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Quality Characteristics of Smoked Barracuda Fish (*Sphyraena jello*) Processed with Different Concentrations of Corncob Liquid Smokes

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ABSTRACT Smoking with liquid smoke is one of the alternative processing methods with lower temperatures and is expected to reduce protein and amino acid damage. This study aimed to determine the effect of different concentrations of corncob liquid smoke on the quality of both chemical and sensory of smoked barracuda fish. The method used was smoking barracuda fish with four different concentrations of corncob liquid smoke, namely 0%, 2.5%, 5%, and 7.5% at a temperature of 40-50°C (1 hour), 50-60°C (1 hour), and 70-80°C (2 hours). The main parameter observed was amino acid profile, and supporting parameters were protein, water, phenol content, and organoleptic. The results show that amino acids in barracuda fish increased with increasing concentration of liquid smoke. The highest amino acid was generated by a 7.5% concentration of corncob liquid smoke with an essential amino acid value of lysine 22,035.56 mg/kg. Meanwhile, the highest non-essential amino acid was glutamic acid with a value of 36,868.31 mg/kg. The immediate concentration of 7.5% overall gave the best results with water content 48.11 ± 1.94%, protein content 25.43 ± 0.05%, phenol content 0.188 ± 0.00%, and organoleptic with a confidence interval of $8.367<\mu<88.666$.

Keywords: Amino acid profile; liquid smoke; phenol content; smoked barracuda fish

INTRODUCTION

Barracuda fish (*Sphyraena jello*) is a fishery commodity that has economic value in Indonesia. The open market for barracuda fish is relatively high because it has delicious meat so the public often consumes it. Smoking is one of the fish processing that uses high temperatures. Hot smoking is a method commonly used in traditional smoking. The temperature used for hot smoking is 80-100°C (Wicaksono *et al*., 2014). The hot smoking method causes frequent cases of nutritional damage due to uncontrolled heating and the smoking process.

Proteins have complex structures that are important to their function. This structure has numerous levels including primary, secondary, tertiary, and quaternary (Bischof & He, 2006). When the protein is heated, protein is transformed from an ordered "native" to a less-ordered state due to the rearrangement of hydrogen bonding without any change to covalent bonds. Protein denaturation results in decreased solubility, loss of biological activity, increased viscosity and protein susceptibility to attack by proteolytic enzymes. The use of high temperatures can have a positive effect on the properties of the protein, but if the heating is not controlled it can cause a decrease in the value of protein and amino acids.

An alternative smoking method that can be used is liquid smoke. Liquid smoke is the result of condensation from wood containing phenol, organic acids, and carbonyl. These three compounds play a role in improving the properties of smoked fish products, antimicrobial and antioxidant. In the traditional method, the smoke directly hits the product at a high temperature. In contrast, the temperature used in smoking with liquid smoke is lower, but the smoking time is longer (Swastawati *et al*., 2018). The smoking method with liquid smoke that uses a lower temperature is expected to prevent damage to protein and amino acids caused by the high temperatures (Cieslik *et al*., 2018), the heating method can affect amino acid changes in fish. The proteolytic reactions that occur during low heating can cause the increased formation of free amino acids.

Smoking at 70°C for 4-6 hours increases almost all of the amino acids. Smoking causes an increase in amino acids in pike fish (*Northern pike*) with the most dominant amino acids being glutamic acid, aspartic acid, lysine, and leucine. Glutamic acid increased from 2.06 to 2.70, aspartic acid increased from1.40 to 1.83, lysine increased from 1.26 to 1.64, and leucine increased from 1.11 to 1.44 g/100g (Cieslik, 2017).

The addition of liquid smoke in different concentrations is hypothesized to increase amino acids. This is presumably due to the phenol and acid content in liquid smoke which causes the opening of the three-dimensional arrangement of the protein molecule into a random structure. The unfolding of protein folds makes it easier for digestive enzymes to hydrolyze and break down proteins into amino acid monomers.

Based on this description, it is necessary to determine the effect of liquid smoke concentration on smoked barracuda fish's amino acid profile and its effect on protein, water, phenol content, and organoleptic values.

