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

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Abstract

Vermicelli is the main material to prepare Hue Beef rice vermicelli (Bun bo Hue) that is a very famous Vietnamese dish originated from Hue city, the former imperial capital of Vietnam. In the process of vermicelli strands prepared from rice, the different duration of soaking rice in water definitely influences on quality and flavor for the end-product. It means that soaking step made change most biochemical properties of rice material leading to finally change quality in that 2-AP and other volatile compounds will make specific flavors for the end-product.

In order to clearly demonstrate these changes, the 2 Acetyl 1 Pyrroline (2-AP) was extracted from Pandan leaf and used as a standard to identify and quantify these changes including the key constituent 2 AP and other volatile compounds in rice before and after soaking rice in water for 2, 3, and 5 days under the bun traditional process. Preliminary research on two Vietnamese rice varieties: KDM, OM 3212, and two Korea rice varieties: Chucheong (milled rice), Black rice (mixture of

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Key words: Hue Beef rice vermicelli (Bun bo Hue), volatile compound, vermicelli, 2 Acetyl 1 Pyrroline (2-AP), Pandan leaf, SPME/GC-FID (Solid Phase Micro-Extraction/Gas Chromatography- Flame Ionization Detector), SDE/GCMS (Simultaneous Distillation Extraction/Mass Spectrometry).

several varieties) showed that 2-AP and other volatile compounds have been changed clearly in the process.S

1. Introduction

Vietnam's main food is rice. In the country, there are many different 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 rice was found out in pandans leaf (Pandanus amaryllifolius). The 2-AP has been also found in white bread, and flowers (Vallaris glabra) (Varaporn Laksanalamai et al. 1993). Due to pandan leaf contains specific constituents with very high content, 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 (Phan Phuoc Hien et al., 2011).

In order to develop aromatic rice production in Viet Nam, reliable and practical methods to assess volatile compounds in aromatic rice are required to evaluate and select the better variety. In response to this demand, during the past 7 years, two quantitative methods were set up and operated at the Physiochemical laboratory in Nong Lam University, Vietnam. The first is Solid Phase Micro-Extraction coupling with both Gas Chromatography (SPME/GC) and Mass Spectrometry (SPME/MS), and the second is SDE (Simultaneous Distillation Extraction) coupling with GCFID 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 the 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. The change of the key compound 2-AP in rice material in Bun processing was quantified and presented in this paper.

2. Materials and Methods

2.1. Materials

- Pandan leaf (Pandanus amaryllifolius).

- Rice varieties:

* From Vietnam: OM6162, K.D. Mali;

* From Korea: Milled rice, and Black rice.

- 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)

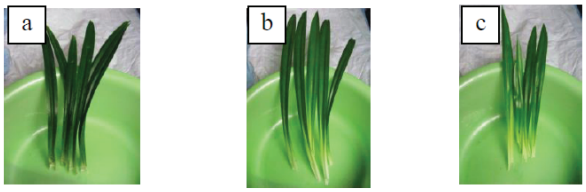

Fig 1. Pandan leaves were classified into three types

2.2 Extraction methods

a. Simultaneous Distillation-Extraction (SDE)

The steam distillationsolvent 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 tetradecane as internal standard. Duration of extraction is 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 -18C 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 (divinylbenzene/Carboxen/polydimethylsiloxane) 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 80^0C 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 400C 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 40^0C to 115^0C at a rate of 3^0C/min then from 115^0C to 220^0C at 30^0C/min and finally maintained at 220^0C for 5 min. The detector port was maintained at 250^0C. Concentration of 2-AP in samples was identified and quantified in the section 3.1 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 250^0C. An Agilent 6980 gas chromatography equipped with a DB-WAX fused silica capillary column (60 m$\times$ 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 injector temperature were respectively maintained at 260^0C and 250^0C. He at 2 ml/min was the carrier gas. The column was maintained at 220^0C for 15 min. Source temperature was 150^0C 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.

