

## Identification of Potent Odorants in Different Cultivars of Snake Fruit [*Salacca zalacca* (Gaert.) Voss] Using Gas Chromatography–Olfactometry

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Three cultivars of snake fruits, Pondoh Hitam, Pondoh Super, and Gading, were freshly extracted using liquid–liquid extraction. The aroma compounds of the three samples were analyzed by GC–MS and GC–olfactometry using the nasal impact frequency (NIF) method. A total of 24 odor-active compounds were associated with the aroma of snake fruit. Methyl 3-methylpentanoate was regarded as the character impact odorant of typical snake fruit aroma. 2-Methylbutanoic acid, 3-methylpentanoic acid, and an unknown odorant with very high intensity were found to be responsible for the snake fruit's sweaty odor. Other odorants including methyl 3-methyl-2-butenate (overripe fruity, ethereal), methyl 3-methyl-2-pentenoate (ethereal, strong green, woody), and 2,5-dimethyl-4-hydroxy-3[2]-furanone (caramel, sweet, cotton candy-like) contribute to the overall aroma of snake fruit. Methyl dihydrojasmonate and isoeugenol, which also have odor impact, were identified for the first time as snake fruit volatiles. The main differences between the aroma of Pondoh and Gading cultivars could be attributed to the olfactory attributes (metallic, chemical, rubbery, strong green, and woody), which were perceived by most of the panelists in the Pondoh samples but were not detected in the Gading samples. This work is a prerequisite for effective selection of salak genotypes with optimal aroma profiles for high consumer acceptance.

**KEYWORDS:** Aroma; volatiles; gas chromatography–mass spectrometry; gas chromatography–olfactometry; snake fruit; stir bar sorptive extraction

### INTRODUCTION

Salak [*S. zalacca* (Gaert.) Voss] has been known as snake fruit originally coming from Indonesia and other Southeast Asian countries. The fruit has been used as fresh fruit in these areas for a long time. In Indonesia, there are about 30 cultivars of snake fruit, such as Pondoh and Bali. The popularity of snake fruit as a commercial fruit has increased significantly since the discovery of snake fruit cv. Pondoh in the 1980s, which was planted at a village in Yogyakarta province. Today, three types of cv. Pondoh are known, including Pondoh Super and Pondoh Hitam. Another cultivar of snake fruit planted in Yogyakarta is Gading. Snake fruit cv. Pondoh has different flesh characters compared with snake fruit cv. Bali. Besides this positive attribute the cultivar cv. Pondoh is characterized by the highest aroma

intensity, including overripe and sweaty impressions. Outside Indonesia this kind of aroma is usually not preferred.

As a promising export fruit commodity, cv. Pondoh is playing very important roles. To enhance the fruit acceptance by developing new accessions by breeding, it is important to characterize the aromatic profiles of the flesh of these fruits. The volatiles of unknown cultivars have already been reported (1) as well as the qualitative and quantitative changes in the volatile compounds of the Pondoh cultivar during the maturation process (2).

To the best of our knowledge, the aroma impact compounds responsible for the characteristic odor of this fruit still have not been investigated. Therefore, the topic of the present investigation is to identify the impact odorants of salak by mass spectrometry (MS) and gas chromatography–olfactometry (GC–O). In addition, the aroma profiles of the fresh flesh of three cultivars of snake fruit are compared. This work is a prerequisite for effective selection of salak genotypes with optimal aroma profiles for high consumer acceptance, that is, cultivars with typical fresh fruity aroma and low overripe and sweaty attributes.

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## MATERIALS AND METHODS

**Snake Fruit Samples.** Two cultivars of Pondoh snake fruits called Pondoh Super and Pondoh Hitam, and also a local cultivar called Gading, at a stage of 5.5–6 months after pollination were collected from the growers at Sleman, Yogyakarta. The fruits were transported by plane to Germany and were analyzed immediately after arrival.

**Overall Sensory Evaluation.** The overall flavor impressions of the three salak cultivars were defined by an experienced panel. After tasting of the fresh peeled fruit flesh, the sensory impressions were fixed in an open discussion with the six panelists with the aim of describing the flavor using identical terms.

