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Liquid smoking as a method for addressing polycyclic aromatic hydrocarbons (PAH) in traditional *masmin*

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ABSTRACT

Masmin is a traditional smoked and dried product from Lakshadweep Islands, Union territory of India. Intensive smoking practiced during production of *masmin* leads to chances of accumulation of carcinogenic compounds like polycyclic aromatic hydrocarbons (PAH) in the product. The aim of this study was to develop liquid smoked *masmin* which has matching flavour with traditional *masmin* and lower content of PAH. Response surface methodology was used for standardisation of the products. It was observed that spraying cooked tuna loins with a commercial liquid smoke "SMOKEZ ENVIRO 24PB" (diluted in 1:6 proportion with distilled water and added with 3.5% (w/v) salt for 150 min at a flow rate of 1 l h^{-1} in a multi-functional smoke kiln at a chamber temperature of 60°C was effective in giving desirable flavour for the product. Analysis of PAH content in traditional *masmin* showed values exceeding the national and international standards. However, liquid smoked *masmin* had a PAH content which is well within the recommended level. Shelf life evaluation of the products revealed that they have shelf life of more than 12 months at ambient temperature.

Keywords: Fish, *Masmin*, Seafood, Smoking, Tuna

Introduction

Smoking as a food processing method during ancient days has made significant contributions for ensuring food safety as a cheap and effective way for preserving food commodities, especially seafood. The technology is still widely used due to the preference for typical flavour of the products (Holley and Patel, 2005). However, recent advances in scientific knowledge and health concerns have raised few ambiguities regarding the technology, especially with regard to the higher content of polycyclic aromatic hydrocarbons (PAH) in products thereof (US EPA, 2002; Scientific Committee on Food, 2002; European Union, 2011; FSSAI, 2011).

Masmin is a traditional smoked and dried fishery product from Lakshadweep islands produced from skipjack tuna (*Katsuwonus pelamis*). The product enjoys a huge demand locally as well as in countries like Sri Lanka, Singapore and Myanmar. The method of preparation of the product has been passed down through generations and the process is followed even today with very little modifications. Skipjack tuna loins are cooked in seawater and allowed to cool in the same water for about 6 h. After cooling, the loins are smoked for 4 to 5 h, followed by sun drying for about 10 days. This smoking and drying cycle is repeated several times until the final product is formed (Yathavamoorthi *et al.*, 2010). *Masmin* resembles a dark piece of wood and can be stored for a period of one year with proper packaging. However, no scientific study has been conducted to evaluate the exact shelf life of the product due to the minor variation in processing steps between the islands. In most cases the production date of the commodity is unknown. Being a heavily smoked product, *masmin* also possesses considerable chance for accumulation of a large amount of PAH. The crude processing practiced in production also adds to chances of further PAH contamination from other sources.

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 (Sanders and Wise, 1997). The main health concern about PAH 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. European Union (2011) has set a maximum level of 30 µg kg^{-1} for "PAH4" [sum content of PAH *viz.,* benzo (a) pyrene, chrysene, benzo (a) anthracene and benzo (b) fluoranthene] in smoked fish and fishery products, while maintaining a separate maximum level of 5 µg kg^{-1} 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}. The present study is an attempt to address the PAH contamination in *masmin* by replacing the traditional smoking practices with liquid smoking.

Use of liquid smoke is a more controllable and consistent process compared to traditional smoking practices. Liquid smoke includes either smoke condensate dissolved in water, oil or smoke extracts in organic solvents. Smoke condensate can also be absorbed on solids such as spices, salt, sugars, starch or protein, thus resulting in dry or powdered forms (Toth and Potthast, 1984). Major advantages of liquid smoking are applicability in variety of foods and reduced environmental pollution. The technology also offers considerable flexibility in mode of application *viz.* spraying, soaking and mixing (Maga, 1988). Spray application was used in the present study, considering the nature of the product and appropriateness of the commercial liquid smoke used. Lower content of PAH resulting from intensive refining and filtration practiced during production of liquid smoke adds edge to the technology (Da porto, *et al*., 2006; Swastawati *et al*., 2007). In this context, the aim of this study is to investigate the possibility of developing liquid smoked *masmin* with matching flavour of traditional *masmin* and comparison of PAH content in the product to evaluate the effectiveness of the technology. The study also intends to explore the shelf life of liquid smoked *masmin* in monolayer (low density polyethylene) and laminate pouches (polyester/ polyethylene laminate). Choice of the materials was based on their wide spread use in the dry fish industry.