Fig 2: System of the SPME extraction

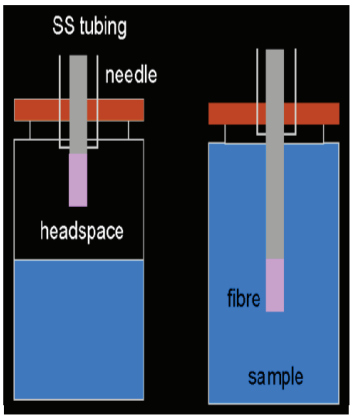

Fig 3: Adsorption phase in SPME extraction

3. RESULTS AND DISCUSSIONS

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 minutes and 9.498 minutes respectively wherein

it 2-AP will be used as a standard Rt to identify and quantify 2-AP in aromatic rice.

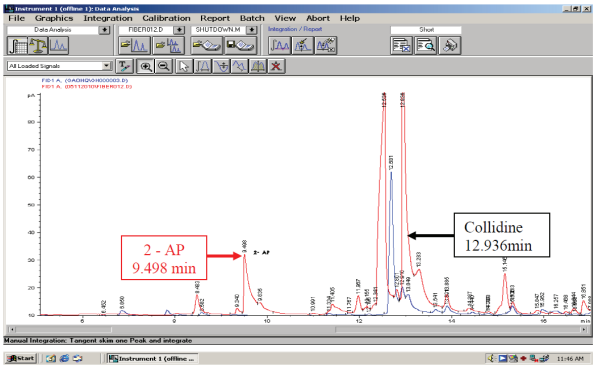

Figure 4: GC-FID chromatograph of 2-AP and other volatile compounds in pandan leaf

Quantification of 2-AP in pandan leaf

Content of 2 - AP in pandan leaf extracted by SDE was calculated as follows:
$[2-AP]_{SDE}(\mu g/kg) = \frac{A}{RF} \times \frac{d}{m}$, 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 follows:

Sample	Peak area (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 1: Peaks areas of collidine and 2-AP in pandan leaves recorded by GC-FID and GCMS.

RF of 2 - AP was calculated under the external standard collidine as follows:
$RF_{collidine} = \frac{14009069}{0.01} = 1400906900(pA * s/\mu g)$.
whereby collidine mass injected into GCFID was 0.01 μg.
By this way, 2-AP content of the pandan leaves was quantified (Table 2).

3.2. Identification of 2-AP in Korean rice varieties

By SDE and SPME coupling with GC-FID, rice samples from Korea have been extracted and analyzed by the same conditions, analytical result showed that there is no 2-AP peak in the rice sample at the Rt (9,498 minutes) as the 2-AP

Pandan leaves	Content 2 – AP (ng/kg)
Young pandan leaf	2,075,72
Mature pandan leaf	737,818
Old pandan leaf	1,134,134

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

peak of Pandan leaf. It means that the Korea rice variety is not aromatic (fig 5).

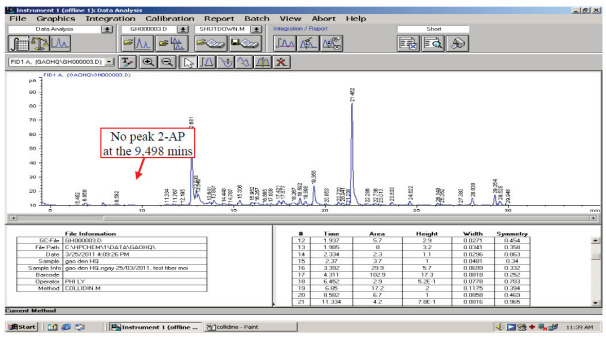

Fig 5. Volatile compounds in Chucheong rice from Korea recorded by GC-FID showed that it has no peak 2-AP at the Rt 9,498 minutes.

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:

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

GC-FID chromatograph of OM 6162 variety showed that this is a aromatic rice variety because of the peak 2-AP was identified clearly at the Rt 9,678 minute in the fig 6.

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

In this experiment, rice variety Khao Dawk Mali (KDM) is not soaked in water to compare with the same KDM sample to be soaked in water for 8 hours, 24 hours, and 48 hours under traditional vermicelli processing in Hanoi, HoChiMinh and southern provinces in Viet Nam.

