International Journal of Secondary Metabolite
2019, Vol. 6, No. 4, 292–301

https://dx.doi.org/10.21448/ijsm.650609

Published at http://www.ijate.net http://ijsm.ijate.net/en/ Research Article

Fragrance Component Analysis for Nebulvapours of European Anchovy Oils by Using Colorimetric Printing and Electronic Nose

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Abstract: Analysis of odor components about biochemicals find the wide space in the evaluation of flavor parameters and anchovies as biological materials. Food dye solutions as printer's inks were sprayed on to the fabric throughout the printing operation and skin oil vapors of anchovy were simultaneously sent to the paper hopper of printer intensely via a nebulizer device. Before and after dyeing process, images of tela fabric were taken by smartphone and analyzed by software in the smartphone for the purpose of determination of colorimetric fragrance component concentrations and visual odor profile in range of visible region. The ten major ingredient contents (with relative percentages) (aldehyde compounds intensely such as 2,4-heptadienal (23%), (E,E)-2,4-nonadienal (17%)) of anchovy skin essential oils were determined. For colorimetric printing analysis via smartphone, LOD and LOQ were 1 ppm and 3 ppm, respectively. Methodology can be used in the analysis of toxic components that interact with foods.

ARTICLE HISTORY

Received: July 17, 2019
Revised: October 15, 2019
Accepted: November 15, 2019

KEYWORDS

Fragrance Components,
Anchovy Oils,
Printing,
Electronic Nose,
Food Dye

1. INTRODUCTION

Analysis of odor components in foods and chemicals are important in the evaluation of flavor parameters. As biological materials, anchovies stand out with their pungent odor. Anchovies process is located in the top position in Turkey's seafood industry [1]. According to the Turkish statistical data in 2018, while the most produced sea fish in Turkey by fishing was anchovy with 27%, the rate of anchovies used in oil production was 16% [2]. Microbial deterioration, due to the growth of microorganisms naturally present in fish, can occur. As microorganisms grow, they produce nutrients and metabolites such as sulfuric compounds, aldehydes, ketones, esters, trimethylamine and the total volatile base nitrogen [3]. Previous studies reported an increase in protein and relative RNA content of anchovy, a decrease in carbohydrate and relative lipid content of anchovy as the anchovy larvae was growing [4, 5]. In another study, silver ion high-performance liquid chromatography was used to examine the diversity and variations of the molecular triglyceride species derived from fish oils extracted from the laboratory [6]. The selected fish oils were separated and the fatty acid distribution of the fractions was determined by gas-liquid chromatography. Trace amounts of wax esters, free fatty acids or sterols were available in fish oils. The South African anchovy oils contained

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ISSN-e: 2148-6905 /© IJSM 2019

very small amounts of monoenoic acids. The lipids were visualized by spraying with copper (II) sulfate [6].

Smartphone and printer technology stands out in many studies. For example; in immunohistochemical breast cancer study, the differentiation of positive (brown color) and negative (blue color) stained cells by manual counting was evaluated by 3D printed adapter and smartphone used to obtain microscopic images of stained tissue slides [7]. Smartphone biosensors are cost-effective, portable devices that can be used in colorimetric analysis. Smartphone colorimetric readers allow the analysis of photographs of liquid samples taken using a camera by means of software programs [8]. With the combination of digital camera, printer, scanner, desktop or portable computer and proprietary software, the way of captured, identified and dimensioned of image can be changed naturally [9].

Azo group paints have been widely used in cosmetic, tattoo, food and beverages, pharmaceuticals, printing inks, plastics, leather and paper industries [10]. Colorimetric indicators (eg, labels, prints) could interact with target compounds such as CO_2, O_2, ammonia gas and aldehydes to exhibit visible color changes [11, 12]. Synthetic food dyes such as Tartrazine, Allura Red, Brilliant Blue were classified according to the US Food and Drug Administration (FDA) test criteria [13]. Certified food dyes (such as Tartrazine, Allura Red, Brilliant Blue) have been commonly used synthetic compounds because of their color and shelf life stability, so as not to alter food tasting and color [14]. Synthetic paints, which are less expensive and more stable than their natural counterparts, have been used in many commercial products [15]. Food dye mixtures (Tartrazine, Allura Red, Brilliant Blue) were applied to plates such as SiO_2, TiO_2 by inkjet printers used as office chromatography concept, and the separation performance was measured using a smartphone [16]. Enzyme ratios for enzyme systems were obtained by a two-dimensional code design color distribution by a method which uses pressure on inkjet paper [17]. A new colorimetric method based on polyaniline film for the development of intelligent packaging was defined as a chemical sensor for color change of various essential volatile amines, which were microbial degradation products on the surface of the fish [18]. In other study, colorimetric indicators have been created with ink-jet printing technology to develop a CO_2 sensitive colorimetric indicator [19].