Materials and methods

Traditional masmin

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

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. Selection of the same was based on pre-run trials.

Standardisation of process parameters for the production of commercial liquid smoked (CMLS) masmin

Experimental design and statistical models

All the spraying and drying applications for the study were carried out in a multi-functional smoke kiln (CS700EL, KERRES Anlagensysteme GmbH, Germany). The kiln consisted of a stainless steel chamber with digital controls for adjusting the process parameters and 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. After completing the treatments, loins were dried in a kiln at 60°C to moisture content below 10%; cooled and then packed.

Input variables selected for spray application were; duration of exposure (spraying duration of liquid smoke), salt content in liquid smoke, chamber temperature (temperature inside the spraying chamber), flow rate (quantity of liquid smoke being sprayed) and dilution of liquid smoke. Input variables and their broad range of application for the study (Table 1) were arrived upon from pre-run trials. Sensory score, total phenolic content and salt content of the product were the dependent variables identified for the study.

Table 1. Input variables and their levels of application by spraying, for CMLS products

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 h^{-1})	x_4	1	2	3
Dilution (%)	x_5	1:2 (50%)	1:3 (33.3%)	1:6 (16.6%)

Central composite design was used for the analysis 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 and Montgomery, 2002).

$$Y = \beta_o + \sum_i \beta_i x_i + \sum_{ii} \beta_{ii} x_{ii}^2 + \sum_i \sum_{j,i<j} \beta_i \beta_j x_i x_j + e, i \neq j \quad (1)$$

where, Y = response variable, β_0 = intercept, β_i = linear regression coefficients, β_{ii} = quadratic regression coefficients, β_{ij} = interaction regression coefficients and e = error term. Desirability score was computed for multiresponse optimisation of input process parameters. While computing desirability score, the predicted values of response variables were either maximised or minimised; 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 optimised conditions were confirmed with validation studies.

Pre-processing operations

Fresh skipjack tuna 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 (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. After the pre-processing operations, the loins were exposed to spray application with varying input variable combinations in separate batches.

Determination of total phenolic content (TPC)

TPC of *masmin* was determined using Folin-Ciocalteu method as described by Ismail *et al*. (2004) with slight modifications. *Masmin* samples were scraped into thin flakes using a knife and were powdered in a mechanical grinder. Sample (5 g) was soaked in 50 ml distilled water for 12 h with intermediate swirling. Prior to analysis, the extract was filtered using Whatman grade-1 filter paper. One millilitre from this extract was pipetted out into a test tube and 0.75 ml of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) was added and mixed. The mixture was allowed to stand at room temperature for 5 min. Then, 0.75 ml of sodium carbonate solution (6% diluted in distilled water) 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^{-1}) was plotted. Total phenolic content was calculated from the calibration curve after correcting dilution of the sample.

Determination of salt content

About 1g of homogenised sample was weighed into an Erlenmeyer flask. To this, 25 ml distilled water, 5 ml nitric acid and 40 ml standard silver nitrate 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 silver nitrate with 0.1 N ammonium thiocyanate. Sodium chloride content (%) of the sample was calculated using the following equation:

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

Determination of overall acceptability for standardisation

Sensory analysis of the products was carried out by a five member expert panel using an 8 point hedonic scale prescribed by Meilgaard *et al*. (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. 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.

Reagents and 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 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 and 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 and FLT) and Heavy PAH (sum content of PAH with higher molecular weight than Light PAH *viz.,* BaA, CHR, BbF, BkF, BaP, ICP, BgP and DhA) was calculated from the data.

