Biogenic Amine, Fatty Acid, and Volatile Compound Contents in Ivorian Traditionally Fermented Fish “Adjuevan”

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ABSTRACT: This study identified biogenic amines, fatty acids, and volatile compounds in adjuevan, an Ivorian traditionally salted and fermented fish. Samples were obtained from two processing methods (method 1: entire fish adjuevan; method 2: both sides filleted adjuevan) with the fish species Galeoides decadactylus. Biogenic amines found in freshly produced adjuevan were histamine, putrescine, cadaverine, tyramine, β-phenyl ethylamine, and spermidine. Among these, the most prevalent were β-phenyl ethylamine and cadaverine. Biogenic amine contents varied according to the processing method but remained lower than levels considered hazardous for human health. The major fatty acids present in adjuevan from method 1 were docosahexaenoic acid, palmitic acid, and oleic acid. In adjuevan from method 2, the major fatty acids were oleic acid, stearic acid, and palmitic acid. The omega (ω)-3/ω-6 ratio was 8.87 and 4.12 for adjuevan from methods 1 and 2, respectively. Most of the fatty acids are considered healthy fats, making adjuevan a useful food for treating and preventing lifestyle diseases. The volatile compounds found composed of 19 aldehydes, 12 alcohols, 7 esters, 7 ketones, 3 furans, 10 aromatic compounds, and 7 acids with aldehyde, alcohol, and ester compounds as the predominant groups. Among the aldehydes, 2,4-heptadienal (E,Z), octanal, and 2-octenal (E) were most prevalent in adjuevan from method 1, whereas 2-nonenal (E), 2,4-heptadienal (E,Z), and octanal were most prevalent in adjuevan from method 2.

Keywords: adjuevan, biogenic amines, fatty acids, fish, volatile compounds

INTRODUCTION

Adjuevan, uncooked, salted, and fermented fish, is a traditional and popular fermented fish from Côte d’Ivoire. This fermented fish is mostly produced in river and sea coastal areas and is appreciated first for its flavour and second as a source of protein. Adjuevan is used as a condiment in urban and rural areas in Côte d’Ivoire and in neighboring countries (Kouakou et al., 2013).

Fish fermentation consists of transforming organic substances into simpler compounds, such as peptides, amino acids, and other nitrogenous compounds, either by the action of microorganisms or endogenous enzymes. Peptides and amino acids are important contributors to the flavour and aroma of fermented products. However, fermented products belong to the most common sources of biogenic amines, mainly histamine, tyramine, putrescine, and cadaverine (Rabie et al., 2009). Biogenic amines are low-molecular weight nitrogenous compounds that are formed in foodstuffs mainly by microbial decarboxylation of the precursors of amino acids. Intake of dietary biogenic amines in a normal diet is not considered harmful because healthy individuals can readily metabolize amines by acetylation and oxidation reactions mediated by the enzymes monoamine oxidase, diamine oxidase, and polyamine oxidase (Proestos et al., 2006). However, excess consumption of these amines can induce severe toxicological effects and can produce various physiological symptoms. Thus, excessive intake of histamine may cause dilatation of peripheral blood vessels, hypotension, urticaria, flushing, and headache. High amounts of tyramine in food could also induce headache and hypertension (Rai et al., 2013). In addition, amines are precursors of carcinogenic N-nitroso compounds. Histamine higher than 100 mg/kg may cause slight, intermediate and intensive poisoning (Parente et al., 2001). Therefore, the presence...
of biogenic amines significantly influences the food quality and safety (Smit et al., 2005). Nout (1994) demonstrated that the maximum allowable level of histamine and tyramine in food should 50–100 mg/kg and 100–800 mg/kg, respectively, whereas Carelli et al. (2007) showed that histidine content must not exceed 200 mg/kg in brine-processed fishery products. In the European Union, 100 mg/kg has been established as an acceptable level for histamine (Council of European Communities, 1991). However, there aren’t any recommendations about acceptable levels of the others compounds, such as putrescine and cadaverine.

Fish is also known to contain certain polyunsaturated fatty acids that can regulate prostaglandin synthesis and induce wound healing. Omega (ω)-3 and ω-6 polyunsaturated fatty acids (PUFA) have been shown to have positive effects on cardiovascular diseases and cancers (Connor, 1997). However, due to their high degree of unsaturation, PUFA are extremely sensitive to lipid oxidation during food processing and/or storage. Several factors may affect their oxidative status and the color of fish products: the quality of the raw material, the salting and drying procedures adopted, the physical state of the matrix, and the storage conditions (time, temperature, and light exposure). The oxidative changes to the lipid components influence the sensory quality of the fish products.

