60 µg/kg of BaP in different hot and cold smoked fish from commercial smokehouses, while in black sea sprat (*Clupeonella cultriventris*), smoked in an electrostatic apparatus supplied with smoke generated at 250–300°C, the content of BaP was 1.7µg/kg.

El-Saeid (2010) has reported the levels of selected PAHs in four types of barbecued meat commonly consumed in Egypt. It was observed that, grilling of meat in charcoal oven resulted in higher concentrations of PAHs than that was roasted in an electric oven.

In an attempt to reduce PAH levels in charcoal grilled meat, two treatments, preheating (steam and microwave) and wrapping (aluminum foil and banana leaf) have been investigated. Using these pre-treatments before charcoal grilling, resulted in reduced levels of carcinogenic PAH in grilled meat samples (Farhadian, Jinap, Hanifah, & Zaidul, 2011).

The highest concentration of PAH in smoked products is immediately after finishing the smoking and then it decreases due to light decomposition and interaction with present compounds. However, PAHs also penetrate into smoked products, where they are protected from light and oxygen, and after some time, the concentration stabilizes at a certain constant level (Simko & Knezo, 1992).

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Chapter

2 Development of Indigenous Liquid Smoke

Contents

The major objective of this study was to develop a low cost and effective method for production of an indigenous liquid smoke suitable for production of *masmin* and *masmin* based products. As reviewed earlier, dried coconut husk is the major smoke source used in traditional *masmin* production. Hence, to exactly mimic the flavour, it was imperative to use the same as smoke source in liquid smoke production also. However, the influence of smoke source characteristics (using coconut husk as such, coconut fibre alone or coconut fibre powder alone as smoke source) on the composition of the liquid smoke was worth investigating. Influence of these variations on the composition (total phenolic content, total carbonyls, titratable acidity, pH and PAH content) of the developed liquid smokes was investigated and compared with that of a commercial liquid smoke to arrive at the optimum reliable source.

2.1 Materials & Methods

2.1.1 Production of indigenous liquid smoke

The apparatus (Figure 1 & Plate 1) consisted of an incineration chamber, a Davies double surface vertical condenser, a submersible pump and a steam generator. A layer of burning charcoal was laid at the bottom of the

incineration chamber to supply the heat for pyrolysis. The smoke source was then fed in to the incineration chamber and allowed to smoulder in constant contact with charcoal layer. Steam produced from the steam generator was introduced in to the incineration chamber through an inlet port situated at the top. Amount of steam entering the chamber was controlled with a knob fixed on top of the steam generator. The steam inlet was so adjusted that it directs the entire steam in to the condenser without being trapped inside the incineration chamber. Smoke generated in the incineration chamber along with steam was allowed to pass through the condenser maintained at 3±2°C by continuous circulation of chilled water using the submersible pump. The co-movement of smoke and steam inside the setup allows proper mixing of smoke components. Upon reaching the condenser, this steam-smoke mixture gets condensed and the liquefied smoke is collected through the stop cock. The steam generator knob was adjusted to produce 3 L condensate per hour. The condensed liquid was allowed to cool, double filtered through Whatman No: 1 filter paper and then concentrated to half of its initial volume by evaporation. Evaporation can be hastened by using a flash evaporator. However, considering the limited facilities available in the islands, a process modification was also attempted, wherein unconcentrated liquid smoke produced in a batch was used for substituting potable water in the steam generator of the setup. Height of liquid column in the steam generator prior to switching on was noted. Time taken for half of the liquid column to evaporate was arrived upon by intermediate measurements for the first run and thereafter followed without changes (in the setup mentioned above, it took 3 hrs for a batch of liquid smoke (18 L) to concentrate to half of its volume). This method has the advantage of production of steam for the setup and simultaneous

concentration of liquid smoke with minimal energy requirements. Smoke source and charcoal in the incineration chamber were replenished as and when required. Produced liquid smoke was stored in amber coloured glass bottles. This method is having a capacity of generating 1.5 L of concentrated liquid smoke per hour. Freshly produced concentrated liquid smoke was allowed to stabilize for 18 hrs. The sediments formed during stabilization were removed by decanting the liquid smoke prior to use/analysis. Freshly produced liquid smoke had a yellow colouration (Plate 2 to Plate 4) and showed a gradual change to brown colour during stabilization and storage.

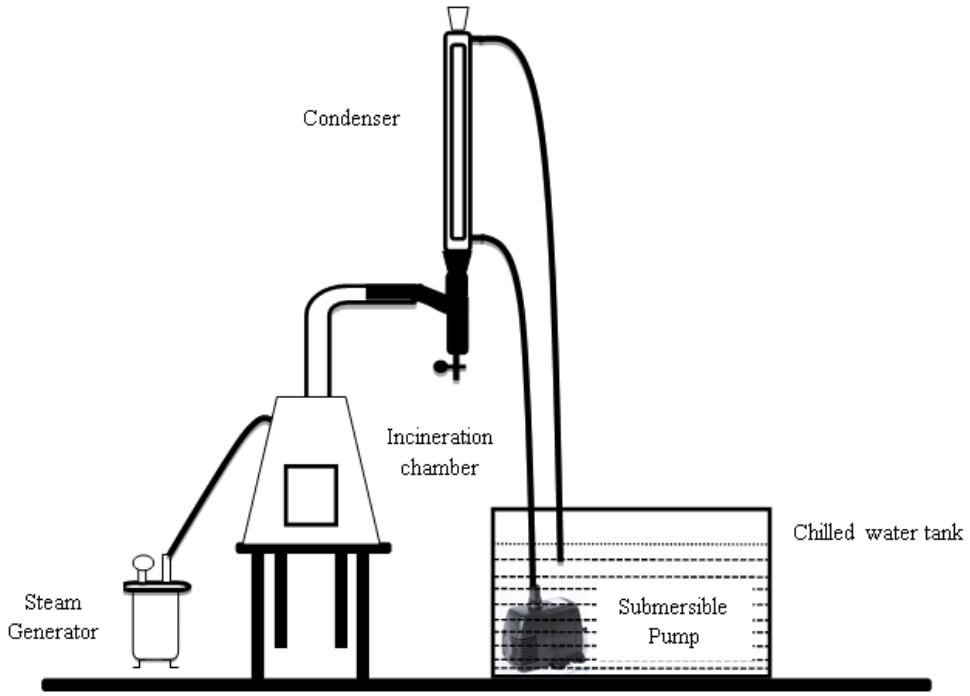

Figure 1 Schematic of the indigenous liquid smoke production unit

Plate 1 Indigenous liquid smoke production unit

Plate 2 Liquid smoke produced from coconut husk

Plate 3 Liquid smoke produced from coconut fibre

Plate 4 Liquid smoke produced from coconut fibre powder

2.1.2 Commercial liquid smoke used for the study

"SMOKEZ ENVIRO 24PB" produced by Red Arrow International LLC, USA with beech wood flavour was used for the study (Plate 5). Choice of the same was based on pre-run trials and widespread availability in India.

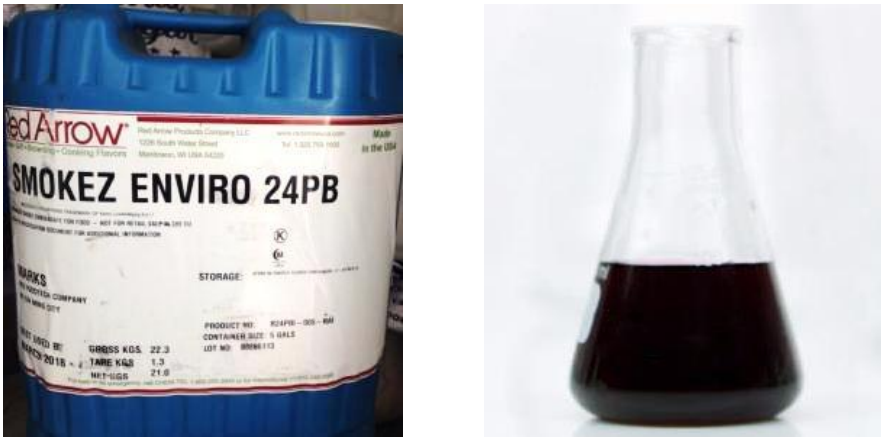

Plate 5 Commercial liquid smoke used for the study

2.1.3 Reagents & Chemicals

All the chemicals used in this study were of analytical grade and acetonitrile was of HPLC grade, all obtained from Merck Millipore (Billerica, MA, USA). Water was purified with a Cascada BIO water System (Pall Corporation, NY, USA). PAH standard EPA 610 Polynuclear Aromatic Hydrocarbons Mixture (mixture of 16 EU priority PAH- acenaphtene-ACE, acenaphthylene-ACY, anthracene-ANT, benzo(a)anthracene-BaA, benzo (a)pyrene-BaP, benzo(b)fluoranthene-BbF, benzo(g,h,i)perylene-BgP, benzo (k)fluoranthene-BkF, chrysene-CHR, dibenzo(a,h)anthracene-DhA, fluoranthene-FLT, fluorene-FLR, indeno(1,2,3-cd)pyrene-IcP, naphthalene-NAP, phenanthrene-PHE, pyrene- PYR) was obtained from Supelco (Bellefonte, PA, USA).

2.1.4 Estimation of total phenolic contents (TPC) in liquid smoke

TPC of liquid smoke was measured using Folin-Ciocalteu method as described by Amin, Zamaliah, and Chin (2004) with slight modifications.

2.1.4.1 Reagents:

1) Standard gallic acid solution: 0.5 mg/ml stock standard solution of gallic acid was prepared by dissolving 250 mg of dry gallic acid in 1 ml ethanol and then diluting to 500 ml with distilled water. The stock solution was stored at 4°C. Working standards between 0.01 and 0.05 mg/ml were prepared by diluting the stock solution with distilled water
2) Folin-Ciocalteu reagent: Diluted 10-fold with distilled water
3) Sodium carbonate solution: 6% (w/v) in distilled water
4) Liquid smoke sample: 1 ml indigenous liquid smoke or 0.1 ml commercial liquid smoke was diluted to 50 ml using distilled water. 1 ml from each was used for analysis.

Pipetted out 1 ml of the sample into a test tube and 0.75 ml of Folin-Ciocalteu reagent was added and mixed. The mixture was allowed to stand at room temperature for 5 min. Then, 0.75 ml sodium carbonate solution was added to the mixture and mixed gently. After standing at room temperature for 90 min, the absorbance was read at 725 nm using spectrophotometer. A blank was also run substituting 1 ml distilled water for the sample. The standard calibration curve of gallic acid (0.01–0.05 mg/ml) was plotted. Calculated, total phenolic content from the calibration curve after correcting dilution of the sample.

2.1.5 Estimation of total carbonyls in liquid smoke

Determination of total carbonyls in liquid smoke was performed according to the method prescribed by JECFA (2001).

2.1.5.1 Principle:

The carbonyls present in liquid smoke were converted to hydrazone derivatives by reaction with 2, 4-dinitrophenylhydrazine (2, 4-DNPH) in acid medium at 60°C. A red pigment was formed on adjusting the pH and the absorbance was read at 430 nm and expressed as heptaldehyde.

2.1.5.2 Reagents:

1. Carbonyl free ethanol: To 1L of ethyl alcohol, 5-10 g of aluminium dust and 8-10 g potassium hydroxide were added and refluxed for 1 hr. The contents were subsequently distilled, first and last 50 ml of the distillate was discarded.
2. 2, 4-DNPH; Twice recrystallized from carbonyl free methanol (Prepared in the same manner as carbonyl free ethanol): 2, 4-DNPH powder (1% w/v) and few drops of concentrated hydrochloric acid in carbonyl free methanol was refluxed for 2-3 hrs and allowed to cool. The solution was filtered through Whatman grade-42 filter paper; filtrate was transferred to a petri dish and dried in a hot air oven at 60°C for 3 hrs.
3. Saturated 2, 4-DNPH solution: 0.05% w/v in toluene, prepared one day in advance and allowed to settle overnight. Insoluble crystals of 2, 4-DNPH was present. The upper layer was filtered prior to use. Prepared fresh weekly.
4. Potassium hydroxide (KOH) solution: 4% w/v in carbonyl free ethanol, prepared fresh daily.
5. Trichloroacetic acid (TCA) solution: 4% w/v in toluene, stable at room temperature.
6. Heptaldehyde standard solution: 30 µg/ml in toluene.
7. Liquid smoke solution: 1 ml indigenous liquid smoke or commercial liquid smoke (0.1 ml) was diluted to 50 ml with carbonyl free ethanol, of

which 5 ml is again diluted to 50 ml with a 10% carbonyl free ethanol-90% toluene solution.

Pipetted 1 ml diluted liquid smoke solution in to a 25 ml volumetric flask and 1 ml of toluene in to another flask to serve as blank. Added to each volumetric flask: 1ml of toluene, 2 ml of trichloroacetic acid solution and 2 ml of saturated 2, 4-DNPH solution. Covered the flask with glass stoppers and heated for 30 min at 60°C in a water bath, immediately cooled in an ice bath. Added 5 ml of KOH solution and diluted to 25 ml with carbonyl free ethanol. Allowed the colour to develop and exactly 10 min after addition of KOH absorbance was read at 430 nm. Corrected absorbance for the blank and constructed a calibration curve using the standard heptaldehyde solution in the range of 1.5-30 µg/ml in toluene, plotting absorbance versus concentration. Calculated percentage carbonyls (as heptaldehyde) from the calibration curve after correcting for dilution of the sample.

2.1.6 Determination of titratable acidity and pH of liquid smoke

Titratable acidity of liquid smoke was determined as per JECFA (2001). 1 ml liquid smoke was pipetted in to a 250 ml beaker, diluted with 100 ml distilled water and titrated with 0.1 N sodium hydroxide solution to an equivalence point of pH 8.15, as determined using a pH meter (Cyber Scan PC 510 model, Eutech instruments, Singapore) equipped with a probe (EC-FC72522-01B). Calculated titratable acidity (Y) as percentage by weight of acetic acid using the following equation (1 ml of 0.1 N sodium hydroxide is equivalent to 60.05 mg acetic acid).

$$Y = \frac{Amount\ of\ titre\ (ml) X\ Normality\ of\ NaOH\ X\ 60.05}{Sample\ volume\ (ml)\ X\ 1000}\ X\ 100$$

For determination of pH, an aliquot of 20 ml liquid smoke was taken in a beaker and pH at 25°C was recorded using the pH meter.

2.1.7 Determination of Polycyclic Aromatic Hydrocarbon content in liquid smoke

2.1.7.1 Extraction of PAHs from liquid smoke

Procedure described by APHA (2005) was used for extraction of PAHs. 200 ml indigenous liquid smoke along with 50 ml 1% brine was taken in a separating funnel and shaken vigorously. For the extraction of PAHs from commercial liquid smoke 100 ml aliquots were used. The solution was thrice extracted with 50 ml portions of methylene chloride. Pooled the extract and filtered through anhydrous sodium sulphate. Evaporated the solvent completely and reconstituted in 2 ml cyclohexane.

2.1.7.2 Chromatographic clean-up and HPLC analysis

Chromatographic clean-up was performed on a 20 mm ID column filled with 3 g anhydrous sodium sulphate above 20 g silica gel (60-120 mesh) activated overnight at 120°C. Before loading the sample, column was pre-eluted with 40 ml n-Hexane and covered with aluminium foil. The cyclohexane extract was loaded in to the column and the container was rinsed with additional 2 ml portions of cyclohexane. Completely opened the stop cock and just before the lower meniscus of cyclohexane layer reached the sodium sulphate layer, 50 ml n-Hexane was added to the column and discarded. Eluted the column with 50 ml 2:3 mixture of methylene chloride and n-Hexane. Collected in a flat bottom flask and concentrated the contents to a lower volume and added 4 ml acetonitrile. Again concentrated to a volume less than 1 ml and reconstituted in 5 ml and 10 ml acetonitrile for indigenous and commercial liquid smoke, respectively.

The extract was subsequently analysed in a HPLC (Plate 6) fitted with a reverse phase PAH C18 column, S-5 µm; 250 X 3.0 mm (Waters, Germany),

using a gradient elution programme with a mixture of acetonitrile and water which started at 40% acetonitrile, reaching 100% in 28 min and held at 100% during the next 17 min at a flow rate of 1.5 ml/min. For the PAH determination, the following detection parameters were used: fluorescence detector (Ex/Em) 280/330 nm (NAP, ACE & FLR) 246/370 nm (PHE), 250/406 nm (ANT) 280/450 nm (FLT) 270/390 nm (PYR) 265/380 nm (BaA & CHR) 290/430 nm (BbF, BkF & BaP) 290/410 (DhA & BgP) and 300/500 nm (IcP). PAH content (µg/L) in different samples was calculated by external standard calibration.

PAH4 (sum content BaP, BaA, BbF & CHR), Light PAH (sum content of PAH having molecular weight less than 216.3 Da viz- NAP, ACE, FLR, ANT, PHE, PYR & FLT) and Heavy PAH (PAH with higher molecular weight than Light PAH viz. BaA, CHR, BbF, BkF, BaP, ICP, BgP & DhA) were calculated from the data.

Plate 6 HPLC used for PAH analysis

2.1.8 Statistical analysis

Composition of coconut husk liquid smoke (CH), coconut fibre liquid smoke (CF), coconut fibre powder liquid smoke (CP) and commercial liquid smoke (CL) (Table 1) were compared by multivariate ANOVA (IBM SPSS Statistics version 20). Results are reported as mean ± standard deviation. Means were compared using Tukey's multiple comparison test and independent sample t-test at 5 % level of significance.

2.2 Results & Discussions

2.2.1 Total phenolic content

Analysis of total phenolic content showed that, CH had a significantly higher phenolic content (1518 ± 184 mg/L) compared to CF (1037 ± 110 mg/L) and CP (834 ± 48.23 mg/L) ($p<0.05$) (Table 1). No significant difference was observed between the TPC of CF and CP ($p>0.05$). CL showed a significantly higher TPC (20047 ± 193 mg/L) compared to all the indigenously developed liquid smokes ($p<0.05$). The commercial liquid smoke used for the study was a thick solution with strong burning aroma and was meant to be diluted and applied on foods. Whereas indigenous liquid smokes were supposed to be directly applied on foods without any further dilution.

Swastawati, Agustini, Darmanto, and Dewi (2007) have reported phenolic content of 335 and 4812 mg/L in liquid smoke produced from corn cob and lamtoro wood, respectively. Liquid smokes prepared from mixed dried hardwood sawdust had a total phenolic content of 9900–11100 mg/L (Ramakrishnan & Moeller, 2002). Montazeri, Alexandra, Brian, Mary, and Charles (2013) have reported a TPC content of 3220 mg/L in a full-strength commercial liquid smoke preparation. In a related study, we observed a TPC

content of 1348 mg/L in unconcentrated coconut husk liquid smoke produced using the same setup (Nithin *et al*., 2016).

Phenols and phenolic esters are produced during the pyrolysis of lignin (Maga, 1988). Coconut husk is a thick, coarse, bulky and fibrous material consisting of strands of fibro vascular bundles or 'coir' embedded in a non-fibrous parenchymatous "corky" connective tissue and is reported to have a lignin content of 33%. Lignin degradation during pyrolysis extends over a temperature range of 300- 450°C (Girrard, 1992). Fenner and Lephardt (1981) while examining pyrolysis of kraft pine lignin also observed that maximum lignin decomposition occurred at a pyrolysis temperature of 385°C. During the production of CH, smouldering of coconut husk would have resulted in formation of a layer of burning charcoal in the exterior layer of the husk. This charcoal layer would have facilitated more efficient heat transfer to the adjacent fibre layers of the husk which could be the reason behind higher phenolic content in CH compared to other indigenous liquid smokes.

2.2.2 Total carbonyl content

Carbonyl content in all the indigenous liquid smokes (Table 1) were homogenous in nature ($p>0.05$). However, the same in CL (26.33 ± 4.04 %) was significantly higher than all the indigenous liquid smokes ($p<0.05$). Among the indigenous liquid smokes CH showed the highest carbonyl content (3.74 ± 0.75 %) followed by CP (1.22 ± 0.3 %) and CF (1.16 ± 0.24 %). Carbonyls are thought to be originated from the thermal decomposition and rearrangement of cellulose and hemicellulose via classical carbohydrate degradation schemes (Kim, Kurata, & Fujimaki, 1974). Coconut husk consist of a well-defined polymeric structure of cellulose (28%) and hemicellulose (38%) (Pollard, Fowler, Sollars, & Perry, 1992).

Despite of their contribution to aroma of food matrices, carbonyl compounds, especially aldehydes contribute to colour and the texture of smoked foods (Vincent *et al*., 2007). Development of the characteristic colour of smoked foods is reported to be the results of maillard reaction, in which the carbonyl groups present in the smoke react with the amino groups present on the food surface to produce a brown colouration (Maga, 1988). Guillen and Ibargoitia (1998) reported that these reactions between carbonyls and proteins can also impart greater firmness to meat products, thereby affecting the texture of the products.

2.2.3 pH and titratable acidity

pH of the liquid smoke is an important parameter in determining its quality. Further it denotes the level of decomposition of the organic material used as smoke source. Generally, a lower pH value is preferred for liquid smoke as it adds to the storage life, antimicrobial properties and organoleptic characteristics. No significant difference was observed between the three indigenous liquid smokes in terms of pH and titratable acidity ($p>0.05$). Commercial liquid smoke showed the lowest pH (2.99 ± 0.41) and highest titratable acidity (2.71 ± 0.17 %) ($p<0.05$). Titratable acidity in indigenous liquid smokes varied from 1.88 % (in CH) to 2.08 % (in CF). Aqueous liquid smokes have been generally reported to be very acidic in nature, having a pH of 2.5 or less and a titratable acidity of 3% (Chiu, 1983). Achmadi, Mubarik, Nursyamsi, and Septiaji (2013) have reported a pH of 3.2 in redistilled liquid smoke from oil-palm shells. pH of 1.23 to 2.13 has been reported in liquid smoke produced from coconut husk (Susy & Abrina, 2013). The authors also found an inverse relation of pH with pyrolysis temperature. Lombok, Setiaji, Trisunaryanti, and Wijaya (2014) reported a pH of 4.10 to 6.20 in liquid smoke produced from coconut shell. They found that, distillation temperature

also shows an inverse relation with the pH of the liquid smoke. It can be inferred that during pyrolysis and distillation at higher temperatures, vaporisation of acids with high boiling point like acetic acid take place, which results in increased acidity.

2.2.4 Polycyclic Aromatic Hydrocarbon content

Total PAH content (Table 1) in all the indigenous liquid smoke were homogenous in nature ($p>0.05$). CL had a significantly higher total PAH content compared to others (215 ± 15.45 µg/L) ($p<0.05$). Among the indigenous liquid smokes, CP had the highest total PAH content (8.23 ± 1.47 µg/L). CH recorded the lowest total PAH content (0.64 ± 0.13 µg/L). Swastawati, Agustini, Darmanto, and Dewi (2007) had observed a total PAH content of 3.04 µg/L in liquid smoke produced from Lamtoro wood (*Leucaena leucocephala*), whereas no PAH were detected in liquid smoke produced from corn cob. Carla, Sabrina, and Susi (2006) have reported a total PAH content of 3.8 µg/L in liquid smoke produced from fermented marc. Aqueous liquid smokes contains lesser amount of PAH compared to oil based liquid smokes because of the vigorous refining processes practiced during their production and due to the lipophilic nature of the PAH compounds (Stołyhwo & Sikorski, 2005; Visciano, Perugini, Conte, & Amorena, 2008).

PAHs show wide variations in their molecular mass ranging from Indene (116 Da) to Dibenzopyrenes (302 Da) (Toth & Potthast, 1984). The Light PAHs (Containing two or three rings) of molecular mass below 216 Da, are not regarded as carcinogenic since they are relatively volatile, soluble and more degradable than Heavy PAH. BaP has been accepted as a marker for the occurrence of carcinogenic PAH in food (Scientific Committee on Foods, 2002). Maximum tolerable levels of BaP in variety of foodstuffs have been

specified by European Union (2011), wherein a maximum limit of 5 µg/kg has been set for smoked fish and fishery products. Further, the regulation also recommends that sum content of BaP, BaA, BbF and CHR (PAH4) in smoked fish and fishery products should not exceed 30 µg/kg. EU regulation No. 2065/2003 has recommended that the level of BaP and BaA in a primary smoke condensate should not exceed 10 and 20 µg/L, respectively (EC, 2003). FSSAI (2011) has fixed 5 µg/kg as the maximum level of benzo(a)pyrene in smoked fishery products marketed in India.

All the PAHs detected in CH and CL were coming under the category of Light PAHs. In the case of CF and CP, Light PAHs constituted 99.4 and 95 % of the total PAHs, respectively. Significant difference was observed in Heavy PAH content of CF and CP ($p<0.05$). Heavy PAHs constituted 0.6 and 5 % of the total PAH content in CF and CP, respectively. All the Heavy PAHs detected in CF and CP was coming under the category of PAH4. BaA was detected only in CF samples. CHR was the PAH4 compound found in highest quantities in CP, BbF was found in the lowest concentration. PAH compounds BaP, PYR, CHR and BbF were detected only in CP. FLR and PHE were detected in all the liquid smoke samples. FLR was the highest individual PAH detected in CL. NAP, ACE & PHE were the highest individual PAHs present in indigenous liquid smokes.

PAHs are thought to be generated predominantly from incomplete combustion of organic materials (Dyremark, Westerholm, Overvik, & Gustavsson, 1995; Wu, Wong, Lee, Shi, & Ong, 1997). While using coconut powder and coconut fibre as smoke source, due to the increased surface area compared to coconut husk, there is limited air supply between the smoke source and charcoal layer. The combustion taking place under such conditions will be largely incomplete, which might have been the reason behind higher PAH content in CP and CF.

Table 1 Composition of indigenous and commercial liquid smoke

Composition	CH	CF	CP	CL
Total phenolic content (mg/L)	1518 ± 184[a]	1037 ± 110[b]	834 ± 48.23[b]	20047 ± 193[c]
Total carbonyl content (%)	3.74 ±0.75[a]	1.16 ± 0.24[a]	1.22± 0.3[a]	26.33 ± 4.04[b]
pH	4.06 ± 0.64[a]	3.75± 0.47[a]	3.81± 0.23[a]	2.99± 0.41[b]
Acidity (% acetic acid)	1.88 ± 0.07[a]	2.08 ± 0.12[a]	1.94 ± 0.08[a]	2.71 ± 0.17[b]
Polycyclic aromatic hydrocarbons (µg/L)				
NAP	ND	2.68 ±0.421[a]	ND	60.25 ± 3.645[b]
ACY	ND	2.4 ± 0.589[a]	0.08 ± 0.0025[b]	4.30 ± 0.456[c]
FLR	0.12 ± 0.034[a]	0.73 ± 0.234[a]	0.09 ± 0.00257[a]	64.06 ± 4.28[b]
PHE	0.43 ± 0.084[a]	1.37 ± 0.189[a]	3.29 ± 0.46[a]	50.22 ± 3.98[b]
ANT	0.06 ± 0.0096[a]	ND	2.03 ± 0.396[a]	33.17 ± 2.58[b]
FLT	0.03 ± 0 .0072[a]	ND	0.77 ± 0.167[b]	3.01± 0.512[c]
PYR	ND	ND	1.54 ± 0.351	ND
BaA	ND	0.04 ± 0.0089[a]	0.14 ± 0.038[b]	ND
CHR	ND	ND	0.20 ± 0.066	ND
BbF	ND	ND	0.03 ± 0.016	ND
BkF	ND	ND	ND	ND
BaP	ND	ND	0.07 ± 0.0012	ND
Light PAH	**0.64 ± 0.13[a]**	**7.18 ± 1.43[a]**	**7.79 ± 1.38[a]**	**215 ± 15.45[b]**
Heavy PAH	**ND**	**0.04 ± 0.009[a]**	**0.44 ± 0.09[b]**	**ND**
Total PAH	**0.64 ± 0.13[a]**	**7.22 ± 1.44[a]**	**8.23 ± 1.47[a]**	**215 ± 15.45[b]**
PAH4	**ND**	**0.04 ± 0.009[a]**	**0.44 ± 0.09[b]**	**ND**

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

2.3 Conclusion

The results obtained shows that, variation in source characteristics can significantly influence the composition of resulting liquid smoke. Significant difference was observed in terms of total phenolic content and Heavy PAH content. Liquid smoke produced from coconut husk showed the highest

phenolic content. Lowest phenolic content was observed in liquid smoke produced from coconut fibre powder. Coconut fibre powder liquid smoke showed the highest Heavy PAH content among the indigenous liquid smokes. None of the Heavy PAHs were detected in liquid smoke produced from coconut husk. Source characteristics were found to have no influence on total carbonyls, pH and acidity of liquid smokes. Significant difference was observed between indigenous and the commercial liquid smoke in all the analysed parameters. Commercial liquid smoke showed higher phenolic content, carbonyl content and PAH content than indigenous liquid smokes. All the liquid smoke used in this study had a PAH content far below the approved limit by European Union and BaP was detected only in liquid smoke produced from coconut husk powder. Among the indigenous liquid smokes, due to the higher phenolic content and lesser PAH, liquid smoke produced from coconut husk was found to be of superior quality and hence was selected for the ensuing studies.

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Chapter

3

Standardization of Process Parameters for the Production of Liquid Smoked *Masmin* and *Masmin* Based Products

Contents

Spraying, soaking and mixing with food are the three major ways of application of liquid smoke in foods. Production parameters of 12 liquid smoked products viz; indigenous liquid smoked (INDLS) *masmin* by spraying, INDLS *masmin* by soaking, INDLS *masmin* flakes by spraying, INDLS *masmin* flakes by soaking, INDLS *masmin* powder by spraying, INDLS *masmin* powder by soaking, commercial liquid smoked (CMLS) *masmin* by spraying, CMLS *masmin* by soaking, CMLS *masmin* flakes by spraying, CMLS *masmin* flakes by soaking, CMLS *masmin* powder by spraying and CMLS *masmin* powder by soaking were standardized using Response Surface Methodology (RSM). Spraying and soaking were the methods applicable for producing liquid smoked *masmin* and *masmin* powder. In the case of *masmin* flakes, all the three methods were applicable. Standardization of process parameters for the production of liquid smoked *masmin* flakes by mixing was not done with RSM since there was only one independent variable, ie: quantity

of liquid smoke. Hence, process standardization of INDLS & CMLS *masmin* flakes by mixing was carried out as a separate study. Products from optimum combinations were compared with each other to arrive at the best treatment in indigenous and commercial liquid smoked products.

3.1 Materials & Methods

Representative samples of traditional *masmin* were collected from different islands of Lakshadweep and kept sealed in metalized polyester polythene pouches (12µ polyester/10µ Aluminium foil/300 gauge LDPE) until analysis.

3.1.1 Multi-functional smoke kiln

All the smoking, spray application of liquid smoke and drying processes for the study were carried out in a multi-functional smoke kiln (CS700EL, KERRES Anlagensysteme GmbH, Germany). The unit (Plate 7) consisted of a stainless steel chamber with digital controls for adjusting the process parameters. The unit was capable of operating in eight different modes viz: fast drying, slow drying, hot smoking, cold smoking, cooking, dry cooking, liquid smoking (by spraying) and showering.

Plate 7 Multi-functional Smoke kiln

3.1.2 Experimental design and statistical models

Input variables and their broad range of application for the study were arrived upon from pre-run trials. Input variables selected for spray application were; duration of exposure (duration of spraying), salt content in liquid smoke, chamber temperature (temperature of the spraying chamber), flow rate (quantity of liquid smoke being sprayed) and dilution of liquid smoke (applicable only for commercial liquid smoke). Duration of exposure (duration of soaking), salt content in liquid smoke and dilution of liquid smoke (applicable only for commercial liquid smoke) were the input variables

selected for soaking treatments. A summary of the entire experiment is given in Figure 2 and Figure 3. Input variables and their levels of application for commercial and indigenous liquid smoke are given in Table 2 to Table 5. Sensory score, total phenolic content and salt content of the product were the dependent variables identified for the study.

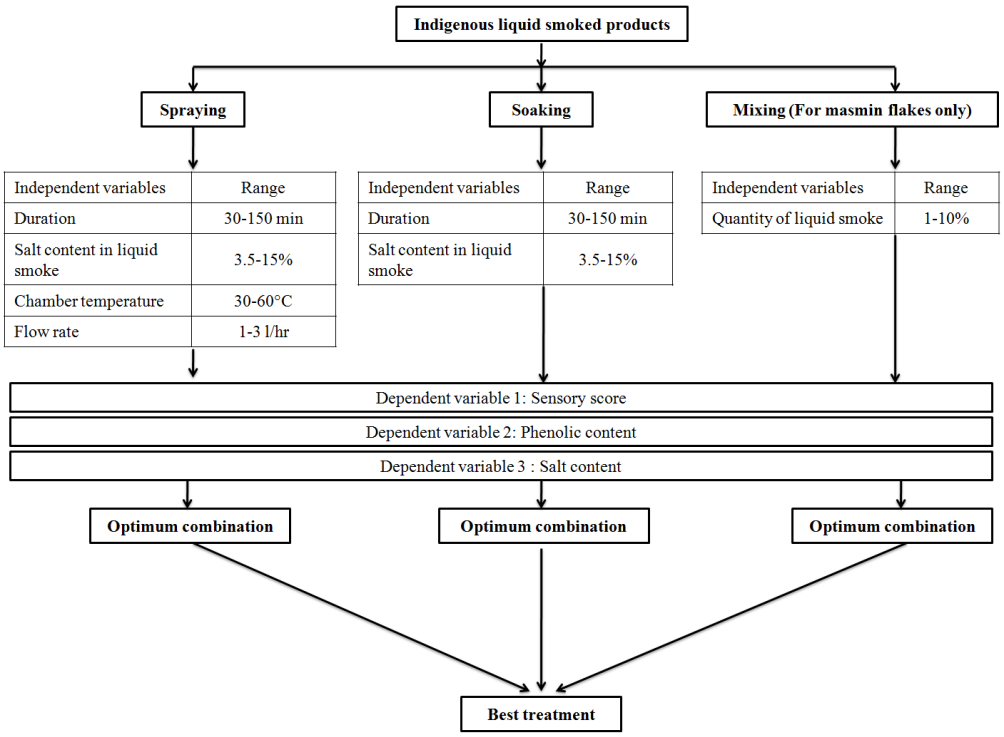

Figure 2 Summary of experiments for production of INDLS products

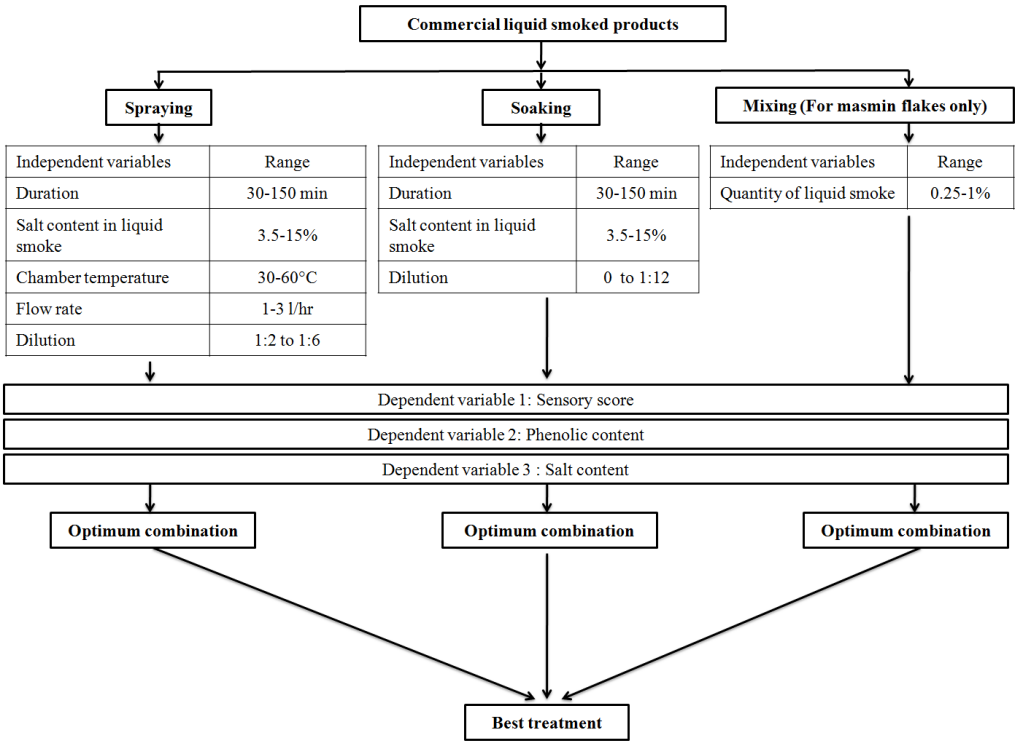

Figure 3 Summary of experiments for production of CMLS products

Table 2 Input variables and their levels of application for CMLS products by spraying

Input variables	Code	Levels		
		-1	0	1
Duration of exposure (min)	x_1	30	90	150
Salt content in liquid smoke (%)	x_2	3.5	9.25	15
Chamber temperature (°C)	x_3	30	45	60
Flow rate (L/hr)	x_4	1	2	3
Dilution (%)	x_5	1:2 (50%)	1:3 (33.3%)	1:6 (16.6%)

Table 3 Input variables and their levels of application for CMLS products by soaking

Input variables	Code	Levels		
		-1	0	1
Duration of exposure (min)	x_1	30	90	150
Salt content in liquid smoke (%)	x_2	3.5	9.25	15
Dilution (%)	x_3	0 (0%)	1:12 (8.3%)	1:6 (16.6%)

Table 4 Input variables and their levels of application for INDLS products by spraying

Input variables	Code	Levels		
		-1	0	1
Duration of exposure (min)	x_1	30	90	150
Salt content in liquid smoke (%)	x_2	3.5	9.25	15
Chamber temperature (°C)	x_3	30	45	60
Flow rate (L/hr)	x_4	1	2	3

Table 5 Input variables and their levels of application for INDLS products by soaking

Input variables	Code	Levels		
		-1	0	1
Duration of exposure (min)	x_1	30	90	150
Salt content in liquid smoke (%)	x_2	3.5	9.25	15

Central composite design was used for the study and the model was fitted using Design Expert 7.1.5 (Stat-Ease, lnc., Minneapolis MN, USA). Quadratic model in the following equation was used to break up the total variability into variability due to linear, quadratic and interaction effect of process parameters and error (Myers & Montgomery, 2002).

$$Y = \beta_o + \sum_i \beta_i x_i + \sum_{ii} \beta_{ii} x_{ii}^2 + \sum_i \sum_{j,i<j} \beta_i \beta_j x_i x_j + e, i \neq j$$

Where, "Y" is response variable, "β_o" is intercept, "β_i" is linear regression coefficients, "β_{ii}" is quadratic regression coefficients, "β_{ij}" is interaction regression coefficients and "e" is error term. Desirability score was computed for multiresponse optimization of input process parameters. While computing desirability score, the predicted values of response variables were either maximized or minimized; otherwise target values were fixed for response variables. The optimum combination of process parameters was chosen where the desirability score was close to the value of 1. Desirability scores obtained for the optimized conditions were confirmed with validation studies. Products from optimum combinations were compared with each other to arrive at the best tratments in indigenous and commercial liquid smoked products.

3.1.2.1 Sample preparation for standardization of liquid smoked *masmin*

3.1.2.1.1 Pre-processing operation

Fresh skipjack tuna (Plate 8) were purchased from landing centers at Thoppumpady, Kerala and transported to the laboratory in iced condition with a fish to ice ratio of 1:1 (w/w). The fishes were beheaded, eviscerated, washed in chilled potable water (Plate 9) (bigger fishes if used were manually split in to two halves along the vertebral column) and then cooked in 5% salt water with a fish to water ratio of 1:5 for 90 min. The pieces were allowed to cool in the same water. After sufficient cooling, skin and bones were removed and the loins separated manually (Plate 10).

Plate 8 Skipjack tuna

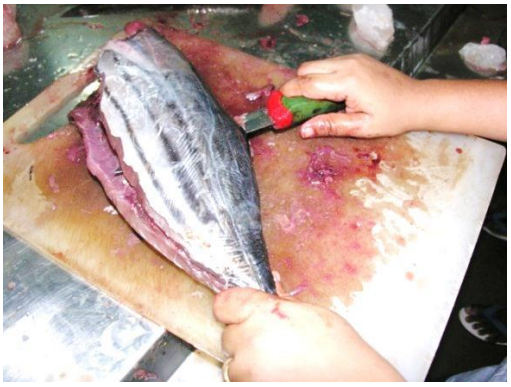

Plate 9 Beheading and gutting of tuna

Plate 10 Cooked and cleaned loins

After the pre-processing operations, the loins were exposed to different treatment combinations in separate batches. Mass to liquid ratio of 1:5 was used for soaking trials (Plate 11), for spraying operations (Plate 12) 5 kg cooked loins were processed in a batch. After completing the treatments, loins were dried in the multipurpose smoker cum drying kiln at 60°C to moisture content below 10%. Dried loins (Plate 13) after cooling were packed in metallized polyester polythene pouches and stored at room temperature until further analysis.

Plate 11 Soaking trials for production of *masmin*

Plate 12 Spraying trials for production of *masmin*

Plate 13 Liquid smoked *masmin*

3.1.2.2 Sample preparation for standardization of liquid smoked *masmin* flakes

3.1.2.2.1 Pre-processing operation

The fishes (skipjack tuna) were washed in potable water, beheaded, gutted, de-skinned and meat was separated by removing bones. The meat was washed with potable water then minced with 3% salt in a pre-cooled bowl chopper (for producing liquid smoked *masmin* flakes by mixing, liquid smoke in various proportions were also blended with the meat during this step).

Mince was then stuffed in to metallic moulds (20 cm x 5cm x 4cm) and steam cooked for 30-40 min at atmospheric pressure. Cooked meat blocks (Plate 14) were removed from the mould with the help of a thin knife.

Plate 14 Cooked meat blocks for production of *masmin* flakes

The meat blocks were then subjected to different treatments in separate batches (Plate 14 & Plate 16) and then subsequently dried at 60°C until the moisture content reached below 30% (the drying process took 18-24 hrs). This smoked and dried meat blocks were made to thin flakes with the help of an exclusively designed *masmin* flaking machine (Plate 18) and the flakes were further dried at 60°C to moisture content below 10%. Dried flakes (Plate 17) after cooling were packed in metallized polyester polythene pouches and stored at room temperature until further analysis.

Plate 15 Soaking trials for production of *masmin* flakes

Plate 16 Spraying trials for production of *masmin* flakes

Plate 17 Liquid smoked *masmin* flakes

3.1.2.2.2 *Masmin* flaking machine

A *masmin* flaking machine was specially designed and fabricated at Sun Ray Industries, C-98, Industrial area, Yadavagiri, Mysore-570020. The machine was meant to chop the *masmin* blocks to uniform thin flakes. It is a self-standing SS 304 steel made machine equipped with a 220V, AC 50Hz (Single Phase) variable frequency drive motor with provisions for adjusting the RPM. The chopping mechanism consisted of removable circular high carbon steel blade heads. *Masmin* blocks were fed in to the machine through inlet port situated on top and pressed against the rotating blades with stainless

steel handle on top. Flakes were collected in a tray kept at the outlet. 300 to 360 RPM was found to be optimum for flaking *masmin* blocks with moisture content of 30%.

Plate 18 *Masmin* flaking machine

3.1.2.3 Sample preparation for standardization of liquid smoked *masmin* powder

Cooked loins were prepared as described in the pre-process operations for *masmin* (section 3.1.2.1.1). The loins were then manually shredded to thin strips by hand (Plate 19), exposed to different treatments in separate batches (Plate 20 & Plate 21) and subsequently dried to moisture content below 10 %. The dried strips were then powdered by using a mechanical grinder, cooled (Plate 22) and then packed in metallized polyester polythene pouches and stored at room temperature until further analysis.

Plate 19 Cooked and shredded loins

Plate 20 Soaking trials for production of *masmin* powder

Plate 21 Spraying trials for production of *masmin* powder

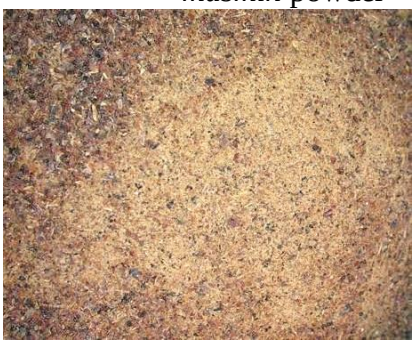

Plate 22 Liquid smoked *masmin* powder

3.1.3 Determination of total phenolic content

TPC of *masmin* and *masmin* based products were determined using Folin-Ciocalteu method as described by Amin, Zamaliah, and Chin (2004) with slight modifications. *Masmin* (scraped in to thin flakes using a knife) and *masmin* flakes were ground in a mechanical grinder. *Masmin* powder was used as such for analysis. Approximately 5 g sample was soaked in 50 ml distilled water for 12 hrs with intermediate swirling. Prior to analysis, the extract was filtered using Whatman grade-1 filter paper. 1 ml from this extract was used for analysis. Further analysis was carried out as per the method described in section 2.1.4.

3.1.4 Determination of salt content

About 1g of homogenized sample was weighed into an Erlenmeyer flask. To this, 25 ml distilled water, 5 ml HNO_3 and 40 ml standard $AgNO_3$ solution (0.1 N) were added. The contents of the flask were boiled for 15 min and after sufficient cooling, titrated against standard ammonium thiocyanate (0.1 N) using ferric alum as indicator. The end point was denoted by a reddish brown colour. A blank was also run by titrating 40 ml 0.1 N $AgNo_3$ with 0.1 N ammonium thiocyanate. Calculated sodium chloride content (%) of the sample from the following equation.

$$Sodium\ chloride\ (\%) = \frac{(\text{Blank} - \text{Titre value})\ 58.45}{\text{Wt. of the sample}} \text{X}\ 100$$

3.1.5 Determination of overall acceptability

Sensory analysis of the products was carried out by a five member expert panel using an 8 point hedonic scale prescribed by Meilgaard, Civille,

and Carr (1999) with slight modifications. The panel consisted of individuals who were familiar with the use of *masmin* and *masmin* based products and experts in the field of smoked foods. Score of 8 in the scale denoted "No difference from control" and 1 denoted "Extremely large difference from control". Samples were provided to the panelist in coded plates along with a known control. In the case of liquid smoked *masmin,* traditional *masmin* was used as the control. For the standardization of liquid smoked *masmin* flakes, traditional *masmin* was made to thin flakes and used as control. Powdered traditional *masmin* was used as control for developing liquid smoked *masmin* powder. Panelists were asked to score on appearance, colour, odour, flavour, smoke taste, saltiness and mouth feel of the samples in comparison to the controls. Overall acceptability was calculated by taking the average of scores obtained for each attribute.

SENSORY EVALUATION SCORE CARD FOR STANDARDIZATION OF PRODUCTS

Assessor: …… Date: ……

(Please score the sample characteristics by placing the relevant score)
An honest expression of your personal feeling will help us.

ATTRIBUTES	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
Appearance				
Colour				
Odour				
Flavour				
Smoke Taste				
Saltiness				
Mouth feel				
Overall acceptability				

Please score the sample characteristics according to the following scale

QUALITY GRADE DESCRIPTION	SCORE
No difference from control from control	08
Very slight difference from control	07
Slight difference from control	06
Moderate difference from control	05
Moderately large difference from control	04
Large difference from control	03
Very large difference from control	02
Extremely large difference from control	01

Comments:

Signature:

3.2 Results & Discussions

3.2.1 Standardization of process parameters for production of indigenous liquid smoked *masmin* by spraying

Second order response regression model was found to be best fitted for explaining the variability in dependent variables as a function of input variables, with R^2 values 0.74, 0.79 and 0.97 for sensory score, total phenolic content and salt content, respectively. Response surface plots of different response variables showing the effect of input variables are given in Figure 4 to Figure 9. Regression coefficients of fitted model along with R^2 values are given in Table 6.

Linear regression coefficients of salt content in liquid smoke and flow rate showed a significant influence on sensory score of the samples ($p<0.05$). Interaction effect of duration of exposure (duration of spraying) and salt content in liquid smoke also showed a significant influence on sensory score ($P<0.05$). A sharp increase in sensory score was noted in samples produced from increased duration of exposures. This is expected due to higher phenolic content and saltiness in samples processed with longer duration of exposures. Importance of phenolic compounds in imparting flavour to smoked foods has been well explored (Maga, 1988; Kjhallstrand & Petersson, 2001; Serot, Baron, Knockaert, & Vallet, 2004; Swastawati, Agustini, Darmanto, & Dewi, 2007). Traditional *masmin* has been reported to contain salt content in the range of 4.1 to 6.75% and an approximate total phenolic content of 235 ppm (David, Rajagopalasamy, & Sugumar, 1990; Nair, Nair, Joseph, & Cyriac, 1994). From the response surface plots, it can be seen that these values were reached at a salt content of 3.5 to 6.38 in liquid smoke and a flow rate of 2.25 to 3 L/hr. Chamber temperature did not show any significant influence on the

response variables ($p>0.05$). However, a slight decreasing trend in sensory score was observed with an increase in chamber temperature from 30 to 60°C.

Duration of exposure was found to have a significant influence on the total phenolic content of the samples ($p<0.05$). An increasing trend in total phenolic content was noted with increased duration of exposures. TPC of the samples showed a proportionate increase with flow rate; however the effect was not found to be statistically significant ($p>0.05$). Chamber temperature and salt content in the samples were not found to have any influence on TPC of the samples ($p>0.05$). On the contrary, Serot, Baron, Knockaert, and Vallet, (2004) have reported that increase in chamber temperature significantly increased the phenol deposition in product during liquid smoking.

Duration of exposure, flow rate and salt content in liquid smoke had a significant linear effect on the salt content of the product ($p<0.05$). A proportionate increase in salt content was observed with an increase in these parameters. Product salt content was also found to be significantly influenced by the quadratic effect of salt content in liquid smoke and interaction effect of duration of exposure and salt content in liquid smoke ($p<0.05$). However, chamber temperature did not show any significant influence on the salt content of the sample ($p>0.05$).

Based on the desirability score, spraying cooked loins with indigenous liquid smoke containing 4% salt for 155 min at flow rate of 3 L/hr and chamber temperature of 60°C was found to give the product a matching flavour with traditional *masmin*. The corresponding desirability score was 0.97.

