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Odor-Active Compounds in Jackfruit (*Artocarpus heterophyllus* Lam.) and Cempedak (*Artocarpus integer* (Thunb.) Merr.)

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Contents

1. Summary

Tropical fruits have gained increasing popularity in recent years. Examples of fashionable tropical fruits include jackfruit, the fruit of *Artocarpus heterophyllus* Lam., and cempedak, the fruit of the cempedak tree (*Artocarpus integer* (Thunb.) Merr.). Both trees are evergreen and belong to the family Moraceae. While the jackfruit tree is spread all over the tropics, the cempedak tree is only of local importance. The fruits are multiple fruits whose leathery skin encloses a yellow pulp. The pulp covers the seeds, which are arranged around a peduncle. The overall aroma of jackfruit pulp is dominated by sweet, fruity, malty, and cheesy odor notes, while the overall aroma of cempedak pulp additionally exhibits a characteristic sulfury, oniony, smear cheese-like odor note, which is not perceived in jackfruit pulp. To clarify the molecular background of the overall aroma of both fruits and especially the characteristic sulfury odor note of cempedak pulp, the volatiles were isolated from the fruit pulps by solvent extraction and solvent-assisted flavor evaporation (SAFE). The odor-active compounds were determined by aroma extract dilution analysis (AEDA). Results revealed 48 odor-active compounds in jackfruit pulp and 49 odorants in cempedak pulp with flavor dilution (FD) factors between 1 and ≥8192. Five and six additional odor-active compounds were detected in jackfruit and cempedak pulp, respectively, by headspace gas chromatography-olfactometry (GC-O). The structures of 44 out of 53 and 45 out of 55 detected odorants could be assigned in jackfruit pulp and cempedak pulp, respectively. In both fruits, high FD factors were found for ethyl 3-methylbutanoate (fruity), ethyl (2*E*)-3-phenylprop-2-enoate (fruity), 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF; caramel-like), 2-acetyl-1-pyrroline (cooked rice-like), an unknown, meaty smelling compound, 3-methylbutanoic acid (cheesy), hexanoic acid (sweaty), hexanal (green, grassy), 2-/3-methylbutan-1-ol (malty), and 3-(methylsulfanyl)propanal (cooked potato-like). Additionally, in jackfruit pulp high FD factors were determined for ethyl butanoate (fruity) and methyl 3-methylbutanoate (fruity). In cempedak pulp, an unknown, sulfury smelling compound, methyl hexanoate (fruity), octanal (citrusy), butan-1-ol (malty), an unknown, fruity smelling compound, and 2-phenylethanol (floral) were additional highly potent odor-active compounds. Among the odor-active compounds in cempedak pulp, the three compounds 2-(methylsulfanyl)propane, 2-(methylsulfanyl)butane, and 2-(methylsulfanyl)pentane attracted particular attention, as their odor represented the characteristic sulfury, oniony, smear cheese-like note in cempedak pulp aroma. The odor of none of the three compounds was perceived during AEDA in the jackfruit pulp. Thus, these compounds were suggested to play a crucial role for the aroma difference between cempedak pulp and jackfruit pulp. As 2-(methylsulfanyl)butane and 2-(methylsulfanyl)pentane are chiral compounds with one stereogenic center, their enantiomeric distributions in cempedak pulp were determined by enantio-GC. The respective enantiomers all show the same odor. In cempedak pulp, the (*S*)-isomer of 2-(methylsulfanyl)butane and 2-(methylsulfanyl)pentane dominated with 67% and 59%, respectively. To clarify the role of the 2-(methylsulfanyl)alkanes for the overall aroma of both fruits, the identified compounds were quantitated using isotopically substituted odorants as internal standards and odor activity values (OAVs) were calculated to estimate the odor contribution of the individual compounds. In jackfruit pulp, 35 odor-

active compounds had an OAV >1, in cempedak pulp, an OAV >1 was calculated for 41 compounds. The highest OAVs in jackfruit pulp were obtained for ethyl 3-methylbutanoate, ethyl hexanoate, 3-methylbutanal, and 2-methylpropanal. In cempedak pulp, ethyl 3-methylbutanoate, 3-methylbutanal, and ethyl hexanoate showed the highest OAVs. The most pronounced difference in the OAVs between jackfruit pulp and cempedak pulp was obtained for the 2-(methylsulfanyl)alkanes. In contrast to cempedak pulp, none of these five compounds occurred in odor-active amounts in jackfruit pulp. Successful aroma reconstitution experiments confirmed that all odorants contributing to the overall aroma of jackfruit pulp and cempedak pulp were correctly identified and quantitated. Finally, an omission test confirmed that the 2-(methylsulfanyl)alkanes were key odorants of cempedak pulp and vitally contributed to the aroma difference between cempedak pulp and jackfruit pulp.

2. Zusammenfassung

Tropische Früchte erfreuen sich auch hierzulande immer größerer Beliebtheit. Zu den angesagten tropischen Früchten zählen unter anderem Jackfrucht und Cempedak. Sie wachsen an immergrünen Bäumen, die der Pflanzenfamilie Moraceae angehören. Während der Jackfruchtbaum (*Artocarpus heterophyllus* Lam.) mittlerweile überall in den Tropen kultiviert wird, ist der Cempedakbaum (*Artocarpus integer* (Thunb.) Merr.) nur von lokaler Bedeutung. Bei den Früchten der beiden Bäume handelt es sich botanisch korrekt um einen Fruchtverband. Unter der ledrigen Schale der beiden tropischen Früchte sind die Samen um einen Pedunkulus angeordnet. Das Fruchtfleisch umhüllt jeweils die einzelnen Samen.

Das Aroma des Fruchtfleisches der Jackfrucht wird von süß-fruchtigen, malzigen und käsigen Noten dominiert, während das Aroma des Fruchtfleisches der Cempedak zusätzlich noch eine schweflige, zwiebelige Note aufweist, die sehr intensiv und charakteristisch ist und im Fruchtfleisch der Jackfrucht nicht wahrnehmbar ist. Um den molekularen Hintergrund dieser Aromanoten aufzuklären, wurden die flüchtigen Bestandteile mittels Lösungsmittelextraktion und Solvent-Assisted Flavor Evaporation (SAFE) aus dem Fruchtfleisch isoliert und die geruchsaktiven Verbindungen durch Aromaextraktverdünnungsanalyse (AEVA) ermittelt. Die AEVA ergab 48 Geruchsstoffe im Fruchtfleisch der Jackfrucht und 49 in dem der Cempedak. Die Flavor Dilution (FD) Faktoren lagen dabei zwischen 1 und ≥8192. Zusätzlich wurden fünf weitere Geruchsstoffe im Fruchtfleisch der Jackfrucht und sechs weitere geruchsaktive Verbindungen im Fruchtfleisch der Cempedak bei der GC-O-Analyse statischer Headspace-Proben ermittelt. Im Fruchtfleisch der Jackfrucht konnte die Struktur von 44 geruchsaktiven Verbindungen aufgeklärt werden. Im Fruchtfleisch der Cempedak konnte bei 45 Verbindungen die Struktur zugeordnet werden. Hohe FD-Faktoren in beiden Früchten wiesen Ethyl-3-methylbutanoat (fruchtig) und Ethyl-(2*E*)-3-phenylprop-2-enoat (fruchtig), 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-on (HDMF; Karamell), 2-Acetyl-1-pyrrolin (gekochter Reis), eine unbekannte, fleischig riechende Verbindung, 3-Methylbuttersäure (käsig), Hexansäure (schweißig), Hexanal (grün, grasig), 2-/3-Methylbutan-1-ol (malzig) und 3-(Methylsulfanyl)propanal (gekochte Kartoffel) auf. Im Jackfruchtisolat wurden zusätzlich Ethylbutanoat (fruchtig) und Methyl-3-methylbutanoat (fruchtig) als potente Geruchsstoffe identifiziert, während im Isolat der Cempedak darüber hinaus eine unbekannte, schweflig riechende Verbindung, Methylhexanoat (fruchtig), Octanal (Citrus), Butan-1-ol (malzig), eine unbekannte, fruchtig riechende Verbindung und 2-Phenylethanol (blumig) hohe FD-Faktoren aufwiesen. Unter den geruchsaktiven Verbindungen im Cempedakisolat erweckten vor allem die drei Verbindungen 2-(Methylsulfanyl)propan, 2-(Methylsulfanyl)butan und 2-(Methylsulfanyl)pentan besondere Aufmerksamkeit, da deren Geruchsqualität die charakteristische, schweflige, zwiebelige Aromanote von Cempedakfruchtfleisch widerspiegelte. Diese typische Geruchsqualität konnte während der GC-O-Analyse des Jackfruchtisolates nicht wahrgenommen werden. Da es sich bei 2-(Methylsulfanyl)butan und 2-(Methylsulfanyl)pentan um chirale Verbindungen mit je einem Stereozentrum handelt, wurde mittels Enantio-GC das Enantiomerenverhältnis in Cempedak bestimmt. Die jeweiligen Enantiomere zeigen die gleiche Geruchsqualität. In Cempedakfruchtfleisch dominierten die (*S*)-Isomere von 2-(Methylsulfanyl)butan und 2-(Methylsulfanyl)pentan mit 67 % bzw. 59 %.

Um die Rolle der 2-(Methylsulfanyl)alkane abschließend zu klären, wurden die Verbindungen mit Hilfe von isotopensubstituierten Standardverbindungen quantifiziert und Odor Activity Values (OAVs) berechnet, um den Aromabeitrag der einzelnen Verbindungen abzuschätzen. Während in Jackfrucht 35 geruchsaktive Verbindungen einen OAV >1 aufwiesen, wurde in Cempedak für 41 Verbindungen ein OAV >1 berechnet. Die höchsten OAVs in Jackfrucht zeigten die Verbindungen Ethyl-3-methylbutanoat, Ethylhexanoat, 3-Methylbutanal und 2-Methylpropanal. In Cempedak wiesen Ethyl-3-methylbutanoat, 3-Methylbutanal und Ethylhexanoat die größten OAVs auf. Der auffälligste Unterschied bei den OAVs der beiden Früchte war für die schweflig, zwiebelig riechenden 2-(Methylsulfanyl)alkane festzustellen. In Cempedak wurde für all diese Verbindungen ein OAV >1 ermittelt, während sie in Jackfrucht Konzentrationen unterhalb ihrer jeweiligen Geruchsschwelle aufwiesen. Erfolgreiche Rekonstitutionsversuche zeigten, dass alle aromarelevanten Verbindungen korrekt identifiziert und quantifiziert wurden. Ein Omissionsversuch bestätigte, dass die 2-(Methylsulfanyl)alkane Schlüsselaromastoffe des Fruchtfleisches der Cempedak sind und eine entscheidende Rolle für den Aromaunterschied der beiden Früchte spielen.

3. Abbreviations and Nomenclature

Abbreviations:

AEDA	aroma extract dilution analysis
ASTM	American Society for Testing and Materials
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CI	chemical ionization
CP	cempedak
EI	electron ionization
Et	ethyl
FFAP	free fatty acid phase
FD	flavor dilution
FID	flame ionization detector
GC	gas chromatography
GC-GC-MS	two-dimensional heart-cut gas chromatography-mass spectrometry
GC×GC-MS	comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
GC-O-enantio GC-O/MS	two-dimensional heart-cut gas chromatography-olfactometry-mass spectrometry in combination with a chiral column in the second dimension
HS-GC-O	static headspace gas chromatography-olfactometry
JF	jackfruit
M^+	molecular ion
Me	methyl
MS	mass spectrometry
NMR	nuclear magnetic resonance
NIST	National Institute of Standards and Technology
OAV	odor activity value

OTV	odor threshold value
RI	retention index
SAFE	solvent-assisted flavor evaporation
SIDA	stable isotope dilution assay
SMe	methylsulfanyl
SPME	solid phase microextraction

Nomenclature:

2-acetyl-1-pyrroline	1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethanone
HDMF	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one
linalool	3,7-dimethylocta-1,6-dien-3-ol
MDMF	4-methoxy-2,5-dimethylfuran-3(2*H*)-one
γ-octalactone	5-butyldihydrofuran-2(3*H*)-one
sotolon	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one
vanillin	4-hydroxy-3-methoxybenzaldehyde

4. Introduction

4.1 Molecular Sensory Science

4.1.1 Odor-Active Compounds

Besides look, freshness, healthiness, and freeness of contaminants, the aroma is one of the most important quality attributes of food products for the consumers. The aroma is caused by a multitude of various odor-active compounds. Odor-active compounds are volatile compounds, which are recognized by ~ 400 types of olfactory receptors in the human nose. Together with the gustatory compounds detected on the tongue, the odorants essentially contribute to the overall sensory impression of a food.

Volatility is an elementary requirement for odorants, because only compounds which are sufficiently volatile can be released from food into the ambient air and then can get into the nose. Therefore, odorants are normally low molecular weight compounds with a molar mass below 300 g/mol, as the volatility depends on the molecular weight and the polarity of the odorants.[1]

Besides the volatility, an odorant must be able to bind to olfactory receptor proteins and activate them. Thus, a functional group and hydrophobic regions in the molecule are typically required. The odorants either attain the nose during inhaling through the nostril (orthonasally) or they are released during consumption and reach the olfactory receptor cells of the olfactory epithelium in the nasal cavity during exhaling from behind (retronasally) (Figure 1).[1]

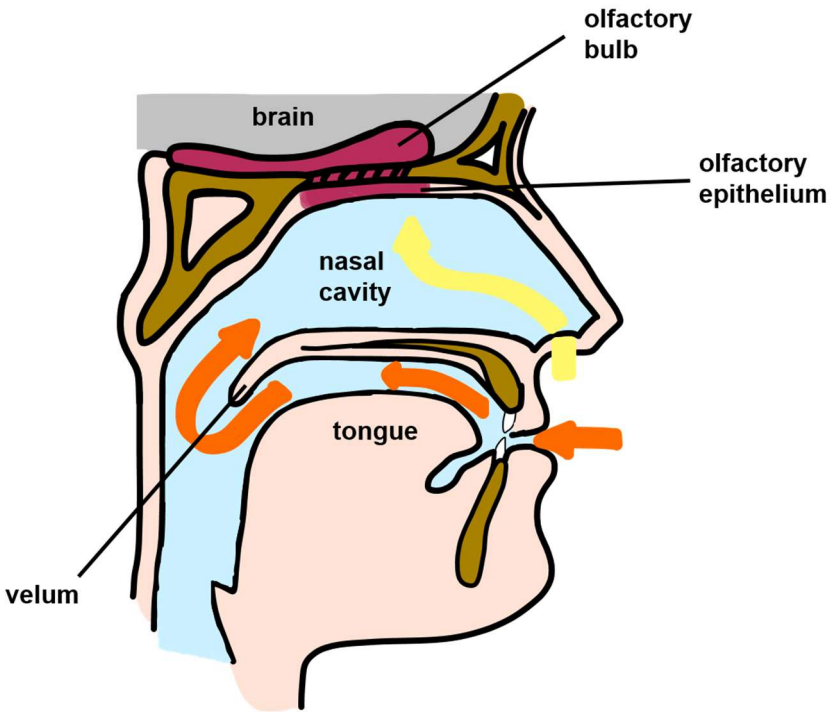

Figure 1: Orthonasal and retronasal odor perception.

Then the odorants bind to G-protein-coupled olfactory receptors in the membrane of the olfactory receptor cells' cilia in the olfactory epithelium. The binding of the odorants to the receptors causes conformation changes, which lead to the release of the α-subunit of the heterotrimeric G-protein complex. This activates adenylatcyclase, which converts cellular ATP to cyclic AMP (cAMP). The cAMP opens ion channels of the receptor cells in the cell membrane causing its depolarization. The depolarization generates a neural impulse, which is transmitted via the axon of the olfactory neuron to the olfactory bulb. In the olfactory bulb, axons of receptor cells with the same receptor type are grouped together in a glomerulus. Activation of a certain set of glomeruli results in a specific activation pattern, which is transmitted via mitral-cells to higher regions of the brain. There, the patterns are interpreted as a specific odor impression (Figure 2). An individual odorant can activate one or more receptors.[2–10]

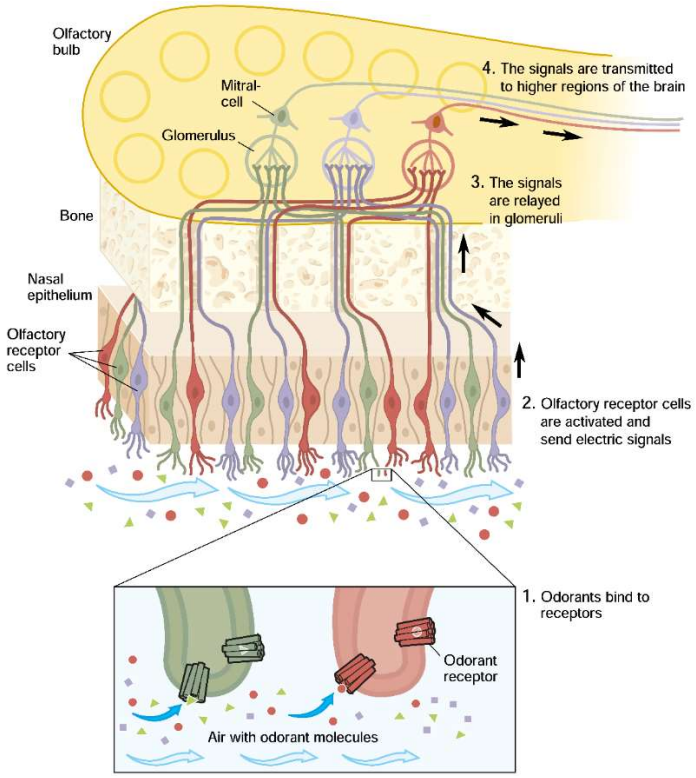

Figure 2: Function of olfactory system.[3]

To generate such an intracellular reaction cascade, a certain minimum concentration of the odorants is required. Hence, every odorant has a compound specific odor threshold. The odor threshold value (OTV) in the food matrix is influenced by its concentration in the food and the specific release characteristics affected by the food matrix and its chemical properties. The OTVs in a specific food matrix vary extremely for different odorants. For example, cheesy smelling butanoic acid shows an OTV of 2400 µg/kg[11] in water, whereas the OTV of (2*E*,6*Z*)-nona-2,6-dienal, which exhibits a cucumber-like odor note, is 0.0045 µg/kg[11] in the same matrix. Compounds whose

concentrations in the food exceed their respective OTVs may generally contribute to the aroma of the food.

The aroma is evoked by a multitude of different odorants. However, only a few of these odorants are relevant for the characteristic aroma. These odorants are called key odorants. Key odorants which appear in a range of different types of food are called generalists, whereas others are unique to a specific type of food and are named individualists.[12] By means of the sensomics concept, these key odorants can be identified.