Odor is one of the most important parameters when evaluating the freshness of food. Each product has a characteristic profile of volatile compounds and, therefore, its own characteristic odor [20]. The odor caused by fish degradation was attributed to amines (eg; trimethylamine, dimethylamine, ammonia, histamine, putresine, cadaverine), short chain carbonyls, sulfur compounds, aldehydes [21]. DNA-based oligo deoxy floroside was measured using the digital values of red, blue, green, luma signal channels under epifluorescence microscopy by staining for fluorescence via inkjet printing of various dyes on cellulose paper for detection of volatiles resulting from food spoilage [22]. Shima company has improved the print quality for knitwear and knitted fabrics with a new inkjet printer [23]. Aerosols produced from a variety of solutions with high quality programmed print modes using an inkjet printer were characterized by an aerodynamic particle sizer for determining of particle sizes [24]. In patients with cystic fibrosis, inhalation of natural oils by nebulizers was attempted to treat chronic lung infections [25]. Five different formulation containing *Breu* essential oils were evaluated using an inhalation chamber and GC-FID combined with nebulizer for sedative and antinociceptive activities in mice [26].

This study aimed to develop a new method that is practical, fast and cheap and that can be applied accurately and sensitivity in terms of detection limits alternative to expensive GC-MS application. Furthermore, the method will be used in the analysis of foreign volatile components like formaldehyde that can interact with foods. In this respect, nebulvapor safety risk parameters will be minimized at the environment in terms of analysis. Odor components will be measured qualitatively and quantitatively. Without mass detection, density of volatile components, which have low and high molecular weights, will be visualized with smartphone cumulatively by food staining technique via a printer. The study can shed light on the evaluation of the waste fish skin oil profile and can be applied with 3-D printer technology combined with different technologies like confocal laser scanning microscope in the future.

2. MATERIAL and METHODS

2.1. Chemicals and Materials

Hydrochloric acid (reagent grade, 37%, 25 mL) (CAS No: 7647-01-0) was purchased from Sigma- Aldrich for acidic hydrolysis of anchovy skins for the purpose of obtaining essential oils. Tartrazine (≥85%, 100 g, CAS No:1934-21-0), which is an anionic, hydrophilic azo dye with an orange-yellow color used in fabrics, foods and cosmetics, was purchased from Sigma- Aldrich. Among the other azo food dyes, Allura red (100 g, 80%, E129, CAS No:25956-17-6), which is an effective scatterer in luminescence spectroscopy, was purchased from Merck. Brilliant Blue food dye (E133, blue powder, 85%, 100 g, CAS No: 3844-45-9) was purchased from Sigma- Aldrich. 100% Polyester tela fabric (1 m^2) was purchased from Manufacturer, Trading Company (Guangdong, China (Mainland)) for the purpose of printing paper. Dried anchovy skins (500 g) were purchased from Shandong Kingsun Foods Co., Ltd. (Shandong, China (Mainland)) for essential oil analysis because of the thought with higher oil yield from Turkey anchovies. All dye reagents were solved with ultrapure water produced by using Milli-Q® IQ 7003/7005 Ultrapure Lab Water System (Merck).

2.2. Instruments and Analysis

EPSON A4 size flatbed printer machine (with heat function, print speed:20 ppm, printing size: 300 mm x 210 mm, 90 pieces x 6 colours Nozzle, high quality USB data line and software settings) that is one kind of inkjet printer which can print on almost materials, such as plastic, metal, glass, wood, stone, leather, and fabric, was purchased from Shenzhen Bettsens Industrial Co. Ltd. (Guangdong, China (Mainland)) for printing on tela fabric by using the food dyes as inks. Vivocare Steamy Compressor Jet Nebulizer Device (particle size <4 µm, Bayer company, Germany) was used for vaporizing of European anchovy skin oils (120 mL). Colorful printing distribution with food dyes of European anchovy volatiling oil components on tela fabric captured by smartphone (Samsung Galaxy S5 (Seoul, South Korea)).

