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Identification of volatile organic compounds with reference to water activity in salt cured and sun dried Indian mackerel (*Rastrelliger kanagurta*) by Headspace Gas Chromatography and Mass Spectrometry (HS-GCMS)

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ABSTRACT

The volatile organic compounds (VOCs) released from salt cured and sun dried Indian mackerel (*Rastrelliger kanagurta*) was studied by headspace gas chromatography and mass spectrometry (HS-GCMS). Volatile profile from the stage of salt curing till sun drying upto 4 days were profiled which revealed diversifications in their production and occurrence. Alcohols *viz*., 1-Penten 3-ol, 1-Octen 3-ol and 3-Methyl butanol were dominating in the volatile profile from salt curing stage to 2 days of sun drying. Aldehyde hexanal was first detected after first day sun dried samples which peaked in 2nd day dried fish. The presence of various esters were detected in samples dried for two days or more. The moisture content (%) and water activity (a_w) of the samples were found to be influential in production and diversity in the profile of VOCs as the percentage of volatiles decreased with the reduction of these parameters. These VOCs detected are reportedly responsible for the odour, aroma and other flavour characteristics of dry fish in general and their mechanism of production is discussed.

Keywords: Headspace GCMS, Indian mackerel, Salt curing and sun drying, Volatile organic compounds, Water activity

Introduction

Drying is a common method to preserve food which is applicable to protein rich food like meat and fish. It is a widely used food processing technology not only to conserve food, but also to minimise the postharvest loss, where accessibility to preserving facilities and methodologies are limited. This is very much applicable in case of fisheries sector, where it contributes significantly towards food security especially in low income food deficient countries by extending 22% of animal protein. Unlike other food stuffs, fish has a unique odour having desirable and undesirable aroma which are due to the volatile organic compounds released due to various biochemical reactions that takes place after harvest. Traditional processing of fish such as salting and drying influences this aroma composition (Chung *et al.,* 2007).

Analysis of volatiles from solid substances encounters a variety of problems which are solved by the use of headspace gas chromatography (HS-GC). This technique is based on principle of sampling analytes from the atmosphere around the sample (Marsili, 2002). The advantage of this method is the elimination of organic solvents resulting in a chromatogram without solvent peak which ensures no masking of eluting analytes. Aliquots of the gas phase over the sample is sampled using a gas tight syringe after equilibration attained between analyte concentration in the sample and headspace in a sealed vial. Injection of sample aliquot to the gas chromatograph however takes place at a temperature and pressure above ambient conditions (Slack *et al.,* 2003). Since the vapour phase thus collected does not contain any macromolecular or ionic interfering substances from the sample matrix, it can be directly transferred to GC for analysis. In modern analytical chemistry, gas chromatography coupled with mass spectrometry (GCMS) is considered as a sensitive and efficient analytical technique to profile the volatile characteristics of seafood (Elmore, 2008). Automated headspace sampling in conjugation with GCMS is an effective method to characterise fish volatiles as demonstrated by Duflos *et al.* (2006).

The present study was undertaken with an aim to isolate and identify volatile organic compounds released during curing and the successive drying stages and to explore the influence of water activity (a_w) on production of volatile organic compounds (VOCs). The fish species used for the study was Indian mackerel, *Rastrelliger*

kanagurta. Selection of the species was based on their wide spread availability along the Indian coast and due to the large market preference as dry fish.

Materials and methods

Samples of Indian mackerel were procured from local fish markets in Kochi, Kerala. Fish samples at different drying stages used for the study were prepared by dry salting method. Dry salting was done with 25% (w/w) salt for 24 h. The samples were then sun dried for four days with intermittent sampling, designated as salt cured fish without drying (SCF), salt cured and 1 day dried fish (SCDF 1D), salt cured and 2 days dried fish (SCDF 2D), salt cured and 3 days dried fish (SCDF 3D) salt cured and 4 days dried fish (SCDF 4D) and used for further analysis.