MATERIALS AND METHODS

Materials

The materials used in this study were barracuda fish (*Sphyraena jello*) from Kobong Fish Market, Central Java, Indonesia, with an average length of 40-45 cm and corncob

liquid smoke from Asap Cair Multiguna company. The equipmentused in this research is Ultra Performance Liquid Chromatography (Acquity), oven (Binder), analytical balance (Fujitsu FS-AR210), and Kjeldigester (Buchi K-446).

Methods

Sample

Barracuda fish were weeded, filleted, and washed with clean water. The solutions were prepared from liquid smoke, salt, and water. The fish samples were then immersed in a 3% salt solution and corncob liquid smoke with varying concentrations of 0%, 2.5%, 5%, and 7.5% for 15 minutes. Furthermore, smoking is carried out in an oven at a temperature of 40-50°C (1 hour), 50-60°C (1 hour), and 70-80°C (2 hours). The smoked fish is then directly analyzed for its water content and sensory in the analysis laboratory, the Faculty of Fisheries and Marine Sciences. While the amino acids were analysed in Saraswati Laboratory, Bogor.

Amino acid analysis

Testing the amino acid profile using the Ultra Performance Liquid Chromatography (UPLC) method (The National Standardization Agency of Indonesia, 1992). The test consisted of several stages. The sample is weighed as much as 0.1 g, crushed and put into a closed test tube. The sample solution was added with 5-10 ml of 6 N HCl hydrolyzed in an oven at 110°C for 22 hours, then cooled at room temperature and transferred to a 500 ml volumetric flask. Then, distilled water was added to the mark and filtered with a 0.45 L filter and 10 L pipette, added 70 L ACCQ Fluor Borate and vortexed. Then 20 L of Flour a reagent was added and vortexed, then allowed to stand for 1 minute and incubated for 10 minutes at 55°C. Then the sample was injected into the UPLC as much as 1 L with chromatographic conditions using the ACCQ-Tag Ultra C18 column, temperature 49°C, mobile phase composition system gradient detector PDA, flow rate 0.7 L/min and wavelength 260 nm. The amino acid content in the ingredients can be calculated by the formula:

$$\text{Amino acid (\%)} = \frac{sample\ area\ x\ C\ x\ Fp\ x\ BM}{standard\ area\ x\ sample\ weighht}\ x\ 100\%$$

Information:
C : Standard concentration of amino acids (µg/ml),
FP : Dilution factor,
BM : The molecular weight of each amino acid (g/mol).

Protein content analysis

Protein content was analyzed based on (The National Standardization Agency of Indonesia, 2006). The analysis was carried out by testing the protein content of smoked fish using the Kjeldahl method. The sample was weighed as much as 2 grams and put into a digestion flask. Next, 2 tablets of Kjeldahl, and 15 ml of concentrated H_2SO_4 were added slowly and allowed to stand for 10 minutes in an acid chamber. Destruction was carried out at 410°C for ± 2 hours or until the solution was clear, then allowed to stand until it reached room temperature and added 50-75 ml of distilled water. Erlenmeyer containing 25 ml of 4% H_3BO_3 solution containing indicator as a container for the distillate was prepared. The flask containing the destruction results was installed in a series of steam distillation apparatuses, and then 50-75 ml of sodium hydroxide-thiosulfate solution was added. Then distillation is carried out and the distillate is accommodated in an Erlenmeyer until the volume reaches a minimum of 150 ml (the distillate will turn yellow). The distillate results were titrated with standardized 0.2 N HCl until the colour changed from green to neutral grey. The water content is calculated by the formula:

$$\text{Protein content (\%)} = \frac{(Vp - Vb)\ x\ N\ HCl\ x\ 14{,}007\ x\ FK}{Sample\ weight\ (g)}$$

Information:
Vp : HCl volume required for sample titration (ml),
Vb : HCl volume required for blank titration (ml),
N : HCl solution normality,
Fk : Conversion factor (6.25 for fishery products).

Moisture content analysis

The water content was analyzed based on (The National Standardization Agency of Indonesia, 2015). The analysis was carried out on the level of smoked fish using the gravimetric method. The procedure for testing the moisture content is that the oven is conditioned at the temperature to be used until it reaches a stable condition. The empty cup is then placed in the oven for at least 2 hours. The empty cup was transferred to desiccators for about 30 minutes until it reached room temperature and weighed the empty weight (Ag). Samples that have been finely weighed as much as ± 2 g into a cup (Bg). The cup that has been filled with the sample is put in an oven at a temperature of 95°C – 100°C. The cup is then transferred using a clamp into a desiccator for ± 30 minutes and then weighed. The water content is calculated by the formula:

$$\text{Moisture content (\%)} = \frac{B - CB - C}{B - AB - A}\ x\ 100\%$$

Information:
A : Weight of the cup (g),
B : Weight (cup +sample) before drying (g),
C : Weight of sample (cup +sample) after drying (g).

