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RESEARCH ON THE CHANGE OF 2-AP AND OTHER VOLATILE COMPOUNDS IN PROCESSING BUN FROM RICE

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ABSTRACT

Vermicelli is the traditional dish of Vietnam which is the main material to prepare "Hue Beef rice vermicelli" (Bun bo Hue), a very famous specialty originated from Hue city, the former imperial capital of Vietnam. Flavor and taste are important attributes of vermicelli. This study was carried out to determine the change in 2-AP and other volatile compounds as influenced by different durations of soaking rice in water during vermicelli processing. In order to identify and quantify the amount of 2-AP and other volatile compounds 2-AP extracted from pandan leaves was used as standard. Results indicated that 2-AP and other volatile compounds clearly changed in the vermicelli processing process and soaking for 12 hours was recommended.

Keywords: Rice vermicelli, 2- Acetyl - 1 Pyrroline (2-AP), volatile compounds.

Nghiên cứu sự thay đổi cấu tử thơm 2-AP và các chất bay hơi khác trong qui trình chế biến bún từ gạo

TÓM TẮT

Bún là nguyên liệu chính để chế biến "Bún Bò Huế", đây là một món ăn đặc sản của Việt Nam có nguồn gốc từ thành phố Huế, trước đây là kinh đô của Việt Nam. Hương vị đặc trưng của sợi bún quyết định tới chất lượng bún sản phẩm. Để làm rõ những sự thay đổi này, đề tài nghiên cứu đã tiến hành chiết xuất cấu tử chính tạo nên mùi thơm 2 Acetyl- 1 Pyrroline từ lá dứa và sử dụng nó như là chất chuẩn để định tính và định lượng sự thay đổi này bao gồm cấu tử chính 2-AP và các cấu tử bay hơi khác trong gạo ngâm nước theo quy trình chế biến bún truyền thống. Kết quả chứng tỏ rằng cấu tử thơm 2-AP và những cấu tử bay hơi khác đã biến đổi một rất rõ rệt trong quá trình chế biến này. Trên cơ sở đó đưa ra khuyến cáo ngâm gạo trong 12 giờ quy trình chế biến bún.

Từ khóa: Bún, cấu tử bay hơi, 2 Acetyl - 1 Pyrroline (2-AP).

1. INTRODUCTION

In Vietnam, there are many rice varieties in that the most interesting is aromatic rice (Phan Phuoc Hien et al., 2009). The key aromatic constituent 2-Acetyl-1-Pyrroline (2 - AP) in aromatic rice was found out in pandan leaf (*Pandanus amaryllifolius*), also existed in white bread, and flowers (*Vallaris glabra*) (Varaporn Laksanalamai et al., 1993). Due to pandan leaf contains this specific constituent with very high content as compared with aromatic rice (Phan Phuoc Hien, 2011), it is often used to enhance the appealed flavor of foods in many countries such as Indonesia, Philippines, Malaysia, Thailand, Vietnam and Burma, especially in rice cooked and sweet cakes (Varaporn Laksanalamai and Sarath Ilangantileke, 1993).

In order to develop aromatic rice production in Viet Nam, reliable and practical methods to assess 2-AP and other volatile compounds in aromatic rice are required to evaluate and select the better varieties. In response to this demand, during the past 7 years, two modern methods have been fitted up and operated at the Physiochemical laboratory in Nong Lam University, Ho Chi Minh City, Vietnam. The first is Solid Phase Micro-Extraction coupling with Gas Chromatography (SPME/GC) and Mass Spectrometry (SPME/MS), and the second is SDE (Simultaneous Distillation Extraction) also

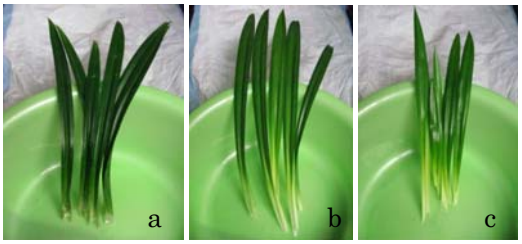
coupling with GC and GCMS. SPME/GC enables for estimation of 2-AP low concentration like aromatic rice. The SDE method is suitable for extraction of the 2-AP high concentration materials like Pandan leaf (Phan Phuoc Hien, 2011). Based on the two methods we studied for extraction and quantitative analysis of 2-AP in the pandan leaf and used it as the standard for qualitative and quantitative analysis of 2-AP in aromatic rice and other medicinal plants such as Thien Nien Kien *Homalomena aromatica* (Phan Phuoc Hien et al., 2011).