4.1.2 The Identification of the Key Odorants with the Sensomics Concept

The following steps are applied to identify the key odorants according to Schieberle 1995[13] and Grosch 2001[14] (Figure 3):

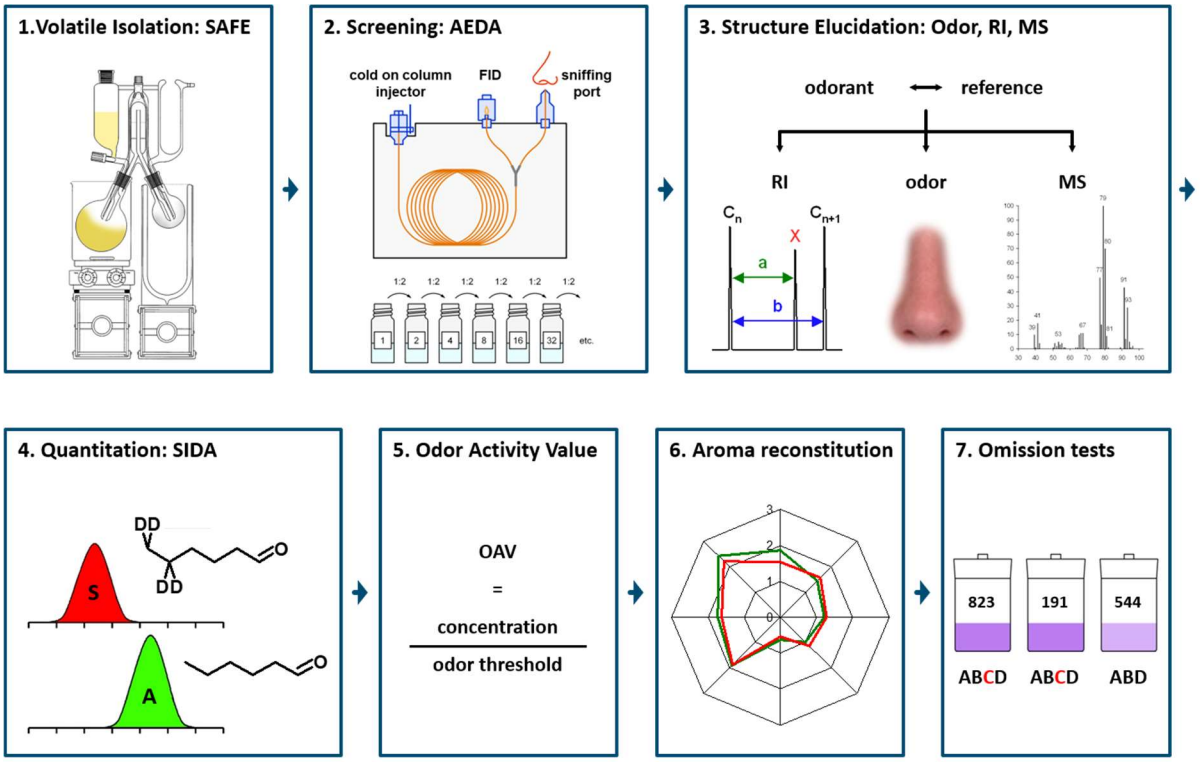

Figure 3: Identification of the key odorants according to Schieberle 1995[13] and Grosch 2001[14] (illustrations: Martin Steinhaus).

The first step of the identification of the key odorants is the gentle isolation of the volatile compounds to obtain a representative aroma isolate of the food. Therefore, the extraction is accomplished with low boiling solvents e.g. diethyl ether or dichloromethane. After solvent extraction, volatiles are separated from non-volatile compounds by using solvent-assisted flavor evaporation (SAFE)[15] (Figure 4). For the separation of the volatiles during SAFE, the volatile compounds are evaporated under

high vacuum and subsequently recondensed with the help of liquid nitrogen. After SAFE, the distillate is gently concentrated by using a Vigreux column and a Bemelmans microdistillation device.[16] During the SAFE and concentration approach, the temperature is maintained ≤ 40 °C. The comparatively low temperature reduces the risk of the degradation of odorants and avoids the formation of artifacts. It is important to check if the aroma of the extract, the distillate, and the concentrated volatile isolate still fully reflect the characteristic overall aroma of the food. Nevertheless, odorants with a lower boiling point than the solvent are lost during the concentration of the distillate. Hence, the gas phase above the food needs to be additionally analyzed via static headspace-gas chromatography-olfactometry (HS-GC-O).

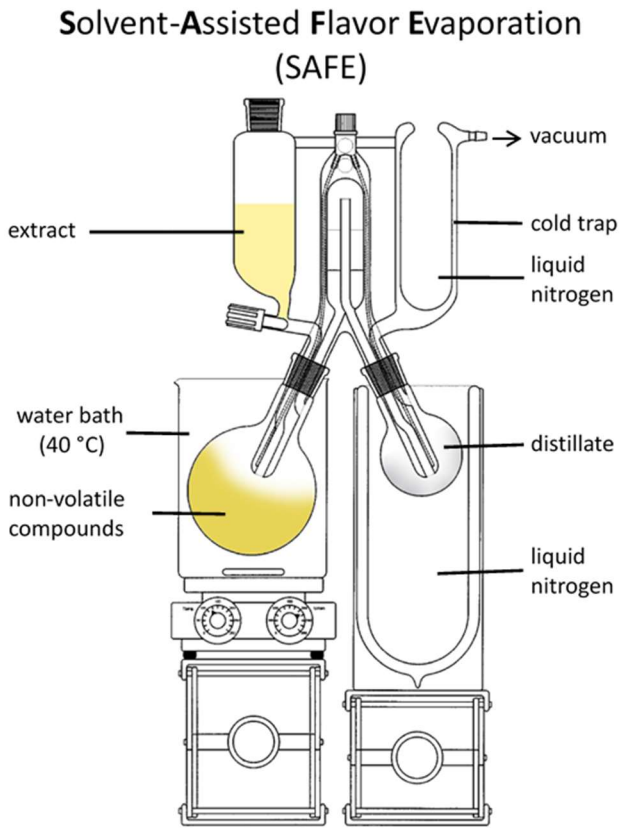

Figure 4: SAFE equipment to separate volatiles from non-volatile compounds (illustration: Martin Steinhaus).

The gas chromatography-olfactometry (GC-O) analysis is fundamental to prevent overlooking important odor-active trace compounds. A defined volume of the volatile isolate or of the gas phase above the food is applied to GC-O to differentiate between odor-active compounds and the multitude of odorless volatiles. During the GC-O analysis, the volatiles are separated on the GC capillary column. The end of the column is connected to a Y-shaped glass splitter which divides the column effluent into two equal parts. One part is transferred to the flame ionization detector (FID), which is connected to a recorder. The other part is directed to a heated exit called sniffing port.

During a GC-O run, the FID signal is plotted by a recorder, while a trained panelist places his nose closely above the sniffing port and evaluates the effluent. Whenever the panelist perceives an odor at the sniffing port, he notes the odor description as well as the associated retention time. During HS-GC-O, cyro focussing of the injected volatiles sampled from the gas phase above the food is performed before separating them by GC (Figure 5).

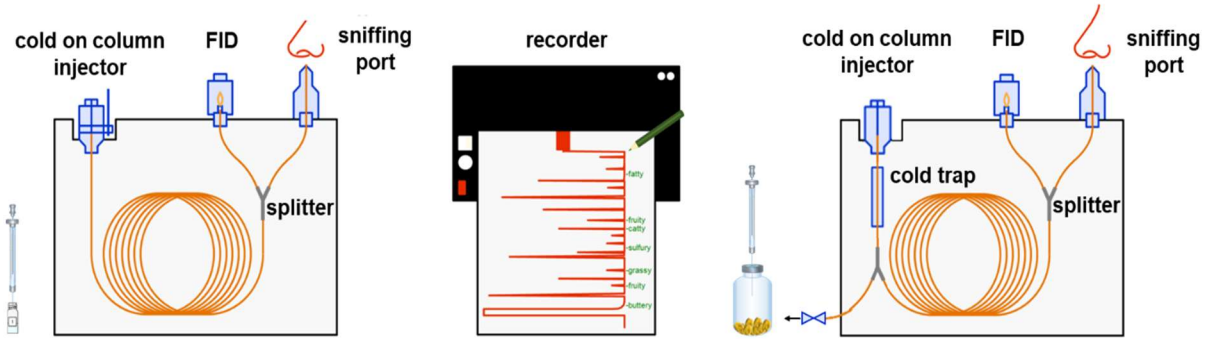

Figure 5: Schemes of a basic GC-O/FID system (1) and a basic HS-GC-O system (2) (illustrations: Martin Steinhaus, Johanna Grimm).

An aroma extract dilution analysis (AEDA)[17] is applied to rank the odorants according to their odor potency in the food volatile isolate. The initial volatile isolate is stepwise diluted 1:2 with solvent to obtain the dilutions of 1:2, 1:4, 1:8, 1:16 etc. Each diluted sample is analyzed by GC-O. Diluting is continued until no odor can be perceived at the sniffing port anymore. A flavor dilution (FD) factor, which is defined as dilution factor of the highest diluted sample in which the respective odorant is detected by GC-O analysis, is assigned to each odor-active compound. For static headspace dilution analysis, the initial injection volume is stepwise reduced by a factor of two. Finally, each odor-active compound is assigned an FD factor, which represents the ratio of the initial injection volume to the lowest injection volume in which the odorant has been detected during the HS-GC-O analysis (Figure 6).

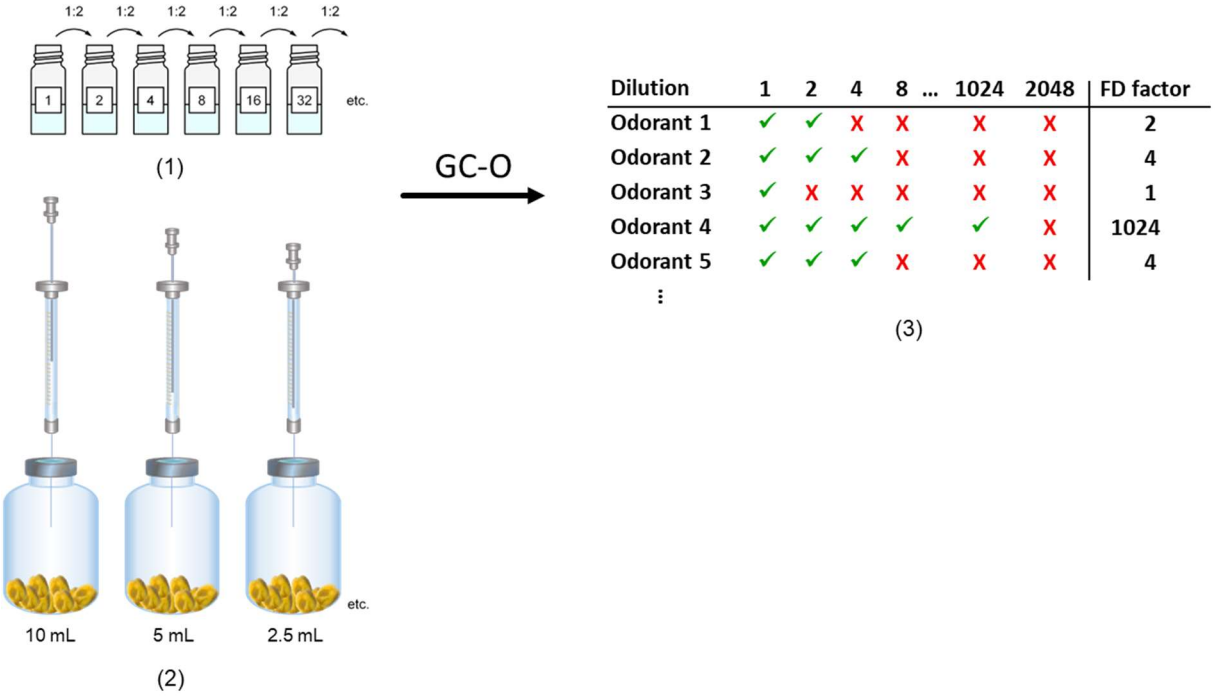

Figure 6: AEDA and static headspace dilution analyses: stepwise dilution of the volatile isolate (1), stepwise reduction of the gas phase volume (2), and FD factor calculation (3) (illustrations: Martin Steinhaus, Johanna Grimm).

Structure elucidation of the odorants detected during AEDA and HS-GC-O dilution analysis is performed by comparing specific parameters with the respective parameters of authentic reference compounds analyzed in appropriate dilution under the same conditions. The parameters are the odor perceived at the sniffing-port, the retention index (RI), and the mass spectrum. The odor is acquired during GC-O analysis. RIs are determined by co-chromatography of the volatile isolate on two capillary columns of different polarities with a mixture of *n*-alkanes according to Kováts.[18] Mass spectra are generated by gas chromatography-mass spectrometry (GC-MS) in electron ionization (EI) and chemical ionization (CI) modes. To avoid mass spectral interferences by coeluated compounds during structure assignment with GC-MS, acid/base extraction as well as fractionation of the compounds according to their polarity by silica gel chromatography can be done. Furthermore, thiols can be selectively isolated from the food isolate by mercurated agarose gel.[19] After fractionation, the odorants are localized in the individual fractions by GC-O and finally their mass spectra are determined in the fractions by GC-MS. In case of chiral compounds, the determination of the enantiomeric ratio is necessary, because the odor as well as the OTV of the isomers can be significantly different.[20–22] If a reference compound for structure elucidation is not commercially available, it has to be synthesized. The structure of this synthesized compound has to be confirmed by means of nuclear magnetic resonance (NMR) spectrometry.

Based on AEDA, the contribution of the individual odorants to the aroma of the food cannot be finally evaluated, because GC-O analysis does not consider matrix effects

and the unequal volatility of the different compounds as compounds are totally evaporized during analysis. By contrast, HS-GC-O dilution analysis considers matrix effects and volatility, but low-volatile compounds are discriminated during the HS-GC-O analysis because of adsorption phenomena.[1] Therefore, further assays based on the exact quantitation of the odorants are necessary. For quantitation, stable isotope dilution assays (SIDA)[23] can be used. In SIDA, deuterated- or ^{13}C-substituted analogues[24] of the target odorants are added to the food at the beginning of the workup as internal standards. Because the physical and chemical properties of the target odorants nearly accord with their corresponding isotopically substituted analogues, losses during the workup are compensated. If a part of the target analyte is lost during the workup, the loss of the standard is of the same degree. Correspondingly, the ratio of the target analyte to its isotopically substituted standard, which is finally determined by GC-MS, remains unchanged (Figure 7). The concentration of the target analyte in the food is calculated from the ratio of the areas of the target analyte and the corresponding standard, the amount of the added standard, and the amount of sample with the help of a calibration line equation which has previously been obtained from the analysis of target analyte and standard mixtures in different concentration ratios.

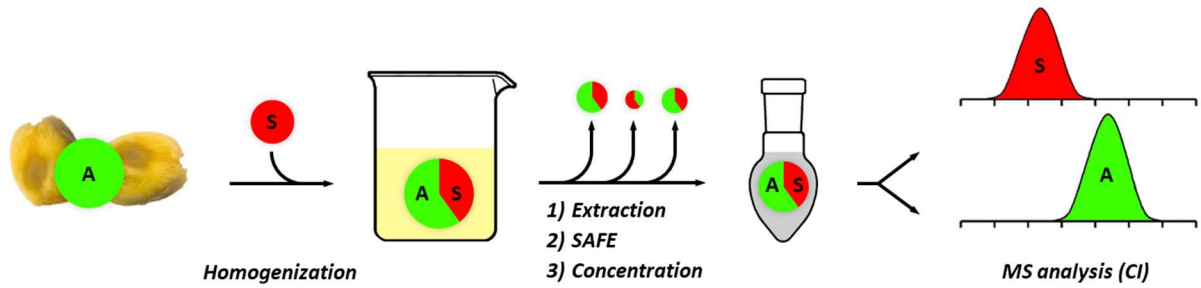

Figure 7: Schematic representation of the application of SIDA (illustrations: Martin Steinhaus).

In the next step of the approach for the identification of the key odorants, the concentration of each odorant is compared to its OTV. This is done to assess the odor potency of each odorant and approximate the contribution of the individual compounds to the overall aroma of the food. Therefore, the odor activity value (OAV), which is defined as ratio of the exact concentration of the odorant to its OTV, is calculated (Figure 8). [25, 26] The OTV is determined orthonasally according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste threshold by a forced choice ascending concentration series method of limits.[27] The matrix employed should be as similar as possible to the original food matrix. Generally, those odorants may contribute to the overall aroma of the food whose concentrations exceed their respective OTVs and thus exhibit OAVs ≥1. However, additive effects, synergisms, and antagonisms are not considered in the concept of odor activity values. However in most cases, odorants with an OAV <1 do not contribute to the overall aroma of the food.

$$\mathrm{OAV} = \frac{\text{odorant concentration } \left(\frac{\mu g}{kg}\right)}{\text{odor threshold value } \left(\frac{\mu g}{kg}\right)}$$

Figure 8: Calculation of the OAV.

During GC-O, the odor-active compounds are perceived separately, whereas during consumption of food, the odorants are evaluated together as a mixture. Therefore, the relative importance of the individual odorants is influenced by additive effects, synergisms, and antagonisms during food consumption.[1] To verify that all important odorants contributing to the overall aroma have been identified and quantitated in the food, an aroma reconstitution is performed. For this purpose, odorants for which OAVs >1 have been calculated are added in their corresponding concentration to a model solution mimicking the original food matrix. The food matrix should represent the composition of the food including the content of water, lipids, sugars, and the pH value of the food. The aroma model solution is orthonasally compared to the original food in a quantitative olfactory profile analysis by using a trained panel (Figure 9). If the olfactory profile of the food and the aroma model solution show a good agreement, it can be assumed that all odorants essential for the aroma of the food have correctly been identified and quantitated.

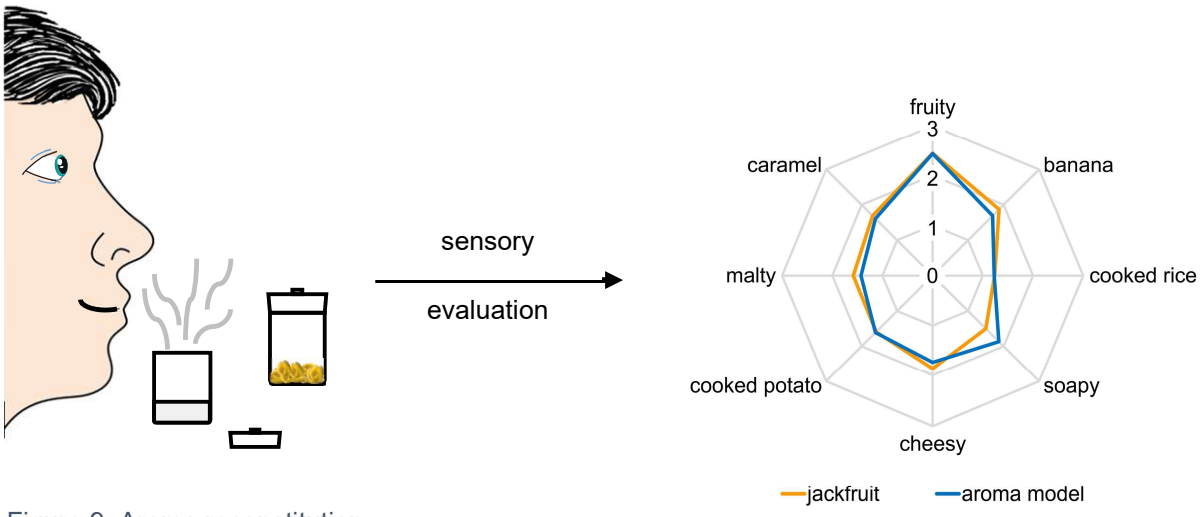

Figure 9: Aroma reconstitution.

If the aroma reconstitution has been successful, an omission test is performed as final step in the approach for the identification of the key odorants. In an omission test, an individual odorant or a group of odorants is omitted. The incomplete aroma model is orthonasally compared to the complete aroma model in a 3-alternative forced choice test. If the result of the 3-alternative forced choice test shows significant difference between the two model solutions, the omitted odorant or the group of odorants is

evidently relevant for the olfactory profile of the complete aroma model. If this is the case for a single odorant, this odorant is classified as key odorant of the food.[28, 29]

4.2 Tropical Fruits

Fruits are parts of plants which emerge from flowers and which usually enclose the seeds until they are ripe. Fruits often fall off the plants and so they satisfy an important role in the spread of the plants.[30] Fruits are often assigned to different types based on their genetic, morphological, and functional characteristics. Some of the main types are legumes, capsules, drupes, and citrus fruits as well as berries, multiple fruits, and nuts.

Tropical fruits have their origin in the tropics and semi-tropics and mostly grow on evergreen trees. Their diversity is huge. As part of an international program for the research of plant resources of Southeast Asia, 700 plant species whose fruits are utilized as food were discovered in this area alone.[30] The fruits grow either on cultivated plants or on wild plants. In Europe, however, only a limited variety of tropical fruits like banana, pineapple, avocado, mango, and passion fruit is found in common supermarkets. Reasons are a limited shelf-life of many tropical fruits, which excludes transport by ship, and the high price for air transport.