The images of color distribution of volatiling components on tela fabric were further analyzed by ImageJ combined with application of Zoner Photo Studio 17 PRO (Brno; Czech Republic) software for color intensity acquiring using for all three channels (R, G and B) in a region on the photograph responding to diameter of the dots on tela fabric matrix [27, 28]. Dried anchovy fish skins (500 g) were powdered via a blender (Model SHB 3062; Sinbo, Istanbul, Turkey). Powders of dried European anchovy fish skins (500 g) were hydrodistilled via a Clevenger-type device (Sesim Kimya Laboratuvar, Ankara, Turkey) by using 980 mL distilled water and 20 mL hydrochloric acid (37%) for 4 h according to modified method of Tural and Turhan [5].

Essential oils of anchovies extracted with acidic hydrolysis were collected to amber coloured vials (Merck, volume 4 mL, glass, 15 mm × 45 mm) and stored in the refrigerated incubator (FOC 215I, Velp Scientifica, TempSoft™ dedicated software for times and temperatures) at 4 °C until used. With electronic nose (PERES foodsniffer (Swiss Technology) uses four sensing nozzle including temperature, humidity, ammonia and volatile organic compound sensors), which measures the levels of volatile organic compounds and works in conjunction with a smartphone, vaporizing chemical compositions of European anchovy essential oils were analyzed safely [29].

2.3. Method Validation

Validation parameters (linear range, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision) were applied for determination of visualizing concentration limits and tela fabric matrix effects about food dye printing applications for analyzing of European anchovy essential oils. The linear regressions (slopes, correlation coefficient (r^2)) of drawn calibration curves by using ImageJ program were used to assess the linear range for concentration measurements based on the color intensity of the painted essential oils on tela fabric with trying triplicate smartphone applications.

The LOD and LOQ (detection limits) were determined by the average of standard deviation of the calibration curves by created via Zoner Photo Studio 17 program in terms of 3 times and 10 times respectively in which the smallest point zones of pictures painted with food dyes taken with the help of smartphone for vaporizing European anchovy essential oil test levels.

To determine the accuracy and precision, in intra-day and inter-day conditions, replicated applications for assessment of visual color intensity with smartphone, were evaluated. In addition, matrix effects caused by dye solvent or tela fabric were eliminated by creating stable zone areas. Color intensity processing data depending on concentration with captured of light signals via smartphone was correctly analyzed by removing dark blind spot signals and colorless zone areas. For validation, databases of painted zone photographs on tela fabric of volatiling components of European anchovy skins were processed by the algorithm in smartphone [30].

2.4. Statistical Analysis

Statistical analysis was carried out by using Microsoft Excel (Microsoft Office Corporation, 2010, Redmond, Washington) and SPSS Version 21.0 software program. All statistical analyses were reported significantly (p<0.05) with standard deviation. Data were processed by ANOVA test.

Table 1. Detection limits and linear working range based on colorimetric color intensity of total dyed zone areas (average particule size: 3 A°) containing essential oil components on tela fabric.

For Total Pointed Picture Areas of Dyed Vaporizing Odor Components Including of Anchovy Oils					
Detection Limits (± SD, n:3)		Linear Working		Color Intensity Recovery (%)	
LOD (ppm)	LOQ (ppm)	Range (n:3)	Equation (r^2: 0.9996)	Intra Day	Inner Day
1+-0.01	3+-0.03	1 ppm-5 ppm	y = 2.08x	96	90

SD: Average Standart Deviation, n: analysis repetition, ppm: parts per million, 95 % confidence interval, critical ratio: p<0.05

3. RESULTS and DISCUSSION

Optimized loaded liposomes were more delivered effectively by an air-jet nebulizer, than a vibrating- nebulizer during 10 min period via the abbreviated impactor in a study [31]. Similar to this conclusion, in this study, with applying in parallel of jet nebulizer, anchovy oil vapors were sent on tela fabric surface in effective rate successfully. Schneider et al. and Sunoj et al. reported that the method based on visualizing phenological comparisons of plants was developed by using a standard ColorChecker chart with six different color schemes coded ImageJ plugin named ‘ColorCal’ (version 1.52d, with [3 × 3] color calibration matrix). The use of three basic color corrections (red, yellow and blue) was sufficient for quality color calibration [32, 33].

In this study, painted colored zone areas of anchovy essential oils were formed on the tela fabric by using tartrazine, allura red and brilliant blue food dyes (250 mg /mL, aquatic form each other) separately via a printer. Pictures of these spot painted fragments were taken with the smartphone. The visible region (400-700 nm) color scale (a standard ColorChecker chart color schemes) generated by 3 basic color patches (red, yellow, and blue) in the smartphone was used as a calibrator. The black and bright light signals and small zone areas (zone radius < 2 A°) were eliminated with the help of the algorithm in the smartphone. Calibration graphs of the color intensities of the zone fields in the captured images were created (Figure 1).