To the best of our knowledge, only a few reports are available on the safety and sensorial quality of African fermented fish products, particularly Ivorian adjuevan. Therefore, the objective of the present study was to determine the contents of biogenic amines, fatty acids, and volatile compounds in adjuevan samples after production.

**MATERIALS AND METHODS**

**Adjuevan production**

The Ivorian fermented fish adjuevan was produced according to two traditional methods. Fresh fish of the sea species G. decadactylus (size of 218–305 cm) were used as the raw material. In method 1, the entire fish was allowed to deteriorate in air for 48 h in a jar or bowl covered with plastic. The fish were then eviscerated, washed, and dry salted at around 35% (w/w) salt. The salted fish was arranged in a jar, covered with plastic or small plates weighted down with stones, and allowed to ferment for 5 days. Thereafter, the fermented fish were sun dried on racks or nets for 4 days at ambient temperature (28–32°C). For method 2, the entire fish was allowed to deteriorate in air for 24 h, following which it was gutted, filleted along both sides, washed, and dry salted at 20–35% (w/w) salt. Fermentation was carried out outside at room temperature (28–32°C), simultaneously with sun drying on racks or nets, for at least 7 days.

**Sampling**

Adjuevan samples were collected according to the two main traditional processing methods from local producers randomly identified at Assinie and Abidjan-Vridi in the district of Abidjan, Southern Côte d'Ivoire. For each processing method (methods 1 and 2), four local producers were selected (two producers at Assinie and two producers at Vridi). Sampling consisted of collecting 1 kg of fermented fish in triplicate for each production series, with a total of 48 samples per production method. Samples were placed into sterile plastic bags, stored in an icebox filled with ice, and transported to the laboratory for analysis.

**Biogenic amines analysis by high-performance liquid chromatography (HPLC)**

Internal standard (1,7-diaminoheptane) and biogenic amine standard (putrescine, histamine, cadaverine, spermidine, and β-phenyl ethylamine and spermine) solutions (50 mg/50 mL concentration), dansyl chloride solution (10 mg/mL), and biogenic amines extracted from fermented fish adjuevan were separately prepared according to the method of Shukla et al. (2010). About 5 g of adjuevan samples were used for extraction. Derivatization of biogenic amines in the extracts and standards for the HPLC analysis was carried out according to the method used by Shukla et al. (2010).

The HPLC system equipped with a detector SPD-20A (λ=254 μm, Shimadzu, Kyoto, Japan) and LC software was used to analyse the biogenic amines. The mobile phases were ammonium acetate (0.1 M, solvent A) and acetonitrile (solvent B). Separation of the compounds was carried out using a column 5 OBD, 250×4.6 mm conditioned with the mixture of 50% solvent A and 25% solvent B for 90% in 19 min gradient elution. The sample volume injected was 2 μL (for generating data in triplicate).

**Determination of fatty acid composition**

**Fatty acid extraction and esterification:** Total lipid (TL) extraction was carried out by an automatic liquid-solid extraction system Soxtec type (Avanti Polar Lipids, Alabaster, AL, USA) for 2 h at 135°C with 65 mL of solvent (petroleum ether, Merck KGaA, Darmstadt, Germany) added to 6 g of adjuevan. Fatty acid constituents in the TL were converted to fatty acid methyl esters by adding sodium methoxide with five grains of pumice to a flask containing three drops of TL. The mixture was heated at 100°C for 10 min then 3 mL of hydrochloric methanol was added. When the mixture had cooled to room temperature, 10 mL of hexane (Merck KGaA) and 10 mL of distilled water were added to the mixture in the flask. The hexane phase containing the fatty acid methyl esters was recovered for analysis by gas chromatography (GC).
Identification and quantification of fatty acids: A GC (FOCUS, Thermo Fisher Scientific, Waltham, MA, USA) equipped with an ionization detector was used to identify fatty acids. TL (1 μL) was injected and separated by a CIL 88 CP Variant column (50 m, internal diameter 0.25 mm and a thickness of phase 0.20 μm, Global Variome shared LOVD, Leiden, The Netherlands). The injector and the detector were used at a temperature of 250°C. The flow rate of the carrier gas (helium) was 1 mL/min. Fatty acids were separated according to the following temperature program: initial temperature 150°C, temperature increase at a rate of 5°C/min up to 250°C, and an isothermal hold at 250°C for 10 min. Fatty acids were identified by comparing their relative retention times with those of a spectrum from a control solution of salmon oil fatty acids. The fatty acid methyl esters were quantified and identified by comparing the retention times with authentic ones. The results were analysed with GC ChromCard software (Brechbuehler AG, Schlieren, Switzerland), which was used to calculate the weight percentages of various free fatty acids based on peak areas formed by each acid.