Tropical fruits include jackfruit and cempedak. Both belong to the mulberry family (Moraceae) and to the genus Artocarpus, which includes about 50 species with milky latex.[31] The word *Artocarpus* has its origin in the Greek words ‘artos’ and ‘karpos’, which mean ‘bread’ and ‘fruit’ and refer to the importance of the breadfruit *Artocarpus communis* in the natives’ diet.[31]

4.2.1 Jackfruit

4.2.1.1 Botanical Information about Jackfruit

The jackfruit tree (*Artocarpus heterophyllus* Lam.) is an evergreen tropical tree, which has its origin in South Asia, but nowadays it can be found in the entire tropics. The tree grows in a warm, humid, frost-free climate with average temperatures of 25−30 °C.[31] The tree shows a straight trunk with branches near the base and can reach a height of 25 m.[31] The leaves are oval, glossy and simultaneously leathery. All parts of the plant generate a milky latex. The male flowers build oblong clusters while female flowers are borne elliptically or round. For its fruit, timber and medical uses, the jackfruit tree is still respected by farmers in India and all over Southeast Asia.[31]

Figure 10: Jackfruit tree (photo: Michaela Jonas).

The jackfruit, which is also called jak, jaca, nangka (Javanese, Malay), jacquier (French), langka (Filipino), khanoar (Khmer), makmi, khanum, banum (Thai), or mit (Vietnamese), is cauliflorous, which means the fruit grows directly on the trunk or on old branches (Figure 10). The jackfruit is a multiple fruit derived from the coalescence of individual fruits of a whole inflorescence and can reach a length of ~90 cm, a diameter of ~50 cm, and a weight of ~50 kg.[32] With these dimensions, jackfruit is one of the largest tree fruits. However, these dimensions can vary according to the variety. A jackfruit tree can bear more than 2000 kg fruits per year. There are regionally different harvest seasons. Individual fruits can ripen the whole year.[30] The skin is leathery, yellow, green or brownish, and covered with hexagonal nubs (Figure 11 (1)). In the middle of the fruit, there is a central fibrous peduncle. In the ripe state, the interior of the fruit is filled with pulp representing a fully developed perianth and enclosed in narrow, thin, tough ribbons[32] (Figure 11 (2), (3)). The pulp (Figure 11 (4)) covers single, smooth, oval, light-brown seeds, which are surrounded by a thin white membrane and can reach a length of 4 cm[32] (Figure 11 (5), (6)). There may be 100–500 seeds in one fruit. The seeds can be eaten roasted or boiled. The roasted or dried seeds can be processed to flour for baking, too. The yellow pulp is edible and intensely fragrant and represents about a third of the total volume of the fruit. The ripe fruit pulp is eaten fresh, canned, dried, or as jam, syrup, and ice cream, while the unripe fruit pulp is cooked like a vegetable. For example, in South India and Malaysia, the preparation of a curry dish form the unripe jackfruit is common. In Germany, the popularity of jackfruit

increased within the last few years, in particular because the unripe jackfruit pulp can be used as vegan meat alternative due to its similar texture.

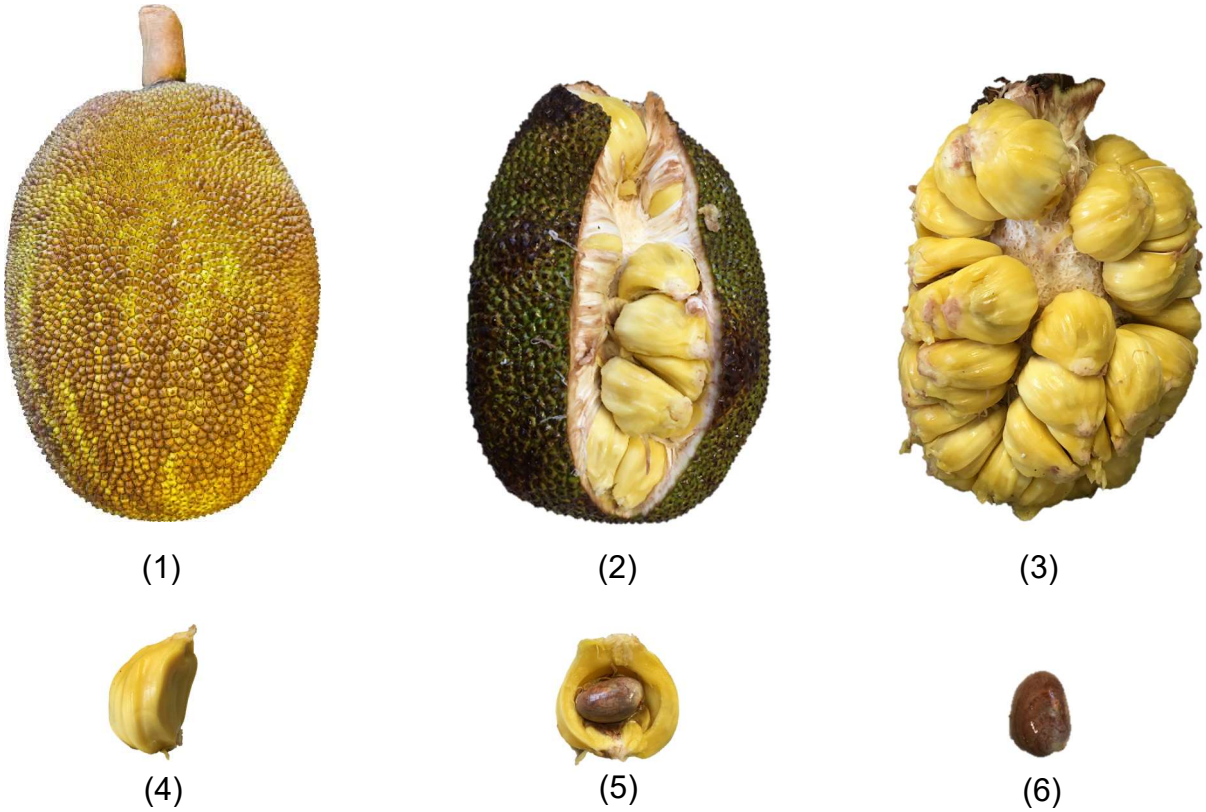

Figure 11: Whole fruit (1) (photo: Martin Steinhaus); opened fruit with ribbons and pulp (2); pulp arranged around the peduncle (3); yellow pulp (4); pulp with seed (5); seed (6).

4.2.1.2 Composition of Jackfruit

According to Souci et al.,[33] water predominates the composition of jackfruit pulp (74.6 g) (Table 1). Furthermore, according to Paull et al.,[31] jackfruit is rich in carbohydrates (25.4 g), vitamin A (66 IU), and it is a moderate protein (1.6 g) source (Table 2).

Table 1: Average Composition of 100 g Jackfruit Pulp according to Souci et al.[33]

energy	78	kcal
water	74.6	g
protein	1.1	g
fat	0.45	g
available carbohydrates	15.3	g
total fiber	4.15	g
minerals	1.0	g
sodium	2.0	mg
potassium	407	mg
calcium	27	mg
iron	600	µg
phosphorus	38	mg
retinol equivalent	8.9	µg
total carotinoids	62	µg
β-carotene	45	µg
cryptoxanthin	17	µg
vitamin B1	30	µg
vitamin B2	110	µg
nicotinamide	600	µg
vitamin C	9	mg
glucose	6000	mg
fructose	1700	mg
sucrose	6900	mg
starch	710	mg
cellulose	1300	mg

Table 2: Average Composition of 100 g Jackfruit Pulp according to Paull et al.[31]

energy	301	kcal
water	rest	
protein	1.6	g
fat	0.2	g
carbohydrates	25.4	g
fiber	5.6	g
ash	2.2	g
sodium	48	mg
potassium	292	mg
calcium	37	mg
iron	1.7	mg
phosphorus	26	mg
riboflavin	0.06	mg
niacin	0.4	mg
vitamin A	66	IU
vitamin C	7.9	mg

4.2.1.3 Jackfruit Volatiles

The aroma of jackfruit pulp combines fruity, sweet, malty, and cheesy odor notes. A few studies have been performed on jackfruit volatiles before.[34–41] Swords et al.[34] were the first to isolate the jackfruit pulp volatiles by solvent extraction followed by vacuum distillation. They identified 20 volatiles in the distillate by GC-MS. Among the identified volatiles were 16 esters and four aliphatic alcohols.

In the previous study by Rasmussen[35], a total of 21 volatile compounds were identified in an extract of jackfruit pulp obtained by solvent extraction, vacuum distillation, and GC-MS analysis. Besides alcohols like butan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, 2-methylpropan-1-ol, and propan-1-ol, the majority of compounds were esters like methyl 3-methylbutanoate, ethyl 3-methylbutanoate, butyl 3-methylbutanoate, 2-methylbutyl butanoate, and propyl 3-methylbutanoate.

Another study[36] revealed 45 volatiles after using simultaneous distillation/extraction combined with a Likens-Nickerson type apparatus and GC-MS. Among them, alcohols and esters represented the largest compound groups with 45.8% and 31.9% of the total volatiles. Other important compound groups were carboxylic acids (13.5%) and carbonyl compounds (6%). The main compounds were 3-methylbutanoic acid, 3-methylbutan-1-ol, 3-methylbutyl 3-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, butan-1-ol, 3-phenylpropanal, 3-phenylpropan-1-ol, propyl 3-methylbutanoate, methyl 3-methylbutanoate, methyl hexanoate, pentane-2,3-dione, and butan-1-ol.[36]

By using water extraction, vacuum distillation, solvent extraction, and GC-MS analysis, 61 compounds were detected in jackfruit pulp by Selvaraj et al.[37] Among them were 23 esters, 15 alcohols, nine aldehydes, five acids, four ketones, one ether, and four miscellaneous compounds. The main volatiles were ethyl 3-methylbutanoate, 3-methylbutan-1-ol, 3-methylbutyl acetate, 2-phenylethanol, 3-methylbutyl 3-methylbutanoate, and 2-methylpropyl 3-methylbutanoate.[37]

Maia et al.[38] analyzed volatiles of two varieties of jackfruit. Among a total of 39 compounds were 24 esters. Therefore, esters were the predominant compound group in jackfruit pulp. The main compounds were 3-methylbutyl 3-methylbutanoate, butyl 3-methylbutanoate, palmitic acid, ethyl 3-methylbutanoate, butyl acetate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, and 3-methylbutan-1-ol, which were isolated by simultaneous distillation/extraction and characterized by GC-MS analysis.

Ong et al.[39] analyzed flavor changes in jackfruit during ripening and determined 23 compounds by solvent extraction and GC-MS. In this study, the esters were identified as indicators of ripeness.

By application of SPME-GC-MS of jackfruit pulp, Ong et al.[40] revealed 37 volatiles including 20 esters, five alcohols, nine aldehydes, two ketones, and one ether in five jackfruit cultivars. So far, this study has been the only one to quantitate the detected volatiles. Ethyl 3-methylbutanoate, 3-methylbutyl acetate, butan-1-ol, propyl 3-methylbutanoate, 2-methylpropyl 3-methylbutanoate, 2-methylbutan-1-ol, and

butyl 3-methylbutanoate appeared in all five jackfruit varieties in relatively high concentrations.

Most of the published studies focused on the structural identification of the major volatiles, whereas little is currently known about the contribution of individual compounds to the overall aroma of the fresh pulp. Fraga et al.[41] were the first to have a closer look at the odor-active compounds. Gas chromatography-olfactometry was applied to identify the most odor-active compounds among the diversity of volatiles. An AEDA revealed the highest FD factors for ethyl 3-methylbutanoate (512), ethyl butanoate (256), butyl butanoate (128), and butyl 3-methylbutanoate (128). The FD factors of the other 52 detected odorants were comparatively low (≤ 16).

4.2.2 Cempedak

4.2.2.1 Botanical Information about Cempedak

Like the closely related jackfruit tree, the cempedak tree (*Artocarpus integer* (Thunb.) Merr.) is an evergreen tropical tree native to Southeast Asia. The cempedak tree is an understory tree and only of local importance. It is spread in Indonesia, Malaysia, New Guinea, as well as parts of Thailand, India, and Australia.[31, 32] In contrast to the jackfruit tree, the leaves, young branchlets, and buds of the cempedak tree have brown hairs. The cempedak tree is cauliflorous. It is also called champedak, bankon, baroh (Malay/Indonesian), champada (Thai), kathal, kathar (Hindi), campedak, comedak (Javanese), and mit to nu (Vietnamese).

Figure 12: Cempedak grows directly on the trunk or old branches (photo: Fitrah[42]).

The fruit is a multiple fruit and is cylindrically shaped. With dimensions of ~35 cm (length) and ~15 cm (diameter),[32] it is clearly smaller than jackfruit. The cempedak grows directly on the trunk or old branches (Figure 12). The skin of the cempedak is leathery, warty, and yellow-brownish colored (Figure 13 (1)). In the center of the fruit, there is a peduncle, which is slenderer than the one in jackfruit. Like in jackfruit, there are slim and tough ribbons between the skin and the central peduncle (Figure 13 (2)). Numerous seeds are attached to the peduncle (Figure 13 (3)). Each seed (Figure 13 (6)) is enclosed by the pulp, which is colored pale yellow to deep orange (Figure 13 (5)). The pulp (Figure 13 (4)) is tender, juicy, and softer than the one of jackfruit. It is usually eaten fresh, but sometimes it is also prepared with rice. The roasted seeds are edible, too. The harvest period is from July to October depending on the rain period.[43]

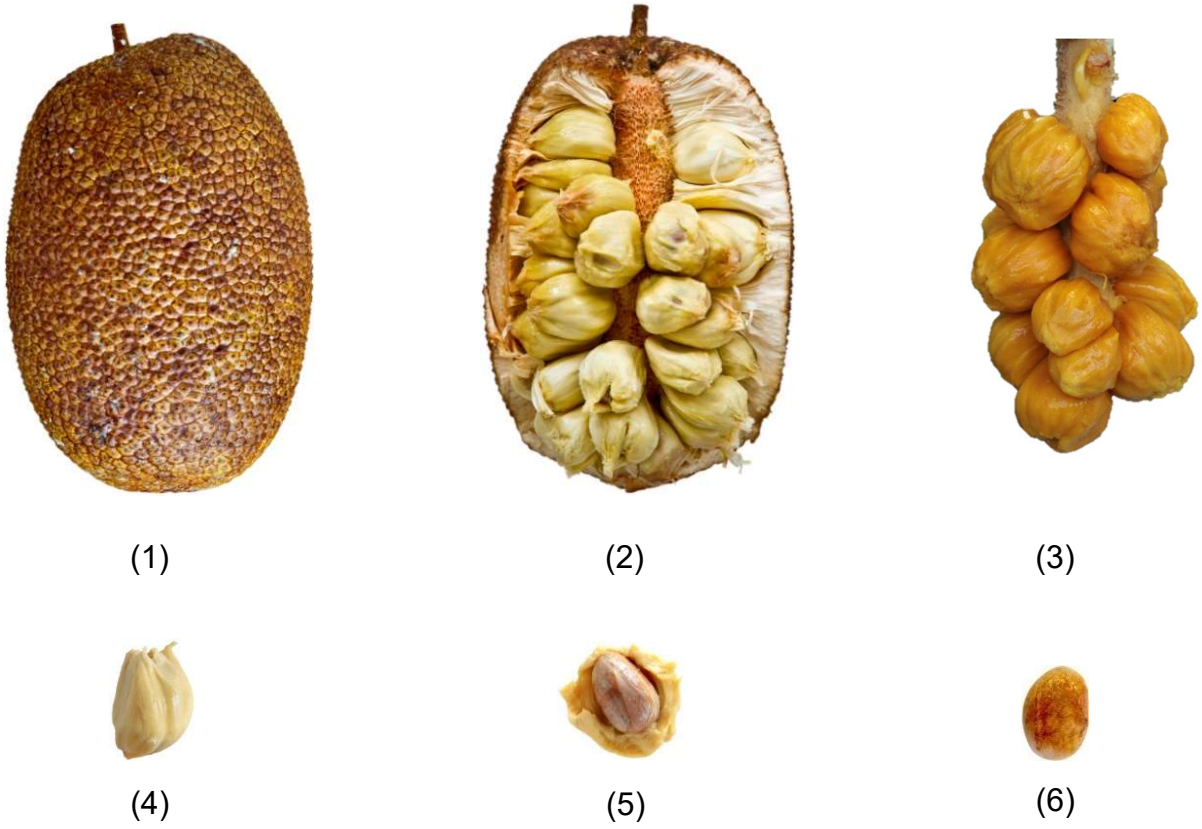

Figure 13: Whole fruit (1); opened fruit with ribbons and pulp (2); pulp arranged around the peduncle (3); pulp (4); pulp with seed (5); seed (6) (photos: Martin Steinhaus).

4.2.2.2 Composition of Cempedak

According to Paull et al.,[31] water predominates the composition of cempedak pulp with a value of 67 g/100 g (Table 3).

Table 3: Average Composition of 100 g Cempedak Pulp.[31]

water	67	g
energy	490	kcal
protein	2.5	g
fat	0.4	g
carbohydrates	25.8	g
fiber	3.4	g
ash	1.2	g
calcium	40	mg
iron	1.1	mg
phosphorus	5	mg
potassium	246	mg
sodium	25	mg
riboflavin	0.15	mg
niacin	0.5	mg
vitamin A	48	IU
vitamin C	17.7	mg

4.2.2.3 Cempedak Volatiles

The cempedak pulp is soft, juicy, and intensely fragrant. The aroma of cempedak pulp combines fruity, sweet, malty, and cheesy odor notes, and has a very unique note, which is described as sulfury, smear cheese-like, and somewhat oniony and distinguishes the aroma of cempedak pulp from the aroma of jackfruit pulp. The aroma of cempedak pulp is more intense than the aroma of jackfruit pulp. However, only a few studies have been concerned with cempedak pulp volatiles.[36, 44, 45]

The first study on cempedak volatiles[36] revealed 54 compounds after using simultaneous distillation/extraction combined with a Likens-Nickerson type apparatus. 43 out of 54 volatile compounds could be identified according to their mass spectra and Kováts indices in comparison to those of authentic reference compounds. Nine

compounds were tentatively identified on the basis of mass spectra matching to those of an MS library. Alcohols (nine compounds) and carboxylic acids (ten compounds) represented the largest compound groups with 37.4% and 32.2% of the total volatiles. Other important compound groups were aldehydes and ketones (14.6% of the total volatiles), esters (6.7% of the total volatiles), sulfur compounds (1.7% of the total volatiles) and nitrogen containing compounds (0.7% of the total volatiles). The main individual volatile compounds in the cempedak pulp were 3-methylbutanoic acid (28.2% of the total volatiles), 3-methylbutan-1-ol (24.3% of the total volatiles), 3-hydroxybutan-2-one (6.3% of the total volatiles), 2-phenylethanol (5.8% of the total volatiles), 2-methylpropan-1-ol (4.2% of the total volatiles), ethyl 3-methylbutanoate (3.8% of the total volatiles), pentane-2,3-dione (2.5% of the total volatiles), and methyl 3-methylbutanoate (2.1% of the total volatiles). The study of Wong et al.[36] focused on the structural identification of the major volatiles, whereas only little is known about the contribution of the individual volatiles to the aroma of the fresh pulp.

Wijaya et al.[44] tested different extraction methods including maceration, vacuum distillation, steam distillation, and simultaneous distillation/extraction on cempedak pulp and evaluated the intensity of the characteristic aroma. The best results were obtained by maceration using dichloromethane. 64 volatile compounds were revealed by GC-MS. 20 compounds could be identified. Among them, the major volatiles were 3-methylbutyl 3-methylbutanoate, 3-methylbutan-1-ol, 3-methylbutanoic acid, hexan-2-ol, octanoic acid, hexan-3-ol, and butyl 3-methylbutanoate. Furthermore, this study applied GC-O to the cempedak volatiles. The GC-O analysis suggested that 3-methylbutyl 3-methylbutanoate and butyl 3-methylbutanoate were character impact compounds of cempedak pulp. However, the authors did not substantiate their results with quantitation, calculation of OAVs, and reconstitution experiments.