In the process of vermicelli strands (Bun) prepared from rice, step of soaking rice in water with different duration definitely influences on quality and flavor for the end-product "Bun". In reality, this step made change biochemical properties of rice material leading to finally change 2-AP and other volatile compounds in rice. In order to demonstrate clearly these changes, SDE and SPME coupling with GC-FID and GCMS were used to identify, quantify and presented in this paper.

2. MATERIALS AND METHODS

2.1. Materials

The pandan (*Pandanus amaryllifolius*) leaves (Fig. 1) were used to extract 2-AP as standard. Rice materials include two varieties from Vietnam, OM 6162, *Khao Dawk Mali* (KDM) and two varieties from Korea, Chucheong variety (milled rice), and Black rice (mixture of several varieties).



Pandan leaves (collected from Di An District, Binh Duong, Vietnam) are classified into 3 types:
old leaf (a), young leaf (b), and mature leaf (c)

Fig 1. Three types of Pandan leaves: old leaf (a), young leaf (b), and mature leaf (c)

For the identification and quantification of change in 2-AP and other volatile compounds, milled rice of KDM was soaked in water for 12 and 48 hours; non-soaked rice served as control.

2.2. Extraction methods

a. Simultaneous Distillation-Extraction (SDE)

The steam distillation-solvent extraction was used as a reference for 2AP and other volatile compounds quantification. Extraction was performed using Godefroot apparatus (Godefroot et al., 1981) on 20g of brown rice with dichloromethane as solvent and collidine as internal standard. Duration of extraction was 30 minutes from apparition of the first drop of water in the bottom of the condensed tube. Volatile compound extracts were then concentrated to 0.3 ml by drying under a nitrogen flow at room temperature and stored at -18°C prior to GC/FID and GCMS analysis (Phan Phuoc Hien et al, 2009; 2010; 2011).

b. Solid Phase Micro Extraction (SPME)

Extraction of volatile fractions in rice was performed by using a Supelco VB/Carboxen/PDMS (divinylbenzène/ Carboxen/ polydiméthylsiloxane) fiber 3.5 g of milled rice with 500 µl of water were placed in a 10 ml vial. As for rice samples analysed by SPME-GC, collidine was added as an internal standard. The solution was equilibrated at 80°C for 5 minutes then the fiber was introduced in the headspace surrounding rice at the same temperature for 15 minutes (Phan Phuoc Hien, 2009; 2010; 2011).

2.3. Analysis methods

a. Quantification of 2AP concentration by GC-FID

The extracts obtained by the SDE and SPME were analysed by using a Hewlet Packard 5890 Series II gas chromatograph with a flame ionisation detector (GC-FID). The column was a non-polar DB-5 (J&W Scientific) capillary column (length 60m, 0.32mm, film

thickness 0.25 µm). Helium was used as carrier gas at a flow rate of 1.9 ml/min at 25^0C. The injection was performed in splitless mode first (5 min for SPME and 2 min for SDE), then in split mode to the end of the cycle (38.5 min for SPME and 70 min for SDE). After warming the column at 40^0C for 5 minutes, the following temperature programs were applied:

- For SDE: from 40^0C to 220^0C at a rate of 3^0C/min and finally maintained at 220^0C for 5 min;

- For SPME: from 40°C to 115°C at a rate of 3°C/min then from115°C to 220°C at 30°C/min and finally maintained at 220◦C for 5 min. The detector port was maintained at 250^0C. Concentration of 2-AP in samples s is identified and quantified in the 3.1 section in this paper.

b. Volatile compounds analysis by SPME coupling with Mass Spectrometry (SPME-MS)