Solid phase micro-extraction (SPME) and identification of volatile compounds
About 1.5 g of each sample of fermented fish was used for SPME analysis. To quantify the extracted compounds, 40 μL of a solution of hexanol (5 g/L) diluted 1/1,000 was added as an internal standard. The fiber used was a SPME fiber polydimethylsiloxane-ivinylbenzene (65 mm). Before the first use, the fiber was preconditioned at 250°C for 30 min to remove any impurities that may have been absorbed. The different extraction and desorption temperature parameters were previously optimized. Samples were incubated in an oven where they were stirred at 250 trs/min for 5 min until the fiber adsorbed the compounds present in the headspace (60°C for 30 min). Once extracted, the compounds were desorbed from the fiber in a heated high-temperature and analyzed by GC–mass spectrometry (MS). Analysis of the aromas from the fermented fish adjuevan was performed using a chromatograph GC-MS Agilent 6890N type automatic injection mode on a polar capillary column DBWAX 122-7032 J & W 30 m long with a 0.25 mm internal diameter and a film thickness of 0.25 microns (Agilent Technologies, Santa Clara, CA, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C and sample injection was carried out as follows: the column temperature was programmed to increase from 40°C (holding time 3°C/min) to 170°C at a rate of 10°C/min from 170 to 240°C. The chromatograph was coupled to an Agilent 5973 mass spectrometer (Agilent Technologies) operating mode Network electron impact source with internal ionization 70 eV. The column temperature was initially held at 40°C for 3 min and subsequently programmed to 230°C.

The volatile compounds were identified based on comparing mass spectra, Kovats retention index (RI), and retention times with those obtained for standard compounds. RIs were determined for SPME-GC by using a series of n-alcanes C8 ~ C20 (Supelco Inc., Bellefonte, PA, USA) and comparing values with those previously reported in the literature and those listed on several authentic online databases with published data (http://www.flavornet.org). To improve the detection limits, selected ion monitoring mode was used to obtain RIs. The perception threshold and odour description reported in the present study were also referred from the database mentioned above.

Statistical analyses
Data were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni test (Dunn), using the Statistica software (TIBCO Software Inc., Palo Alto, CA, USA). P<0.05 were considered significant.

RESULTS AND DISCUSSION

Biogenic amine compounds in adjuevan
The biogenic amines found in the adjuevan samples were histamine, putrescine, cadaverine, tyramine, β-phenyl ethylamine, and spermidine (Fig. 1). These amines belong to the most important group of biogenic amines generally found in food. As can be seen for both kind of adjuevan, the dominant biogenic amines were β-phenyl ethylamine and cadaverine, followed by putrescine, histamine, and tyramine. The content of β-phenyl ethylamine varied from
3.4 to 6.6 mg/kg in samples produced with method 1, and from 5.8 to 10.0 mg/kg in samples produced with method 2. The contents of cadaverine were 2.2~3.7 mg/kg and 4.7~5.9 mg/kg, respectively, for adjuevan produced from methods 1 and 2. These values were higher than those reported by Restuccia et al. (2015) for tuna and mullet samples, which only reached 1.37 mg/kg (β-phenyl ethylamine) and 6.43 mg/kg (cadaverine) after six months of storage. Cadaverine, although not toxic himself, can increase the negative effect of other amines by interfering with detoxification enzymes that metabolize them (Stratton et al., 1991). β-Phenyl ethylamine can induce effects such as blushing, headaches, and increases in blood pressure (Papageorgiou et al., 2018). Since β-phenyl ethylamine contents of 30 mg/kg in food are considered toxic, it can be stated that adjuevan just at the end of production does not represent a health risk for consumers with regard to the values obtained for this biogenic amine.

Histamine is the biogenic amine most frequently studied for its toxicity and for its rapid accumulation, especially in fish (Prester, 2011). The histamine content in both adjuevan products was lower than 2 mg/kg. These results are in the same range as tuna and mullet bottarga samples that have been stored for six months (Restuccia et al., 2015). On the contrary, Keda et al. (2007) reported higher values of 6~305 mg/kg in fish-nukazuke, one of the traditional and popular fermented fish products in Japan. This difference may be due to the fermentation period, which lasted one week for adjuevan and last one to two years for fish-nukazuke. Furthermore, as the formation of high levels of histamine have been reported to be dependent on the fish species (Prester et al., 2009), this finding may mean that G. decadactylus is less implicated in histamine poisoning than Scomber japonicas (mackerel) and Sardinops melanostictus (sardine). It is also well known that in addition to the hygienic quality of the raw material and the availability of free amino acids, the principal factors contributing to accumulation of histamine in foods are pH, water activity, NaCl concentration, packaging, and temperature during production (Restuccia et al., 2015). According to Parente et al. (2001), a histamine intake of 8~40 mg, 40~100 mg, and higher than 100 mg may cause slight, intermediate, and intensive poisoning, respectively. These data suggest that histamine levels found in adjuevan at the end of production are much lower than those considered hazardous for human health.