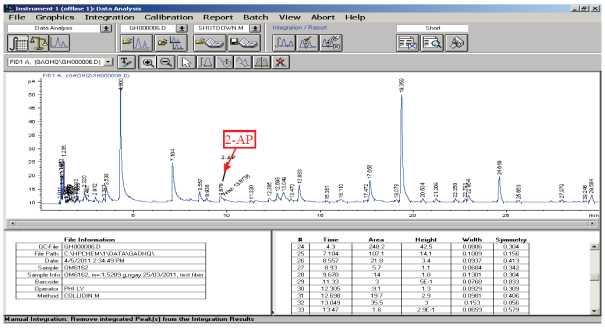

Fig 6. SPME/GC-FID chromatograph of OM 6162 exposed the peak 2-AP at the Rt 9,678 minutes

By the GCMS analysis, 38 volatile compounds were recorded in that the key compound 2-AP was identified and quantified. The analytical results showed that:

- KDM is an aromatic rice;
- There are the changes of volatile compounds between the control treatment (not to be soaked in water) and the treatment soaked in water for 8 hours in Bun processing (table 3).

Table 3: The 38 volatile compounds in KDM were recorded by GCMS in the two treatments: not to be soaked and soaked in water for 8 hours.

N°	*Rice soaked in water for 8 hours*	*Rice not soaked in water*
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

Particularly, if KDM rice is soaked in water for over 2 days, the 2-AP and other volatile compounds will be mostly lost. This can be seen the qualitative results recorded by GCMS in the chromatograph (fig 7) and quantitative results in the table 3 as follows:

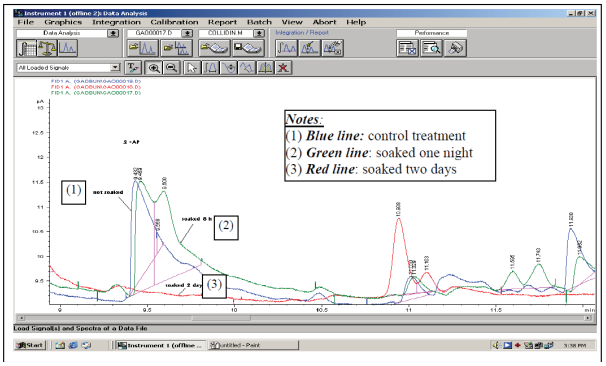

Fig. 7 GC-FID chromatograph found out the change of 2-AP and other volatile compounds of three treatments: KDM not to be soaked, to be soaked one night, and 2 days.

Data in table 2 and illustration in the figure 7 showed that:

- 2-AP content in the control treatment is 2.439.731 ppb;

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

Treatment (KDM rice sample)	*Rice sample mass (g)*	*Area of 2-AP (pA*S)*	*2-AP content (ppb)*
not soaked in water	1.5030	21.8000	2.439731
soaked in water 12 hours	1.5029	14.7000	1.664187
soaked in water 48 hours	1.0500	0.0000	0.000000

- 2-AP content in the rice soaked in water for one day is 1.664.187 ppb: decreased 31.79%;

- 2- AP content in the rice soaked in water for two days is completely lost.

4. Conclusion

By SDE and SPME methods, 2-AP from pandan leaf and aromatic rice varieties were extracted, then identified and quantified by GC-FID in that the 2-AP extracted from Pandan leaf is used as a standard to identified and quantified 2-AP in aromatic rice.

Based on these results, the changes of 2-AP and some other volatile compounds in Bun processing from rice were determined by SDE/GC-FID, SDE/GCMS and SPME-GC-FID and SPME/MS. In the traditional Bun process, step soaking rice in water is very important and influences definitely on flavor and quality of the end-product. Initial studies showed that most of volatile compounds in aromatic rice were significantly changed after soaking in water for 8 hours, one day and two days. In the research, the rice varieties from Korea were tested to compare with the Vietnamese rice. Analytical results found out that soaking duration in water of both Vietnam and Korea rice varieties not to be over 2-3 days is appropriate for natural weather conditions in Hanoi, HCM and southern regions of Vietnam. For natural condition in Ho chi Minh City, if soaked duration is longer than 2 days, 2-AP and other good volatile compounds will be remarkably reduced or completely lost by the rotten and lactic acid fermentation process.

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