Determination of moisture content and water activity (a_w)

Moisture content of fish samples used for analysis at each stage was determined using a moisture analyser (Mettler Toledo MJ 33-Switzerland). The water activity (a_w) of the samples were determined prior to the GCMS analysis with a water activity meter (Lab Swift-aw Switzerland). The values are expressed as mean ± standard deviation obtained from five replicates.

Headspace-Gas Chromatography Mass Spectrometry (HS-GCMS)

Agilent GC system 7890A, equipped with a 5975C Inert MS Detector with triple-axis detector and G1888 network headspace sampler was used for the headspace analysis of the volatiles. The chromatographic column was HP-5MS capillary column (30m × 250 µm × 0.25 µm thickness of 5% phenyl-95% methyl siloxane). High pure helium was used as the carrier gas with a flow rate of 1 ml min^{-1}. Two grams of finely powdered samples were placed in 20 ml flat bottom headspace vials fitted with PTFE/silicone septum and crimp cramp and used for analysis. The volatile compounds were identified by comparing and matching mass spectra fragment with the standard mass spectra of NIST MS 08 spectral library with Mass Spectral Search Program Version 2.0. The volatiles were confirmed with a spectral default fit value of 90% and above. The samples were run in duplicate. The relative content (%) of the identified volatiles was calculated using the following formula:

Relative content (%) of compound = Single constituent area/ total area x 100

GC and headspace conditions

The GC oven temperature was set to 40ºC initially and held for 7 min, further heated up to 100ºC at the rate of 10ºC min^{-1} and held for 3 min and then ramped to 200°C at a rate of 10°C min^{-1}, where it was held for 6 min. Temperature was increased from 200 to 230°C at 20°C min^{-1} and held for 2 min. The injector was operated in splitless mode with line temperature of GC at 250°C and MS transfer line temperature was 230°C. The carrier gas flow rate was 1ml min^{-1}. Two microlitre each of the samples were injected in splitless mode. The headspace conditions were programmed as 80°C (oven temperature), 90°C (loop temperature) and 100°C (transfer line temperature). Vial equilibration, pressurisation, loop fill, loop equilibrium and inject were set at 15.0, 0.20, 0.20, 0.05, and 1min, respectively. The temperature for MS quad and MS source were programmed at 230°C and 150°C respectively. All analyses were performed at an ionisation energy of 70 eV. Data were obtained by full scan of mass spectra within the range of 29-550 (*m/z*).

Statistical analysis

The data analysis was performed in IBM SPSS Statistic version 20. One-way ANOVA at 5% level of significance was performed to compare the means of percentage of individual VOCs, classes of VOCs, moisture percentage and water activity. Tukey's multiple comparison tests were used for *post-hoc* analysis. The results are expressed as mean±standard deviation.

Results and discussion

Isolation and identification of Headspace Volatile Organic Compounds (HS-VOC_S)

The HS-GCMS study results revealed that the volatile composition responsible for the odours changed significantly as the drying process progressed. The total ion chromatogram obtained from the analysis of the dried fish samples of various days are given in Fig. 1(a-e). Six volatile compounds were detected during the progressive stages of curing and drying and based on their structure and general properties, the compounds are classified in 3 groups *viz*., 3 alcohols, 1 aldehyde and 2 esters. Alcohols were the dominating class of volatiles in the stages of salt curing and early drying, the relative content of which was statistically significant from other stages of drying ($p<0.05$) and reduced as the drying progressed (Table 1). 1-Penten 3-ol, 3-Methyl butanol and 1-Octen 3-ol are the isolated and identified alcohols (Table 2). The percentage of 1-Penten 3-ol was peaked in SCDF 1D ($p<0.05$) and gradually reduced as the drying process progressed (Fig. 2). The presence of 1-Penten 3-ol is reported in studies on canned tuna (Kim and Lindsay, 1992) and salmon volatiles (Girard and Durance, 2010) and referred as an indicator of early oxidation in fish oil and omega-3-rich fish by Nordvi *et al.* (2007). It was observed in GCMS analytical studies of cold smoked salmon (Jorgensen *et al.,* 2001) and reported at high levels in headspace extracts of other fishes and sea food (Elmore, 2008). The compound