**Isolation of Volatiles by Liquid–Liquid Extraction.** The flesh (200 g) of each cultivar was homogenized for 1 min in 300 mL of NaCl solution (18.6% w/v). The homogenate was centrifuged at 4 °C for 30 min at 3000 rpm to give a supernatant. To obtain a clear juice, the supernatant was filtered through filter paper. A portion (250 mL) of the filtrate was subjected to a fluid–fluid extractor apparatus (3) after the addition of an internal standard. The volatile compounds were extracted by 30 mL of 1,1,1-trichlorofluoromethane (Freon F11) from the aqueous solution for 20 h at room temperature. The Freon fraction was concentrated with a Vigreux column directly before analyses.

**Isolation of Volatiles by Stir Bar Sorptive Extraction (SBSE).** An aliquot of 10 mL of supernatant prepared as mentioned above was pipetted in a 20 mL glass headspace vial and saturated by adding 3 g of solid NaCl. A stir bar (0.5 mm film thickness, 10 mm length; Gerstel GmbH, Mühlheim an der Ruhr, Germany) coated with PDMS was used. The stir bar was placed in the 20 mL vial. The vial was sealed with a crimp cap and stirred at 300 rpm at room temperature for 45 min. After removal from the sample, the stir bar was rinsed with distilled water, dried with a tissue, and transferred in a glass thermal desorption tube for GC-MS analysis.

**Gas Chromatography–Mass Spectrometry.** For liquid samples a Hewlett-Packard GC-MS system (GC 5890 plus and MSD 5972) equipped with a split–splitless injector at 250 °C was used. SBSE was carried out with a system from Gerstel GmbH. The GC-MS was equipped with a Gerstel TDU and CIS4. Thermal desorption and cold injection were carried out under typical SBSE conditions described in ref 4. The MS detector temperature was 280 °C. A polar column (HP INNOWax, 0.25 mm i.d.  $\times$  30 m length  $\times$  0.5  $\mu$ m film thickness) was used with the following temperature program: 45 °C held for 5 min, then raised to 200 °C at a rate of 2 °C/min, and held for 30 min. The flow rate of the carrier gas (He) was 1.0 mL/min. A volume of 1  $\mu$ L of each sample was injected with the split ratio of 1:3 and 1:50, respectively. For compound identification the Wiley and NIST libraries were used.

**Gas Chromatography–Olfactometry. Instrument and Operation.** The sniffing sessions were conducted in an air-conditioned room (22 °C). A Hewlett-Packard GC 6890 series II gas chromatograph equipped with a polar fused silica column (HP INNOWax, 0.25 mm i.d.  $\times$  15 m length  $\times$  1.0  $\mu$ m film thickness) was used with split injection. Both injector and FID temperatures were at 250 °C. The oven program was the following for all runs: 45 °C for 5 min, then raised to 210 °C at a rate of 10 °C/min, and finally held for 5 min. Each sniffing run continued for 25 min. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. A volume of 1  $\mu$ L of snake fruit extract was injected at a split flow of 5 mL/min. The column outlet was connected either with the FID via transfer line (monitor run) or with the sniffing port equipped with a heated transfer line of identical length and a glass funnel. The complete sniffing session for one variety consists of two FID analyses, one with a boiling point sample for calculation of retention indices and one monitor run with the salak extract for retention time adjustment to the GC-MS run of the same extract. After the column outlet had been switched to the sniffing port, six separate runs followed, carried out by six trained panelists.

**Data Processing.** To determine potentially odor-active compounds of the snake fruit extracts, the nasal impact frequency (NIF) method (5) was performed with modifications (6). Panelists recorded the perception of an odor by pressing a button as long as the smell could be received. Moreover, each panelist was encouraged to describe the odor quality of each perception. The signals were registered by the HP