SPME fiber was directly introduced in the GC/MS injector operating with splitless mode for 4 minutes at 250°C. An Agilent 6980 gas chromatography equipped with a DB-WAX fused silica capillary column (60 m × 0.25 mm d.i.; film thickness = 0.25 µm) coupled with a Agilent 5973N mass spectrometer was used for the GC/MS analysis. The transfer line and the

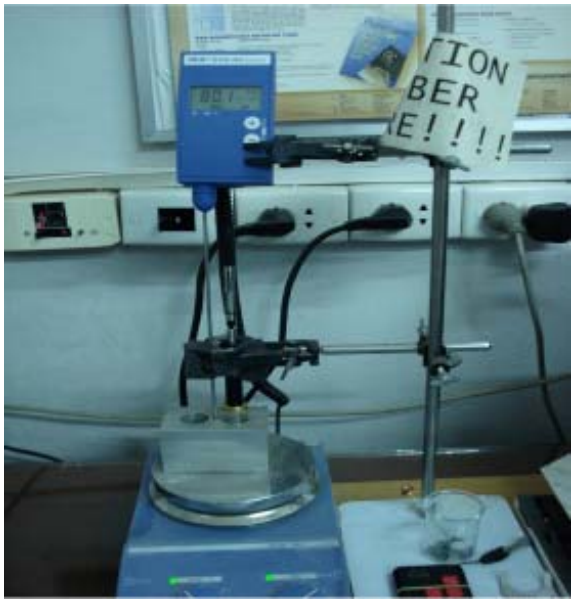


Fig 2. System of the SPME extraction

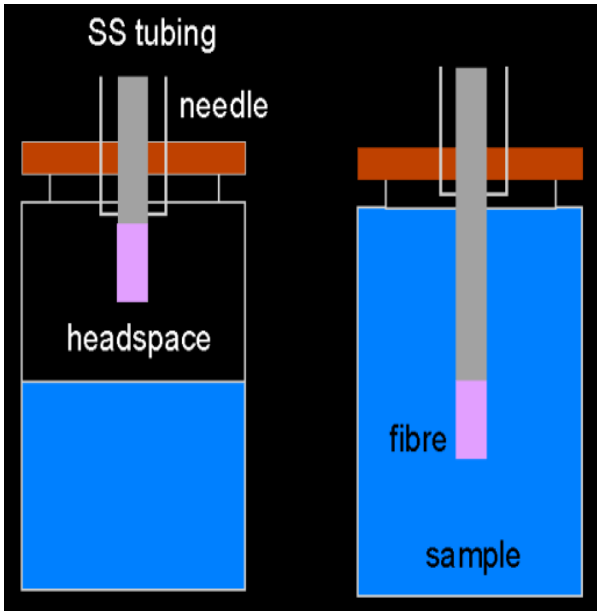


Fig 3. Adsorption phase in SPME extraction

injector temperature were respectively maintained at 260°C and 250°C. He at 2 ml/min was the carrier gas. The column was maintained at 220°C for 15 min. Source temperature was 150°C and the mass spectra were scanned at 70 eV in the m/z range from 40 to 200 at 8.17 scans/second. The global signal registered between 2.8 and 10 minutes was transformed by using the Pirouette® software.

3. RESULTS AND DISCUSSION

3.1. Extraction, identification, and quantification of 2-AP in Pandan leaf

In this experiment, response factor (RF) of collidine has been used to identify and quantify 2-AP in pandan leaf that was extracted by SDE and then analyzed by GC-FID. By this method, retention time (Rt) of collidine and 2-AP in pandan were detected at 12.936 minute and 9.498 minute respectively wherein this 2-AP will be used as a standard to identify and quantify 2-AP in aromatic rice (Phan Phuoc Hien, 2009; 2010; 2011).