Buttara et al.,[45] however, analyzed odorants in two cempedak cultivars, namely Tongtapan and Sainumpung, by using AEDA, stable isotope dilution quantitation, calculation of OAVs, aroma reconstitution, and omission tests. The application of GC-O combined with AEDA revealed 23 and 17 odorants for the two cultivars. Among them, ethyl 3-methylbutanoate, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, 3-methyl-butanal, ethyl 2-methylbutanote, 3-(methylsulfanyl)propanal, 2-acetyl-1-pyrroline, and 3-methylbutanoic acid showed the highest FD factors. High OAVs were determined for 3-methylbutanal, ethyl 3-methylbutanoate, methyl 3-methylbutanoate, octanal, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and hexanal in at least one of the two cultivars. According to the reconstitution and omission experiments, Buttara et al.[45] suggested 3-methylbutanal, octanal, ethyl 3-methylbutanoate, methyl 3-methylbutanoate, and 2-acetyl-1-pyrroline as key odorants in cempedak pulp. However, none of these odorants could explain the typical sulfury, smear cheese-like, oniony odor note. Nevertheless, the organosulfur odorants 3-(methylsulfanyl)propanal, 3-(methylsulfanyl)butanal, 3-(methylsulfanyl)butan-1-ol, dimethyl trisulfide, and 2-(methylsulfanyl)ethanal were found in cempedak pulp. However, according to the reconstitution experiments, 3-(methylsulfanyl)propanal, 3-(methylsulfanyl)butanal, 3-(methylsulfanyl)butan-1-ol did not contribute to the overall aroma. Dimethyl trisulfide

as well as 2-(methylsulfanyl)ethanal were only tentatively identified and not quantitated. Thus, their aroma contribution remained unclear.

5. Objectives

As detailed in the introduction, there is a number of tropical fruits which are not widely spread in Germany, among them jackfruit, the fruit of *Artocarpus heterophyllus* Lam., and cempedak, the fruit of cempedak *Artocarpus integer* (Thunb.) Merr. Both fruits exhibit very characteristic aroma properties. The aroma of ripe jackfruit pulp combines fruity, sweet, malty, and cheesy odor notes, whereas the more intense aroma of cempedak pulp additionally shows a typical sulfury, smear cheese-like, oniony odor note. Only a few studies had been concerned with jackfruit and cempedak pulp volatiles. In particular, the knowledge about the contribution of individual odor-active compounds to the aroma of both fruits was sparse and the molecular background of the typical sulfury, smear cheese-like, oniony odor note distinguishing the aroma of jackfruit and cempedak pulp was unclear.

The objective of the current study was to identify the major odorants in jackfruit pulp and cempedak pulp with emphasis on the compounds contributing to the sulfury, smear cheese-like, and oniony odor note of cempedak pulp by using the sensomics concept. The concept includes (1) odorant screening by AEDA and static headspace dilution analysis, (2) exact quantitation of the major odorants by using GC-MS in combination with stable isotopologues of the odorants as internal standards, (3) calculation of OAVs, and finally (4) aroma reconstitution and omission experiments based on the natural odorant concentrations as proof of success.

6. Results and Discussion

The present thesis is based on three peer reviewed publications. Two were published in an international scientific journal and one as a book chapter. Copies of the publications, summaries including the author contributions, as well as the publishers' permissions for article reuse are included in the appendix.

6.1 Odorant Screening

For screening, jackfruit and cempedak were purchased from the German internet shop *Tropenkost* (www.tropenkost.de). The fruits were grown in Thailand and handpicked by local farmers when they were almost fully ripe. The fruits were delivered by airfreight within two days. If necessary, the fruits were stored at room temperature to reach full ripeness, which was indicated by softening and release of the characteristic odor. Each fruit was opened with a knife and the seeds were removed from the bulbs by hand. For the analyses, the fresh pulp was flash frozen and cryomilled.

For AEDA, the jackfruit and cempedak volatiles were isolated from the respective cryomilled fruit pulp via solvent extraction and SAFE. The SAFE distillates were concentrated using a Vigreux-column. The concentrates were subjected to AEDA. The results revealed 48 odorants in jackfruit pulp and 49 odorants in cempedak pulp. FD factors ranged from 1 to ≥8192 (Table 4).[46–48] On the basis of the comparison of the RI and the odor perceived at the sniffing port during AEDA with published data,[49, 50] preliminary structure assignments were achieved. The preliminary assignments were confirmed by analyzing the corresponding reference compounds in appropriate dilution in parallel with the fruit isolates by GC-O using two separation systems of different polarity (DB-FFAP and DB-5). Based on the identity of RIs and odor descriptions between the fruit odorants and the reference compounds, the structures of 39 out of 48 jackfruit pulp odorants (**2**−**5**, **7**−**10**, **12**−**21**, **23**−**28**, **30**, **33**−**35**, **41**−**46**, **48**, **49**, **51**−**53**) and the structures of 39 out of the 49 cempedak pulp odorants (**2**−**5**, **7**, **8**, **10**, **13**−**21**, **23**−**31**, **33**−**35**, **41**−**46**, **48**, **49**, **51**−**53**) could be assigned. Final structure confirmation was achieved by the comparison of the mass spectra of the fruit pulp odorants in EI and CI mode to the mass spectra of the reference compounds as obtained by GC-MS analysis. To avoid any overlap of mass spectra, the fruit isolate was fractionated into five fractions according to their polarity by silica gel chromatography before GC-MS analysis. Another experiment was performed to selectively isolate the thiols by using mercurated agarose gel. Each fraction was separately analyzed by GC-O for the localization of the previously detected odorants and by GC-MS in parallel with the reference compounds. Using this approach, 31 and 33 out of the 39 and 39 previously assigned structures were confirmed in jackfruit and cempedak pulp, respectively.

The highest FD factor (≥8192) was detected for the fruity smelling ethyl 3-methylbutanoate (**8**) in both fruits. The odorant had been found in jackfruit pulp[34–38, 40, 41] and cempedak pulp[36, 45] before. High FD factors in jackfruit (JF) and cempedak (CP) were also found for the caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF, **46**; FD JF ≥8192, FD CP 4096), the cheesy, sweaty

smelling 3-methylbutanoic acid (**35**; FD JF 256, FD CP 512), the grassy smelling hexanal (**10**; JF FD 256, CP FD 256), the sweaty smelling hexanoic acid (**41**; JF FD 256, CP FD 256), the malty smelling 2-/3-methylbutan-1-ol (**16**/**15**; FD JF 128, FD CP 256), the cooked potato-like smelling 3-(methylsulfanyl)propanal (**25**; JF FD 128, CP FD 128), and the fruity smelling ethyl (2*E*)-3-phenylprop-2-enoate (**49**; JF FD 128, CP FD 128). In both fruits, these odorants had been reported previously[34–41, 44, 45] except for 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**46**) in jackfruit pulp and ethyl (2*E*)-3-phenylprop-2-enoate (**49**) in cempedak pulp.

High FD factors only in jackfruit pulp were determined for the unknown compound **37** (meaty, seasoning-like; FD 2048), methyl 3-methylbutanoate (**4**; fruity; FD 256), and ethyl butanoate (**5**; fruity; FD 128). In cempedak pulp, the FD factors were lower. Methyl 3-methylbutanoate had been identified in jackfruit pulp[35–38, 40, 41] and cempedak pulp[36, 44, 45] before. Ethyl butanoate had been reported in jackfruit[34, 35, 37, 38, 40, 41] before, but not in cempedak. The two banana-like smelling compounds, butyl acetate (**9**) and 3-methylbutyl acetate (**12**), were only found in jackfruit pulp. This could indicate a role for the aroma difference between the two fruits. However, both compounds had been reported in jackfruit pulp before.[34–41] Moreover, the odorants **40** (fatty; FD 16) and **50** (seasoning-like; FD 16) were only detected in jackfruit pulp during AEDA.

High FD factors in cempedak pulp were additionally obtained for 2-acetyl-1-pyrroline (**21**; popcorn-like; FD 4096), the unknown compound **22** (sulfury; FD 1024), methyl hexanoate (**14**; fruity; FD 512), octanal (**18**; citrusy, soapy; FD 256), butan-1-ol (**13**; malty; FD 128), the unknown compound **32** (fruity; FD 128), and 2-phenylethanol (**44**; floral; FD 128). In jackfruit pulp, the FD factors of these odorants all were lower. All these compounds had been reported in jackfruit pulp[36–41] and cempedak pulp[36, 44, 45] before. The caramel-like odor of 4-methoxy-2,5-dimethylfuran-3(2*H*)-one (MDMF, **31**), the seasoning-like odor of compound **47**, the citrusy, floral odor of linalool (**29**), and the sulfury, oniony odor of compounds **1** and **6** could only be detected in cempedak pulp during AEDA. Whereas MDMF had been reported in cempedak before,[44, 45] linalool was determined for the first time. The compounds **1** and **6** particularly attracted attention, because their odor clearly represented the characteristic oniony, smear cheese-like, sulfury odor note of cempedak pulp. Both compounds could not be detected in jackfruit pulp. Therefore, the two compounds **1** and **6** were suggested to play a major role for the aroma of cempedak pulp. However, with a value of 32 their FD factors were comparatively low.

Table 4: Odorants of Jackfruit (JF) Pulp and Cempedak (CP) Pulp obtained by AEDA.

no.	odorant[a]	odor[b]	RI[c] FFAP	RI[c] DB-5	FD factor[d]	
					JF	CP
1	2-(methylsulfanyl)butane	oniony, sulfury, cempedak	<1000	786	–	32
2	ethyl 2-methylpropanoate[e,f]	fruity	<1000	759	16	1
3	butane-2,3-dione	butter	<1000	<700	4	8
4	methyl 3-methylbutanoate	fruity	1013	931	256	64
5	ethyl butanoate	fruity	1028	809	128	32
6	2-(methylsulfanyl)pentane	oniony, sulfury, cempedak	1041	870	–	32
7	ethyl 2-methylbutanoate	fruity	1044	849	64	32
8	ethyl 3-methylbutanoate	fruity	1061	859	≥8192	≥8192
9	butyl acetate	banana	1067	820	4	–
10	hexanal	green, grassy	1073	800	256	256
11	unknown	skunky	1103	839	1	64
12	3-methylbutyl acetate	banana	1115	881	4	–
13	butan-1-ol	malty	1140	<700	64	128
14	methyl hexanoate	fruity	1180	928	16	512
15	3-methylbutan-1-ol[g]	malty	1207	742	128	256
16	2-methylbutan-1-ol[g]	malty	1207	742	128	256
17	ethyl hexanoate	fruity	1225	1002	1	32
18	octanal	citrusy, soapy	1279	1005	64	256
19	3-hydroxybutan-2-one	butter	1280	716	4	4
20	1-octen-3-one[e, f]	mushroom	1290	979	32	32
21	2-acetyl-1-pyrroline[f]	cooked rice	1332	921	128	4096
22	unknown	sulfury	1365	–	4	1024
23	nonanal	citrusy, soapy	1390	1100	4	4
24	acetic acid	vinegar	1454	<700	32	64
25	3-(methylsulfanyl)propanal	cooked potato	1457	910	128	128
26	decanal	citrusy, soapy	1485	1208	1	32
27	3-isobutyl-2-methoxypyrazine[e, f]	bell pepper	1512	1181	4	4
28	(2*E*)-non-2-enal[e, f]	fatty	1530	1163	16	32
29	linalool	citrusy, floral	1544	1102	–	8
30	(2*E*,6*Z*)-nona-2,6-dienal[e, f]	cucumber	1580	1159	4	4
31	MDMF	caramel	1589	1060	–	64
32	unknown	fruity	1610	1224	4	128
33	butanoic acid	cheesy, sweaty	1620	815	64	16
34	phenylacetaldehyde	floral, honey	1645	1046	2	32
35	3-methylbutanoic acid	cheesy, sweaty	1665	871	256	512

Table 4, continued

no.	odorant[a]	odor[b]	RI[c] FFAP	RI[c] DB-5	FD factor[d]	
					JF	CP
36	unknown[h]	citrusy, soapy, green	1690	1550	32	16
37	unknown[i]	meaty, seasoning	1724	1025	2048	128
38	unknown	green	1759	−	16	32
39	unknown	cooked potato	1769	1170	32	32
40	unknown	fatty	1800	−	16	−
41	hexanoic acid	sweaty	1844	1010	256	256
42	2-methoxyphenol[f]	smoky	1863	1091	8	16
43	ethyl 3-phenylpropanoate	fruity	1887	1352	32	16
44	2-phenylethanol	floral	1916	1116	32	128
45	*γ*-octalactone	coconut	1920	1260	8	4
46	HDMF[f]	caramel	2047	1068	≥8192	4096
47	unknown	seasoning	2068	−	−	16
48	4-methylphenol	fecal, horse stable	2095	1098	8	8
49	ethyl (2*E*)-3-phenylprop-2-enoate	fruity	2120	1469	128	128
50	unknown	seasoning	2150	−	16	−
51	sotolon[e]	seasoning	2203	1109	32	32
52	2-phenylacetic acid	floral, honey	2589	1264	8	2
53	vanillin	vanilla	2600	1409	16	32

[a]Each odorant was identified by comparing its retention index on two GC capillaries of different polarity (DB-FFAP, DB-5), its mass spectrum obtained by GC-MS, as well as its odor perceived at the sniffing port during GC-O to data obtained from authentic reference compounds analyzed in parallel. [b]Odor perceived at the sniffing port during GC-O. [c]Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [d]Flavor dilution factor; dilution factor of the highest dilution of the jackfruit pulp isolate or cempedak pulp isolate in which the odorant was detected during GC-O analyses. [e]An unequivocal mass spectrum of the compound could not be obtained by GC-MS analysis of the cempedak pulp isolate. Identification was based on the remaining criteria detailed in footnote a. [f]An unequivocal mass spectrum of the compound could not be obtained by GC-MS analysis of the jackfruit pulp isolate. Identification was based on the remaining criteria detailed in footnote a. [g]3-methylbutan-1-ol and 2-methylbutan-1-ol were not separated on the GC column used for AEDA. FD factors of 128 (JF) and 256 (CP) were determined for the mixture of both compounds. [h]Mass spectral data allowed for the tentative identification of the compound as an octenol, however, positions of the double bond and the hydroxy group have not been elucidated. [i]The compound could be enriched by mercurated agarose gel and thus was a thiol, but no further information on its structure was gained.

The retention indices and the oniony, sulfury odor of the compounds **1** and **6** did not match any data in the databases.[49, 50] However, the GC-MS analysis resulted in the mass spectra depicted in Figure 14.[48, 51] The comparison of the mass spectra with MS database[52] spectra suggested compound **1** to be 2-(methylsulfanyl)butane. The structure assignment could be confirmed by analyzing the reference compound by GC-O and GC-MS in parallel to the cempedak pulp. However, comparing the mass spectrum of compound **6** with MS database spectra did not result in a hit. The

comparison with the mass spectrum of compound **1** suggested that compound **6** was a homologue differing from **1** in an additional methylene group. The fragmentation pattern in the mass spectrum suggested that compound **6** was either 2-(methyl-sulfanyl)pentane or 3-methyl-2-(methylsulfanlyl)butane (Figure 14).

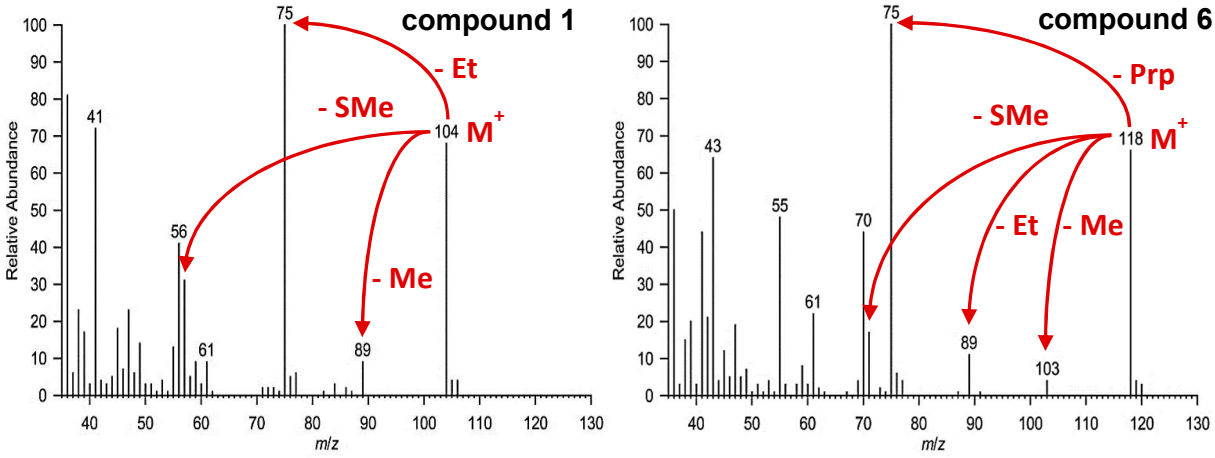

Figure 14: Mass spectra of compounds **1** and **6**.

Both compounds were commercially not available. Therefore, the compounds had to be synthesized. Synthesis was accomplished by tosylation of the corresponding alcohols followed by substitution of the tosylate with methanethiolate (Figure 15).[48, 51] The structures of both synthesized compounds were confirmed by NMR spectrometry.

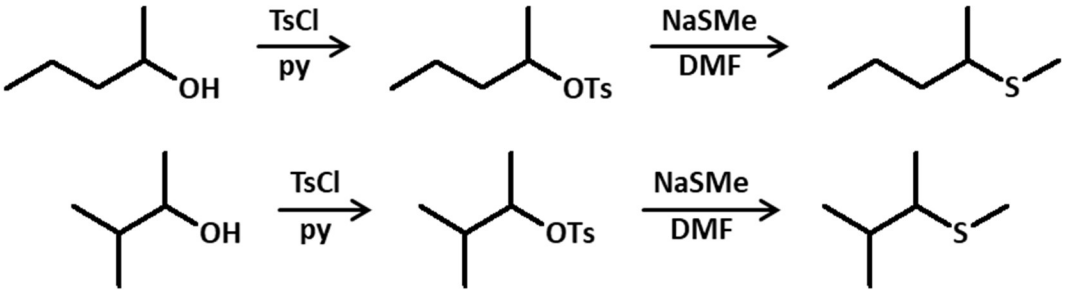

Figure 15: Synthesis of 2-(methylsulfanyl)pentane and 3-methyl-2-(methylsulfanyl)butane.

2-(Methylsulfanyl)pentane and 3-methyl-2-(methylsulfanyl)butane were analyzed by GC-O and GC-MS in comparison to the cempedak pulp volatiles. Based on the RI, the odor description, and the mass spectrum, compound **6** was identified as 2-(methylsulfanyl)pentane.

2-(Methylsulfanyl)butane and 2-(methylsulfanyl)pentane are chiral compounds with one stereogenic center. As enantiomers can differ in their odor and OTVs, a GC-O-enantioGC-O/MS analysis was performed. After synthesis of the enantiopure

compounds from the enantiopure alcohols, *R*/*S* ratios of 33/67 for 2-(methylsulfanyl)butane and 41/59 for 2-(methylsulfanyl)pentane were determined (Figure 16, Table 5).[48, 51] The odor qualities of the isomers were almost identical, but they differed in their OTVs (Table 5).[51] 2-(Methylsulfanyl)butane and 2-(methyl-sulfanyl)pentane had not been reported as fruit odorants before.

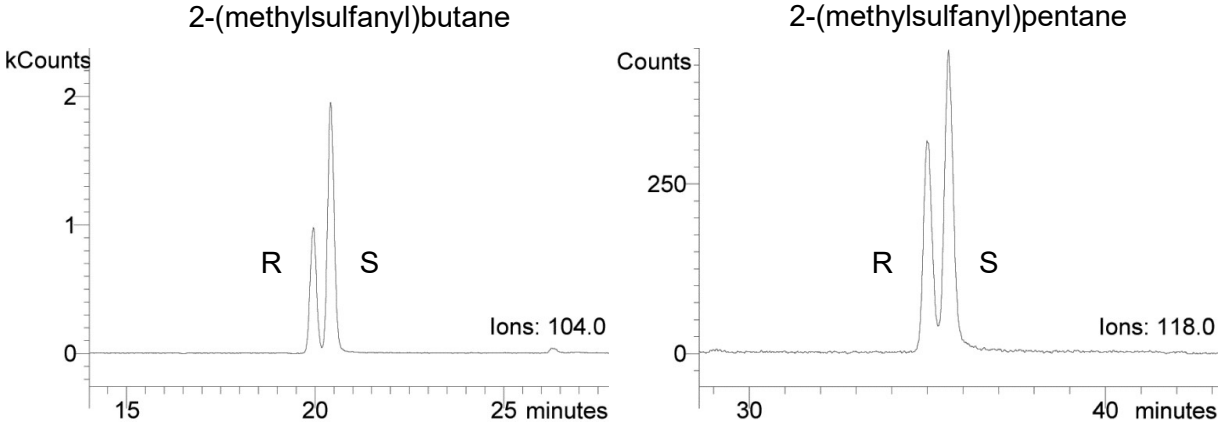

Figure 16: GC-O-enantioGC-O/MS analysis of cempedak pulp on a chiral BGB 176 column.