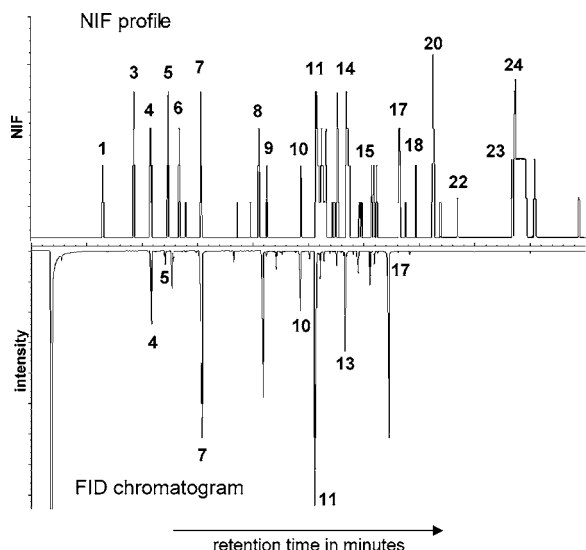
Chemstation via an A/D converter as square signals. The six single sniffing runs were accumulated to one coincident response chromatogram, a so-called NIF profile (5), using a specially designed macro (6). The resulting NIF profile can be handled by the HP Chemstation, for instance, to integrate peaks. Odors detected by only one or two judges were considered as olfactorical noise and rejected. Odor-active components were defined as those that were detected at identical retention times by at least three panelists. It is known that the perception and description of odor qualities is highly individualized. Therefore, after the sniffing session, an open discussion with the six panelists was held with the aim of describing the odors using identical terms.

## RESULTS AND DISCUSSION

**Overall Sensory Evaluation.** The overall sensory description of the fresh snake fruits was evaluated by a panel. The aroma was characterized with the following characteristics: fruity, chemical, cheesy, overripe banana-like, earthy, caramel, pleasant, vanilla, thinner, and unpleasant. The taste impressions were described as chemical, unpleasant, nutty, apricot, thinner, and astringent (especially the Gading cultivar). It seemed that salak had an interestingly unique exotic flavor, especially for the non-native consumer. Generally, the impression of overripe, cheesy, and sweaty character and astringent taste might be a constraint for the acceptability of snake fruit, especially for European consumers. Therefore, salak genotypes with a light fruity, pleasant aroma in combination with low astringent taste should have best preference.

**Identification of Aroma-Active Compounds.** Constituents of the snake fruit extracts that could be identified by the GC-MS were in agreement with the published ones (1, 2). The chromatograms of Freon extracts are very detailed and contain more than 100 discrete peaks. The components were dominated by the carboxylic acids and their methyl esters. Methyl 3-methylpentanoate, methyl 3-methyl-2-pentenoate, methyl 3-hydroxy-3-methylbutanoate, 2-methylbutanoic acid, and 3-methylpentanoic acid were found as the major components. The other esters that were abundantly detected included methyl 3-methyl-2-butenoate, methyl 3-hydroxy-3-methyl pentanoate, methyl hexanoate, and methyl 3-hydroxy-3-methylbutanoate. The last two, however, were found only in smaller amounts in Gading cultivar. Some alcohols, furanones, and aldehydes also could be found among the identified compounds. Some terpenes, although in small amount, could also be detected using SBSE sample preparation. One big and one medium peak remained unknown. Pondoh Super and Pondoh Hitam showed similar patterns in their chromatograms. The Gading cultivar had slight differences, especially for the ratio of several components.

**Figure 1** shows the NIF profile (top) versus the FID gas chromatogram (bottom, inverted) of a snake fruit extract. The importance of the olfactometric analysis in the study of the aromatic profile again was proved here (7, 8). Some evidence showed that minor compounds might be able to play an important role in influencing the flavor. Many of the major compounds such as methyl 3-hydroxy-3-butanoate, methyl hexanoate, methyl 3-methylhydroxypentanoate, or the large unknown peak produced little to no olfactory responses. On the other hand, some of the intense olfactory responses were found in regions with little FID signals, that is, peaks 1, 3, 6, 17, 18, and 20 and some others. As shown on the mirrored signal between the FID and olfactory response (**Figure 1**), this technique even allowed the detection of components that were unable to be detected by the FID. For example, peak 8, with cooked potato aroma description, usually corresponded to the presence of methional. No peak in the FID chromatogram correlated with this compound. This phenomenon has been



**Figure 1.** Comparison of NIF profile and FID chromatogram (monitor run) of salak cv. Pondoh Hitam (S1). Compound numbers refer to Table 1.

previously reported (7). Because methional has a very low olfactory threshold of 0.2 ng/L in water (7), it might be recognized by the panelists even in very low concentration. This small olfactory active peak might be beneath the large methyl 3-hydroxy-3-methylbutanoate peak. The GC-MS spectrum indicated that this large nearest peak has more than one compound.