Table 6 Regression coefficients for coded factors of INDLS *masmin* produced by spraying

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+2.76	+180.05	+7.91
Duration of exposure	x_1	-0.44	+120.25*	+1.82*
Salt content in liquid smoke	x_2	-0.96*	+16.29	+3.63*
Chamber temperature	x_3	+0.11	+15.87	+0.15
Flow rate	x_4	+1.11*	+39.06	+1.89*
Duration of exposure X Salt content in liquid smoke	x_1x_2	-1.21*	+13.60	+1.08*
Duration of exposure X Chamber temperature	x_1x_3	-9.562	+5.45	+0.38
Duration of exposure X Flow rate	x_1x_4	+0.73	+0.16	+0.18
Salt content in liquid smoke X Chamber temperature	x_2x_3	-0.12	+6.28	-0.15
Salt content in liquid smoke X Flow rate	x_2x_4	+0.23	-4.01	+0.47
Chamber temperature X Flow rate	x_3x_4	+0.70	+9.45	-0.40
Duration of exposure^2	x_1^2	-0.89	+72.08	+0.31
Salt content in liquid smoke^2	x_2^2	-0.061	+15.81	+0.66*
Chamber temperature^2	x_3^2	+0.21	-1.40	-0.029
Flow rate^2	x_4^2	-0.27	-5.34	-0.76
	R^2	0.74	0.79	0.97

* Significant at 5% level of significance

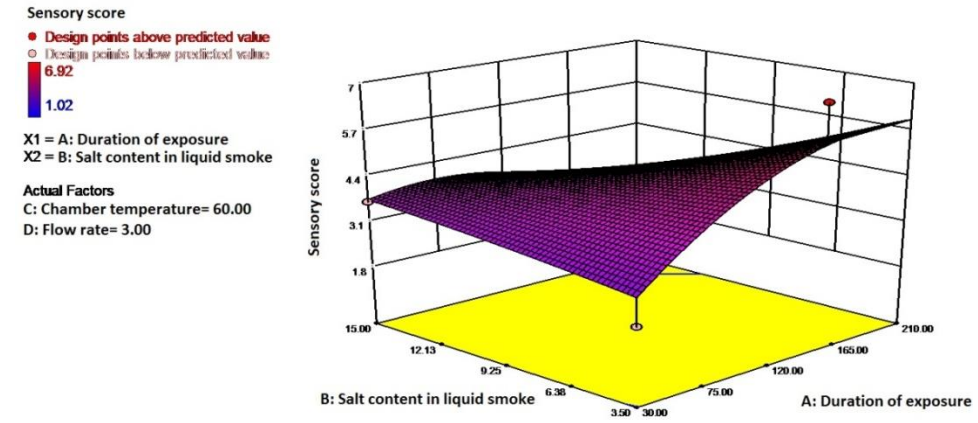

Figure 4 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of INDLS *masmin* produced by spraying

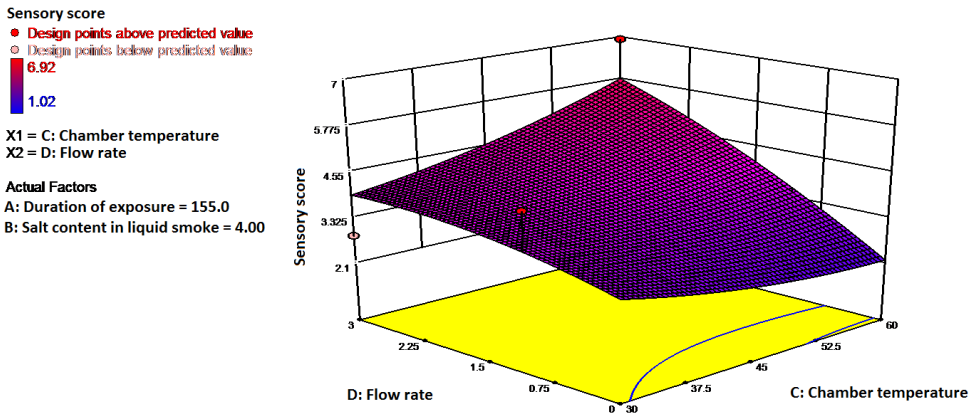

Figure 5 Response surface plots for the effect of flow rate and chamber temperature on sensory score of INDLS *masmin* produced by spraying

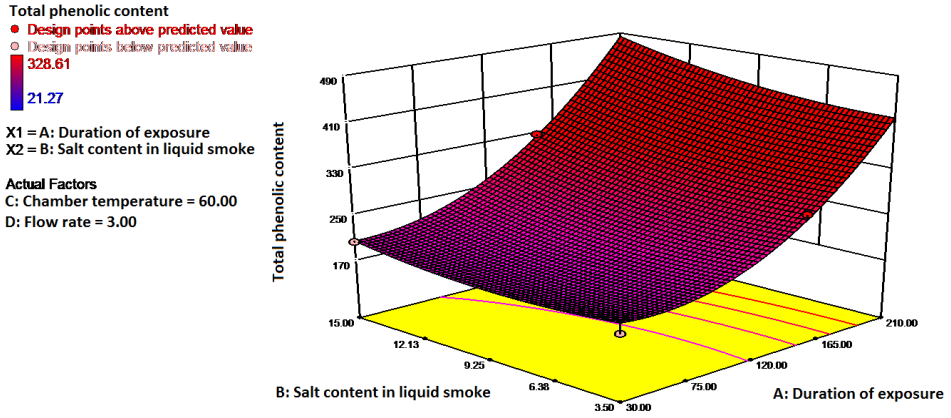

Figure 6 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of INDLS masmin produced by spraying

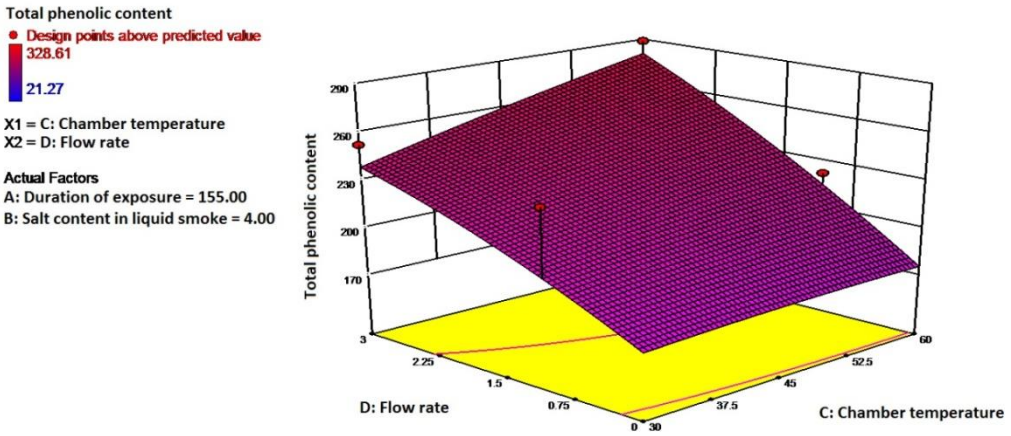

Figure 7 Response surface plots for the effect of flow rate and chamber temperature on total phenolic content of INDLS masmin produced by spraying

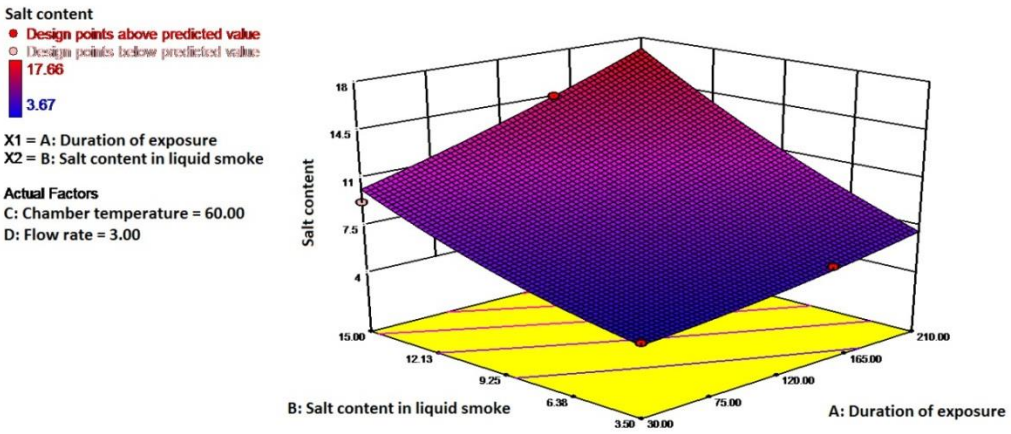

Figure 8 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of INDLS *masmin* produced by spraying

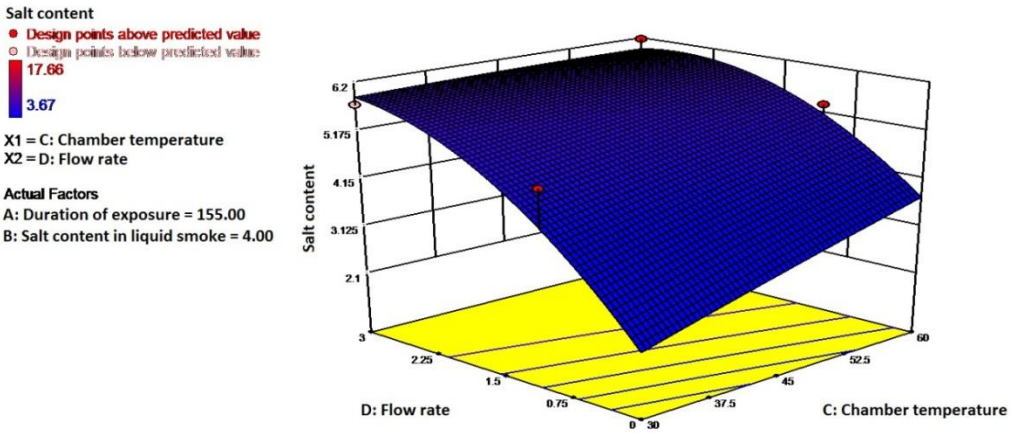

Figure 9 Response surface plots for the effect of flow rate and chamber temperature on salt content of INDLS *masmin* produced by spraying

3.2.2 Standardization of process parameters for production of indigenous liquid smoked *masmin* by soaking

Quadratic model was found to be best fitted for explaining the variability in sensory score, TPC and salt content. Response surface plots for the effect of input variables on the response variables are given in Figure 10 to Figure 12. Table 7 shows the regression coefficients of fitted model along with R^2 values.

The quadratic, linear and interaction regression coefficients for duration of exposure (duration of soaking) and salt content in liquid smoke were not found to have a significant influence on the sensory score of the products ($p>0.05$).

A sharp increase in phenolic content of the product was observed with increase in duration of exposure. Increase in salt content of the liquid smoke also resulted in minimal increase in phenolic content. However, these influences were not found to be statistically significant ($p>0.05$).

Salt content in liquid smoke was found to have an obvious linear effect on the salt content of the final product ($p<0.05$). Higher duration of exposures resulted in a corresponding increase in salt content of the samples. Interaction and quadratic regression coefficients of the independent variables were not found to be significant for the salt content ($p>0.05$).

Based on the desirability score, soaking cooked loins with indigenous liquid smoke with a salt content of 7.5% for 60 min was found to give the desirable results. The corresponding desirability score was 0.80.

Table 7 Regression coefficients for coded factors of INDLS *masmin* produced by soaking

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+4.07	+295.79	+7.03
Duration of exposure	x_1	-9.582	+63.70	+0.86
Salt content in liquid smoke	x_2	-0.55	+18.72	+1.62*
Duration of exposure X Salt content in liquid smoke	x_1x_2	+0.15	-0.98	+0.36
Duration of exposure^2	x_1^2	-0.072	-10.72	-0.016
Salt content in liquid smoke^2	x_2^2	-0.29	+4.58	-0.32
	R^2	0.13	0.74	0.90

* Significant at 5% level of significance

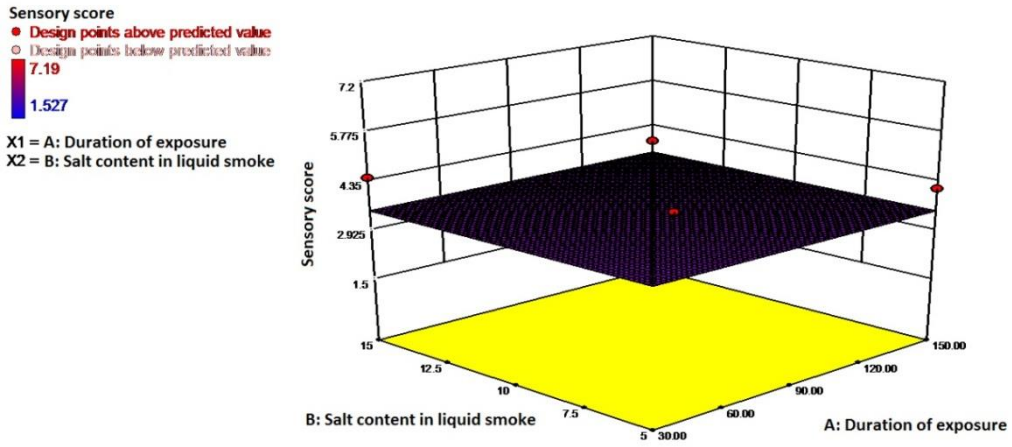

Figure 10 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of INDLS *masmin* produced by soaking

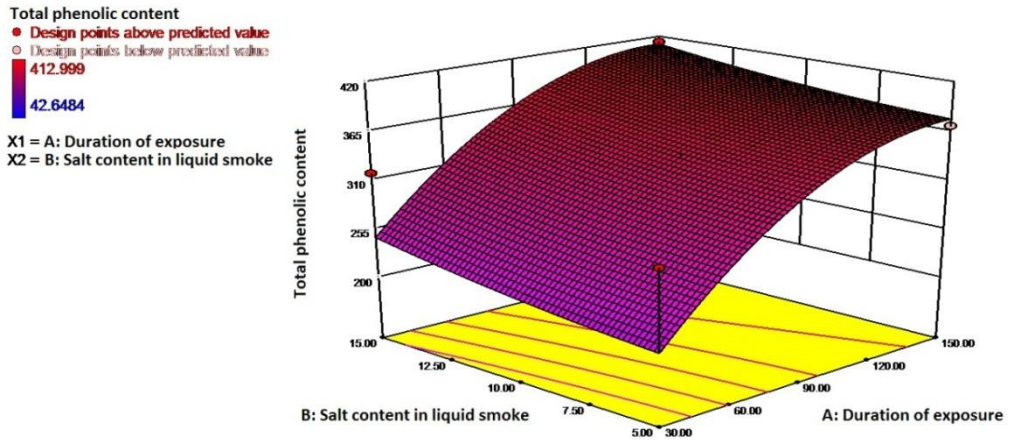

Figure 11 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of INDLS *masmin* produced by soaking

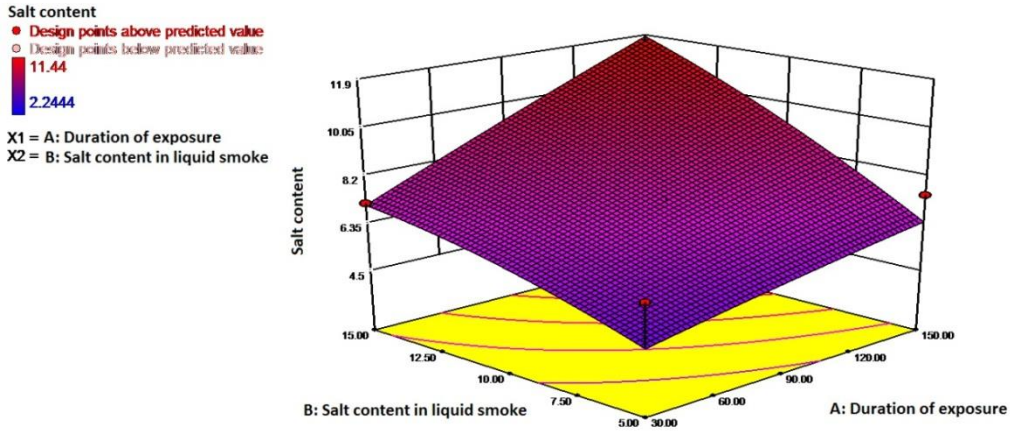

Figure 12 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of INDLS *masmin* produced by soaking

3.2.3 Standardization of process parameters for production of indigenous liquid smoked *masmin* flakes by spraying

Quadratic model was found to be best fitted for explaining the variability in sensory score, total phenolic content and salt content with R^2 values of 0.64, 0.78 and 0.86, respectively. Regression coefficients for the fitted model are given in Table 8. Response surface plots for the study are given in Figure 13 to Figure 18.

Salt content in liquid smoke was found to have a significant linear influence of the sensory acceptability of the products ($p<0.05$). However, regression coefficients for chamber temperature was not found to be significant for sensory score ($p>0.05$). Increase in duration of exposure resulted in a decrease in the sensory acceptability. This is expected to be due to the bitter flavour associated with higher absorption of phenols.

Phenolic content of the product was found to be significantly influenced by the linear effect of duration of exposure and flow rate ($p<0.05$). An increase in flow rate from 0 to 3 L/hr resulted in a linear increase in TPC of the product. A sharp increase in phenolic content was observed in samples produced by applying duration of exposures above 120 min. Chamber temperature and salt content in liquid smoke did not put forth any significant changes in TPC of the product ($p>0.05$).

Duration of exposure, flow rate and salt content in the liquid smoke was found to have significant influence on salt content of the product ($p<0.05$). An increase in level of these independent variables produced a corresponding increase in the salt content of the product.

Based on the desirability score, spraying cooked blocks with indigenous liquid smoke containing 3.5% salt for 30 min at flow rate of 1.25 L/hr and chamber temperature of 60°C was found to give the desired result. The corresponding desirability score was 0.91.

Table 8 Regression coefficients for coded factors of INDLS *masmin* flakes produced by spraying

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+2.42	+223.82	+9.93
Duration of exposure	x_1	-0.72	+92.51*	+2.98*
Salt content in liquid smoke	x_2	-0.74*	+26.16	+2.17*
Chamber temperature	x_3	+0.13	+13.41	+0.31
Flow rate	x_4	+0.36	+57.67*	+2.85*
Duration of exposure X Salt content	x_1x_2	-0.33	+24.74	-0.51
Duration of exposure X Chamber temperature	x_1x_3	-0.51	+4.81	+0.16
Duration of exposure X Flow rate	x_1x_4	+0.78	+7.54	-0.20
Salt content in liquid smoke X Chamber temperature	x_2x_3	-0.31	-0.30	-0.088
Salt content in liquid smoke X Flow rate	x_2x_4	+0.087	+3.21	+0.12
Chamber temperature X Flow rate	x_3x_4	-0.60	-1.54	+0.067
Duration of exposure^2	x_1^2	+0.60	+43.83	+1.71
Salt content^2	x_2^2	+0.21	-1.31	+0.14
Chamber temperature^2	x_3^2	+0.044	+4.98	-0.091
Flow rate^2	x_4^2	-0.51	-20.75	-0.96
	R^2	0.64	0.78	0.86

* Significant at 5% level of significance

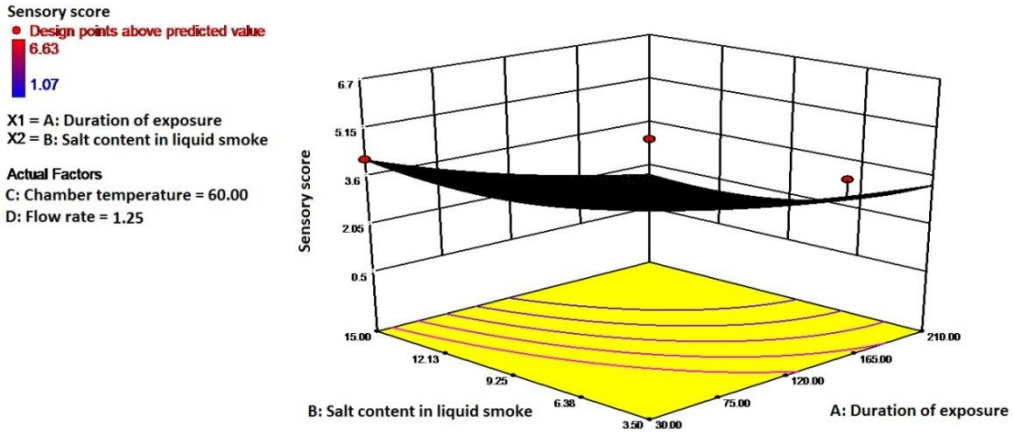

Figure 13 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of INDLS *masmin* flakes produced by spraying

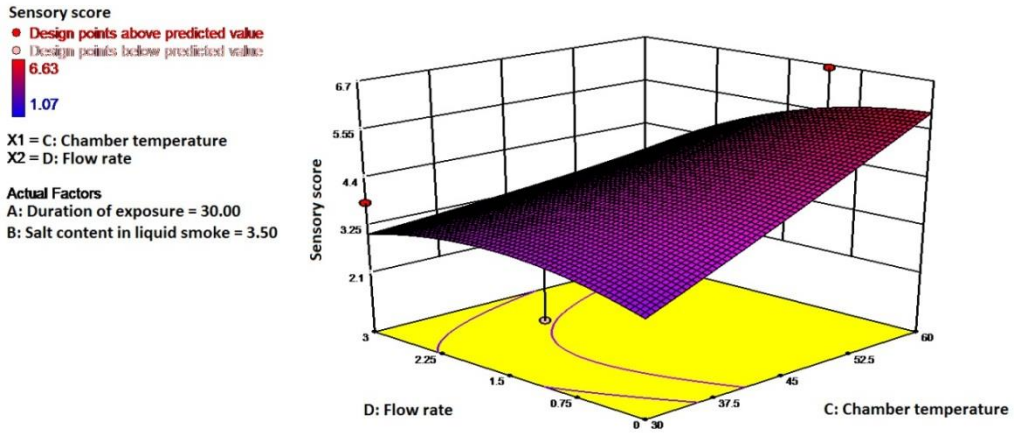

Figure 14 Response surface plots for the effect of flow rate and chamber temperature on sensory score of INDLS *masmin* flakes produced by spraying

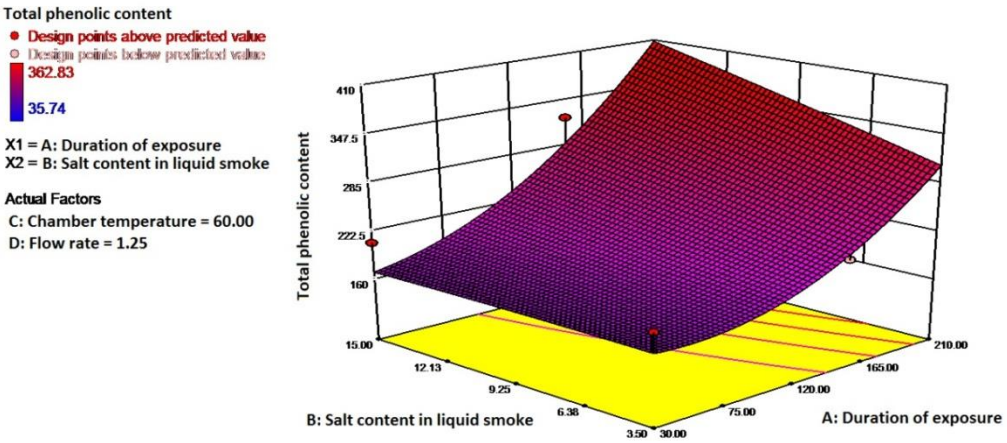

Figure 15 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of INDLS *masmin* flakes produced by spraying

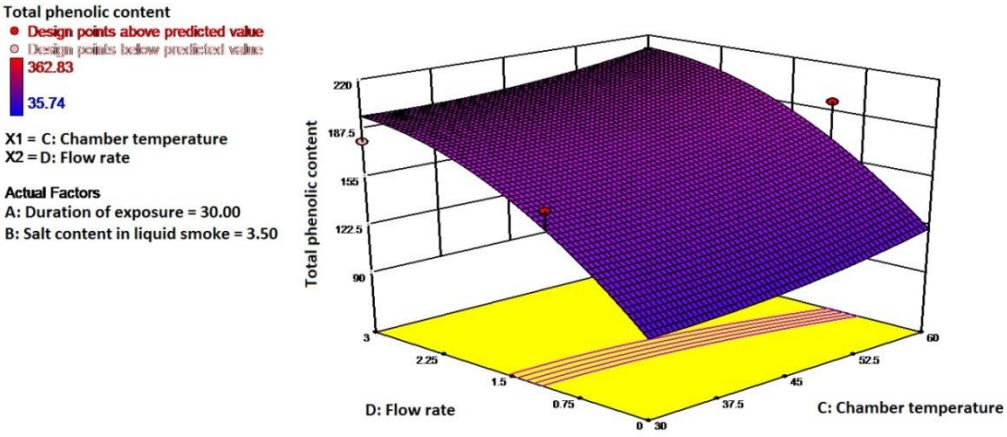

Figure 16 Response surface plots for the effect of flow rate and chamber temperature on total phenolic content of INDLS *masmin* flakes produced by spraying

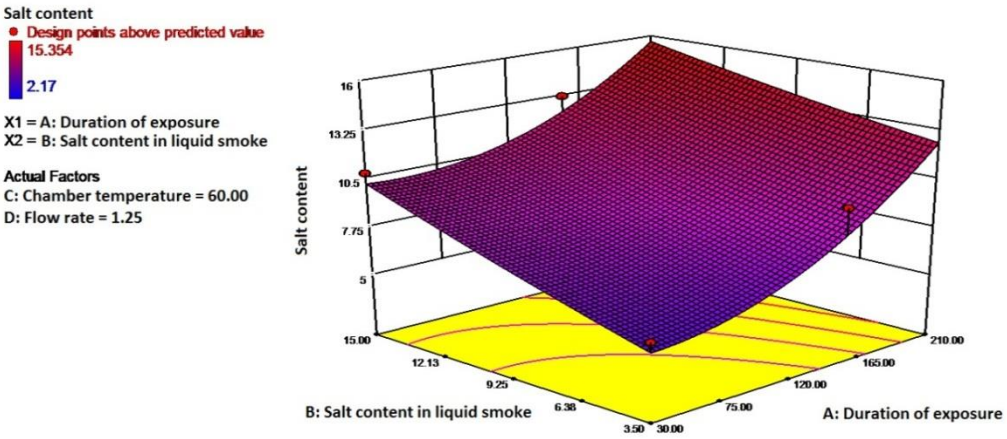

Figure 17 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of INDLS *masmin* flakes produced by spraying

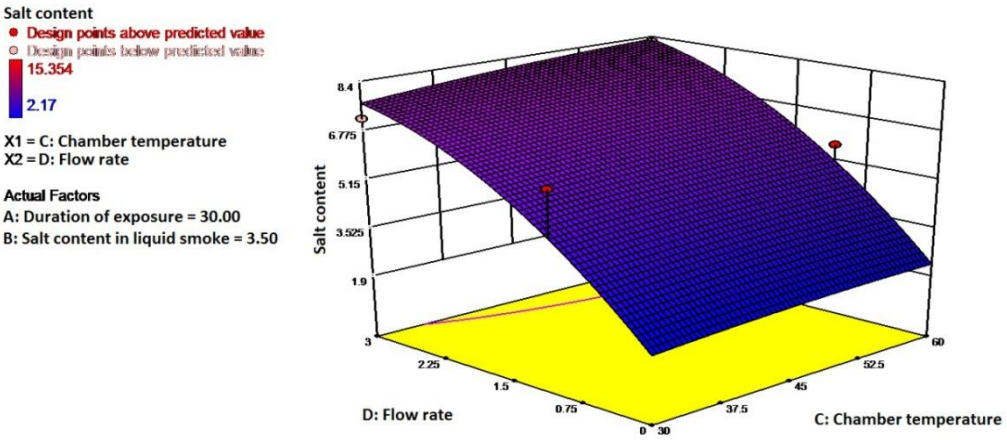

Figure 18 Response surface plots for the effect of flow rate and chamber temperature on salt content of INDLS *masmin* flakes produced by spraying

3.2.4 Standardization of process parameters for production of indigenous liquid smoked *masmin* flakes by soaking

Quadratic model was found to be best fitted for explaining the variability in sensory score, total phenolic content and salt content with R^2 values of 0.86, 0.84 and 0.87, respectively. Regression coefficients of fitted model along with R^2 values are given in Table 9. Response surface plots of different response variables showing the effect of input variables are given in Figure 19 to Figure 21.

Linear and quadratic regression coefficients for duration of exposure showed a significant influence on sensory score of the product ($p<0.05$). An increasing trend in sensory score was observed in samples produced from 30-90 min of exposure time, and thereafter the value showed a decreasing trend. Quadratic effect of salt content in liquid smoke also showed a significant influence on the sensory acceptability of the products ($p<0.05$). Samples produced around a salt content of 9.25% received higher sensory acceptability; further increase in salt content resulted in lower sensory score.

Phenolic content of the product was found to be significantly influenced by linear effect of duration of exposure ($p<0.05$). Increase in duration of exposure resulted in an increased absorption of phenols. Salt content in liquid smoke was not found to influence the phenolic content ($P>0.05$).

Linear regression coefficient of salt content in liquid smoke showed a significant influence on salt content of the product ($p<0.05$). Increase in salt level in liquid smoke resulted in a proportionate increase in salt content of the product.

Based on the desirability score obtained, soaking cooked blocks in indigenous liquid smoke containing 7.5% salt for 70 min was found to give the desired flavour for the product. The corresponding desirability score was 0.98.

Table 9 Regression coefficients of coded factors of INDLS *masmin* flakes produced by soaking

Input variables	**Coded factors**	**Sensory score**	**Total phenolic content**	**Salt content**
	Intercept	+4.31	+213.68	+6.56
Duration of exposure	x_1	+0.53*	+46.43*	+1.12
Salt content in liquid smoke	x_2	-3.863	+30.62	+3.39*
Duration of exposure X Salt content in liquid smoke	x_1x_2	-0.28	+0.041	+0.35
Duration of exposure^2	x_1^2	-0.29*	-3.20	+0.051
Salt content in liquid smoke^2	x_2^2	-1.12*	-5.47	+0.15
	R^2	0.86	0.84	0.87

* Significant at 5% level of significance

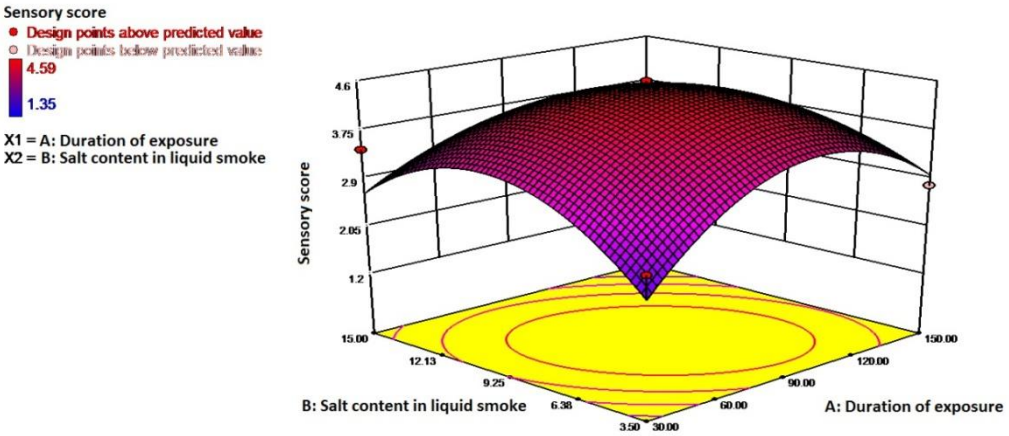

Figure 19 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of INDLS *masmin* flakes produced by soaking

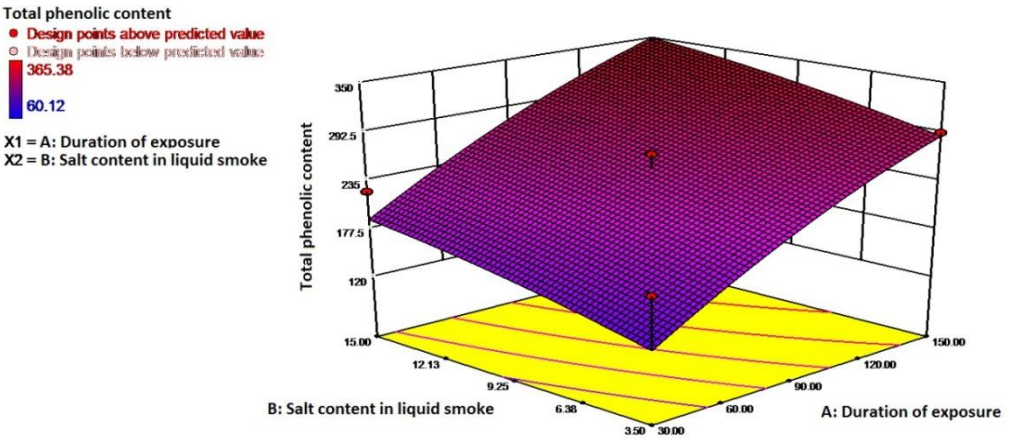

Figure 20 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of INDLS *masmin* flakes produced by soaking

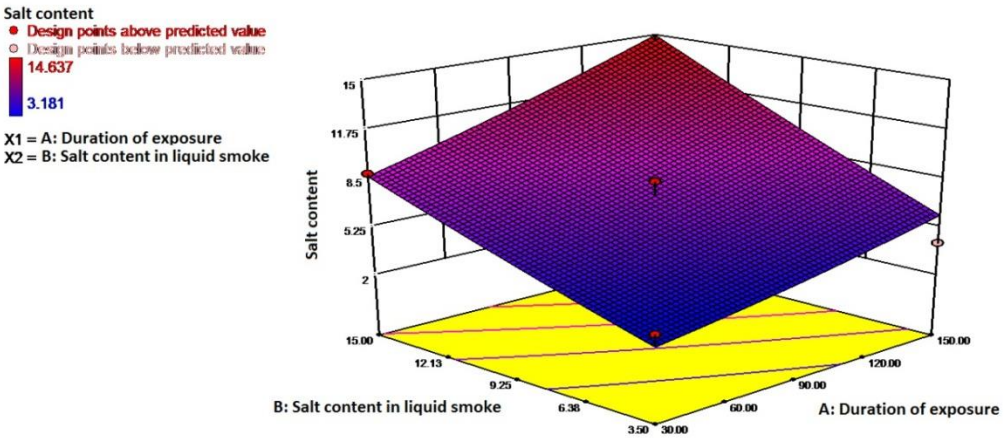

Figure 21 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of INDLS *masmin* flakes produced by soaking

3.2.5 Standardization of process parameters for production of indigenous liquid smoked *masmin* flakes by mixing

Indigenous liquid smoke, at different levels was mixed with fish mince during preparation of *masmin* flakes. Quantity of liquid smoke used and respective total phenolic content, salt content and sensory score of the samples are given in Table 10. Significant difference was observed in the total phenolic content of samples produced from different levels of liquid smoke ($p<0.05$). Based on the sensory score obtained, *masmin* flakes produced by mixing indigenous liquid smoke at 2.5% level was found to be superior. Salt content of the product was not found to be influenced by the variation in quantity of liquid smoke ($p>0.05$).

Table 10 Standardization of process parameters for production of INDLS *masmin* flakes by mixing

Quantity of liquid smoke (%)	Total Phenolic Content (mg/L)	Salt content (%)	Sensory score
1.00	74.12±10.27[a]	6.37±0.84[a]	2.65±0.97[a]
2.50	187.32±26.74[b]	5.51±0.72[a]	6.32±0.82[b]
5.00	319.65±41.37[c]	6.41±1.28[a]	3.94±0.95[a]
7.50	447.34±48.13[d]	5.14±0.59[a]	1.63±0.52[a]
10.00	524.39±62.39[e]	4.77±0.71[a]	1.37±0.27[c]

* Significant at 5% level of significance

3.2.6 Standardization of process parameters for production of indigenous liquid smoked *masmin* powder by spraying

Quadratic model was found to be best fitted for explaining the variability in sensory score, total phenolic content and salt content with R^2 values of 0.55, 0.75 and 0.88, respectively. Response surface plots showing the influence of input variables on the response variables are given in Figure 22 to Figure 27. Regression coefficients of the fitted model along with R^2 values are given in Table 11.

Salt content in liquid smoke was found to have a significant linear effect on the sensory acceptability of the products ($p<0.05$). Sensory acceptability of the product showed a decreasing trend with increase in flow rate. Samples produced by employing higher duration of exposures and chamber temperatures received higher sensory acceptability. However, these effect was not found to be statistically significant ($p>0.05$).

Linear regression coefficient of duration of exposure showed a significant influence on the phenolic content of the product ($p<0.05$). Phenolic content of the product showed an increasing trend with increase in duration of

exposure. Even though not statistically significant ($p>0.05$), an increase in flow rate and chamber temperature, positively influenced the TPC content.

Salt content of the product was found to be linearly influenced by duration of exposure, salt content in liquid smoke and flow rate ($p<0.05$). An increase in level of these factors resulted in an increased salt content in the product. Chamber temperature did not put forth any significant influence on the salt content ($p>0.05$).

Based on the desirability score obtained, spraying the cooked and shredded loins with indigenous liquid smoke with a salt content of 3.5% for 165 min at chamber temperature of 60°C and flow rate of 0.75 L/hr was found to be optimum. The corresponding desirability score was 0.82.

Table 11 Regression coefficients for coded factors of INDLS *masmin* powder produced by spraying

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+3.14	+210.62	+8.45
Duration of exposure	x_1	+0.52	+149.59*	+3.89*
Salt content in liquid smoke	x_2	-0.89*	+15.30	+2.99*
Chamber temperature	x_3	+0.27	+25.04	+0.18
Flow rate	x_4	-0.051	+49.17	+1.99*
Duration of exposure X Salt content in liquid smoke	x_1x_2	-0.98	+1.92	+0.53
Duration of exposure X Chamber temperature	x_1x_3	+0.036	+5.99	-0.14
Duration of exposure X Flow rate	x_1x_4	-0.89	+2.87	-0.52*
Salt content in liquid smoke X Chamber temperature	x_2x_3	+0.077	+0.31	+0.23
Salt content in liquid smoke X Flow rate	x_2x_4	+0.77	-6.34	-1.56
Chamber temperature X Flow rate	x_3x_4	-0.49	-3.72	+0.22
Duration of exposure^2	x_1^2	-0.22	+75.01	+1.90
Salt content in liquid smoke^2	x_2^2	+0.19	+19.47	+0.47
Chamber temperature^2	x_3^2	+0.20	-1.63	+0.055
Flow rate^2	x_4^2	-0.49	+10.18	+0.22
	R^2	0.55	0.75	0.88

* Significant at 5% level of significance

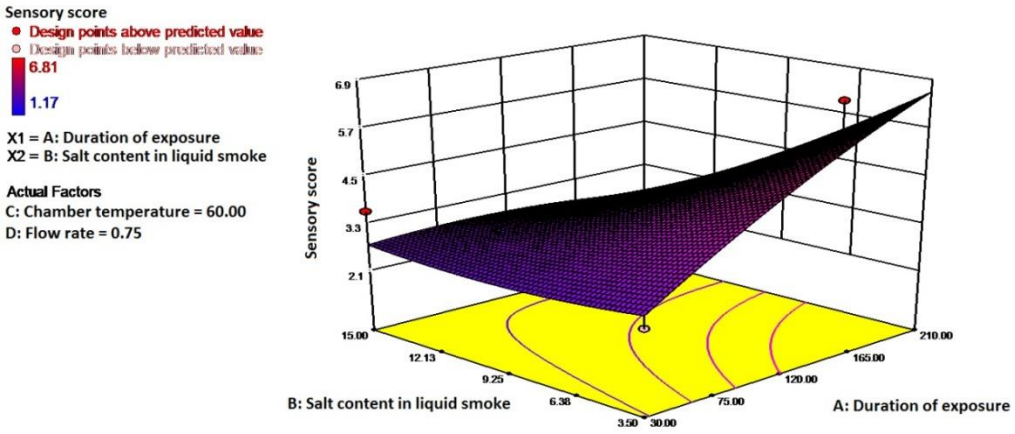

Figure 22 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of INDLS *masmin* powder produced by spraying

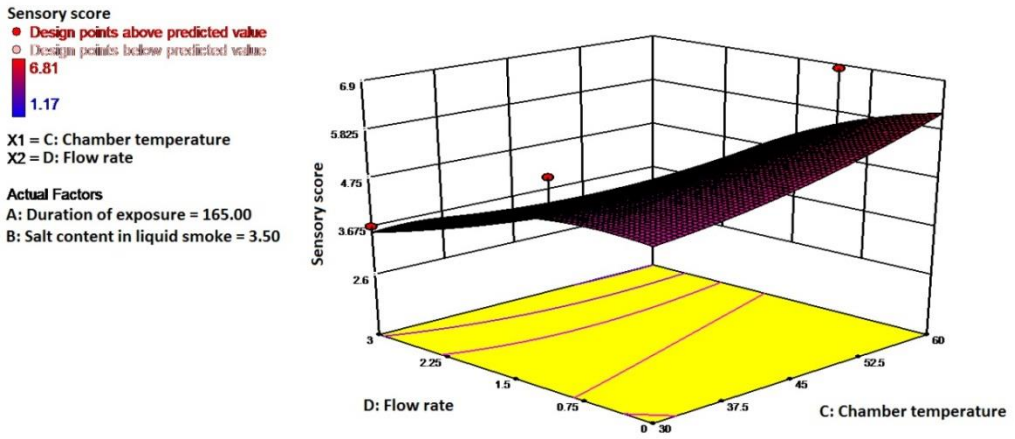

Figure 23 Response surface plots for the effect of flow rate and chamber temperature on sensory score of INDLS *masmin* powder produced by spraying

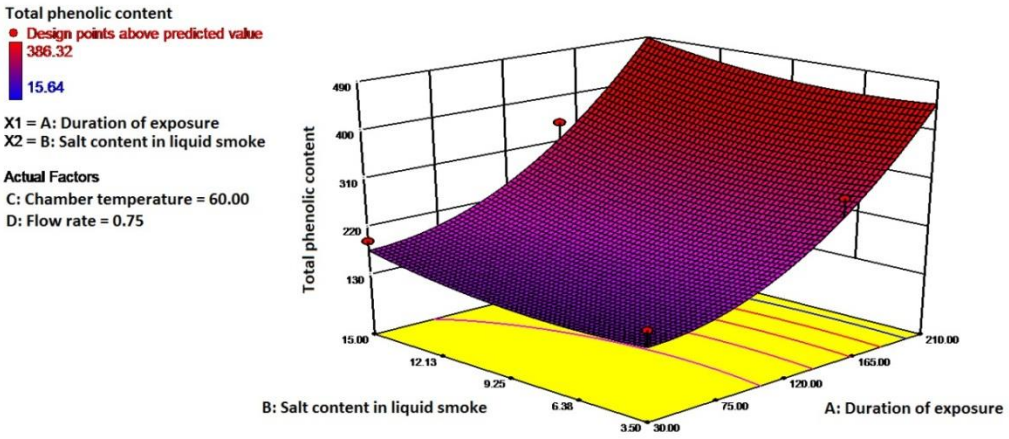

Figure 24 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of INDLS *masmin* powder produced by spraying

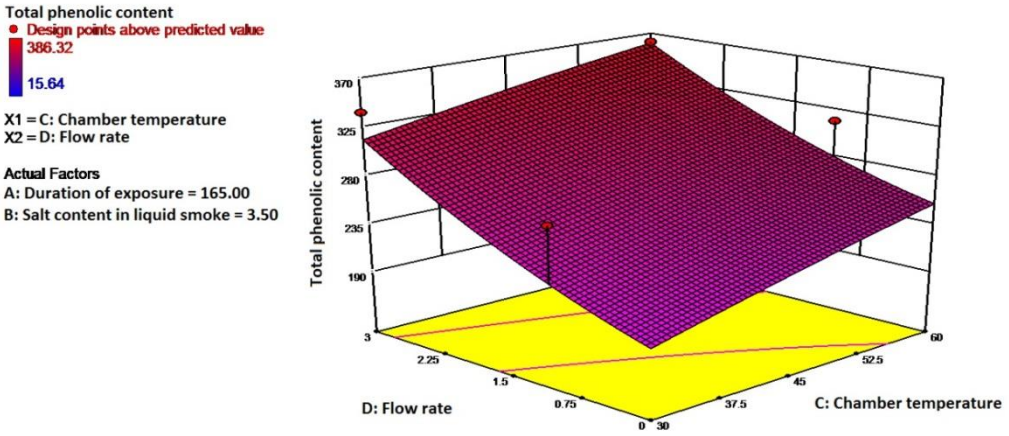

Figure 25 Response surface plots for the effect of flow rate and chamber temperature on total phenolic content of INDLS *masmin* powder produced by spraying

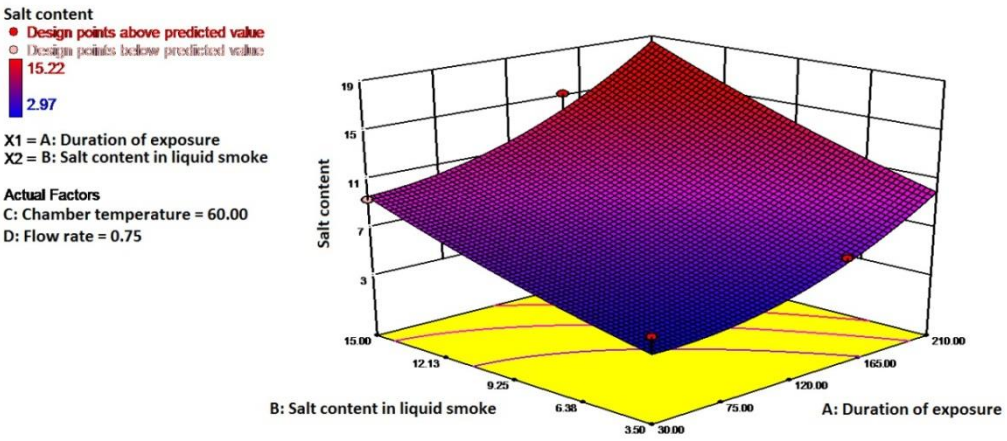

Figure 26 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of INDLS *masmin* powder produced by spraying

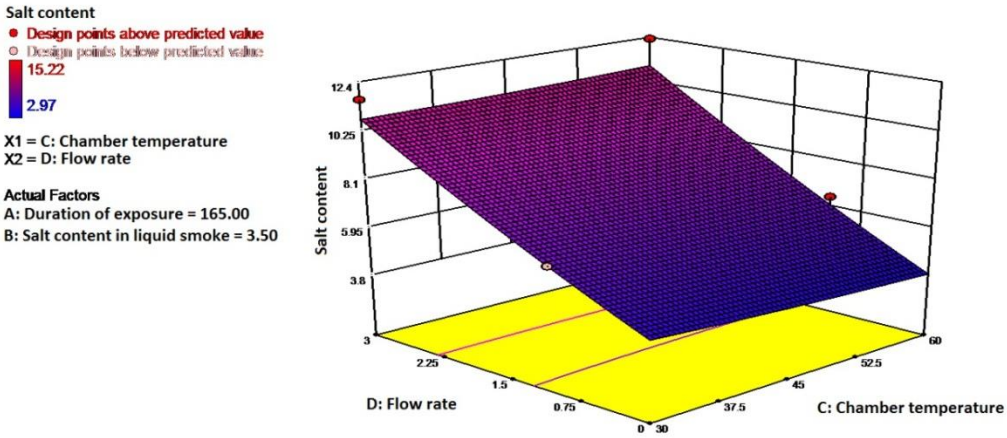

Figure 27 Response surface plots for the effect of flow rate and chamber temperature on salt content of INDLS *masmin* powder produced by spraying

3.2.7 Standardization of process parameters for production of indigenous liquid smoked *masmin* powder by soaking

Quadratic model was found to be best fitted for explaining the variations in sensory score, TPC and salt content with R^2 values of 0.73, 0.77 and 0.96, respectively. Regression coefficients of fitted model along with R^2 values are given in Table 12. Response surface plots depicting the variation in response variables with respect to input variables are given in Figure 28 to Figure 30.

Linear, interaction and quadratic regression coefficients of the input variables were not found to have a significant influence on the sensory score of the product ($p>0.05$). However, with increase in duration of exposures and salt content in liquid smoke, a hike in sensory acceptability was observed.

Linear regression coefficient for duration of exposure showed a significant influence on total phenolic content of the product ($p<0.05$). Phenolic content was not found to be influenced by salt content in liquid smoke ($P>0.05$).

Duration of exposure showed a significant linear effect on the salt content of the product ($p<0.05$). An increase in salt content of the samples was noted with increase in salt level in liquid smoke.

Based on the desirability score obtained, soaking the cooked and shredded loins in indigenous liquid smoke with a salt content of 3.5% for 150 min. was found to give the desirable results. The corresponding desirability score was 0.92.