Table 5: Chromatographic and Sensory Properties as well as Distribution of 2-(Methylsulfanyl)butane and 2-(Methylsulfanyl)pentane Enantiomers in Cempedak Pulp.

odorant	odor[a]	RI[b] BGB 176	OTV in air[c] (ng/L)	enantiomeric ratio[d]
(2*R*)-2-(methylsulfanyl)butane	oniony, sulfury, cempedak	718	1	33%
(2*S*)-2-(methylsulfanyl)butane	oniony, sulfury, cempedak	725	2	67%
(2*R*)-2-(methylsulfanyl)pentane	oniony, sulfury, cempedak	802	2	41%
(2*S*)-2-(methylsulfanyl)pentane	oniony, sulfury, cempedak	804	5	59%

[a]Odor as perceived at the sniffing port during GC-O [b]Retention index; calculated by linear interpolation from the retention time of the compound and the retention times of adjacent *n*-alkanes on the chiral 2,3-dimethyl-6-*tert*-butyldimethylsilyl-β-cyclodextrine-based BGB 176 column used for GC-O-enantioGCO/MS analysis. [c]Odor threshold value in air [d]enantiomeric ratio in the cempedak pulp isolate.

The screening by AEDA does not cover odorants with boiling points below the boiling point of the solvent used for the extraction. These highly volatile compounds are lost during the concentration of the SAFE distillate. To screen for the highly volatile odorants, GC-O in combination with static headspace sampling was applied. The sampled volume of the headspace above the two cryomilled fruit pulps was successively reduced before headspace GC-O analysis until no odorants were perceived at the sniffing-port. The odorants were structurally assigned by comparing their GC-O and GC-MS data to those of the reference compounds. The approach revealed additional five odorants in jackfruit pulp and additional six odorants in cempedak pulp (Table 6).[46, 47]

The malty smelling compounds **57** and **58**, showing the highest FD factor of 32 in the headspace of both fruits, were identified as 2-methylbutanal (**57**) and 3-methylbutanal

(**58**). Odorant **54** was assigned to dimethyl sulfide (asparagus-like, putrid). For dimethyl sulfide, a lower FD factor was found in jackfruit pulp (2) than in cempedak pulp (32). The malty smelling compounds **55** and **56** were determined as 2-methylpropanal (**55**) and butanal (**56**). In jackfruit pulp, 2-methylpropanal exhibited a higher FD factor (16) than in cempedak pulp (8), whereas for butanal, a lower FD factor was found in jackfruit pulp (2 vs. 16). The retention index and the oniony, sulfury odor of odorant **59**, which was only detected in cempedak (FD 16), did not match any data in the databases.[49, 50] As its odor clearly represented the characteristic smear cheese-like, oniony, sulfury odor note of cempedak pulp, similar to the compounds **1** and **6**, compound **59** also attracted special attention. GC-MS analysis of the headspace of cempedak pulp resulted in a mass spectrum of this compound, which matched the mass spectrum of 2-(methylsulfanyl)propane in the MS database.[52] GC-O and GC-MS analysis of a 2-(methylsulfanyl)propane reference confirmed the structure assignment. This odorant had not yet been identified in cempedak pulp before. 2- and 3-Methylbutanal, on the other hand, had been reported as jackfruit[40] and cempedak[45] volatiles before. Except for butanal in jackfruit pulp[40], the other highly volatile odorants were detected for the first time in jackfruit pulp and cempedak pulp.

Table 6: Additional Odorants Detected in the Headspace of Jackfruit and Cempedak Pulp.

no.	odorant[a]	odor[b]	RI[c] DB-5	FD factor[d]	
				JF	CP
54	dimethyl sulfide	asparagus, putrid	518	2	32
55	2-methylpropanal	malty	559	16	8
56	butanal	malty	594	2	16
57	2-methylbutanal[e]	malty	655	32	32
58	3-methylbutanal[e]	malty	655	32	32
59	2-(methylsulfanyl)propane	oniony, sulfury, cempedak	674	–	16

[a]Each odorant was identified by comparing its retention index on the DB-5 capillary, its mass spectrum obtained by GC-MS, as well as its odor perceived at the sniffing port during GC-O to data obtained from authentic reference compounds analyzed under equal conditions. [b,c]cf. Table 4. [d]Flavor dilution factor; calculated as ratio of the initial injection volume (10 mL) to the lowest injection volume in which the odorant was detected during GC-O analyses. [e]2-Methylbutanal and 3-methylbutanal were not separated on the GC column used for static headspace GC; an FD factor of 32 was determined for the mixture of both compounds.

In summary, the combination of the screening by AEDA and by static headspace dilution analysis resulted in 53 odorants in jackfruit pulp, among which 44 could be identified, whereas in cempedak pulp 55 odorants were determined, among which 47 could be assigned a structure. 2-(Methylsulfanyl)propane, 2-(methylsulfanyl)butane, and 2-(methylsulfanyl)pentane were suggested as major contributors to the typical sulfury, smear cheese-like, oniony odor note distinguishing the aroma of cempedak pulp from the aroma of jackfruit pulp.

6.2 Odorant Quantitation and OAV Calculation

To identify the character impact compounds of jackfruit pulp and cempedak pulp and to unequivocally clarify the role of the 2-(methylsulfanyl)alkanes, quantitation of the identified odorants, calculation of OAVs, and aroma reconstitution experiments were performed. The fruit pulps were cryomilled and stable isotopically substituted analogues of the odorants were added as internal standards to compensate for losses during the workup. The mixture was stirred with solvent and insoluble material was removed. The volatiles were separated from the non-volatiles using SAFE. The distillates were concentrated and the concentrates were subjected to analysis by heart-cut GC-GC-MS (CI) or GC×GC-MS (EI). The concentrations were calculated by means of a calibration line equation from the amount of the fruit pulp, the amount of the added standards, and the integrated peak areas of analytes and their corresponding isotopically substituted analogues. All odorants which had been structurally identified in at least one of the two fruits were quantitated in both fruit pulps. For the quantitation of the 2-(methylsulfanyl)alkanes, the isotopically substituted 2-[($^2H_3$)methylsulfanyl]-alkanes were synthesized from the corresponding 2-aclohols by tosylation, thioacetylation, reduction, and trideuteromethylation with ($^2H_3$)methyl iodide according to the approach detailed by Polster and Schieberle.[53] The concentrations of the individual enantiomers of 2-(methylsulfanyl)butane and 2-(methylsulfanyl)pentane were calculated from the sum of the concentrations of both enantiomers obtained by GC-MS analysis and the respective enantiomeric ratios.

The results of the quantitations revealed odorant concentrations ranging from 0.0161 µg/kg to 101000 µg/kg in jackfruit pulp and from 0.0391 µg/kg to 40700 µg/kg in cempedak pulp (Table 7).[46, 47]

The highest concentration in jackfruit pulp was determined for 3-methylbutanoic acid. High concentrations in jackfruit pulp were additionally obtained for 3-hydroxybutan-2-one (**19**; 44600 µg/kg), acetic acid (**24**; 9940 µg/kg), 3-methylbutan-1-ol (**15**; 5460 µg/kg), HDMF (**46**; 3100 µg/kg), 2-methylbutan-1-ol (**16**; 2190 µg/kg), ethyl 3-methylbutanoate (**8**; 1710 µg/kg), hexanoic acid (**41**; 1560 µg/kg), ethyl butanoate (**5**; 1370 µg/kg), octanal (**18**; 1130 µg/kg), and butanoic acid (**33**; 1110 µg/kg). Ong et al.[40] were the only ones to have published quantitative data on odorants in jackfruit pulp. For most odorants, Ong et al.[40] reported lower concentrations. This applied for hexanal (22–33 µg/kg), ethyl butanoate (102–27 µg/kg), 3-methylbutanal (119–176 µg/kg), ethyl 3-methylbutanoate (238–986 µg/kg), butanal (109–159 µg/kg), methyl 3-methylbutanoate (132–388 µg/kg), 2-methylbutanal (115 µg/kg), 3-methylbutan-1-ol (711–1753 µg/kg), and 2-methylbutan-1-ol (469–9006 µg/kg). In contrast, concentrations of 3-methylbutyl acetate (499–8284 µg/kg), ethyl 2-methylbutanoate (174 µg/kg), and butyl acetate (241–988 µg/kg) were higher. Only butan-1-ol was reported in the same concentration range (359–721 µg/kg) as in the current study.

Like in jackfruit pulp, the highest concentration in cempedak pulp was obtained for 3-methylbutanoic acid (**35**; 40700 µg/kg). High concentrations were found for acetic acid (**24**; 23300 µg/kg), 3-hydroxybutan-2-one (**19**; 21200 µg/kg), ethyl 3-methylbutanoate (**8**; 9400 µg/kg), 3-methylbutan-1-ol (**15**; 6950 µg/kg), butan-1-ol (**13**;

6060 µg/kg), hexanoic acid (**41**; 5770 µg/kg), HDMF (**46**; 2920 µg/kg), 2-methylbutan-1-ol (**16**; 2290 µg/kg), ethyl hexanoate (**17**; 1450 µg/kg), and octanal (**18**; 1270 µg/kg). The only other study on cempedak volatiles which reported quantitative data was published by Buttara et al.[45] They also found high concentrations of 3-methylbutanoic acid, 3-methylbutan-1-ol, ethyl 3-methylbutanoate, HDMF, and octanal. Additionally, high concentrations were determined by Buttara et al.[45] for methyl 3-methylbutanoate, MDMF, hexanal, and hexan-1-ol.

To assess the odor potency, an OAV was calculated for each individual odorant from its concentration in the fruit pulp and its OTV in water (Table 7).[46, 47] 35 previously quantitated compounds featured OAVs ≥1 in jackfruit pulp. In cempedak pulp, OAVs ≥1 were obtained for 41 odorants (Table 7). Thus, their concentrations in the pulp were beyond their respective OTVs in water.

Among the 35 compounds which revealed an OAV ≥1 in jackfruit pulp, the highest OAV of 74000 was obtained for the fruity smelling ethyl 3-methylbutanoate (**8**). Additionally high OAVs were found for ethyl butanoate (**5**; OAV 1800), 3-methylbutanal (**58**; OAV 1500), 2-methylpropanal (**55**; OAV 1400), butanal (**56**; OAV 550), octanal (**18**; OAV 330), hexanal (**10**; OAV 290), methyl 3-methylbutanoate (**4**; OAV 280), 3-methylbutanoic acid (**35**; OAV 210), ethyl 2-methylbutanoate (**7**; OAV 190), dimethyl sulfide (**54**; OAV 180), 2-methylbutanal (**57**; OAV 170), (2*E*,6*Z*)-nona-2,6-dienal (**30**; OAV 110), and 2-acetyl-1-pyrroline (**21**; OAV 100). Additional 21 odorants showed OAVs ranging from 82 to 1.7 (**2**–**3**, **9**, **12**, **15**–**17**, **19**–**20**, **23**–**28**, **34**, **43**, **46**, **49**, **51**, **52**), whereas the concentrations of 16 odorants (**1**, **6**, **13**, **14**, **29**, **31**, **33**, **41**, **42**, **44**, **45**, **48**, **53**, **59**) remained below their respective OTVs. The OAV data suggested that the fruity odor note of the aroma of jackfruit pulp is caused by ethyl 3-methylbutanoate (**8**; OAV 74000), ethyl butanoate (**5**; OAV 1800), methyl 3-methylbutanoate (**4**; OAV 280), and ethyl 2-methylbutanoate (**7**; OAV 190). 3-Methylbutanoic acid (**35**; OAV 210) was considered to contribute to the sweaty and cheesy odor note. Furthermore, 2-/3-methylbutanal (**57**; OAV 170 / **58**; OAV 1500), 2-methylpropanal (**55**; OAV 1400), and butanal (**56**; OAV 550) could provoke the malty odor note.

Like in jackfruit pulp, the highest OAV in cempedak pulp was shown for the fruity smelling ethyl 3-methylbutanoate (**8**; OAV 410000). The high OAV confirmed the results of the AEDA, in which ethyl 3-methylbutanoate showed the highest FD factor. Further high OAVs were found for 3-methylbutanal (**58**; OAV 1700), ethyl hexanoate (**17**; OAV 1200), butanal (**56**; OAV 620), (2*E*,6*Z*)-nona-2,6-dienal (**30**; OAV 530), (2*S*)-2-(methylsulfanyl)butane (**1b**; OAV 480), (2*R*)-2-(methylsulfanyl)butane (**1a**; OAV 470), 2-acetyl-1-pyrroline (**21**; OAV 450), ethyl butanoate (**5**; OAV 390), octanal (**18**; OAV 370), dimethyl sulfide (**54**; OAV 370), 2-methylbutanal (**57**; OAV 350), ethyl 2-methylbutanoate (**7**; OAV 170), 2-methylpropanal (**55**; OAV 160), hexanal (**10**; OAV 150), and 2-(methylsulfanyl)propane (**59**; OAV 110). Furthermore, 25 odorants (**2**–**4**, **6a**, **6b**, **13**, **15**, **16**, **19**, **20**, **23**–**29**, **34**, **35**, **41**–**44**, **46**, **49**) showed OAVs ranging from 83 to 1.2. OAVs <1 were determined for ten odorants (**9**, **12**, **14**, **31**, **33**, **45**, **48**, **51**–**53**) in cempedak pulp (Table 7). In accordance to this study, Buttara et al.[45] found high OAVs for ethyl 3-methylbutanoate, 3-methylbutanal, and octanal. Additionally, Buttara

et al.[45] determined high OAVs for methyl 3-methylbutanoate, HDMF, and hexanal, whereas these compounds showed lower OAVs in the present study. The OAV data of the present study suggested that the fruity odor note in cempedak pulp is caused by ethyl 3-methylbutanoate (**8**; OAV 410000). 3-Methylbutanal (**58**; OAV 1700), butanal (**56**; OAV 620), and 2-methylbutanal (**57**; OAV 350) were considered to contribute to the malty odor note. The cheesy odor note of the cempedak pulp could be provoked by 3-methylbutanoic acid (**35**; OAV 83). The characteristic oniony, sulfury, smear cheese-like, and cempedak-like odor note could be caused by (2*S*)-2-(methylsulfanyl)butane (**1b**; OAV 480), (2*R*)-2-(methylsulfanyl)butane (**1a**; OAV 470), 2-(methylsulfanyl)propane (**59**; OAV 110), whereas (2*R*)-2-(methylsulfanyl)pentane (**6a**; OAV 4.3), (2*S*)-2-(methylsulfanyl)pentane (**6b**; OAV 2.5) were found to be much less potent in cempedak pulp.

The data revealed higher OAVs in cempedak pulp than in jackfruit pulp for the majority of the quantitated odorants, namely for 27 odorants. In contrast, 17 odorants exhibited higher OAVs in jackfruit pulp than in cempedak pulp. Seven compounds did not show an appreciable difference in the OAVs between the two fruits. This was in agreement with the more intense aroma of cempedak pulp. Considerably higher OAVs in cempedak pulp were determined for ethyl 3-methylbutanoate (**8**; OAV 410000 vs. 74000), ethyl hexanoate (**17**; OAV 1200 vs. 82), (2*E*,6*Z*)-nona-2,6-dienal (**30**; OAV 530 vs. 110), and 2-acetyl-1-pyrroline (**21**; OAV 450 vs. 100). However, substantially higher OAVs were found in jackfruit pulp than in cempedak pulp for ethyl butanoate (**5**; OAV 1800 vs. 390), 2-methylpropanal (**55**; OAV 1400 vs. 160), methyl 3-methylbutanoate (**4**; OAV 280 vs. 46), 3-methylbutanoic acid (**35**; OAV 210 vs. 83), and hexanal (**10**; OAV 290 vs. 150). 3-Methylbutyl acetate (**12**) and butyl acetate (**9**), which were detected during AEDA in jackfruit pulp, but not in cempedak pulp, consequently showed concentrations exceeding their OTVs in jackfruit pulp, whereas in cempedak pulp OAVs were <1. In contrast, the concentrations of the 2-(methylsulfanyl)alkanes (**1a**, **1b**, **6a**, **6b**, **55**) exceeded their respective OTVs in cempedak pulp, whereas in jackfruit pulp OAVs were <1. These results were to be expected from the AEDA results. The 2-(methylsulfanyl)alkanes were confirmed to play a major role in the difference between jackfruit pulp and cempedak pulp.

Table 7: Concentrations, OTVs, and OAVs of Major Jackfruit and Cempedak Pulp Odorants.

no.[a]	odorant	concentration[b] (µg/kg)		OTV[c] (µg/kg)	OAV[d]	
		JF	CP		JF	CP
8	ethyl 3-methylbutanoate	1710	9400	0.023	74000	410000
5	ethyl butanoate	1370	293	0.75	1800	390
58	3-methylbutanal	759	862	0.50	1500	1700
55	2-methylpropanal	689	80.6	0.49	1400	160
56	butanal	482	541	0.87	550	620
18	octanal	1130	1270	3.4	330	370
10	hexanal	700	368	2.4	290	150
4	methyl 3-methylbutanoate	613	101	2.2	280	46
35	3-methylbutanoic acid	101000	40700	490	210	83
7	ethyl 2-methylbutanoate	25.1	21.7	0.13	190	170
54	dimethyl sulfide	55.0	111	0.30	180	370
57	2-methylbutanal	248	532	1.5	170	350
30	(2*E*,6*Z*)-nona-2,6-dienal	0.488	2.39	0.0045	110	530
21	2-acetyl-1-pyrroline	5.41	23.8	0.053	100	450
17	ethyl hexanoate	98.3	1450	1.2	82	1200
19	3-hydroxybutan-2-one	44600	21200	590	75	36
2	ethyl 2-methylpropanoate	6.14	0.291	0.089	69	3.3
3	butane-2,3-dione	61.7	67.3	0.96	64	70
46	HDMF	3100	2920	87	36	34
20	1-octen-3-one	0.547	0.126	0.016	34	7.9
43	ethyl 3-phenylpropanoate	66.1	5.66	2.1	31	2.7
25	3-(methylsulfanyl)propanal	12.1	5.61	0.43	28	13
15	3-methylbutan-1-ol	5460	6950	220	25	32
12	3-methylbutyl acetate	111	1.78	7.2	15	<1
23	nonanal	29.3	48.7	2.8	10	17
9	butyl acetate	153	4.97	27	5.6	<1
49	ethyl (2*E*)-3-phenylprop-2-enoate	7.53	5.69	1.7	4.4	3.3
52	2-phenylacetic acid	291	49.9	68	4.3	<1
51	sotolon	5.64	1.37	1.7	3.3	<1
28	(2*E*)-non-2-enal	0.521	0.780	0.19	2.8	4.1
27	3-isobutyl-2-methoxypyrazine	0.0160	0.0391	0.0062	2.6	6.3
34	phenylacetaldehyde	9.53	25.9	5.2	1.8	5.0
24	acetic acid	9940	23300	5600	1.8	4.2
16	2-methylbutan-1-ol	2190	2290	1200	1.8	1.9

Table 7, continued

no.[a]	odorant	concentration[b] (µg/kg)		OTV[c] (µg/kg)	OAV[d]	
		JF	CP		JF	CP
26	decanal	15.8	78.3	9.3	1.7	8.4
1b	(2*S*)-2-(methylsulfanyl)butane	<0.1	667[e]	1.4[f]	<1	480
1a	(2*R*)-2-(methylsulfanyl)butane	<0.1	329[e]	0.7	<1	470
59	2-(methylsulfanyl)propane	<0.5	210	1.9	<1	110
13	butan-1-ol	478	6060	590	<1	10
6a	(2*R*)-2-(methylsulfanyl)pentane	<0.2	5.65[e]	1.3	<1	4.3
29	linalool	0.569	2.45	0.58	<1	4.2
42	2-methoxyphenol	0.338	2.69	0.84	<1	3.2
6b	(2*S*)-2-(methylsulfanyl)pentane	<0.2	8.14[e]	3.3[g]	<1	2.5
44	2-phenylethanol	103	227	140	<1	1.6
41	hexanoic acid	1560	5770	4800	<1	1.2
53	vanillin	1.87	49.1	53	<1	<1
31	MDMF	0.143	29.9	56	<1	<1
45	γ-octalactone	1.04	1.75	6.5	<1	<1
33	butanoic acid	1110	548	2400	<1	<1
48	4-methylphenol	0.427	0.633	3.9	<1	<1
14	methyl hexanoate	0.414	10.4	90	<1	<1

[a]Numeration according to Tables 4 and 6. [b]Mean of duplicates, triplicates, or quadruplicates. [c]Odor threshold value in water. [d]Odor activity value; calculated as ratio of concentration to OTV. [e]Concentrations of the (*R*)- and (*S*)-enantiomers calculated from the concentrations of the sum of both enantiomers and the enantiomeric distribution. [f]Pure (2*S*)-2-(methylsulfanyl)butane was not available; therefore, the OTV in water was approximated from the OTV in water of (2*R*)-2-(methylsulfanyl)butane and the ratio of the OTVs in air of both enantiomers (2 ng/L / 1 ng/L). [g]Pure (2*S*)-2-(methylsulfanyl)pentane was not available; therefore, the OTV in water was approximated from the OTV in water of (2*R*)-2-(methylsulfanyl)pentane and the ratio of the OTVs in air of both enantiomers (5 ng/L / 2 ng/L).

