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Effect of PLA/Gelatin/PBAT-Based Three-Layer Film with Added *Physalis* Leaf Extract on Shelf-Life Extension of Fish Meat Powder[§]

Running head: Applications of PLE added PLA/Gelatin/PBAT-Based Three-Layer Film

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SUMMARY

Research background. Nowadays, there is growing interest in active packaging infused with natural extracts due to safety concerns and consumer preferences. *Physalis angulata* is a medicinal and edible species of Solanaceae family and rich in phenolic compounds. Phenolic compounds, the secondary metabolites are synthesized and stored in all plant tissues, which acts as a plasticizers/filler material can be used to enhance the

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interfacial interaction between the two biopolymers and prevent moisture and gas transmission from the food product and prolong the shelf life to some degree.

Experimental approach. Effect of fish gelatin based (Poly lactic acid (PLA)/Gelatin/Poly butylene adipate-co-terephthalate (PBAT)) three-layer film incorporated with and without *Physalis* leaf extract (PLE) on quality changes of fish meat powder stored at 27–30 °C (30 days) were investigated, in comparison with uncovered samples and those covered with polyethylene (PE) film, PLA, PBAT and gelatin films. Fish meat powder was sealed in a cylindrical bottle covered with developed films. The storage characteristics such as moisture content, pH, peroxide value (PV), thiobarbutric acid reactive substances (TBARS), total volatile base-nitrogen (TVB-N), changes in colour, sensory properties and volatile compounds of fish meat powder that packed with the developed film were analysed.

Results and conclusions. Moisture content of meat powder was lower for sample covered with film (P/G/B-PLE-7%) throughout the storage period. However, the sample covered with PE film exhibited highest PV amongst others ($p<0.05$). TBARS, TVB-N as well as volatile compounds lowered for the samples covered with P/G/B-PLE-7% at 30th day of storage. PLE incorporation into three-layer film improved the properties of the film and prolonged the shelf life of fish meat powder. Thus, the addition of PLE into three-layer film might serve as biodegradable active packaging and could be a promising substitute to commercial plastic films.

Novelty and scientific contribution. This is the first report on examining the chemical changes of fish meat powder covered with PLA/Gelatin/PBAT three-layer film incorporated with PLE. This will provide better understanding about the role of three-layer film containing *Physalis* leaf extract on shelf life extension of fish meat powder.

Keywords: three-layer film; fish skin gelatin; *Physalis* leaf extract; bioplastics; lipid oxidation; volatile compounds

INTRODUCTION

Food is being externally preserved through packaging during all supply chains, including storage, transit and distribution. Food products, especially meat and meat products, need to be packed and preserved from both intrinsic and extrinsic factors. Food packaging should withstand environmental contaminants as well as other factors such as temperature, odour, physical damage, dust, shocks, humidity and microorganisms. Packaging must

enhance the shelf life of preserved food product and reduce food loss and wastage (*1*). The usage of traditional chemical preservatives in food formulation has been reduced due to safety concerns. Nowadays, novel natural additives have received much interest as a promising substitute alternative to chemical additives (*2*), particularly in the form of active additives incorporated in packaging.

Gelatin, a highly effective biopolymer known for its exceptional film-forming properties and versatility among animal proteins (*3*,*4*). Gelatin can be employed as the outstanding material for the food packaging which has high oxygen barrier and UV blocking properties (*5*). Hence, the gelatin based packaging films can prevent the lipid oxidation in seafoods and further extend the shelf life due to the remarkable oxygen barrier property (*6*). However, the gelatin films have inadequate (mechanical and water vapour permeability (WVP)) barrier properties mostly due to its hydrophilicity. The recent study has found that the inclusion of copolymers lowered the stiffness and enhanced the barrier properties of gelatin film (*7*).

PLA (Poly lactic acid), an easily degradable polyester with outstanding attributes such as hydrophobicity, high mechanical strength, biocompatibility and thermal plasticity, comparable to many petroleum-based polymers (*8*). PLA, a renewable plastic (*9*) has been used in a wide range of fields including commercial packaging and pharmaceutical as well as biological applications. However, it has certain constraints, most notably weak durability and high brittleness (*10*).

PBAT (Poly butylene adipate-co-terephthalate), an emerging biodegradable polymer that shows great potential as a substitute for packaging applications. This could be attributed to its outstanding processability, exceptional elongation at break, biocompatibility, superior thermal properties and excellent flexibility (*11*). PBAT, a flexible synthetic aliphatic-aromatic copolyester, exhibits tensile properties comparable to low-density polyethylene (LDPE) while offering excellent thermal and mechanical characteristics *(12)*. However, its commercial use is limited due to its high cost and relatively greater water vapor permeability compared to conventional films (*13*).

The natural compounds present in plant extracts and essential oils are significant for human health promotion and food preservation (*14*). Natural extracts are thus of great interest to food makers as potential substitutes for synthetic additives (*15*). The numerous fruit and plant parts of agricultural crops have been proven to be a reliable supply as fillers in packaging application. Plant extracts have attracted a lot of interest, as they have a higher level of phenolic components, which have excellent cross-linking ability, antioxidant and antibacterial

functions. Food packaging films developed with the addition of natural extracts showed modified barrier properties and bioactivities of films (*16*). The main purpose of packaging systems is to restrict the transfer of moisture and gases either from or to the product.

A crucial barrier that measures a film's resistance to water vapor called WVP (water vapor permeability) (*17*). Reduced water vapor transfer between food (internal) and the environment (external) due to lower WVP of packaging is preferable to extend the shelf life of the product (*18*). Incorporating the extract, which possesses hydrophobic properties, might enhance the film's overall hydrophobicity, thereby reducing its WVP (*16*). Incorporation of polyphenols rich extract with gelatin nanocomposite films lowered the levels of WVP and moisture content of stored fish meat powder (*19*). Green tea extract incorporated with PLA significantly lowered the levels of TVB-N in salmon (smoked) (*20*). The significant reduction of the upsurge in the pH, TBARS and TVB-N has been recorded in golden pompano fillets covered with gelatin film (*21*). The presence of basil leaf essential oil with gelatin nanocomposite film significantly showed reduction of pH, PV, TBARS and TVB-N in sea bass slices (*22*). Thus, the aim of this study focused on the chemical changes of fish meat powder covered with PLA/Gelatin/PBAT three-layer film incorporated with and without *Physalis* leaf extract during the storage period of 30 days (27–30 °C).