Table 12 Regression coefficients of coded factors of INDLS *masmin* powder produced by soaking

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+2.45	+274.92	+11.03
Duration of exposure	x_1	+0.68	+62.61*	+1.75*
Salt content in liquid smoke	x_2	-0.55	+27.07	+2.74*
Duration of exposure X Salt content in liquid smoke	x_1x_2	-1.34	+10.21	+0.81
Duration of exposure^2	x_1^2	+0.62	-41.26	-1.13
Salt content in liquid smoke^2	x_2^2	+0.99	+24.72	-1.54*
	R^2	0.73	0.77	0.96

* Significant at 5% level of significance

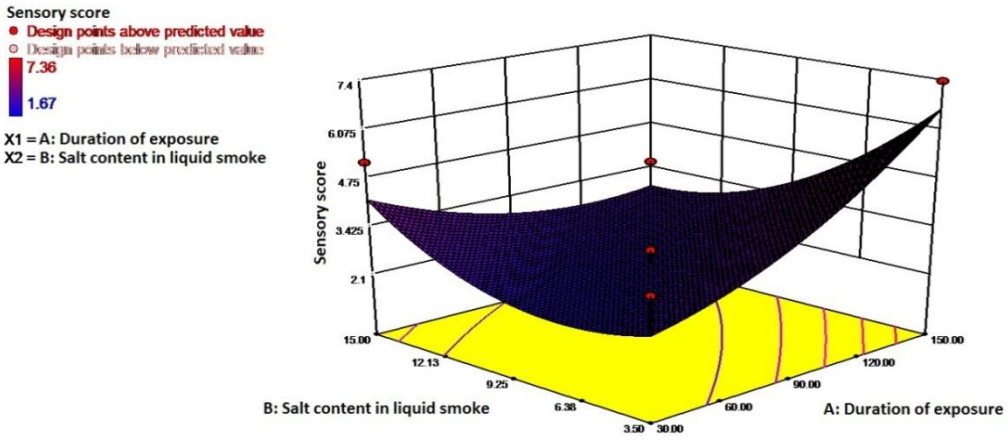

Figure 28 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of INDLS *masmin* power produced by soaking

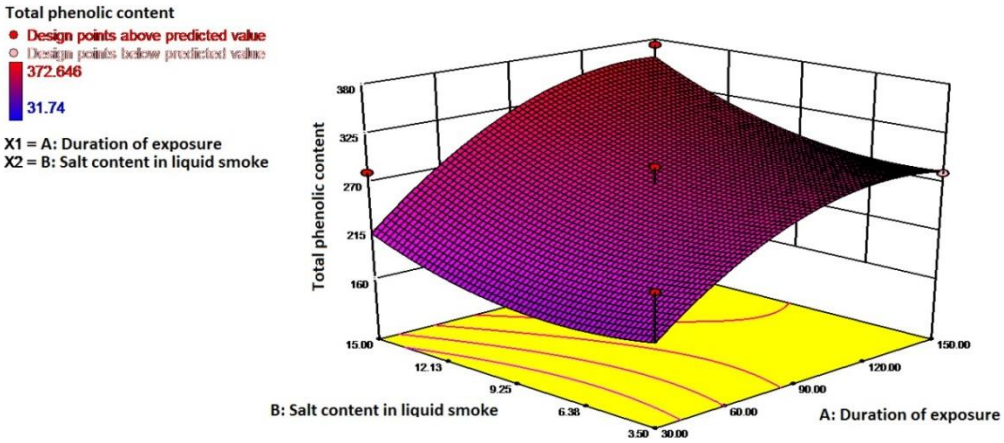

Figure 29 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of INDLS *masmin* powder produced by soaking

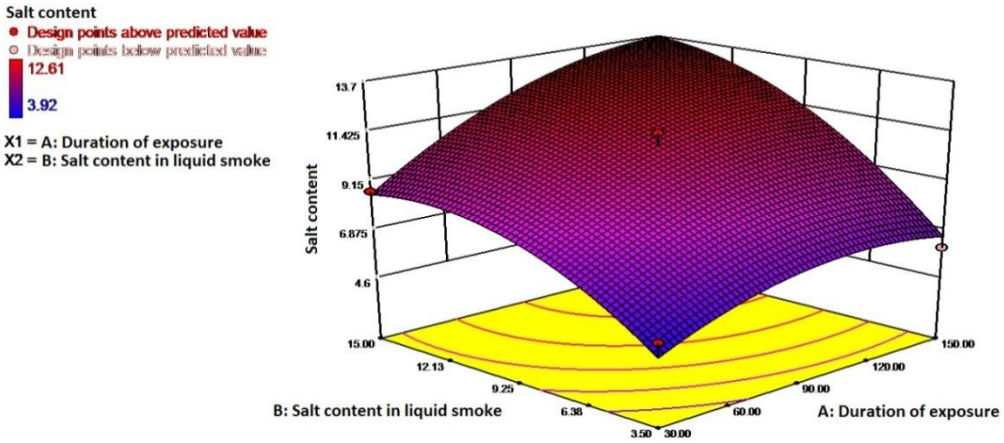

Figure 30 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of INDLS *masmin* powder produced by soaking

3.2.8 Standardization of process parameters for production of CMLS *masmin* by spraying

Quadratic model was found to be best fitted for sensory score, total phenolic content and salt content with R^2 values of 0.91, 0.78 and 0.96, respectively. Response surface plots of sensory score, total phenolic content and salt content for varying levels of input variables are given in Figure 31 to Figure 39. Regression coefficients for coded factors with R^2 values are given in Table 13.

Interaction between duration of exposure and salt content in liquid smoke was found to have a significant influence on sensory score of the product ($P<0.05$). Experimental runs with longer duration of exposures resulted in higher sensory acceptability. Salt content in liquid smoke showed an inverse relation with sensory acceptability of the product. Interaction regression coefficients for dilution and salt content in liquid smoke also showed a significant influence on sensory acceptability of the samples. Samples processed with a dilution of 16.6% (1:6) received higher sensory acceptability. Lowering the dilution resulted in low sensory acceptability. It is presumed that, reducing the dilution results in reduced pumpability of the commercial liquid smoke due to the viscous nature and hence results in reduced phenol deposition on the product. This might have resulted in lesser smoky aroma in the product and thereby reducing the sensory score. Linear regression coefficient of the independent variables did not show any significant influence on sensory score of the product ($p>0.05$).

Dilution of liquid smoke showed a significant linear effect on the phenolic content of the product ($p<0.05$). Maximum phenol deposition was

observed at a dilution of 33.3% (1:3), further increase or decrease in dilution resulted in lower phenolic deposition in the product.

Salt content of the product was found to have a linear relationship with duration of exposure, salt content in liquid smoke, flow rate and dilution ($p<0.05$). There was a proportionate increase in salt content of the product with increase in duration of exposure. Absorption of salt in the product was more or less constant for samples produced with salt levels of 3.5 to 9.25%, thereafter an increase in salt absorption was noted at higher levels of salt in liquid smoke. However, this increased absorption in salt content resulted in a low sensory acceptability. A gradual increase in salt content was observed with increase in flow rate. Absorption of salt in the product was higher at lower dilutions (1:2) and thereafter further dilution of liquid smoke resulted in lower salt content in the product.

Based on the desirability score obtained, spraying the cooked loins with commercial liquid smoke (diluted in 1:6 proportion with distilled water and added with 3.5% (w/v) salt) for 150 min at a flow rate of 1L/hr in the multi-functional smoke kiln at a chamber temperature of 60°C was found to give desirable flavour for *masmin*. The corresponding desirability score was 0.98.

Table 13 Regression coefficients for coded factors of CMLS *masmin* produced by spraying

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+4.02	+171.54	+7.71
Duration of exposure	x_1	+0.24	+26.26	+1.34*
Salt content in liquid smoke	x_2	-0.25	+20.87	+0.81*
Chamber temperature	x_3	-0.12	+24.10	+0.21
Flow rate	x_4	+0.27	+24.48	+0.61*
Dilution	x_5	-3.33	-42.08*	+0.64*
Duration of exposure X Salt content in liquid smoke	x_1x_2	-0.86*	-13.25	-0.40
Duration of exposure X Chamber temperature	x_1x_3	-0.18	+11.45	+0.37
Duration of exposure X Flow rate	x_1x_4	-0.36	+3.62	+0.14
Duration of exposure X Dilution	x_1x_5	-0.26	+22.51	+0.18
Salt content in liquid smoke X Chamber temperature	x_2x_3	-0.15	-1.90	-0.043
Salt content in liquid smoke X Flow rate	x_2x_4	+0.11	-2.72	+0.22
Salt content in liquid smoke X Dilution	x_2x_5	+0.82*	+2.85	-0.69*
Chamber temperature X Flow rate	x_3x_4	-0.43	-0.38	+0.99*
Chamber temperature X Dilution	x_3x_5	-0.17	+17.27	+0.066
Flow rate X Dilution	x_4x_5	+0.25	-5.86	-0.11
Duration of exposure^2	x_1^2	-0.45	+30.47	-0.56
Salt content in liquid smoke^2	x_2^2	-0.54	-53.16	+1.11
Chamber temperature^2	x_3^2	-0.64	+23.28	+1.14*
Flow rate^2	x_4^2	+1.08	+75.63	-1.06
Dilution^2	x_5^2	+0.49	-48.11	-0.97
	R^2	0.91	0.78	0.96

* Significant at 5% level of significance

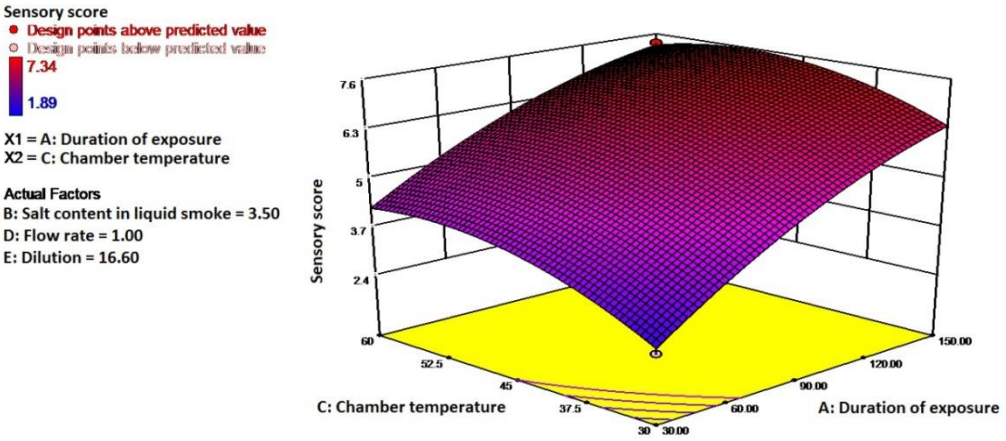

Figure 31 Response surface plots for the effect of chamber temperature and duration of exposure on sensory score of CMLS *masmin* produced by spraying

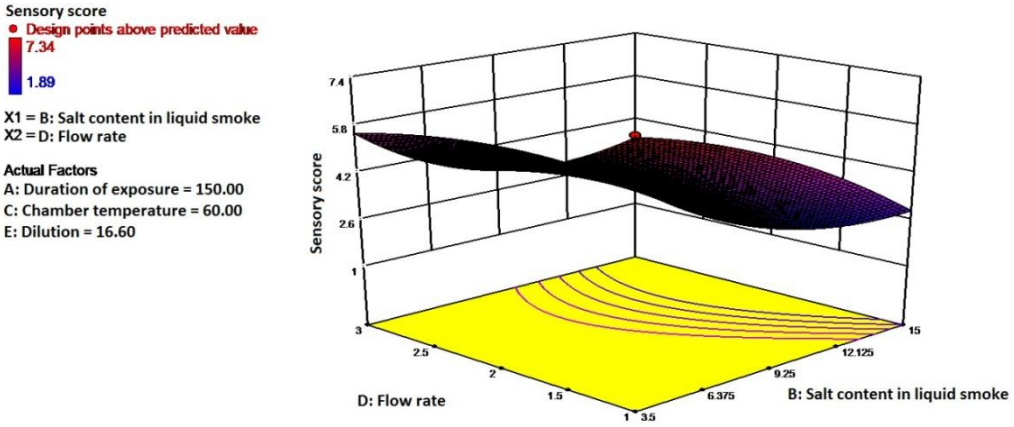

Figure 32 Response surface plots for the effect of flow rate and salt content in liquid smoke on sensory score of CMLS *masmin* produced by spraying

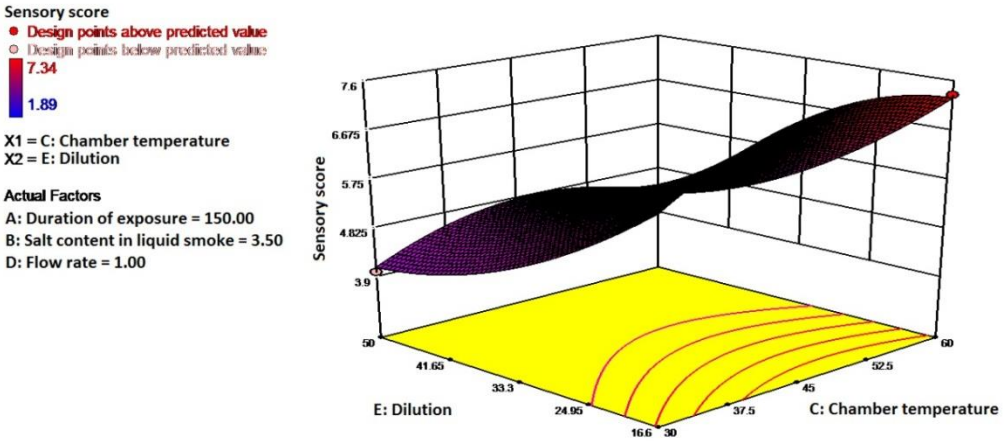

Figure 33 Response surface plots for the effect of dilution and chamber temperature on sensory score of CMLS *masmin* produced by spraying

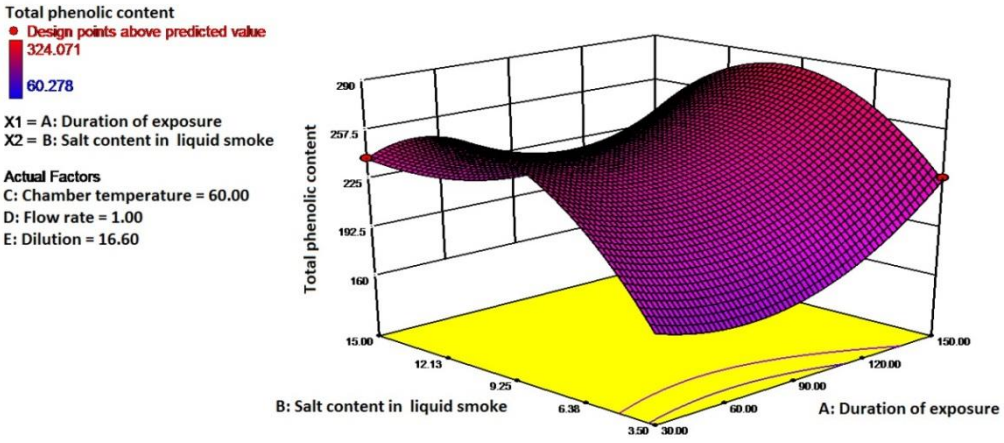

Figure 34 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of CMLS *masmin* produced by spraying

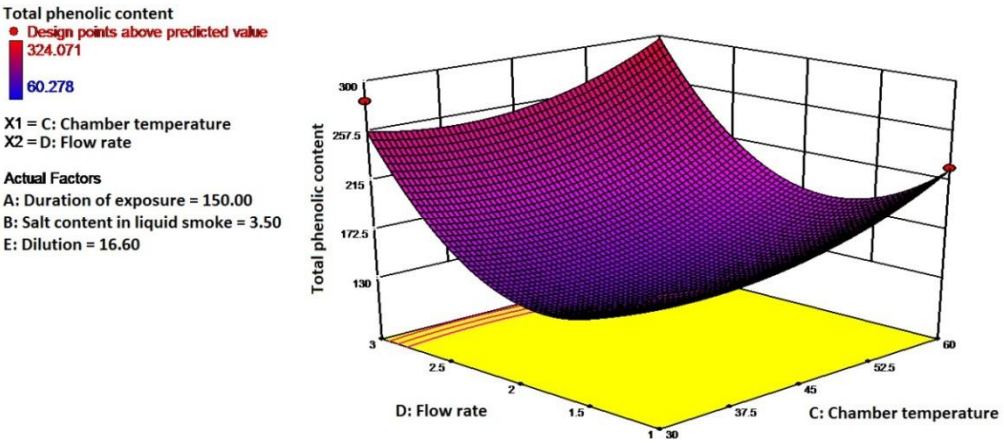

Figure 35 Response surface plots for the effect of flow rate and chamber temperature on total phenolic content of CMLS *masmin* produced by spraying

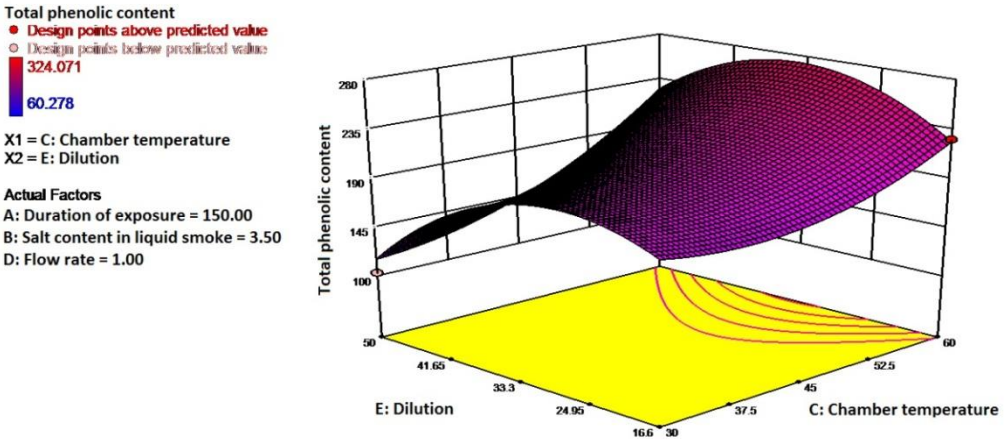

Figure 36 Response surface plots for the effect of dilution and chamber temperature on total phenolic content of CMLS *masmin* produced by spraying

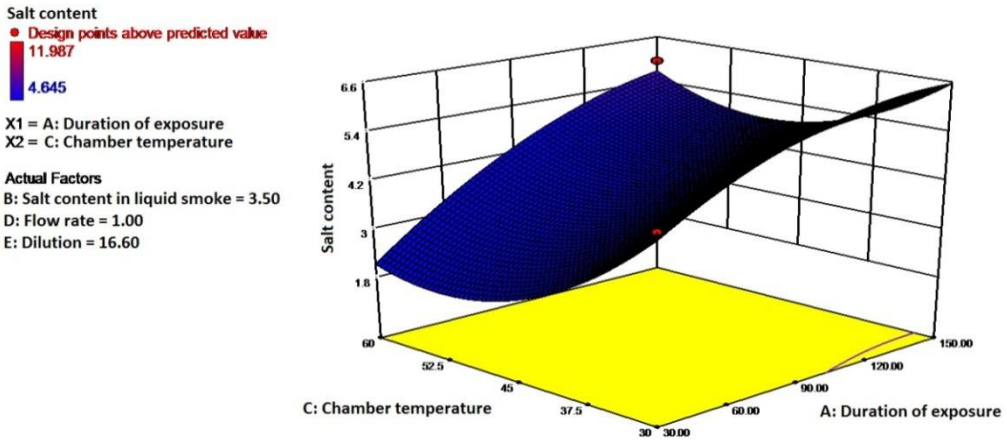

Figure 37 Response surface plots for the effect of chamber temperature and duration of exposure on salt content of CMLS *masmin* produced by spraying

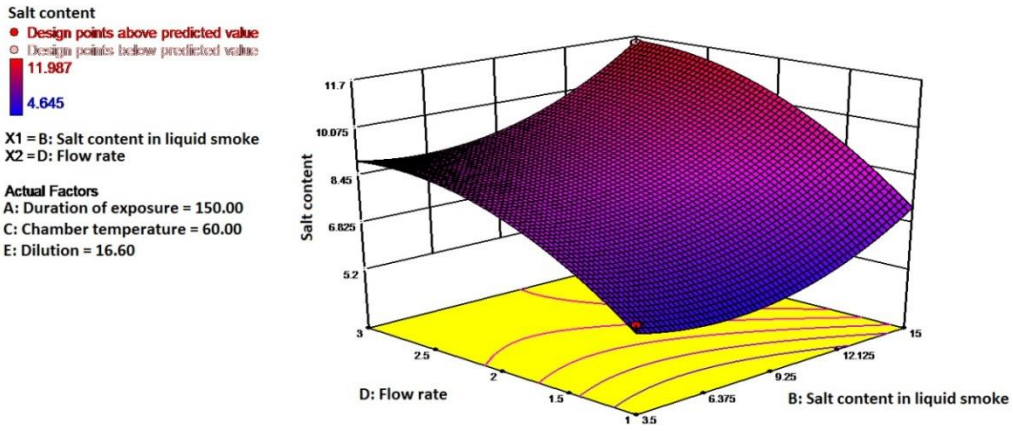

Figure 38 Response surface plots for the effect of spraying quantity and salt content in liquid smoke on salt content of CMLS *masmin* produced by spraying

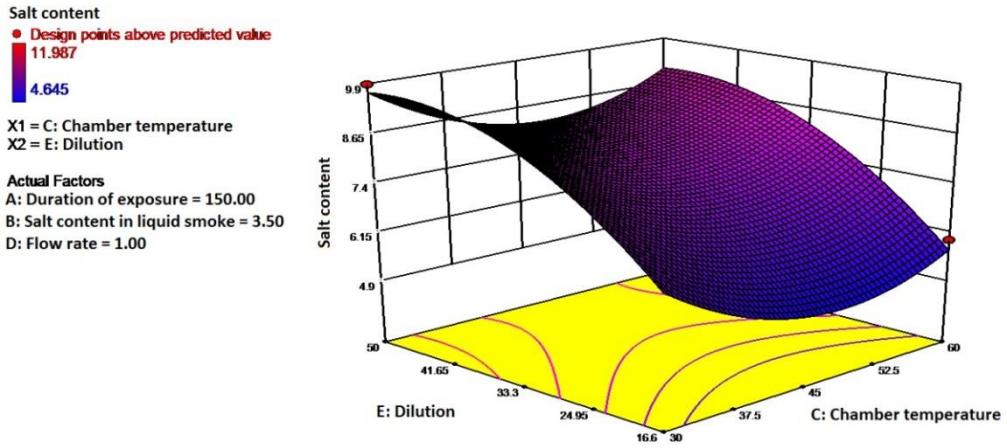

Figure 39 Response surface plots for the effect of dilution and chamber temperature on salt content of CMLS *masmin* produced by spraying

3.2.9 Standardization of process parameters for production of commercial liquid smoked *masmin* by soaking

Quadratic model was found to be best fitted for sensory score, total phenolic content and salt content with R^2 values of 0.99, 0.98 and 0.97, respectively. Response surface plots of sensory score, total phenolic content and salt content for varying levels of input variables are given in Figure 40 to Figure 45. Regression coefficients for coded factors along with R^2 values are given in Table 14.

Linear effect of salt content and dilution of liquid smoke had a significant effect on the sensory score ($p<0.05$). A gradual increase in sensory score was observed with increase in salt content from 3.5% to 9.25% after that the same showed a decreasing trend. Higher dilutions in liquid smoke produced better acceptability in the product. This is expected to be due to soothing smoke aroma associated with a moderate phenolic content. Sensory acceptability of the products were significantly influenced by the interaction

between duration of exposure and salt content in liquid smoke ($p<0.05$). Exposure durations from 30-90 min showed a corresponding increase in sensory acceptability of the products, further increase in duration resulted in lower sensory acceptability. Quadratic effects of all the three independent variable were found to significantly influence the sensory score ($p<0.05$).

Linear and interaction effect of duration of exposure and dilution showed a significant influence on total phenolic content ($p<0.05$). Higher duration of soaking resulted in better absorption of phenols. Increase in dilution of liquid smoke showed a negative impact on the phenolic content. Quadratic regression coefficient of dilution was also found to be significant in terms of TPC.

Linear and quadratic effect of duration of exposure was found to be significantly influencing the salt content of the product ($p<0.05$). Increased duration of soaking in liquid smoke resulted in better salt absorption in the product. Salt content in liquid smoke showed an obvious linear relationship with salt content in the product ($p<0.05$). Quadratic regression coefficient for dilution had a significant effect on salt content ($p<0.05$). Higher salt absorption was observed at lower dilutions.

Based on the desirability score obtained, soaking the cooked loins in commercial liquid smoke diluted at 1:12 ratio (8.3%) and added with 9.25% salt for 90 min. was found to be optimum for production of CMLS *masmin*. The corresponding desirability score was 0.997.

Table 14 Regression coefficients for coded factors of CMLS *masmin* produced by soaking

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+6.56	+218.20	+5.76
Duration of exposure	x_1	-0.054	+155.24*	+1.31*
Salt content in liquid smoke	x_2	-0.22*	+9.77	+3.06*
Dilution	x_3	+0.71*	-601.71*	-0.48
Duration of exposure X Salt content in liquid smoke	x_1x_2	-0.40*	+3.65	+0.30
Duration of exposure X Dilution	x_1x_3	+0.055	-251.20*	+0.62
Salt content in liquid smoke X Dilution	x_2x_3	-0.16	-22.35	-0.47
Duration of exposure^2	x_1^2	-1.68*	-95.42	+1.63*
Salt content in liquid smoke^2	x_2^2	-1.66*	+103.44	+0.81
Dilution^2	x_3^2	-3.19*	+761.39*	+1.74*
	R^2	0.99	0.98	0.97

* Significant at 5% level of significance

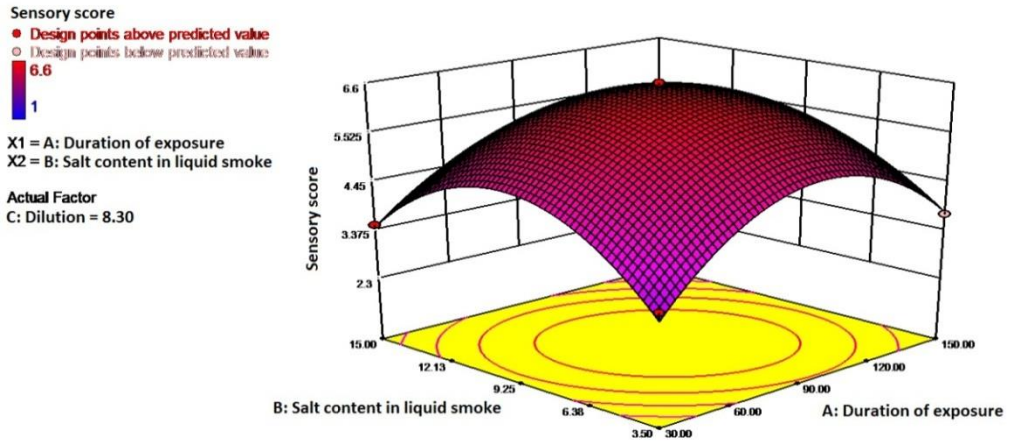

Figure 40 Response surface plots for the effect of salt content in liquid smoke and soaking time on sensory score of CMLS *masmin* produced by soaking

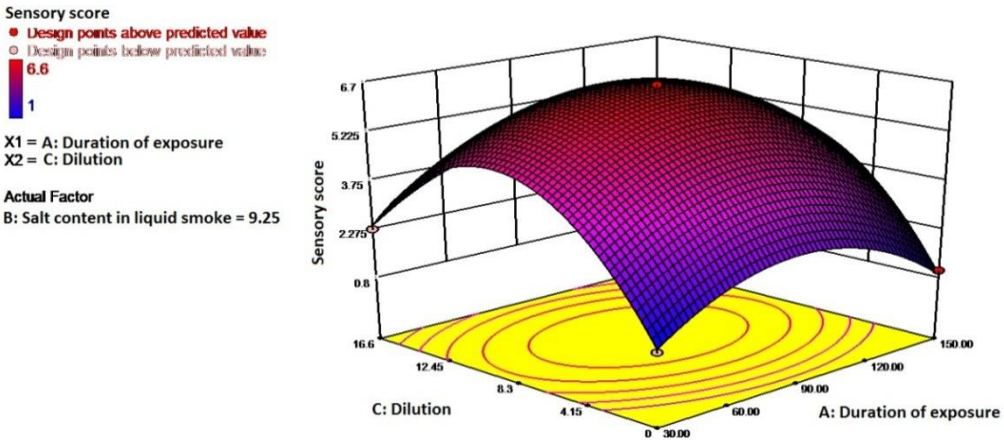

Figure 41 Response surface plots for the effect of dilution and duration of exposure on sensory score of CMLS *masmin* produced by soaking

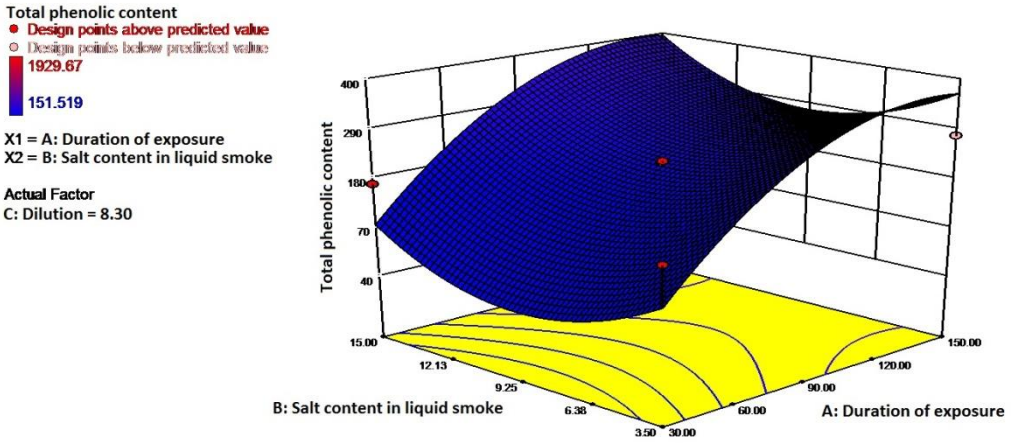

Figure 42 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of CMLS *masmin* produced by soaking

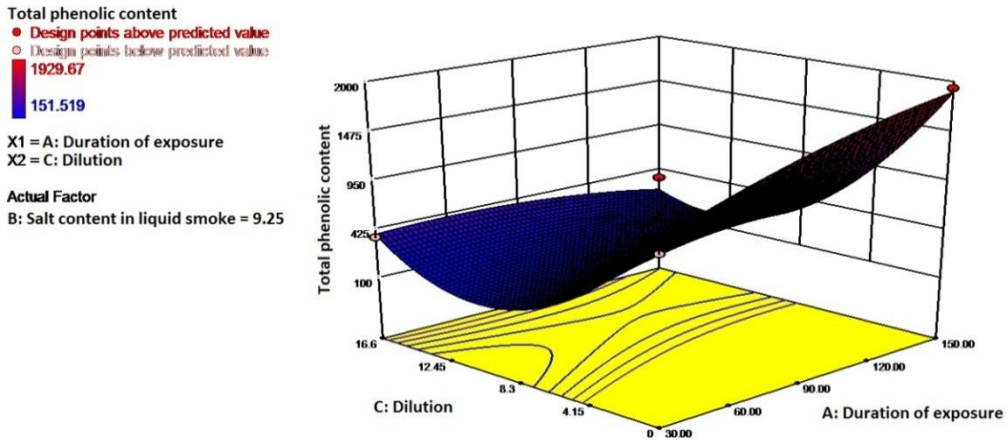

Figure 43 Response surface plots for the effect of dilution and duration of exposure on total phenolic content of CMLS *masmin* produced by soaking

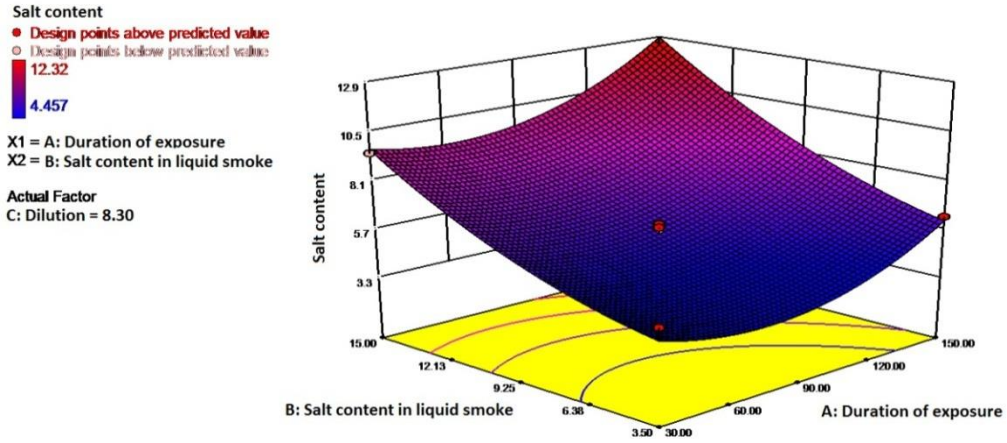

Figure 44 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of CMLS *masmin* produced by soaking

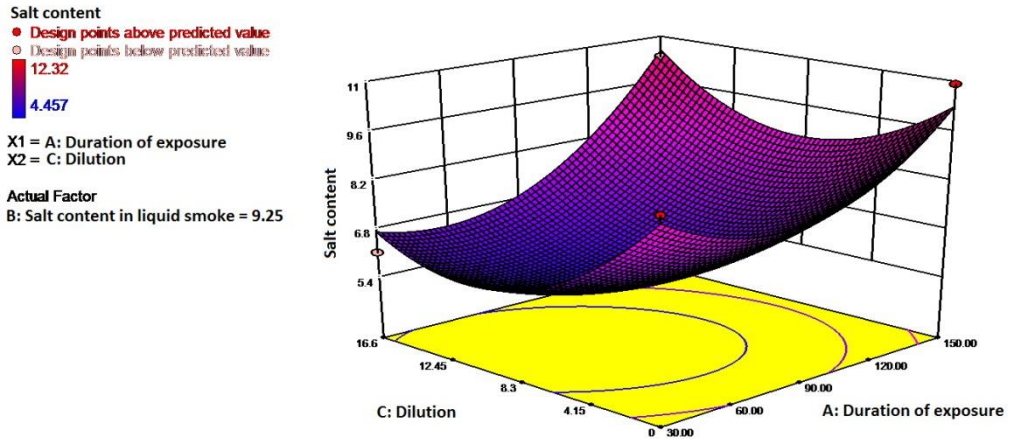

Figure 45 Response surface plots for the effect of dilution and duration of exposure on salt content of CMLS *masmin* produced by soaking

3.2.10 Standardization of process parameters for production of commercial liquid smoked *masmin* flakes by spraying

Quadratic model was found to be best fitted for sensory score, phenolic content and salt content with R^2 values of 0.67, 0.81 and 0.94. Response surface plots for the effect of input variables on response variables are given in Figure 46 to Figure 54. Regression coefficients for coded factors along with R^2 values are given in Table 15.

None of the independent variables had a significant effect on sensory score of the products ($p>0.05$). Phenolic content of the product was found to be significantly influenced by the linear effect of duration of exposure ($p<0.05$). A gradual increase in phenolic content was observed with increase in duration of exposures. Duration of exposure and salt content in liquid smoke had a significant linear effect on the salt content of the products ($p<0.05$). Both these factors showed a positive correlation with the salt content. Quadratic effect of flow rate was also found to be significantly influencing the salt content of the products ($p<0.05$).

An increasing trend in salt content was evident in samples processed with flow rate of 1-2 L/hr. However, further increase in flow rate showed a decrease in salt content of the product. This is expected to be due to the probable washing out of salt deposited on the surface of meat blocks at higher flow rate. Based on the desirability score obtained, spraying the cooked blocks with commercial liquid smoke (diluted in 1:3 proportion with distilled water and added with 9.25% (w/v) salt) for 90 min at a flow rate of 1 L/hr and a chamber temperature of 45°C was found to give the desirable flavour. The corresponding desirability score was 0.89.

Table 15 Regression coefficients for coded factors of CMLS *masmin* flakes produced by spraying

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+3.60	+182.17	+8.42
Duration of exposure	x_1	-0.27	+33.44*	+0.70*
Salt content in liquid smoke	x_2	-0.059	+11.59	+1.21*
Chamber temperature	x_3	+0.21	+2.06	-0.26
Flow rate	x_4	+0.19	+15.00	+0.17
Dilution	x_5	+0.057	+20.05	-0.32
Duration of exposure X Salt content in liquid smoke	x_1x_2	-0.021	-6.25	+7.576
Duration of exposure X Chamber temperature	x_1x_3	-0.29	-10.09	-0.057
Duration of exposure X Flow rate	x_1x_4	-0.14	+7.46	-0.18
Duration of exposure X Dilution	x_1x_5	-0.16	+3.13	+0.13
Salt content in liquid smoke X Chamber temperature	x_2x_3	-0.045	-19.43	-0.34
Salt content in liquid smoke X Flow rate	x_2x_4	+0.12	-22.61	-0.25
Salt content in liquid smoke X Dilution	x_2x_5	-0.14	-3.00	+0.36
Chamber temperature X Flow rate	x_3x_4	-2.375	-10.49	+0.26
Chamber temperature X Dilution	x_3x_5	-0.42	+16.67	+0.26
Flow rate X Dilution	x_4x_5	+0.065	+7.99	+0.35
Duration of exposure^2	x_1^2	+0.49	+26.18	+0.68
Salt content in liquid smoke^2	x_2^2	-2.01	-28.30	-0.17
Chamber temperature^2	x_3^2	-0.86	-19.40	-0.36
Flow rate^2	x_4^2	+1.98	+9.75	-1.15*
Dilution^2	x_5^2	-0.46	+27.44	+0.44
	R^2	0.67	0.81	0.94

* Significant at 5% level of significance

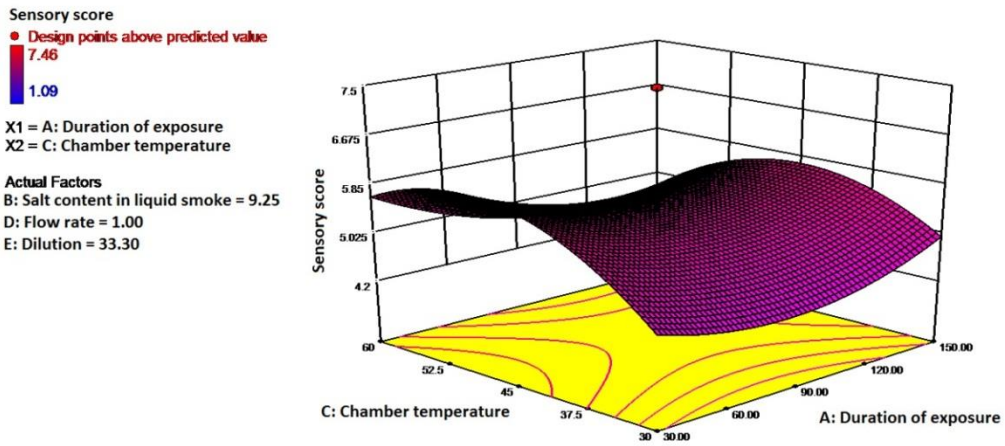

Figure 46 Response surface plots for the effect of chamber temperature and duration of exposure on sensory score of CMLS *masmin* flakes produced by spraying

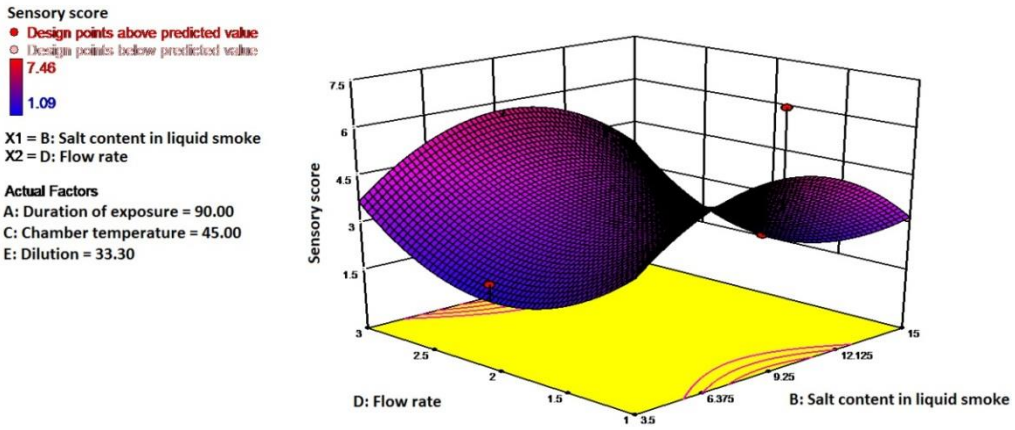

Figure 47 Response surface plots for the effect of flow rate and salt content in liquid smoke on sensory score of CMLS *masmin* flakes produced by spraying

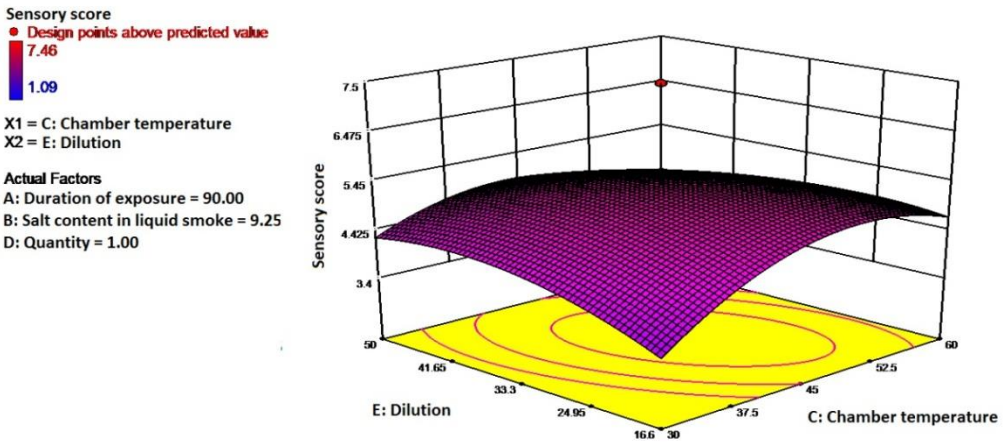

Figure 48 Response surface plots for the effect of dilution and chamber temperature on sensory score of CMLS *masmin* flakes produced by spraying

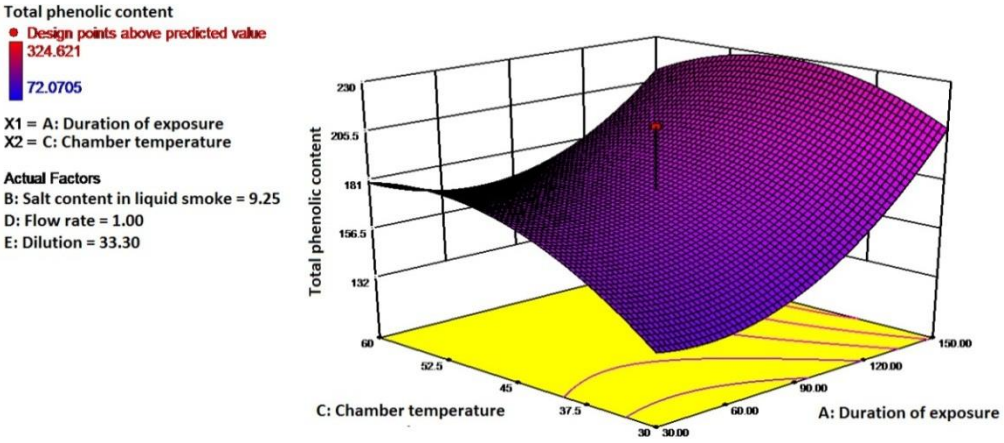

Figure 49 Response surface plots for the effect of chamber temperature and duration of exposure on total phenolic content of CMLS *masmin* flakes produced by spraying

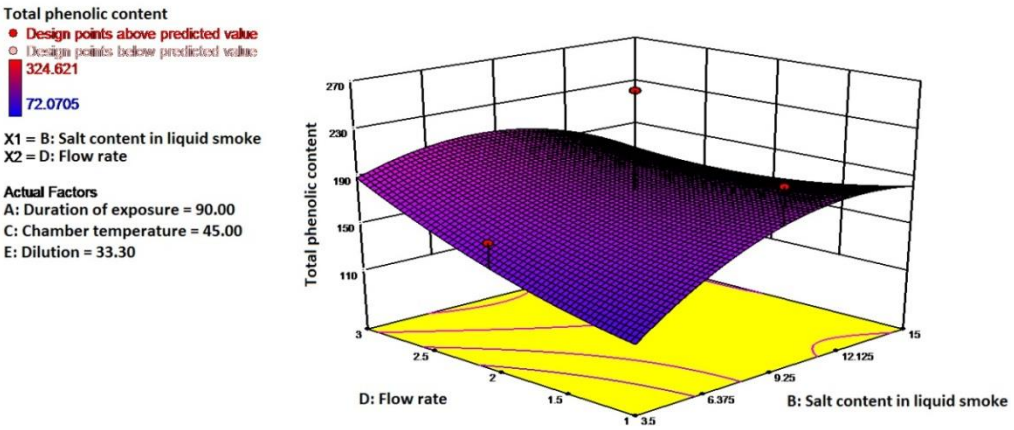

Figure 50 Response surface plots for the effect of flow rate and salt content in liquid smoke on total phenolic content of CMLS *masmin* flakes produced by spraying

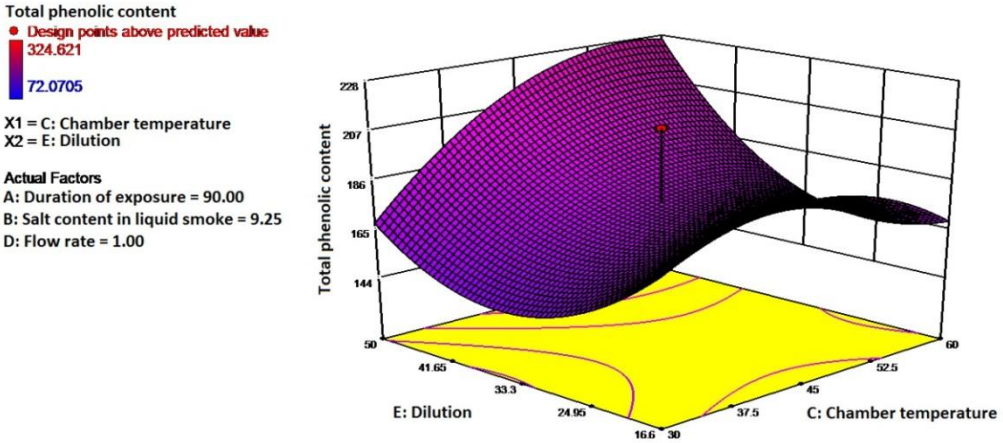

Figure 51 Response surface plots for the effect of dilution and chamber temperature on total phenolic content of CMLS *masmin* flakes produced by spraying

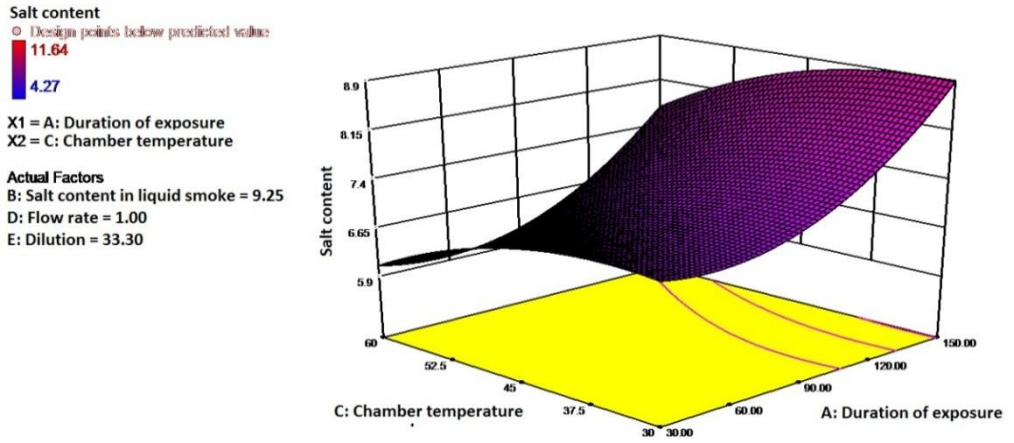

Figure 52 Response surface plots for the effect of chamber temperature and duration of exposure on salt content of CMLS *masmin* flakes produced by spraying

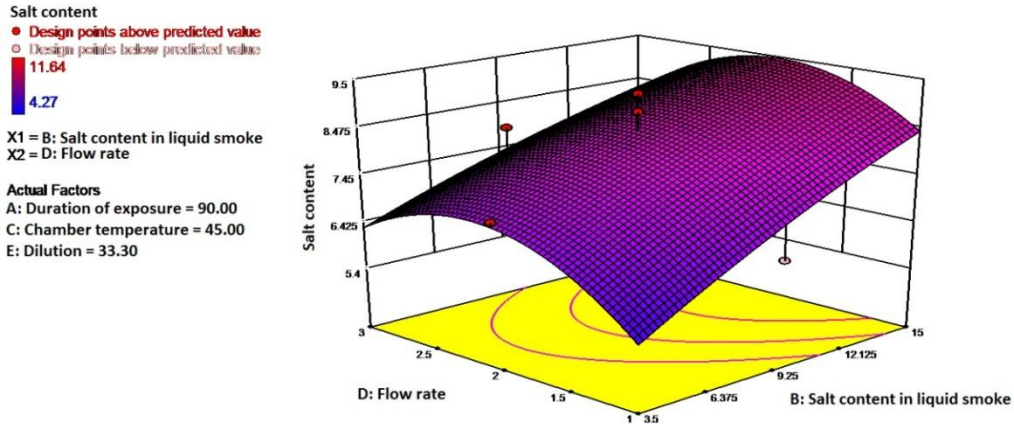

Figure 53 Response surface plots for the effect of flow rate and salt content in liquid smoke on salt content of CMLS *masmin* flakes produced by spraying

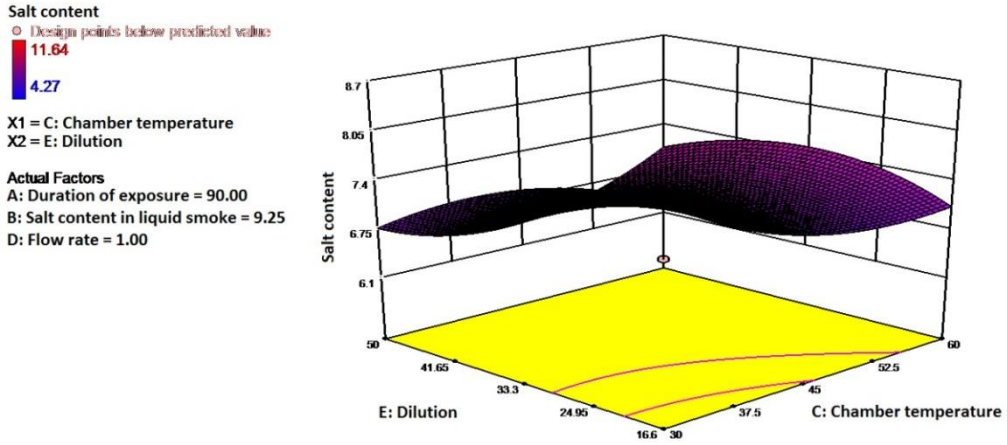

Figure 54 Response surface plots for the effect of dilution and chamber temperature on salt content of CMLS *masmin* flakes produced by spraying

3.2.11 Standardization of process parameters for production of commercial liquid smoked *masmin* flakes by soaking

Quadratic model was found to be best fitted for explaining the effect of input variables on sensory score (R^2=0.98) and phenolic content (R^2=0.96) of the products. However, only linear model was found suitable for salt content (R^2=0.88). Regression coefficients of fitted model along with R^2 values are given in Table 16. Response surface plots for the effect of input variables on response variables are given in Figure 55 to Figure 60.

Dilution and salt content in liquid smoke had a significant linear effect on sensory score of the products ($p<0.05$). Dilution of 1:12 (8.3%) was found to be optimum in terms of sensory score. This is attributed to the bitter flavour in samples produced at lower dilutions due to higher phenolic content. Salt content of 9.25% in liquid smoke yielded desired results. Quadratic effect of the three independent variables was also found to be significant for sensory score ($p<0.05$).

Linear regression coefficients of duration of exposure and dilution showed a significant linear effect on phenolic content of the products ($p<0.05$). Total phenolic content of the products were directly proportional to the duration of exposure. An indirectly proportional relation was found between TPC and dilution of liquid smoke.