6.3 Aroma Reconstitution and Omission Experiments

To prove that all important odorants contributing to the aroma were identified and quantitated in the fruits, aroma reconstitution experiments were performed. Aqueous aroma model solutions were prepared, which contained 35 and 39 compounds for which OAVs ≥1 had been calculated in jackfruit pulp[46] and cempedak pulp,[47] respectively. The concentrations of the compounds corresponded to their natural concentrations in the fruits. To mimic the fruit matrix, the model solution was based on water and contained the major sugars and organic acids. As no quantitative data on sugars and organic acids were available for cempedak pulp, the cempedak matrix was approximated by using jackfruit pulp data.[39] As (2*S*)-2-(methylsulfanyl)butane and (2*S*)-2-(methylsulfanyl)pentane were not available as reference compounds, their odor contribution in the cempedak aroma model solution was approximated by racemic 2-(methylsulfanyl)butane and (2*R*)-2-(methylsulfanyl)pentane, respectively, in amounts that corresponded to the same OAVs. The model solutions were orthonasally compared to the respective fruit pulps in a quantitative olfactory profile analysis by using a sensory panel and descriptors representing the characteristic odor notes of the fruits. The panelists rated the intensities of the predefined descriptors on a scale from 0 to 3 in 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate and 3 = strong. Furthermore, the panelists evaluated the similarity of the aroma model solution and the fruit pulp on a scale from 0 to 3 with 0 = no similarity, 1 = weak similarity, 2 = the fruit can be recognized, 3 = identical.

The predefined descriptors and corresponding references for jackfruit pulp were “cooked potato-like” (3-(methylsulfanyl)propanal), “malty” (3-methylbutan-1-ol), “caramel-like” (HDMF), “cheesy” (3-methylbutanoic acid), “fruity” (ethyl 3-methylbutanoate), “banana-like” (3-methylbutyl acetate), “cooked rice-like” (2-acetyl-1-pyrroline), and “soapy” (octanal). The results showed a good agreement between the olfactory profile of the aroma model solution and the olfactory profile of the original fresh jackfruit pulp (Figure 17). A balanced combination of fruity, banana-like, cooked rice-like, soapy, cheesy, cooked potato-like, malty, and caramel-like odor notes represented the overall olfactory profiles. The intensity of the fruity odor note was rated slightly higher than the other ones. The intensity of the soapy odor note was rated a little higher in the aroma model solution, whereas the intensities of the banana-like, malty, and cheesy odor notes were evaluated a little bit lower. However, the similarity was rated 2.9 out of 3. The excellent agreement between the aroma of the model solution and the fresh jackfruit pulp proved that all important odorants of jackfruit pulp were correctly identified and quantitated even though some odorants detected during AEDA remained unidentified.

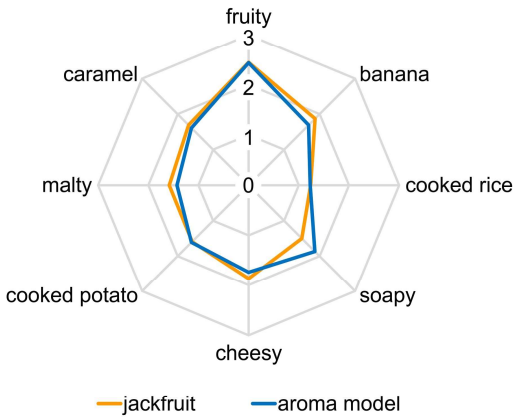

Figure 17: The olfactory profile of the fresh jackfruit pulp in comparison to the olfactory profile of the jackfruit pulp aroma model solution (35 compounds, OAVs ≥1).[46] Panelists rated the intensity of each descriptor on a scale from 0 to 3 in 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

For the evaluation of cempedak pulp and its model solution, the descriptors “sulfury, oniony, cempedak-like” (2-(methylsulfanyl)butane), “cooked potato-like” (3-(methylsulfanyl)propanal, “malty” (3-methylbutan-1-ol), “caramel-like” (HDMF), “cheesy” (3-methylbutanoic acid), “fruity” (ethyl 3-methylbutanoate), “banana-like” (3-methylbutyl acetate), “cooked rice-like” (2-acetyl-1-pyrroline), and “soapy” (octanal) were predefined. The olfactory profiles of fresh cempedak pulp and the complete aroma model solution (Figure 18) were dominated by the oniony, sulfury, cempedak-like and cheesy odor notes. Furthermore, both olfactory profiles were moderately affected by fruity, caramel-like, malty, cooked potato-like, banana-like, and cooked rice-like odor notes and weakly influenced by a soapy odor note. The olfactory profile of the complete cempedak pulp model solution showed a good agreement with the olfactory profile of the original fresh cempedak pulp (Figure 18). However, the intensities of the oniony, sulfury, cempedak-like odor note and the cheesy odor note were rated a little bit higher in the fresh cempedak pulp than in the complete cempedak pulp aroma model solution. The similarity of the two samples was rated 2.6 by the panelists. This nevertheless confirmed that the most important odorants were correctly identified and quantitated in cempedak pulp, even though some of the odorants detected during AEDA could not be identified. To demonstrate that the 2-(methylsulfanyl)alkanes are important for the characteristic aroma of the cempedak pulp and contribute to the difference between jackfruit pulp aroma and cempedak pulp aroma, an incomplete cempedak pulp aroma model solution from which the 2-(methylsulfanyl)alkanes were omitted was additionally included in the quantitative olfactory profile analysis. A fourth sample consisted of fresh jackfruit pulp (Figure 18). The intensity of the oniony, sulfury odor note, which is characteristic for cempedak pulp, was rated lower in the olfactory profile of the incomplete cempedak pulp aroma model solution than in the olfactory profile of the fresh cempedak pulp and in its complete aroma model solution. Instead, the intensity of this typical cempedak odor note in the olfactory profile of the incomplete aroma model solution was close to its intensity in the olfactory profile of jackfruit pulp.

Therefore, the data confirmed that 2-(methylsulfanyl)alkanes are key odorants in cempedak pulp and contribute to the difference between cempedak pulp and jackfruit pulp.

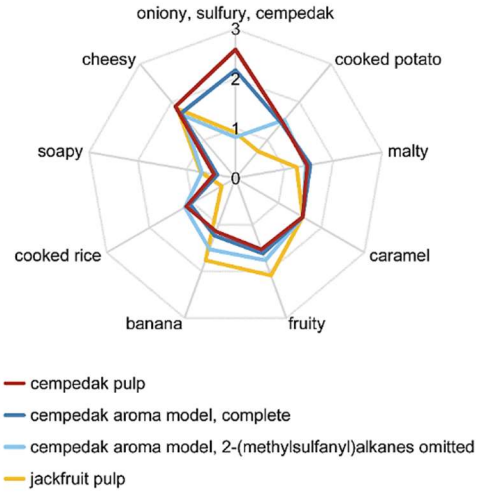

Figure 18: The olfactory profile of the fresh cempedak pulp in comparison to the olfactory profile of the cempedak pulp aroma model solution (39 compounds, OAVs ≥1), the olfactory profile of the cempedak aroma model solution from which the 2-(methylsulfanyl)alkanes were omitted, and the olfactory profile of the fresh jackfruit pulp.[47] Panelists rated the intensity of each descriptor on a scale from 0 to 3 in 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

To summarize, application of AEDA and static headspace dilution analysis, quantitation of odor-active compounds, calculation of OAVs, reconstitution experiments of jackfruit pulp and cempedak pulp, as well as an omission experiment demonstrated that the important odorants contributing to the aroma of the fruits were correctly identified and quantitated. In particular, the data showed that 2-(methyl-sulfanyl)alkanes are key odorants in cempedak pulp, and that they are responsible for the characteristic oniony, sulfury note of cempedak pulp, and vitally contribute to the aroma difference between cempedak pulp and jackfruit pulp.

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8. Appendix

8.1 Publication 1: Characterization of the Major Odor-Active Compounds in Jackfruit Pulp

8.1.1 Bibliographic Data

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8.1.2 Publication Reprint

For the reprint of publication 1, please turn to the next page.

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Characterization of the Major Odor-Active Compounds in Jackfruit Pulp

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Supporting Information

ABSTRACT: An aroma extract dilution analysis applied to the volatiles isolated from jackfruit (*Artocarpus heterophyllus* Lam.) pulp by solvent extraction and solvent-assisted flavor evaporation resulted in the detection of 48 odorants with flavor dilution (FD) factors between 1 and ≥8192. Application of gas chromatography-olfactometry to static headspace samples revealed additional five highly volatile odorants. The structures of 44 out of the 53 detected odorants could be assigned. These 44 compounds were quantitated using stable isotopically substituted odorants as internal standards, and their odor activity values (OAVs) were calculated as the ratio of the natural concentrations in jackfruit pulp and the odor threshold values in water. High OAVs were in particular obtained for ethyl 3-methylbutanoate (74000), ethyl butanoate (1800), 3-methylbutanal (1500), and 2-methylpropanal (1400). An aroma model solution based on the natural concentrations of the 35 compounds for which OAVs > 1 had been calculated fully mimicked the characteristic jackfruit pulp aroma.

KEYWORDS: *jackfruit, Artocarpus heterophyllus Lam, aroma extract dilution analysis, AEDA, stable isotopically substituted odorants, aroma reconstitution*

INTRODUCTION

The jackfruit tree, *Artocarpus heterophyllus* Lam., is an evergreen tropical tree in the mulberry family Moraceae. It is native to South Asia but grown in the entire tropics today. The cylindrically shaped multiple fruits may reach a length of 90 cm and a width of 50 cm and grow from the trunk and old branches.[1] The skin is leathery, yellow, green, or brownish colored and covered with characteristic hexagonal nubs. In the ripe state, the room between the skin and the central fibrous peduncle is filled with a mixture of slim, tough ribbons and numerous seeds. Each seed is enclosed in a bulb-like, fleshy, and edible pulp of yellow color, sweet taste, and delicious aroma. The pulp is mainly eaten fresh but can also be processed into preserves such as canned pulp, dried pulp, jam, or syrup.

The aroma of ripe jackfruit pulp combines sweet, fruity, malty, and cheesy notes. Although some studies on jackfruit volatiles have been published, little is currently known on the contribution of individual compounds to the overall aroma of the fresh pulp. Most studies were solely focused on the structural identification of major volatiles without any assessment of their odor activity. In the pioneering study published in 1978, Swords et al. isolated jackfruit pulp volatiles by vacuum distillation and solvent extraction and identified 19 volatiles by GC-MS, among which were 15 esters and 4 aliphatic alcohols.[2] Further studies utilized fruits grown in Malaysia,[3−6] India,[7] and Brazil.[8,9] This led to a total number of ∼200 different jackfruit volatiles revealed so far. Among the major jackfruit volatiles reported in the different studies were alcohols such as butan-1-ol,[2−7,9] 2-methylbutan-1-ol,[3,6,9] 3-methylbutan-1-ol,[2−9] and 2-phenylethanol,[4,7,9] esters such as butyl acetate,[2,3,5,6,8,9] 2-methylbutyl acetate,[3,6,8] 3-methylbutyl acetate,[3−8] ethyl butanoate,[2,3,6−9] ethyl 2-methylbutanoate,[6,8,9] methyl 3-methylbutanoate,[3,4,6−9] ethyl 3-methylbutanoate,[2−4,6−9] propyl 3-methylbutanoate,[2−9] butyl 3-methylbutanoate,[2−9] 2-methylpropyl 3-methylbutanoate,[2−4,6−9] 2-methylbutyl 3-methylbutanoate,[3] 3-methylbutyl 3-methylbutanoate,[2,4−9] and methyl hexanoate,[7,9] as well as acids such as 3-methylbutanoic acid.[4,5,7,9] Only in a single case, gas chromatography-olfactometry (GC-O) was applied to identify the most odor-active compounds among the bulk of irrelevant volatiles and application of an aroma extract dilution analysis (AEDA)[10] revealed the highest flavor dilution factors (FD factors) for ethyl 3-methylbutanoate (512), ethyl butanoate (256), butyl butanoate (128), and butyl 3-methylbutanoate (128) whereas FD factors of all other 52 detected odorants were comparably low (≤16).[9] Our own preliminary GC-O experiments resulted in 26 odor-active compounds among which some odorants were previously unknown in jackfruit pulp.[11]

Considering the fragmentary knowledge on the odor activity of individual volatiles illustrated by the literature overview detailed above, the aim of the present study was to identify the major odorants in jackfruit pulp by using a systematic approach including (1) odorant screening by AEDA and dilution analysis of static headspace samples, (2) exact quantitation using stable isotopologues of the odorants as internal standards, (3) calculation of odor activity values (OAVs), and finally (4) aroma reconstitution based on the natural odorant concentrations as proof of success.

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MATERIALS AND METHODS

Jackfruit Pulp. Fruits grown in Thailand were purchased from a German Internet shop. The fruits were handpicked by local farmers when almost fully ripe and sent to Germany by airfreight within 2 days. Fruits were carefully opened with a knife, seeds were removed from the bulbs by hand, and the jackfruit pulp was immediately used for analysis.

Reference Odorants. **1**, **2**, **4**−**8**, **10**, **12**−**16**, **18**, **22**−**27**, **29**−**31**, **37**−**44**, **46**, **47**, and **49**−**51** were purchased from Sigma-Aldrich (Taufkirchen, Germany), **3** was obtained from Lancaster (Mühlheim, Germany), **11** and **48** were from Merck (Darmstadt, Germany), **17**, **52**, and **53** were from Alfa Aesar (Karlsruhe, Germany), **21** was from Acros Organics (Geel, Belgium), and **19** was synthesized according to a previously published procedure.[12] **30** was freshly distilled before use.

Stable Isotopically Substituted Odorants. ($^{13}C_4$)-**17** was obtained from Toronto Research Chemicals (Toronto, Canada). ($^{2}H_3$)-**22**, ($^{2}H_8$)-**43**, ($^{13}C_2$)-**47**, and ($^{2}H_6$)-**49** were purchased from Sigma-Aldrich, ($^{2}H_5$)-**40** was from CDN Isotopes (Quebec, Canada), and ($^{13}C_2$)-**42** was obtained from aromaLAB (Planegg, Germany). The following isotopically substituted odorants were synthesized as detailed in the literature: ($^{13}C_4$)-**2**,[13] ($^{2}H_3$)-**4**,[14] ($^{13}C_2$)-**7**,[15] ($^{2}H_{11}$)-**10**,[16] ($^{2}H_4$)-**8**,[14] ($^{2}H_2$)-**13**,[17] ($^{2}H_{2-5}$)-**19**,[18] ($^{2}H_4$)-**24**,[19] ($^{2}H_3$)-**25**,[20] ($^{2}H_2$)-**27**,[21] ($^{13}C_2$)-**30**,[22] ($^{2}H_2$)-**31**,[17] ($^{2}H_2$)-**37**,[23] ($^{2}H_3$)-**38**,[24] ($^{2}H_2$)-**41**,[25] ($^{13}C_2$)-**46**,[26] ($^{2}H_3$)-**48**,[27] ($^{2}H_2$)-**52**,[28] and ($^{2}H_2$)-**53**.[28] Compounds ($^{2}H_3$)-**1**, ($^{2}H_3$)-**3**, ($^{2}H_5$)-**5**, ($^{2}H_3$)-**6**, ($^{2}H_3$)-**12**, ($^{2}H_3$)-**15**, ($^{2}H_5$)-**39**, and ($^{2}H_5$)-**44** were prepared from the corresponding acids and (2,2,2-$^{2}H_3$)ethanol, ($^{2}H_5$)ethan(^{2}H)ol, or ($^{2}H_3$)methan(^{2}H)ol (all Sigma-Aldrich) using a general approach for ester synthesis detailed recently.[29] ($^{2}H_4$)-**11**, ($^{2}H_4$)-**51**, ($^{2}H_4$)-**16**, and ($^{2}H_4$)-**21** were prepared from but-3-yn-1-ol, oct-3-yn-1-ol, and non-3-yn-1-ol (all Sigma-Aldrich), respectively, using the method detailed for the synthesis of ($^{2}H_4$)hexanal from 5-hexyn-1-ol via ($^{2}H_4$)hexan-1-ol.[14] ($^{2}H_4$)-**18** was synthesized by reacting ($^{2}H_4$)-**8**[14] and vinyl magnesium bromide[30] followed by oxidation of the obtained ($^{2}H_4$)oct-1-en-3-ol using Dess-Martin periodinane (Sigma-Aldrich).[31] ($^{2}H_3$)-**23** and ($^{2}H_6$)-**50** were prepared from 3-[($^{2}H_3$)methylsulfanyl]propan-1-ol[32] and 2-($^{2}H_3$)methyl(3,3,3-$^{2}H_3$)propan-1-ol (CDN Isotopes), respectively, by Dess-Martin oxidation.[31] ($^{2}H_2$)-**26** was synthesized from non-3-yn-1-ol (Sigma-Aldrich) by deuteration with lithium aluminum deuteride (Sigma-Aldrich)[33] and oxidation of the obtained ($^{2}H_2$)non-2-en-1-ol using Dess-Martin periodinane.[31] ($^{2}H_2$)-**29** was prepared by using the approach published for the synthesis of ($^{2}H_2$)-**31**[17] but starting from 3-buten-1-ol (Sigma-Aldrich) instead of 3-methyl-3-buten-1-ol.

Miscellaneous Chemicals and Reagents. Dichloromethane, diethyl ether, and pentane were purchased from VWR (Darmstadt, Germany). Before use, they were freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings. Silica gel 60 (0.040−0.63 mm) was obtained from VWR and purified as detailed previously.[34] Mercurated agarose gel was prepared from Affi-Gel 10 (Bio-Rad, Munich, Germany).[35]

GC-O/FID System. A gas chromatograph GC 8000 Series (Fisons Instruments, Mainz, Germany) was equipped with a cold on-column injector, a flame ionization detector (FID), and a tailor-made sniffing port.[36] The column was either a DB-FFAP, 30 m × 0.32 mm ID, 0.25 μm film, or a DB-5, 30 m × 0.32 mm ID, 0.25 μm film (both Agilent, Waldbronn, Germany). The carrier gas was helium at 50 kPa (DB-FFAP) and 40 kPa (DB-5). The injection volume was 1 μL. The initial oven temperature of 40 °C was held for 2 min, followed by a gradient of 6 °C/min. Final temperatures of 230 °C (DB-FFAP) and 240 °C (DB-5) were held for 5 min. The end of the column was connected to a Y-shaped glass splitter, which divided the column effluent into two equal parts. Two deactivated fused silica capillaries (50 cm × 0.25 mm ID) conveyed the effluent halves to the FID (base temperature 250 °C) and the sniffing port (base temperature 230 °C), respectively.