MATERIALS AND METHODS

Chemicals

Fish skin gelatin, PBAT and PLA were purchased from Vihn Hoan (Dong Thap Province, Vietnam), Jinhui Zhalong High Technology Co., Ltd. (Shanxi, China) and NaturTec (Chennai, India), respectively. Trichloroacetic acid and 2-thiobarbituric acid were procured from Himedia Laboratories Pvt. Ltd. (Maharastra, India). Glycerol, chloroform, sodium hydroxide, potassium iodide, sodium thiosulfate, starch, acetic acid, boric acid, perchloric acid, sodium sulfate and hydrochloric acid were procured from Chemspure Pvt. Ltd. (Chennai, India).

Extraction of ethanolic extract from Physalis angulata

Ethanolic extraction was done (*7*,*23,24*) and obtained extract was referred as *Physalis* Leaf Extract (PLE).

Preparation of three-layer film from gelatin and bioplastics incorporated with PLE

Fish skin gelatin based three-layer film (P20/G60/B20) were prepared using fish gelatin and bioplastics (PLA and PBAT) at 4 % (*m/V*) as defined by Murugan *et al.* (*7*) with the incorporation of PLE at 0 and 7 % for each layer and named as P/G/B-PLE-0% and P/G/B-PLE-7%. For control gelatin, PLA and PBAT films were prepared by casting GFFS, PFFS and BFFS on a plastic (GFFS) and glass petri plate (PFFS and BFFS) with solid content of 4 % (*m/V*).

Study on shelf-life extension of fish meat powder using gelatin based three-layer films with and without PLE

Preparation of fish meat powder

Fresh fish (mackerel) fillets were obtained from Pulicat landing centre, Tiruvallur, India and dried (60 °C for 10 h) in a tray dryer (Everflow Scientific Instruments, Chennai, India). The dried sample was then uniformly ground using a blender and named as "fish meat powder."

Storage of fish meat powder with and without different films

Sample (25 g) was sealed in cylindrical aluminium cups (30 mm of diameter), which was covered with PE, PLA, PBAT and gelatin films also with the both sides of three-layer films incorporated with and without PLE. The rubber gaskets and silicone vacuum grease were used to seal the cups. Meat powder was stored at 27-30 °C (25±5 % RH). For the control, meat powder was uncovered in aluminium cups. Polyethylene (PE) film had a thickness of 0.032 mm and developed films in the range of 0.0485 to 0.0645 mm. The samples were analysed for 30 days with interval of 5 days. For the evaluation of volatile compounds and colour, the meat powder stored for 0 and 30 days was used.

Analyses

Moisture content

The moisture content of preserved fish meat powder was analysed as per the AOAC method (*25*).

pH

pH measurement of fish meat powder was done as previously documented (*26*).

Peroxide value (PV)

Sample (10 g) was homogenized with chloroform (50 mL) and then filtered. Glacial acetic acid (15 mL) and potassium iodide solution (10 %, 10 mL) were added to the filtrate and kept in the dark (10 min). Then, distilled water (50 mL) and starch solution (1 mL) were added (*25*). The liberated iodine was titrated against $Na_2S_2O_3$ (0.02 N) until the disappearance of blue colour occurred. Chloroform extract (15 mL) was taken in pre-weighed beaker, and the solvent was evaporated in the water bath. The sample was incubated (100 °C) and further cooled in desiccator. The weight of fat deposited in a beaker was determined and the amount of iodine liberated per gram of fat was analysed and expressed as milli equivalent of peroxides/kg fat (PV/(mmol/kg)).

Thiobarbutric acid reactive substances (TBARS)

Sample (20 g) was added with cold trichloro acetic acid, TCA (20 %, 50 mL) and rinsed with distilled water and filtered. Then, filtrate was collected and termed as TCA extract. TCA extract (5 mL) was mixed with 0.01M TBA (5 mL). TCA (10 %, 5 mL) was referred to as blank solution. TCA, TBA and blank solutions were heated (100 °C for 30 min) (*27*). The sample's absorbance at 532 nm was measured using a spectrophotometer, and the TBARS value was expressed as mg malondialdehyde (MDA)/kg of sample (*w*(MDA)/(mg/kg)).

Total Volatile Base-Nitrogen (TVB-N)

Sample (10 g) was homogenized with perchloric acid (50 mL) to precipitate the protein and followed by centrifugation (4000 rpm for 5 min) to obtain supernatant. Supernatant (5 mL) was allowed for distillation in Kjeldhal apparatus for 10 min (*25*). The distillate was collected in a digestion tube added with boric acid (10 mL) and mixed indicator (2-3 drops). The solution was titrated against HCl until the pink colour appeared and the values were expressed as (*w*(TVB-N)/%).

Colour

Colour of fish meat powder was assessed by using Chromameter (MiniScan EZ 4000, Hunter lab, Reston, VA, USA). Δ*E** (total difference in colour) was computed using the following Eq. 1 (*28*).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad /1/$$

Sensory analysis

Colour, odour and OAL (overall likeness) was performed with 20 untrained panelists who were familiar with the fish (mackerel) consumption by using a 9-point hedonic scale (*29*). The test was conducted on 0, 15 and 30th day of storage.

Analysis of volatile compounds

The volatile compounds in the samples stored at day 0 and the chosen samples that were covered with PE, P/G/B-PLE-7% and control film (uncovered) stored for 30 days were measured using a SPME-GCMS (Solid-phase micro extraction gas chromatography mass spectrometry) (*30*).