Biogenic amines are commonly produced by microbially decarboxylation of amino acids in food. Kouakou et al. (2012) demonstrated that several free amino acids essential for human health (including arginine, tryptophan, histidine, isoleucine, lysine, threonine, methionine, phenylalanine, leucine, valine, and alanine) are present as major compounds in adjuevan. Cadaverine, β-phenyl ethylamine, tyramine, spermidine, putrescine, and histamine are derived from respective decarboxylation of lysine, phenylalanine, tyrosine, arginine, ornithine, and histidine by the Enterobacteriaceae, Lactobacillus, Bacillus, Staphylococcus, and Micrococcus species (Tsai et al., 2006). Levels of putrescine, tyrosine, and spermidine detected in the adjuevan samples were in the following ranges: 1.0~2.9 mg/kg (putrescine), 0.3~2.5 mg/kg (tyrosine), and 0.4~1.1 mg/kg (spermidine). Putrescine and spermidine do not have adverse effects on human health but may aggravate adverse effects of histamine and tyramine since they compete for some of the mechanisms involved in their detoxification (Straub et al., 1995). Tyrosine can induce hypertension and has been identified as the major mutagen precursor. However, we detected tyrosine at levels much lower than the maximum allowable level in foods of 100~800 mg/kg, as identified Nout (1994). Thus, our findings support that no safety concerns are related with both kinds of adjuevan just after their production.

**Fatty acid composition of adjuevan**

The fatty acid composition of adjuevan from the fish species G. decadactylus were just studied at the end of production. By GC analysis, several fatty acid components were detected and quantified in adjuevan samples from both method 1 (entire fish) and method 2 (filleted fish). The data obtained are reported in Table 1. Adjuevan from method 1 was characterized by a content of fatty acids in the order PUFA> saturated fatty acids (SFA)> mono-unsaturated fatty acids (MUFA), whereas adjuevan fatty acid contents derived from method 2 were MUFA>SFA>PUFA. The most abundant SFA in adjuevan samples from method 1 was palmitic acid (C16:0) (22.04 g/100 g) followed by stearic acid (C18:0) (11.69 g/100 g). The contrary was observed for adjuevan from method 2. Stearic and palmitic acid contents were 11.69 g/100 g and 10.65 g/100 g, respectively. This difference could be due to processing. In the first instance, fish were allowed to ferment for 5 days then were sun dried on racks or nets for 4 days. In the second, fish were filleted along both sides and fermentation was carried out simultaneously with sun drying on racks or nets, for at least 7 days. The stearic and palmitic acid contents determined in this study are higher than those reported by Restuccia et al. (2015) for tuna and mullet bottarga samples. The high level of stearic acid may be beneficial to people consuming adjuevan as it lowers the “bad” low-density lipoprotein (LDL)-cholesterol and is therefore considered a healthy saturated fat. On contrary, palmitic acid raises the levels of total cholesterol and LDL-cholesterol without affecting the levels of “good” high-density lipoprotein (HDL)-cholesterol (Hunter et al., 2010). Fortunately, when other fatty acids, such as linoleic acid, are eaten at the same
Table 1. Fatty acid contents of adjueva from the fish species Galeoides decadactylus produced using two processing methods (unit: g/100 g)