Determination of PAH content

Extraction of PAH was carried out according to Takatsuki *et al.* (1985). *Masmin* samples were aseptically scrapped into thin flakes and then ground in a mechanical

grinder. Twenty five grams of the sample was refluxed in a round bottom flask along with 200 ml ethanol, 35 ml 50% aqueous potassium hydroxide, 2 g sodium sulphite and few glass beads for 2 h and subsequently cooled to 40°C. One hundred and fifty milliliter of 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 re-extracted thrice 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 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 extract was loaded into the column and the container was rinsed with additional 2 ml portions of n-Hexane. The stop cock was completely opened and just before the lower meniscus reached the sodium sulphate layer, 50 ml n-Hexane was added to the column and discarded. The column was eluted 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 4 ml acetonitrile was added, again concentrated to a volume of less than 1 ml and finally reconstituted in 10 ml acetonitrile.

The extract was subsequently analysed in a HPLC 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^{-1}. For the PAH determination, the following detection parameters were used: fluorescence detector (Ex/Em) 280/330 nm (NAP, ACE and 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 and BaP), 290/410 nm (DhA and BgP) and 300/500 nm (IcP). PAH content (µg l^{-1}) in different samples was calculated by external standard calibration.

Packing materials used for the study

Pouches made of 90 µ low density polyethylene (LDPE) and laminate of 12 µ polyester and 300 gauge polyethylene (PEST/PE) was used for the study.

Shelf life evaluation of liquid smoked masmin

Determination of total volatile base nitrogen (TVBN) and tri-methyl amine-nitrogen (TMA-N) was carried out according to Conway (1950) and expressed as $mgN_2 100\ g^{-1}$ of the sample. Thiobarbituric acid (TBA) was estimated according to Tarladgis *et al*. (1960) and expressed as mg malonaldehyde per kg of the sample. Sensory analysis of the products during shelf life evaluation was carried out by a five member expert panel. The panel evaluated the samples using a 9-point hedonic scale (Meilgaard *et al*., 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. Yeast and mould analysis was performed according to AOAC (2012). Average counts were calculated and expressed as decimal logarithm of, log 10 cfu g^{-1} of the sample. Limit of 100 cfu g^{-1}, under 2-class sampling fixed by FSSAI (2016) was taken as the microbial rejection criterion. Samples were analysed in triplicates and compared statistically by multivariate ANOVA (IBM SPSS Statistics version 20).

Results and discussion

Standardisation of process parameters for production of commercial liquid smoked masmin

Response surface plots of sensory score, total phenolic content and salt content for varying levels of input variables are given in Fig. 1 to 9. 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. Regression coefficients for coded factors with R^2 values are given in Table 2. 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 1 l h^{-1} in the multi-functional smoke kiln at a chamber temperature of 60°C was found to give desirable flavour for the product. The corresponding desirability score was 0.98. Predicted values for response variables are given in Table 3. Result of the validation study indicated that the value of each response variable was in the range of predicted values (Table 3).

Interaction between duration of exposure and salt content in liquid smoke was found to have a significant