A similar effect occurred at the high boiling point area, such as peaks 23 and 24, which have strong olfactory intensity but do not correlate with any peaks. In the case of peaks 21 and 22, the compounds are tentatively identified by using the result of SBSE as well as the aroma description in comparison with the previously reported data (9–11). The SBSE was used in parallel with the liquid extraction for MS identification. In general, chromatograms after SBSE give similar results but with more detailed chromatograms in the region of high retention times. Therefore, it was possible to identify the substances methyl dihydrojasmonate (21) and isoeugenol (22) in SBSE extracts but not in liquid extracts.

The result of GC-O is summarized in Table 1. The unique flavor of snake fruit might be due to these various characters of olfactory responses. The responses were varied from pleasant notes, such as flowery, fruity, and sweet caramel, to unpleasant note, such as cheesy, stinky wet cloth, and sweaty, and also light flavor notes including green, fresh, and cooked potato as well as heavy ones such as overripe, warm, spicy, pungent, and woody.

Methyl 3-methylpentanoate (fruity, sweet, typical snake fruit) was found to be the compound that is responsible for the snake fruit flavor because most of panelists were able to recognize it with a similar impression, “typical salak”. This compound has been also reported by Supriyadi et al. (2) as having snake fruit character odor quality. The other compound, methyl 3-methyl-2-pentanoate, which was reported also as having snake fruit character (2), could also be detected by most of the panelists, although its role as a fruit character odorant seems to be questionable because unspecific aroma descriptors (woody, solvent-like or chemical, strong green, stale/spoilage) were obtained. Another olfactory peak that contributes to typical snake fruit aroma might be methyl 3-methylbutanoate, which has been described as snake fruit-like and cheesy typical snake fruit (12). It was clearly recognizable in the Pondoh Super cultivar but not in the Gading cultivar and almost undetected in the Pondoh

Hitam cultivar, possibly due to its higher threshold compared to the existing amount.

Cheesy, sweaty, stinky wet cloth or socky, overripe fruit, and sour butter categories were the predominant characters in these results. 2-Methylbutanoic acid (13, 14) and 3-methylpentanoic acid were two of the more intense aroma compounds active in all cultivars, which might be responsible for this kind of character. Together with 3-hexenoic acid and presumably another carboxylic acid with a longer chain (higher boiling point), they are responsible for the unpleasant aroma of the snake fruit claimed as sweaty odor already by Supriyadi et al. (2). The lack of corresponding flavor notes of 3-methylpentanoic acid, which has been reported as herbaceous (11), remains as a question. It is possible that the accompanying peak, 3-methyl-2-butenic acid, was also responsible for the observed olfactory responses. In the future, reducing this category of aroma should be a challenge in improving the acceptability of snake fruit.

Caramel, sweet, warm, and burnt characters were also well-detected aroma categories. The presence of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), although this component has been detected only as a small peak by FID detection, provided a strong cotton candy aroma (9, 14–16). The other compound that should be responsible for this aroma impression was 4,5-dimethyldihydro-2(3*H*)-furanone. This compound has been described as warm, woody, smoke, burnt caramel, and sharp spicy.

Furthermore, it is interesting to note that almost all panelists detected a specific strong aroma like lovage (very similar to the well-known liquid seasoning produced by Maggi Corp.). This compound has been suspected as an artifact, but it has been proven by blank runs that the origin is the fruit homogenate. It seems likely that methyl anthranilate corresponds to this olfactory impact. The presence of this compound in fruit is not surprising (12). The reported odor description of this compound, however, which is described as fruity, grape (10), or foxy (9), prevents a positive identification.

Also, the olfactory peak 3 was identified tentatively. This peak corresponded well with a compound identified as methyl pentanoate. However, on the basis of the olfactory perception (10) such as metallic, chemical, rubber, and strong green, these characters are more likely to come from the smaller peak, (*E*)-2-methyl-2-butenal.