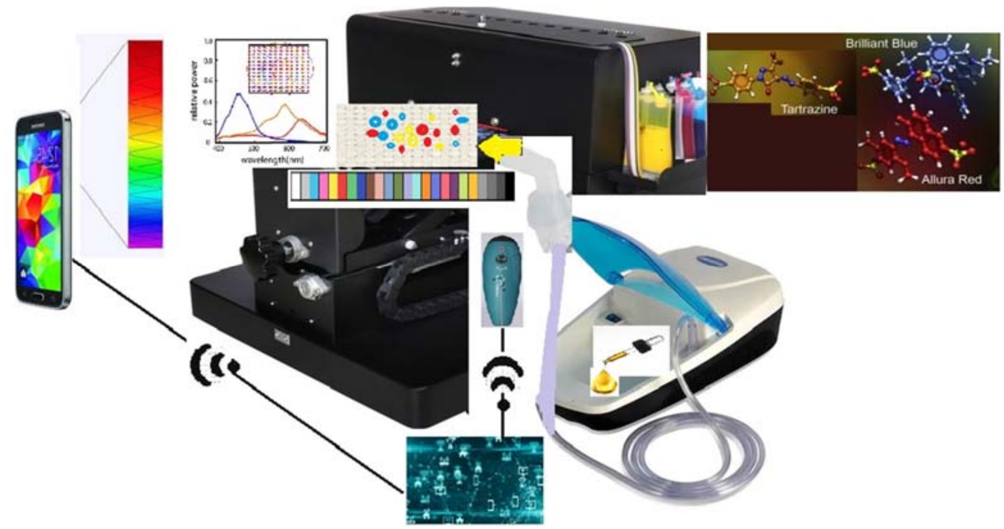

Figure 1. Systematic analysis of nebulous vapors of anchovy essential oils by fabric dye printing combined with electronic nose and smartphone applications.

Detection limits (LOD: 1 ppm, LOQ: 3 ppm) were respectively determined as 3 times and 10 times of the average ratios (slope / standard deviation) obtained from the calibration graphs with triplicate smartphone applications. Linear range was found between 1 ppm-5 ppm (Table 1). For intra-day and inter-day conditions, the differences in color intensity measurements between the wet or dried dyed images of volatile European anchovy oils on tela fabric constituted a standard deviation of approximately 0.03, so it was statistically significant ($p< 0.05$). The light signaling sent from the device by connected to the smartphone device calibration was stable. The method was found to be accurate and precise because recovery of color intensity was high compared with optimal values of color stability in the studies about colorimetric determination of streptomycin with the help of anthocyanin dye and iron (III) in bioethanol fuel via smartphone [34, 35]. A previous study investigated the desirable odor healing of chicken oil after lipoxygenase treatment by the techniques of gas chromatography and sensory evaluation. The amounts of volatile compounds (ethyl acetate, pentanal, 2-pentyl furan, E-2- heptenal and nonanal) found in modified chicken oil was higher than in the original chicken oil [36].

Also, in this study, after essential oil vapor components of dried European anchovy skin powders (500 g) had been painted with food dyes via printer, electronic nose (PERES foodsniffer (Swiss Technology), with combined smartphone odor component library program) was used for odor components analysis of oil vapors, simultaneously.

The data acquisition time was 120 s. 13 odorant components (methane-thiobis, thiophene, toluene + butanoic acid ethyl ester, hexanal, 1-hexanol, 1-octen-3-one, 1-octen-3-ol, dimethyltrisulfide, octanal, 1-nonen-3-ol, (E)- 2-nonenal and 2 unknown compounds) of 144 volatile compounds were detected in European seabass flesh via GC-MS in another study [37].

Also, in this study, electronic nose (PERES foodsniffer) is an artificial effective detection equipment simulates the olfactory function of the mammalian nose and is effectively used for food analysis, adsorbed the odor molecules by the sensors and generated signals from sensors were evaluated by connecting to volatile molecules for principal component analysis via smartphone signal processing system and component library (containing volatile molecules database). Electronic nose working principle was explained in a lot of studies [38, 39]. Especially, in a study, electronic nose was applied for analysis of the content of nebulizer vapors in wood vinegar extract of black garlics successfully [40].