Quantification of 2-AP in pandan leaf:

Content of 2 - AP in pandan leaf extracted by SDE was calculated as follows:

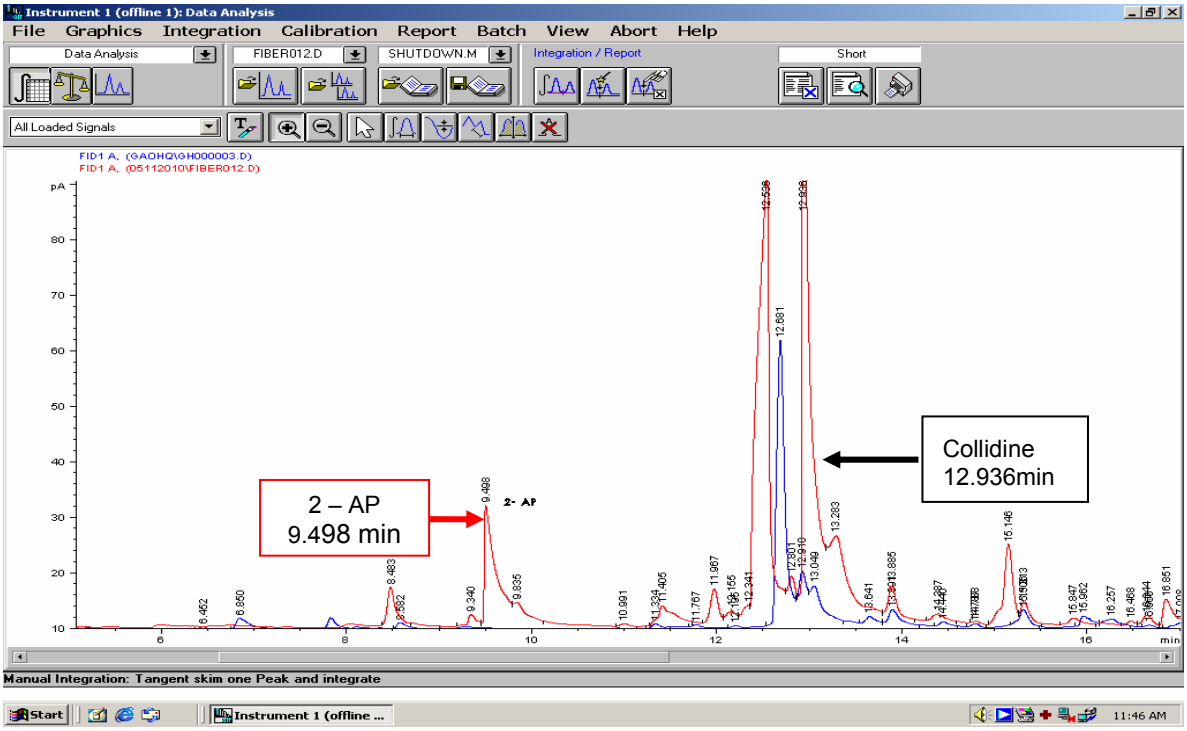


Figure 4. GC-FID chromatograph of 2-AP and other volatile compounds in pandan leaf

$[2\text{ - AP}]_{SDE}$ (g/kg) = $\frac{A}{RF}$, in which:

- A: Area of the 2 - AP peak

- RF: Response factor under the external standard collidine

- d: diluted concentration of sample

- m: sample mass analyzed (kg)

The peak areas were quantified as table 1. RF of 2 - AP was calculated under the external standard collidine as follows:

$$RF_{collidine} = \frac{14009069}{1.01} = 1400906900\ (pA*s/\mu g).$$

Whereby collidine mass injected into GCFID was 0.01 µg. By this way, 2-AP content of the pandan leaves was quantified (Table 2).

Table 1. Peak areas of collidine and 2-AP in pandan leaves recorded by GC-FID and GCMS

Samples	Peaks'areas (pA*s)
Collidine	14009069
2-AP in young pandan leaf	58157862
2-AP in mature pandan leaf	20672313
2-AP in old pandan leaf	31776315

Table 2. Content of 2 - AP (ng/kg) in the pandan leaves quantified by SDE-GCFID

Pandan leaves	Content 2 - AP (ng/kg)
Young pandan leaf	2.07572
Mature pandan leaf	737.818
Old pandan leaf	1.134134

3.2. Identification of 2-AP in Korea rice varieties

By SPME coupling with GC-FID, rice samples from Korea were extracted and analyzed by the same conditions as described above.

The analytical results showed that there is no 2-AP peak in the Korea rice samples at the Rt (9.498 minute) as the 2-AP peak of Pandan leaf. It means that the Korea rice varieties are not aromatic (Fig 5).