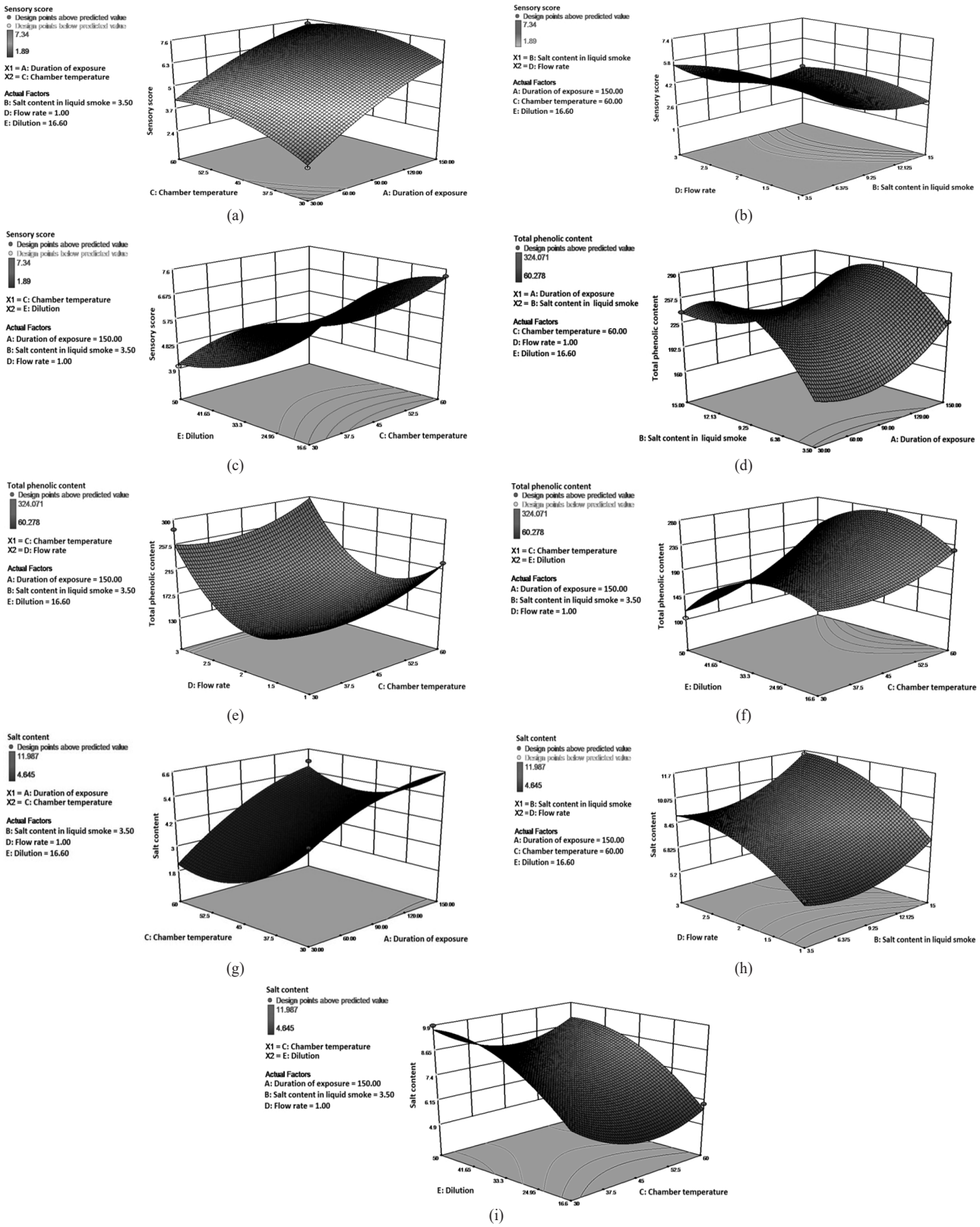

Fig. 1. Response surface plots for the effect of (a): chamber temperature and duration of exposure on sensory score, (b):flow rate and salt content in liquid smoke on sensory score, (c): dilution and chamber temperature on sensory score, (d): salt content in liquid smoke and duration of exposure on total phenolic content, (e): flow rate and chamber temperature on total phenolic content, (f): dilution and chamber temperature on total phenolic content, (g): chamber temperature and duration of exposure on salt content, (h): spraying quantity and salt content in liquid smoke on salt content, (i): dilution and chamber temperature on salt content, in CMLS *masmin*

Table 2. Regression coefficients for coded factors of CMLS *masmin*

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

Table 3. Predicted values for response variables and result of the validation study

	Total phenolic content (ppm)	Salt content (%)	Sensory score (1-8)
Predicted values	226.46	5.95	7
Result of the validation study	189.21±31.14	6.31±1.03	7.56±0.77

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.