Statistical analyses

A one-way ANOVA was performed, and the means were analysed using Duncan's Multiple Range Test for comparison using SPSS software v. 26.0 (*31*).

RESULTS AND DISCUSSION

Changes in moisture content of fish meat powder covered with different films during storage

The moisture content of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the 30 days of storage period (27–30 °C) (Fig. 1a). The moisture content of sample at 0th day was 2.78 %. The fish meat powder preserved with films exhibited lower water absorption rate throughout the storage period, irrespective of film types than the uncovered sample ($p<0.05$). However, the higher moisture content detected for fish meat powder preserved with gelatin film ($p<0.05$). In three-layer films, the presence of PLA and PBAT might prevent the entry of water molecules into the film matrix. This was plausibly due to the hydrophobicity of those bioplastics (*32*). In contrast, gelatin film showed poor resistance against water, due to its hydrophilicity (*33*). Cervera *et al.* (*34*) stated that the higher moisture content found in hydrophilic film was plausibly due to the interactions between the water vapor and hydrophilic polymer matrix. Therefore, there was an increase in the permeability of water vapor through the matrix of gelatin film. Generally, all samples were found to be increased moisture content due to the augmented environmental moisture absorption through the packing material (*34*). The sample covered with film, in which PLA side

of three-layer (P/G/B-PLE-0 and 7 %) film exposed to the environment showed lower moisture content than PBAT side (B/G/P-PLE-0 and 7 %) as the outer sider, irrespective of the addition of PLE. In general, P/G/B-PLE-7% exhibited the lowest moisture content of the sample throughout the 30 days of storage when compared to other bio-based films. This was plausibly due to the organized film matrix formed by the effective H-bonding between the -OH group of PLE with the polymeric chains and the "tortuous pathway" (*35*). However, the lowermost moisture content observed for PE covered samples throughout the 30 days of storage. PE had better resistance to water than the protein-based films (*36*). Thus, the findings indicated that the WVP of fish gelatin film enhanced by the presence of bioplastics and left extract (PLE).

Changes in pH of fish meat powder covered with different films during storage

pH of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the 30 days of storage period (27–30 °C) (Fig. 1b). pH of all meat powder samples gradually increased during storage. However, the highest pH recorded for sample uncovered with any films throughout the entire storage time ($p<0.05$). The formation of alkaline substances (ammonia) and the enzymatic activities of microbes were responsible for higher pH (*37*). However, the lower pH reported for fish slices packed with gelatin based film (*22*). Microbiological quality of meat/meat powder was directly correlated with the pH (*38*). A high pH value indicated that the meat powder probably contaminated with a large number of spoilage organisms. Therefore, the samples covered with the enhanced three-layer films showed the improved quality compared to the uncovered and gelatin film covered samples.

Fig. 1

Changes in PV of fish meat powder covered with different films during storage

The lipid oxidation is a significant chemical indicator to assess the quality related with lipid oxidation of fish. PV of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the 30 days of storage period (27–30 °C). PV of samples was found to be 8.5 PV/(mmol/kg) at 0^{th} day of storage (Fig. 2a). Most fish smell rancid when PV was above 20 PV/(mmol/kg). For a good fish, the PV values should be

below 10 PV/(mmol/kg) (*39*). In general, PV of all products gradually rose during the storage period, and it confirmed the synthesis of primary lipid oxidation products (*40*). The free radical formation could induce the formation of hydroperoxides in the oxygen-rich environment. Hydroperoxides were the primary oxidation products that initiated the oxidative changes in mackerel meat powder (*41*). The sample covered with P/G/B-PLE-7% film yielded the lower PV amongst all films throughout the storage of 30 days. This was mostly due to the complex formation between the presence of phenolic compounds of PLE and gelatin in the film structure. However, the samples uncovered with any films exhibited the highest peroxide value throughout the storage. Gelatin had the excellent oxygen barrier property in general that superior to commercial PE films (*19*). Thus, the gelatin based films resulted in the lower PV for fish meat powder. The previous reports stated that the sample preserved with gelatin and gelatin based films observed lower PV (*36, 42*). Abreu *et al.* (*43*) documented that the blue shark (*Prionace glauca*) samples coated with films containing barley husks exhibited lower PV than their control counterpart. The similar observations of lowered PV reported for PLA films added with green leaf extract (*20*). The presence of PLA as an outer layer effectively prevents the water vapor permeability than PBAT (*7*) and thus also resulted in the lowered PV when compared to PBAT side of the same film. Thus, the incorporation of *Physalis* leaf extract with gelatin (protein) based films effectively suppressed the primary lipid oxidation and further prevented rancidity in fish products.

Changes in TBARS Value of fish meat powder covered with different films during storage

TBARS value of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the 30 days of storage period (27–30 °C) are depicted in Fig. 2b. Malondialdehyde (MDA), a primary aldehyde produced during lipid oxidation of food and expressed as TBARS. MDA involved in the breakdown of PUFA in hydroperoxides produced during the oxidation of lipids (*44*). The uppermost TBARS value recorded for uncovered samples, irrespective of storage days ($p<0.05$). TBARS value is commonly used to assess the level of secondary lipid oxidation in meat and meat products (*45*). The major products from secondary lipid oxidation had been identified as alcohols, saturated and unsaturated aldehydes (*46*). P/G/B-PLE-7% showed the lowest TBARS value when compared to other gelatin based films. TBARS value of sample covered with P/G/B-