Phenol content analysis

Testing phenol levels by weighing 5 g of samples that have been tested crushed and then put into an Erlenmeyer volume of 100 ml, diluted with distilled water to a volume of 100 ml using a measuring flask. The solution was filtered/centrifuged until a clear solution was obtained. Next 1 ml solution was put into a test tube and 0.5 ml of Follis Denis was added. Then 1 ml of saturated Na_2CO_3 solution was added and allowed t stand for 10 minutes. Furthermore, distilled water was added to a volume of 10 ml and then vortexed until homogeneous. The absorbance of the sample is read using a spectrophotometer at a wavelength of 730 nm. The results are recorded and then calculated using the phenol standard curve.

Organoleptic test

Organoleptic tests on fish were immediately carried out using a scoresheet that refers to (The National Standardization Agency of Indonesia, 2013). The number of panellists in the organoleptic test of smoked fish was 30 panellists. Panellists will assess the quality of smoked fish products based on the parameters of appearance, smell, taste, texture, mould, and slime then give a value from numbers: 3-9 based on product specifications.

Statistical analysis

The design of this study used a completely randomized design. Parametric data were analyzed using analysis of variance (ANOVA). If the analysis shows significantly different results, then proceed with the Tukey mean test. Non-

parametric data were analyzed using Kruskal Wallis, if it shows significantly different results, then the Mann-Whitney test can then be carried out by using SPSS version 16.0 for windows (SPSS Inc., Chicago, USA) with three replications.

RESULTS AND DISCUSSION

Amino acid

Amino acids are substances that bind to each other to form peptide bonds (Sumandiarsa *et al.*, 2020). Amino acid test results showed that smoked barracuda fish (*Sphyraena jello*) had complete amino acid content. The results of the amino acid profile are presented in Table 1.

Table 1. Amino acid profile of smoked barracuda fish processed using liquid smoke with different concentrations.

Amino acid	0%	2,5%	5%	7,5%
Essential				
L-Lysine	20190.17	20650.00	20871.51	22035.56
L-Leucine	18474.49	19114.97	20340.99	22030.37
L-Arginine	18746.73	15881.40	17981.13	19956.02
L-Threonine	13990.91	13275.52	12455.77	15466.44
L-Valine	13282.51	12436.83	12029.13	14218.19
L-Phenylalanine	9010.35	12506.28	11939.99	12200.62
L-Isoleucine	10340.80	10783.11	11532.14	12560.74
L-Histidine	5450.79	6681.07	7008.14	7334.80
Non-Essential				
L-Glutamic acid	32201.84	36282.33	34237.18	36868.31
L-Aspartic acid	18999.11	21895.33	20118.35	21591.86
L-Alanine	16545.30	15460.01	17394.76	16925.47
Glycine	13182.03	11975.60	13232.32	13621.76
L-Serine	10283.01	10899.10	11566.16	12770.55
L-Tyrosine	7204.35	10038.07	10182.76	10238.52
L-Proline	8605.03	8043.46	8824.37	9215.69
Total amount	**216,507.42**	**225,923.08**	**229,714.70**	**247,034.90**
Essential	109,486.75	111,329.18	114,158.80	125,802.74
Non-Essential	107,020.67	114,593.90	115,555.90	121,232.16

The test results showed that barracuda fish (*Sphyraena jello*) has a complete amino acid content, both essential and non-essential amino acids. The test results show that lysine is the highest essential amino acid in smoked barracuda (20,190.17 - 22,035.56 mg/kg). The highest lysine was in the addition 7.5% liquid smoke concentration and the lowest was in the control treatment. The concentration of liquid smoke used is directly proportional to the value of the lysine content. The greater of liquid smoke concentration, the greater value of the lysine content (Megawati & Swastawati, 2014). While the highest non-essential amino acid value found in smoked barracuda fish was glutamic acid with the highest value at the addition of 7.5% liquid smoke, which was 36,868.31 mg/kg.