| Fatty acid               | Method 1 | Method 2  |
|--------------------------|----------|-----------|
| C14:0 Myristic acid      | 1.26±0.10a | 2.50±0.32b |
| C15:0 Pentadecanoic acid | 1.31±0.40a | 0.85±0.21a |
| C16:0 Palmitic acid      | 22.04±1.31b | 10.65±0.03a |
| C17:0 Heptadecanoic acid | 1.52±0.80a | 1.19±0.70a |
| C18:0 Stearic acid       | 8.55±0.13a | 11.69±0.81b |
| C20:0 Arachidic acid     | 1.06±0.61b | 0.15±0.52a |
| C16:1 (ω-7) Palmitoleic acid | 8.22±0.21a | 9.27±0.43a |
| C17:1 (ω-7) Heptadecenoic acid | 0.53±0.5a | 0.31±0.01a |
| C18:1 (ω-9c) Oleic acid  | 16.80±0.82a | 25.14±0.72b |
| C20:1 (ω-3) Eicosenoic acid | 3.14±0.91b | 1.20±0.21a |
| C22:1 (ω-9) Erucic acid  | 3.54±0.50a | 5.49±0.73b |
| C18:3 (ω-6) Linoleic acid| 0.71±0.32 | nd         |
| C18:3 (ω-3) γ-Linolenic acid | 1.09±1.20a | 2.53±1.49a |
| C20:3 (ω-3) Eicosatrienoic acid | 0.53±0.03 | nd         |
| C20:5 (ω-3) Eicosapentaenoic acid | 6.14±0.71b | 1.65±0.51a |
| C22:3 (ω-3) Docosatrienoic acid | 8.37±0.42a | 6.75±2.06a |
| C22:5 (ω-3) Docosapentaenoic acid | 23.0±0.91b | 9.50±0.31a |
| Total lipid              | 5.84      | 7.21      |
| Saturated fatty acids (SFA)| 35.74     | 27.04     |
| Monounsaturated fatty acids | 31.38      | 39.62     |
| Polyunsaturated fatty acids | 43.61     | 26.10     |
| Unsaturated fatty acids (UNSAT) | 74.99 | 65.72     |
| SFA/UNSAT ratio          | 0.48      | 0.41      |
| ω-3/ω-6 ratio            | 8.87      | 4.12      |

Values are expressed as means±SD for three independent trials.
Different letters (a,b) in the same row indicate significant differences within the same parameter (P<0.05).
nd, not detectable.

time, they can offset some of the effects of palmitic acid on cholesterol (French et al., 2002).

The main MUFAs in both types of adjueva are oleic acid (16.80 g/100 g and 25.14 g/100 g, respectively) and palmitoleic acid (8.22 g/100 g and 9.27 g/100 g, respectively). The next most common MUFAs were erucic acid (1.20 ∼ 3.14 g/100 g) and vaccenic acid (1.41 ∼ 2.83 g/100 g). Our results show that of the two processing methods used in this study, higher MUFA contents were extracted from adjueva using method 2. This finding is in concordance with results reported by Majumdar et al. (2009) on Shidal (salt-free fermented fish product of India) where the most abundant MUFAs were oleic acid (C18:1 ω-9c) and palmitoleic acid (C16:1 ω-7). Other studies have reported that marine fish contain oleic acid, which is a dominant fatty acids among MUFAs (Aydın et al., 2013; Koizumi and Hiratsuka, 2009). The high level of oleic acid in sea fish may be related to its role in the energy metabolism of spawning fish during gonad development (Huynh et al., 2007). Palmitoleic acid is a lipokine, an adipose tissue-derived hormone involved in regulating insulin and systemic metabolic homeostasis associated with type 2 diabetes, obesity, atherosclerosis, and inflammatory disorders (Goldberg et al., 2009). These MUFAs have hypocholesterolemic effects but they do not decrease HDL-cholesterol, which protects against cardiovascular diseases (Romero et al., 2013).

The ω-6 PUFAs in adjueva were composed mainly of linoleic acid (3.54 ∼ 5.49 g/100 g) with very low amounts of linolelaidic acid (0.17 ∼ 0.22 g/100 g), and ω-3 PUFAs showed the most abundant fatty acid (C18:3 ω-3) and palmitoleic acid (C16:1 ω-7). Other studies have reported that marine fish contain oleic acid, which is a dominant fatty acids among MUFAs (Aydın et al., 2013; Koizumi and Hiratsuka, 2009). The high level of oleic acid in sea fish may be related to its role in the energy metabolism of spawning fish during gonad development.
Table 2. Aldehydes of adjuevan from the fish species Galeoides decadactylus produced using two processing methods (unit: mg/100 g)