PLE-7% observed lower than control gelatin film (0.183 to 1.007 *w*(MDA)/(mg/kg)) ($p<0.05$) throughout the storage time. However, the TBARS value recorded higher for fish meat powder that preserved with PE and bio-plastic films and also for uncovered sample. Siripatrawan and Noiha (*47*) documented that the pork sausages packed with green tea extract incorporated chitosan film observed lower TBARS value than control film. The augmented TBARS value was plausibly caused due to the sample dehydration and the enhanced lipid oxidation (*48*). Reduced TBARS values may be associated with the depletion of volatile lipid oxidation products, particularly those with lower molecular weights. Lipid oxidation can be accelerated by various mechanisms, including singlet oxygen production, the presence of reactive oxygen, and the enzymatic or non-enzymatic generation of free radicals (*49*). The potential of gelatin film was crucial to serve as a gas barrier that prolonged the food's shelf life (*50*). In contrast, polymeric films with hydrophobic nature (PLA, PBAT and PE) had low barrier ability to oxygen, whereas those films possessed excellent barrier capacity to water vapor. Nevertheless, protein-based films were impermeable to oxygen (*3*). Zanardi *et al.* (*51*) reported that the acceptable limit of TBA was less than 2 *w*(MDA)/(mg/kg). During 30 days of storage, the TBARS value of fish meat powder covered and uncovered with films was within permissible limits ($p<0.05$), expect the uncovered sample at day 30. Incorporation of polyphenols rich PLE likely induced protein cross-linking in the gelatin film, resulting in a more compact structure. This might more effectively limit the oxygen migration through the film. This was evidenced with the lowered rate of lipid oxidation. Therefore, the result suggested that PLE incorporated gelatin based three-layer film reduced the secondary lipid oxidation and preserved the quality of product at an acceptable level.

Changes in TVB-N content of fish meat powder covered with different films during storage

TVB-N content of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the 30 days of storage period (27–30 °C) are illustrated in Fig. 2c. Generally, fish products easily break down into TVB-N or volatile bases with unpleasant odour that include nitrogen compounds such as ammonia, amines and other volatile bases throughout the storage time (*52*). The enzymatic activity and microbiological growth had well connected to TVB-N accumulation (*53*). TVB-N value of all samples at day 0 was 63 *w*(TVB-N)/%, indicated the freshness of meat powder. A value of 50 to 70 mg of

w(TVB-N)/% for fish reported as a maximum limit and the fish with the value exceeding the limit considered as inedible. However, for salted and dried fish, the value should not greater than 100-200 *w*(TVB-N)/% (*39*). TVB-N content of all fish meat powder samples augmented as the storage time upsurged ($p<0.05$). However, the rate of upsurge in TVB-N content was diverse amongst samples which covered with various films. A rapid augmentation noticed for uncovered fish meat powder throughout the storage, which recorded a maximum value of 124.25 *w*(TVB-N)/% amongst all samples at day 30. This value was comparably higher than the samples preserved with gelatin based films during the storage of 30 days ($p<0.05$). The sample preserved with P/G/B-PLE-7% exhibited the lowest TVB-N concentration (85.75 *w*(TVB-N)/%). The lower TVB-N value observed was plausibly due to more rapid decrease in bacterial population/lower ability of bacteria to oxidatively de-aminate NPN compounds. The related results documented for the samples preserved with gelatin based films (*21, 22*) Thus, the findings suggested that PLE incorporated three-layer film slowed the formation of volatile base compounds and inhibited microbial growth.

Fig. 2

Changes in colour of fish meat powder covered with different films during storage

Colour of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the 0 and 30th day of storage period (27–30 °C) are presented in Table 1. *L*, a*, b** and *ΔE**-values of fish meat powder preserved with various films increased when compared to those found at 0th day of storage. Lightness of uncovered sample augmented at 30th day of storage when compared to that day 0. The lowest *a**-value (redness) recorded for the sample preserved with PE at day 30 ($p<0.05$). The uncovered samples, those preserved with PE and gelatin films exhibited higher yellowness (b^*-value) when compared to other fish meat powder ($p<0.05$). The increased *b**-value plausibly be due to the Maillard reaction allied with enhanced lipid oxidation, particularly for the uncovered (control) sample (*50*). Nagarajan *et al.* (*19*) stated that glycation of carbonyl groups such as aldehydes produced during lipid oxidation with amino groups of proteins can cause non-enzymatic browning process. Lipid oxidation more likely related with browning reaction. Upsurged PV and TBARS values of samples suggested that the browning probably linked to the upsurged lipid oxidation. The similar increased rate of *a** and *b**-values detected in fish

powder for control (uncovered) sample (*50, 19*). Salmon meat preserved with gelatin-lignin film had better *L*, a** and *b**-values throughout the storage when compared to the control sample (54). The total colour difference (Δ*E**-value*)* of samples increased at 30th day. This report correlated with the lower lightness (*L**-value) of fish meat powder preserved with bioplastics and three-layer films. Therefore, the films had an impact on the colour of samples that most probably connected to the lipid oxidation and Maillard reaction.

Table 1

Changes in sensory properties of fish meat powder covered with different films during storage

The likeness score of fish meat powder preserved with PE film, gelatin film, PLA film, PBAT film and PLA/Gelatin/PBAT based three-layer films incorporated with 0 and 7 % of PLE in comparison with the uncovered sample during the storage of 0, 15 and 30th day are depicted in Fig. 3. The score for colour, odour and OAL (overall likeness) observed lower throughout the storage period of 30 days ($p<0.05$). The uncovered samples of fish meat powder exhibited the deterioration as indicated by the production of off-odour with colour changes after 15 days of storage might be due to the microbial growth and lipid oxidation. The sample covered with P/G/B-PLE-7% had higher scores than other samples. Gelatin and three-layer films with and without PLE exhibited better overall likeness than other films ($p<0.05$). This might be due to the barrier ability of gelatin films to oxygen. This reconfirmed from the results of lower PV and TBARS value (Fig. 2a and Fig. 2b) observed for the sample covered with gelatin based films, especially for P/G/B-PLE-7%. The addition of *Physalis* leaf extract improved the odour, colour and overall quality of the fish meat powder over the storage period of 30 days. Notably, incorporation of oregano oil (0.05 %, *V/m*) obtained better likeness score and augmented the shelf-life of fish from 11 to 26 days (*55*). Thus, the three-layer film incorporated with PLE could minimize lipid oxidation, thereby prolonging the shelf-life of fish products.