Linear effect of duration of exposure and salt content in liquid smoke was found to be significant for salt content in the product ($P<0.05$). Based on the desirability score obtained, soaking the cooked blocks in commercial liquid smoke diluted at 1:12 ratio and added with 7% salt for 90 min was found to be optimum for producing CMLS *masmin* flakes by soaking. The corresponding desirability score was 0.99.

Table 16 Regression coefficients of coded factors of CMLS *masmin* flakes produced by soaking

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+6.48	+156.18	+7.80
Duration of exposure	x_1	-0.26	+145.37*	+1.28*
Salt content in liquid smoke	x_2	-0.59*	+25.84	+3.50*
Dilution	x_3	+0.85*	-474.79*	-0.15
Duration of exposure X Salt content in liquid smoke	x_1x_2	-0.32	-2.75	NA
Duration of exposure X Dilution	x_1x_3	-0.36	-222.74*	NA
Salt content in liquid smoke X Dilution	x_2x_3	-0.27	-28.73	NA
Duration of exposure^2	x_1^2	-1.73*	-67.56	NA
Salt content in liquid smoke^2	x_2^2	-1.53*	+43.37	NA
Dilution^2	x_3^2	-3.00*	+613.03*	NA
	R^2	0.98	0.96	0.88

* Significant at 5% level of significance
NA - Not Analyzed

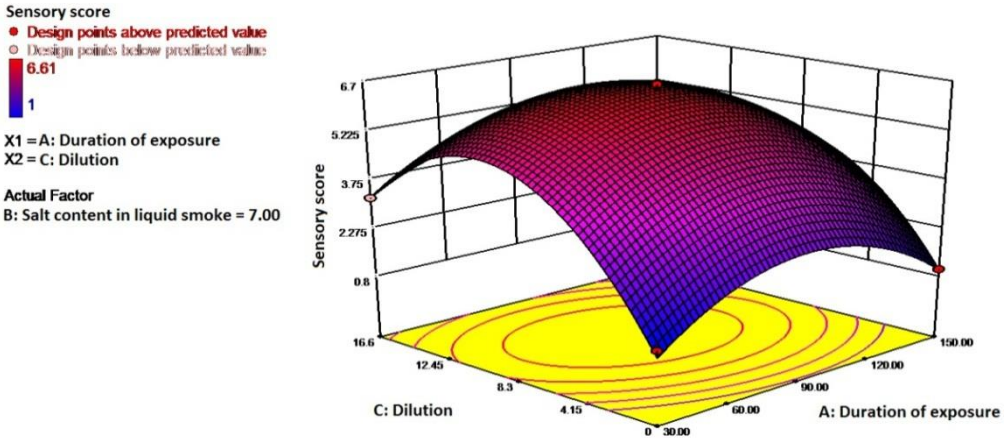

Figure 55 Response surface plots for the effect of dilution and duration of exposure on sensory score of CMLS *masmin* flakes produced by soaking

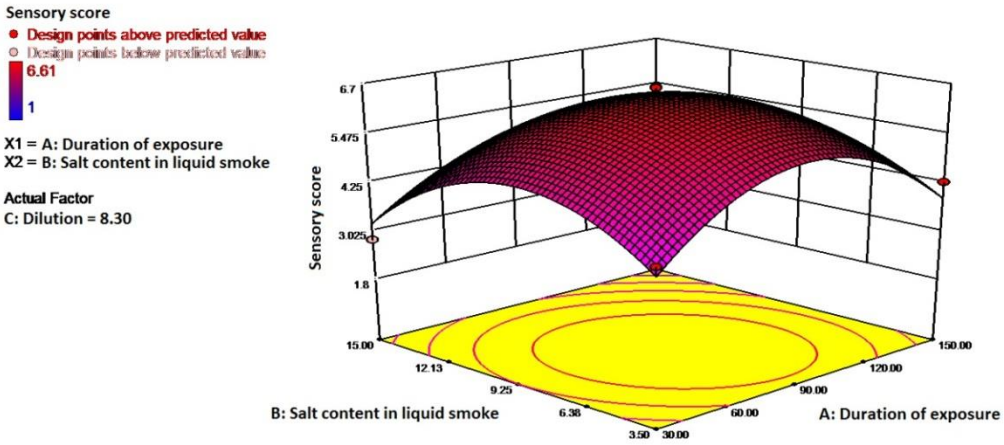

Figure 56 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of CMLS *masmin* flakes produced by soaking

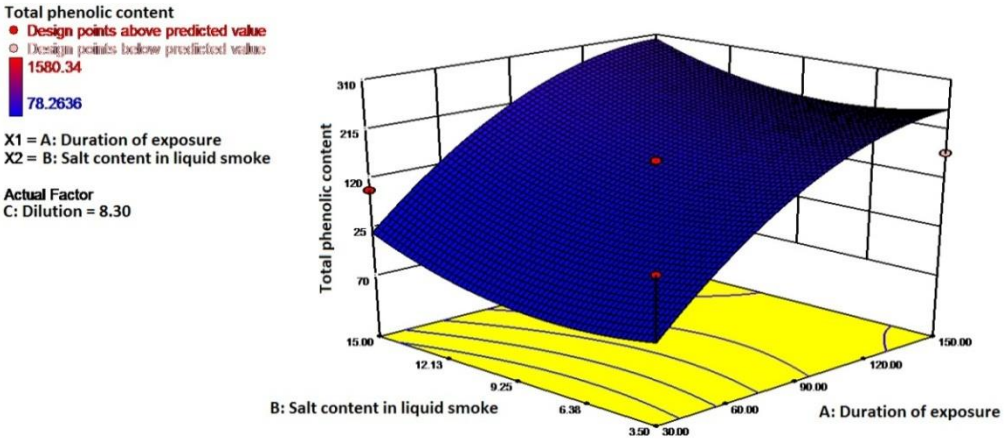

Figure 57 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of CMLS *masmin* flakes produced by soaking

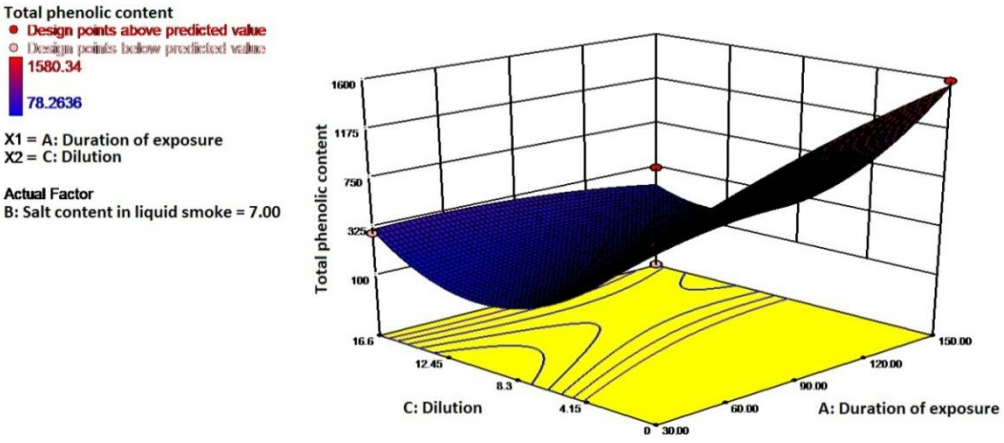

Figure 58 Response surface plots for the effect of dilution and duration of exposure on total phenolic content of CMLS *masmin* flakes produced by soaking

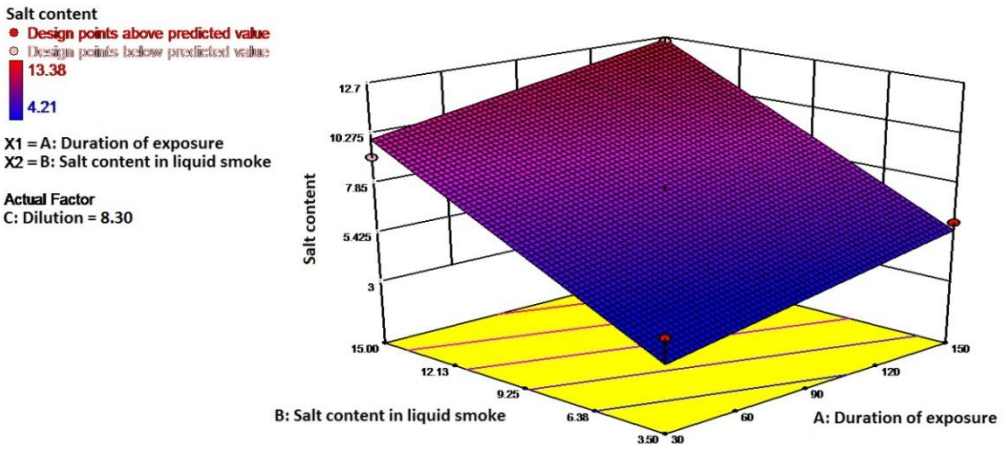

Figure 59 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of CMLS *masmin* flakes produced by soaking

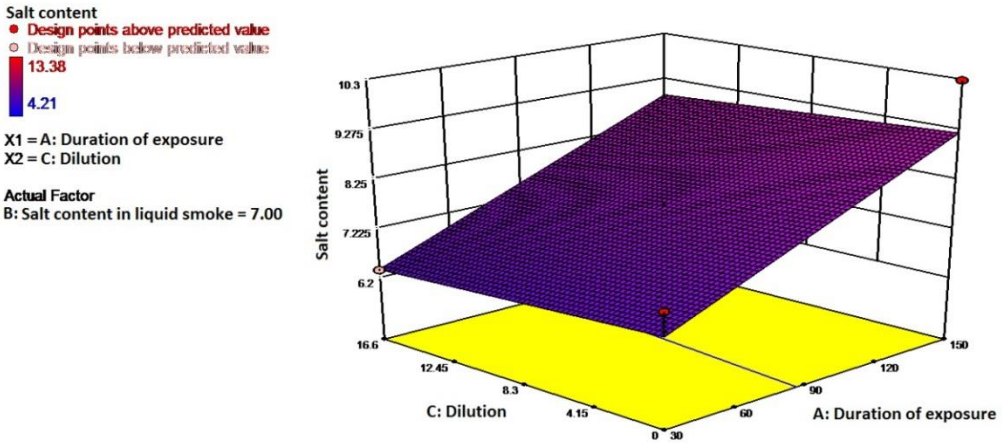

Figure 60 Response surface plots for the effect of dilution and duration of exposure on salt content of CMLS *masmin* flakes produced by soaking

3.2.12 Standardization of process parameters for production of commercial liquid smoked *masmin* flakes by mixing

Quantity of liquid smoke used and respective total phenolic content, salt content and sensory score of the samples are given in Table . Significant difference was observed in the total phenolic content of samples produced from different levels of liquid smoke ($p<0.05$). Based on the sensory score obtained, *masmin* flakes produced by mixing commercial liquid smoke at 0.5% level was found to be superior.

Table 17 Standardization of process parameters for production of CMLS *masmin* flakes by Mixing

Quantity of liquid smoke (%)	Total Phenolic Content (mg/L)	Salt content (%)	Sensory score
0.25	140.24 ± 10.25^{a}	4.25 ± 0.75^{a}	3.99 ± 1.29^{a}
0.5	224.79 ± 18.34^{b}	5.24 ± 0.57^{a}	6.99 ± 1.14^{b}
0.75	341.37 ± 25.17^{c}	5.25 ± 0.69^{a}	4.91 ± 0.97^{a}
1	567.20 ± 36.97^{d}	6.28 ± 0.51^{a}	2.87 ± 1.03^{a}

* Significant at 5% level of significance

3.2.13 Standardization of process parameters for production of commercial liquid smoked *masmin* powder by spraying

Quadratic model was found to be best fitted for sensory score, phenolic content and salt content of the product. Response surface plots for the effect of input variables on response variables are given in Figure 61 to Figure 69. Regression coefficients of fitted model along with R^2 values are given in Table 18.

Duration of exposure and dilution of liquid smoke showed a significant linear influence on the sensory acceptability of the products ($p<0.05$). Increase in duration of exposure and decrease in dilution of the liquid smoke was found

to have a negative impact on the sensory score. Interaction between salt content in liquid smoke and chamber temperature was found to be significantly influencing the sensory acceptability of the products ($p<0.05$). Quadratic regression coefficient for salt content in liquid smoke was also found to be significant for sensory acceptability ($p<0.05$). Salt content of 3.5% in liquid smoke was found to give the desired sensory characteristics to the products.

Linear effect of duration of exposure, chamber temperature, flow rate and dilution had significant influence on the total phenolic content ($p<0.05$). A proportionate increase in the phenolic content was observed with increasing duration of exposure and decreasing dilution. Flow rates above 2.5 L/hr resulted in lower phenol deposition and correspondingly lower sensory score. Higher deposition of phenols was observed in samples processed around a chamber temperature of 45°C.

Duration of exposure, flow rate and salt content in liquid smoke had a significant linear influence on the salt content of the product ($p<0.05$). A proportionate increase in salt content was observed with increase in these factors. Quadratic regression coefficient for duration of exposure was also found to be significant for salt content in the product ($p<0.05$).

Based on the desirability score obtained, spraying commercial liquid smoke (diluted in 1:6 proportion with distilled water and added with 3.5% (w/v) salt) for 30 min at a flow rate of 1L/hr in the multi-functional smoke kiln at a chamber temperature of 30°C was found to give desirable flavour. The corresponding desirability score was 0.89.

Table 18 Regression coefficients for coded factors of CMLS *masmin* powder produced by spraying

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+2.72	+285.32	+6.57
Duration of exposure	x_1	-0.62*	+42.16*	+2.10*
Salt content in liquid smoke	x_2	-0.44	+7.38	+0.95*
Chamber temperature	x_3	-0.31	+9.71*	+0.030
Flow rate	x_4	-0.10	+20.60*	+0.75*
Dilution	x_5	-0.67*	+30.19*	-0.41
Duration of exposure X Salt content in liquid smoke	x_1x_2	+0.072	+0.61	-0.44
Duration of exposure X Chamber temperature	x_1x_3	-0.073	+5.77	-0.18
Duration of exposure X Flow rate	x_1x_4	+0.38	+10.68*	+0.13
Duration of exposure X Dilution	x_1x_5	+0.25	+9.61*	+0.47
Salt content in liquid smoke X Chamber temperature	x_2x_3	+0.65*	-6.98	-7.132
Salt content in liquid smoke X Flow rate	x_2x_4	+0.037	-2.83	-0.11
Salt content in liquid smoke X Dilution	x_2x_5	+0.27	-5.10	+0.39
Chamber temperature X Flow rate	x_3x_4	+0.11	+0.61	-0.13
Chamber temperature X Dilution	x_3x_5	+0.32	+6.06	-0.041
Flow rate X Dilution	x_4x_5	+0.26	+6.65	-0.041
Duration of exposure^2	x_1^2	-0.39	+33.73*	+1.57*
Salt content in liquid smoke^2	x_2^2	-1.40*	+16.59	+1.04
Chamber temperature^2	x_3^2	+0.43	-29.12*	+1.595
Flow rate^2	x_4^2	+0.98	-19.81	-0.58
Dilution^2	x_5^2	+0.44	-7.87	+0.77
	R^2	0.88	0.98	0.96

* Significant at 5% level of significance

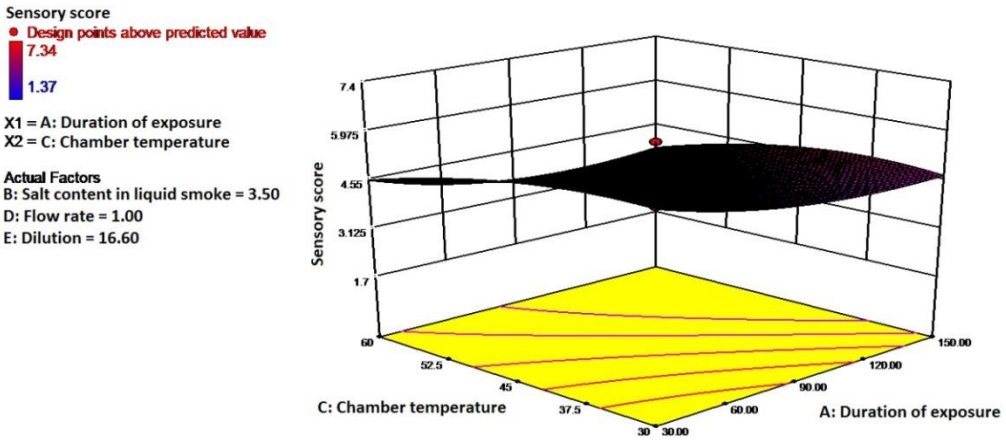

Figure 61 Response surface plots for the effect of chamber temperature and duration of exposure on sensory score of CMLS *masmin* powder produced by spraying

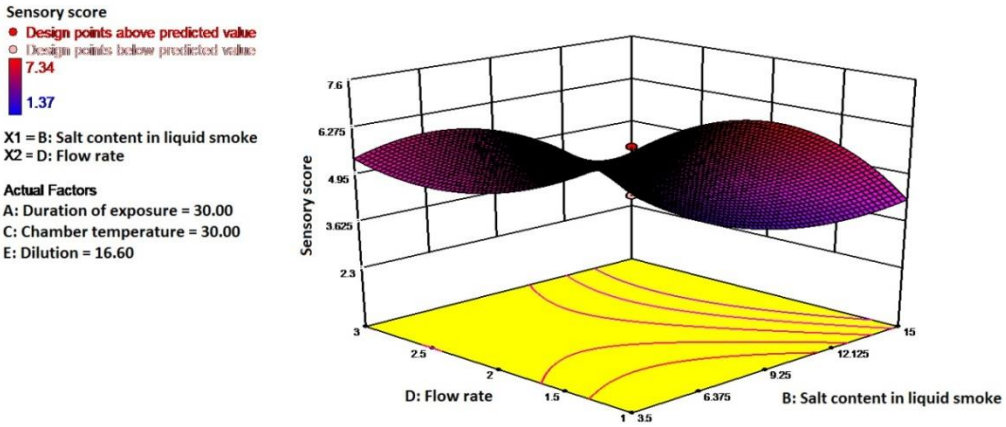

Figure 62 Response surface plots for the effect of flow rate and salt content in liquid smoke on sensory score of CMLS *masmin* powder produced by spraying

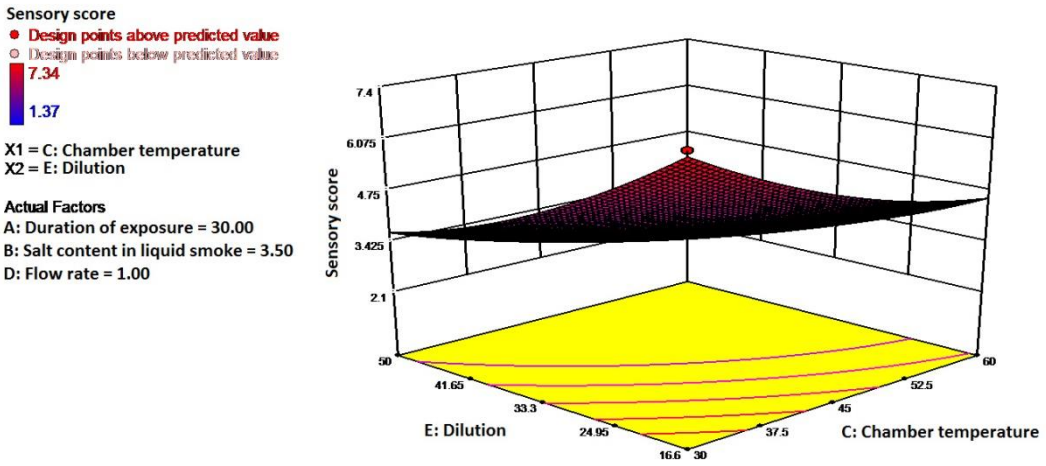

Figure 63 Response surface plots for the effect of dilution and chamber temperature on sensory score of CMLS *masmin* powder produced by spraying

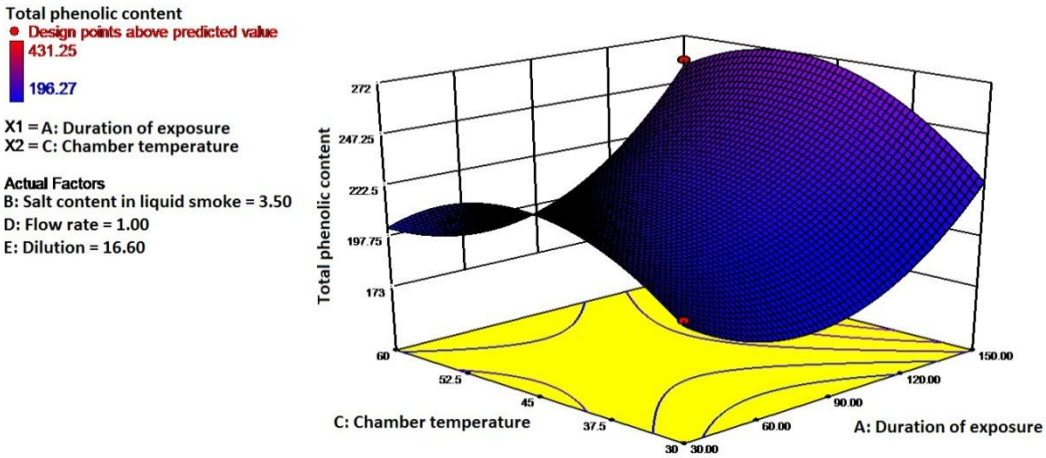

Figure 64 Response surface plots for the effect of chamber temperature and duration of exposure on total phenolic content of CMLS *masmin* powder produced by spraying

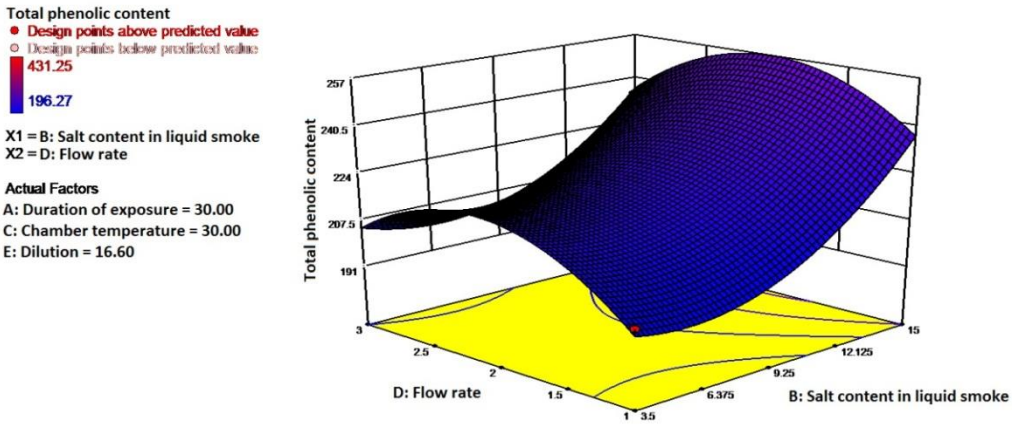

Figure 65 Response surface plots for the effect of flow rate and salt content in liquid smoke on total phenolic content of CMLS *masmin* powder produced by spraying

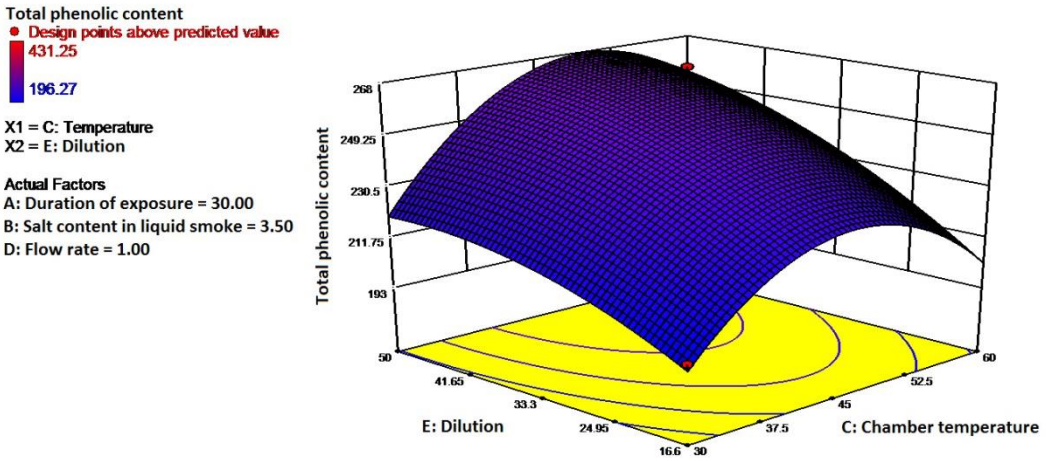

Figure 66 Response surface plots for the effect of dilution and chamber temperature on total phenolic content of CMLS *masmin* powder produced by spraying

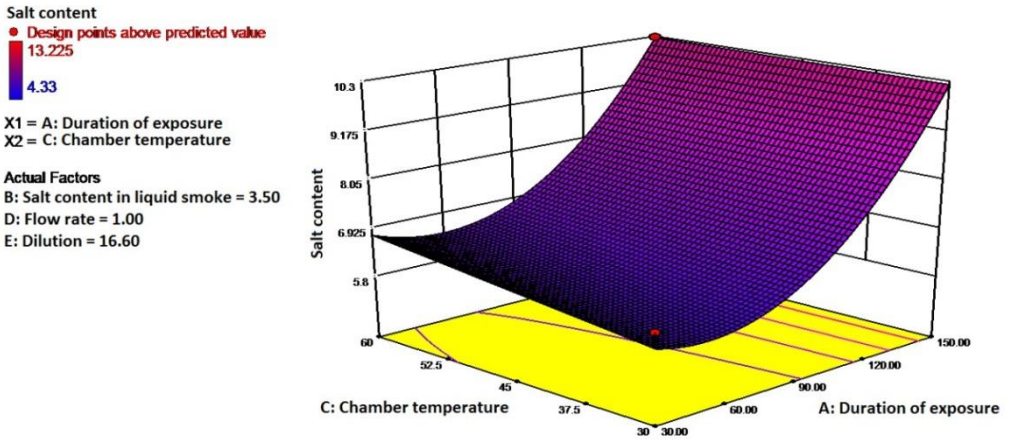

Figure 67 Response surface plots for the effect of chamber temperature and duration of spraying on salt content of CMLS *masmin* powder produced by spraying

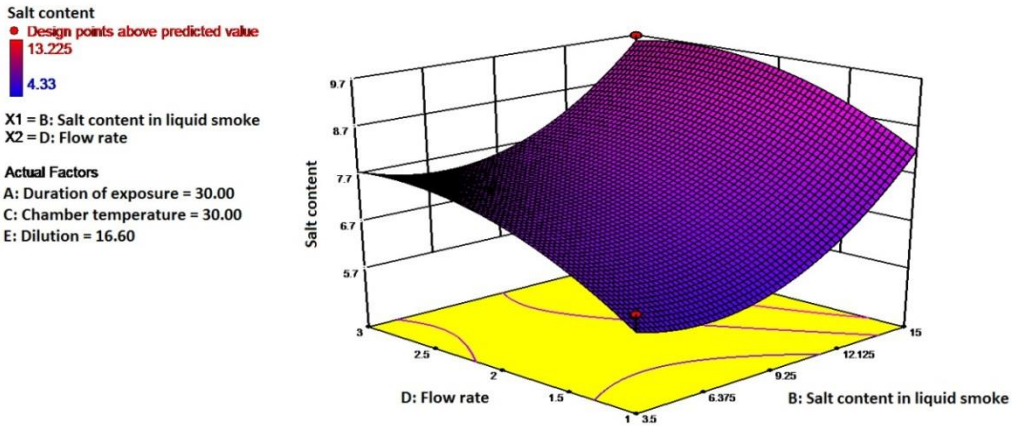

Figure 68 Response surface plots for the effect of flow rate and salt content in liquid smoke on salt content of CMLS *masmin* powder produced by spraying

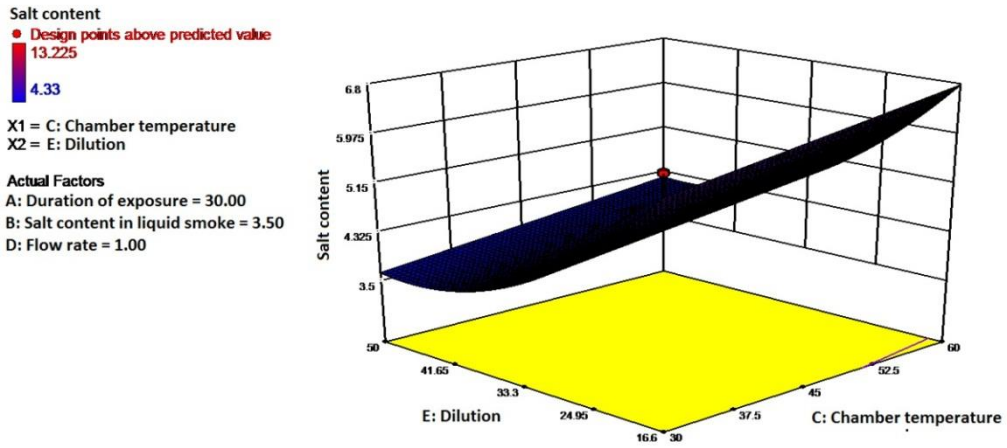

Figure 69 Response surface plots for the effect of dilution and chamber temperature on salt content of CMLS *masmin* powder produced by spraying

3.2.14 Standardization of process parameters for production of commercial liquid smoked *masmin* powder by soaking

Quadratic model was found to be best fitted for explaining the variability in dependent variables as a function of input variables. Response surface plots for the influence of input variables on the response variables are shown in Figure 70 to Figure 75. Regression coefficients of fitted model with R^2 values are given in Table 19.

Among the input variables, quadratic effect of dilution was found to have a significant effect on the sensory score and phenolic content of the products ($p<0.05$). Increase in dilution was found to cause lower absorption of phenols. However, this minimal deposition of phenols resulted in good sensory acceptability. Duration of exposure had a significant linear effect on the phenolic content and salt content of the products ($p<0.05$). A linear increase in these dependent variables was observed with increase in duration of exposure. Quadratic regression coefficient of dilution was also found to be significant for total phenolic content of the product ($p<0.05$). TPC of the

product was also found to be influenced by the interaction between duration of exposure and dilution ($p<0.05$). Based on the desirability score obtained, soaking the shredded loins in commercial liquid smoke (diluted at 15% and added with 7% salt) for 30 min was found to be optimum for the production of CMLS *masmin* powder. The corresponding desirability score was 0.86.

Table 19 Regression coefficients for coded factors of CMLS *masmin* powder produced by soaking

Input variables	Coded factors	Sensory score	Total phenolic content	Salt content
	Intercept	+3.88	+202.22	+8.22
Duration of exposure	x_1	-0.92	+152.32*	+2.46*
Salt content in liquid smoke	x_2	-0.35	+20.03	+2.85*
Dilution	x_3	+0.67	-607.25*	+0.22
Duration of exposure X Salt content in liquid smoke	x_1x_2	+0.000	+29.60	+1.14*
Duration of exposure X Dilution	x_1x_3	-0.60	-179.60*	+0.90
Salt content in liquid smoke X Dilution	x_2x_3	-0.16	-14.97	+0.23
Duration of exposure^2	x_1^2	+0.52	+27.78	+1.36*
Salt content in liquid smoke^2	x_2^2	-0.52	+21.90	-0.21
Dilution^2	x_3^2	-2.21*	+779.20*	+0.52
	R^2	0.82	0.99	0.97

* Significant at 5% level of significance

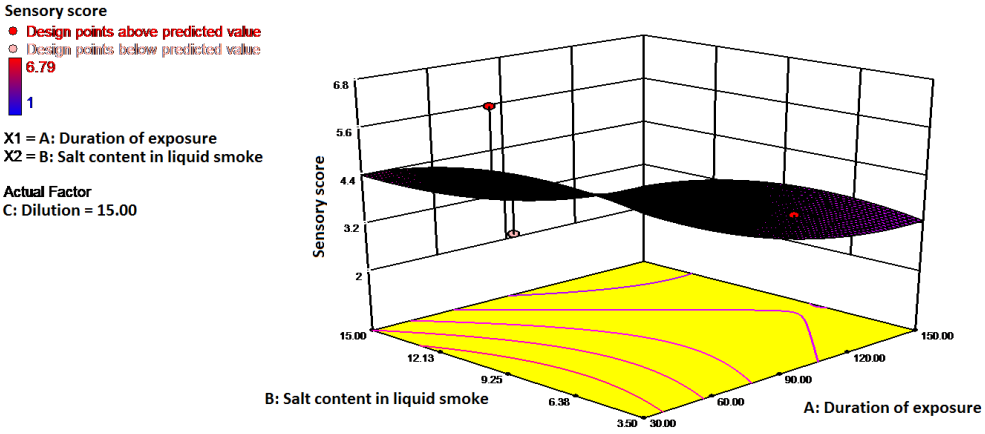

Figure 70 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on sensory score of CMLS *masmin* powder produced by soaking

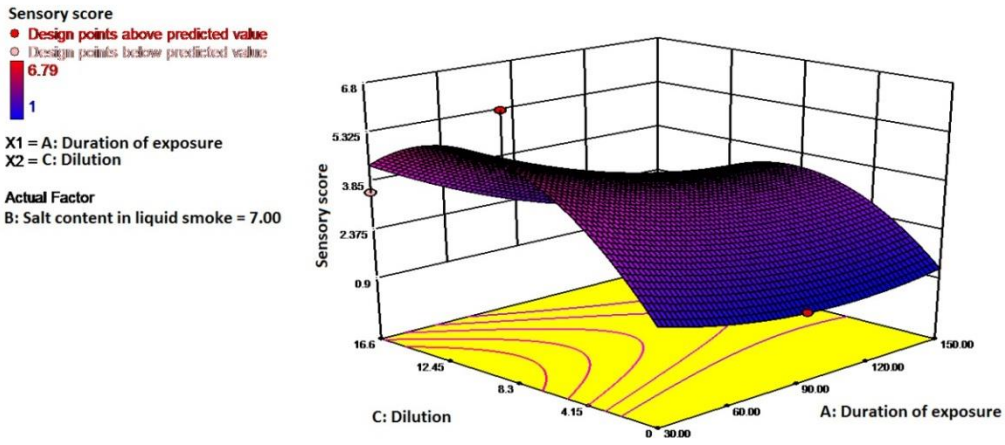

Figure 71 Response surface plots for the effect of dilution and duration of exposure on sensory score of CMLS *masmin* powder produced by soaking

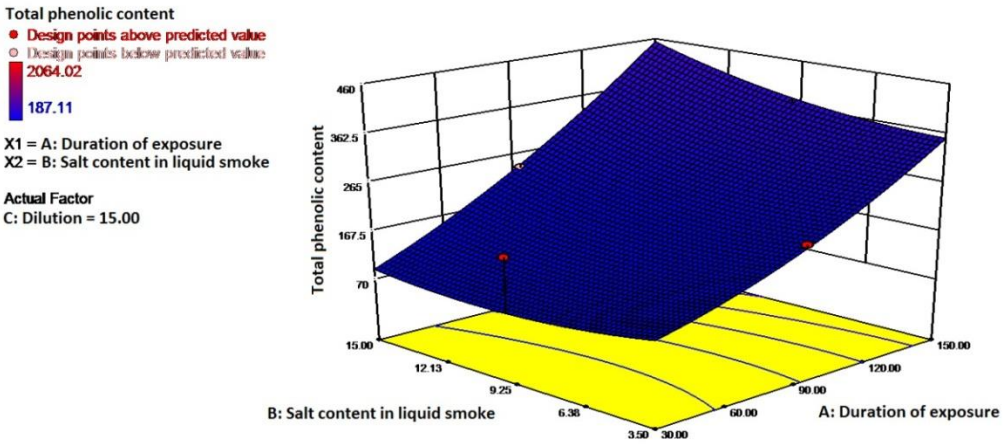

Figure 72 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on total phenolic content of CMLS *masmin* powder produced by soaking

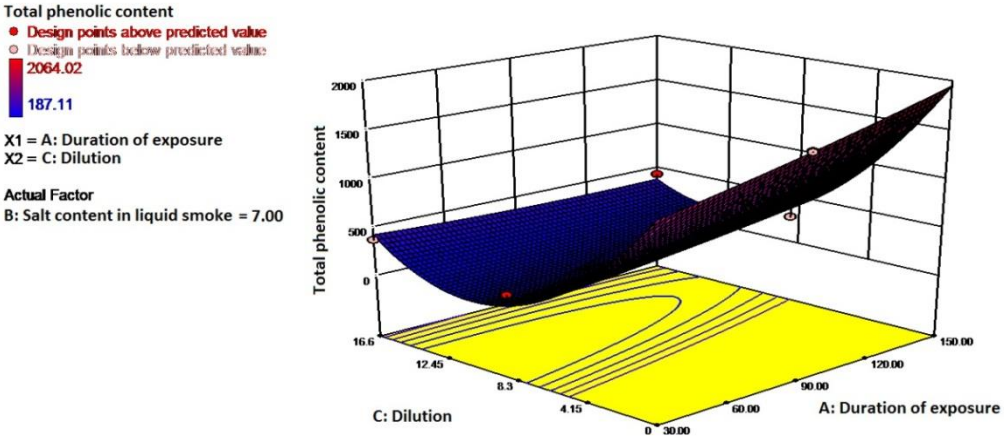

Figure 73 Response surface plots for the effect of dilution and duration of exposure on total phenolic content of CMLS *masmin* powder produced by soaking

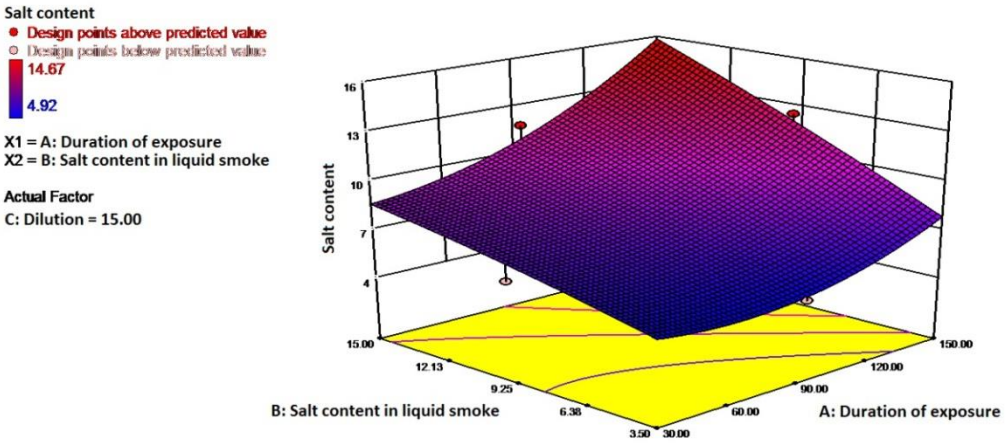

Figure 74 Response surface plots for the effect of salt content in liquid smoke and duration of exposure on salt content of CMLS *masmin* powder produced by soaking

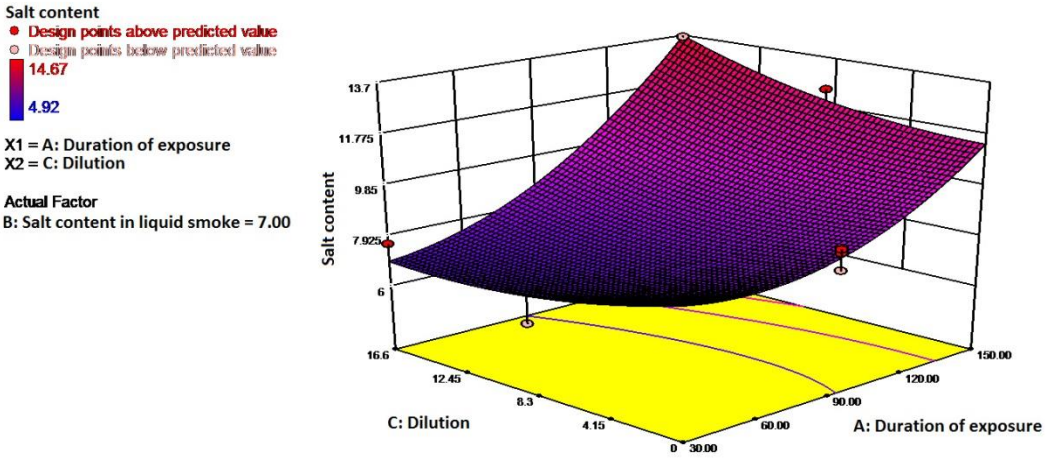

Figure 75 Response surface plots for the effect of dilution and duration of exposure on salt content of CMLS *masmin* powder produced by soaking

3.2.15 Validation study and selection of best treatments

3.2.15.1 Indigenous liquid smoked products

Standardized treatments for each product and the predicted values for response variables are given in Table 20. Validation study was conducted to affirm the repeatability of predicted values. Result of the validation study

(Table 21) indicated that the value of each response variable was in the range of predicted values. Samples produced by the standardized treatments were compared with each other and treatment which received higher sensory score was selected as the best treatment.

Table 20 Standardized treatments and predicted values for the production of INDLS products

Product	Treatment	Input variables				Predicted values		
		Duration of exposure (min)	Salt content in liquid smoke (%)	Chamber temperature (°C)	Flow rate (L/hr)/ Quantity (%)	Total phenolic content (ppm)	Salt content (%)	Sensory score (1-8)
Masmin	Spraying	155	4	60	3	288.65	6.17	5.7
	Soaking	60	7.5	-	-	294	6.24	4.25
Masmin flakes	Spraying	30	3.5	60	1.25	177.84	5.72	5.52
	Soaking	70	7.5	-	-	224.13	6.12	4.43
	Mixing	-	-	-	2.50 %	NA	NA	NA
Masmin powder	Spraying	165	3.5	60	0.75	313.64	6.41	6.11
	Soaking	150	3.5	-	-	283.7	6.56	6.63

NA – Not Applicable

Table 21 Result of the validation study for the production of INDLS products

Product	Treatment	Input variables				Result of the validation study		
		Duration of exposure (min)	Salt content in liquid smoke (%)	Chamber temperature (°C)	Flow rate (L/hr)/ Quantity (%)	Total phenolic content (ppm)	Salt content (%)	Sensory score (1-8)
Masmin	Spraying	155	4	60	3	276.47 ± 22.65	6.51 ± 0.96	6.32±0.78
	Soaking	60	7.5	-	-	315.28 ± 45.81	6.11 ± 0.57	6.59±0.88*
Masmin flakes	Spraying	30	3.5	60	1.25	208.16 ± 28.22	6.08 ± 1.09	6.71±0.99*
	Soaking	70	7.5	-	-	194.31 ± 31.97	5.89 ± 0.85	5.59±0.97
	Mixing	-	-	-	2.50 %	219.48 ± 21.84	5.97 ± 0.80	6.17±1.07
Masmin powder	Spraying	165	3.5	60	0.75	324.17 ± 4 5.71	5.71 ± 0.76	6.82±0.67
	Soaking	150	3.5	-	-	302.54 ± 38.04	6.21 ± 0.98	7.42±0.95*

*Selected as best treatment

3.2.15.2 Commercial liquid smoked products

Standardized treatments for each product and the predicted values for response variables are given in Table 22. Result of the validation study (Table 23) indicated that the value of each response variable was in the range of predicted values.

Table 22 Standardized treatments and predicted values for the production of CMLS products

		Input variables					Predicted values		
Product	**Treatment**	**Duration of exposure (min)**	**Salt content in liquid smoke (%)**	**Chamber temperature (°C)**	**Flow rate (L/hr)/ Quantity (%)**	**Dilution (%)**	**Total phenolic content (ppm)**	**Salt content (%)**	**Sensory score (1-8)**
Masmin	Spraying	150	3.5	60	1	16.6	226.46	5.95	7
	Soaking	90	9.25	-	-	8.3	218.37	6	6.5
Masmin flakes	Spraying	90	9.25	45	1	33.3	176.92	7.09	5.38
	Soaking	90	7	-	-	8.3	155.89	6.37	6.37
	Mixing	-	-	-	0.5 %		NA	NA	NA
Masmin powder	Spraying	30	3.5	30	1	16.6	201.29	6.35	6.7
	Soaking	30	7	-	-	15	187.11	6.07	5.17

NA – Not Applicable

Table 23 Result of the validation study for the production of CMLS products

		Input variables					Result of the validation study		
Product	**Treatment**	**Duration of exposure (min)**	**Salt content in liquid smoke (%)**	**Chamber temperature (°C)**	**Flow rate (L/hr)/ Quantity (%)**	**Dilution (%)**	**Total phenolic content (ppm)**	**Salt content (%)**	**Sensory score (1-8)**
Masmin	Spraying	150	3.5	60	1	16.6	189.21±31.14	6.31±1.03	7.56±0.77*
	Soaking	90	9.25	-	-	8.3	224.97±18.21	5.76±0.94	6.78±0.53
Masmin flakes	Spraying	90	9.25	45	1	33.3	197.38±26.14	7.13±1.14	7.24±1.26*
	Soaking	90	7	-	-	8.3	165.89±27.18	5.87±0.85	6.78±0.73
	Mixing	-	-	-	0.5 %	-	187.38±22.36	6.24±0.72	7.12±0.61
Masmin powder	Spraying	30	3.5	30	1	16.6	216.92±36.28	6.12±0.75	7.28±0.68*
	Soaking	30	7	-	-	15	198.34±25.62	5.87±0.88	6.28±1.37

*Selected as best treatment

3.3 Conclusion

Process parameters for the production of 12 liquid smoked *masmin* and *masmin* based products were standardized. In the case of INDLS *masmin* and *masmin* powder soaking in liquid smoke was found to be the best mode of application. Spraying with liquid smoke was found ideal for production of INDLS *masmin* flakes. Spraying was found to be the best mode of application for all the CMLS products.

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Chapter

4 Effect of Liquid Smoking on the Polycyclic Aromatic Hydrocarbon Content of *Masmin* and *Masmin* Based Products

Contents

Having standardized the protocol for developing liquid smoked *masmin* and *masmin* based products, the aim of this study was to understand the effectiveness of liquid smoking in reducing the PAH content in the developed products. PAH content in traditional *masmin* was compared with that of indigenous (INDLS) and commercial liquid smoked (CMLS) *masmin*. In the case of liquid smoked *masmin* flakes, smoked *masmin* flakes were used as control. Improved (IMP) *masmin* powder was used as controls for liquid smoked *masmin* powder.

4.1 Materials & Methods

4.1.1 Traditional *masmin*

Representative samples of traditional *masmin* were collected from different islands of Lakshadweep. The samples were kept in sealed metalized polyester polythene pouches (12µ polyester/10µ Aluminium foil/300 gauge LDPE) until analysis.

4.1.2 Production of liquid smoked *masmin*

4.1.2.1 Production of indigenous liquid smoked *masmin*

Cooked loins prepared after pre-processing operations (section 3.1.2.1.1) were soaked in indigenous liquid smoke for 60 min with a salt content of 7.5% (w/v). After soaking, the loins were dried in the multi-functional smoke kiln at 60°C to moisture content below 10% (drying requires approximately 48 hrs). Dried loins after cooling were packed in metallised polyester polythene pouches and stored at room temperature (28±2°C).

4.1.2.2 Production of commercial liquid smoked *masmin*

Cooked loins prepared after pre-processing operations (section 3.1.2.1.1) were sprayed with the commercial liquid smoke (diluted in 1:6 proportion with distilled water and salted at 3.5% level (w/v)) for 150 min at a flow rate of 1L/hr in the multi-functional smoke kiln at a chamber temperature of 60°C. After this, the loins were dried in the same kiln at 60°C to moisture content below 10 %. Dried loins after cooling were packed in metallised polyester polythene pouches and stored at room temperature.

4.1.3 Production of smoked *masmin* flakes

Cooked meat blocks after pre-processing operations (section 3.1.2.2.1) were smoked in the multi-functional smoke kiln for two hour at 60°C. Coconut husk powder with a moisture content of 20-30 % was used as the smoke source. Smoked blocks were subsequently dried in the same kiln at 60°C to a moisture content of 30% (the drying process took 18-24 Hrs.). Smoked and dried meat blocks (Plate 23) were flaked to thin flakes by using *masmin* flaking machine. The flakes were again dried at 60°C to moisture content below 10 % (8-10 hrs of drying). Dried flakes after cooling (Plate 24) were

packed in metallised polythene pouches and stored at room temperature until further analysis.

Plate 23 Smoked meat blocks

Plate 24 Smoked *masmin* flakes

4.1.4 Production of indigenous liquid smoked *masmin* flakes

Cooked meat blocks were prepared as described in the pre-processing operations for smoked *masmin* flakes (section 3.1.2.2.1) and then sprayed with 3.5% (w/v) salted indigenous liquid smoke in the multi-functional smoke kiln at 60°C with a flow rate of 1.25 L/hr for 30 min. The liquid smoked blocks were then dried in the same kiln at 60°C to an average moisture content of 30% and then flaked by using the *masmin* flaking machine. The flakes were further dried at 60°C to moisture content below 10%. Dried flakes after cooling were packed in metallised polyester polythene pouches and stored at room temperature until further analysis.

4.1.5 Production of commercial liquid smoked *masmin* flakes

Cooked meat blocks were prepared as described in the case of smoked *masmin* flakes (section 3.1.2.2.1) and then sprayed with the commercial liquid smoke diluted with potable water at 1:3 ratio and added with 9.25 % (w/v) salt at a kiln temperature of 45°C for 90 min with a flow rate of 1 L/hr. The liquid smoked blocks were then dried in the same kiln at 60°C to an average

moisture content of 30 % and then flaked and packed in the same method followed for smoked *masmin* flakes and INDLS *masmin* flakes.

4.1.6 Production of improved *masmin* powder

Cooked loins were prepared as described in the pre-processing operations for liquid smoked *masmin* (section 3.1.2.1.1) and were scrambled to thin strips by hand; spread in perforated trays and smoked in the multi-functional smoke kiln for two hours at 60°C and then subsequently dried at 60°C to moisture content below 10 %. The dried strips were then powdered by using a mechanical grinder, cooled and then packed in metallised polyester polythene pouches and stored at room temperature until further analysis.

4.1.7 Production of indigenous liquid smoked *masmin* powder

Cooked loins were prepared as described in the pre-processing operations for liquid smoked *masmin* (section 3.1.2.1.1) and scrambled to thin strips by hand. The strips were then soaked in 3.5% (w/v) salted indigenous liquid smoke for 150 min, drained and then dried at 60°C to moisture content below 10 %. The dried strips were then powdered by using a mechanical grinder, cooled and then packed in metallised polyester polythene pouches and stored at room temperature until further analysis.