| Aldehydes                  | RI  | Processing methods            | Odour description                  |
|----------------------------|-----|-------------------------------|-----------------------------------|
|                            |     | Method 1                      | Method 2                          |
| Ropanal                    | 801 | 117.63<sup>b</sup>            | 4.58<sup>a</sup>                  | Sour, chemical                    |
| Pentanal                   | 1,014 | 107.65<sup>b</sup>           | 21.20<sup>a</sup>                | Malt, pungent                     |
| Hexanal                    | 1,090 | 52.86<sup>b</sup>            | 5.17<sup>a</sup>                 | Fat                              |
| 2-Methyl-2-pentenal        | 1,132 | 221.30<sup>b</sup>           |                                   |                                  |
| Heptanal                   | 1,157 | 137.26<sup>b</sup>           | 80.75<sup>a</sup>                | Fats, rancid                      |
| 2-Hexenal (E)              | 1,190 | 66.19<sup>a</sup>            | 129.49<sup>b</sup>               | Green, leaf                       |
| 4-Heptenal (Z)             | 1,213 | 9.11<sup>a</sup>             | 122.71<sup>b</sup>               | Fatty, creamy                     |
| 4-Heptenal (E)             | 1,255 | 60.33<sup>a</sup>            | 104.97<sup>b</sup>               | Fatty, rancid                     |
| Octanal                    | 1,266 | 504.20<sup>a</sup>           | 156.98<sup>a</sup>               | Fatty, pungent                    |
| Nonanal                    | 1,387 | 102.99<sup>b</sup>           | 6.59<sup>a</sup>                 | Green, fatty                      |
| 2-Octenal (E)              | 1,426 | 216.08<sup>b</sup>           | 16.42<sup>a</sup>                | Green                            |
| 2,4-Heptadienial (E,Z)     | 1,483 | 582.61<sup>b</sup>           | 178.37<sup>a</sup>               | Fatty, nuts                       |
| 2,4-Heptadienial (E,E)     | 1,500 | 85.03<sup>b</sup>            | 29.15<sup>a</sup>                | Fatty, fish                       |
| 2,6-Nonadienial (E,Z)      | 1,537 | 3.08<sup>a</sup>             | 1.06<sup>a</sup>                 | Wax, green                        |
| 2-Nonenal (E)              | 1,550 | 45.96<sup>a</sup>            | 279.68<sup>b</sup>               | Fatty, cucumber                   |
| 2,4-Nanodienial (E)        | 1,631 | 32.77<sup>a</sup>            | 105.69<sup>b</sup>               | Geranium, serving                 |
| 2,4-Decadienial (E,E)      | 1,632 | 48.48<sup>b</sup>            | 4.31<sup>a</sup>                 |                                  |
| 2-Decenal (Z)              | 1,674 | 1.08<sup>a</sup>             | 17.28<sup>a</sup>                |                                  |
| 2-Undecenal               | 1,720 | 63.12<sup>b</sup>            | 26.21<sup>a</sup>                | Sweet, fat                        |
| **Total**                  |      | 2,457.73                     | 1,296.3                           |

Different letters (a,b) in the same row indicate significant differences within the same parameter (P<0.05).
RI, Kovalts retention index.
Table 3. Alcoholic compounds of adjuevan from fish species Galeoides decadactylus produced using two processing methods (unit: mg/100 g)

| Alcohols                  | RI   | Method 1     | Method 2     | Odour description               |
|---------------------------|------|--------------|--------------|---------------------------------|
| Ethanol                   | 938  | 28.20        | 78.16        | Alcoholic                       |
| Propanol                  | 1,065| 88.37        | 67.98        | Green, pungent                  |
| 2-Penten-1-ol (E)         | 1,308| 371.38       | 109.6        | Fleshy                          |
| 1-Penten-3-ol             | 1,141| 305.02       | 58.68        | Solvent                         |
| 3-Hexanol                 | 1,214| 25.76        | 18.69        | Fish, grassy                     |
| 2-Methyl-1-butanol        | 1,219| 114.65       | 258.01       | Balsamic                        |
| 1-Pentanol                | 1,228| 129.71       | 285.01       | Spicy, fruits                   |
| 1-Hexanol                 | 1,232| 1.39         | 34.70        | Green, mustard                  |
| 1-Octen-3-ol              | 1,462| 246.02       | 4.29         | Fishy                            |
| 1-Heptanol                | 1,468| 4.43         | 1.86         | Green chemical                  |
| 1-Nonanol                 | 1,670| 29.73        | 5.08         | Oily                            |
| Total                     |      | 1,620.09     | 912.97       |                                 |

Different letters (a, b) in the same row indicate significant differences within the same parameter (P<0.05). RI, Kovats retention index.