Fig. 3

Changes in volatile compounds of fish meat powder covered with the selected films during storage

The volatile components of fish meat powder at day 0 and the sample preserved with 7 % PLE incorporated PLA-side of three-layer film and PE film in comparison to the uncovered sample after the storage period for 30 days are represented in Table 2. Fish meat powder had volatile compounds including aldehyde (benzaldehyde, nonanal, octanal and hexanal), alcohol

(1-pentanol, ethanol, 1-octene-3-ol, 1-octanol, 1-heptanol, 2-penten-1-ol and 1-penten-3-ol) at day 0. Amongst all alcohols, 1-penten-3-ol and 1-heptanol found for fish meat powder (17.48 and 15.18×10^7, respectively), whilst benzaldehyde (42.81×10^7) and nonanal (16.08×10^7) from aldehydes had appeared at 0^{th} day. Aldehydes have low threshold values, are the primary contributors to off-flavour and used as the key indicators of lipid oxidation (*36*). The numerous aldehydes such as nonanal, octanal, pentanal and hexanal developed during oxidation (*36*). Hexanal and benzaldehyde were the two most prevalent aldehydic compounds in fish meat powder, followed by nonanal and octanal. Nagarajan *et al.* (19) reported that both hexanal and nonanal were secondary oxidation products of linolenic acid. Hexanal could be the good indicator of rancidity in meats detected in the covered PE film and uncovered samples at 30^{th} day. However, the lowest level observed in the sample preserved with P/G/B-PLE-7%. The similar result reported for fish meat powder preserved with gelatin based nanocomposite film containing EECH (*19*). The presence of aldehydes such as nonanal and octanal was non-detectable from the meat powder covered with P/G/B-PLE-7% at day 30. Aldehydes were most abundant in samples that preserved in an open condition. Hemoglobin-catalyzed nonanal was the cause of the oxidized oil odour. The presence of carbonyl compound, *e.g.* octanal accountable for fishy odour (19). However, the volatile compounds such as nonanal and octanal disappeared at day 30. Alcohols, the secondary by-products of the breakdown of hydroperoxide. Ethanol was not detectable at day 0 but appeared at day 30. The lower level of ethanol reported in sample covered with P/G/B-PLE-7% film compared to other samples. Benzaldehyde detected in all the samples at 30^{th} day of storage. However, the sample covered with P/G/B-PLE-7% film showed the lower level. The abundance of 1-penten-3-ol, 1-octene-3-ol and 2-penten-1-ol found maximum in the uncovered sample. Nevertheless, after 30^{th} day, the meat powder preserved with P/G/B-PLE-7% film exhibited lowest abundance. The compounds such as 1-octanol, 1-heptanol and 1-pentanol were not detected at day 30. This was plausibly due to their volatilization during storage. Aliphatic alcohols such as 1-octene-3-ol and 1-pentene-3-ol had musty flavour associated with the oxidative degradation of lipids (*19*). All fish species had familiar for containing A8-carbon alcohols. In addition, the important hydro-peroxides of fatty acids can be decomposed to form 1-alkanals (such as hexanal and pentanol) and 1-alkanols (19).

Acids (butanoic acid, propanoic acid and 3-methyl-butanoic acid) and alkyl pyrazine (2,3,5-trimethyl pyrazine, 2,3,5,6-tetramethyl pyrazine and 2,3-dimethyl-5-ethyl pyrazine) also detected in the samples with the different abundance, depending on the covered films. Alkyl

pyrazines, pyrazine-based compounds with various patterns of substitution. The naturally occurring, strong aromatic alkyl pyrazines typically have an extremely low threshold for odour. In general, alkyl pyrazines produced during the cooking of a variety of foods via Maillard reactions (36). The pyrazines often detected in greater concentrations in the uncovered and PE film covered samples than the sample covered with P/G/B-PLE-7% film after 30 days of storage. Heptane was non-detectable at 0^{th} day of storage, nevertheless it detected at day 30, which found highest of 41.08×10^7 for control and lowest (40.14×10^7) had reported in the sample covered with P/G/B-PLE-7 % film than that of other samples. The sample covered with any film exhibited the lower concentration of all acids. In general, TBARS values correlate well with the result of volatile compounds. Therefore, it reaffirmed that the films developed from fish skin gelatin added with the bioplastics and 7 % PLE might delay the oxidative degradation and inhibited the development of unpleasant odour from fish meat powder by blocking the passage of gas, light and water vapor.

Table 2

CONCLUSION

Three-layer film incorporated with 7 % *Physalis* leaf extract could effectively retard the moisture permeation and lipid oxidation of fish meat powder when compared to PE film, gelatin film, PLA film, PBAT film and control (uncovered) sample. Gelatin based three-layer film incorporated with 7 % PLE significantly lowered the deterioration by maintaining the quality of fish meat powder as reconfirmed by lower PV, TBARS and TVB-N values. PLA, gelatin and PBAT layers of developed three-layer films could serve as the external (outer layer), middle and internal (food contact) layers, respectively for food packaging. Thus, the PLE incorporated three-layer film could gradually replace the non-biodegradable plastic films in near future.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY

Data were not shared.

ETHICS AND CONSENT TO PARTICIPATE

Not applicable.

AUTHORS' CONTRIBUTION

G. Murugan and M. Nagarajan designed the work and G. Murugan, E. Govindhasami and M. Nagarajan conducted the experiments. G. Murugan drafted the article and S. Benjakul, M. Nagarajan, B. Subbiah, M. Dhanushkodi, G. Pandi and N.M Stephen interpreted and evaluated the data and carried out the review and editing. S. Benjakul and M. Nagarajan conceptualised the study and provided resources, supervised, offered funding and critically revised the manuscript. All authors revised and approved the final version of the article.