4.1.8 Production of commercial liquid smoked *masmin* powder

Cooked loins were prepared as described in the pre-processing operations for liquid smoked *masmin* (section 3.1.2.1.1) and scrambled to thin strips by hand. The strips were then spread in perforated trays and sprayed with commercial liquid smoke diluted at 1:6 ratio (with potable water) and salted at 3.5% (w/v) level for 30 min. The chamber temperature for the process was 30°C with a flow rate of 1 L/hr. The liquid smoked strips were then dried

at 60°C to moisture content below 10 %. The dried strips were then powdered by using a mechanical grinder, cooled and then packed in metallised polyester polythene pouches and stored at room temperature until further analysis.

4.1.9 Determination of Polycyclic Aromatic Hydrocarbon content in *masmin* and *masmin* based products

Extraction of PAH from *masmin* and *masmin* based products were carried out according to Takatsuki, Suzuki, Salo, and Ushizawi (1985). *Masmin* samples were aseptically scrapped in to thin flakes and then ground in a mechanical grinder. *Masmin* flakes and *masmin* powder were directly used for analysis. 25 g of the sample was refluxed in a round bottom flask along with 200 ml ethanol, 35 ml 50 % aqueous KOH, 2 g sodium sulphite and few glass beads for 2 hrs and subsequently cooled to 40°C. 150 ml n-Hexane was added to the flask in portions with gentle swirling. The contents were transferred to a 500 ml separating funnel containing 150 ml 1% brine. The round bottom flask was rinsed with 10 ml portions of n-Hexane and transferred to the separating funnel. After vigorous shaking, the flask was allowed to stand for phase separation. Lower n-Hexane layer was collected and the remaining aqueous layer was thrice re-extracted with 150 ml portions of n-Hexane. The n-Hexane layers were pooled and washed with 100 ml distilled water and filtered through anhydrous sodium sulphate. The extract was concentrated to 3-5 ml by flash evaporation. Chromatographic clean-up and HPLC procedure were carried out as described for analysis of PAH in liquid smoke (section 2.1.7.2). All the chemicals used in this study were of analytical grade and acetonitrile was of HPLC grade, all obtained from Merck Millipore (Billerica, MA, USA). Water was purified with a Cascada BIO water System (Pall Corporation, NY, USA). PAH standard EPA 610 Polynuclear Aromatic Hydrocarbons Mixture (mixture of 16 EU priority

PAHs: acenaphtene-ACE, acenaphthylene-ACY, anthracene-ANT, benzo(a) anthracene-BaA, benzo(a)pyrene-BaP, benzo(b)fluoranthene-BbF, benzo(g,h,i) perylene-BgP, benzo(k)fluoranthene-BkF, chrysene-CHR, dibenzo(a,h) anthracene-DhA, fluoranthene-FLT, fluorene-FLR, indeno(1,2,3-cd)pyrene-IcP, naphthalene-NAP, phenanthrene-PHE, pyrene- PYR) was obtained from Supelco (Bellefonte, PA, USA). PAH4 (sum content BaP, BaA, BbF & CHR), Light PAH (sum content of PAH having molecular weight less than 216.3 Da viz- NAP, ACE, FLR, ANT, PHE, PYR & FLT) and Heavy PAH (PAH with higher molecular weight than Light PAH viz. BaA, CHR, BbF, BkF, BaP, ICP, BgP & DhA) was calculated from the data.

4.1.10 Statistical analysis

Samples were analysed in triplicates and compared statistically by multivariate ANOVA (IBM SPSS Statistics version 20). Independent sample T test was used for comparing two different means.

4.2 Results & Discussions

4.2.1 Effect of liquid smoking on the PAH content of *masmin*

PAH content in traditional *masmin*, commercial liquid smoked *masmin* & indigenous liquid smoked *masmin* is presented in Table 24. Significant difference was observed between PAH content in traditional and liquid smoked *masmin* ($p<0.05$). Traditional *masmin* showed the highest total PAH content (480.9±102.4 µg/kg) followed by CMLS *masmin* (109.54±17.45 µg/kg) and INDLS *masmin* (43.89±10 µg/kg). Silva, Adetunde, Oluseyi, Olayinka, and Alo (2011) have reported a total PAH content of 1320.9 µg/kg in catfish smoked with firewood. Total PAH content up to 1200 µg/kg has been reported in cold smoked rainbow trout (Hattula, Elfving, Mroueh, & Luoma, 2001).

No significant difference was observed between the PAH content in commercial and indigenous liquid smoked *masmin* ($p>0.05$), except in the case of BaA ($p<0.05$). PAH viz. NAP, ACE, DhA, and IcP were not detected in any of the samples. Highest individual PAH present in traditional *masmin* were PHE and PYR. BaA and PHE were the predominant PAHs present in commercial liquid smoked *masmin*. In the case of indigenous liquid smoked *masmin*, PHE and BgP were found in highest concentration. Visciano, Perugini, Conte, and Amorena (2008) while investigating the PAH content in rainbow trout processed by traditional flue gas smoking and by liquid smoke flavouring observed that ANT, FLR and PYR were the highest individual PAH present in the samples.

Significant difference was observed between traditional and liquid smoked *masmin* in terms of Heavy PAH content ($p<0.05$). Traditional *masmin* showed the highest Heavy PAH deposition (72.4±21.46 µg/kg) followed by CMLS (39.69±7.93 µg/kg) and INDLS *masmin* (16.62±5.02 µg/kg). No significant difference was observed between the Heavy PAH content in INDLS and CMLS liquid smoked samples ($p>0.05$).

Light PAH containing two or three rings are relatively volatile, soluble and are more degradable than higher molecular weight compounds. Hence they are not considered carcinogenic. Significant difference was observed between traditional and liquid smoked *masmin* samples in terms of Light PAH content ($p<0.05$). In traditional *masmin* they constituted 85 % of the total PAHs present. Total Light PAH content in indigenous and commercial liquid smoke *masmin* was homogenous in nature ($p>0.05$) each contributing 62 % and 64%, respectively.

Significant difference was observed between the PAH4 content in traditional and liquid smoked *masmin* ($P<0.05$). Traditional *masmin* showed

the highest PAH4 content (70.35±20.94 µg/kg) followed by CMLS *masmin* (28.53±5.08 µg/kg) and INDLS *masmin* (6.24±1.38 µg/kg). PAH4 content in commercial and indigenous liquid smoked *masmin* was homogenous in nature ($p>0.05$). PAH4 content in traditional *masmin* was exceeding the limit of 30 µg/kg set by European Union (2011).

BaP content in traditional *masmin* was found to be 14.55±5.61 µg/kg, which also was exceeding the regulatory limit of 5 µg/kg fixed by European Union (2011). Karl and Leinemann (1996), while comparing the PAH content in traditional kiln smoked fishery products reported a BaP content of 3.9µg/kg in eel muscle while the same in smoked halibut muscle was 3.6µg/kg. Stumpe-Vıksna, Vadims, Agnese, and Andris (2008) investigated the influence of wood type used for smoking on the formation of PAH. Eight common hard wood species (apple, alder, maple, hazel, plum, aspen, bird cherry and rowan) and two soft wood species (spruce and juniper) were used for the study. Wide variations in BaP content (from 6.04 to 35.07µg/kg) and total PAH (from 47.94 to 470.91µg/kg) were observed among different wood species.

BaP concentration was below the detection limit in both commercial and indigenous liquid smoked *masmin* samples. Similar results were obtained by Hattula, Elfving, Mroueh, and Luoma (2001) who found that use of liquid smoking for imparting smoke flavour in rainbow trout fillets was effective in reducing the PAH content by three fold without major difference in the sensory profile. However, Visciano, Perugini, Conte, and Amorena (2008) while comparing the PAH content in rainbow trout smoked with traditional flue gas smoking and with two commercial liquid smoked flavourings, did not find any significant difference between the treatments. It can be presumed that, in the later study the fillets after flue gas smoking were stored in frozen condition which could have resulted in a washing out of PAH during thawing

process before analysis, whereas in the former one, samples were vacuum packed and chill stored. Carla, Sabrina, and Susi (2006) reported that the level of BaP in the distillates obtained from smoked marc was lower than 0.03µg/kg.

Table 24 PAH content (µg/kg) in traditional and liquid smoked *masmin*

PAH	Traditional *masmin*	CMLS *masmin*	INDLS *masmin*
NAP	ND	ND	ND
ACE	ND	ND	ND
FLR	11.55 ± 4.21^{a}	5.29 ± 1.24^{ab}	1.17 ± 0.22^{b}
PHE	154.37 ± 24.215^{a}	24.64 ± 2.98^{b}	14.23 ± 2.65^{b}
ANT	68.51 ± 12.31^{a}	17.54 ± 1.83^{b}	3.92 ± 0.82^{b}
FLT	35.32 ± 8.71^{a}	11.10 ± 1.1^{b}	0.3 ± 0.056^{b}
PYR	138.75 ± 31.56^{a}	11.27 ± 2.37^{b}	7.65 ± 1.32^{b}
BaA	39.47 ± 10.11^{a}	25.73 ± 4.39^{a}	5.39 ± 1.16^{c}
CHR	13.79 ± 4.52^{a}	1.66 ± 0.45^{b}	0.61 ± 0.18^{b}
BbF	2.54 ± 0.7^{a}	1.15 ± 0.24^{b}	0.24 ± 0.044^{b}
BkF	2.05 ± 0.52^{a}	0.60 ± 0.18^{b}	0.12 ± 0.061^{b}
BaP	14.55 ± 5.61	ND	ND
DhA	ND	ND	ND
BgP	ND	10.55 ± 2.67^{a}	10.26 ± 3.58^{a}
IcP	ND	ND	ND
Total PAH	**480.9 ± 102.4^{a}**	**109.54 ± 17.45^{b}**	**43.89 ± 10.1^{b}**
Light PAH	**408.5 ± 81^{a}**	**69.85 ± 9.52^{b}**	**27.27 ± 5.06^{b}**
Heavy PAH	**72.4 ± 21.46^{a}**	**39.69 ± 7.93^{b}**	**16.62 ± 5.02^{b}**
PAH4	**70.35 ± 20.94^{a}**	**28.53 ± 5.08^{b}**	**6.24 ± 1.38^{b}**

Different superscripts (a, b, c) in the same row indicate significant difference between treatments means ($p < 0.05$). ND-Not detected

4.2.2 Effect of liquid smoking on the PAH content of *masmin* flakes

Comparative PAH content in smoked and liquid smoked *masmin* flakes is given in Table 25. Treatments applied were found to have significant influence on the PAH content ($p<0.05$). Total PAH content were highest in

smoked *masmin* flakes (135.41±33.93 µg/kg) followed by CMLS (67.99±19.30 µg/kg) and INDLS (14.05±3.92 µg/kg) samples. Smoked *masmin* flakes had a significantly higher concentration of Light PAH (109.64±26.22 µg/kg) compared to liquid smoked samples. PHE was the Light PAH found in higher concentration in all the samples. Heavy PAH and PAH4 content of smoked *masmin* flakes and CMLS samples were homogenous in nature ($p>0.05$). INDLS samples had a significantly lower Heavy PAH (4.40±0.87 µg/kg) and PAH4 deposition (1.78±0.40 µg/kg) compared to other samples ($p<0.05$). BaA and BbF was the dominant Heavy PAH in smoked *masmin* flakes; in the case of liquid smoked samples BaA and BgP dominated the list. All the Heavy PAH present in smoked *masmin* flakes samples were belonging to the PAH4 category. BaP, BkF, DhA, IcP, NAP and ACE were not detected in any of the samples.

Table 25 PAH content (µg/kg) in smoked and liquid smoked *masmin* flakes

PAH	Smoked *masmin* flakes	CMLS *masmin* flakes	INDLS *masmin* flakes
NAP	ND	ND	ND
ACE	ND	ND	ND
FLR	3.62 ± 1.25^{a}	2.38 ± 0.48^{ab}	0.72 ± 0.12^{b}
PHE	43.93 ± 12.40^{a}	15.28 ± 3.17^{b}	5.35 ± 1.90^{b}
ANT	13.52 ± 3.82^{a}	12.97 ± 5.08^{a}	1.51 ± 0.33^{b}
FLT	11.13 ± 2.18^{a}	6.78 ± 1.18^{b}	0.12 ± 0.04^{c}
PYR	37.45 ± 6.57^{a}	7.99 ± 1.70^{b}	1.95 ± 0.66^{b}
BaA	23.67 ± 7.10^{a}	14.96 ± 5.53^{a}	1.53 ± 0.34^{b}
CHR	1.92 ± 0.57^{a}	0.80 ± 0.22^{b}	0.25 ± 0.06^{b}
BbF	0.17 ± 0.04^{a}	0.28 ± 0.06^{b}	ND
BkF	ND	ND	ND
BaP	ND	ND	ND
DhA	ND	ND	ND
BgP	ND	6.55 ± 1.88^{a}	2.63 ± 0.47^{b}
IcP	ND	ND	ND
Total PAH	**135.41 ± 33.93^{a}**	**67.99 ± 19.30^{b}**	**14.05 ± 3.92^{c}**
Light PAH	**109.64 ± 26.22^{a}**	**45.40 ± 11.61^{b}**	**9.65 ± 3.05^{b}**
Heavy PAH	**25.77 ± 7.71^{a}**	**22.58 ± 7.69^{a}**	**4.40 ± 0.87^{b}**
PAH4	**25.77 ± 7.71^{a}**	**16.04 ± 5.81^{a}**	**1.78 ± 0.40^{b}**

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

4.2.3 Effect of liquid smoking on the PAH content of *masmin* powder

PAH content of IMP and liquid smoked *masmin* powder is given in Table 26. The data showed a significant difference between IMP and liquid smoked samples in terms of total PAH, Light PAH, Heavy PAH and PAH4 ($p<0.05$). However no significant difference was observed between the same among liquid smoked samples. Even though not statistically significant, INDLS samples had higher deposition of PAHs in comparison to CMLS samples. This is attributed to the difference in method of production as the INDLS samples were produced by soaking cooked loins in liquid smoke for 150 min, which resulted in a higher deposition of PAHs. Whereas, CMLS samples were exposed to mild spray application with diluted liquid smoke for 30 min only. PHE was the individual PAH present in higher quantities in all the three samples. While comparing the PAH concentration in IMP *masmin* powder and traditional *masmin,* it can be seen that the improved method was effective in reducing the total PAH, Heavy PAH and PAH 4 content in traditional *masmin*. However application of liquid smoking was found to bring forth further reduction in PAH content.

Table 26 PAH content (µg/kg) in smoked and liquid smoked *masmin* powder

PAH	IMP *masmin* powder	CMLS *masmin* powder	INDLS *masmin* powder
NAP	ND	ND	ND
ACE	ND	ND	ND
FLR	6.92 ± 1.77^{a}	0.43 ± 0.06^{b}	0.84 ± 0.21^{b}
PHE	74.45 ± 14.25^{a}	4.14 ± 1.01^{b}	21.46 ± 3.03^{b}
ANT	20.85 ± 3.52^{a}	3.98 ± 0.57^{b}	2.90 ± 0.94^{b}
FLT	17.51 ± 2.51^{a}	1.15 ± 0.33^{b}	0.19 ± 0.03^{b}
PYR	29.45 ± 5.76^{a}	3.72 ± 0.56^{b}	6.40 ± 1.29^{b}
BaA	26.18 ± 3.94^{a}	2.39 ± 0.33^{b}	2.83 ± 0.74^{b}
CHR	1.66 ± 0.41^{a}	0.30 ± 0.07^{b}	0.76 ± 0.23^{b}
BbF	1.41 ± 0.29^{a}	0.18 ± 0.03^{b}	0.32 ± 0.06^{b}
BkF	0.45 ± 0.17^{a}	ND	0.18 ± 0.05^{b}
BaP	ND	ND	ND
DhA	ND	ND	ND
BgP	0.21 ± 0.09^{a}	2.77 ± 0.64^{b}	6.46 ± 1.02^{c}
IcP	ND	ND	ND
Total PAH	$\mathbf{179.09 \pm 32.71^{a}}$	$\mathbf{19.05 \pm 2.96^{b}}$	$\mathbf{42.33 \pm 7.6^{b}}$
Light PAH	$\mathbf{149.18 \pm 27.81^{a}}$	$\mathbf{13.42 \pm 2.53^{b}}$	$\mathbf{31.79 \pm 5.5^{b}}$
Heavy PAH	$\mathbf{29.91 \pm 4.9^{a}}$	$\mathbf{5.63 \pm 0.43^{b}}$	$\mathbf{10.55 \pm 2.1^{b}}$
PAH4	$\mathbf{29.25 \pm 4.64^{a}}$	$\mathbf{2.86 \pm 0.43^{b}}$	$\mathbf{3.91 \pm 1.03^{b}}$

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

4.3 Conclusion

It is evident from the present study that traditional *masmin* is having a heavy deposition of PAHs which is far exceeding the current regulatory limits. It is understood that adoption of hygienic handling and controlled smoking as practiced for production of IMP *masmin* or *masmin* powder can significantly

reduce the PAH content in traditional *masmin*. However, liquid smoking is effective in further reduction in PAH content. Both commercial and indigenous liquid smokes were effective in lowering the PAH content in *masmin*. Among the liquid smokes, indigenous liquid smoke was found to be more effective.

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Chapter

5 Influence of Process Modifications on the Nutritional Value of *Masmin* and *Masmin* Based Products

Contents

Traditional *masmin* has been reported to be rich in protein, essential amino acids and polyunsaturated fatty acids (PUFA) (Yathavamurthi, Mumthaz, Jinu, Bindu, Suseela, & Gopal, 2010). Present study aims to explore, whether the process modifications (by adopting improved methods or liquid smoking) are significantly influencing the nutritional profile (proximate composition, amino acid profile and fatty acid profile) of *masmin* and *masmin* based products in comparison to traditional *masmin*. It is also intended to explore, whether the mild processing conditions applied in improved and liquid smoked *masmin* and *masmin* based products is having a protective effect on the nutritional value of the same.

5.1 Materials & Methods

5.1.1 Production of improved *masmin*

Cooked loins were prepared as described in section 3.1.2.1.1 and smoked in the multi-functional smoke kiln for two hours at 60°C and then subsequently dried at 60°C to moisture content below 10 %, cooled and then

packed in metallized polyester polythene pouches and stored at room temperature until further analysis.

Traditional *masmin* samples were collected from different islands of Lakshadweep. Liquid smoked *masmin* & *masmin* based products, improved *masmin* powder and smoked *masmin* flakes were prepared as described in section 4.1.2 to 4.1.8.

5.1.2 Determination of moisture content

Analysis was performed according to AOAC (2000). About 5 g of the homogenized sample was weighed in to pre-weighed petri dishes. The dishes were placed in a hot air oven maintained at 105 ± 1°C for 12 hrs. The same was then cooled in a desiccator and the difference in weight due to evaporation of water was noted. Percentage moisture content was calculated by the following equation.

$$Moisture\ (\%) = \frac{\text{Difference in weight (g)}}{\text{Weight of the sample (g)}} \text{X } 100$$

5.1.3 Determination of crude protein

Analysis was performed according to AOAC (2000). About 1-2 g of sample was taken in a 100 ml Kjeldahl flask. Few glass beads, a pinch of digestion mixture (8 parts K_2SO_4 and 1 part $CuSO_4$) and 20 ml concentrated H_2SO_4 were added to the flask. The sample was digested inside a smoke hood by keeping the flask in inclined position over a heating coil. Heating was continued until the sample became clear and colourless. Cooled and transferred the digested sample to a 100 ml standard flask and made up to the volume by adding distilled water in small quantities with intermittent shaking and cooling.

Distilled 2 ml of the made-up solution, along with 5 ml 40 % NaOH in a Micro-Kjeldahl distillation apparatus. Ammonia liberated during distillation was collected in a 100 ml conical flask containing 10 ml 2% boric acid with few drops of tachiro's indicator. Distillation was continued till 4th min from the point at which the boric acid solution turns its colour from pink to green. Amount of ammonia liberated was determined by titrating with 0.01 N H_2SO_4. Total nitrogen content was calculated from the following equation.

$$Total\ Nitrogen = \frac{\text{Vol. of 0.01 N } H_2SO_4 \text{ X 0.14 X Vol. made up}}{\text{Wt. of the sample X Vol. of sample distilled X 1000}} \text{ X 100}$$

Crude protein content was calculated by multiplying total nitrogen content with a conversion factor of 6.25 and expressed as percentage.

5.1.4 Determination of crude fat

Analysis was performed according to AOAC (2000). About 2-3 g of moisture free sample was taken in an extraction thimble plugged with cotton. Placed the extraction thimble in the soxhlet apparatus attached to a pre-weighed receiving flask and poured petroleum ether (40-60°C boiling point) washing into the thimble. Connected the extraction unit and receiving flask to the soxhlet condenser and the flask was heated using a boiling water bath. The water bath was so adjusted that the solvent siphons over 5-6 times per hour. Extraction was continued for 16-20 hrs or till the solvent inside the soxhlet apparatus became clear. After completing the extraction, the receiving flask was removed, dried in a hot air-oven maintained at 100 ± 1°C, cooled in a desiccator and weighed. The crude fat was calculated from the following equation and expressed as percentage.

$$Crude\ fat\ (\%) = \frac{\text{Wt.of fat}}{\text{Wt.of sample}} \text{ X 100}$$

5.1.5 Determination of ash content

Analysis was performed according to AOAC (2000). About 1-2 g of the moisture free sample was transferred into a pre-heated, cooled and weighed silica crucible and the sample was carbonized by burning at low red heat and was placed in a muffle furnace at 550°C for about 4 hrs until a grey / white ash results. Crucibles were cooled in a desiccator and weighed and percentage of ash was calculated using the following equation.

$$Ash\ content\ (\%) = \frac{\text{Wt. of ash}}{\text{Wt. of sample}} \text{X } 100$$

5.1.6 Determination of amino acid composition

Method prescribed by Ishida, Fujita, and Asai (1981) was used for the analysis

5.1.6.1 Reagents:

1) 6 N hydrochloric acid
2) HPLC buffers
3) Buffer A: Dissolved 13.31 g tri sodium citrate in 70 ml ethanol, to this added citric acid monohydrate (12.8 ml), NaCl (3.74 g) and Brij (4 ml). Adjusted the pH to 3.2 and finally made up to 1 L with distilled water.
4) Buffer B: Dissolved tri sodium citrate (117.6 g) and boric acid (24.8 g) in 500 ml distilled water. To this added 4N NaOH (45 ml) and adjusted the pH to 10 and made up to 2 L with distilled water.
5) O-pthalaldehyde (OPA) buffer: Dissolved sodium carbonate (40.7 g), boric acid (13.57 g) and potassium sulphate (18.8 g) in distilled water and made up to 1 L with water.

6) O-pthalaldehyde reagent: Dissolved OPA (80 mg), ethanol (1.4 ml), 2-mercaptoethanol (0.2 ml) and brij (0.15 ml) in distilled water and made up to 200 ml in OPA buffer.

7) Sodium hypochlorite solution: 0.2 ml sodium hypochlorite diluted to 200 ml in OPA buffer.

5.1.6.2 Sample preparation:

100 mg homogenized sample was taken in a heat stable test tube; added 6N HCI (10 ml) and heat sealed the tube after filling with pure nitrogen gas. Digested the contents of the tube at 110°C for 24 hrs in a hot air oven. Cooled and filtered the contents in to a round bottom flask using Whatman No.1 filter paper. The tube was rinsed with distilled water and filtered. Pooled the extract and evaporated 2-3 times in a vacuum flash evaporator with 50 ml portions of distilled water to remove traces of HCI. The residue was dissolved in buffer A and injected in to HPLC.

5.1.6.3 HPLC Analysis

Amino acid analysis was carried out with non-switching flow method and fluorescence detection after post-column derivatization with o-phthalaldehyde. The sample was filtered through a membrane filter of 0.45 µm and injected 20 µl of this to an amino acid analyser (Hitachi L-2130 Elite La Chrom) equipped with cation exchange column packed with a strongly acidic cation exchange resin i.e., styrene di vinyl benzene co polymer with sulphinic group. The column used was Na type i.e., lSC- 07/S 1504 Na having a length of 19 cm and diameter 5 mm. The instrument was equipped with a fluorescence detector (L-2485). The mobile phase of the system consisted of two buffers, Buffer A and buffer B. A gradient system was followed for the

effective separation of amino acids. The oven temperature was maintained at 60°C. Total run was programmed for 62 min.

5.17.4 Quantification of amino acids

Concentration of individual amino acid was calculated by the following formula

$$\text{Amino acid (AA) conc. (}\mu\text{ mol)} = \frac{\text{Conc. of std AA (}\mu\text{ mol) X Area of sample AA}}{\text{Area of std AA}}$$

Concentration of each amino acid (µg) was calculated by multiplying individual concentration with its molecular weight. Individual amino acid concentration was expressed as g /100 g protein.

5.1.7 Estimation of Tryptophan

Method prescribed by Sastry & Tammura (1985) was used for the analysis

5.1.7.1 Reagents:

1) 5 % Sodium hydroxide solution
2) 6 N Hydrochloric acid
3) 2.5 % Sucrose solution
4) 80 % Thioglycolic acid solution
5) 50% Sulphuric acid solution
6) Tryptophan standard:

   Stock solution- 1 mg/ml solution in 0.1 N HCl

   Working standard- 1 ml of the stock in 100 ml of 0.1 N HCl to get 10 µg/ml solution

5.1.7.2 Estimation:

About 300 mg of finely homogenized sample was weighed in to a heat stable test tube. Added 10 ml 5 % NaOH in to the test tube and sealed the tube after filling with nitrogen gas. Contents of the tube were digested by keeping at 110°C for 24 hrs in a hot air oven. Neutralised the contents by adding 6N HCl and total volume was made up to 100 ml. filtered the contents through Whatman No.1 filter paper.

Added 2.5% sucrose (0.1 ml) and 0.6% thioglycolic acid (0.1 ml) successively in to a test tube containing 50% H_2SO_4 (4 ml). The tube was kept immersed in a water bath maintained at 45-50°C for 5 min and cooled. 0.1-0.8 ml of sample was added to the test tube and mixed. The volume was made up to 5 ml with 0.1N HCl and left aside for 5 min. the colour intensity was measured in a spectrophotometer at 500 nm. A blank was also run, Corrected absorbance for the blank and constructed a calibration curve using the standard tryptophan solution plotting absorbance versus concentration. Calculated concentration of tryptophan (µg) from the calibration curve and expressed as g /100 g protein.

5.1.8 Determination of fatty acid composition

Extraction and purification fatty acids were done according to Folch, Lees, and Sloane-Stanley (1957), followed by esterification as per Metcalfe, Schmitz, and Pelka (1966).

5.1.8.1 Extraction and purification fatty acids

About 100 g of finely powdered sample was mixed with appropriate volume of chloroform-methanol mixture (2:1) for two minutes using a high speed stirrer. Filtered the extract under slight vacuum using a Buckner funnel

with Whatman No.1 filter paper. The extraction and filtration was repeated thrice. Pooled the extract in to a separating funnel and added 20 % of the volume water. Shaken well and allowed to separate overnight. Collected the lower layer and filtered through anhydrous sodium sulphate. Evaporated the extract to dryness under nitrogen and the lipids were quantified gravimetrically.

5.1.8.2 Esterification and gas chromatographic analysis

To 100 mg of extracted fat/oil, 5 ml of 0.5 N Methanolic NaOH was added and refluxed for 5 min in a water bath under a stream of nitrogen. Esterification was done by refluxing with 6 ml of BF3- methanol for 5 min. After cooling, 6 ml saturated NaCl was added. This solution was extracted thrice with petroleum ether and washed with double distilled water and filtered through anhydrous sodium sulphate, evaporated and made up to 1 ml in petroleum ether for further analysis. The corresponding fatty acid methyl esters were analysed in a Perkin Elmer Autosystem XL Gas Chromatograph attached to flame ionization detector (FID). An Elite 225 capillary column measuring 30 m length and 0.25 mm inner diameter with a film thickness of 0.25 μm was used for the analysis. Helium at a flow rate of 0.5 ml/min was used as the carrier gas. Oven temperature was initially held at 110°C for 4 min and was programmed to increase to 240°C at a rate of 2.7°C/min, held at 240°C for 3 min and then programmed to increase to 280°C and held for 5 min. FID was maintained at 275°C. The total run time was about 41.28 min.

5.1.9 Statistical analysis

Samples were analysed in triplicates and compared statistically by multivariate ANOVA (IBM SPSS Statistics version 20).

5.2 Results & Discussions

5.2.1 Influence of process modifications on the nutritional value of *masmin*

5.2.1.1 Influence on proximate composition

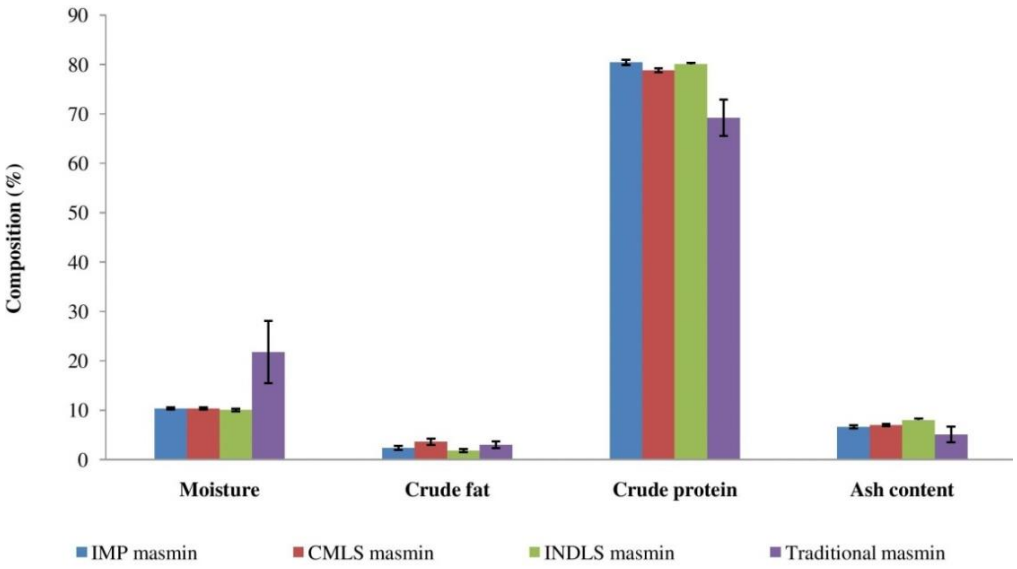

Figure 76 Comparative proximate composition of *masmin*

Comparative proximate composition of the *masmin* samples are given in Figure 76. Traditional *masmin* samples showed significantly higher moisture content (21.74±6.31%) compared to IMP (10.31±0.24%) and liquid smoked samples (10.05±0.27% for INDLS *masmin* and 10.36±0.23% for CMLS *masmin*) ($p<0.05$). Similar values for traditional and IMP *masmin* were reported by Yathavamurthi, Mumthaz, Jinu, Bindu, Suseela, and Gopal (2010) & Antony, Muraleedharan, and Mukundan (2003). Lack of standardized production protocols, insufficient drying, improper packing and storage of traditional *masmin* are expected to be the reasons behind the higher moisture content. Moisture content in IMP and liquid smoked samples were

homogenous in nature ($p>0.05$). Crude protein content in traditional *masmin* (69.19±3.67%) was significantly low when compared to other samples ($P<0.05$). The same is expected to be due to the relative variation in composition due to higher moisture content in traditional *masmin*. Crude protein content of IMP (80.44±0.51%), INDLS (80.08±0.27%) and CMLS (78.86±0.40%) *masmin* were homogenous in nature ($p>0.05$). Among the liquid smoked samples, CMLS samples had a higher fat content (3.61±0.64%) than INDLS *masmin* (1.82±0.35%) ($p<0.05$). Among all the samples, INDLS *masmin* showed the highest ash content (7.98±0.32%) ($p<0.05$), which is expected to be the result of higher salt deposition on the surface of loins during soaking in indigenous liquid smoke.

5.2.1.2 Influence on amino acid profile

Comparative amino acid profile of traditional *masmin*, IMP *masmin* and liquid smoked *masmin* is given in Table 27. In all the samples, glutamic acid was the amino acid found in highest concentration followed by aspartic acid. Amino acids found in least concentration were cysteine and arginine. Essential amino acids contributed 45% of the total amino acids in traditional *masmin*. Total essential amino acids in IMP *masmin* was 47% of the total amino acids. The same in CMLS and INDLS *masmin* was 49% and 50%, respectively. However, statistically significant difference was not observed between the samples in terms of total essential amino acids ($p>0.05$). This is in agreement with Yathavamurthi, Mumthaz, Jinu, Bindu, Suseela, and Gopal (2010), who while comparing the quality characteristics of IMP *masmin* and traditional *masmin* reported that even though minor variations in individual amino acids existed, total essential amino acid content was homogenous in nature.

In comparison to traditional *masmin*, IMP *masmin* showed significantly higher retention of lysine, histidine and proline ($p<0.05$). The longer cooking and smoking durations applied during production of traditional *masmin* is expected to be the reason for such loss in amino acids. Awuah, Ramaswamy, and Economides (2007) have reported that severe heating at high temperatures can bring about changes in amino acids like lysine, L-arginine and L-histidine. Lysine due to its highly reactive amino group is the most chemically modified amino acid. Tooley and Lowrie (1974) found about 25 % loss in lysine during thermal processing. Processing conditions can also influence sulfur containing amino acids which are not stable under heat processing (Donoso, Lewis, Miller, & Payne, 1962). Castrillon, Navarro, and Garcia-Arias (1996) observed losses of histidine, cysteine, threonine and leucine in canned tuna. Sikorski (2001) has reported loss of threonine in heat treated samples. Thermal degradation of tryptophan has been reported by Rakowska, Werner, Szkilladziowa, Nadolna, and Zielinska (1975) & Friedman and Cuq (1988). Sreenath and Ravishankar (2007) reported a comparison of lysine retention among heat processed samples. The results showed that samples processed at shortest time had better retention of lysine. Seet and Brown (1983) reported retention of 80-85% lysine in canned tuna processed at shorter duration of heating. Dvorak and Vognarova (1965) have also reported that short smoking time did not influence the lysine content.

Several authors have reported loss in the total lysine content during smoking (Chen & lssenberg, 1972; Hoffman, Barranco, Francis, & Disney, 1977). Major reason for the same is attributed to the denaturation at elevated temperature during smoking (Bhuiyan, Ackman, & Lall, 1986; Eyo, 2001; Adebowale, Dongo, Jayeola, & Orisajo, 2008) and due to the action of formaldehyde from smoke, which reacts with s-NH group of lysine to reduce

its availability (Carton, Bocker, Ofstad, Sorheim, & Kohler, 2009). Akintola, Brown, Bakare, Osowo, and Omolola (2013) have reported that availability of lysine can also be reduced due to maillard reaction taking place in smoked foods. Smoking time, smoke temperature, storage time and water activity during storage are the primary factors influencing lysine content (Maga, 1988). Akande, Oladosu, and Tobor (1998) have reported that lysine reduction is directly proportional to the temperature and duration of smoking. Di Cesare, Senesi, and Moioli (1980) have reported loss of lysine in smoked fillets of *Sardine pilchardus*. Similar results were also reported by Petrichenko and Dolzhenko (1981) in muscle proteins of Cyprinids and by Cattaneo, Balzaretti, Basilico, Beretta, and Cantoni (1983) in smoked *Salmo gardneri*. Losses up to 44% was reported in uncured beef smoked at 65°C for 10 hour whereas samples processed without smoking lost only 15% lysine (Chen & lssenberg, 1972). Clifford, Tang, and Eyo (1980) reported a 25% loss of available lysine on the surface and a 12% loss at the centre of hot smoked fish fillet.

Liquid smoked *masmin* showed higher retention of histidine, lysine, and tryptophan compared to traditional *masmin*. In addition to these commonly retained amino acids, INDLS *masmin* was found to have higher retention of cysteine, threonine and proline compared to IMP and traditional *masmin*. Significantly higher retention of arginine was found in the case of CMLS *masmin* compared to IMP *masmin*. No significant difference was observed between the four samples in terms of aspartic acid, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine ($p>0.05$).

Table 27 Amino acid profile (g/100 g protein) of traditional, IMP and liquid smoked *masmin*

Amino acid	Traditional *masmin*	IMP *masmin*	CMLS *masmin*	INDLS *masmin*
Aspartic acid	10.85 ±1.07[a]	11.14 ±1.15[a]	10.27 ±1.32[a]	10.17 ±1.52[a]
Threonine	4.97 ±0.47[a]	4.37 ±0.65[a]	5.17 ±0.32[ab]	6.54 ±0.60[b]
Serine	7.59 ±0.88[a]	7.31 ±0.91[a]	6.97 ±0.86[a]	6.41 ±0.65[a]
Glutamic acid	14.34 ±1.69[a]	14.68 ±1.32[a]	14.49 ±1.01[a]	13.41 ±1.54[a]
Proline	0.81 ±0.10[a]	0.96 ±0.16[ab]	0.71 ±0.09[a]	1.25 ±0.10[b]
Glycine	8.29 ±0.71[a]	7.94 ±0.94[a]	7.68 ±0.63[a]	7.14 ±0.55[a]
Alanine	9.82 ±1.20[a]	8.27 ±0.90[a]	8.15 ±1.15[a]	8.12 ±1.11[a]
Cysteine	0.67 ±0.08[ab]	0.52 ±0.06[a]	0.62 ±0.05[ab]	0.70 ±0.04[b]
Valine	7.95 ±0.74[a]	7.42 ±0.71[a]	7.08 ±0.68[a]	7.18 ±0.67[a]
Methionine	3.61 ±0.43[a]	2.95 ±0.30[a]	3.19 ±0.39[a]	3.08 ±0.32[a]
Isoleucine	5.62 ±0.49[a]	6.23 ±0.46[a]	6.38 ±0.66[a]	6.44 ±0.28[a]
Leucine	10.03 ±0.88[a]	9.87 ±1.13[a]	9.99 ±1.28[a]	9.17 ±0.97[a]
Tyrosine	1.43 ±0.12[a]	1.86 ±0.17[a]	1.71 ±0.14[a]	1.79 ±0.26[a]
phenylalanine	3.48 ±0.29[a]	3.79 ±0.32[a]	3.66 ±0.37[a]	3.80 ±0.46[a]
Histidine	6.45 ±0.55[a]	7.75 ±0.38[b]	8.61 ±0.54[b]	8.72 ±0.50[b]
Lysine	2.56 ±0.20[a]	3.72 ±0.24[b]	3.97 ±0.24[b]	4.68 ±0.29[c]
Arginine	0.71 ±0.07[c]	0.31 ±0.06[a]	0.53 ±0.05[b]	0.36 ±0.05[a]
Tryptophan	0.78 ±0.08[a]	0.87 ±0.07[ab]	0.92 ±0.12[ab]	1.07 ±0.13[b]

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

5.2.1.3 Influence on fatty acid profile

Comparative fatty acid profile of traditional and liquid smoked *masmin* is given in Table 28. All the samples were found to be rich in poly and mono unsaturated fatty acids. Among PUFA, Docosahexaenoic acid (DHA) and arachidonic acid was found to be present in higher concentration. Oleic acid was the monounsaturated fatty acid (MUFA) present in higher concentration. Palmitic acid and stearic acid were the saturated fatty acids (SFA) found in higher concentration. Similar results have been reported by Yathavamurthi, Mumthaz, Jinu, Bindu, Suseela, and Gopal (2010) in traditional and improved *masmin*; Stephen, Jeya Shakila, Jeyasekaran, and Sukumar (2010) in raw skipjack tuna; and by Gopakumar and Nair (1972), Bhuiyan, Ackman, and

Lall. (1986). Beltran and Moral (1990), Sanchez- Muniz, Viejo, and Medina (1992) & Bandarra, Batista, Nunes, Empis, and Christie (1997), in many marine fish species.

Among the individual fatty acids, palmitoleic acid and arachidonic acid showed significant difference among treatments ($P<0.05$). Significantly higher concentration of palmitoleic acid was found in IMP *masmin* and CMLS *masmin*. Higher concentration of arachidonic acid was found in INDLS *masmin*. However, such differences did not influence the Total SFA, Total MUFA and Total PUFA contents ($P>0.05$). Stephen, Jeya Shakila, Jeyasekaran, and Sukumar (2010) while investigating the effect of different heat processing (cooking, frying, canning and microwave heating) on chemical changes in tuna has also reported that the PUFA content in cooked samples were more or less same as that of their raw counterparts. In a comparative study between traditional *masmin* and IMP *masmin*, Yathavamurthi, Mumthaz, Jinu, Bindu, Suseela, and Gopal (2010) has found significantly higher retention of eicosapentaenoic acid (EPA) and DHA in IMP *masmin* compared to traditional *masmin*. However, such difference was not observed in the present study. This contrast in the data is expected to be due to the lack of standardized protocols for production of traditional *masmin* among different islands of Lakshadweep and due to the alterations in fatty acid content of traditional *masmin* during storage.

The major factors expected to influence the fatty acid composition of the samples in the present study were 1) cooking duration 2) treatments applied (smoking method and duration) and 3) drying process. Gallardo, Aubourg, and Perez-Martin (1989) observed an increase in PUFA and a decrease in SFA and MUFA content of albacore tuna during pre-cooking. Medina, Aubourg, and Perez-Martin (1995) also reported that pre-cooking and subsequent removal of exuded liquid can greatly increase the level of FFA in tuna meat. It can be noted that, unlike in pre-cooking operations for canning,

the cooking process followed in *masmin* production is by immersing the fish in boiling brine and sufficient time is given for cooling and stabilization by allowing the meat to remain in the same brine. Hence it can be presumed that, the loss of exuded water during cooking will be marginally compensated during the cooling period and as a result only minimal changes in fatty acid content was observed in *masmin*. Tanaka, Nagashima, and Taguchi (1985) while investigating the quality changes in canned mackerel found that, longer thermal processing time results in increased FFA formation. In traditional *masmin* preparation, cooking time inconsistently varies from 1-6 hrs. For production of IMP and liquid smoked *masmin*, the cooking time was invariably fixed as 90 min. However, this difference in cooking duration was not found to influence the fatty acid content in the sample ($p>0.05$).

The treatments applied (smoking method and duration) were not found to influence the fatty acid content in the sample ($p>0.05$). Venugopal (2006) reported that even though brining, smoking and refrigerated storage caused a reduction in total lipids, they were not found to influence the EPA and DHA content in seafood. Stołyhwo, Kolodziejska, and Sikorski (2006) while investigating the changes in PUFA content of raw and smoked Atlantic mackerel (*Scomber scombrus*) and Baltic sprats (*Sprattus sprattus*) observed no significant changes in the content of EPA and DHA. In the experiments of Cha, Park, Kim, Jeong, Chung, and Kim (2001), no significant changes in the concentration of EPA and DHA were found in stored seasoned-dried Pacific saury treated with liquid smoke. However, Beltran and Moral (1989) has reported that hot smoking of sardine fillets resulted in small, although statistically significant decreases in the contents of EPA (by 11.5%) and DHA (by 12.9%).

The alternate drying and smoking cycles employed in the production of traditional *masmin* and controlled drying practiced for the production of improved and liquid smoked *masmin* did not put forth any significant influence on the fatty acid content ($p>0.05$). According to Tabara, Ueki, Ito, Watanabe,

Kan, and Hiraoka (1998), drying of horse mackerel, frog flounder, Japanese whiting, hard clam, arkshell and scallop, under various conditions, did not induce significant changes in the contents of EPA and DHA.

Table 28 Fatty acid profile (% of total fatty acids) of traditional, IMP and liquid smoked *masmin*

Fatty acids		Traditional *masmin*	IMP *masmin*	CMLS *masmin*	INDLS *masmin*
Saturated fatty acids (SFA)					
C16:0	Palmitic acid	19.15 ±1.42[a]	18.61 ±1.68[a]	20.38 ±1.32[a]	19.71 ±1.53[a]
C18:0	Stearic acid	11.87 ±0.96[a]	12.79 ±1.31[a]	11.02 ±1.73[a]	13.93 ±1.37[a]
C17:0	Heptadecanoic acid	1.57 ±0.12[a]	1.87 ±0.94[a]	2.68 ±0.20[a]	1.78 ±0.41[a]
	Total SFA	32.59 ± 2.50[a]	33.27 ± 3.92[a]	34.08 ± 3.24[a]	35.41 ± 3.31[a]
Monounsaturated fatty acids (MUFA)					
C16:1	Palmitoleic acid	0.92 ±0.11[a]	1.67 ±0.19[c]	1.32 ±0.15b[c]	0.96 ±0.14[ab]
C18:1n9	Oleic acid	14.67 ±1.36[a]	13.28 ±1.13[a]	13.42 ±1.65[a]	14.12 ±1.42[a]
	Total MUFA	15.49 ± 1.47[a]	14.95 ± 1.33[a]	14.74 ± 1.80[a]	15.08 ± 1.56[a]
Polyunsaturated fatty acids (PUFA)					
C20:5n-3	EPA	3.79 ±0.27[a]	4.03 ±0.19[a]	3.94 ±0.34[a]	3.18 ±0.53[a]
C22:6n-3	DHA	38.21 ±4.38[a]	37.25 ±3.29[a]	36.65 ±2.24[a]	34.55 ±3.68[a]
C18:2n-6	Linoleic acid	3.53 ±0.42[a]	3.68 ±0.64[a]	4.03 ±0.58[a]	3.18 ±0.27[a]
C20:4	Arachidonic acid	6.09 ±0.39[a]	6.56 ±0.42[a]	6.28 ±0.24[a]	8.15 ±0.87[b]
	Total PUFA	51.62 ± 5.45[a]	51.52 ± 4.53[a]	50.89 ± 3.40[a]	49.06 ± 5.35[a]

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

5.2.2 Influence of process modifications on the nutritional value of *masmin* flakes

5.2.2.1 Influence on proximate composition

Graph comparing the proximate composition of smoked and liquid smoked *masmin* flakes is shown as Figure 77. CMLS samples showed significantly higher moisture (7.73±0.13 %) and lipid content (2.44±0.25 %) when compared to INDLS and smoked *masmin* flakes ($p<0.05$). Crude protein

content in smoked *masmin* flakes (83.09±0.70 %) and INDLS *masmin* flakes (83.73±0.58 %) was homogenous in nature (p>0.05) and was found to be significantly higher than CMLS samples. No significant difference was obtained between the treatments in terms of ash content (p<0.05).

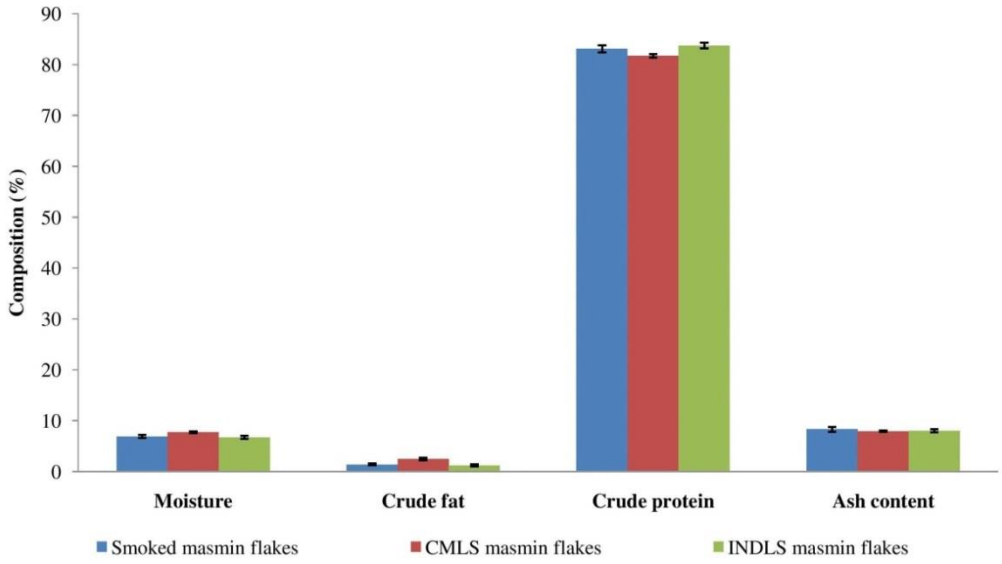

Figure 77 Comparative proximate composition of *masmin* flakes

5.2.2.2 Influence on amino acid profile

Amino acid profile of smoked and liquid smoked *masmin* flakes is shown in Table 29. As in the case of traditional *masmin* glutamic acid was the amino acid found in highest concentration in all the samples. Cysteine and arginine were the amino acids found in least concentration.

Significant difference was observed between smoked *masmin* flakes and CMLS samples in the case of lysine, tyrosine and tryptophan content (p<0.05). Among them, CMLS samples showed higher retention of lysine and tyrosine. Comparison of INDLS and smoked *masmin* flakes showed that they were homogenous in nature (P>0.05) except in the case of lysine, wherein

INDLS samples showed 40% higher retention (P<0.05). The major reason behind this difference between liquid smoked and smoked *masmin* flakes is expected to be the long-term direct exposure of smoke in the case of later. CMLS *masmin* flakes showed higher retention of proline and tyrosine compared to INDLS samples (p<0.05). Essential amino acids contributed 46 % of the total amino acids in CMLS and INDLS *masmin* flakes. In the case of smoked *masmin* flakes essential amino acid composition were 44 %. However, this difference was not statistically significant (P>0.05).

Table 29 Amino acid profile (g/100g protein) of smoked and liquid smoked *masmin* flakes

Amino acids	Smoked *masmin* flakes	CMLS *masmin* flakes	INDLS *masmin* flakes
Aspartic acid	11.38 ± 1.19[a]	11.41 ± 1.23[a]	11.57 ± 1.62[a]
Threonine	5.13 ± 0.41[a]	5.41 ± 0.75[a]	5.51 ± 0.78[a]
Serine	6.88 ± 0.94[a]	6.55 ± 0.51[a]	6.97 ± 0.74[a]
Glutamic acid	15.27 ± 1.45[a]	14.53 ± 1.83[a]	14.33 ± 2.04[a]
Proline	1.10 ± 0.19[ab]	1.27 ± 0.11[a]	0.91 ± 0.09[b]
Glycine	7.87 ± 0.92[a]	6.92 ± 0.61[a]	7.91 ± 0.78[a]
Alanine	10.35 ± 1.03[a]	9.67 ± 0.86[a]	9.82 ± 1.15[a]
Cysteine	0.87 ± 0.10[a]	0.66 ± 0.08[a]	0.78 ± 0.07[a]
Valine	7.96 ± 0.91[a]	8.09 ± 0.70[a]	8.17 ± 0.62[a]
Methionine	2.61 ± 0.28[a]	2.21 ± 0.18[a]	2.50 ± 0.36[a]
Isoleucine	5.17 ± 0.77[a]	5.48 ± 0.39[a]	4.94 ± 0.51[a]
Leucine	10.37 ± 1.40[a]	10.40 ± 0.95[a]	10.63 ±1.11[a]
Tyrosine	0.78 ± 0.10[b]	1.63 ± 0.17[a]	0.91 ± 0.15[b]
phenylalanine	3.51 ± 0.42[a]	4.06 ± 0.63[a]	3.63 ± 0.32[a]
Histidine	6.57 ± 0.77[a]	6.72 ± 0.50[a]	6.66 ± 0.82[a]
Lysine	1.81 ± 0.25[b]	2.43 ± 0.17[a]	2.55 ± 0.20[a]
Arginine	0.68 ± 0.09[a]	0.53 ± 0.07[a]	0.52 ± 0.05[a]
Tryptophan	1.25 ± 0.14[b]	1.66 ± 0.19[a]	1.40 ± 0.11[ab]

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means (p < 0.05). ND-Not detected

5.2.2.3 Influence on fatty acid profile

Comparative fatty acid profile of IMP and liquid smoked *masmin* powder is given in Table 30. No significant difference was obtained between the fatty acid content of smoked and CMLS *masmin* flakes ($p>0.05$). INDLS *masmin* flakes showed a significantly higher retention of stearic acid and lower retention of EPA compared to other two samples ($p<0.05$).