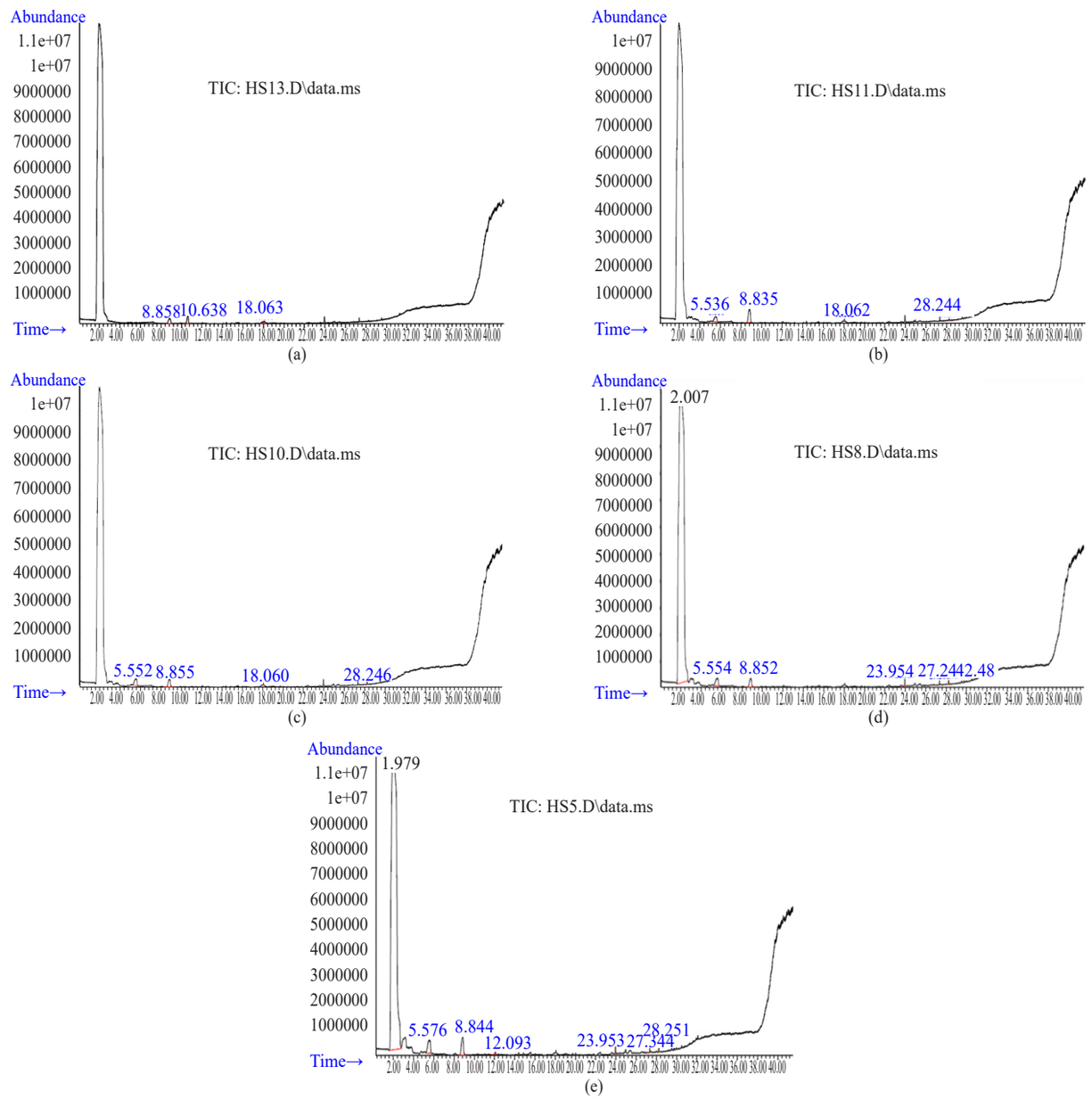

Fig. 1. Total ion chromatogram of HS-VOCs isolated from *Rasrelliger kanagurta* at different drying stages. (a) SCF; (b) SCDF 1D; (c) SCDF 2D; (d) SCDF 3D; (e) SCDF 4D