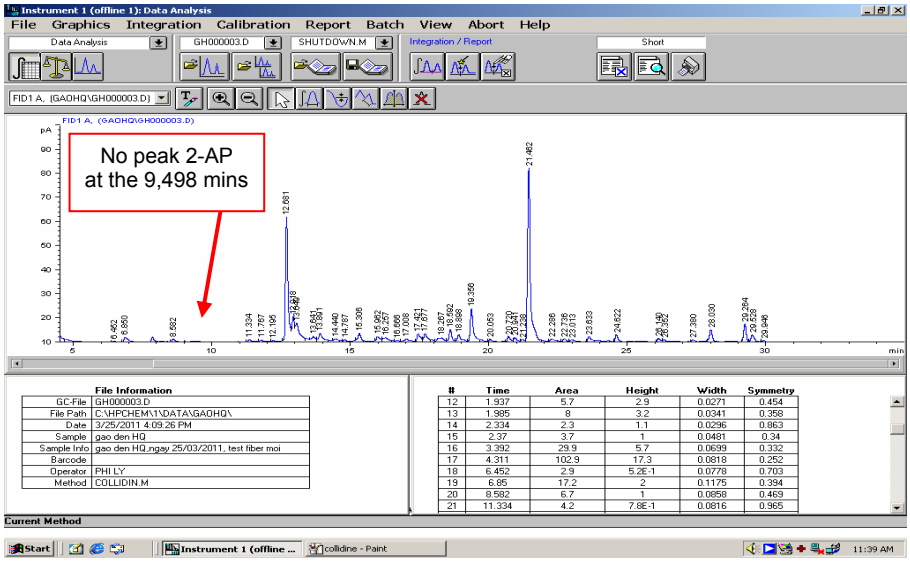


Fig 5. Volatile compounds in Chucheong rice from Korea recorded by GC-FID showed that it has no peak 2-AP at the Rt 9.498 minute

3.3. Identification of 2-AP in OM rice from Cuulong Rice Research Institute, Viet Nam

GC-FID chromatograph of OM 6162 variety recorded in Figure 6 showed that OM 6162 is an aromatic rice variety because its peak 2-AP was identified clearly at the Rt 9.678 minute.

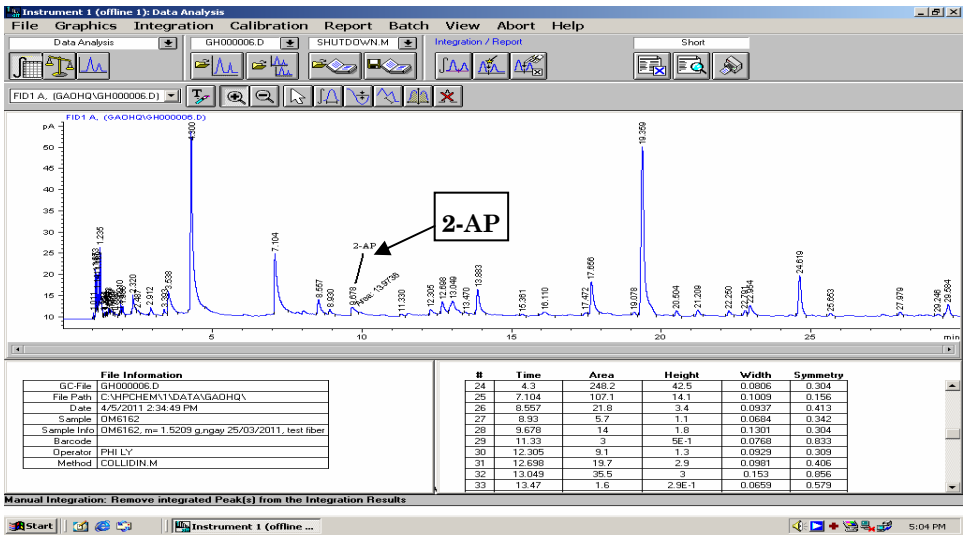


Fig 6. SPME/GC-FID chromatograph of OM 6162 exposed the peak 2-AP at the Rt 9.678 minute