Overall, both essential and non-essential amino acids increased and decreased at each concentration of liquid smoke. However, the total amino acid value increased with the addition of liquid smoke concentration. This is presumably due to the phenol and acid content in the liquid smoke, which changes the protein composition and increases protein digestibility to increase amino acids. The increase of amino acids is due to the acetic acid content in the liquid smoke. The acidic nature of liquid smoke can donate H+ ions so that it can cause amino acids to be at the isoelectric point and in the form of amphoteric (zwitter ions). Amino acids at this point can release hydrogen bonds that form secondary, tertiary, and quaternary structures. So that due to the breakdown of the complex structure, it produces a primary structure of amino acids that are bound by peptide bonds and increases the value of amino acids.

Phenol and acid compounds in liquid smoke also play a role in maintaining the availability of amino acids because they have antibacterial properties. Amino acids are very easily damaged by bacterial activity which can cause rot and cause unpleasant odours. According to Sumpono (2018), this antibacterial property is related to the content of compounds in liquid smoke, namely phenol and acetic acid. Phenol compounds can damage the cytoplasmic membrane causing leakage of the membrane this can interfere with bacterial growth and can even cause death. Acetic acid also has an important role in liquid smoke because it can cause destabilization of various functions and structures of cell components phenolic compounds can damage the cytoplasmic membrane, causing leakage of the membrane so that which can interfere with bacterial growth and even cause death.

Overall essential amino acids in smoked barracuda fish with the liquid smoke method showed higher yields than in traditional smoking fish. Research by Usydus (2009) showed that the amino acids in mackerel with traditional smoking were 1.57 g/100g lysine, 1.40 g/100g ppm leucine, and 1.12 g/100g arginine. This value is lower than the research

results, respectively 2.20 g/100g, 2.20 g/100g, and 1.99g/100g. The amino acid value of smoked barracuda fish also increased compared to fresh barracuda fish. The amino acids lysine, leucine, and arginine in fresh barracuda fish were 1.95 g/100g, 1.63 g/100g, 1.23 g/100g (Pradana, 2013) and increased by 13%, 35%, and 62%.

Protein content

The protein content analysis showed an increase in protein content along with an increase in the concentration of liquid smoke. The lowest protein content was in the control and the highest protein content was in the treatment with the addition of 7.5% liquid smoke. The increase in protein content is in line with the increase in amino acid values along with the increase in the concentration of liquid smoke.

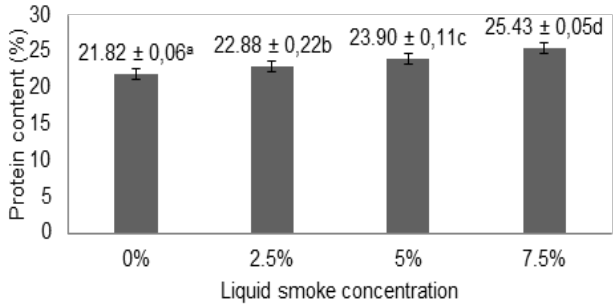

Figure 1. The protein content of smoked barracuda fish.

Protein content has an inverse relationship with water content. The percentage of protein increases with water loss during the smoking process. At a concentration of 7.5%, the protein content has the highest value of 25.43%, while the water content has the lowest value of 48.11%. Acid compounds in liquid smoke cause proteins to lose their water-binding properties so that water can easily come out of the material. Budiarti *et al*. (2016) that the acidic nature of liquid smoke can cause water in fish meat to come out. This is directly proportional to the concentration of liquid smoke where the higher the concentration of liquid smoke, the more acid content so that it can release more water.

The increase in protein content is also caused by the protein denaturation process which causes the protein to lose its solubility. This causes coagulation or clumping so that protein is left in the meat and causes protein levels to increase (Gomez-Guillen *et al*., 2000). The use of liquid smoke in products can cause water to come out of fish meat due to the acidity of the liquid smoke which can cause the insoluble protein of the meat. The higher protein content of smoked catfish was caused by protein denaturation in smoked fish (Martinez *et al*., 2007; Khamidah *et al*., 2009). According to Sanny *et al*. (2013), liquid smoke has a high osmotic pressure so that it can draw water from fish and cause protein denaturation and coagulation resulting in shrinkage of fish meat and separate proteins. The results of the protein coagulation process are usually able to form the desired characteristics. Factors that can affect the occurrence of protein coagulation are heat, shaking, pH, salt and sugar.