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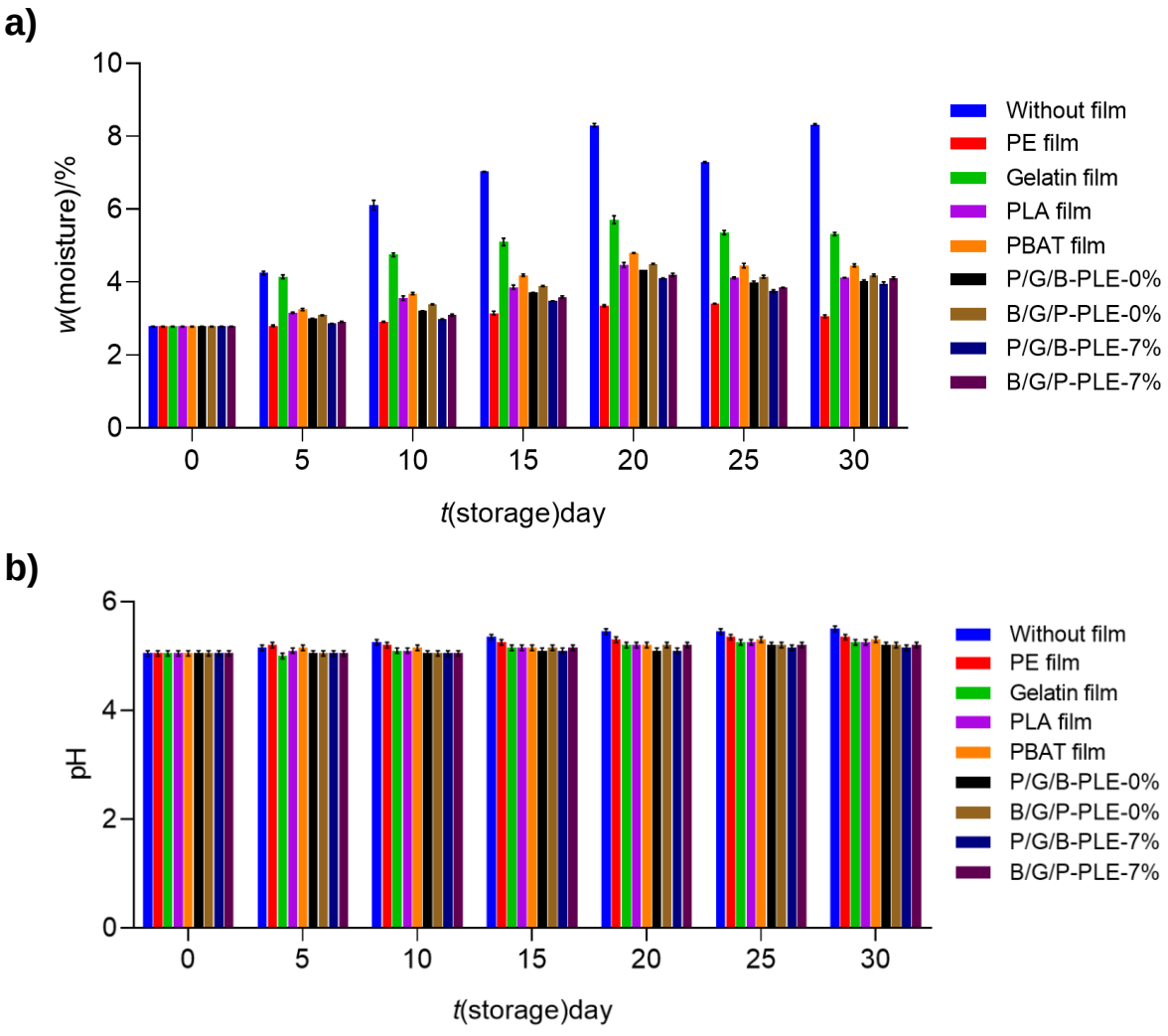
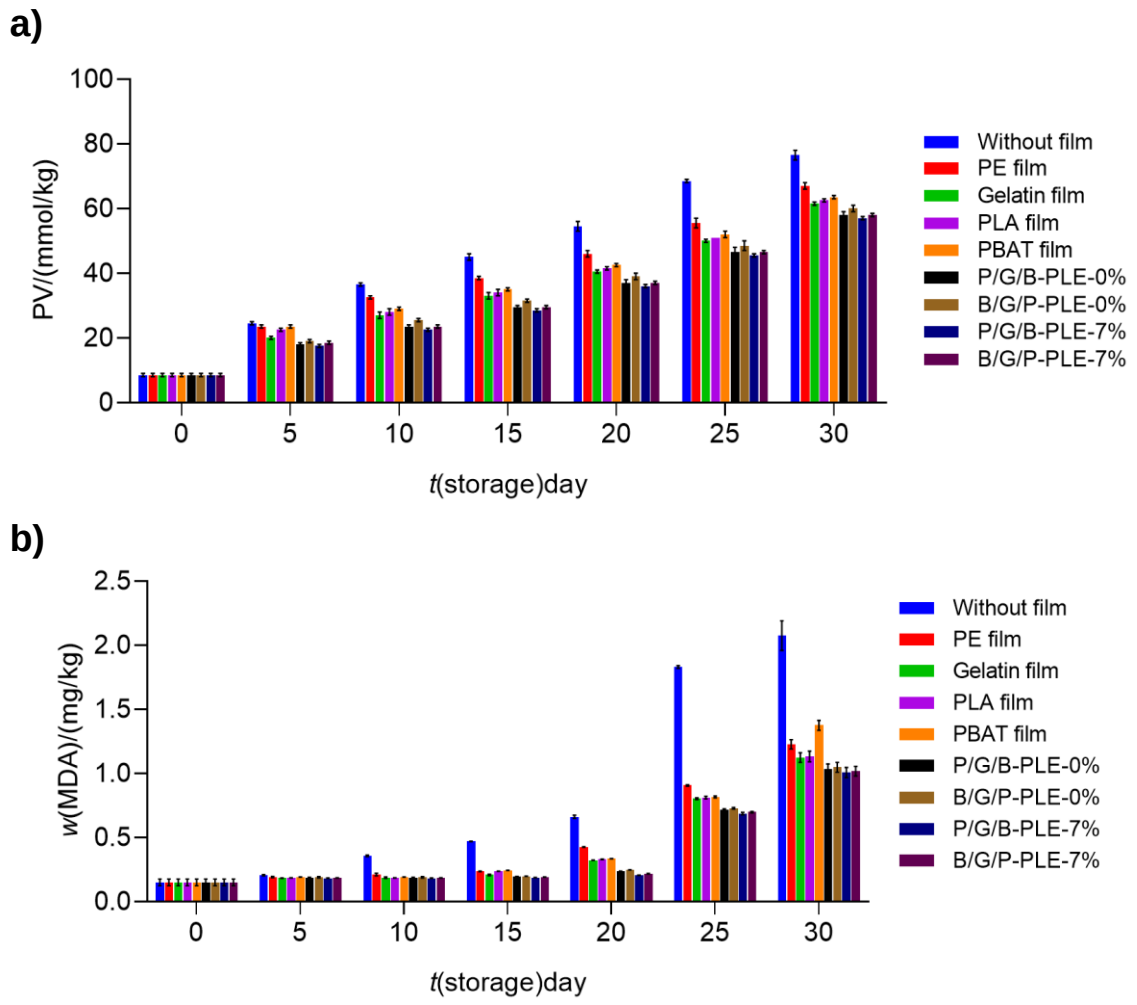