Table 1. Relative content (%) of VOCs detected in salt cured and dried Indian mackerel

Samples	a_w (Mean ± SD)	Moisture % (Mean ± SD)	Percentage of VOCs		
			Alcohols	Aldehydes	Esters
SCF	0.8354 ± 0.0016^a	53.47 ± 0.29^a	99.17 ± 1.17^a	ND	ND
SCDF 1D	0.7318 ± 0.0010^b	43.80 ±0.53^b	68.55 ± 3.51^b	29.68 ±3.316^a	1.78 ± 0.194^a
SCDF 2D	0.7226 ± 0.0008^c	37.36 ± 0.36^c	48.03 ± 1.26^c	47.80 ±1.104^b	2.29 ± 0.291^b
SCDF 3D	0.7186 ± 0.0005^d	34.37 ± 0.33^d	2.32 ± 0.045^d	2.17 ± 0.143^c	0.21 ± 0.022^c
SCDF 4D	0.718 ± 0.0000^d	32.55 ± 0.24^e	1.18 ± 0.052^d	1.31 ± 0.037^c	0.17 ± 0.057^c

Different superscripts in same column indicate significant difference between treatment means ($p<0.05$)

is responsible for fresh fish odour and emerges from lipid oxidation. Alcohols generally may be formed by decomposition of secondary hydroperoxides of fatty acids and a rearrangement and cleavage of hydroperoxides from linoleic or arachidonic acids could yield 1-penten-3-ol (Wurzenberger *et al.,* 1986). Decomposition product of n-3PUFA 2,4-heptadienal, also yields the compound as reported by Olsen *et al.* (2005). The compound has been reported to have different types of odour/ aroma/flavour such as fishy and grassy (Tao *et al.,* 2014); burnt, meaty and pungent odour (Giri *et al.,* 2010); solvent, vegetal, spicy aroma (Girad and Durance, 2000) and fatty, hay or grass flavour (Cha *et al.,* 1999).

1-Octen 3-ol, was abundant in SCF ($p<0.05$) (Table 2) and reduced with progress in drying (Fig. 2). The occurrence of 1-Octen 3-ol in cod fish was determined by Bjorkevoll *et al.* (2008), in mackerel by Duflos *et al.* (2006) and reported that oxidation of arachidonic acid by enzymatic action of 12 lipoxygenase produce 1-Octen 3-ol and other short chain carbonyls. Phospholipid membranes in vertebrates contain fatty acids such as linoleic acid or arachidonic acid in abundance and autolysis of arachidonic acid by the skin enzyme system was found to produce 1-Octen-3-ol in ayu fish *Plecoglossus altivelis* (Zhang *et al.,* 1992). The compound is characterised by a fermented mushroom odour (Frank *et al.,* 2009) and is well known as mushroom alcohol. It has an aroma like earthy, herbaceous and spicy as described by Girard and Durance (2010) and like fresh fish as reported by Leduc *et al.* (2012). In salted codfish 1-Octen 3-ol and1-Penten 3-ol are regarded as the key aroma volatiles (Bjorkevoll *et al.,* 2008).

3-Methyl butanol was detected only in SCF (Table 2, Fig. 2). Joffraud *et al.* (2001) and Jorgensen *et al.* (2001) reported the presence of 3-Methyl butanol in GCMS analysis of cold smoked salmon, Duflos *et al.* (2006) in mackerel and Jin *et al.* (2015) in seafood squids. As a result of protein degradation by microbial spoilage and growth of *Pseudomonas* sp., methyl butanol is produced in fish and also by the action of *Shewanella putrefaciens* (Polo *et al.,* 2014). Oxidative deamination of free amino acid precursors of leucine by Ehrlich mechanism also results in the production of this compound (Olafsdottir *et al.,* 2005). Chi-tang (2001) reported that Strecker degradation of amino acids generates the compound. *Moraxella phenylpyruvica*, *Staphylococcus xylosus* and *Staphylococcus starnosus transforme* degrade amino acid leucine to 3-Methyl-butanol. According to Boumba *et al.* (2008), degradation of amino acids such as threonine, leucine, isoleucine and valine by the action of yeast also produce 3-Methyl butanol. The compound has a pungent, solvent aroma (Girard and Nakai, 1993) and odour like balasmic, burnt malt (Giri *et al.,* 2010).