Flowery was also important in the whole aroma composition of snake fruits. There are two types of flowery: one is perfumery flowery, as shown by peak 21 (methyl dihydrojasmonate), and the other is heavy flowery (stinky flowery), like the olfactory response at peaks 12 and 24. The existence of sulfur compounds such as tetrahydro-2-methylthiophene (peak 12) in this fruit should contribute to its specific characteristic flavor. There were also mixed notes of flowery, spicy, and smoke (tobacco) aroma that are probably due to the presence of isoeugenol (12). The presence of these compounds has not been reported before (1, 2).

It should be noted that some peaks are still unknown. As mentioned before, the cheesy sweaty odor was very intense during the GC-O measurement. Because it had a longer after-odor, it may be covering the olfactory response of the other surrounding compounds. The presence of a large number of methyl esters of C5 and C6 branched-chain alkanic, alkenic, and hydroxyalkanoic acids with their isomers (1, 2) is another challenge to obtaining all of the olfactometry descriptions. In fact, this phenomenon is rarely found even in some tropical fruits reported previously (8, 17–19). Most of the unknown compounds are present at low concentrations but have high odor

Table 1. Results of GCO Analyses

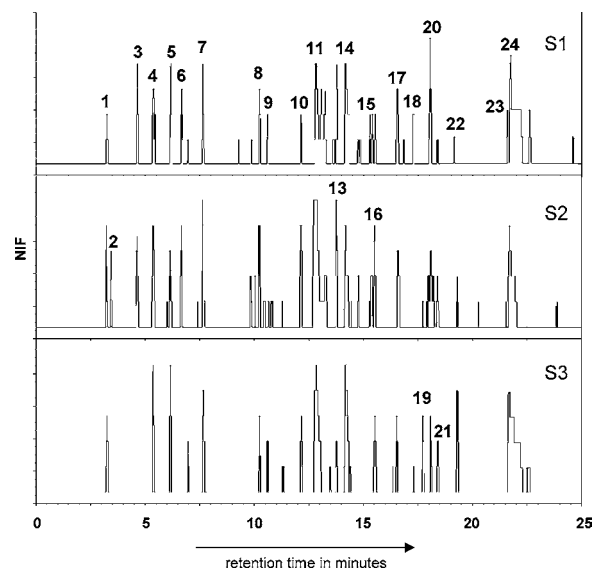
no.	RT <sup>b</sup> (min)	RI <sup>c</sup>	compound	odor description	ident <sup>d</sup>	NIF cultivar <sup>a</sup>		
						S1	S3	S4
1	3.25	1011	methyl 2-methylbutanoate	fruity, fresh	1, 2, 3	5	4	4
2	3.46	1020	methyl 3-methylbutanoate	cheesy, snake-fruit like	1, 3	1	4	nd
3	4.63	1085	2-methyl-2-butenal	metallic, rubbery, green	1, 3	5	5	nd
4	5.34	1127	methyl 3-methylpentanoate	fruity, sweet, typical snake-fruit	1, 2, 3	5	5	6
5	6.16	1184	methyl 3-methyl-2-butenate	overripe fruit, fruity, green/ethereal	1, 3	5	5	6
6	6.66	1209	unknown	pyrazine, metallic, chemical, woody, strong green		4	5	nd
7	7.63	1262	methyl 3-methyl-2-pentenoate	ethereal, woody, strong green, spoiled	1, 2, 3	5	6	5
8	10.24	1463	methional	cooked potato	2	4	5	4
9	10.59	1478	unknown ester ( <i>m/z</i> 41, 55, 87)	fruity, sweet, typical snake-fruit (weak)	1	3	2	3
10	12.13	1591	4,5-dimethylidihydro-2(3 <i>H</i> )-furanone	warm, woody, burnt caramel, sharp spicy	1, 2	3	5	4
11	12.78–13.24	1658	2-methylbutanoic acid	cheesy, unpleasant overripe, sweaty, sour, buttery	1, 2, 3	5	6	6
12	13.75	1741	tetrahydro-2-methyl thiophene	stinky flowery, almond, stale	1	5	6	3
13	14.16	1780	3-methylpentanoic acid, 3-methyl-2-butenic acid	unpleasant riped cheese, rancid, pungent	1, 3	5	5	6
14	14.80–14.93	1835	2-methyl-2-butenic acid	marzipan, unpleasant	1, 2, 3	3	3	1
15	15.28–15.36	1890	phenylethyl alcohol	flowery, floral, drop acid (honey), fragrant	1, 2, 3	3	3	nd
16	15.54	1909	( <i>E</i> )-3-hexenoic acid	stinky wet cloth, sharp pungent, vinegar, sweaty	1, 2, 3	3	5	4
17	16.54	2020	2,5-dimethyl-4-hydroxy-3(2 <i>H</i> )-furanone	caramel, sweet, cotton candy	1, 2, 3	4	5	4
18	17.29	2113	unknown	chemical, burnt		3	1	3
19	17.71	2215	unknown	spicy, clove, Christmas cake		nd	2	4
20	18.04	2226	methyl anthranilate	strong, lovage, celery leaves, "Maggi"	1, 2, 3	6	5	3
21	18.38	2276	methyl dihydrojasmonate	flowery, perfumery	2, 4	2	3	3
22	19.27	2378	isoeugenol	spicy, cooked meals, smoky	2, 4	3	3	5
23	21.54	>2600	unknown	vanilla-like		3	2	nd
24	21.67	>2600	unknown	stinky flowery, silage		5	5	5