The phenol content also influences protein denaturation in liquid smoke. The value of phenol is directly proportional to the protein. The highest phenol content was 0.118% at a concentration of 7.5%. Phenol at low levels interacts with proteins to form phenol protein complexes. The bond between protein and phenol is weak and undergoes decomposition immediately. Free phenol will penetrate the cell, causing precipitation and protein denaturation (Handrianto, 2016).

Moisture content

The water content analysis showed that the higher of liquid smoke concentration, the lower of water content value. The highest water content was in the control treatment or without the addition of liquid smoke, while the lowest water content was in the treatment with a 7.5% liquid smoke concentration. All test results show the water content of smoked barracuda fish is appropriate with Indonesian standards that the maximum water content of smoked fish is 60% (The National Standardization Agency of Indonesia, 2013).

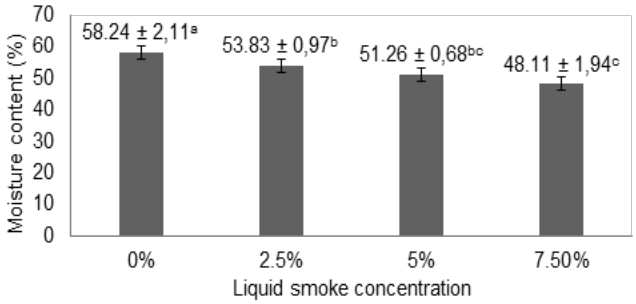

Figure 2. The moisture content of smoked barracuda fish.

The lower water content, is inversely proportional to the concentration of liquid smoke, due to the nature of liquid smoke, which can remove the water content in fish. Acid compounds in liquid smoke cause protein denaturation and proteins lose their biological properties in binding water in the material so that water can easily come out of the material (Widiastuti *et al*., 2019).

The salting process also causes the reduced water content in smoked barracuda. Salt and acid diffuse into the fish muscle, changing the protein properties and lowering the pH value. The low pH value can cause protein denaturation (Serdaroglu *et al*., 2015). The interaction between myofibril proteins and salts can cause protein denaturation, causing texture changes and reducing water holding capacity.

Phenol content

The phenol level analysis showed that the sample with the 7.5% liquid smoke treatment had the highest phenol content, while the lowest was in the control treatment. Phenol content increases with the addition of the concentration of liquid smoke. It is due to the phenol being the main component in liquid smoke. Corncob liquid smoke has the main component of carbonyl, dominated by phenol and its derivatives (Swastawati *et al*., 2014).

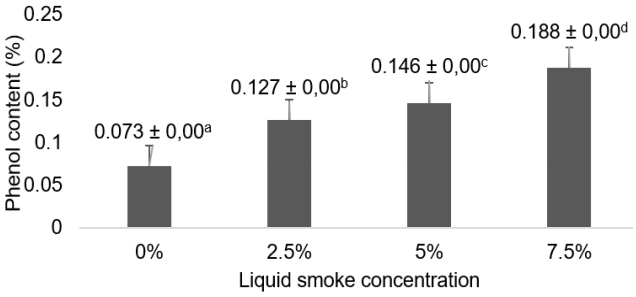

Figure 3. Phenol content of smoked barracuda fish.

Phenol is an antioxidant compound found in liquid smoke to

extend the shelf life of smoked products. Besides that, phenol gives a distinctive taste and colour to processed products. However, the higher the phenol content can cause a carcinogenic effect (Swastawati, 2007). The safe limit of phenol levels in smoked fish during storage for consumption is 0.02 – 1.00% (Davidson & Branen, 1981). All of the smoked barracuda samples contained phenol below 1.00%, so it was safe for consumption.

The type of fuel strongly influences phenol content in liquid smoke. This study uses liquid smoke with fuel from corncob. The phenol content in corncob liquid smokes is 1.38% lower than in coconut shells, which were 3.04% (Nurhazisa et al., 2018).

Organoleptic

The organoleptic test is an assessment technique using the five senses as a parameter. The organoleptic test acts as early detection in assessing quality to determine deviations and changes in the product (Bernadeta, 2012). The results of the organoleptic assessment of smoked barracuda fish including appearance, odour, taste, and texture parameters are presented in Table 2.