During a GC-O run, a panelist placed his nose closely above the sniffing port and evaluated the effluent. Whenever an odor was detected, the position as well as the odor quality were marked in the FID chromatogram plotted by a recorder. Linear retention indices (RI) were calculated from the retention times of the odorants and the retention times of adjacent *n*-alkanes by linear interpolation.[37]

GC-MS System. A HP 5890 Series II gas chromatograph (Hewlett-Packard, Heilbronn, Germany) equipped with a cold on-column injector was connected to an MAT 95 sector field mass spectrometer (Finnigan, Bremen, Germany). Helium with a constant flow of 1.9 mL/min was the carrier gas. The columns used with this system were equivalent to those used in the GC-O/FID instrument, and the same temperature programs were applied. Mass spectra were generated in the electron ionization (EI) mode at 70 eV and a scan range of m/z 35−300. Data analysis was accomplished by using the Xcalibur software (Thermo).

Heart-Cut GC-GC-MS System. A Trace gas chromatograph Ultra (Thermo) was equipped with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), a cold on-column injector (Thermo), and a GC column which was either a DB-FFAP capillary, 30 m × 0.32 mm ID, 0.25 μm film or a DB-5 capillary, 30 m × 0.25 mm ID, 1 μm film (both Agilent). Helium at 100 kPa constant pressure was used as carrier gas. The injection volume was 1−2 μL. The initial oven temperature of 35−40 °C was held for 2−10 min, followed by a gradient of 6−40 °C/min to a final temperature of 230 °C. The end of the column was connected to a moving column stream switching (MCSS) system (Thermo). Employing helium at 50 kPa as makeup gas, the MCSS system allowed for the time-programmed transfer of the column eluate via deactivated fused silica capillaries (0.32 mm ID) either simultaneously to an FID (Thermo) and a sniffing port used as monitor detectors or to a second GC column installed in a separate gas chromatograph (CP 3800, Varian, Darmstadt). The capillary to the second column passed through a heated (250 °C) hose connecting the two gas chromatographs and a liquid nitrogen-cooled trap inside the oven of the second gas chromatograph allowing for the refocusing of the heart-cut. The column in the second dimension was either a DB-1701, 30 m × 0.25 mm ID, 0.25 μm film (Agilent), a DB-5, 30 m × 0.25 mm ID, 1 μm film (Agilent), a BGB-174 E, 30 m × 0.25 mm ID, 0.25 μm film (BGB Analytik, Rheinfelden, Germany), or a DB-5, 30 m × 0.25 mm ID, 0.25 μm film (Agilent). The initial temperature of the second oven was 30−40 °C and was held for 2−10 min, followed by a gradient of 6−40 °C/min to a final temperature of 200−230 °C. The end of the column in second dimension was connected to a Saturn 2200 mass spectrometer (Varian) run in EI mode or in chemical ionization (CI) mode. For CI, methanol was used as the reagent gas.

Before a heart-cut analysis, the retention times of the target compounds in the first dimension were determined after injection of reference compounds by using the monitor detectors. During the elution of the target compounds in the subsequent analysis, the effluent of the first column was directed via the MCSS system to the column in the second dimension and the transferred substances were cryofocused in the precooled trap. When the MCSS system had switched back to the monitor detectors, the trap cooling was turned off and the temperature program of the second gas chromatograph was started together with the mass spectrometer. Data analysis was accomplished by using the MS Workstation software (Varian).

GC × GC-MS System. A 6890 Plus gas chromatograph (Agilent) was equipped with a Combi PAL autosampler (CTC Analytics), a KAS4 injector (Gerstel, Mühlheim/Ruhr, Germany), and a GC column DB-FFAP, 25 m × 0.25 mm ID 0.25 μm film (Agilent). Helium at 2.0 mL/min constant flow was used as a carrier gas. The end of the column was connected to a second column, which was a DB-5 capillary, 2 m × 0.15 mm ID, 0.30 μm film (Varian). At the beginning of the second column, a liquid nitrogen-cooled dual stage quad-jet thermal modulator (Leco, Mönchengladbach, Germany) was used to collect the volatiles eluted from the first column in discrete portions (4 s), which then were rechromatographed on the major part of the second column. This part was installed in a secondary oven located inside the primary oven. The end of this column was connected to the MS inlet (250 °C) of a Pegasus III time-of-flight (TOF) mass spectrometer (Leco). Mass spectra were generated in EI mode at 70 eV, a scan range of m/z 35−300, and a scan rate of 100

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spectra/s. Temperature programs were 40 °C (2 min), then 6 °C/min to 230 °C (5 min) for the first oven, and 70 °C (2 min), followed by a gradient of 6 °C/min to 250 °C (5 min) for the second oven. For data analysis, GC Image software (GC Image, Lincoln, NE, USA) was employed.

Static Headspace GC System. A Trace GC Ultra gas chromatograph (Thermo) was equipped with a Combi PAL autosampler (CTC Analytics), a cold on-column injector (Thermo), a liquid nitrogen oven cooling, and a cold trap 915 (Thermo). The carrier gas was helium at 110 kPa. A deactivated fused silica capillary, 0.2 m × 0.53 mm ID (Agilent), was connected to the injector, passed through the cold trap, and ended in a Y-shaped glass splitter. One outlet of the splitter was connected via another piece of deactivated fused silica capillary to a solenoid valve. The other outlet was connected to a DB-5 capillary, 30 m × 0.25 mm ID, 1 μm film (Agilent). The end of this column was connected to two further Y-shaped glass splitters in series, which divided the column effluent via further pieces of deactivated fused silica capillaries into three parts, among which one part (25%) was conveyed to an FID (base temperature 250 °C), the second part (25%) was directed to a sniffing port (base temperature 230 °C), and the third part (50%) was transferred to a Saturn 2100 mass spectrometer (Varian) run in EI or CI mode. For CI, methanol was used as reagent gas. The initial oven temperature of 0 °C was held for 2 min, followed by a gradient of 6 °C/min. At 100 °C, the gradient was increased to 40 °C/min. The final temperature of 230 °C was held for 5 min.

During the injection of headspace samples, the trap was cooled to −150 °C and the solenoid valve was open, allowing for an elevated flow (20 mL/min). After injection, the solenoid valve was closed and the trap was heated to 250 °C to transfer the cryofocused compounds to the DB-5 column. Effluent evaluation was performed as detailed above for GC-O/FID analyses.

Isolation of Jackfruit Volatiles. Jackfruit pulp was flash frozen with liquid nitrogen and ground into a powder using a laboratory mill GM 200 (Retsch, Haan, Germany) at 4000 rpm (2 × 15 s). Under ice-cooling, a portion of the powder (100 g) was added to dichloromethane (300 mL). Anhydrous sodium sulfate (250 g) was added in small portions while the mixture was continuously homogenized using a hand blender made of stainless steel. After magnetic stirring at room temperature (1 h), the homogenate was filtered through sea sand and cotton wool. The residue was washed with dichloromethane (3 × 100 mL) and the organic phases were combined. Nonvolatiles were removed by solvent-assisted flavor evaporation (SAFE)[38] at 40 °C. The SAFE distillate was concentrated to a final volume of 1 mL, first using a Vigreux column (50 × 1 cm) and after that a Bemelmans microdistillation device.[39] The jackfruit volatile isolates were stored at −20 °C before analysis.

AEDA. A jackfruit volatile isolate (1 mL) prepared as described above was analyzed by GC-O using the DB-FFAP column. Analyses were repeatedly carried out by four trained and experienced GC-O sniffers (3 female, 1 male; age 22−46) until results were reproducible. Results were combined into a consensus GC-O chromatogram. Then, the jackfruit volatile isolate was stepwise diluted 1:2 with dichloromethane to obtain dilutions of 1:2, 1:4, 1:8, 1:16, etc. of the initial solution. Diluted samples were also subjected to GC-O analyses (3 sniffers, 2 female, 1 male; age 24−46). Finally, each odorant was assigned a flavor dilution (FD) factor representing the dilution factor of the highest diluted sample in which the odorant was detected during GC-O by any of the three panelists. This approach best compensates for potential specific anosmia and hyposmia of individual sniffers.

Fractionation of Jackfruit Volatiles. A jackfruit volatile isolate (1 mL) was prepared as described above. To remove any dichloromethane, hexane (1 mL) was added and the resulting mixture was concentrated to a volume of 1 mL by using a Bemelmans microdistillation device[39] at a water bath temperature of 52 °C. The concentrate was applied onto a slurry of purified silica gel (8 g) in pentane within a water-cooled (10 °C) glass column (1 cm ID). Elution was carried out with pentane/diethyl ether mixtures of 100 + 0, 90 + 10, 70 + 30, 50 + 50, 0 + 100 (*v* + *v*; 50 mL each). The eluate was collected in 50 mL portions, and each portion was concentrated to 1 mL. Another jackfruit volatile isolate was used to selectively isolate volatile jackfruit thiols by means of mercurated agarose gel.[34]

Static Headspace Dilution Analysis. Jackfruit powder (5 g) prepared as detailed above was placed together with a magnetic stir bar into a 120 mL septum-sealed glass vial. After an equilibration time of 1 h under continuous stirring at room temperature, a portion of the headspace (10 mL) was injected into the static headspace GC system by using a gastight syringe. Static headspace GC-O analyses were carried out by 3 sniffers (2 female, 1 male; age 24−46) until results were reproducible. Results were combined into a consensus GC-O chromatogram. Then, the injected headspace volume was stepwise reduced by a factor of 2 resulting in volumes of ∼5, 2.5, 1.25, 0.63, 0.31, and 0.16 mL. Finally, each odorant was assigned an FD factor representing the ratio of the initial injection volume (10 mL) to the lowest injection volume in which the odorant was detected during headspace GC-O by any of the three panelists.

Odorant Quantitation. For the quantitation of compounds **1**−**8**, **10**−**19**, **21**−**27**, **29**−**31**, **37**−**44**, **46**−**48**, and **50**−**53**, a portion of cryomilled jackfruit pulp (0.5−50 g) was added to solvent (20−150 mL) under ice-cooling. The solvent used was either dichloromethane (**4**−**8**, **10**−**19**, **21**−**27**, **29**−**31**, **37**−**44**, **46**−**48**) or diethyl ether (**1**−**3**, **50**−**53**). Stable isotopically substituted odorants (0.1−125 μg) dissolved in solvent (10 μL−3 mL) were added as internal standards (cf. Supporting Information). Anhydrous sodium sulfate (10−125 g) was added in small portions while the mixture was continuously homogenized using a hand blender made of stainless steel. After magnetic stirring at room temperature (1 h), the homogenate was filtered through sea sand and cotton wool. The residue was washed with solvent and the organic phases were combined. Nonvolatiles were removed by SAFE at 40 °C. The SAFE distillates were concentrated to final volumes between 0.2 μL and 5 mL, and concentrates were analyzed by using the GC × GC-TOFMS system (**47**) or the heart-cut GC-GC-MS system (**1**−**8**, **10**−**19**, **21**−**27**, **29**, **30**, **31**, **37**−**44**, **46**, **48**, **50**−**53**).

For the quantitation of compound **49**, a portion of cryomilled jackfruit pulp (5 g) was placed together with a magnetic stir bar into a 120 mL septum-sealed glass vial. Water (5 mL) and ($^2H_6$)-**49** (1 μg) in water (20 μL) were added. After an equilibration time of 1 h under continuous stirring at room temperature, a portion of the headspace (1.2 mL) was injected into the static headspace GC system.

Peak areas corresponding to the analyte and internal standard were obtained from the extracted ion chromatograms using characteristic quantifier ions. The concentration of each target compound in jackfruit pulp was then calculated from the area counts of the analyte peak, the area counts of the standard peak, the amount of fruit pulp used, and the amount of standard added, by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in five different concentration ratios (5:1, 2:1, 1:1, 1:2, and 1:5). Individual quantifier ions and calibration line equations are available in the Supporting Information.

Odor Thresholds. Odor thresholds were determined orthonasally in pure water according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[40] The trained panel consisted of 15−20 employees of the Leibniz-Institute for Food Systems Biology at the Technical University Munich.

Aroma Reconstitution. Aliquots (0.05 mL−2 mL) of aqueous or ethanolic stock solutions of the reference odorants were combined and made up to a defined volume (10 mL) with water. 0.1 mL of the mixture was added to an aqueous buffer (100 g) that approximated the matrix of jackfruit. The buffer included glucose, fructose, sucrose, malic acid, citric acid, succinic acid, and oxalic acid in the concentrations naturally present in jackfruit[5] and had its pH adjusted to the natural value of 5.5 by addition of aqueous potassium hydroxide. The concentrations of the stock solutions and the volume of the aliquots were adjusted to obtain a final concentration of each odorant in the jackfruit pulp aroma model solution that represented the concentration previously determined in the jackfruit pulp. The

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Table 1. Odorants in the SAFE Distillate Obtained from Jackfruit Pulp

no.	odorant[a]	odor[b]	RI[c] FFAP	RI[c] DB-5	FD factor[d]	previously reported[e]
1	ethyl 2-methylpropanoate[f]	fruity	<1000	759	16	–
2	butane-2,3-dione	buttery	<1000	<700	4	–
3	methyl 3-methylbutanoate	fruity	1013	931	256	3, 4, 6–9
4	ethyl butanoate	fruity	1028	809	128	2, 3, 6–9
5	ethyl 2-methylbutanoate	fruity	1044	849	64	6, 8, 9
6	ethyl 3-methylbutanoate	fruity	1061	859	≥8192	2–4, 6–9
7	butyl acetate	fruity, banana	1067	820	4	2, 3, 5, 6, 8, 9
8	hexanal	green, grassy	1073	800	256	6
9	unknown	skunky	1103	839	1	–
10	3-methylbutyl acetate	fruity, banana	1115	881	4	3–8
11	butan-1-ol	malty	1140	<700	64	2–7, 9
12	methyl hexanoate	fruity	1180	928	16	7, 9
13	3-methylbutan-1-ol[g]	malty	1207	742	128	2–9
14	2-methylbutan-1-ol[g]	malty	1207	742	128	3, 6, 9
15	ethyl hexanoate	fruity	1225	1002	1	9
16	octanal	citrusy, soapy	1279	1005	64	4, 8, 9
17	3-hydroxybutan-2-one	buttery	1280	716	4	4
18	1-octen-3-one[f]	mushroom	1290	979	32	–
19	2-acetyl-1-pyrroline[f]	popcorn	1332	921	128	4, 7
20	unknown	sulfury	1365	–	4	–
21	nonanal	citrusy, soapy	1390	1100	4	9
22	acetic acid	vinegar	1454	<700	32	–
23	3-(methylsulfanyl)propanal	cooked potato	1457	910	128	4, 7
24	decanal	citrusy, soapy	1485	1208	1	8, 9
25	3-isobutyl-2-methoxypyrazine[f]	bell pepper	1512	1181	4	–
26	(2*E*)-non-2-enal[f]	fatty	1530	1163	16	–
27	(2*E*,6*Z*)-nona-2,6-dienal[f]	cucumber	1580	1159	4	–
28	unknown	fruity	1610	1224	4	–
29	butanoic acid	cheesy, sweaty	1620	815	64	9
30	phenylacetaldehyde	floral, honey	1645	1046	2	4, 7
31	3-methylbutanoic acid	cheesy, sweaty	1665	871	256	4, 5, 7, 9
32	unknown[h]	citrusy, soapy	1690	1550	32	–
33	unknown[i]	meaty	1724	1025	2048	–
34	unknown	green	1759	–	16	–
35	unknown	cooked potato	1769	1170	32	–
36	unknown	fatty	1800	–	16	–
37	hexanoic acid	sweaty	1844	1010	256	4, 7, 9
38	2-methoxyphenol[f]	smoky	1863	1091	8	–
39	ethyl 3-phenylpropanoate	fruity	1887	1352	32	9
40	2-phenylethanol	floral	1916	1116	32	4, 7, 9
41	γ-octalactone	coconut	1920	1260	8	–
42	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	caramel	2047	1068	≥8192	–
43	4-methylphenol	fecal, horse stable	2095	1098	8	–
44	ethyl (2*E*)-3-phenylprop-2-enoate	fruity	2120	1469	128	9
45	unknown	spicy	2150	–	16	–
46	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one[f]	seasoning	2203	1109	32	–
47	2-phenylacetic acid	floral, honey	2589	1264	8	9
48	vanillin	vanilla	2600	1409	16	–

[a]Each odorant was identified by comparing its retention indices on two GC capillaries of different polarity (DB-FFAP, DB-5), its mass spectrum obtained by GC-MS, as well as its odor quality as perceived at the sniffing port during GC-O to data obtained from authentic reference compounds analyzed under equal conditions. [b]Odor quality as perceived at the sniffing port during GC-O. [c]Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [d]Flavor dilution factor; dilution factor of the highest dilution of the jackfruit volatile isolate in which the odorant was detected during GC-O analyses by any of three panelists. [e]References in which the compound has been reported as jackfruit pulp volatile; the minus sign (−) indicates compounds that have not been reported before. [f]An unequivocal mass spectrum of the compound could not be obtained in the jackfruit volatile isolates; identification was based on the remaining criteria detailed in footnote *a*. [g]3-Methylbutan-1-ol and 2-methylbutan-1-ol were not separated on the GC column used for AEDA, an FD factor of 128 was determined for the mixture of both compounds. [h]Mass spectral data allowed for the tentative identification of the compound as an octenol, however, positions of the double bond and the hydroxy group have not been elucidated. [i]The compound could be enriched by mercurated agarose gel, thus it was a thiol, but no further information on its structure was gained.

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J. Agric. Food Chem. 2019, 67, 5838–5846

final ethanol concentration in the aroma model solution was below 1 g/L, which is the odor threshold of ethanol in water.

Quantitative Olfactory Profiles. Samples (10 g) of freshly homogenized jackfruit pulp and the jackfruit pulp aroma model solution (35 compounds with OAVs ≥ 1) prepared as described above were placed in cylindrical ground neck glasses (height 7 cm, ID 3.5 cm) with lids (VWR, Darmstadt, Germany). Twelve trained panelists (9 female, 3 male, age 21−48) evaluated the olfactory profile of the two samples orthonasally by rating the intensities of 8 predefined descriptors on a scale from 0 to 3 and 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Individual descriptors were defined by the odor of a reference compound dissolved in water in a concentration 100× above its respective odor threshold value. The 8 descriptors and the corresponding reference compounds were "cooked potato" (3-(methylsulfanyl)propanal), "malty" (3-methylbutan-1-ol), "caramel" (4-hydroxy-2,5-dimethylfuran-3(2*H*)-one), "cheesy" (3-methylbutanoic acid), "fruity" (ethyl 3-methylbutanoate), "banana" (3-methylbutyl acetate), "cooked rice" (2-acetyl-1-pyrroline), and "soapy" (octanal). Additionally, the panelists were asked to rate the similarity of the overall olfactory profile of the model solution to the overall olfactory profile of the jackfruit pulp on a scale from 0 to 3 with 0.1 increments with 0 = no similarity, 1 = weak similarity, 2 = clear similarity, reminiscent of jackfruit, and 3 = identical. Ratings of all panelists were averaged by calculating the arithmetic mean.

RESULTS AND DISCUSSION

Odorant Screening by AEDA. A jackfruit volatile isolate was prepared from fresh pulp by solvent extraction, SAFE, and subsequent concentration. The olfactory profiles of the extract, the distillate, and the concentrated jackfruit volatile isolate were evaluated orthonasally on a strip of filter paper after evaporation of the solvent. Results indicated no substantial changes: at all levels of the isolation procedure, the characteristic aroma of jackfruit pulp was still clearly perceivable.