In the case of aldehyde, hexanal was not detected in SCF, however the production was detected in SCFD 1D and peaked in SCFD 2D ($p<0.05$) (Table 2). This was the only aldehyde detected in all samples, the content of which gradually decreased from 3rd day of drying (Fig. 3). Hexanal is reported in headspace volatile studies of many Mediterranean fish species, freeze dried krill and cold smoked salmon (Giogios *et al.,* 2013; Park *et al.,* 2014) and the compound is regarded as volatile, responsible for fresh fish aroma (Duflos *et al.,* 2006). This aldehyde is formed by oxidation of n-6 PUFA or peroxidation of

Table 2. Relative content (%) of individual VOCs detected in salt cured and dried Indian mackerel

Retention time (min.)	Compounds	Spectral match value (%)	Relative content (%) of individual VOCs identified by MS				
			SCF	SCDF1D	SCDF2D	SCDF 3D	SCDF4D
5.55	Hexanal	96	ND	29.68 ± 3.32[a]	47.80 ± 1.10[b]	2.17 ± 0.029[c]	1.31 ± 0.037[c]
8.85	1-Penten-3ol	94	40.30 ± 0.91[a]	63.69 ± 0.80[b]	45.91 ± 1.17[c]	2.32 ± 0.04d	1.17 ± 0.052[d]
10.63	3-Methyl butanol	96	39.24 ± 0.56	ND	ND	ND	ND
18.06	1-Octen -3ol	96	19.63 ± 0.30[a]	5.29 ± 4.31[b]	2.11 ± 0.088[b]	ND	ND
23.95	Isobutyl ester	94	ND	ND	ND	0.23 ± 0.011[a]	0.21 ± 0 .012[a]
28.24	Propanediyl ester	93	ND	1.78 ± 0.19[a]	2.29 ± 0.29[b]	0.19 ± 0.028[c]	0.13 ± 0.028[c]

Different superscripts in the same row indicate significant difference between treatment means ($p<0.05$)

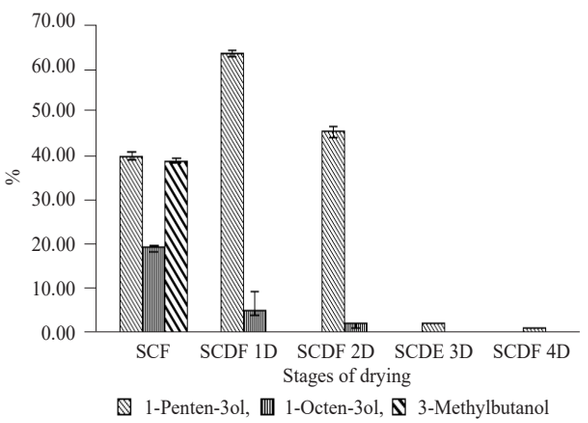

Fig. 2. Relative content of alcohols

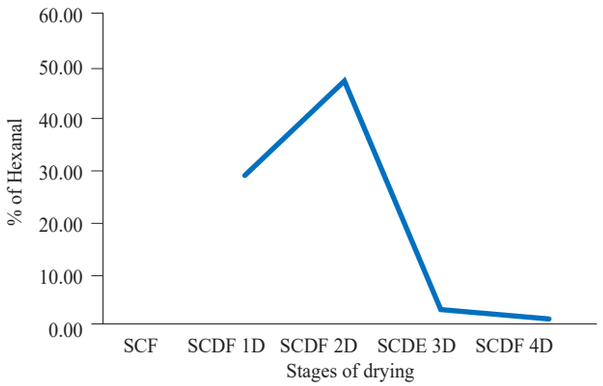

Fig. 3. Relative content of Hexanal

linoleic acid (Bjorkevoll *et al.,* 2008). The compound has a fishy, grassy, leafy, green odour (Giri *et al.,* 2010; Tao *et al.,* 2014) and provides a fresh flavour and aroma to fish.