Table 4. Esters and ketones of adjuevan from the fish species Galeoides decadactylus produced using two processing methods (unit: mg/100 g)

| Compounds                  | RI   | Method 1     | Method 2     | Odour description               |
|---------------------------|------|--------------|--------------|---------------------------------|
| Esters                    |      |              |              |                                 |
| Ethyl acetate             | 892  | 91.39        | 116.01       | Fruity, orange                  |
| Hexanoic acid, ethyl ester| 997  | 57.39        | 353.24       | Fatty, sweet                    |
| Ethyl butanoate           | 1,069| 134.12       | 221.39       | Fruity, apple                   |
| Butyl acetate             | 1,083| 19.88        | 6.77         | Pineapple note                  |
| 3-Methylbutyl butanoate   | 1,269| 74.78        | 315.58       | Sweet, apricot                  |
| Ethyl heptanoate          | 1,357| 250.34       | 70.36        | Banana, fruit                   |
| 2-Phenyl ethyl acetate    | 1,851| 415.39       | 256.49       | Honey, rose                     |
| Total                     |      | 1,043.29     | 1,339.84     |                                 |
| Ketones                   |      |              |              |                                 |
| 2-3-Pentanedione           | 1,072| 274.61       | 5.65         | Cream, butter                   |
| 4-Methyl-penten-2-one     | 1,139| 38.06        | 3.79         | Chemical                        |
| 2-Nonanone                | 1,385| 7.56         | nd           | Mushroom, fresh                 |
| 3.5-Octadecene-2-one (E,E)| 1,588| 175.64       | 343.71       | Tallow, musty                   |
| 2-Undecanone              | 1,628| 88.45        | 280.69       | Frying fat                      |
| Nona-3,5-dien-2-one       | 1,639| 5.84         | 129.94       | Flower, almond                  |
| Acetophenone              | 1,651| 9.13         | 5.71         |                                 |
| Total                     |      | 599          | 769          |                                 |

Different letters (a, b) in the same row indicate significant differences within the same parameter (P<0.05). RI, Kovats retention index; nd, not detectable.
lytic pathway and amino acids via the Ehrlich pathway (Ganguly et al., 2017). The long straight chain alcohols (2-penten-1-ol, 1-penten-3-ol, and 1-hexanol) and branched chain alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) may provide aromas to 


![adjuevan](https://example.com/adjuevan.png), such as that for 


![budu](https://example.com/budu.png), a traditional Malaysian fish sauce (Mohamed et al., 2012).

Esters were found at a lower prevalence that aldehydes and alcohols. Esters are generally formed from short-chain acids and contribute fruity notes to aromas, while those formed from long-chain acids have fat odours (Marušić et al., 2014). The main esters identified in the study were 2-phenyl ethyl acetate (415.39 mg/100 g) and ethyl heptanoate (250.34 mg/100 g) in sample from method 1, and hexanoic acid ethyl ester (353.24 mg/100 g) and 3-methylbutyl butanoate (315.58 mg/100 g) in samples from method 2 (Table 4). In addition, about half of the esters identified were ethyl esters. The presence of these fatty acid ethyl esters justifies that 


![adjuevan](https://example.com/adjuevan.png) contains several medium chain fatty acids capable of producing these desirable compounds. Furthermore, the ester content was higher in the samples from method 2 than in those from method 1. This higher ester content in these samples may be due to the fermentation conditions, which may favour the growth and activity of aerobic and halophilic microorganisms.

Ketones accounted for the second lowest percentage of the all the volatile components in 


![adjuevan](https://example.com/adjuevan.png). However, ketones have low odour thresholds, so they may have a significant effect on the flavour of 


![adjuevan](https://example.com/adjuevan.png). The most prevalent volatile ketones in 


![adjuevan](https://example.com/adjuevan.png) from method 1 were 2,3-pentanedione (274.61 mg/100 g) and 3,5-octadien-2-one (175.64 mg/100 g) (Table 4). In contrast, in 


![adjuevan](https://example.com/adjuevan.png) from method 2, the most prevalent volatile ketones were 3,5-octadien-2-one (E,E) (343.71 mg/100 g) followed by 2-undecanone (280.69 mg/100 g). Such differences between 


![adjuevan](https://example.com/adjuevan.png) types are likely driven by the processing method because ketones like aldehydes, can be produced from fatty acid by oxidation via fermentation processes (Dajanta et al., 2011).

Three furan compounds were also detected in 


![adjuevan](https://example.com/adjuevan.png), with 2-pentylfuran (210.60 mg/100 g) and 2-acetyl furan (248.37 mg/100 g) being the most predominant in 


![adjuevan](https://example.com/adjuevan.png) from methods 1 and 2, respectively (Table 5). Furans greatly intensify the aroma of fermented food. Usually, furans are formed through Amadori rearrangement path-