Fabric images obtained from the high resolution digital camera image acquisition system which are defined at each thread transition point with a multi-zone fuzzy segmentation-based approach [41]. A microfluidic chemistry analyzer which was consisting of a fan-shaped microchip for simultaneous measurements of glucose, triglyceride and total cholesterol from serum samples has been developed with accurate, reliable and reproducible results via smartphone with specific LED light source and camera [42]. Immediately, a thermal inkjet printer heated the liquid in a microfluidic chamber and was successfully applied onto the print cells exiting the nozzle head by removing the droplets to high velocity and providing the necessary pressure drop [43]. For hypothetical testing of drugs seized in forensic cases, a microfluidic device has been developed which permits multiplex detection of various compounds including cocaine, opiates, ketamine and various phenethyl amines by colorimetric reaction by forming hydrophilic channels on chromatographic paper using wax press and thermal lamination [44].

In this study, as different from the others, nebulizer instead of headspace unit was used for evaporation of European anchovy essential oils in inside the printer tela fabric reservoir. Detected volatiling components with PERES foodsniffer were determined as the percentage of concentration with the help of the smartphone analyzing program which uses molecular library database with network by making signal processing.

The ten major ingredient contents (with relative percentages) of European anchovy skin essential oils were 2,4-heptadienal (23%), 2-pentyl furan (8.5%), pentanal (3.5%), (E,E)-2,4-nonadienal (17%), 1,3-octadiene (5%), 2-ethyl-1-hexanol (3%), p-xylene (2.5%), 2-butanone (15%), 2-methyl-1-pentene (7%), (E)-3-undecene (15.5%) (Table 2).

Table 2. Nebulous steam odor components on tela fabric detected by electronic nose.

Number	Detection Time (tsecond)	Component Names	% Relative Quantity
1	15	2,4-heptadienal	23
2	25.5	2- pentyl furan	8.5
3	33.5	pentanal	3.5
4	45	(E,E)-2,4-nonadienal	17
5	54.5	1,3-octadiene	5
6	61.5	2-butanone	15
7	88.5	p-xylene	2.5
8	98	2-ethyl-1-hexanol	3
9	110.5	2-methyl-1-pentene	7
10	120	(E)-3-undecene	15.5
		Total:	100

Blue colored Brilliant Blue dye interacted with aldehyde compounds, found in the highest concentration of these volatile components which are stained with three different color food dyes via molecular dyeing printing technology. Tartrazine and allura red were also azo food dyes with different colors (yellow and red, respectively) tied with high affinity to other group volatile components especially hydrocarbons on tela fabric. The diagram of multi distribution points including inner and outer zone areas of tela fabric visualized information of dying volatile components (Figure 2).

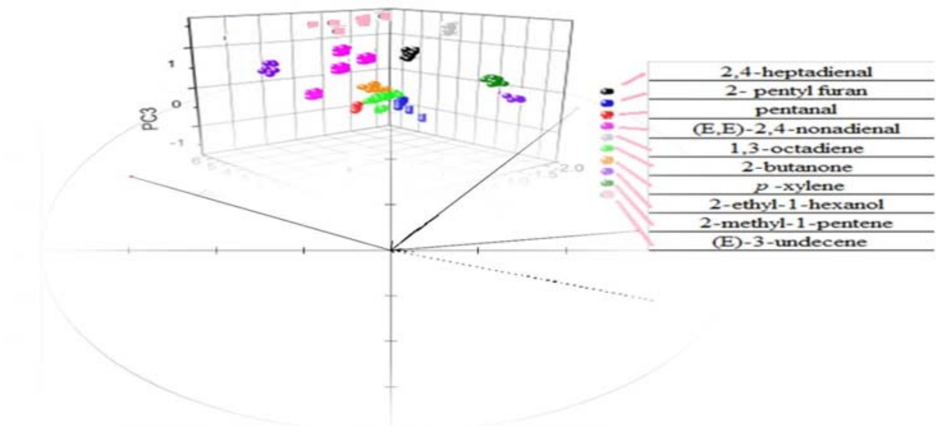

Figure 2. Dyed multi-odor component distribution points including inner and outer zone areas of tela fabric.

4. CONCLUSION

For odor components analyzing of nebulizer vapors of anchovy essential oils, colorimetric printing application and electronic nose detection have been performed firstly and successfully by coordinated with smartphone. Colorimetric printing application method combined with smartphone was validated to determine the colorimetric detection limits of the total volatile components. 10 different compounds (high aldehyde amounts) were detected as relative percentages of concentrations by making multi component analyzes of European anchovy skin essential oil vapors with electronic nose connected smartphone network component library. This methodological study can be used in the analysis of toxic components that interact with foods in the future.

Acknowledgements

I did not receive any grant from funding agencies in the public, commercial, or not- for-profit sectors. I thank my dear family for their moral support.

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