Effect of liquid smoking on the PAH content of masmin

PAH content in traditional *masmin* and CMLS *masmin* is presented in Table 4. It is evident from the study that liquid smoking can bring forth substantial reduction in PAH content of *masmin*. Traditional *masmin* showed considerably higher total PAH deposition (480.9±102.4 µg kg^{-1}) compared to CMLS *masmin* (109.54±17.45 µg kg^{-1}) ($p<0.05$). Highest individual PAH present in traditional *masmin* were PHE and PYR. BaA and PHE were the predominant PAHs present in commercial liquid smoked *masmin*. PAH *viz.,* NAP, ACE, DhA and IcP were not detected in any of the samples. Silva *et al*. (2011) have reported a total PAH content of 1320.9 µg kg^{-1} in catfish smoked with firewood. Total PAH content up to 1200 µg kg^{-1} has been reported in cold smoked rainbow trout (Hattula *et al.,* 2001). Visciano *et al.* (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. Light (molecular weight below 216 Da) PAH containing two or three rings are relatively volatile, soluble and are more degradable than higher molecular weight compounds (Heavy PAHs) and are not considered carcinogenic. Significant difference was observed between traditional and CMLS *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 CMLS *masmin* was 64% of the total PAHs. Heavy PAH content in traditional *masmin* was 72.4±21.46 µg kg^{-1} followed by 39.69±7.93 µg kg^{-1} in CMLS *masmin* ($p<0.05$).

Table 4. Comparative PAH content (µg kg^{-1}) in traditional and CMLS *masmin*

PAH	Traditional *masmin*	CMLS *masmin*
NAP	ND	ND
ACE	ND	ND
FLR	11.55 ± 4.21^{a}	5.29 ± 1.24^{a}
PHE	154.37 ± 24.215^{a}	24.64 ± 2.98^{b}
ANT	68.51 ± 12.31^{a}	17.54 ± 1.83^{b}
FLT	35.32 ± 8.71^{a}	11.10 ± 1.1^{b}
PYR	138.75 ± 31.56^{a}	11.27 ± 2.37^{b}
BaA	39.47 ± 10.11^{a}	25.73 ± 4.39^{a}
CHR	13.79 ± 4.52^{a}	1.66 ± 0.45^{b}
BbF	2.54 ± 0.7^{a}	1.15 ± 0.24^{b}
BkF	2.05 ± 0.52^{a}	0.60 ± 0.18^{b}
BaP	14.55 ± 5.61	ND
DhA	ND	ND
BgP	ND	10.55 ± 2.67
IcP	ND	ND
Total PAH	480.9 ± 102.4^{a}	109.54 ± 17.45^{b}
Light PAH	408.5 ± 81^{a}	69.85 ± 9.52^{b}
Heavy PAH	72.4 ± 21.46^{a}	39.69 ± 7.93^{b}
PAH4	70.35 ± 20.94^{a}	28.53 ± 5.08^{b}

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

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^{-1}) followed by CMLS *masmin* (28.53±5.08 µg kg^{-1}). PAH4 content in traditional *masmin* was exceeding the limit of 30 µg kg^{-1} set by European Union (2011).

BaP content in traditional *masmin* was found to be 14.55±5.61 µg kg^{-1}, which also exceeded the regulatory limit of 5 µg kg^{-1} fixed by European Union (2011) and FSSAI (2011). Karl and Leinemann (1996), while comparing the PAH content in traditional kiln smoked fishery products reported a BaP content of 3.9 µg kg^{-1} in eel muscle while the same in smoked halibut muscle was 3.6 µg kg^{-1}. Stumpe-Vıksna *et al.* (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 35.07 µg kg^{-1}) and total PAH (from 47.94 to 470.91 µg kg^{-1}) were observed among different wood species. BaP content in CMLS *masmin* was found to be below the detection limit. Similar results were obtained by Hattula *et al.* (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. Da porto *et al.* (2006) reported that the level of BaP in the distillates obtained from smoked marc was lower than 0.03 µg kg^{-1}.

Shelf life evaluation of commercial liquid smoked masmin

Total volatile base nitrogen (TVBN)

Changes in TVBN content of CMLS *masmin* packed in PE and PEST/PE during storage is given in Fig. 2. Significant statistical difference was observed between the packing materials used in terms of TVBN content ($p<0.05$). CMLS *masmin* packed in PE showed an initial TVBN content of 32.60±0.68, which reached up to 42.97±0.32 during the 12 months storage (32% increase from the initial value). In the case of samples packed in PEST/PE, the initial value of 28.15±0.71 was raised to 37.58±0.28 (33% increase from initial value). Connell (1990) suggested a limit of 100-200 mg TVBN per 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 reported TVBN values in the range of 49 (in unsalted and dried catfish) to 427 mg%