Similarly, the ester compound was detected initially in SCDF 1D, but peaked significantly in SCDF 2D ($p<0.05$) (Table 2) and their production diversified in SCDF 3D and SCDF 4D and reduced with degree of drying (Fig. 4). The ester compound, propanoic acid (2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester) was detected initially in SCDF 1D and in addition in SCDF 3D, SCDF 4D samples, pentanoic acid (2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester) was also detected (Table 2, Fig. 4). Solid phase micro extraction studies of liquid extract of crab *Ovalipes punctatus* by Bu *et al.* (2013) reported the occurrence of Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester. GCMS detection of Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester was carried out from Chinese spicy beef by Gong *et al.* (2014). Generally, microbial and enzymatic decomposition of lipids results in the production of carboxylic acids and the esterification of alcohols with these carboxylic acids results in ester

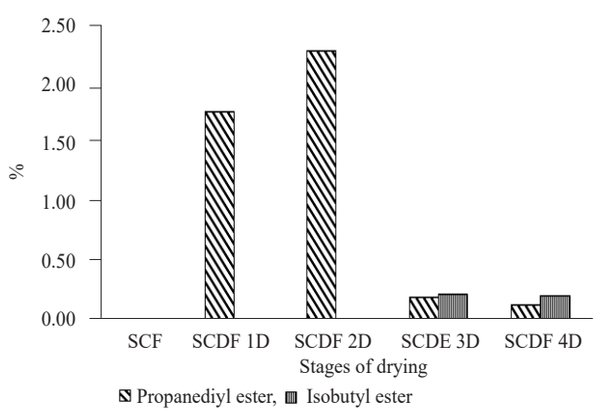

Fig. 4. Relative content of ester compounds

formation (Xie and Liu, 2012). In fermented dairy products, transesterification of triglycerides and ethanol results in the formation of esters (Liu *et al.,* 2004). It is reported that esters are found at the end of maturation process in dry-cured hams and the concentration of NaCl stimulates enzymes, esterases to produce esters (Armenteros *et al.,* 2012). Sabio *et al.* (1998) opined that higher the content of alcohols, higher the concentration of esters as it is formed by the interaction of free fatty acids and alcohols through lipid oxidation. Esters generally have a fruity and floral odour and reported to have sweet fruity and candy like flavour in salted and fermented anchovies (Cha *et al.,* 2006).

Fish and other seafood contain volatile compounds of similar nature responsible for similar aromas apart from their species specific aromas. The aroma composition is affected by all kinds of processing including salting and drying (Chung *et al.,* 2007). Addition of curing agents such as salt during the pre-processing stage of curing and drying affect the volatile proportion (Flores, 1997). The desirable and undesirable odour in fish which changes from point of harvest to spoilage and also during processing are due to enzymatic or auto oxidative reactions. Fish is rich in polyunsaturated fatty acids (PUFA) and lipid oxidation takes place even in chilled and frozen products which yields undesirable aromas by the production of volatile organic compounds (VOCs) (Fratini *et al.,* 2012). Fat act as a precursor for volatile compounds and as aroma compound solvent, while protein degradation lead to the formation of aromatic compounds. Proteins have an effect on flavour and aroma perception due to its interaction with aroma compounds and influence the headspace volatile concentration (Perez-Juan *et al.,* 2008).

Effect of water activity (a_w) on production of VOCs

Influence of (a_w) and moisture content on the production of VOCs are given in Fig. 5. The moisture content and a_w have significant effect on the production of VOC. In SCF, when the a_w was 0.835 and moisture content 53.47%, only production of alcohols was detected which was upto 99.1% and were statistically significant ($p<0.05$) (Table 1). In SCDF 1D, the VOC profile changed with reduction in alcohol (68.55%) and presence of aldehydes (29.68%) and esters (1.78%) were detected when the a_w was reduced to 0.73 and moisture 43.8% respectively. At a_w of 0.72 and moisture of 37.3%, significant increase in the percentage of aldehyde was detected ($p<0.05$) in SCDF 2D, where the alcohol contents were further reduced to 48.03% and esters were also at their peak (2.29%) (Table 1). When moisture content reduced below 34.3% and a_w below 0.71, the percentage of all VOCs detected was very low and was homogenous ($p>0.05$) in SCDF 3D and SCDF 4D (Fig. 5).