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**Table 5. Furans, acids and aromatic compounds of **adjuevan** from the fish species Galeoides decadactylus produced using two processing methods** (unit: mg/100 g)

| Compounds          | RI  | Processing methods          | Odour description       |
|--------------------|-----|----------------------------|-------------------------|
|                    |     | Method 1 | Method 2 |                   |
| Furans             |     |          |          |                   |
| 2-Ethyl-furan      | 903 | 24.37<sup>a</sup> | 28.78<sup>a</sup> | Rubber, pungent  |
| 2-Pentylfuran      | 1,238 | 210.60<sup>b</sup> | 2.50<sup>a</sup> | Green bean like |
| 2-Acetylfuran      | 1,510 | 102.44<sup>a</sup> | 248.37<sup>b</sup> | Smoky, tobacco  |
| Total              |     | 337.4 | 279.64 |                   |
| Aromatic compounds |     |          |          |                   |
| Benzaldehyde       | 1,525 | 426.97<sup>b</sup> | 194.57<sup>a</sup> | Burnt sugar     |
| 4-Ethylbenzaldehyde| 1,728 | 349.55<sup>b</sup> | 137.14<sup>a</sup> | Fruity, anisic  |
| Benzyl alcohol     | 1,889 | 90.85<sup>a</sup> | 194.78<sup>b</sup> | Sweet flower, aromatic |
| 2-Phenylethyl alcohol| 1,931 | 282.07<sup>b</sup> | 16.68<sup>a</sup> | Honey, rosy    |
| Phenol             | 2,042 | 3.31  | nd      | herbal, anisique  |
| 3-Methyl-phenol    | 2,115 | 12.78 | nd      |                   |
| Indole             | 2,330 | 54.51<sup>b</sup> | 3.58<sup>a</sup> | Naphthalene, burned |
| α-Pinene           | 1,019 | 31.58<sup>b</sup> | 2.53<sup>a</sup> | Pine, turpentine|
| α-Thujene          | 1,026 | nd     | 1.37    | Wood, green gras |
| Trimethylamine     | 695  | 218.33<sup>a</sup> | 240.88<sup>a</sup> | Fish            |
| Total              |     | 1,469.95 | 741.31 |                   |
| Acids              |     |          |          |                   |
| Butanoic acid      | 1,645 | 195.29<sup>b</sup> | 148.18<sup>a</sup> | Rancid butter   |
| 2-Methyl-propanoic acid | 773  | 10.42<sup>a</sup> | 7.15<sup>a</sup> | Meat, cheese    |
| 3-Methyl-butanolic acid | 852  | 383.11<sup>b</sup> | 124.24<sup>a</sup> | Rancid, sweet   |
| 4-Methyl-pentanoic acid | 1,848 | 167.54<sup>b</sup> | 73.11<sup>a</sup> | Pungent         |
| Hexanoic acid      | 1,893 | 6.44<sup>a</sup> | 2.96<sup>a</sup> | Pungent         |
| Octanoic acid      | 2,058 | 56.20<sup>b</sup> | 1.81<sup>a</sup> | Cheese          |
| Acetic acid        | 1,443 | 171.03<sup>a</sup> | 258.57<sup>b</sup> | Sour            |
| Total              |     | 990.03 | 616.02 |                   |

Different letters (a,b) in the same row indicate significant differences within the same parameter (P<0.05).

RI, Kováts retention index; nd, not detectable.
ways or are present in dehydrated or fermented carbohydrate condensates (Giri et al., 2010). Oxidation of fatty acids may also produce furans and their derivatives. 2-Pentylfuran and 2-acetyl furan may produce beary, grassy, and smoky smells in both types of adjuevan, whereas Giri et al. (2010) suggested only 2-pentylfuran is the most dominant in *miso*.

We also detected aromatic compounds, dominated by 4-ethyl-benzaldehyde (349.55 mg/100 g for method 1), benzaldehyde (426.97 mg/100 g and 194.57 mg/100 g, respectively for methods 1 and 2) and trimethylamine (240.48 mg/100 g for method 2) in adjuevan (Table 5). Aromatic compounds in several fermented food products are generally produced as a result of aromatic amino acid catabolism, beginning with a transamination step of phenylalanine, tyrosine, and tryptophan. Trimethylamine may give an unpleasant odour to the fermented fish, as reported by Fukami et al. (2004) for the fermented sauce “Moroni”. The major acid compounds in this study were 3-methyl-butyric acid and acetic acid. In general, acetic acid imparts sour odours, and 3-methyl-butyric acid has rancid and sweet aromas. Therefore, adjuevan from method 2 may have a stronger pungent odor than adjuevan produced from method 1. Kouakou et al. (2012) also reported many organic acids in adjuevan, identifying acetic and butyric acids as the most predominant.

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**AUTHOR DISCLOSURE STATEMENT**

The authors declare no conflict of interest.

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