Table 30 Fatty acid profile (% of total fatty acids) of smoked and liquid smoked *masmin* flakes

Fatty acids		Smoked *masmin* flakes	CMLS *masmin* flakes	INDLS *masmin* flakes
Saturated fatty acids (SFA)				
C16:0	Palmitic acid	17.39 ± 1.10^{a}	19.44 ± 1.44^{a}	18.29 ± 2.09^{a}
C18:0	Stearic acid	11.55 ± 1.23^{a}	10.17 ± 1.08^{a}	13.37 ± 1.38^{b}
C17:0	Heptadecanoic acid	1.14 ± 0.14^{a}	1.56 ± 0.20^{a}	1.31 ± 0.34^{a}
	Total SFA	30.08 ± 2.48^{a}	31.17 ± 2.71^{a}	32.97 ± 3.81^{a}
Monounsaturated fatty acids (MUFA)				
C16:1	Palmitoleic acid	0.96 ± 0.12^{a}	1.27 ± 0.19^{a}	1.07 ± 0.15^{a}
C18:1n9	Oleic acid	16.78 ± 1.95^{a}	13.79 ± 1.36^{a}	14.59 ± 1.67^{a}
	Total MUFA	17.74 ± 2.07^{a}	15.06 ± 1.55^{a}	15.66 ± 1.82^{a}
Polyunsaturated fatty acids (PUFA)				
C20:5n-3	EPA	4.59 ± 0.59^{a}	5.76 ± 0.74^{a}	3.49 ± 0.68^{b}
C22:6n-3	DHA	35.29 ± 2.92^{a}	33.64 ± 3.29^{a}	34.94 ± 2.38^{a}
C18:2n-6	Linoleic acid	3.97 ± 0.48^{a}	4.99 ± 0.60^{a}	5.17 ± 0.87^{a}
C20:4	Arachidonic acid	8.27 ± 0.80^{a}	9.50 ± 0.72^{a}	7.68 ± 1.88^{a}
	Total PUFA	52.12 ± 4.79^{a}	53.88 ± 5.34^{a}	51.28 ± 5.81^{a}

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

5.2.3 Influence of process modifications on the nutritional value of *masmin* powder

5.2.3.1 Influence on proximate composition

Proximate composition of smoked and liquid smoked *masmin* powder is shown in Figure 78. No significant difference was observed between the treatments in terms of the parameters analysed ($p > 0.05$). Moisture content, the major variable in proximate composition of *masmin* based products were 6.59±0.44, 7.02±0.12, 6.60±0.27 % in IMP, CMLS and INDLS *masmin* powder, respectively.

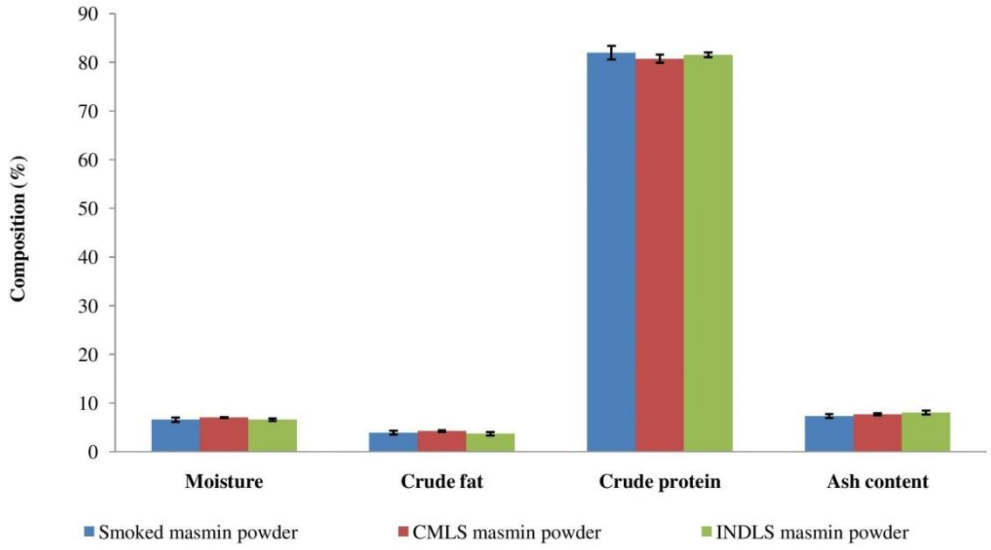

Figure 78 Comparative proximate composition of *masmin* powder

5.2.3.2 Influence on amino acid profile

Amino acid profile of liquid smoked and IMP *masmin* powder is given in Table 31. In comparison to IMP *masmin* powder, CMLS samples showed 55 % higher retention of lysine ($p<0.05$). Significant difference was observed between INDLS *masmin* powder and IMP samples in the content of tyrosine,

lysine and tryptophan ($p<0.05$). Tyrosine (52%) and lysine (80%) retention were higher in INDLS samples; IMP *masmin* powder showed higher retention of tryptophan. No significant difference was observed between the liquid smoked samples in terms of individual amino acids ($p>0.05$). Essential amino acids contributed 44% of the total amino acids in IMP *masmin* powder. In the case of CMLS and INDLS samples the essential amino acid composition was 43% each. No significant difference was observed between the samples in terms of essential amino acid content ($p>0.05$).

Table 31 Amino acid profile (g/100g protein) of IMP and liquid smoked *masmin* powder

Amino acid	IMP *masmin* powder	CMLS *masmin* powder	INDLS *masmin* powder
Aspartic acid	11.45 ± 1.09^{a}	11.30 ± 1.79^{a}	11.41 ± 2.04^{a}
Threonine	5.64 ± 0.61^{a}	5.51 ± 0.87^{a}	5.31 ± 0.77^{a}
Serine	7.12 ± 0.82^{a}	7.41 ± 0.29^{a}	7.54 ± 0.71^{a}
Glutamic acid	15.36 ± 2.21^{a}	15.22 ± 1.37^{a}	14.42 ± 1.48^{a}
Proline	0.98 ± 0.14^{a}	1.05 ± 0.26^{a}	1.28 ± 0.44^{a}
Glycine	7.26 ± 0.47^{a}	7.13 ± 0.76^{a}	7.35 ± 0.21^{a}
Alanine	9.16 ± 1.27^{a}	9.06 ± 1.63^{a}	8.72 ± 2.06^{a}
Cysteine	0.69 ± 0.05^{a}	0.78 ± 0.09^{a}	0.91 ± 0.77^{a}
Valine	7.95 ± 1.02^{a}	7.73 ± 0.83^{a}	7.43 ± 1.37^{a}
Methionine	3.08 ± 0.35^{a}	2.93 ± 0.39^{a}	3.17 ± 0.47^{a}
Isoleucine	5.51 ± 0.45^{a}	5.37 ± 0.74^{a}	5.46 ± 0.92^{a}
Leucine	10.38 ± 1.18^{a}	10.26 ± 1.43^{a}	10.45 ± 0.74^{a}
Tyrosine	0.63 ± 0.06^{a}	0.87 ± 0.12^{ab}	0.96 ± 0.16^{b}
phenylalanine	3.93 ± 0.55^{a}	3.74 ± 0.38^{a}	3.61 ± 0.68^{a}
Histidine	6.92 ± 0.96^{a}	6.76 ± 0.42^{a}	6.58 ± 0.52^{a}
Lysine	2.19 ± 0.21^{b}	3.41 ± 0.32^{a}	3.96 ± 0.57^{a}
Arginine	0.45 ± 0.14^{a}	0.42 ± 0.17^{a}	0.59 ± 0.28^{a}
Tryptophan	1.06 ± 0.07^{a}	0.97 ± 0.09^{ab}	0.82 ± 0.06^{b}

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

5.2.3.3 Influence on fatty acid profile

Comparative fatty acid profile of IMP and liquid smoked *masmin* powder is given in Table 32. Higher retention of palmitoleic acid was seen in CMLS *masmin* powder compared to IMP and INDLS samples ($p<0.05$). INDLS and IMP *masmin* powder showed significantly higher retention of arachidonic acid ($p<0.05$). However, no significant difference was observed between the treatments in terms of total SFA, total MUFA and total PUFA ($p>0.05$).

Table 32 Fatty acid profile (% of total fatty acids) of IMP and liquid smoked *masmin* powder

Fatty acids		IMP *masmin* powder	CMLS *masmin* powder	INDLS *masmin* powder
Saturated fatty acids (SFA)				
C16:0	Palmitic acid	16.37 ± 1.73[a]	18.41 ± 2.28[a]	18.14 ± 2.18[a]
C18:0	Stearic acid	12.13 ±1.67[a]	10.51 ± 1.25[a]	12.61 ± 1.19[a]
C17:0	Heptadecanoic acid	2.61 ± 0.66[a]	1.76 ± 0.97[a]	1.37 ± 0.38[a]
	Total SFA	31.11 ± 4.06[a]	30.68 ± 4.50[a]	32.12 ± 3.74[a]
Monounsaturated fatty acids (MUFA)				
C16:1	Palmitoleic acid	1.12 ± 0.38[b]	1.85 ± 0.27[a]	0.81 ± 0.13[b]
C18:1n9	Oleic acid	15.24 ± 1.18[a]	16.61 ± 1.42[a]	15.82 ± 2.45[a]
	Total MUFA	16.36 ± 1.56[a]	18.46 ± 1.69[a]	16.63 ± 2.58[a]
Polyunsaturated fatty acids (PUFA)				
C20:5n-3	EPA	3.14 ± 0.76[a]	3.79 ± 0.53[a]	4.03 ± 0.32[a]
C22:6n-3	DHA	36.88 ± 4.38[a]	37.15 ± 2.94[a]	35.38 ± 4.55[a]
C18:2n-6	Linoleic acid	4.65 ± 1.27[a]	3.43 ± 0.91[a]	3.62 ± 1.68[a]
C20:4	Arachidonic acid	7.37 ± 0.75[ab]	6.24 ± 0.45[a]	8.54 ± 0.37[b]
	Total PUFA	52.04 ± 7.16[a]	50.61 ± 4.83[a]	51.57 ± 6.92[a]

Different superscripts (a, b, c) in the same row indicate significant difference between treatment means ($p < 0.05$). ND-Not detected

5.3 Conclusion

Liquid smoking and improved smoking methods can positively influence the nutritional value of *masmin* by preserving essential nutrients. Wider variation in the proximate composition of traditional *masmin* was observed because of lack of standardized/uniform protocols for production. Longer cooking, smoking and drying time employed in production of *masmin* results in loss of amino acids like lysine, histidine, arginine and tryptophan. However such losses can be prevented by adoption of liquid smoking or improved smoking methods. Among the new methods, liquid smoking was found to have better retention of amino acids. It was observed that losses of palmitoleic acid and arachidonic acid traditional *masmin* can be prevented by adopting the new methods.

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Chapter

6 Sorption Isotherm Characteristics of Liquid Smoked *Masmin* and *Masmin* Based Products

Contents

Water sorption isotherms are curves describing the relation between water activity (a_w) of foods and their correspondent equilibrium moisture content (EMC) at a given temperature. Determination of sorption isotherm helps in understanding shelf-life & stability of foods, modelling of drying process, selection of packaging materials, optimization of processes parameters and predicting the influence of ingredients (Spiess & Wolf, 1983; Gal, 1987). Multitudinous studies have been conducted in determination of sorption isotherms for a variety of food stuffs viz. Beef (Salwin, 1959), beet root (Iglesias, Chirife & Viollaz, 1976), chicken (Labuza, 1984; Iglesias & Chirife, 1984), rice (Benado & Rizvi, 1985), Soup powder (Gopal, Thankamma, Shenoy, Rao, & Govindan, 1988) potato, carrot, tomato, green pepper, and onion slices (Kiranoudis, Maroulis, Tsami, & Marinos-Kouris, 1993). garlic (Madamba, Driscoll, & Buckle, 1996), cereal grains (Tolaba, Suarez, & Viollaz, 1997), sausages (Singh, Rao, Anjaneyulu, & Patil, 2001) cassava and melon seeds (Aviara & Ajibola, 2002) and starch powders (Al-Muhtaseb, McMinn, & Magee, 2004), corn starch (Guilan, Xiaoguang, Wenfu, & Xiujuan, 2007) tea powder and green tea granules (Sinija & Mishra, 2008).

However, very few such works have been reported in aquatic products, especially in smoked fishery products. Present study is aimed at determination of sorption isotherm for liquid smoked & improved *masmin* and *masmin* based products to spell out the optimum storage conditions and packaging requirements for the products.

Physicochemical properties of a monolayer (Low Density Polyethylene) and a laminate (Polyester/polyethylene) film commonly used in dry fish packaging (Gopal, 2007) was investigated to understand their efficacy in protecting the developed products.

6.1 Materials & Methods

6.1.1 Determination of sorption isotherm

The samples were powdered and sieved through standard test sieves. Sorption characteristics was studied at ambient temperature (28±2°C) by exposing weighed quantities of the sample to different relative humidity ranging from 8 to 97% (see Table 33) in desiccators using appropriate salt solutions (Rockland, 1960). The samples were periodically weighed till they attained constant weight. Equilibrium moisture content was calculated by the following equation and plotted against water activities calculated by dividing the respective relative humidity by a factor of 100. Water activity of a specific product was calculated by linear interpolating its moisture content to respective X axis value. Critical moisture was determined by the development of mould growth.

$$EMC\ (\%) = \frac{(Initial\ moisture\ content\ x\ wt.of\ the\ sample) \pm (Wt.gain\ or\ lose)}{Wt.of\ the\ sample\ \pm (Wt.gain\ or\ lose)}\ x\ 100$$

Table 33 Saturated salt solutions used for the study and respective relative humidity

Salt solutions	Relative Humidity (%)
Potassium hydroxide	8
Potassium acetate	20
Magnesium chloride	32
Sodium dichromate	53
Sodium nitrite	64
Sodium chloride	75
Ammonium sulphate	80
Potassium sulphate	97

6.1.2 Packing materials used for the study

90 µ Low Density Polyethylene (PE) and a laminate of 12 µ Polyester and 300 gauge Polyethylene (PEST/PE) was used for the study

6.1.3 Evaluation of physicochemical properties of packaging materials

6.1.3.1 Determination of tensile strength & elongation at break

Tensile strength is defined as the force parallel to the plane of a specimen required to produce failure in the specimen of specified width and length under specified condition of loading. A Universal Testing Machine (Lloyd instruments LRX plus, UK) (Plate 25) fitted with 500 Newton load cell was used for performing the test as per the method specified by IS:2508 (1984). Sealed specimen were cut into suitable size (15 mm width x 50 mm length) in both machine and cross direction and clamped between the jaws of the testing machine. Test was performed at a pre-adjusted speed of 500 mm/min. Values in

both the machine and cross direction was recorded from minimum of 10 readings and the results are expressed as kg/cm^2. Elongation at break is expressed as percentage of the original length between the reference lines.

Plate 25 Universal testing machine (Lloyd instruments LRX plus, UK)

6.1.3.2 Determination of heat seal strength

Heat seal strength was determined by measuring the force required to pull apart the pieces of a film which have been sealed together. A Universal Testing Machine (Lloyd instruments LRX plus, UK) fitted with 500 Newton load cell was used for performing the test. Method prescribed by ASTM (1973) was used for carrying out the test. Sealed specimen were cut into suitable size (15 mm width x 50 mm length) and clamped between the jaws of the testing machine in such a way that the seal area is located equidistant between the clamps. Test was performed at a pre-adjusted speed of 500 mm/ min. Maximum stress applied to the specimen at yield or breakage was noted as heat seal strength. Values in both the machine and cross direction were

recorded from minimum of 10 readings and the results are expressed as kg/cm^2.

6.1.3.3 Determination of Oxygen Transmission Rate (OTR) (Method: ASTM F 2622-08)

Lyssy OPT-5000 (PBI Dansensor A/S, Ringsted, Denmark) (Plate 26) equipped with a ceramic solid-state oxide sensor was used for measuring oxygen transmission rate. Samples were affixed to a sample holding card with a testing area of 42 cm, which when inserted into the testing area, separates the same in to upper and lower chamber. The lower chamber was continually flushed with the carrier gas (nitrogen, 70.0 cm/min), whereas the upper chamber was flushed with the test gas (oxygen, 70.0 cm/min). This led to a steady-state condition across the sample. Due to the sample's permeability, the lower chamber (gas with high nitrogen content) would be gradually enriched with oxygen from the upper chamber. Oxygen transmission rate was calculated by continuously measuring the increase in oxygen concentration in the lower chamber. Measurements were performed at a constant temperature (23.0±0.5°C). Test results are expressed in $ml/m^2/day$.

Plate 26 Lyssy OPT-5000-Oxygen permeability tester (PBI Dansensor A/S, Ringsted, Denmark)

6.1.3.4 Determination of Water Vapour Transmission Rate (WVTR) (Method: ASTM E 398-03)

Lyssy L80-5000 (PBI Dansensor A/S, Ringsted, Denmark) (Plate 27) was used for determining water vapour transmission rate. The test samples were affixed to a self-adhesive sample card, with an effective film test area of 42 cm and inserted in to the test chamber. Test was performed at 23.0±0.5°C, with 100% RH for the wet chamber of the instrument and 10% for the dry one, yielding to a driving force of 90% RH. Test results are expressed in $g/m^2/day$.

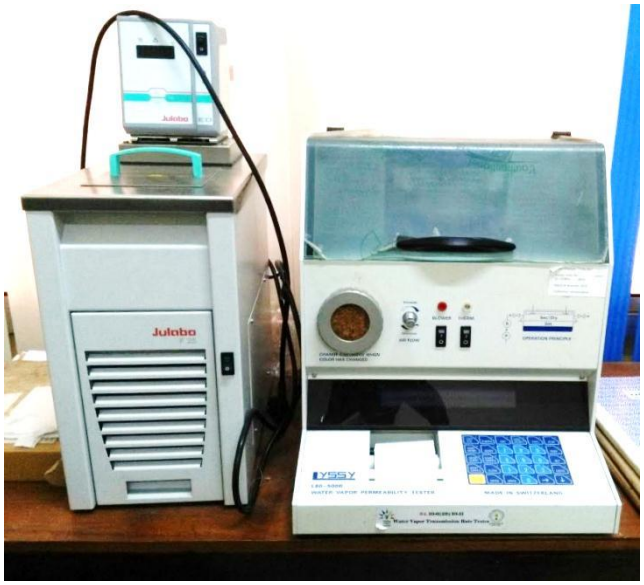

Plate 27 Lyssy L80-5000- Water vapour permeability tester (PBI Dansensor A/S, Ringsted, Denmark)

6.1.4 Statistical analysis

Samples were analysed in triplicates and compared statistically by multivariate ANOVA (IBM SPSS Statistics version 20).

6.2 Results & Discussions

6.2.1 Sorption characteristics of *masmin*

Sorption isotherm of liquid smoked and IMP *masmin* are given in Figure 79 to Figure 81. Moisture content, resulting water activity and critical moisture content of the samples are given in Table 34. Humidity had a significant effect on the sorption characteristics of the sample ($p<0.05$). Initial moisture content in all the three samples were homogenous in nature ($p>0.05$).

However, INDLS *masmin* showed a significantly lower water activity (0.62 ±0.0054) compared to other two samples ($p<0.05$). This is expected to be due to higher retention of salt in the surface layers of INDLS *masmin* as the same is produced by soaking cooked loins in 5% salted liquid smoke. This might have resulted in varied sorption behavior of the samples and hence resulted in lower water activity. Water activity in CMLS and IMP *masmin* were 0.64 ±0.0052 and 0.65 ±0.0048, respectively. These values are in compliance with the maximum water activity of 0.78 allowed in salted dried fish (FSSAI 2016a). Antony, Muraleedharan, & Mukundan (2003) have reported a moisture content of 11.33% in IMP *masmin* which was equivalent to a water activity of 0.55. Another study has reported moisture content of 9.39 % in IMP *masmin* (Yathavamurthi, Mumthaz, Jinu, Bindu, Suseela, & Gopal, 2010). Equilibrium moisture content of 30-34% was critical in *masmin* samples with respect to mould growth.

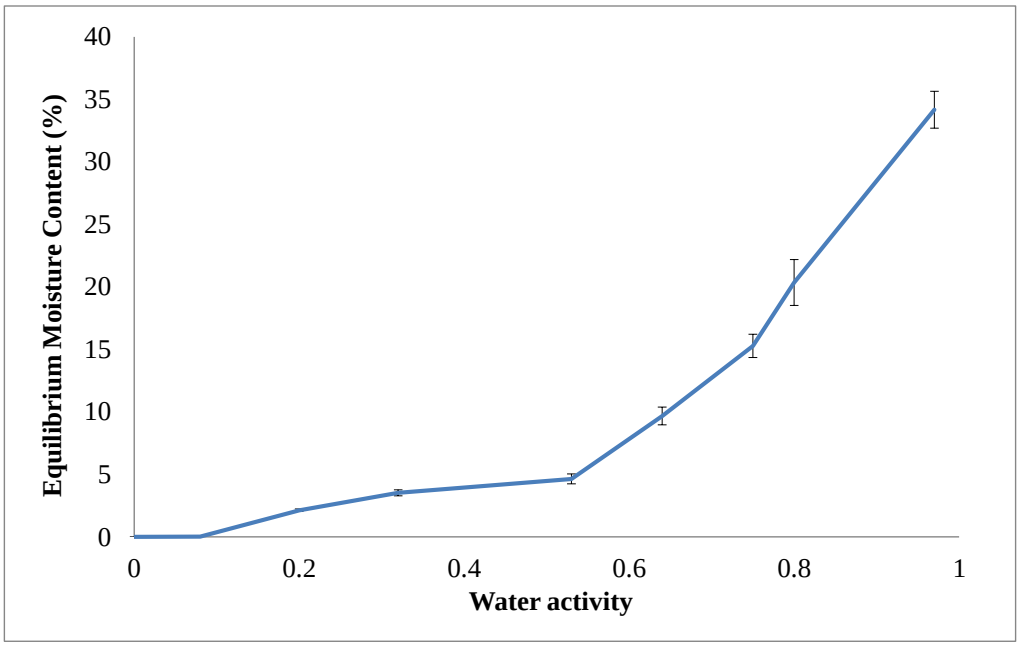

Figure 79 Sorption isotherm for IMP *masmin*

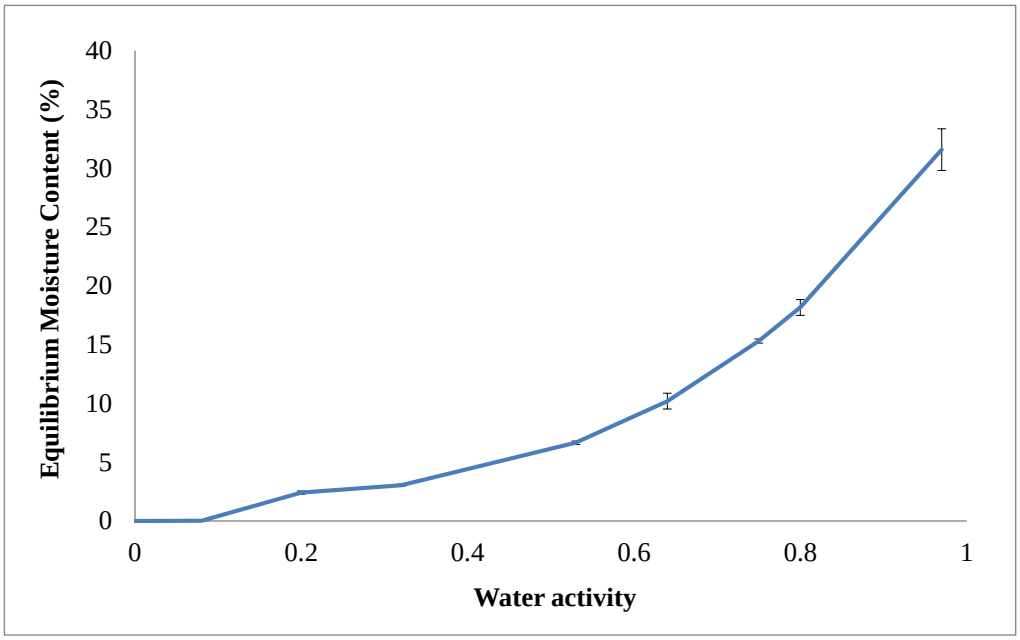

Figure 80 Sorption isotherm for CMLS *masmin*

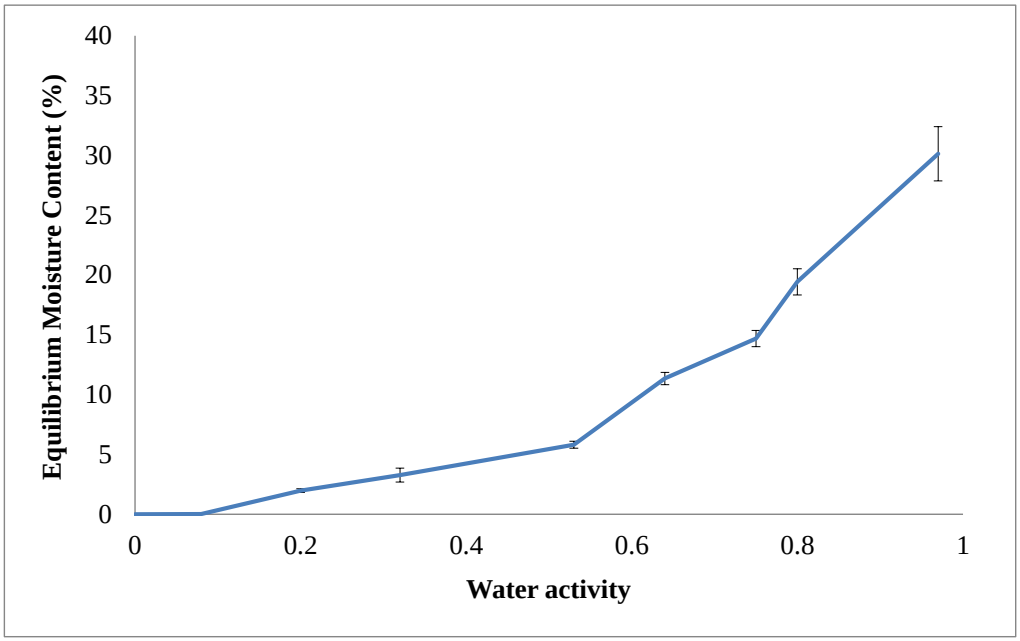

Figure 81 Sorption isotherm for INDLS *masmin*

Table 34 Moisture content, water activity and critical moisture of IMP and liquid smoked *masmin*

Sample	Moisture content (%)	Water activity	Critical moisture (%) at 97% RH
IMP *Masmin*	10.31 ±0.24[a]	0.65 ±0.0048[b]	34.17 ±1.48[a]
CMLS *masmin*	10.36 ±0.23[a]	0.64 ±0.0052[b]	31.59 ±1.76[a]
INDLS *masmin*	10.46 ±0.27[a]	0.62 ±0.0054[a]	30.14 ±2.27[a]

Different superscripts (a, b, c) in the same column indicate significant difference between treatments means ($p < 0.05$).

6.2.2 Sorption characteristics of *masmin* flakes

Sorption characteristics of liquid smoked and smoked *masmin* flakes are given in Figure 82 to Figure 84. Initial moisture content, resulting water activity and critical moisture content of the samples is given in Table 35. Liquid smoke treatment did not show any significant influence on water activity and critical moisture content. However CMLS *masmin* flakes samples showed significantly higher moisture content than other treatments ($p<0.05$). Moisture content between 38-40% was critical in *masmin* flake samples with respect to mould growth.

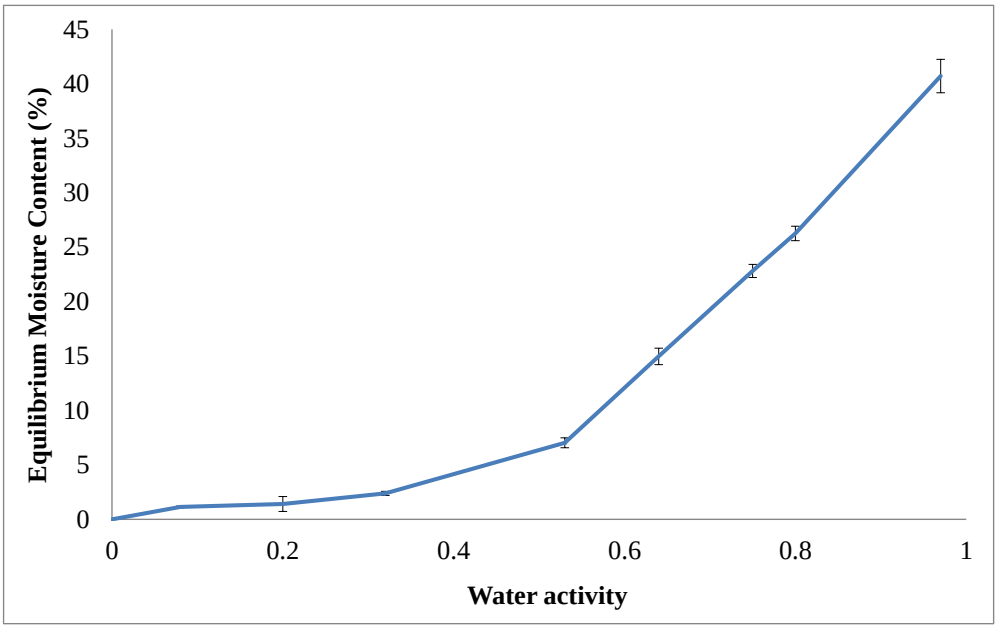

Figure 82 Sorption isotherm for smoked *masmin* flakes

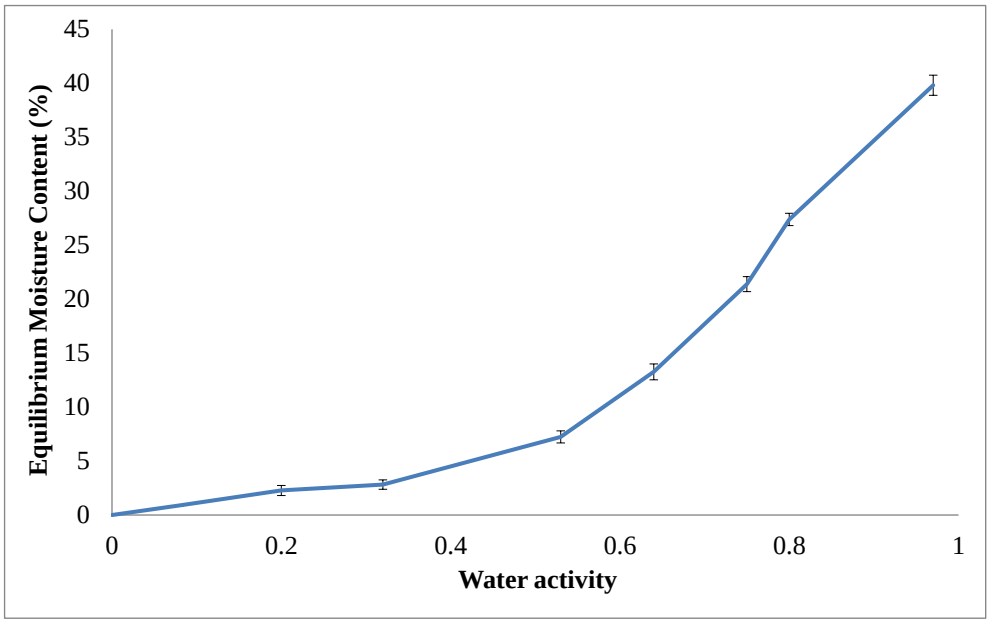

Figure 83 Sorption isotherm for CMLS *masmin* flakes

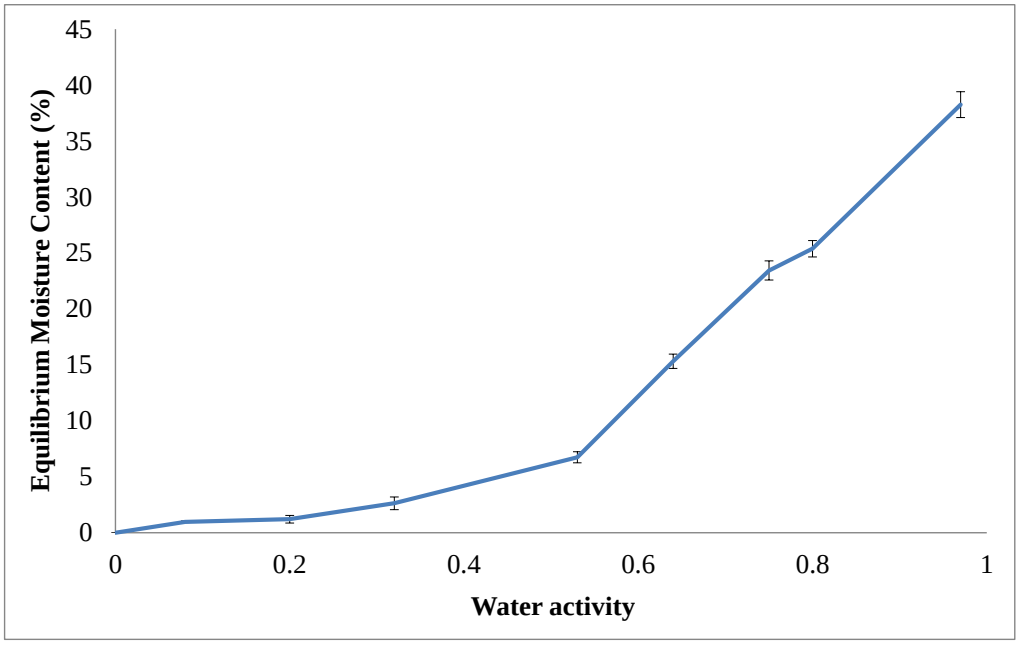

Figure 84 Sorption isotherm for INDLS *masmin* flakes

Table 35 Moisture content, water activity and critical moisture of smoked and liquid smoked *masmin* flakes

Samples	Moisture content (%)	Water activity	Critical moisture (%) at 97% RH
Smoked *masmin* flakes	6.92 ± 0.28[a]	0.52 ± 0.0115[a]	40.71 ±1.54[a]
CMLS *masmin* flakes	7.73 ± 0.12[b]	0.54 ± 0.0030[a]	39.82 ±0.94[a]
INDLS *masmin* flakes	6.73 ± 0.31[a]	0.53 ± 0.0091[a]	38.25 ±1.15[a]

Different superscripts (a, b, c) in the same column indicate significant difference between treatments means ($p < 0.05$)

6.2.3 Sorption characteristics of *masmin* powder

Sorption characteristics of liquid smoked and IMP *masmin* powder is given in Figure 85 to Figure 87. Initial moisture content, resulting water activity and critical moisture content of the same is given in Table 36. No significant difference was observed between the treatments in terms of moisture content and critical moisture content ($p<0.05$). However INDLS *masmin* powder showed

significantly lower water activity compared to other treatments. Moisture content of 39-41% aroused fungal growth in samples.

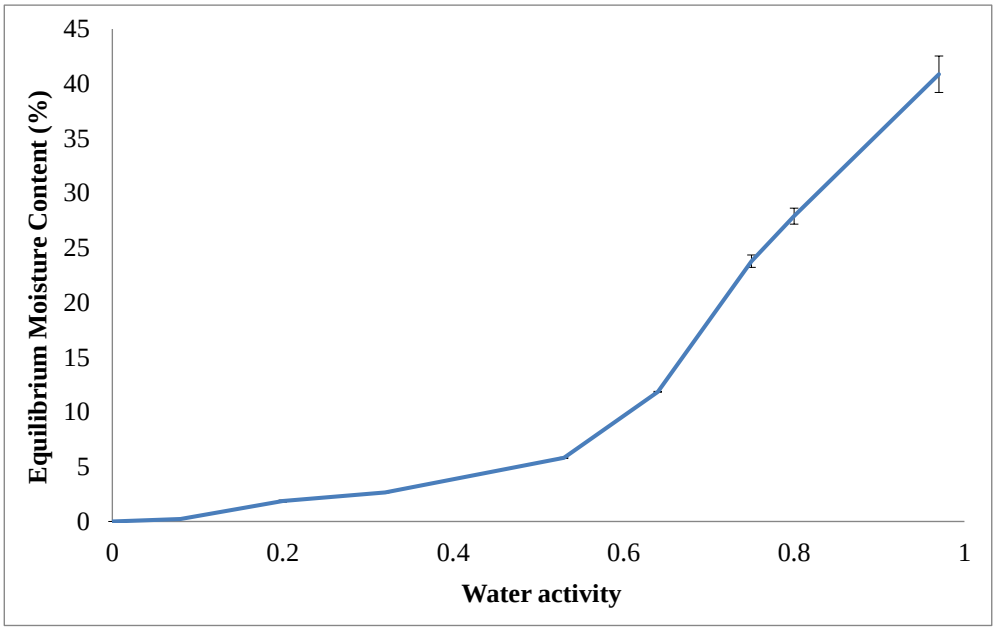

Figure 85 Sorption isotherm for IMP *masmin* powder

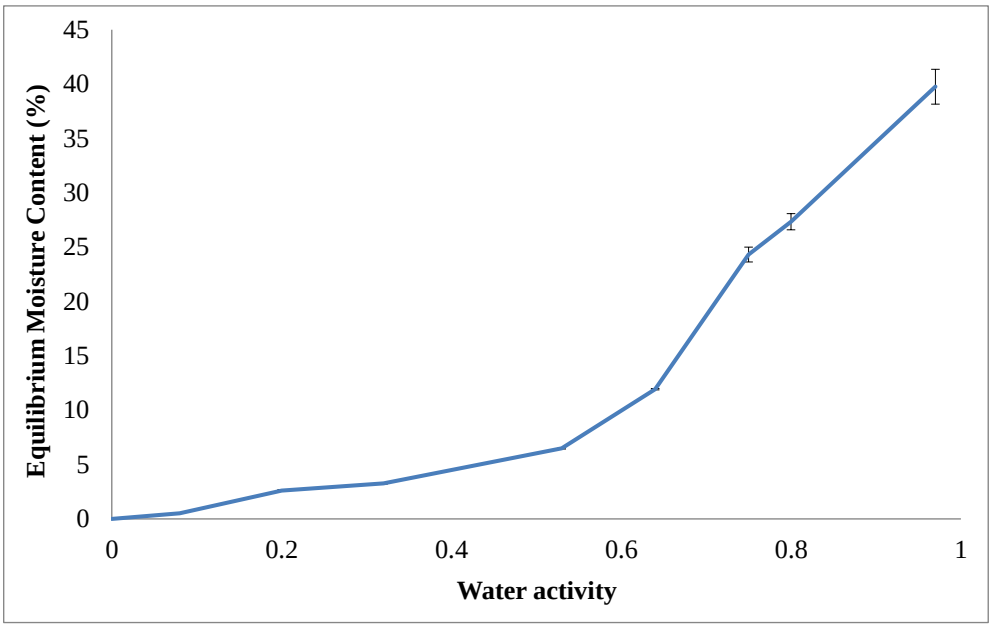

Figure 86 Sorption isotherm for CMLS *masmin* powder

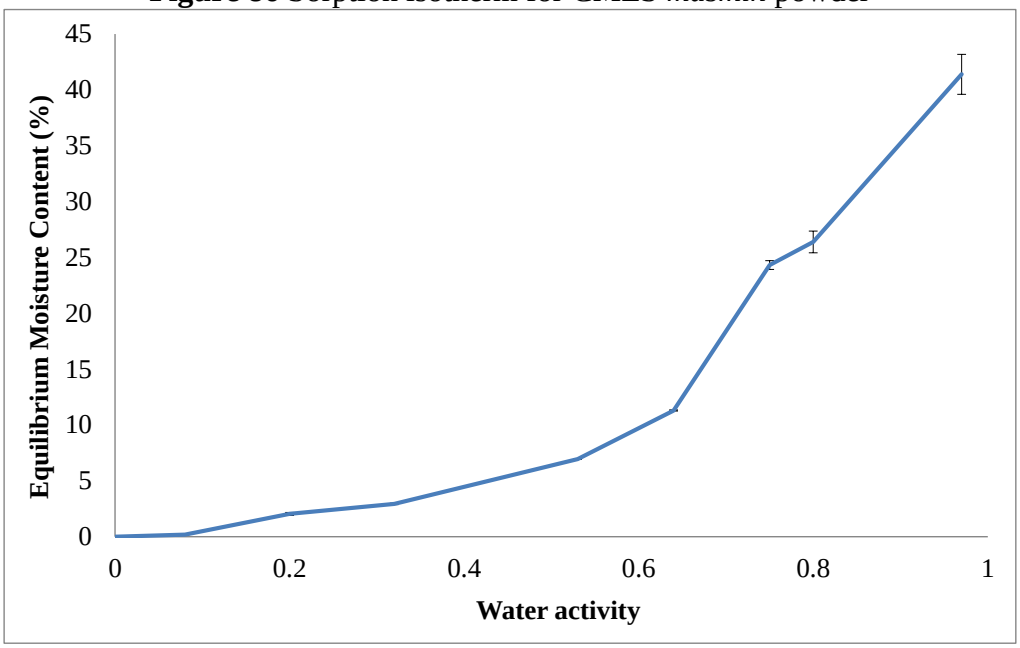

Figure 87 Sorption isotherm for INDLS *masmin* powder

Table 36 Moisture content, water activity and critical moisture of IMP and liquid smoked *masmin* powder

Samples	Moisture content (%)	Water activity	Critical moisture (%) at 97% RH
IMP *masmin* powder	6.59 ±0.44[a]	0.55 ±0.0022[b]	40.87 ±1.66[a]
CMLS *masmin* powder	7.02 ±0.12[a]	0.54 ±0.0025[b]	39.76 ±1.60[a]
INDLS *masmin* powder	6.59 ±0.27[a]	0.51 ±0.0182[a]	41.40 ±1.79[a]

Different superscripts (a, b, c) in the same column indicate significant difference between treatments means ($p<0.05$).

6.2.4 Physicochemical properties of the packaging materials

Inertness, leak proofness, impermeability to oxygen and moisture and low transparency are the major desirable properties for a packing material to be used in dry fish packaging (Gopakumar, 1993). Packaging employed in dry fish industry in India is highly unsatisfactory leaving much to be improved from scientific and hygienic point of view. High density polythene woven

gusseted bags laminated with 100 gauge low density polythene has been recommended for bulk packaging of dry fish (Gopal, 2007). Polyethylene and polypropylene are the most commonly used mono layer films in dry fish packaging. They are cheap, readily available and have good tearing and bursting strength. However they are prone to puncture or damage and have low water vapour, odour and oxygen permeability (Bindu, 2005). Laminates of polyester and polythene is efficient in mitigating these shortfalls of monolayer films and are being successfully used in dry fish industry (Gopal, Nair, Kandoran, Prabhu, & Gopakumar, 1998).

Physiochemical properties of the packaging materials are given in Table 37. PE and PEST/PE films used had a thickness of 360 and 350 gauge respectively. Both the packing materials showed significant difference in terms of the parameters analysed ($p<0.05$). PEST/ PE showed higher seal strength, tensile strength and superior moisture and oxygen barrier properties compared to PE.

Table 37 Physicochemical properties of the packaging materials

Parameter analysed	PE	PEST/ PE
Tensile strength (kg/cm^2)	174.16 ± 0.39 (MD*)[a]	417.11± 0.42 (MD)[b]
	122.86 ± 0.73 (CD*)[a]	327.75 ± 0.65 (CD)[b]
Heat seal strength(kg/cm^2)	105.76 ± 0.92 (MD)[a]	393.23 ± 0.57 (MD)[b]
	95.00 ± 0.67 (CD)[a]	306.34 ± 0.71 (CD)[b]
Elongation at break (%)	716.00 ± 1.65 (MD)[a]	832.72 ± 0.84 (MD)[b]
	965.46 ± 0.94 (CD)[a]	848.48 ± 1.24 (CD)[b]
OTR (ml/m^2/day at 1 atm.)	1931 ± 0.28[a]	116 ± 0.33[b]
WVTR (g/m^2/day)	1.95 ± 0.047[a]	1.51 ± 0.021[b]

Different superscripts (a, b, c) in the same column indicate significant difference between treatments means ($p < 0.05$).
*MD=Machine direction, CD=Cross direction

6.3 Conclusion

Sorption analysis of the developed product showed a sigmoid curve which belong to Type II isotherm as classified by Brunauer, Emmett, and Teller (1938) & Bell and Labuza (2000). This suggests that they are very much sensitive to changes in humidity and require efficient packaging for long term storage. All the samples showed a steep increase in moisture absorption above RH of 53%. Water activity and critical moisture content in *masmin* powder and *masmin* flakes were homogenous in nature ($p>0.05$). However, *masmin* samples showed higher water activity and lower critical moisture content compared to other two samples. Evaluation of physicochemical parameter of the selected packing materials showed that both of them are effective in protecting the products from exposure to undesirable factors. However their true effectiveness can only be affirmed only after a detailed shelf life study.

Chapter

7 Shelf Life Evaluation of Liquid Smoked *Masmin* and *Masmin* Based Products

Contents

Shelf life of the products in two different packaging materials; 90 µ Low Density Polyethylene (PE) and laminate of 12 µ Polyester and 300 gauge Polyethylene (PEST/PE) was investigated. The products were monitored for a maximum period of twelve months; microbiological evaluation was carried out at irregular intervals after the initial six months of storage. Since the production and marketing of traditional *masmin* is a largely decentralized practice, getting freshly prepared *masmin* to be used as appropriate control for the study was not possible. Therefore, IMP *masmin* and IMP *masmin* powder was used as the control for liquid smoked *masmin* and *masmin* powder, respectively. Smoked *masmin* flakes were used as control for liquid smoked *masmin* flakes. The parameters analysed were total volatile base nitrogen, tri-methyl amine-nitrogen, thiobarbituric acid value, overall acceptability and yeast & mould count.

7.1 Materials & Methods

7.1.1 Determination of volatile base nitrogen compounds

Analysis was performed according to the method prescribed by Conway (1950). About 10 g of homogenized sample was extracted with 10% trichloroacetic acid (TCA) by grinding in a mortar; the contents were filtered

in to a 100 ml standard flask through Whatman grade-1 filter paper. Filter paper was thoroughly washed with TCA and the filtrate made up to the volume. This extract was used to measure total volatile base nitrogen and tri-methyl amine-nitrogen in the sample.

7.1.1.1 Determination of total volatile base nitrogen (TVBN)

1 ml of standard 0.01 N sulphuric acid was taken in the inner chamber of a Conway's micro diffusion unit. To the outer chamber 1 ml of TCA extract was added followed by 1 ml of saturated potassium carbonate. The unit was then sealed with glass lid by applying vacuum grease. Gently rotated the unit to mix the solutions in the outer chamber and kept undisturbed overnight. Amount of unreacted acid in the inner chamber was determined by titrating against standard 0.01 N sodium hydroxide with Tashiro's indicator. A blank was also run using 1 ml of 10% TCA instead of TCA extract in the outer chamber. TVBN as mg/100g of the sample was calculated by the following equation.

$$TVBN\ (mg/100g\ of\ sample) = \frac{0.01\ x\ 14\ x\ (blank - test)\ x\ 100\ x\ 100}{Vol.of\ pipetted\ sample\ x\ Wt.of\ the\ sample}$$

7.1.1.2 Determination of Tri-methyl amine-nitrogen (TMA-N)

1 ml of standard 0.01 N sulphuric acid was taken in the inner chamber of a Conway's micro diffusion unit. To the outer chamber 1 ml of TCA extract was added followed by 1 ml neutralized formaldehyde. The unit was kept undisturbed for 3 min to ensure the binding of formaldehyde with all the primary and secondary amines and ammonia contained in the extract. 1 ml saturated potassium carbonate was added to the outer chamber and the unit was sealed with glass lid by applying vacuum grease. Gently rotated the unit to mix the solutions in the outer chamber and kept undisturbed overnight. The

analysis was further carried out as explained in determination of TVBN. TMA-N was calculated and expressed as mg/100g of the sample.

7.1.2 Determination of Thiobarbituric acid (TBA) value

Method prescribed by Tarladgis, Watts, Younathan, & Dugan, (1960) was used for the analysis. About 10g of homogenized sample was mixed with 100 ml 0.2 N HCl and homogenized to make slurry. The slurry was poured into a round bottom flask and connected to the TBA distillation apparatus. Distillation was done until 50 ml of the distillate was collected within 10 minutes. 5 ml of the distillate was taken in a test tube; 5 ml TBA reagent (0.288 g TBA reagent in 100 ml acetic acid and heated gently to dissolve) was added and heated for 35 min in a water bath. A blank was also run with distilled water. Colour developed was measured in a spectrophotometer at 538 nm and TBA value was calculated by multiplying the optical density with a value of 7.8 and expressed as mg malonaldehyde/kg of sample.

7.1.3 Evaluation of overall acceptability

Sensory analysis of the products was carried out by a five member expert panel. The panel consisted of individuals who were familiar with the use of *masmin* and *masmin* based products and experts in the field of smoked foods. The panel evaluated the samples using following 9-point hedonic scale (Meilgaard, Civille, & Carr, 1999). Score of 9 in the scale denoted quality description “likes extremely” and 1 denoted “dislikes extremely”. A score of 4 was considered as the margin of acceptance. Samples were provided to the panelist in coded plates and were asked to score for appearance, colour, odour, flavour, taste and mouth feel of the samples. For the convenience of the panel, *masmin* samples were made in to thin flakes and presented along with a whole piece (to evaluate appearance and colour). Overall acceptability was calculated by taking the average of scores obtained for each attribute.

SENSORY EVALUATION SCORE CARD FOR SHELF LIFE ANALYSIS OF PRODUCTS

Assessor: …… Date: ……

(Please score the sample characteristics by placing the relevant score)
An honest expression of your personal feeling will help us.