<sup>a</sup> Salak cultivars: S1, cv. Pondoh Hitam; S2, cv. Pondoh Super; S3, cv. Pondoh Gading. nd, not detected. <sup>b</sup> Retention time at GC-O. <sup>c</sup> Retention index. <sup>d</sup> Substance identification: 1, MS library search; 2, aroma descriptors; 3, reference data; 4, SBSE-MS and library search.

impacts (extremely low odor thresholds). The unknown substance at peak 6 in **Figure 1** is eluted on the wax column in front of an ester peak (methyl 3-hexenoate). The sensory description as pyrazin-like, metallic, chemical, and green refer to a compound of the pyrazine group. Unknown **12** appears as a defined peak with the following mass fragmentation (*m/z* and intensity): 29 (35%), 41 (52%), 43 (38%), 55 (46%), 87 (100%), 115 (7%). Identification by library search was not successful. The four unknown compounds **18**, **19**, **23**, and **24** belong to odor impressions that appear in chromatogram regions with no defined peaks. Therefore, an expedient mass fragmentation is not available. Further detailed studies regarding substance identification should be carried out.

**Aroma Differences among Cultivars.** The NIF profiles of the three cultivars are shown in **Figure 2**. Pondoh cultivars, consisting of Pondoh Hitam and Pondoh Super, have similar profiles. The Gading cultivar gave a cleaner chromatogram compared to the other two. The Pondoh cultivars revealed more crowded chromatograms, especially at the lower impact response. The main differences between the aroma of Pondoh and Gading cultivars could be attributed to the peaks 2, 3, 6, 15, and 23, which were perceived by almost of the panelists in the Pondoh samples but were not detected in the Gading sample. According to the olfactory results, they contributed metallic, chemical, rubber-like, strong green, woody, flowery, and vanilla-like characters to the Pondoh cultivars.

The main differences between the Pondoh cultivars are shown by the presence of peak 2, which contributes to the salak typical character. This olfactory response could be detected clearly by panelists in Pondoh Super but not in Pondoh Hitam. This response also has not been detected at all in the Gading cultivar. The obtained response is related to methyl 2-methylbutanoate. Higher availability of this compound in Pondoh Super might be related to the stronger snake fruit specific character of this cultivar.



**Figure 2.** NIF profiles of three different salak cultivars. Smell responses with a nasal impact below NIF = 2 were excluded (olfactory noise). Salak cultivars: S1, cv. Pondoh Hitam; S2, cv. Pondoh Super; S3, cv. Pondoh Gading. Compound numbers refer to **Table 1**.

In summary, the significant aroma of snake fruit is not the result of one odor impression. Numerous potent odorants are responsible for the overall flavor impression (18). The results, compared with those obtained by instrumental analysis, contribute to our present knowledge of the aroma composition in snake fruits.

More effort should be aimed at the characterization of unidentified compounds by using different stationary phases and preparative separation to improve their ability to be detected by GC-MS. This could lead to identification of compounds with extremely low detection thresholds that could not be detected in this study. Nevertheless, the obtained knowledge will permit

breeders to develop new snake fruits varieties with optimized aroma and taste properties.

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