Fig. 1. Moisture content: a) and pH b) of fish meat powder covered with PLA/Gelatin/PBAT three-layer films incorporated with PLE with different exposure sides at 0 (P/G/B-PLE-0% and B/G/P-PLE-0%) and 7 % (P/G/B-PLE-7% and B/G/P-PLE-7%) in comparison with uncovered sample and those covered with PE, PLA, PBAT and gelatin films during the storage of 30 days at 27–30 °C



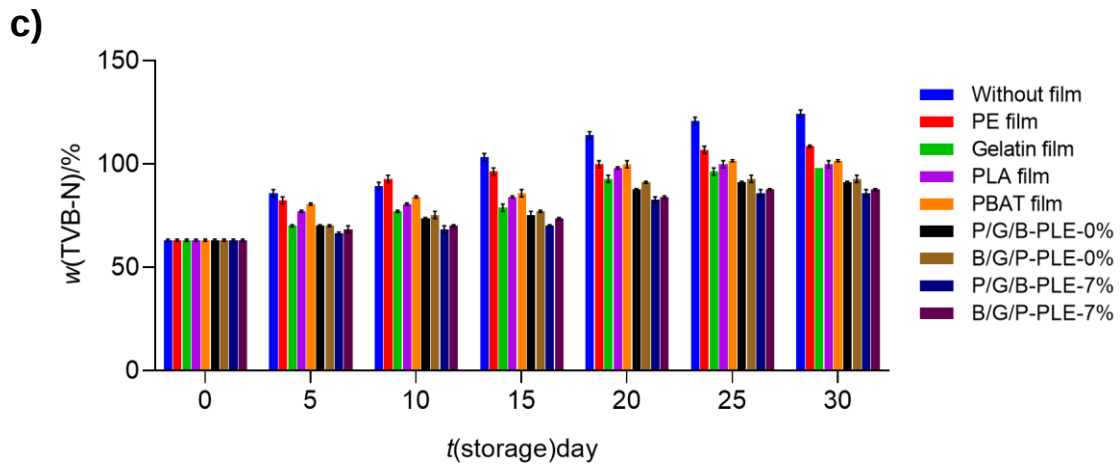


Fig. 2. PV a), TBARS b) and TVB-N c) of fish meat powder covered with PLA/Gelatin/PBAT three-layer films incorporated with PLE with different exposure sides at 0 (P/G/B-PLE-0% and B/G/P-PLE-0%) and 7 % (P/G/B-PLE-7% and B/G/P-PLE-7%) in comparison with uncovered sample and those covered with PE, PLA, PBAT and gelatin films during the storage of 30 days at 27–30 °C