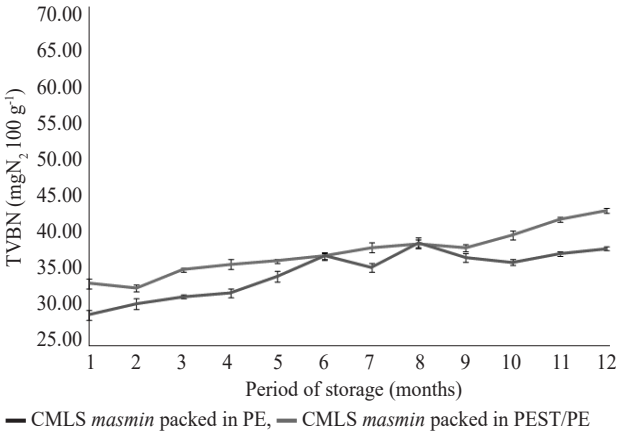

Fig. 2. Changes in TVBN content of CMLS *masmin* packed in PE and PEST/PE

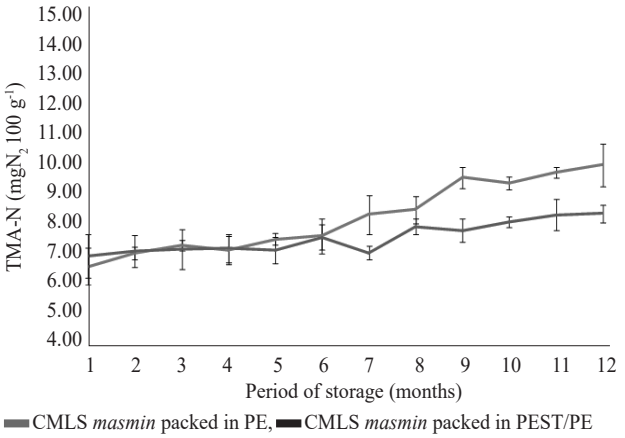

Fig. 3. Changes in TMA-N content of CMLS *masmin* packed in PE and PEST/PE

(in salted and dried mackerel). TVBN values as high as 200±53 mg TVBN 100 g^{-1} has been reported in traditional *masmin* (Kingston *et al.*, 1990). 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 of traditional *masmin*. Gallardo *et al.* (1990) 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. For instance, during traditional *masmin* production, the cooking time usually extends even upto six hours. In the case of improved and liquid smoked *masmin*, the cooking duration does not extend beyond 90 min.

Trimethyl amine nitrogen (TMA-N)

Changes in TMA-N content of CMLS *masmin* packed in PE and PEST/PE during storage is given in Fig. 3. Packaging materials showed a significant influence on the changes in TMA-N content of the samples ($p<0.05$). Highest increase was observed in CMLS *masmin* packed in PE (31% increase from initial value). Samples packed in PEST/PE showed 15% increase from initial value. Limit for TMA-N content in fish for human consumption is fixed as 10-15 mg 100 g^{-1} (Connell, 1980). None of the sample in the present study exceeded this limit. TMA-N content from 9.8 mg100 g^{-1} (in dried *Plectorhinchus schotaf*) to 18.8 mg 100 g^{-1} (in dried *Stolephorus commersonnii*) has been reported by Immaculate *et al.* (2013). Antimicrobial effect of phenolic contents in the commercial liquid smoke is also assumed to be a reason behind the lower TVBN and TMA-N values. The importance of phenolic compounds as antimicrobial (Davidson and Branden, 1981; Kivanc *et al.,* 1991) and antioxidative agents (Toth, 1982; Wittkowski, 1990) in foods has been well documented. In a related study, it was observed that the same commercial liquid smoke at 0.2% concentration was effective in retarding the microbial growth and TVBN formation in tuna sausage (Nithin *et al.,* 2015). Milly *et al.* (2005) reported that nine different commercial liquid smoke fractions have shown antimicrobial properties against a variety of bacteria, yeast and moulds. Sunen *et al.* (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* (Liu *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).