ATTRIBUTES	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
Appearance				
Colour				
Odour				
Flavour				
Taste				
Mouth feel				
Overall acceptability				

Please score the sample characteristics according to the following scale

QUALITY GRADE DESCRIPTION	SCORE
Like extremely	09
Like very much	08
Like moderately	07
Like slightly	06
Neither likes nor dislikes	05
Dislike slightly	04
Dislike moderately	03
Dislike very much	02
Dislike extremely	01

Comments:

Signature:

7.1.4 Yeast & mould analysis

Analysis was performed according to AOAC (2012). Twenty five gram of sample was drawn aseptically and homogenized with 225 ml 0.1 % peptone water in a filter stomacher bag using a Stomacher (400 Circulator, Seward Limited, UK) for 2 min. The homogenized sample was serially diluted using sterile 0.1% peptone water. Duplicates of three consecutive homogenate dilutions were plated on 3M™ Petrifilm™ Yeast & Mould Count Plates, incubated for 48±2 hrs at 25±2°C. Average counts were calculated and expressed as decimal logarithm of Log_{10} cfu/g of the sample. Limit of 100 cfu/g, under 2-class sampling fixed by FSSAI (2016b) was taken as the microbial rejection criteria.

7.1.5 Statistical analysis

Samples were analysed in triplicates and compared statistically by multivariate ANOVA (IBM SPSS Statistics version 20).

7.2 Results & Discussions

7.2.1 Shelf life evaluation of liquid smoked *masmin*

7.2.1.1 Total Volatile Base Nitrogen (TVBN)

Changes in TVBN content of liquid smoked and IMP *masmin* during storage is given in Figure 88 to Figure 90. Significant statistical difference was observed between the treatments and packing materials used in terms of TVBN content ($p<0.05$). It was observed that the TVBN values for all the treatments were homogenous during the initial days of storage ($p>0.05$). Gradual increase in TVBN during further storage is expected to be due to the

microbial catabolism of amino acids in fish muscle resulting in the accumulation of ammonia and other volatile bases (Fraser & Sumar, 1998).

Leroi, Joffraud, Chevalier, and Cardinal (2001) have reported high correlation between shelf life, lactobacilli count and yeast count with TVBN concentration. Highest increase in TVBN content was recorded in IMP *masmin* packed in PE, wherein the value increased from 34.37±0.70 to 68.35±0.66. CMLS *masmin* packed in PEST/PE showed the least changes in TVBN content. Among liquid smoked samples INDLS *masmin* showed higher increase in TVBN content.

The importance of phenolic compounds as antimicrobial (Davidson & Branden, 1981; Kivanc, Akgul & Dogan, 1991) and antioxidative agents (Toth, 1982; Wittkowski, 1985) in foods has been well documented. Higher antimicrobial properties exhibited by the commercial liquid smoke are expected to be due to the presence of some undisclosed additives. In a related study, we found that the same commercial liquid smoke at 0.2 % concentration was effective in retarding the microbial growth and TVBN formation in tuna sausage (Nithin, Ananthanarayanan, Yathavamoorthi, Bindu, Joshy, & Gopal, 2015). Milly, Toledo, and Ramakrishnan (2005) reported that nine different commercial liquid smoke fractions have shown antimicrobial properties against a variety of bacteria, yeast and moulds. Sunen, Fernandez-Galian, and Aristimuno (2001) reported that certain liquid smoke flavourings were effective in retarding the growth of *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Listeria monocytogenes*. Guaiacol, one of the smoke derived phenols has shown antibacterial activity against *Bacillus subtilis* (Hefang *et al*., 2011). Carbonyl compounds such as formaldehyde and acrolein in smoke condensates are also known to exhibit antibacterial properties by penetrating the cell wall and subsequent inactivation of enzymes

on the cytoplasmic membrane and cytoplasm or through interfering with the nutrient uptake (Painter, 1998).

Connell (1990) has suggested a limit of 100-200 mg TVBN /100 g of the sample in salted dried fish. None of the samples in the present study exceeded this value. Vijayan and Surendran (2012), while analysing the quality characteristics of dried fish marketed in north eastern states of India has reported TVBN values in the range of 49 (in unsalted and dried catfish) to 427 mg% (in salted and dried mackerel). TVBN values as high as 200±53 mg TVBN/100 g has been reported in traditional *masmin* (David, Rajagopalasamy, & Sugumar, 1990). The major reason behind such higher values and variability is inferred to be due to the lack of hygienic handling practices during the production, storage and marketing.

Researches also suggest that the heat treatment applied on a product can proportionately influence the TVBN values due to the breakdown of tri-methyl amine oxides at elevated temperatures (Gallardo, Perez-Martın, Franco, Aubourg, & Sotelo, 1990). For instance, during traditional *masmin* production, the cooking time usually extends even up to six hours. In the case of improved and liquid smoked *masmin*, the cooking duration does not extend beyond 90 min.

In all the three treatments, *masmin* samples packed in PEST/PE showed lower TVBN values compared to the same packed in PE. The reason for the same is expected to be due to the good barrier properties of the laminate (Gopal, 2007).

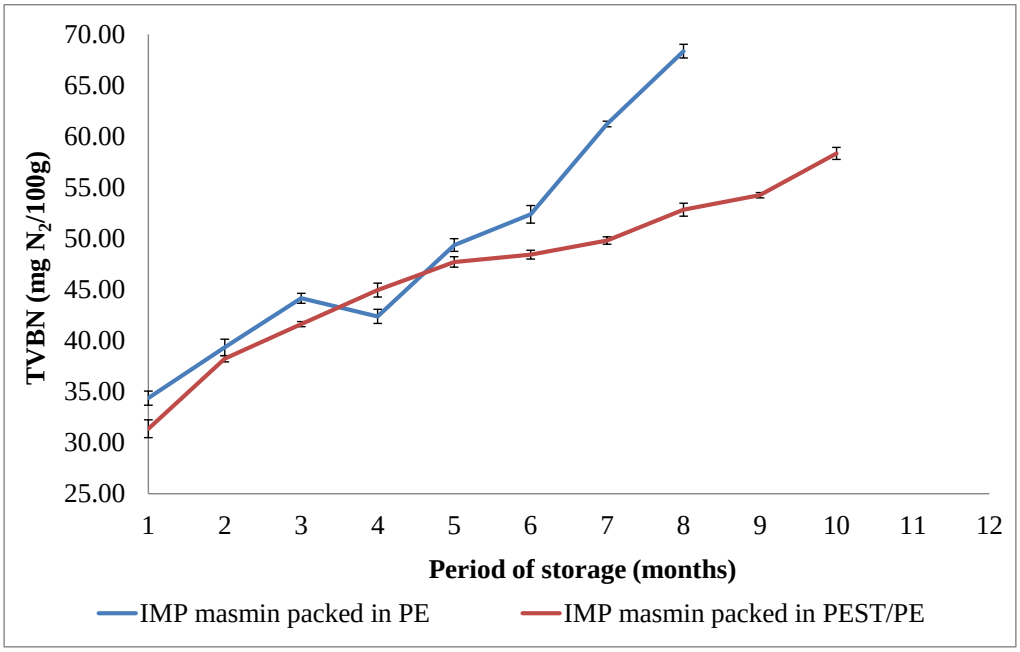

Figure 88 Changes in TVBN content of IMP *masmin* packed in PE and PEST/PE

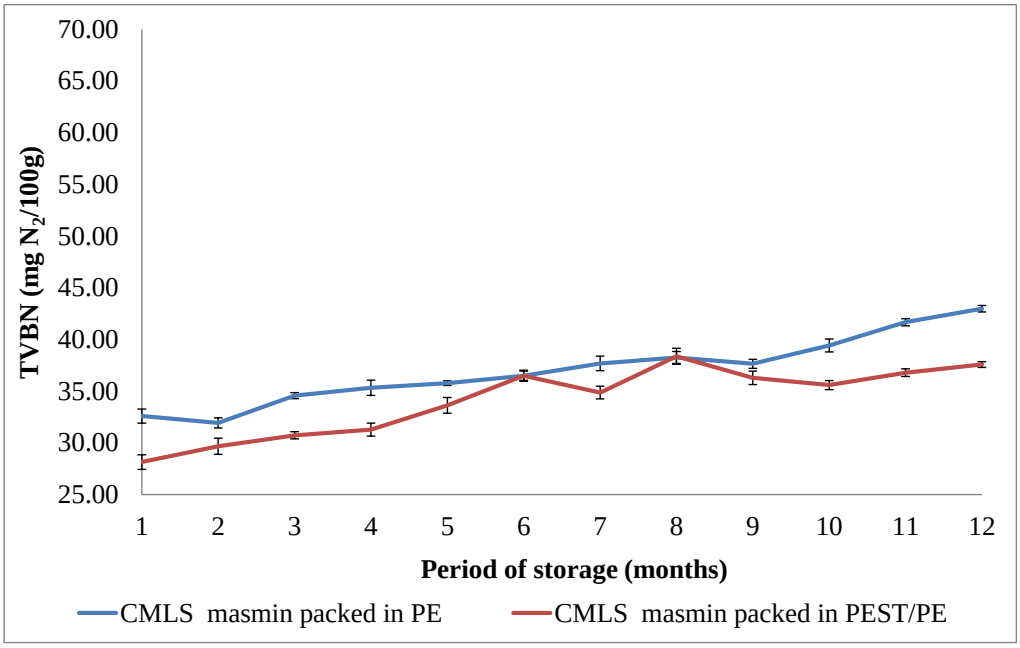

Figure 89 Changes in TVBN content of CMLS *masmin* packed in PE and PEST/PE

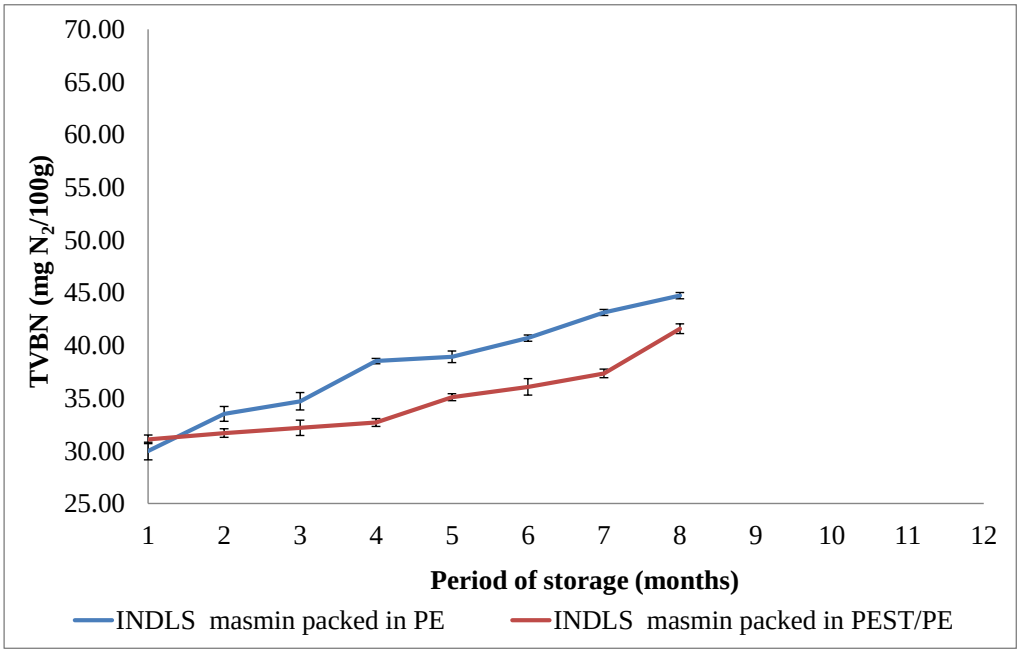

Figure 90 Changes in TVBN content of INDLS *masmin* packed in PE and PEST/PE

7.2.1.2 Tri-methyl amine-Nitrogen (TMA-N)

Changes in TMA-N content of liquid smoked and IMP *masmin* during storage is given in Figure 91 to Figure 93. Both the treatment and packaging materials showed a significant difference in terms of TMA-N content ($p<0.05$). Highest increase in TMA-N content was observed in INDLS *masmin* packed in PE (104% increase from initial value) followed by IMP *masmin* packed in PE (99% increase from initial value). CMLS *masmin* packed in PEST/PE showed the least increase (15% increase from initial value). Among the treatments, samples packed in PE showed significantly higher TMA-N values than the same packed in PEST/PE. Limit for TMA-N content in fish for human consumption is fixed as 10-15 mg/100 g (Connell, 1980). None of the sample in present study exceeded this limit. TMA-N content from 9.8 mg/100g (in dried *Plectorhinchus schotaf*) to 18.8 mg/100g (in dried *Stolephorus commersonii*) has been reported by Immaculate, Sinduja, Velammal, and Jamila (2013).

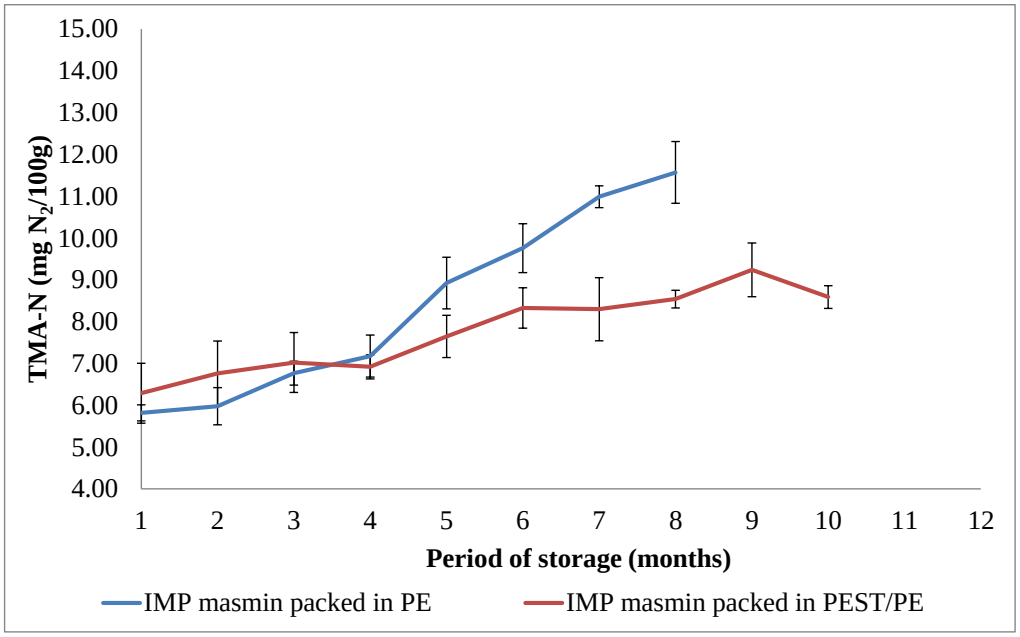

Figure 91 Changes in TMA-N content in IMP *masmin* packed in PE and PEST/PE

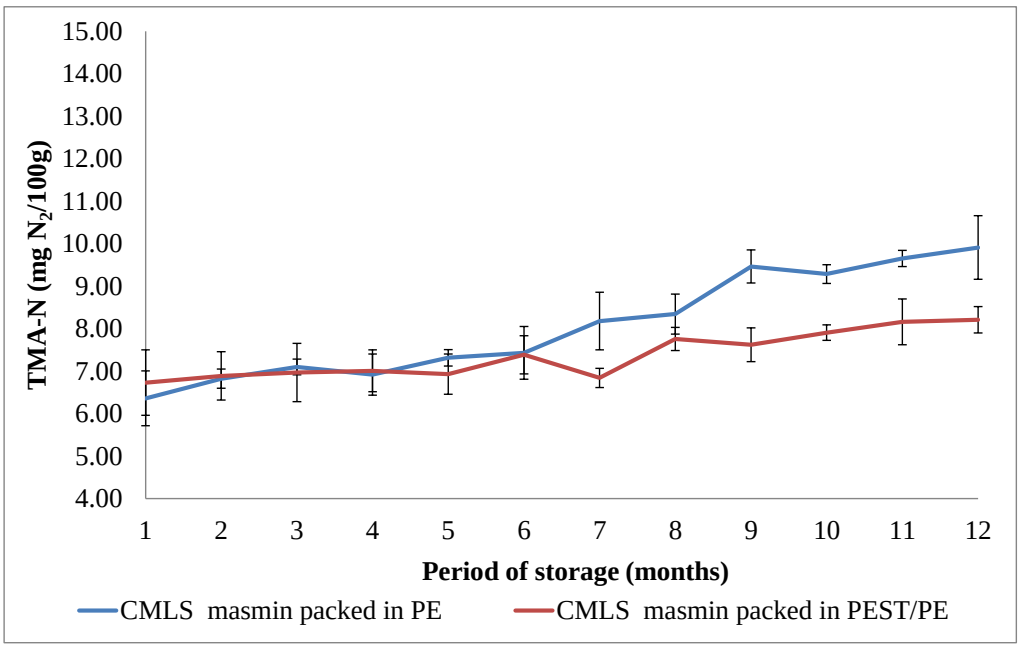

Figure 92 Changes in TMA-N content in CMLS *masmin* packed in PE and PEST/PE

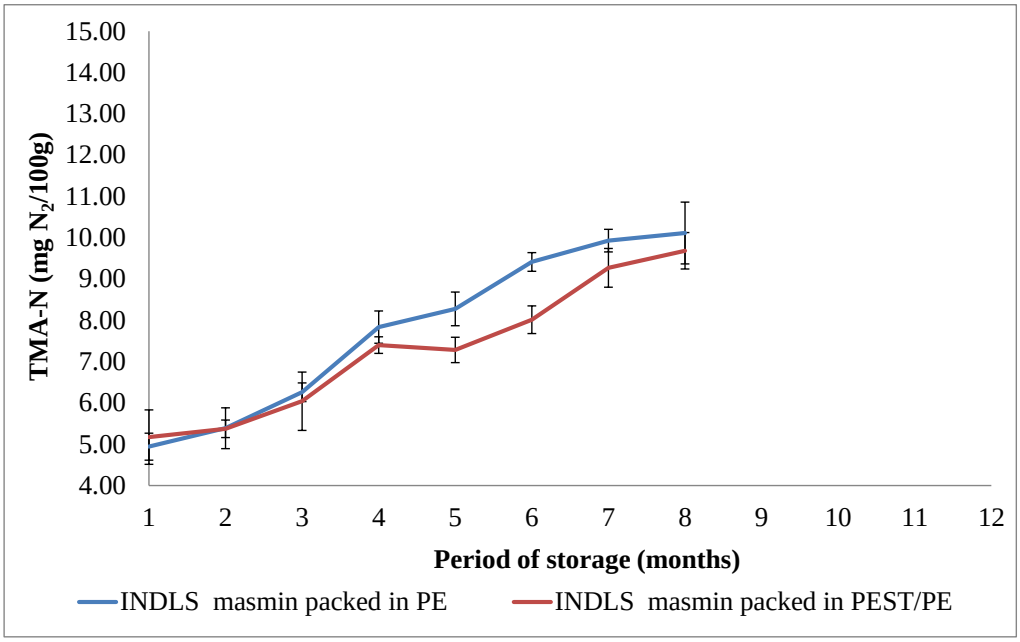

Figure 93 Changes in TMA-N content in INDLS *masmin* packed in PE and PEST/PE

7.2.1.3 Thiobarbituric Acid Value (TBA)

Changes in TBA value of liquid smoked and IMP *masmin* during storage are given in Figure 94 to Figure 96. Significant difference was observed between the treatments in terms of TBA value ($p<0.05$). A gradual increase in TBA values was observed in all the samples throughout the storage period. Highest increase was observed in INDLS *masmin* packed in PE (162% increase from initial value) and PEST/PE (128% increase from initial value). CMLS *masmin* packed in PEST/PE showed the minimal increase (50% increase from initial value) in TBA value.

INDLS *masmin* was produced by soaking the cooked loins in indigenous liquid smoke containing 7.5 % salt for 60 min. Salt has been proven to play a pro-oxidative role in food system (Aubourg & Ugliano, 2002; Connell, 1990; Davis, Goodwin, Smith, & Hole, 1993). This is in agreement

with the findings of Manat (2011), who found that both dry and wet salting resulted in an increased TBA value in tilapia muscle. Kanner, Harel, and Jaffe (1991) reported that this pro-oxidative activity of salt is due to its ability to release iron from heme pigments and other heme binding molecules. This displaced iron may then participate in the initiation of lipid peroxidation (Hultin, 1992). Alternatively chloride ion can be converted to a radical and the same can be added directly to a double bond or abstract hydrogen from fatty acids to initiate oxidation (Kanner & Kinsella, 1983; Hultin, 1992). Comparatively higher duration of direct exposure to salt during the production of INDLS *masmin* could be the reason behind the higher TBA values. Manat (2011) has also reported that longer exposure to brine will cause loss of natural antioxidants from muscle and there by leading to increased chances of oxidation. Dimici and Wada (1994) has reported that mould growth in "*Katsuobushi", a* similar smoked product resulted in increased free fatty acid content. It was observed that INDLS *masmin* samples had comparatively higher microbial load and were rejected on the 8th month of storage. Hence similar scenarios can be expected in the samples and the presence of higher free fatty acids along with reduced anti-oxidative properties would have accelerated the oxidative damage to the product.

According to Connell (1990), 1–2 mg malonaldehyde/kg is usually regarded as the limit beyond which the fish (with a moisture content of 70-80%) will normally develop an objectionable odour/taste. All the samples analysed had a TBA value higher than this limit, which can be the result of concentration of the Thiobarbituric Acid Reactive Substances (TBRS) due to the dehydration of meat (Pikul, Leszczynski, & Kummerow, 1984) and due to the intensity of thermal treatment applied on the product (Koizumi, Wada, & Ohshima, 1987). However none of the panellist reported rancid flavour in the

products during sensory analysis. Several authors have reported similar discrepancies in the TBA values and corresponding sensory characteristics (Boyd, Green, Giesbrecht, & King, 1993; Frankel, 1993 and Koral, Kose, & Tufan, 2009). It has been reported that the reaction of TBA with malonaldehyde is not specific, and reaction with a wide variety of other products may contribute to the absorbance. Several food components including proteins, maillard browning and sugar degradation products also interfere with the reaction (Gordon, 2001). Sun, Faustman, Senecal, Wilkinson, and Furr (2001) evaluated the reactivity of aldehyde with 2-thiobarbituric acid and TBRS in freeze-dried beef during accelerated storage. They reported that aldehydes like propional, butanal and 5-hydroxymethyl-2-furfural produced from maillard reaction has very high affinity to react with thiobarbituric acid and there for interfere with the results of TBA analysis. The presence and importance of aldehydes and maillard reaction in smoked foods is well known (Maga, 1988). Higher TBA values reported in the present samples could be due to the interference of these compounds with analysis. Since the black meat was not removed from the tuna loins during processing, higher proportion of lipids, myoglobin and heme proteins in the black meat (Balachandran, 2001) along with direct exposure to salt also would have increased the concentration of secondary oxidation products.

It was observed that the samples packed in PEST/PE showed lower TBA values compared to the same packed in PE. Reason for the same is expected to be good oxygen barrier properties of the laminate.

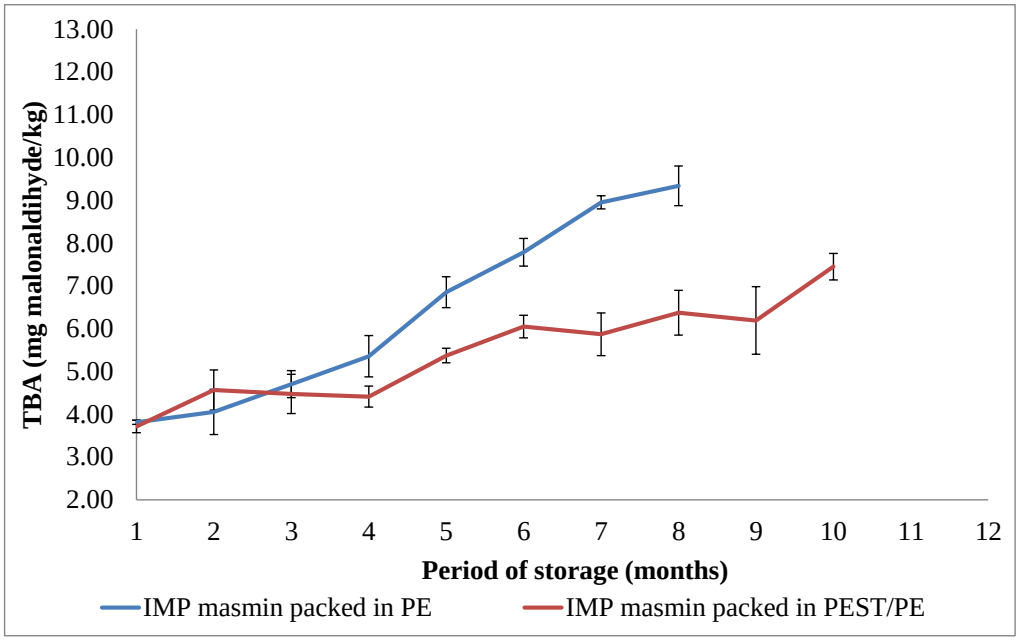

Figure 94 Changes in TBA value of IMP *masmin* packed in PE and PEST/PE

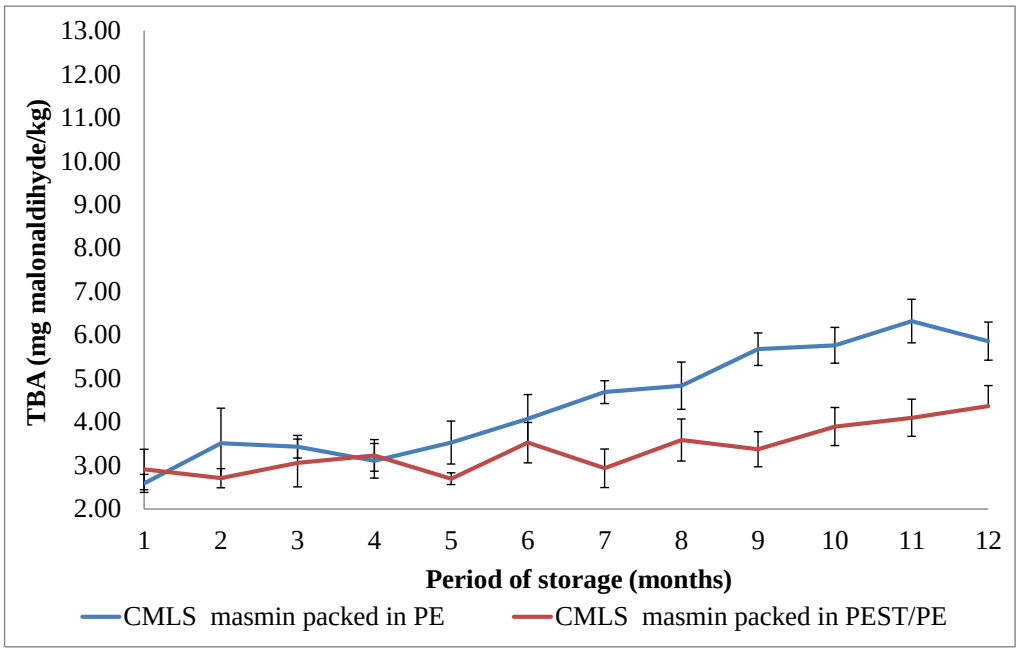

Figure 95 Changes in TBA value of CMLS *masmin* packed in PE and PEST/PE

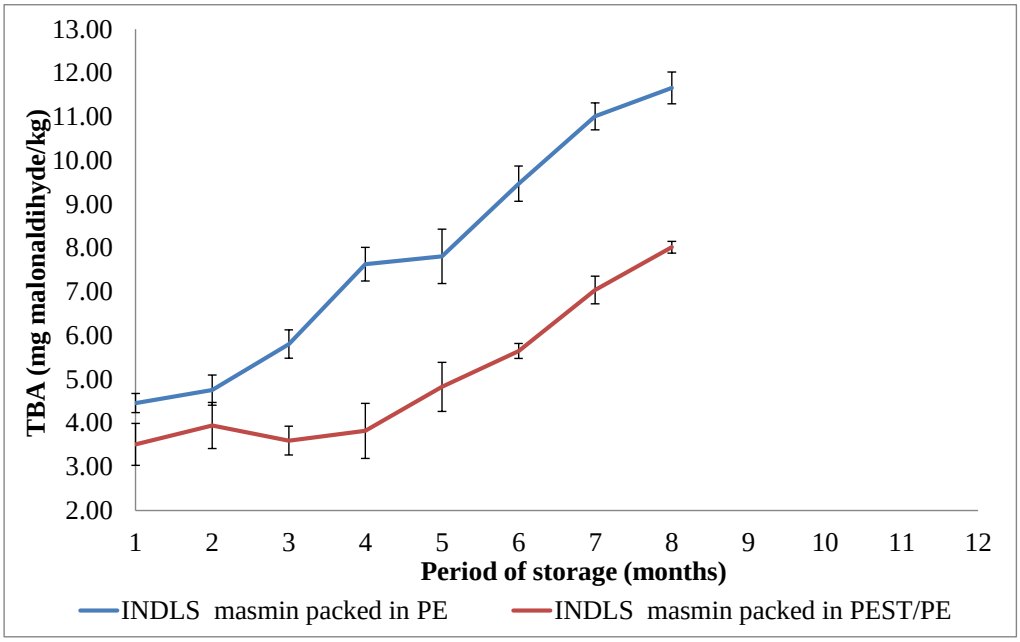

Figure 96 Changes in TBA value of INDLS *masmin* packed in PE and PEST/PE

7.2.1.4 Yeast & mould count and overall acceptability

Result of the yeast and mould analysis showed that, CMLS *masmin* samples packed in PE and PEST/PE were microbiologically safe even after 12 months of storage. IMP *masmin* packed in PE and PEST/PE was microbiologically acceptable till the 8^{th} and 10^{th} month of storage, respectively. Packaging material did not show any significant difference in the shelf life of INDLS *masmin* and both PE and PEST/PE packed samples were acceptable till eighth month of storage. Except in the case of INDLS *masmin*, all other samples packed in PEST/PE were found to be microbiologically superior to the same packed in PE. Antony, Muraleedharan, and Mukundan (2003) have reported shelf life of 10-12 months for improved *mas* fingers and granules. Total bacterial count as high as 6×10^4 has been reported in traditional *masmin* (David, Rajagopalaswami, & Sugumar, 1990). With proper packaging, traditional *masmin* is expected to remain in good condition up to

one year. However, till now no scientific studies have been reported to confirm this. The reason is expected to be difficulty in getting appropriate samples, as the manufacturing date of the product is unknown in most cases.

Changes in overall acceptability of liquid smoked and IMP *masmin* during storage are given in Figure 97 to Figure 99. Significant difference was observed between the three different treatments in terms of overall acceptability ($p<0.05$). INDLS *masmin* samples received lower score compared to the other two samples during the initial days of storage. Towards the end of storage, IMP *masmin* packed in PE started receiving scores close to the limit of acceptability. This is expected to be due to the lower score received for attributes like appearance and mouth feel as a result of moisture absorption. All other samples were found to be sensory wise acceptable even at the point of microbial rejection. CMLS *masmin* samples were sensory wise and microbiologically acceptable even after 12 months of storage. Significant difference was observed between the two packing materials in terms of sensory acceptability ($p<0.05$). After the initial 3-4 months of storage, samples packed in PEST/PE received higher acceptability scores for all three treatments. Textural and flavour changes associated with moisture absorption and consequent microbial action would be the reason behind lower acceptability of samples packed in PE. A gradual decrease in overall acceptability was observed in all the samples during storage ($p<0.05$).

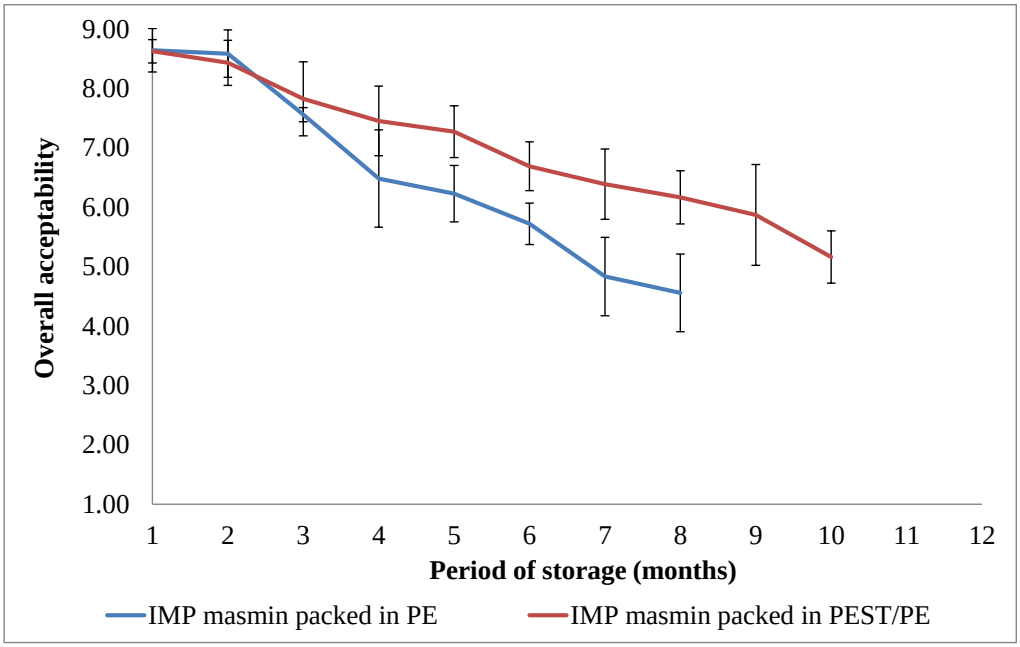

Figure 97 Changes in overall acceptability of IMP *masmin* packed in PE and PEST/PE

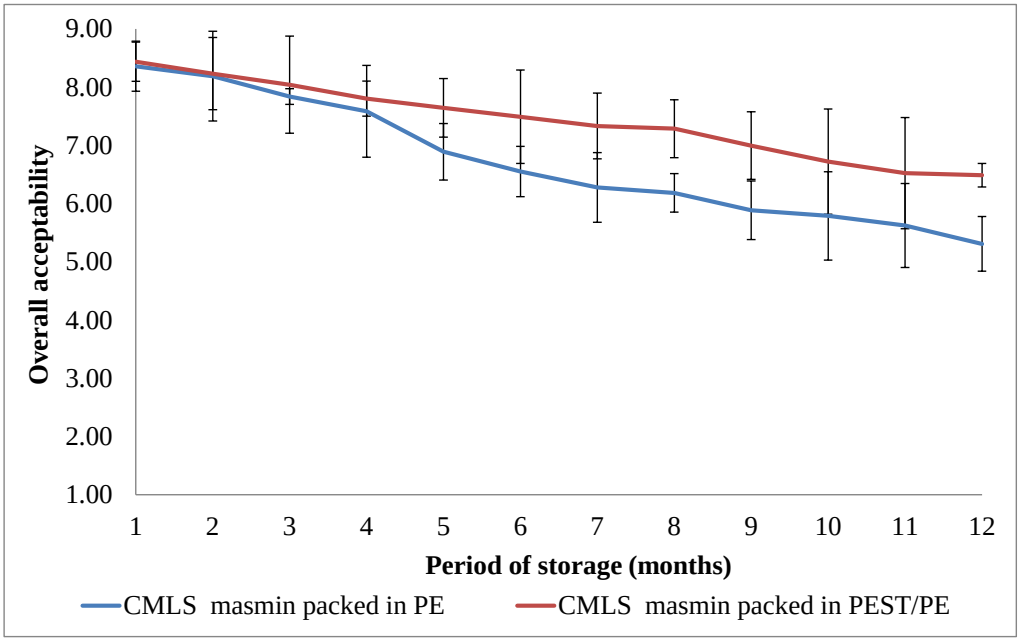

Figure 98 Changes in overall acceptability of CMLS *masmin* packed in PE and PEST/PE

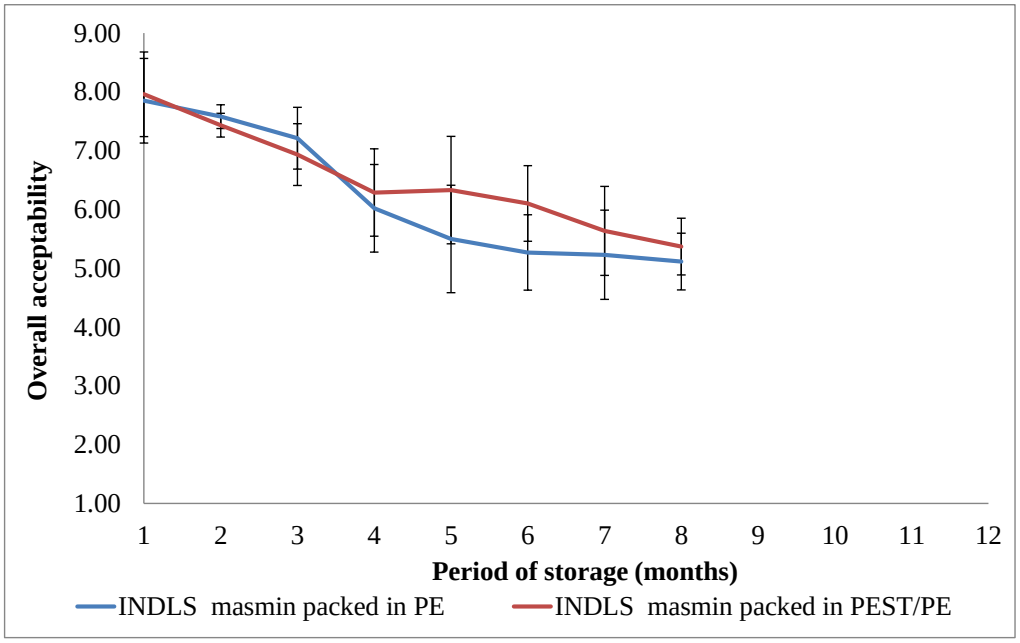

Figure 99 Changes in overall acceptability of INDLS *masmin* packed in PE and PEST/PE

7.2.2 Shelf life evaluation of liquid smoked *masmin* flakes

7.2.2.1 Total Volatile Base-Nitrogen (TVBN)

Changes in TVBN content of liquid smoked and smoked *masmin* flakes during storage are given in Figure 100 to Figure 102. Significant difference was observed between the treatments in terms of TVBN content ($p<0.05$). Highest increase was recorded in INDLS *masmin* flakes packed in PE (87% increase from initial value) followed by the same packed in PEST/PE (86% increase from initial value). Least changes were observed in CMLS *masmin* flakes packed in PEST/PE (45% increase from initial value). Smoked *masmin* flakes packed in PE and PEST/PE showed an increase of 74 and 70% (from initial value), respectively. Packaging material had a significant influence in the TVBN concentration ($p<0.05$). All the samples packed in PEST/PE showed lower increase in TVBN values than the same packed in PE.

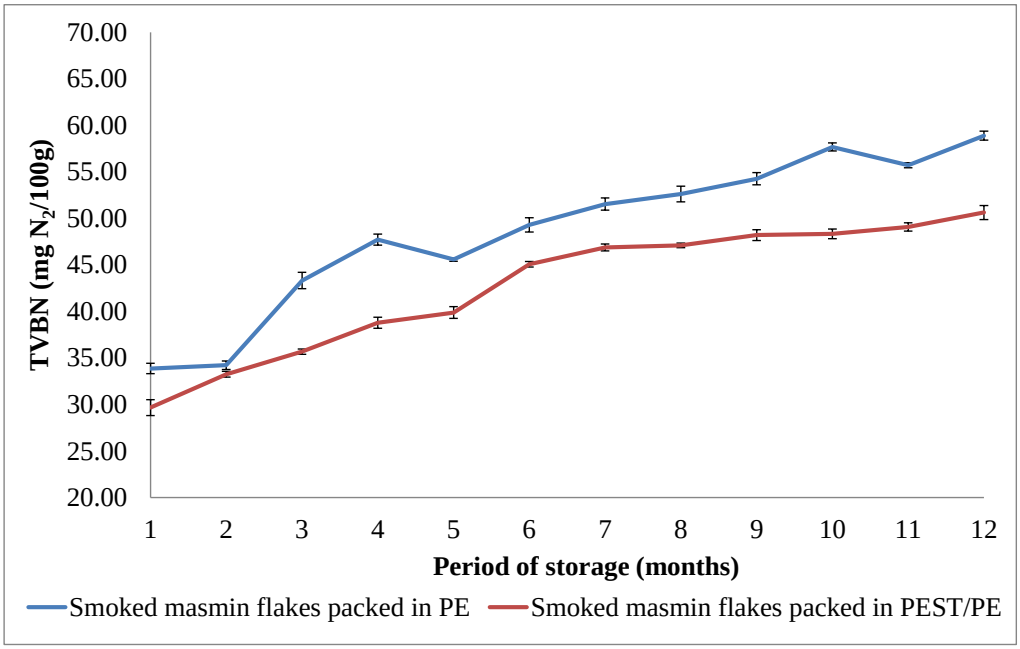

Figure 100 Changes in TVBN content of smoked *masmin* flakes packed in PE and PEST/PE

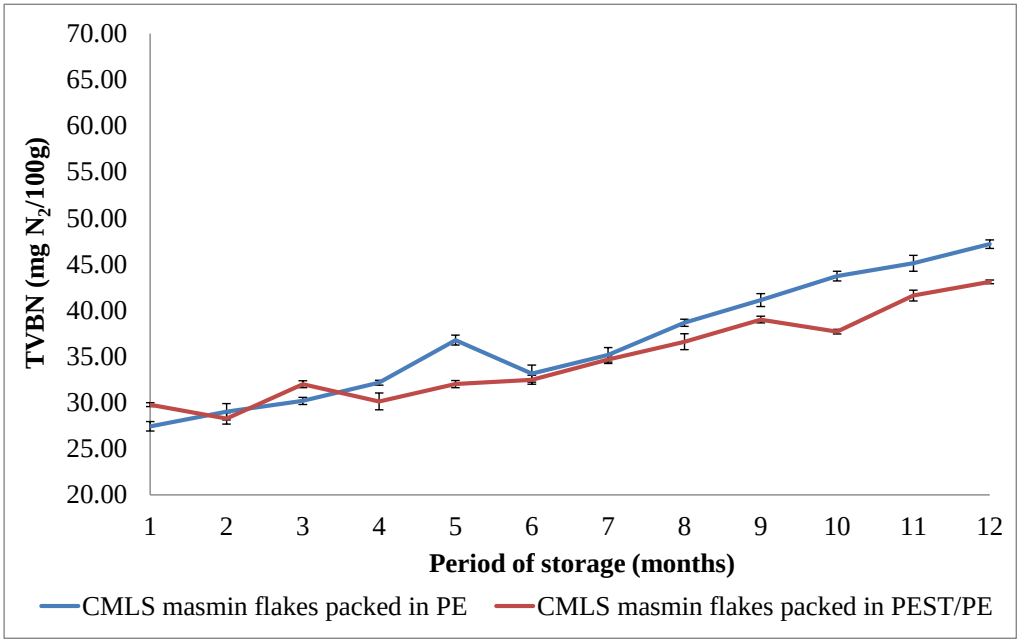

Figure 101 Changes in TVBN content of CMLS *masmin* flakes packed in PE and PEST/PE

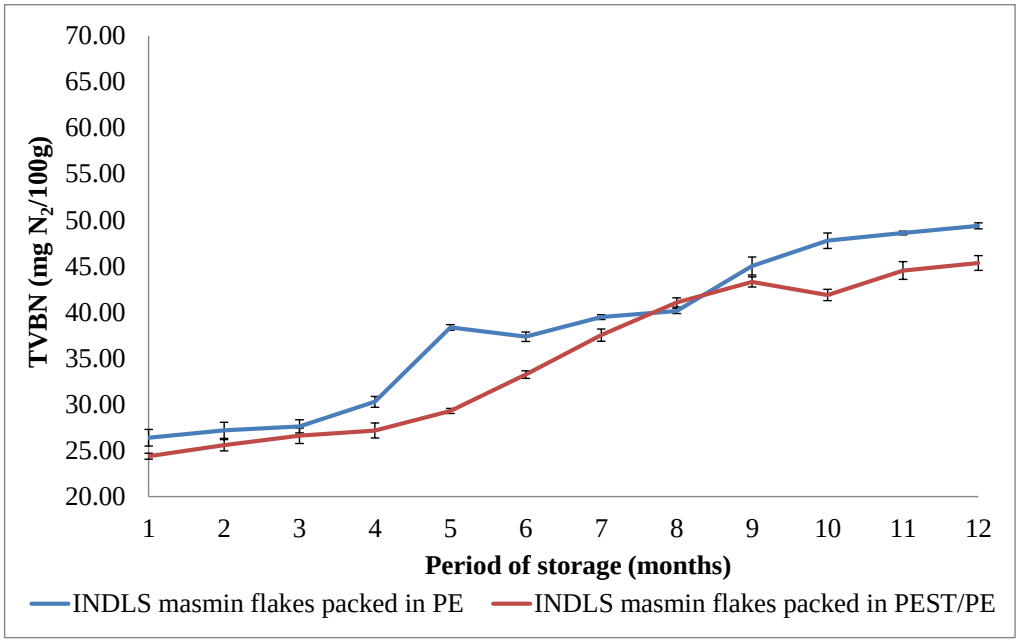

Figure 102 Changes in TVBN content of INDLS *masmin* flakes packed in PE and PEST/PE

7.2.2.2 Tri-methyl amine-Nitrogen (TMA-N)

Changes in TMA-N content of liquid smoked and smoked *masmin* flakes during storage are given in Figure 103 to Figure 105. A trend similar to that of TVBN was observed in the case of TMA-N. Treatments and packaging material showed a significant influence on TMA-N content ($p<0.05$). Highest increase was observed in INDLS *masmin* flakes packed in PE (128% increase from initial value). CMLS *masmin* flakes packed in PEST/PE showed the lowest changes in TMA-N content (43% increase from initial value).

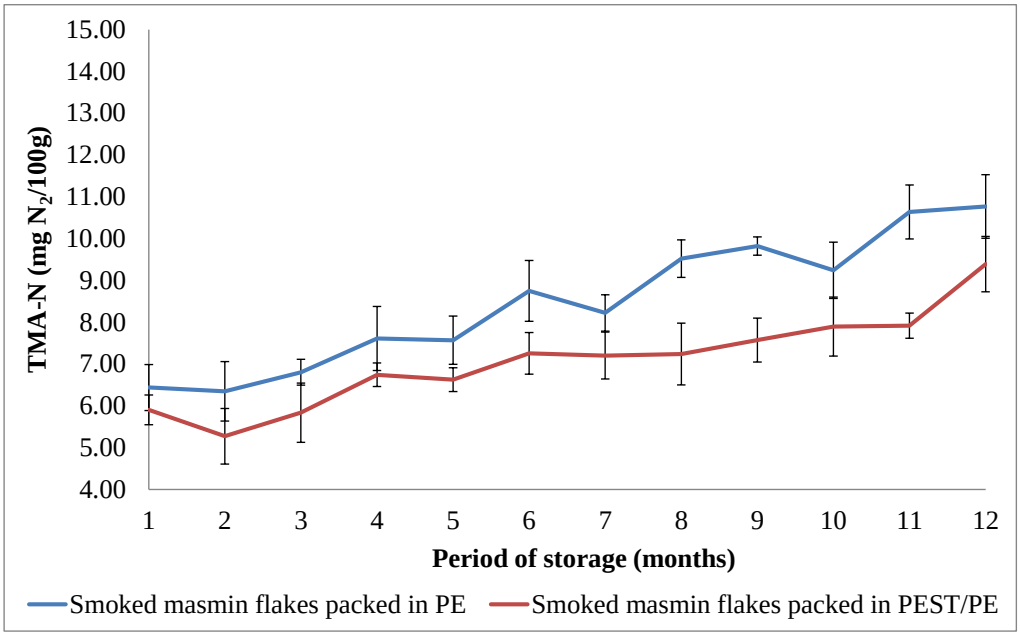

Figure 103 Changes in TMA-N content of smoked *masmin* flakes packed in PE and PEST/PE

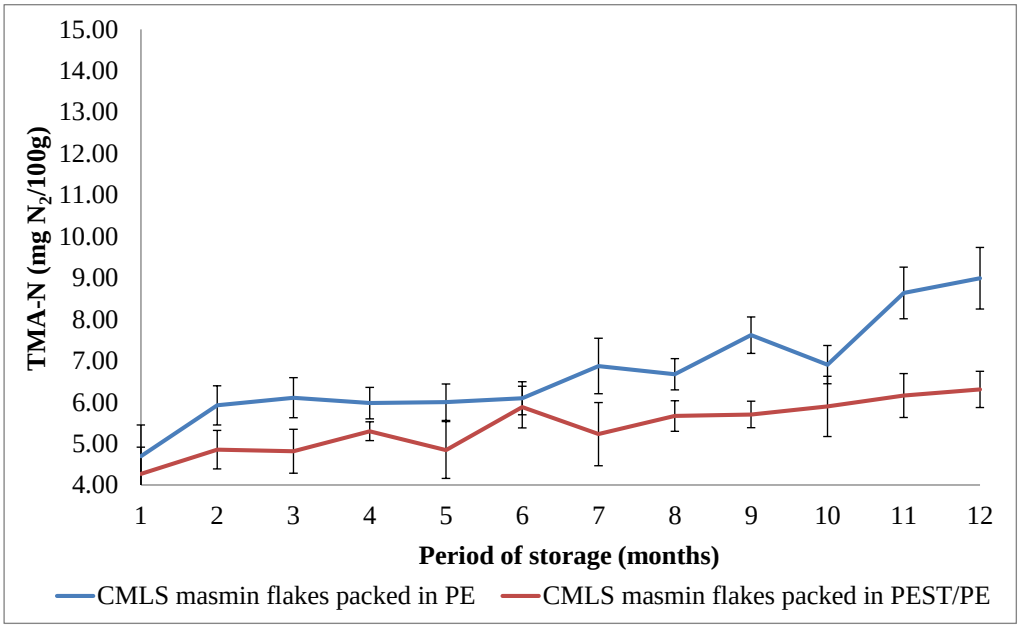

Figure 104 Changes in TMA-N content of CMLS *masmin* flakes packed in PE and PEST/PE

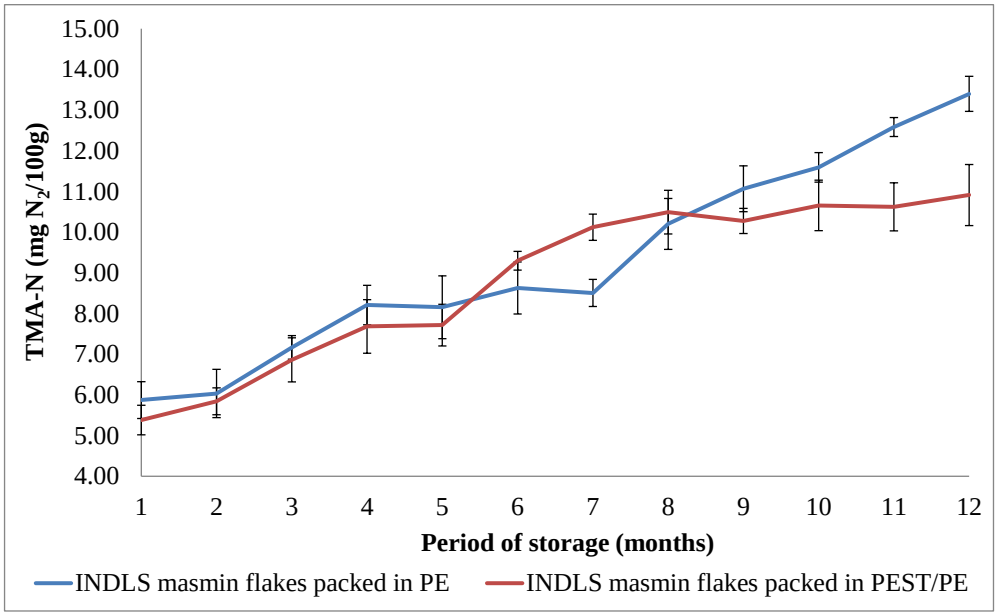

Figure 105 Changes in TMA-N content of INDLS *masmin* flakes packed in PE and PEST/PE

7.2.2.3 Thiobarbituric Acid Value (TBA)

Changes in TBA value of liquid smoked and smoked *masmin* flakes are given in Figure 106 to Figure 108. Significant difference was observed between the treatments and packaging materials used ($p<0.05$). Highest increase in TBA value was observed in INDLS *masmin* flakes packed in PE (154% increase from initial value) followed by the same packed in PEST/PE (117% increase from initial value). Least changes were observed in CMLS *masmin* flakes packed in PEST/PE (48% increase from initial value). Samples packed in PEST/PE showed lower values in terms of TBA. 12 to 79% increase in TBA value was observed in *masmin* flakes compared to corresponding *masmin* samples. This could be due to the lower moisture content and higher retention of TBRS since the meat is cooked in steam, unlike for *masmin* (cooking in brine). Direct cooking in brine results in dilution of TBRS (Aubuorg, Medina, & Pérez-Martín, 1995) and is expected to be the reason behind lower TBA values in *masmin* samples.