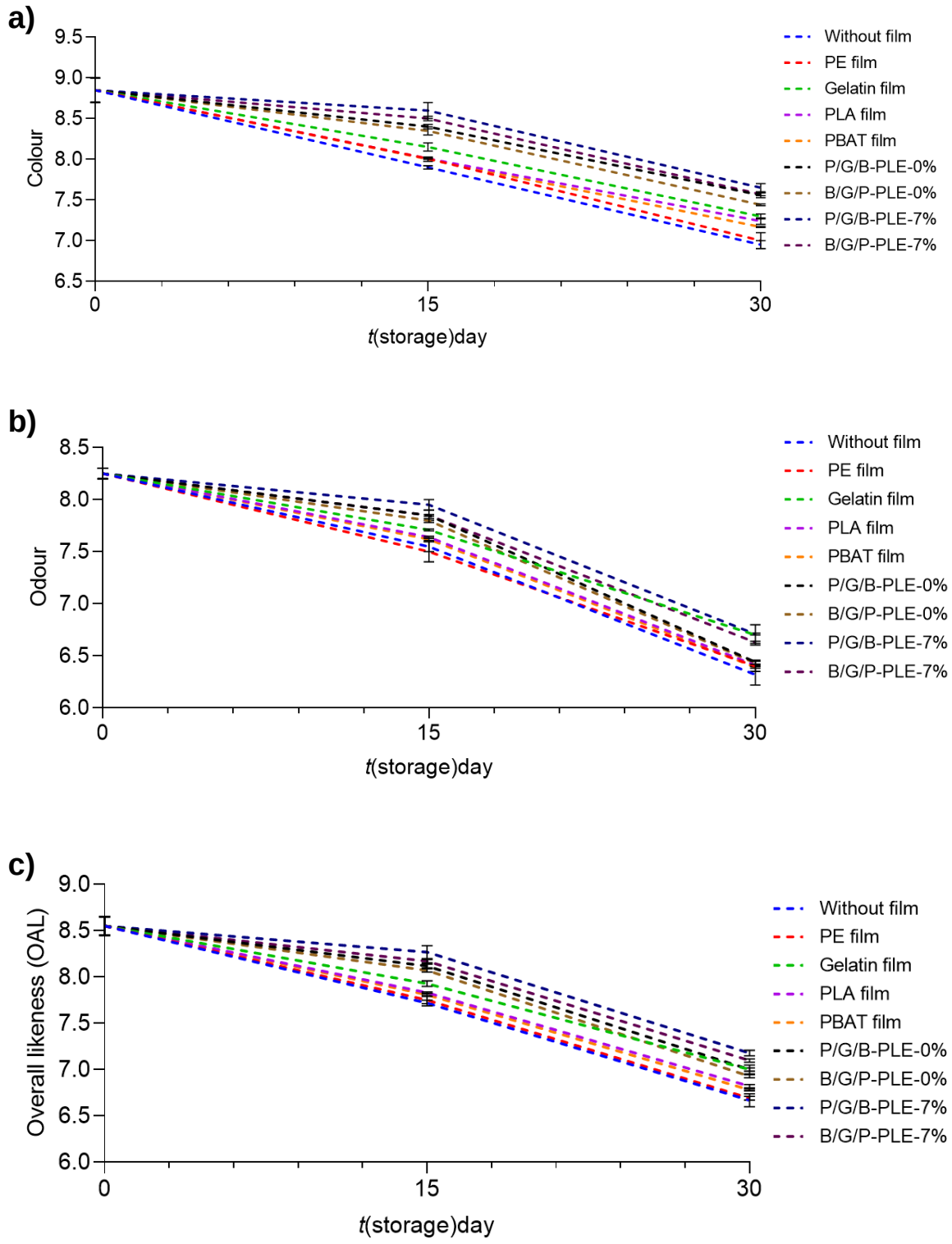


Fig. 3. Sensory analysis [colour a), odour b) and overall likeness c)] of fish meat powder covered with PLA/Gelatin/PBAT three-layer films incorporated with PLE with different exposure sides at 0 (P/G/B-PLE-0% and B/G/P-PLE-0%) and 7 % (P/G/B-PLE-7% and B/G/P-PLE-7%) in comparison with uncovered sample and those covered with PE, PLA, PBAT and gelatin films during the storage of 30 days at 27–30 °C

Table 1. Colour of fish meat powder covered with PLA/Gelatin/PBAT three-layer films incorporated with PLE with different exposure sides at 0 (P/G/B-PLE-0% and B/G/P-PLE-0%) and 7 % (P/G/B-PLE-7% and B/G/P-PLE-7%) in comparison with uncovered sample and those covered with PE, PLA, PBAT and gelatin films during the storage of 30 days at 27–30 °C

	*L**	*a**	*b**	*ΔE**
		0th day		
Sample	(50.29±0.39)	(9.73±0.21)	(34.49±0.22)	(56.25±0.48)
		30th day		
Without film	(53.02±0.51)^{a}	(11.71±0.07)^{a}	(39.43±0.91)^{a}	(57.83±0.98)^{a}
PE film	(49.46±0.62)^{cde}	(9.95±0.69)^{c}	(39.23±5.73)^{a}	(59.99±4.11)^{a}
Gelatin film	(51.54±0.40)^{b}	(11.65±0.17)^{a}	(38.62±0.08)^{ab}	(58.33±0.36)^{a}
PLA film	(48.58±0.79)^{e}	(10.99±0.09)^{b}	(35.17±0.29)^{b}	(58.24±0.41)^{a}
PBAT film	(49.71±0.55)^{cd}	(10.92±0.07)^{b}	(35.50±0.02)^{ab}	(57.55±0.44)^{a}
P/G/B-PLE-0%	(48.91±0.76)^{de}	(10.94±0.11)^{b}	(37.53±1.89)^{ab}	(59.42±1.73)^{a}
B/G/P-PLE-0%	(49.81±0.27)^{cd}	(11.37±0.06)^{ab}	(36.85±0.04)^{ab}	(58.41±0.23)^{a}
P/G/B-PLE-7%	(49.62±0.82)^{cde}	(11.32±0.02)^{ab}	(36.19±0.10)^{ab}	(58.13±0.55)^{a}
B/G/P-PLE-7%	(50.55±0.02)^{bc}	(11.46±0.04)^{a}	(36.55±0.28)^{ab}	(57.68±0.18)^{a}

Mean±SD (*n*=3). Different lowercase superscripts in the same column indicate significant differences in 30th day of storage ($p<0.05$)

Table 2. Volatile compounds of fish meat powder at day 0 and powder covered with PLA/Gelatin/PBAT three-layer film incorporated with PLE (P/G/B-PLE-7%) in comparison with PE film and the control after 30 days of storage at 27–30 °C

Volatile compounds (abundance ×10^7)	*t*(storage)/day			
	0	30		
		Control	PE	P/G/B-PLE-7%
Hexanal	ND	28.43	27.30	1.83
Nonanal	16.08	ND	ND	ND
Octanal	10.82	ND	ND	ND
Benzaldehyde	42.81	37.43	26.11	15.36
Ethanol	ND	55.87	43.29	28.02
1-Heptanol	15.18	ND	ND	ND
1-Octanol	6.42	ND	ND	ND
1-Octene-3-ol	ND	30.84	27.48	4.13
1-Pentanol	9.87	ND	ND	ND
1-Penten-3-ol	17.48	79.47	64.28	58.44
2-Penten-1-ol	7.41	21.78	20.18	14.49
2,3-Dimethyl-5-ethyl pyrazine	ND	60.19	52.42	21.62
2,3,5-Trimethyl pyrazine	ND	161.42	152.18	90.63
2,3,5,6-Tetramethyl pyrazine	ND	532.42	518.34	201.84
Butanoic acid	ND	541.67	218.40	108.69
3-Methyl-butanoic acid	ND	651.42	235.89	52.69
Propanoic acid	ND	64.23	18.39	12.45
Heptane	ND	41.08	41.02	40.14

ND: non-detectable