Thiobarbituric acid value (TBA)

Changes in TBA value of CMLS *masmin* packed in PE and PEST/PE is given in Fig. 4. Significant difference was observed between the samples packed in different packing materials ($p<0.05$). Highest increase in TBA value was observed in CMLS *masmin* packed in PE (126% increase from initial value). Samples packed in PEST/PE showed the minimal increase (50% increase from initial value). Reason for the same is expected to be good oxygen barrier properties of the laminate. According to Connell (1990), 1-2 mg malonaldehyde kg^{-1} is usually regarded as the limit beyond which a fish (with a moisture content of 70-80%) will normally develop an objectionable odour/ taste. Both the samples had TBA value higher than this limit, which could be due to the concentration of the Thiobarbituric acid reactive substances (TBRS) during dehydration of meat (Pikul *et al.,* 1984) and due to the intensity of thermal treatment applied on the product (Koizumi *et al.*, 1987). However during sensory analysis, none of the panelists reported rancid flavour associated

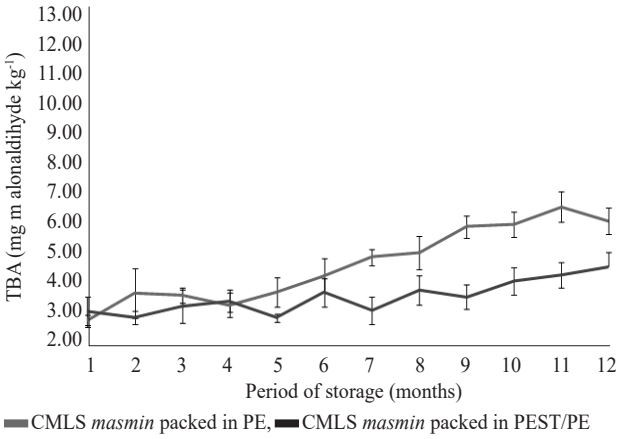

Fig. 4. Changes in TBA content of CMLS *masmin* packed in PE and PEST/PE

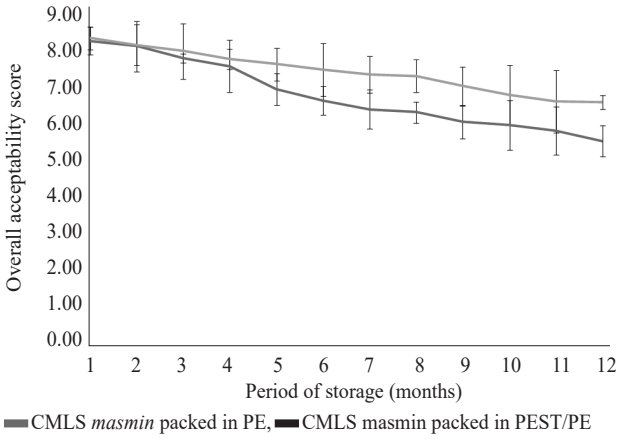

Fig. 5. Changes in overall acceptability of CMLS *masmin* packed in PE and PEST/PE

with the products. Several authors have reported similar discrepancies in the TBA values and corresponding sensory characteristics (Boyd *et al.*, 1993; Frankel, 1993; Koral *et al.*, 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). The presence and importance of aldehydes and maillard reaction in smoked foods have been well documented (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 haeme proteins in the black meat (Balachandran, 2001) along with direct exposure to salt also would have increased the concentration of secondary oxidation products.

Yeast & mould count and overall acceptability

Results 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. Mukundan *et al.* (2003) have reported shelf life of 10-12 months for improved *masmin* fingers and granules. Total bacterial count as high as 6 x 10^4 has been reported in traditional *masmin* (Kingston *et al.*, 1990). Changes in overall acceptability of CMLS *masmin* during storage is given in Fig. 5. A gradual decrease in overall acceptability was observed in all the samples during storage ($p<0.05$). 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. Textural and flavour changes associated with moisture absorption and consequent microbial action would be the reason behind lower acceptability of samples packed in PE.

It is evident from the present study that liquid smoking can be effectively used for mitigating the PAH concerns in smoked products. The developed products have a shelf life comparable to traditional *masmin*. The study is promising towards replication of the technology in other smoked and grilled fishery products. However, the intervention can be made more cost effective by exploring the possibilities of developing indigenous liquid smoke production unit as well as experimenting with other mode of application such as soaking or blending with the food.

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