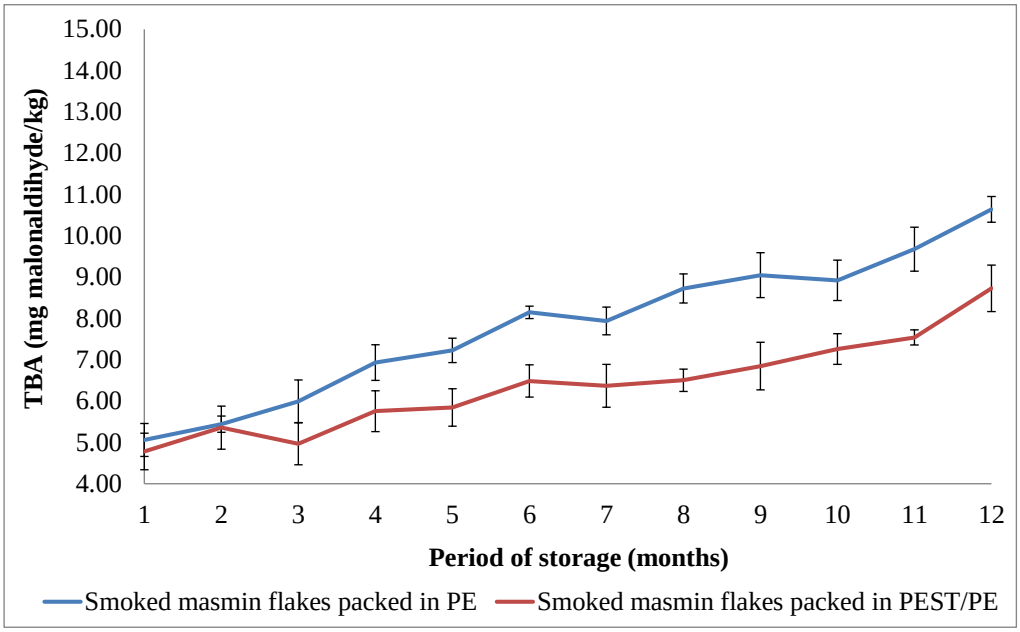

Figure 106 Changes in TBA value of smoked *masmin* flakes packed in PE and PEST/PE

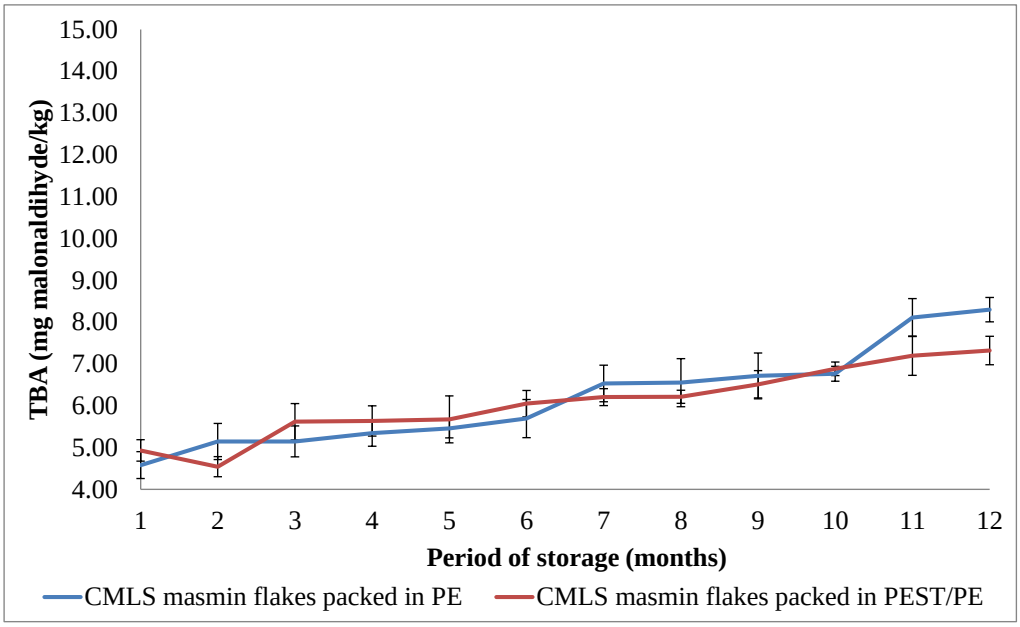

Figure 107 Changes in TBA value of CMLS *masmin* flakes packed in PE and PEST/PE

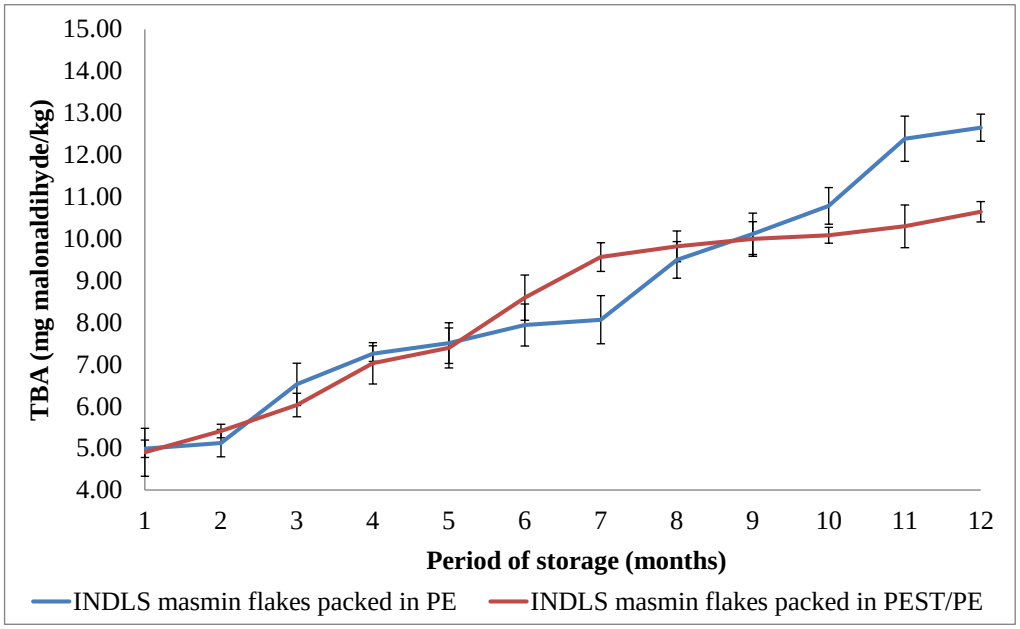

Figure 108 Changes in TBA value of INDLS *masmin* flakes packed in PE and PEST/PE

7.2.2.4 Yeast & mould count and overall acceptability

Result of the yeast and mould analysis showed that all the *masmin* flake samples were microbiologically safe even after 12 months of storage. Changes in overall acceptability of liquid smoked and smoked *masmin* flakes during storage are presented in Figure 109 to Figure 111. Significant difference was observed between the treatments and packaging materials used ($p<0.05$). Highest changes in overall acceptability were observed in INDLS *masmin* flakes packed in PE which recorded a 43% decrease from initial value. Least changes were observed in smoked *masmin* flakes packed in PEST/PE (11 % decrease from initial value) followed by CMLS *masmin* flakes packed in PEST/PE (12% decrease from initial value).

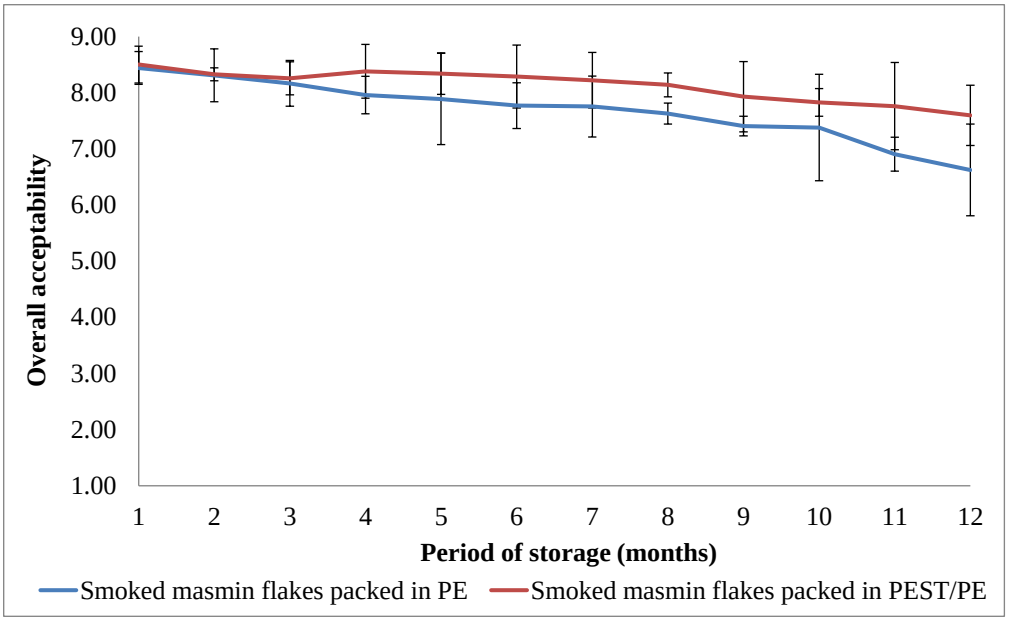

Figure 109 Changes in overall acceptability of smoked *masmin* flakes packed in PE and PEST/PE

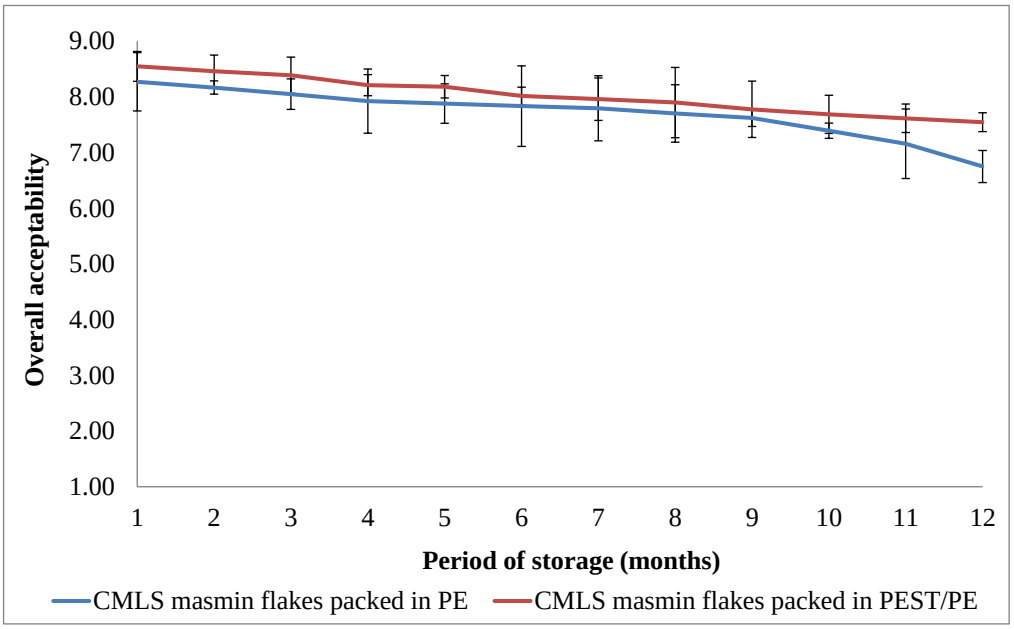

Figure 110 Changes in overall acceptability of CMLS *masmin* flakes packed in PE and PEST/PE

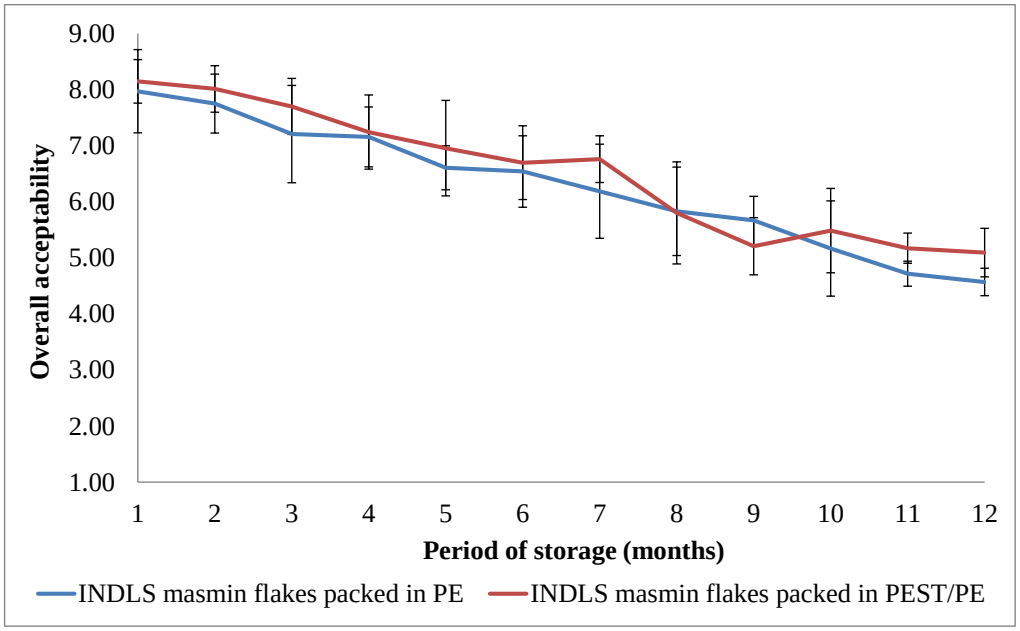

Figure 111 Changes in overall acceptability of INDLS *masmin* flakes packed in PE and PEST/PE

7.2.3 Shelf life evaluation of liquid smoked *masmin* powder

7.2.3.1 Total Volatile Base-Nitrogen (TVBN)

Changes in TVBN content of liquid smoked and smoked *masmin* powder during storage are depicted in Figure 112 to Figure 114. Significant difference was observed between the treatments and packaging materials used ($p<0.05$). Highest increase in terms of TVBN was observed in INDLS *masmin* powder packed in PE (43 % increase from initial value) followed by IMP *masmin* powder packed in PE (38% increase from initial value). All the samples were found to be within the acceptability limit of TVBN for salted dried fish. Rate of increase in TVBN content was minimal in *masmin* powder when compared to *masmin* and *masmin* flake samples. This is expected to be the result of lower microbial action due to low water activity. Samples packed in PEST/PE showed lower changes compared to the same packed in PE.

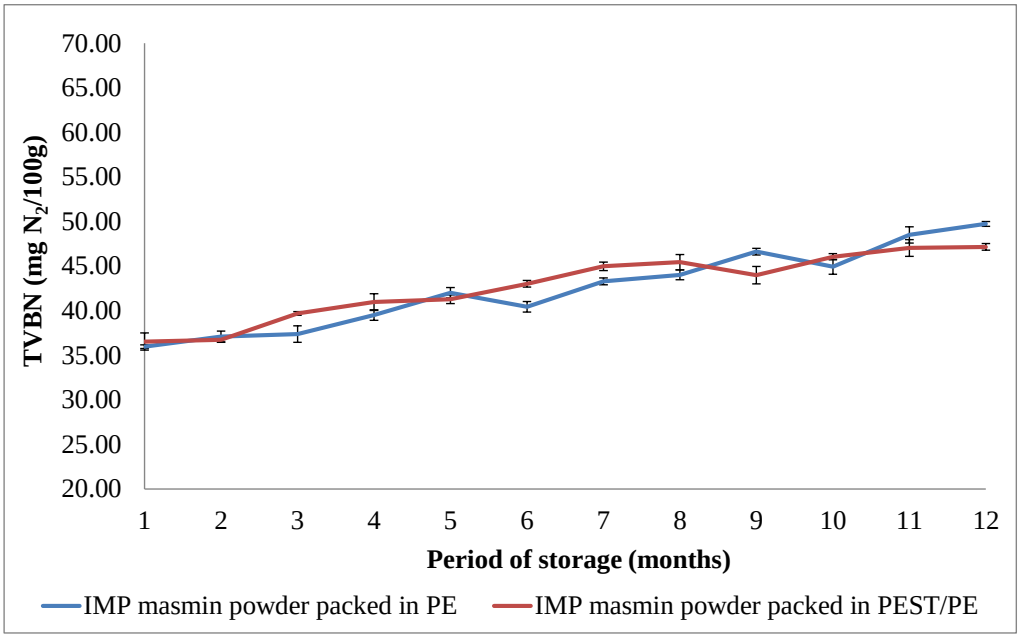

Figure 112 Changes in TVBN content of IMP *masmin* powder packed in PE and PEST/PE

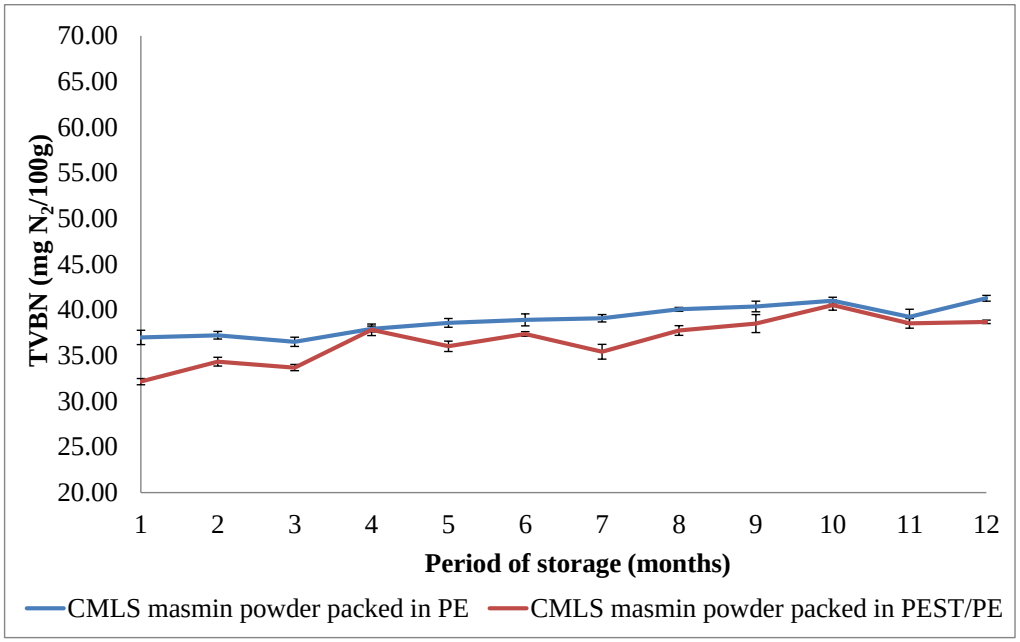

Figure 113 Changes in TVBN content of CMLS *masmin* powder packed in PE and PEST/PE

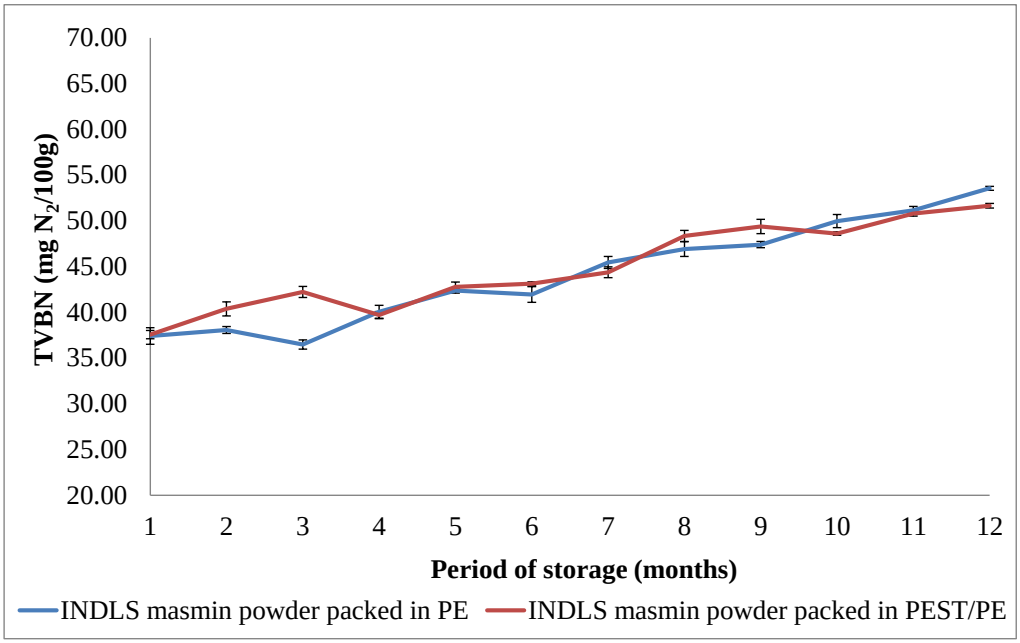

Figure 114 Changes in TVBN content of INDLS *masmin* powder packed in PE and PEST/PE

7.2.3.2 Tri-methyl amine-Nitrogen (TMA-N)

Changes in TMA-N content of liquid smoked and smoked *masmin* powder during storage is given in Figure 115 to Figure 17. Significant difference was observed between the samples in terms of treatments and packaging materials used ($p<0.05$). Unlike in the case of TVBN, highest increase in TMA-N content was observed in IMP *masmin* powder packed in PE (88% increase from initial value) followed by INDLS *masmin* powder packed in PE (66% increase from initial value). Least changes were observed in CMLS *masmin* powder packed in PEST/PE (13% increase from initial value). Samples packed in PEST/PE showed lower changes in terms of TVBN.

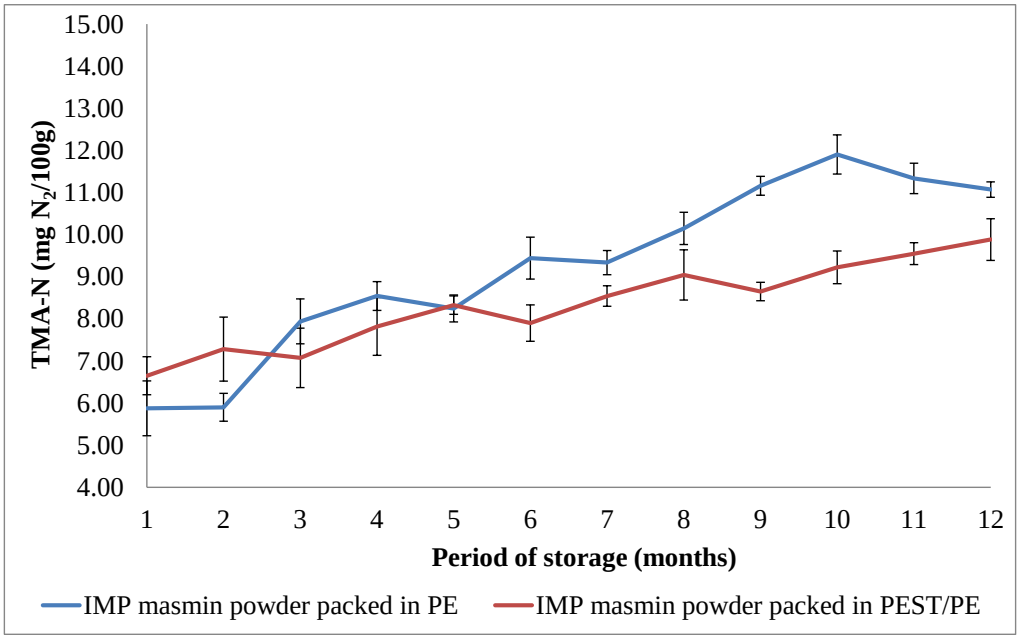

Figure 115 Changes in TMA-N content of IMP *masmin* powder packed in PE and PEST/PE

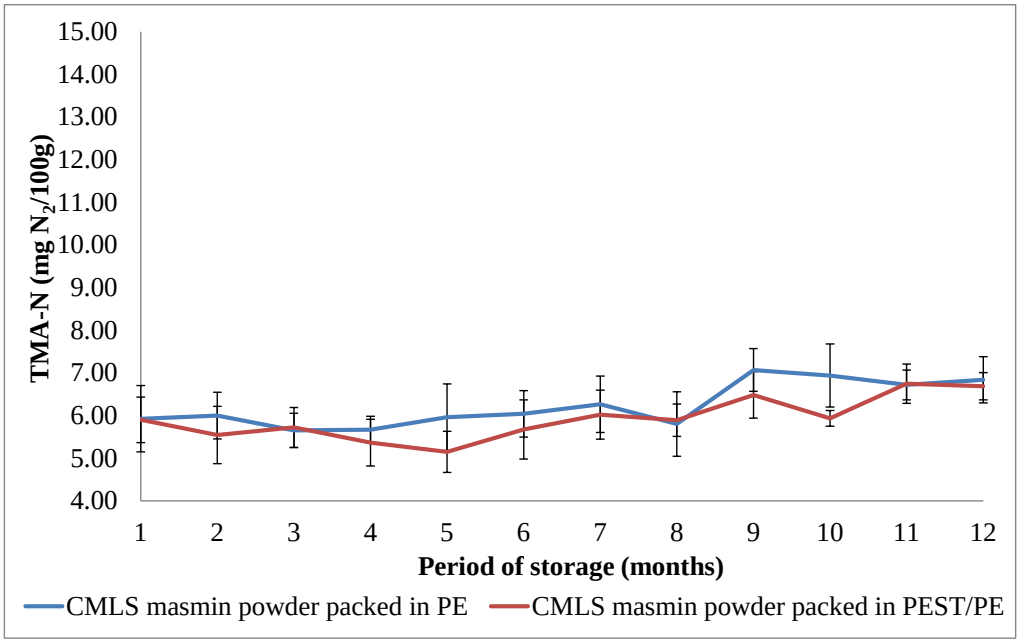

Figure 116 Changes in TMA-N content of CMLS *masmin* powder packed in PE and PEST/PE

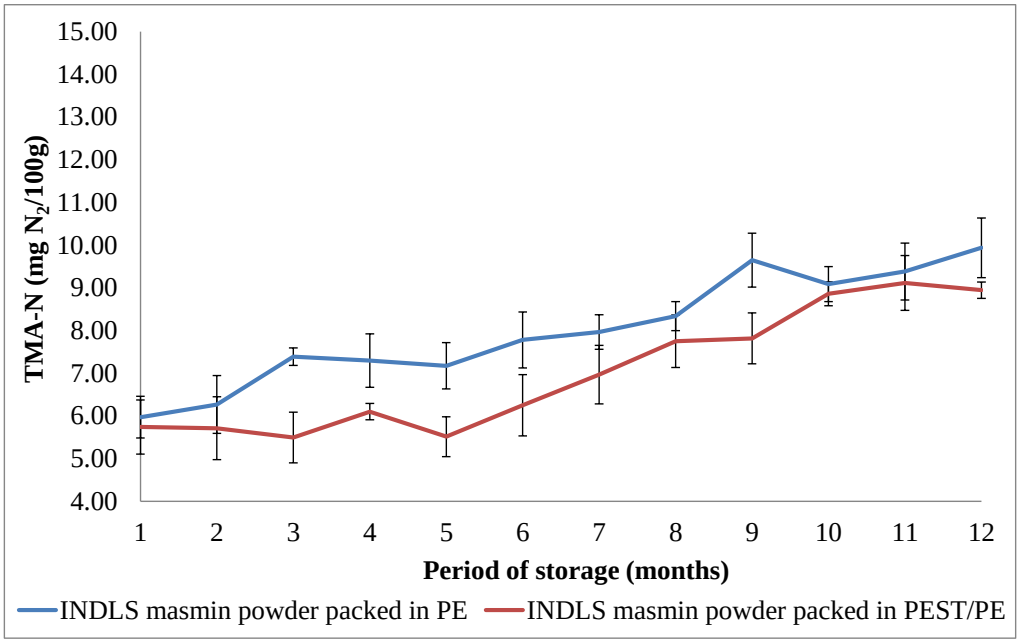

Figure 117 Changes in TMA-N content of INDLS *masmin* powder packed in PE and PEST/PE

7.2.3.3 Thiobarbituric Acid Value (TBA)

Changes in TBA value of liquid smoked and smoked *masmin* powder during storage is given in Figure 118 to Figure 120. Significant difference was observed between the samples in terms of treatments and packaging materials used ($p<0.05$). IMP *masmin* powder packed in PE showed highest increase in TBA value (111% increase from initial value) followed by INDLS *masmin* powder packed in PE (85% increase from initial value). CMLS *masmin* powder packed in PE showed least changes in term of TBA.

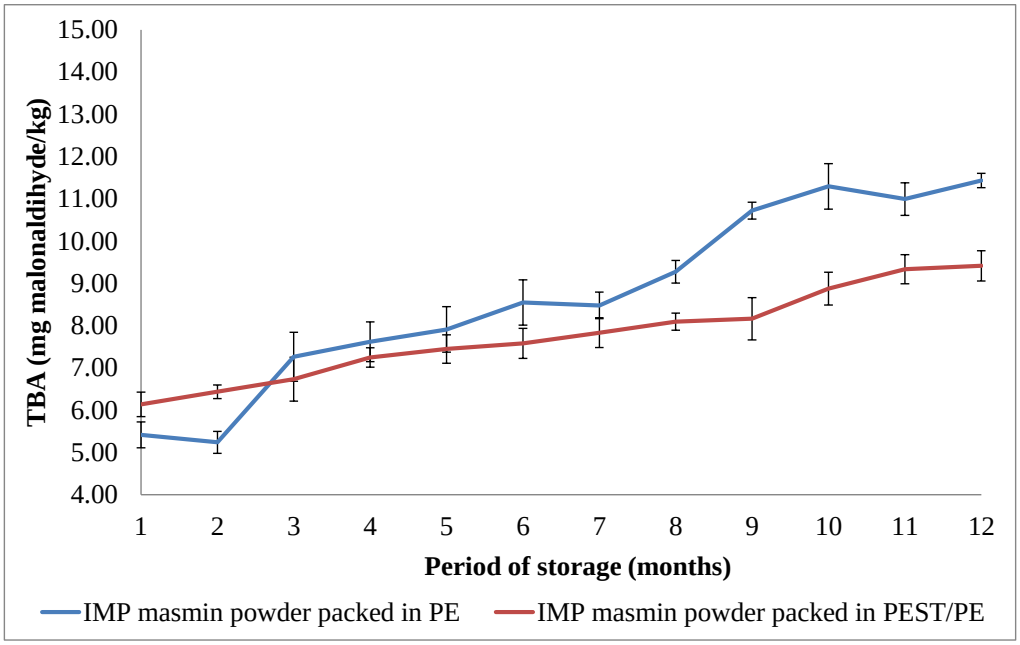

Figure 118 Changes in TBA value of IMP *masmin* powder packed in PE and PEST/PE

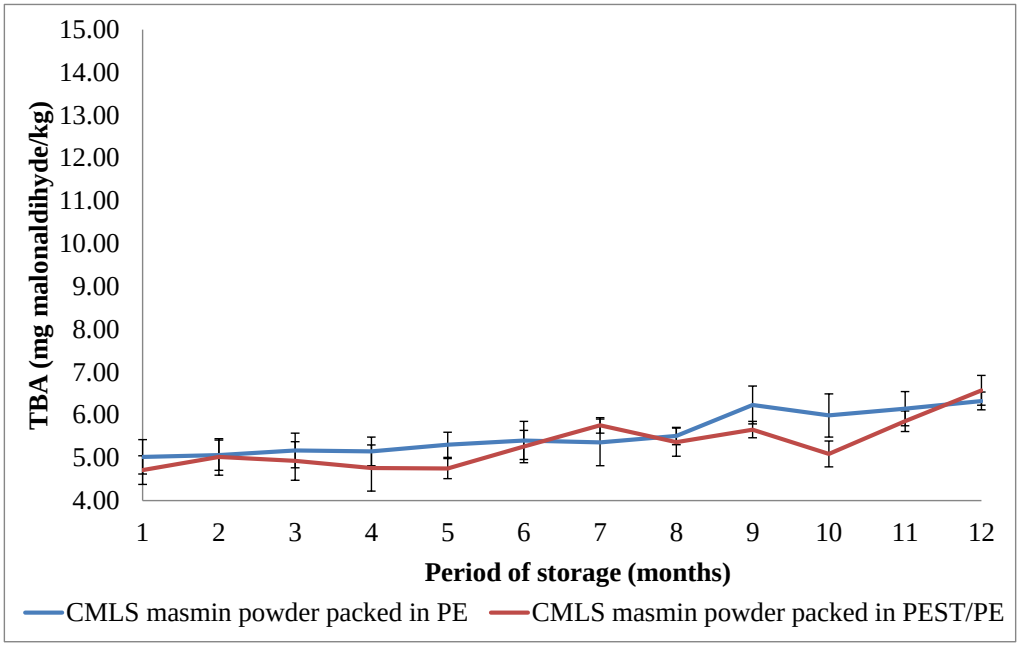

Figure 119 Changes in TBA value of CMLS *masmin* powder packed in PE and PEST/PE

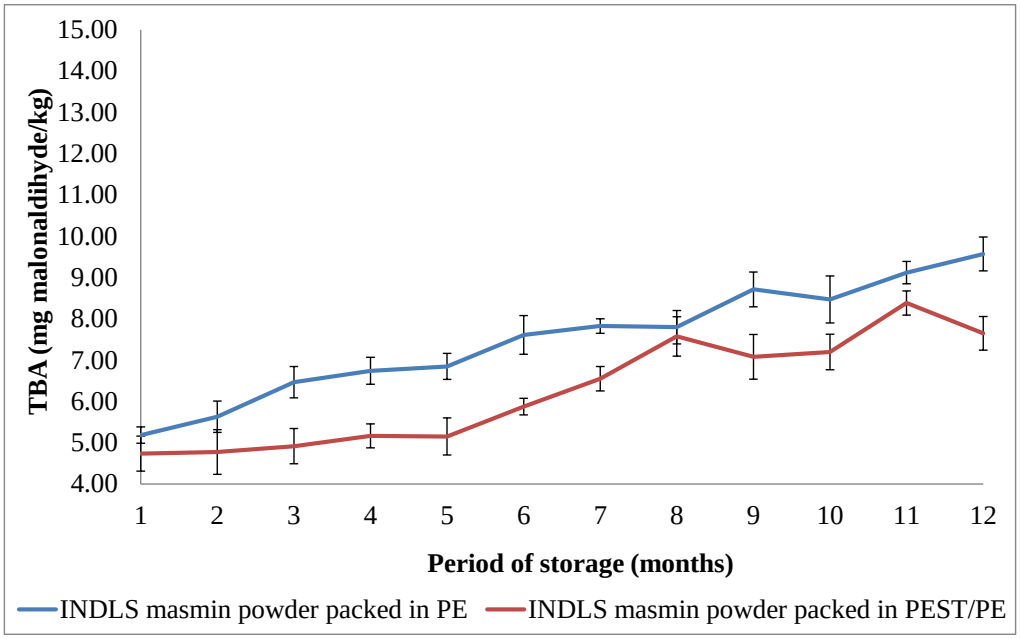

Figure 120 Changes in TBA value of INDLS *masmin* powder packed in PE and PEST/PE

7.2.3.4 Yeast & mould count and overall acceptability

Result of the yeast and mould analysis showed that, all the samples were microbiologically acceptable even after 12 months of storage. Changes in overall acceptability of liquid smoked and smoked *masmin* powder is given in Figure 121 to Figure 123. Significant difference was observed between the samples in terms of treatments and packaging materials used ($p<0.05$). Highest changes in acceptability was observed in INDLS *masmin* powder packed in PE (35% decrease from initial value) followed by CMLS *masmin* powder packed in PE (32% decrease from initial value). Least changes were observed in INDLS *masmin* powder packed in PEST/PE (8% decrease from initial value).

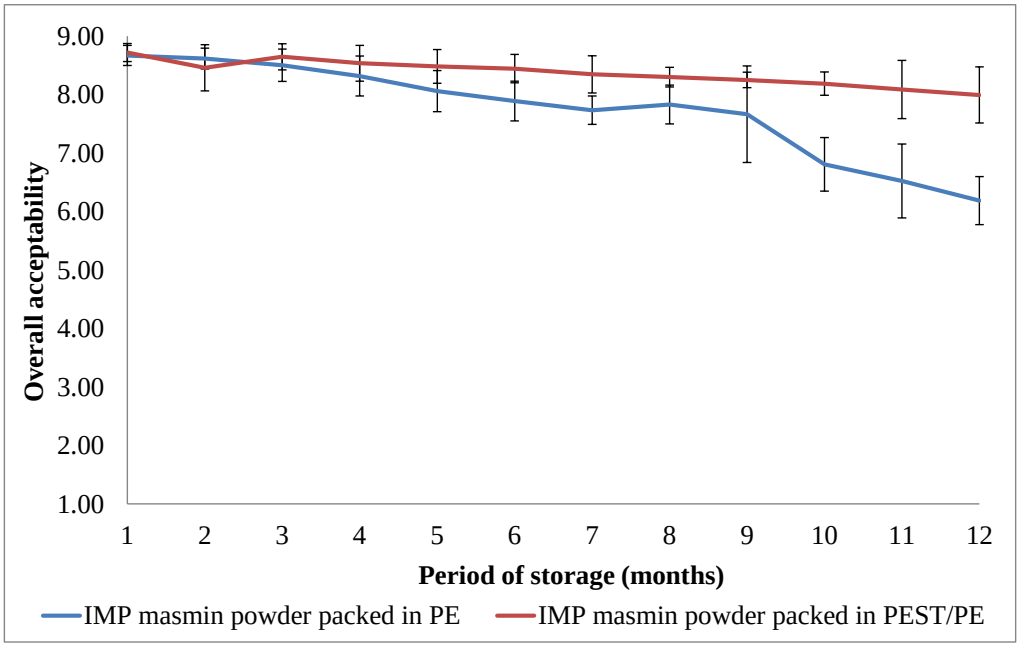

Figure 121 Changes in overall acceptability of IMP *masmin* powder packed in PE and PEST/PE

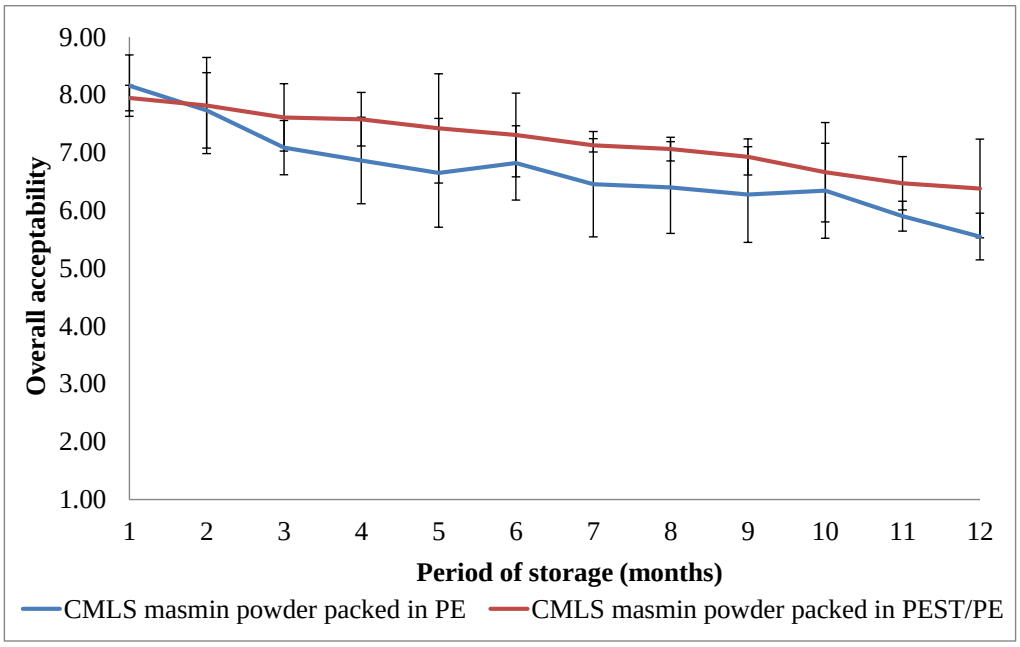

Figure 122 Changes in overall acceptability of CMLS *masmin* powder packed in PE and PEST/PE

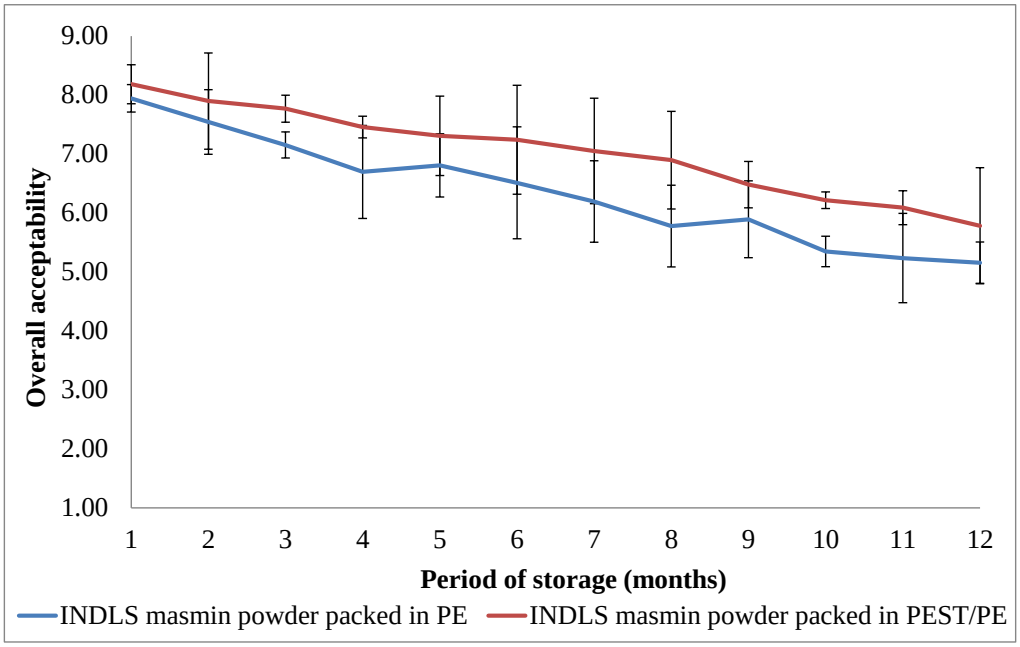

Figure 123 Changes in overall acceptability of INDLS *masmin* powder packed in PE and PEST/PE

7.3 Conclusion

Results of the study indicated that the developed products have shelf stability comparable to commercially available dried products in market. Among the *masmin* samples, CMLS samples showed higher antimicrobial and antioxidative properties and consequently higher shelf life. INDLS liquid smoked *masmin* have a shelf life comparable to IMP *masmin*. All the *masmin* flakes and *masmin* powder samples had a shelf life of more than 12 months. In most cases samples packed in PEST/PE had higher shelf life compared to the same packed in PE.

Summary & Conclusions

The present study was conceptualized with a prime objective of reducing the PAH contamination in traditional *masmin*, by development of *masmin* and *masmin* based convenience product using liquid smoke technology. To ensure a consistent supply of liquid smoke and there by leading to self sufficiency of the industry at Lakshadweep islands, development of a small scale facility for production of liquid smoke was also envisaged.

The indigenous liquid smoke production unit developed under the study had a capacity of generating 1.5 L of concentrated liquid smoke per hour. Influence of smoke source characteristics (using coconut husk as such, coconut fibre alone or coconut fibre powder alone as smoke source) on the composition (total phenolic content, total carbonyls, titratable acidity, pH and PAH content) of the developed liquid smoke was also investigated and compared with that of a commercial liquid smoke to arrive at the optimum reliable source. The results obtained showed that these variations in source characteristics can significantly influence the composition of resulting liquid smoke. Liquid smoke produced from coconut husk showed the highest phenolic content. Lowest phenolic content was observed in liquid smoke produced from coconut fibre powder. Coconut fibre powder liquid smoke showed the highest Heavy PAH content (PAH with molecular weight higher than 216 Dalton; which are potent carcinogens). None of the heavy PAH was detected in liquid smoke produced from coconut husk. Commercial liquid smoke showed higher phenolic content, carbonyl content and PAH content compared to the indigenous liquid smokes. All the liquid smoke produced/used in this study had a PAH content far below the approved limit

by European Union and Benzo(a)pyrene was detected only in liquid smoke produced from coconut fibre powder. Among the indigenous liquid smokes, due to the higher phenolic content and lesser PAH, liquid smoke produced from coconut husk was found to be of superior quality and hence was selected for the further studies.

Process parameters for the production of *masmin* and *masmin* based products using coconut husk liquid smoke was standardised using response surface methodology. Central composite design was used for the study. Sensory score, total phenolic content and salt content of the product were the dependent variables identified for the study. To facilitate a proper comparison, it was imperative to standardize the process parameters for production of commercial liquid smoked *masmin* and *masmin* based products also. Hence, Production parameters of 12 liquid smoked products viz; INDLS *masmin* by spraying, INDLS *masmin* by soaking, INDLS *Masmin* flakes by spraying, INDLS *Masmin* flakes by soaking, INDLS *Masmin* powder by spraying, INDLS *Masmin* powder by soaking, CMLS *masmin* by spraying, CMLS *masmin* by soaking, CMLS *Masmin* flakes by spraying, CMLS*Masmin* flakes by soaking, CMLS*Masmin* powder by spraying and CMLS *Masmin* powder by soaking were standardized by RSM. Results obtained were confirmed with validation studies. Products produced by optimum combinations were compared with each other to arrive at the best treatment in indigenous and commercial liquid smoked products.

Effectiveness of liquid smoking in reducing the PAH content in *masmin* and *masmin* based products was evaluated. Result of the study showed that traditional *masmin* is having heavy deposition of PAH which is far exceeding the current regulatory limits by European Union. It was evident from the study that, adoption of hygienic handling and controlled smoking as

practiced for production of IMP *masmin* can significantly reduce the PAH content. However, liquid smoking was effective in further reduction in PAH content.

Influence of the process modifications (by adopting improved methods or liquid smoking) on the nutritional value of *masmin* and *masmin* based products was also investigated. Wider variations were observed in the proximate composition of traditional *masmin*, which is supposed to be due to the lack of standardized/uniform protocols for production. The results showed that liquid smoking and improved smoking methods can positively influence the nutritional value of *masmin* by preserving essential nutrients. Longer cooking, smoking and drying time employed in production of *masmin* results in loss of amino acids like lysine, histidine, arginine, and tryptophan. However such losses can be prevented by adoption of liquid smoking or improved smoking methods. Among the new methods, liquid smoking was found to have better retention of amino acids. It was also observed that losses of palmitoleic acid and arachidonic traditional *masmin* can be prevented by adopting the new methods.

Sorption isotherm characteristics of the liquid smoked & improved *masmin* and *masmin* based products were investigated to spell out the optimum storage conditions and packaging requirements for the products. Physicochemical properties of a monolayer (90 µ Low Density Polyethylene (PE) and laminate (12 µ Polyester and 300 gauge Polyethylene) film used in dry fish packaging was investigated to understand their efficacy in protecting the developed products. Sorption analysis of the developed product showed a sigmoid curve which belong to Type II isotherm. This suggests that they are very much sensitive to changes in humidity and require efficient packaging for long term storage. All the samples showed a steep increase in moisture

absorption above RH of 53%. Evaluation of physicochemical parameter of the selected packing materials showed that both of them are effective in protecting the products from exposure to undesirable factors.

Actual shelf life of the products under these two packagings; 90 µ Low Density Polyethylene (PE) and laminate of 12 µ Polyester and 300 gauge Polyethylene (PEST/PE) was evaluated. The products were monitored for a maximum period of twelve months; microbiological evaluation (yeast and mould analysis) was carried out at irregular intervals after the initial six months of storage. Limit of 100 cfu/g fixed under 2-class sampling by Food Safety and Standards Authority of India was taken as the microbial rejection criteria. Results of the study indicated that the developed products have shelf stability comparable to commercially available dried products in market. Among the *masmin* samples, CMLS ones showed higher antimicrobial properties and consequently higher shelf life. INDLS liquid smoked *masmin* have a shelf life comparable to IMP *masmin*. All the *masmin* flakes and *masmin* powder samples had a shelf life of more than 12 months. In most cases samples packed in PEST/PE had higher shelf life compared to the same packed in PE.

It is evident from the study that liquid smoking can effectively reduce the PAH contamination in *masmin* without compromising the flavour and nutritional value of the product. Obtained results are promising towards the replication of the technology to other smoked products. Evaluation of specific antimicrobial and antioxidative properties of the developed liquid smoke and identification of the individual constituents and their properties in food will add a new dimension to the study, which can also be explored in future studies.

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