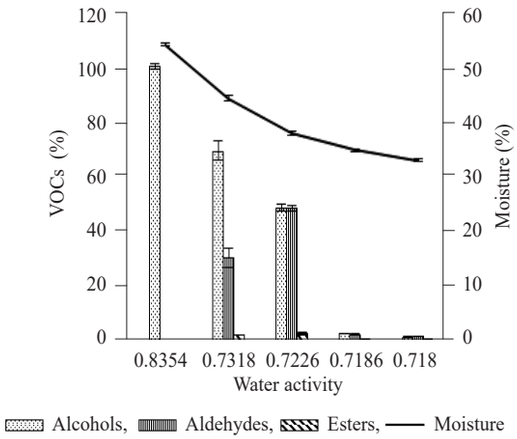

Fig. 5. Effect of water activity (a_w) and moisture content (%) on VOCs detected

The effect of a_w also could explain the profile of VOCs detected from stages of salt curing and further drying. The maximum volatile compound production occurred in the salt curing stage and early stages of drying (SCF, SCDF 1D and SCDF 2D), where the a_w was between 0.83 and 0.72 and moisture content was between of 53.46 and 43.80 (Fig. 5). The compounds were the alcohols *viz*., 1-Penten 3-ol, 1-Octen 3-ol, 3-Methyl butanol, the percentage of which considerably decreased as the water activity reduced (Table 1, Fig. 2). These compounds are mainly the lipid oxidation products (Olafsdottir *et al.,* 2005; Duflos *et al.,* 2006; Nordvi *et al.,* 2007; Bjorkevoll *et al.,* 2008) and or microbial activity product as in the case of 3-Methyl butanol (Polo *et al.,* 2014) and the production might have facilitated by relatively higher content of moisture and a_w.

The production of aldehyde hexanal was detected between a_w of 0.73 and 0.71 (SCDF 1D to SCDF 4D) with a higher percentage at a_w of 0.72 (Fig. 5). Hexanal is oxidation product of fatty acid (Bjorkevoll *et al.,* 2008) and its occurrence at this stage might be due to the lipoxygenase enzyme activity on unsaturated fatty acid as reported by Fakruddin *et al.* (2013) that might have taken place even at this water activity level. The pro oxidant effect of salt in the production of carbonyl compounds in dry cured ham as observed by Perez-Juan *et al.* (2007) also could explain the production and variation of percentage of hexanal in the salt cured and dried fish. Since the compound is considered as a flavour enhancer, the salt content in fish would have prompted the formation of strong ion-dipole interaction between salt ions and water, resulting in the retention of the flavour compounds in meat products as reported by Rabe *et al.* (2003) and would also account for its abundance at water activity (a_w) of 0.72.

According to Fenster *et al.* (2003), the accumulation of esters in cheese are water activity (a_w) dependent. Though the occurrence of an ester was observed initially at a water activity (a_w) of 0.73, the diversification was determined between 0.72 to 0.71, where the moisture content of the samples was 37.3 to 32.5% (Table 1). At low water activity, in the presence of lipases, fatty acids and alcohols are esterified in fish oils (Frankel, 2014). Armenteros *et al.* (2012) reported that esters are found at the end of maturation process in dry cured hams and the concentration of NaCl stimulates enzymes esterases to produce esters. Since in the analysed samples, SCDF 3D and SCDF 4D were with less moisture content and hence with high NaCl concentration, the same effect can be attributed to the ester production in the samples.

The study revealed that the volatile profile of salt cured fish during successive drying stages are in accordance with the reported volatile profile studies of other fishes and seafood. The changes in the emission of volatile odoriferous compounds by various cellular mechanisms can be considered as biomarkers of the particular stage of drying. The composition of VOCs was found to be affected by addition of curing agents such as salt, which also varied with the drying stages, moisture content and water activity (a_w) parameters.

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