3.4. Investigating the changes of 2-AP and other volatile compounds of rice in Bun processing

Table 3. The volatile compounds in non-soaking and 12 hours soaking of Khao Dawk mali recorded by GCMS

N^0	12 hours water soaking	Non-soaking
1	1 - butanol	0
2	hexanal	hexanal
3	1- hexanol	ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-
4	2- heptanone	ethylbenzen
5	heptanal	1-hexanol
6	2- acetyl -1- pyrroline	1-nonanol
7	benzaldehyde	2- acetyl -1- pyrroline
8	1- heptanol	1- heptanol
9	1-octen-3-ol	1-octen-3-ol
10	2 -pentyl-furan	2 -pentyl-furan
11	butanoic acid, butyl ester	5-hepten-2-ol,6-methyl-
12	octanal	octanal
13	2-heptenal	tetradecane
14	benzeneacetaldehyde	benzeneethanol, -dimethyl-
15	butanoic acid, 3-methylbutyl ester	1-hexanol,2-ethyl-
16	2-octenal	2-octen-1-ol
17	ethanone, 1-(1H-pyrrol-2-yl)-	ethanone, 1-(1-cuclohexen-1-yl)-
18	2-octen-1-ol	0
19	1-octanol	1-octanol
20	2-nonanone	
21	propanoic acid, 2-methyl-, pentyl ester	5,9-undecadien-2-one,6,10-dimethyl-
22	2-nonanol	tetradecane,2,6,10-trimethyl-
23	nonanal	nonanal
24	2,4-pentanedione, 3-butyl-	0
25	3-nonen-1-ol	0
26	cyclohexanone, 5-methyl-2-(1-methylethyl)-	cyclohexanol, 1-methyl-4-(1-methylethyl)-
27	2-nonenal	2-undecanone,6,10- dimethyl-
28	1-nonanol	
29	not available in NIST libray of GCMS	Not available in NIST libray of GCMS
30	dodecane	dodecanal
31	decanal	decanal
32	phenol,4-ethyl-2-methoxy-	0
33	2-decenal	2-decenal
34	butanoicacid, heptyl ester	0
35	2-undecanone	2-undecanone
36	undecanal	undecanal
37	pentadecanone, 6,1,14-trimethyl-	2-pentandecanone,6,10,14-trimethyl-
38	n-hexadecanoicacid	0