Table 2. Organoleptic of smoked barracuda fish.

Parameter	Treatments			
	0%	2.5%	5%	7.5%
Appearance	8.13±1.00^{a}	9.00±0.00^{b}	8.73±0.86^{b}	9.00±0.00^{b}
Odour	6.20±0.99^{a}	7.33±0.75^{b}	8.26±0.98^{c}	8.53±1.13^{c}
Taste	5.73±0.98^{a}	7.46±0.86^{b}	8.00±1.01^{c}	8.06±1.01^{c}
Texture	7.53±0.89^{a}	7.93±1.01^{a}	7.80±0.99^{a}	8.46±0.89^{b}

The difference in the concentration of liquid smoke significantly affected the appearance of smoked barracuda fish ($P<0.05$). The resulting colour is glossy brown and the intensity of the colour increases with increasing concentration of liquid smoke. When the product is heated, the carbonyl compound will react with the protein and produce a brownish colour. The best colour in smoked fish is golden and shiny due to the capture of phenolic compounds on the oil surface and the Maillard reaction to form a light brown colour (Toledo, 2007).

The organoleptic test on the odour of smoked barracuda fish showed that smoked fish with control treatment was not suitable for consumption with a value of 6.20. In comparison, smoked fish with liquid smoke is suitable for consumption with a value ranging from 7.33 to 8.53. The smell of smoked fish is produced by reacting carbonyl compound liquid smoke with fat to produce a distinctive aroma (Swastawati *et al*., 2018). The aroma and taste of smoked products are strongly influenced by volatile compounds attached to the meat. Important phenolic compounds such as syringaldehyde and coniferyl aldehyde adhere to food and give smoked fish an intense aroma. The attachment also prevents the loss of aroma over time by evaporation that occurs during storage (Martinez *et al*., 2011).

The organoleptic test on the taste of smoked barracuda fish showed that smoked fish with control treatment was not suitable for consumption with a value of 5.73. The control treatment without the addition of liquid smoke produces a bland taste. At the same time, with smoked fish, the addition of liquid smoke is suitable for consumption with values ranging from 7.46 to 8.06. The long soaking time given produces enough carbonyl compounds. They seep into the components of the fish meat tissue and react with amino acid compounds so that the taste of smoked fish increases (Sulistijowati & Rivai, 2020). In general, the flavour of smoked fish is influenced by phenol compounds. Phenol concentration is used to access the intensity of smoked flavour in fish or meat (Toledo, 2007).

The texture of smoked barracuda showed that smoked fish was suitable for consumption with values ranging from 7.53 to 8.46. This value shows the texture specifications of smoked barracuda fish, which were dense, compact, and tightly intertwined. The highest organoleptic texture value was in the treatment with the addition of 7.5% liquid smoke concentration. It is suspected that the texture value increases with the decrease in water content. The difference in texture values is thought to be due to differences in water content. The higher the water content of smoked fish, the lower the texture value, and vice versa (Isamu *et al*., 2012). The texture of food ingredients is closely related to the water content in the food ingredient. The higher the water content, the softer or mushy the texture. The lower the water content, the higher the texture. It is due to the fish meat will be denser or more rigid as the water content in the fish decreases (Adawyah, 2007).

CONCLUSIONS

Giving corncob liquid smoke to smoked barracuda fish (*Sphyraena jello*) can increase the amino acid value. The addition of 7.5% liquid smoke concentration resulted in the best amino acid profile with a total value of 24.70 g/100g, water content 48.11%, protein 25.43%, phenol 0.188%, and the best organoleptic value $8.36 < \mu < 8.66$. The difference in concentration of corncob liquid smoke applied to smoked barracuda fish (*Sphyraena jello*) tends to increase amino acids, and proteins, and reduce the water content. Further research should be carried out with a greater concentration of liquid smoke or liquid smoke from other materials to determine the changes that may occur.

AUTHORS' CONTRIBUTIONS

All authors have contributed to the final manuscript. The contribution of each author is as follows, FS; devised the main conceptual ideas, supervision, visualization, validation, writing-review and editing. UNW; conceptualization, data curration, formal analysis, methodology, resources, visualization, and writing-original draft preparation. RR; supervision, visualization, validation, writing-review and editing.

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