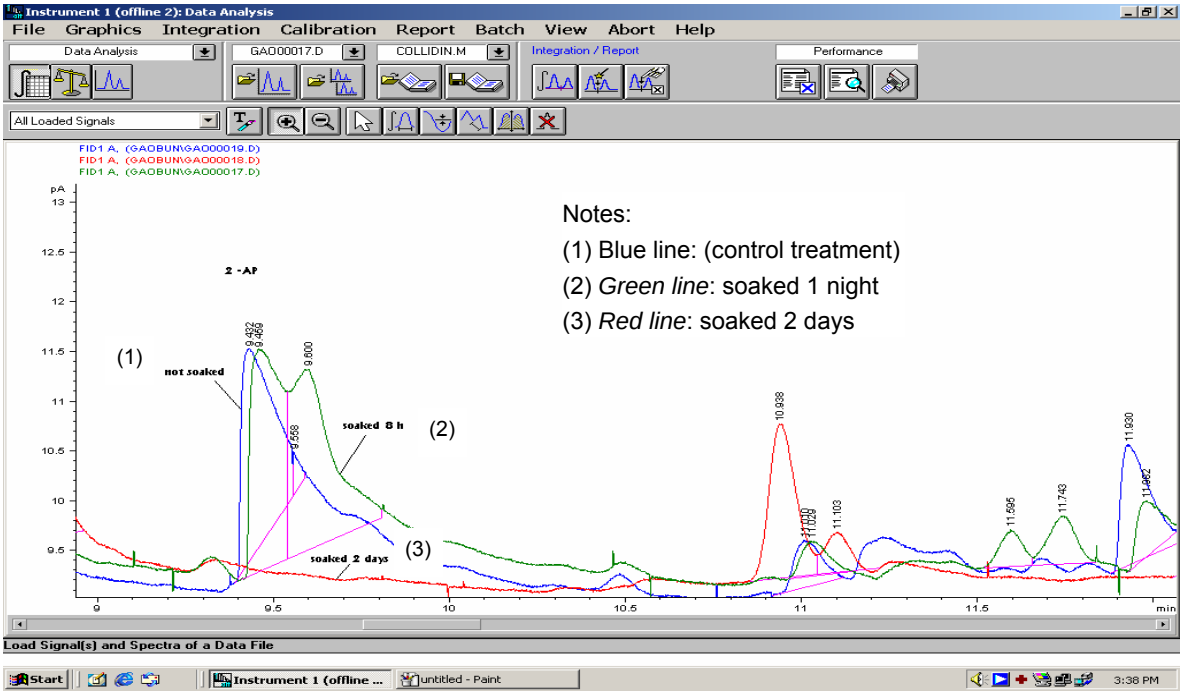


Fig 7. GC-FID chromatograph found out the change of 2-AP and other volatile compounds in three treatments: KDM not to be soaked, to be soaked for 8 hours, and for 2 days.

Table 4. The change of 2-AP content in KDM rice with different soaking durations in Bun processing

No	Treatment (KDM rice sample)	Rice sample mass (g)	Area of 2-AP (pA*S)	2-AP content (ppb)
1	Rice not soaked in water	1.5030	21.8000	2.439731
2	Rice soaked in water 12 hours	1.5029	14.7000	1.664187
3	Rice soaked in water 48 hours	1.0500	0.0000	0.000000

The purpose of this study is to demonstrate the change of 2-AP and other volatile compounds in vermicelli processing from rice. Aromatic rice variety Khao Dawk Mali as confirmed by analysis results with SPME-GCMS was used in the experiment and three treatments were employed as follows:

(1) Rice KDM is not soaked in water (Control treatment)

(2) Rice KDM is soaked in water for 12 hours

(3) Rice KDM is soaked in water for 48 hours

The amount of 2-AP was identified and quantified in both control and treatments. The key aromatic constituent 2-AP still retained after 12 hours soaking of rice but slightly decreased. 29 volatile compounds were detected in the control treatment as compared to 38 volatile compounds when soaked for 12 hours (Table 3).. It means that after 12 hours soaking rice in water 9 new volatile compounds were produced, viz. 1 - butanol, 2-octen-1-ol, 2,4-pentanedione, 3-butyl, 3-nonen-1-ol, phenol, 4-ethyl-2-methoxy, butanoic acid, heptyl ester, n-hexadecanoic acid, 1-nonanol, and 2-nonanone (Table 3). These changes influenced by duration of soaking created new flavor for the end-product.

In contrast to soaking for 12 hours, soaking KDM in water for 48 hours (or two days) ,

resulted in nearly complete loss of 2-AP and other volatile compounds. The qualitative results recorded by GCMS and quantitative results are presented in Figure 7 and table 4, respectively. This might be attributable to the accompanied fermentation process due to long duration soaking.

4. CONCLUSION

By SDE extraction method and use response factor of collidine, 2-AP from pandan leaf was extracted, identified and quantified by GC-FID, GCMS and used as a standard to identify and quantify 2-AP in aromatic rice and its change during vermicelli processing. Based on this method, only cultivar OM 6162 from Cuu long Delta Rice Research Institute and KDM from the institute of agriculture south were identified as aromatic rice.

Different soaking durations clearly change 2-AP and other volatile compounds. Soaking rice for 12 hours increased the volatile compounds (38) as compared to the control (29) The 9 new volatile compounds were 1 - butanol, 2-octen-1-ol, 2,4-pentanedione, 3-butyl, 3-nonen-1-ol, phenol,4-ethyl-2-methoxy, butanoic acid, heptyl ester, n-hexadecanoic acid, 1-nonanol, 2-nonanone and these produce new flavor and specific attribute to the end product. The 2-AP amount was 1.664.187 ppb when soaked for 12 hours and decreased by 31.79% as compared to the control However, if rice soaked for 48 hours 2- AP and many other volatile compounds were reduced or completely lost. These might be explained by biochemical fermentation process in natural conditions.

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