Application of AEDA to the jackfruit volatile isolate revealed 48 odorants with FD factors from 1 to ≥8192 (Table 1). Preliminary structural assignments were achieved by comparing the RIs and odor descriptions of the odorants detected during AEDA to data of reference compounds collected from the literature and compiled in an in-house database. Results were confirmed by analyzing the corresponding reference compounds in an appropriate dilution by GC-O in parallel with the jackfruit volatile isolate on two separation systems of different polarity (DB-FFAP and DB-5). On the basis of identical retention indices and odor properties of the compounds in the jackfruit volatile isolate and authentic reference compounds, the structures of 39 out of the 48 jackfruit odorants (**1**−**8**, **10**−**19**, **21**−**27**, **29**−**31**, **37**−**44**, **46**−**48**) could be assigned. Further confirmation was obtained by GC-MS analysis in the EI and CI modes. To avoid any mass spectral interferences by coeluted compounds, the jackfruit volatile isolate was fractionated into five fractions of different polarity by silica gel chromatography. In another experiment, thiols were selectively isolated from a jackfruit volatile isolate by mercurated agarose gel. Individual fractions were analyzed by GC-O to localize the odorants and then subjected to GC-MS analysis in parallel with appropriately diluted samples of the reference compounds. Using this approach, 31 jackfruit odorants were unequivocally identified (**2**−**8**, **10**−**17**, **21**−**24**, **29**−**31**, **37**, **39**−**44**, **47**, **48**). For compounds **1**, **18**, **19**, **25**, **26**, **27**, **38**, and **46**, mass spectral confirmation failed. No structure proposal was achieved for compounds **9**, **20**, **28**, **32**, **33**, **34**, **35**, **36**, and **45**, neither by comparison of retention indices and odor qualities nor by mass spectral analysis. These compounds thus remained unknown.

With an FD factor ≥8192, the most potent odorants in the jackfruit volatile isolate were fruity smelling ethyl 3-methylbutanoate (**6**) and caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**42**). Ethyl 3-methylbutanoate had been found in jackfruit before.[2−4,6−9] In agreement with our data, the application of AEDA to a jackfruit volatile isolate by Gomes Fraga[9] had also revealed ethyl 3-methylbutanoate as the compound with the highest FD factor. By contrast, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one has not been identified in jackfruit yet. However, it has been reported from the fruit of *Artocarpus integer* (Thunb.) Merr., a closely related species.[4,41] An FD factor of 2048 was determined for the unknown meaty smelling thiol **33**. Further compounds with high FD factors included methyl 3-methylbutanoate (**3**; fruity, FD factor 256), hexanal (**8**; green, grassy, FD factor 256), 3-methylbutanoic acid (**31**; cheesy, FD factor 256), hexanoic acid (**37**; sweaty, FD factor 256), ethyl butanoate (**4**; fruity, FD factor 128), the two chromatographically unseparated odorants 3-methylbutan-1-ol (**13**; malty) and 2-methylbutan-1-ol (**14**; malty) with a combined FD factor of 128, 2-acetyl-1-pyrroline (**19**; cooked rice-like, FD factor 128), 3-(methylsulfanyl)propanal (**23**; cooked potato-like, FD factor 128), and ethyl (2*E*)-3-phenylprop-2-enoate (**44**; fruity, FD factor 128), all of which had been reported as jackfruit volatiles before. Further 35 odorants were detected with FD factors of 1−64. Among them, ethyl 2-methylpropanoate (**1**), butane-2,3-dione (**2**), 1-octen-3-one (**18**), acetic acid (**22**), 3-isobutyl-2-methoxypyrazine (**25**), (2*E*)-non-2-enal (**26**), (2*E*,6*Z*)-nona-2,6-dienal (**27**), 2-methoxyphenol (**38**), γ-octalactone (**41**), 4-methylphenol (**43**), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (**46**), and vanillin (**48**) have previously not been reported as jackfruit volatiles.

Screening for Highly Volatile Odorants by Static Headspace GC-O. An odorant screening by AEDA does not cover compounds with boiling points below the boiling point of the extraction solvent, because these compounds are lost during the concentration step. Therefore, GC-O in combination with a static headspace dilution analysis was applied as a complementary technique to screen the fraction of highly volatile jackfruit pulp compounds for odorants.

Results revealed five additional odorants (Table 2). The most potent among them were the malty smelling odorants 2-methylbutanal (**52**) and 3-methylbutanal (**53**) which were not separated by the column used and thus provided a combined FD factor of 32. The other compounds were 2-methylpropanal (**50**; malty, FD factor 16), butanal (**51**; malty, FD factor 2), and dimethyl sulfide (**49**; asparagus-like, FD factor 2). 2-Methylpropanal and dimethyl sulfide have not been reported as jackfruit volatiles so far.

The combined screenings by AEDA and by static headspace dilution analysis resulted in 53 odor-active compounds among which 44 could be identified and 22 had not been reported in jackfruit pulp before. With 56 odorants, a similar number of odor-active compounds had been reported by Gomes Fraga after application of an AEDA to a jackfruit volatile isolate obtained by a headspace purge-and-trap approach.[9] The outstanding odor potency of ethyl 3-methylbutanoate reported by Gomes Fraga was confirmed by our results, and also ethyl butanoate reported as a major jackfruit pulp odorant by Gomes Fraga was detected with a high FD factor in our AEDA. However, this was not the case for the other two odorants

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J. Agric. Food Chem. 2019, 67, 5838−5846

Table 2. Additional Odorants Detected in the Headspace above Jackfruit Pulp

no.	odorant[a]	odor[b]	RI[c] DB-5	FD factor[d]	previously reported[e]
49	dimethyl sulfide	asparagus	518	2	–
50	2-methylpropanal	malty	559	16	–
51	butanal	malty	594	2	6
52	2-methylbutanal[f]	malty	655	32	6
53	3-methylbutanal[f]	malty	655	32	6

[a]Each odorant was identified by comparing its retention index on the DB-5 capillary, its mass spectrum obtained by GC-MS, as well as its odor quality as perceived at the sniffing port during GC-O to data obtained from authentic reference compounds analyzed under equal conditions. [b]cf. Table 1. [c]cf. Table 1. [d]Flavor dilution factor; calculated as ratio of the initial injection volume (10 mL) to the lowest injection volume in which the odorant was detected during GC-O analyses by any of three panelists. [e]cf. Table 1. [f]2-Methylbutanal and 3-methylbutanal were not separated on the column used for static headspace GC; an FD factor of 32 was determined for the mixture of both compounds.

highlighted by Gomes Fraga, namely butyl 3-methylbutanoate and butyl butanoate, both of which were not among the odor-active compounds detected by us. On the other hand, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one was found with a high FD factor in our study but not reported by Gomes Fraga.

Odorant Quantitation and OAV Calculation. The 44 volatiles identified as odor-active compounds by AEDA and static headspace dilution analysis were subsequently quantitated. In the quantitations of 43 compounds, stable isotopically substituted analogues (cf. Supporting Information) of the target analytes were used as internal standards to fully compensate for losses during the workup. By contrast, 2-methylbutan-1-ol was quantitated simultaneously with 3-methylbutan-1-ol via ($^2H_2$)-3-methylbutan-1-ol as an internal standard.

Results of the quantitations (Table 3) revealed concentrations ranging from 0.0161 μg/kg (3-isobutyl-2-methoxypyrazin, **25**) to 101000 μg/kg (3-methylbutanoic acid, **31**). Next to 3-methylbutanoic acid, high concentrations were also found for 3-hydroxybutan-2-one (**17**; 44600 μg/kg), acetic acid (**22**; 9940 μg/kg), 3-methylbutan-1-ol (**13**; 5460 μg/kg), 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**42**; 3090 μg/kg), 2-methylbutan-1-ol (**14**; 2190 μg/kg), ethyl 3-methylbutanoate (**6**; 1710 μg/kg), hexanoic acid (**37**; 1560 μg/kg), ethyl butanoate (**4**; 1370 μg/kg), octanal (**16**; 1130 μg/kg), and butanoic acid (**29**; 1110 μg/kg).

The only other study on jackfruit volatiles that reported quantitative data was published by Ong et al. in 2008.[6] Quantitations were accomplished using headspace solid phase microextraction in combination with a single internal standard. Data was collected from five different cultivars. The majority of concentrations reported by Ong et al. were lower than the concentrations in our study. Particularly the concentrations of hexanal (22−33 μg/kg), ethyl butanoate (107−127 μg/kg), and 3-methylbutanal (119−176 μg/kg) were clearly below the concentrations determined by us (700 μg/kg, 1370 μg/kg, and 759 μg/kg, respectively). Ong et al. also reported lower concentrations for ethyl 3-methylbutanoate (238−986 μg/kg), butanal (109−159 μg/kg), methyl 3-methylbutanoate (132−388 μg/kg), 2-methylbutanal (115 μg/kg), 3-methylbutan-1-ol (711−1753 μg/kg), and 2-methylbutan-1-ol (469−906 μg/kg). By contrast, Ong et al. found clearly higher concentrations for 3-methylbutyl acetate (499−8284 μg/kg), ethyl 2-methylbutanoate (174 μg/kg), and butyl acetate (241−988 μg/kg). With 359−721 μg/kg, only butan-1-ol was reported in the same range as in our data set (478 μg/kg).

Table 3. Concentrations, Odor Thresholds, and OAVs of Major Jackfruit Pulp Odorants

no.	odorant	concentration[a] (μg/kg)	odor threshold[b] (μg/kg)	OAV[c]
6	ethyl 3-methylbutanoate	1710	0.023	74000
4	ethyl butanoate	1370	0.75	1800
53	3-methylbutanal	759	0.50	1500
50	2-methylpropanal	689	0.49	1400
51	butanal	482	0.87	550
16	octanal	1130	3.4	330
8	hexanal	700	2.4	290
3	methyl 3-methylbutanoate	613	2.2	280
31	3-methylbutanoic acid	101000	490	210
5	ethyl 2-methylbutanoate	25.1	0.13	190
49	dimethyl sulfide	55.0	0.30	180
52	2-methylbutanal	248	1.5	170
27	(2*E*,6*Z*)-nona-2,6-dienal	0.488	0.0045	110
19	2-acetyl-1-pyrroline	5.41	0.053	100
15	ethyl hexanoate	98.3	1.2	82
17	3-hydroxybutan-2-one	44600	590	75
1	ethyl 2-methylpropanoate	6.14	0.089	69
2	butane-2,3-dione	61.7	0.96	64
42	4-hydroxy-2,5-dimethyl-furan-3(2*H*)-one	3090	87	36
18	1-octen-3-one	0.547	0.016	34
39	ethyl 3-phenylpropanoate	66.1	2.1	31
23	3-(methylsulfanyl) propanal	12.1	0.43	28
13	3-methylbutan-1-ol	5460	220	25
10	3-methylbutyl acetate	111	7.2	15
21	nonanal	29.3	2.8	10
7	butyl acetate	153	27	5.6
44	ethyl (2*E*)-3-phenylprop-2-enoate	7.53	1.7	4.4
47	2-phenylacetic acid	291	68	4.3
46	3-hydroxy-4,5-dimethyl-furan-2(5*H*)-one	5.64	1.7	3.3
26	(2*E*)-non-2-enal	0.521	0.19	2.8
25	3-isobutyl-2-methoxypyrazin	0.0160	0.0062	2.6
22	acetic acid	9940	5600	1.8
14	2-methylbutan-1-ol	2190	1200	1.8
30	phenylacetaldehyde	9.53	5.2	1.8
24	decanal	15.8	9.3	1.7
11	butan-1-ol	478	590	<1
29	butanoic acid	1110	2400	<1
37	hexanoic acid	1560	4800	<1
12	methyl hexanoate	0.414	90	<1
38	2-methoxyphenol	0.338	0.84	<1
40	2-phenylethanol	103	140	<1
41	γ-octalactone	1.04	6.5	<1
43	4-methylphenol	0.427	3.9	<1
48	vanillin	1.87	53	<1

[a]Mean of duplicates, triplicates, or quadruplicates; individual data and standard deviations are included in the Supporting Information. [b]Orthonasal odor threshold in water. [c]Odor activity value; calculated as ratio of concentration to odor threshold.

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To assess the odor potency of the individual jackfruit odorants, an OAV was calculated for each compound as a ratio of its concentration in the jackfruit pulp to its orthonasal odor detection threshold in water. For 35 odorants, an OAV > 1 was determined (Table 3), thus their concentrations in jackfruit pulp exceeded their respective thresholds. With an OAV of 74000, fruity smelling ethyl 3-methylbutanoate (**6**) showed the highest value. This conformed to the result of the AEDA, in which **6** exhibited the highest FD factor. High OAVs were also obtained for further fruity smelling esters, namely ethyl butanoate (**4**; OAV 1800), methyl 3-methylbutanoate (**3**; OAV 280), and ethyl 2-methylbutanoate (**5**; OAV 190), for some malty smelling aldehydes such as 3-methylbutanal (**53**; OAV 1500), 2-methylpropanal (**50**; OAV 1400), butanal (**51**; OAV 550), and 2-methylbutanal (**52**; OAV 170), as well as for citrusy, soapy smelling octanal (**16**; OAV 330), green, grassy smelling hexanal (**8**; OAV 290), cheesy, sweaty smelling 3-methylbutanoic acid (**31**; OAV 210), asparagus-like smelling dimethyl sulfide (**49**; OAV 180), cucumber-like smelling (2*E*,6*Z*)-nona-2,6-dienal (**27**; OAV 110), and cooked rice-like smelling 2-acetyl-1-pyrroline (**19**; OAV 100). Additional 21 compounds exhibited OAVs between 1.7 and 82. The concentrations of 9 odorants, however, were below their odor threshold values. These compounds were considered not to be relevant for the overall aroma of jackfruit pulp.

Aroma Reconstitution. To prove that all compounds contributing to the overall aroma of jackfruit pulp were correctly identified and quantitated, an aroma reconstitution experiment was performed. An aqueous aroma model solution was prepared containing the 35 odorants for which OAVs ≥ 1 had been calculated (cf. Table 3). The concentrations of the odorants in the model solution corresponded to their natural concentrations previously determined in the jackfruit pulp (cf. Table 3). Furthermore, the model solution contained the major sugars and acids present in jackfruit pulp and had its pH value adjusted to the pH value of the pulp.

The aroma model solution was orthonasally compared to fresh jackfruit pulp in a quantitative olfactory profile analysis by using a trained sensory panel and 8 descriptors representing the characteristic odor notes perceivable in jackfruit pulp aroma. Results (Figure 1) showed an excellent agreement between the olfactory profile of the model solution and the olfactory profile of the fresh jackfruit pulp. The overall similarity was rated 2.9 out of 3. The profiles were characterized by a balanced combination of fruity, banana-like, cooked rice-like, soapy, cheesy, cooked potato-like, malty, and caramel-like odor notes, whereby the fruity note was rated somewhat stronger than the other notes.

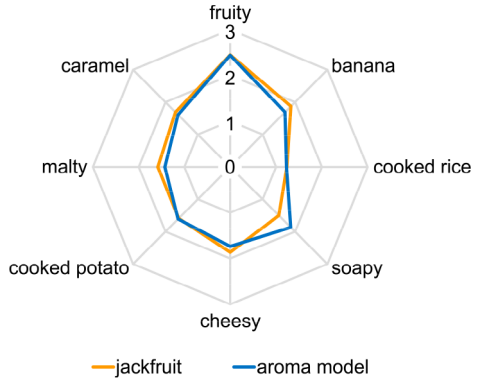

Figure 1. Olfactory profile of jackfruit pulp in comparison to the olfactory profile of the jackfruit pulp aroma model solution (35 compounds, OAVs ≥ 1). Panelists rated the intensity of each descriptor on a scale from 0 to 3 and 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

In summary, this study provided the first comprehensive application of state-of-the-art approaches for the identification of the key compounds contributing to the aroma of jackfruit pulp. AEDA applied to a volatile isolate obtained from jackfruit pulp by an artifact-avoiding procedure including solvent extraction and solvent-assisted flavor evaporation was combined with static headspace GC-O for the additional detection of highly volatile odorants. Thorough structure elucidation allowed for the unequivocal identification of 44 odor-active compounds. Their natural concentrations in jackfruit pulp were determined by using isotopically substituted odorants as internal standards to compensate for any kind of losses during the analytical workup and provide reliable quantitative data. Compounds for which concentrations beyond their respective odor threshold values in water had been determined were successfully used to reconstruct the characteristic jackfruit pulp aroma, providing final evidence that the key compounds of jackfruit pulp aroma were correctly deciphered. Data will provide the basis for further research such as studies on the aroma differences between different jackfruit varieties and studies aimed at the elucidation of the molecular background of aroma changes during jackfruit processing. Data may also be used to aid in the development of new jackfruit cultivars with superior aroma properties by targeted breeding.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b01445.

Quantifier ions and calibration line data used in the quantitations and individual concentration data used for mean calculations and standard deviations (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; CI, chemical ionization; EI, electron ionization; FD factor, flavor dilution factor; FFAP, free fatty acid phase; FID, flame ionization detector; GC-O, gas chromatography-olfactometry; GC-MS, gas chromatography-mass spectrometry; GC × GC-MS, comprehensive two-dimensional gas chromatography-mass spectrometry; ID, inner diameter; MCSS system, moving column stream switching system; OAV, odor activity value; RI,

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retention index; SAFE, solvent-assisted flavor evaporation; TOF, time-of-flight.; 2-acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethanone; Dess-Martin periodinane, 1,1,1-tris-(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one; γ-octalactone, 5-butyldihydrofuran-2(3*H*)-one; vanillin, 4-hydroxy-3-methoxybenzaldehyde.

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J. Agric. Food Chem. 2019, 67, 5838–5846

8.1.3 Summary and Individual Contributions

The jackfruit tree, *Artocarpus heterophyllus* Lam., is a tropical tree in the mulberry family Moracea. It is native to South Asia, but today it is spread throughout the entire tropics. The aroma of the ripe jackfruit pulp combines sweet, fruity, malty, and cheesy odor notes. The aim of the study was to identify the odorants contributing to the aroma of jackfruit pulp.

AEDA of a jackfruit pulp volatile isolate obtained by solvent extraction and SAFE revealed 48 odorants with FD factors ranging from 1 to ≥8192. Additional five odorants were detected by GC-O in combination with static headspace sampling. The structures of 44 out of 53 odorants were assigned. The highest FD factor of ≥8192 was found for the fruity smelling ethyl 3-methylbutanoate and the caramel-like smelling HDMF. Additional high FD factors were obtained for an unknown compound (meaty; FD 2048), methyl 3-methylbutanoate (fruity; FD 256), hexanal (green, grassy; FD 256), 3-methylbutanoic acid (cheesy; FD 256), hexanoic acid (sweaty; FD 256), ethyl butanoate (fruity; FD 128), 2-/3-methylbutan-1-ol (malty; FD 128), 2-acetyl-1-pyrroline (cooked rice-like; FD 128), 3-(methylsulfanyl)propanal (cooked potato-like; FD 128), and ethyl (2*E*)-3-phenylprop-2-enoate (fruity; FD 128).

To substantiate the results of the AEDA, the structurally identified odorants were quantitated by using stable isotopically substituted odorants as internal standards. Furthermore, OAVs were calculated as ratio of the natural concentrations in the jackfruit pulp to the OTVs in water. 35 odorants showed an OAV >1. High OAVs were found for ethyl 3-methylbutanoate (fruity; OAV 74000), ethyl butanoate (fruity; OAV 1800), 3-methylbutanal (malty; OAV 1500), 2-methylpropanal (malty; OAV 1400), butanal (malty; OAV 550), octanal (citrusy, soapy; OAV 330), hexanal (green, grassy; OAV 290), methyl 3-methylbutanoate (fruity; OAV 280), 3-methylbutanoic acid (cheesy; OAV 210), ethyl 2-methylbutanoate (fruity; OAV 190), dimethyl sulfide (asparagus-like; OAV 180), 2-methylbutanal (malty; OAV 170), (2*E*,6*Z*)-nona-2,6-dienal (cucumber-like; OAV 110), and 2-acetyl-1-pyrroline (cooked rice-like; OAV 100). Additional 21 compounds showed concentrations exceeding their respective OTVs, whereas OAVs <1 were calculated for nine odorants. An aroma model solution based on the natural concentrations of 35 compounds for which OAVs >1 had been calculated was olfactorily compared to fresh jackfruit pulp. A balanced combination of fruity, banana-like, cooked rice-like, soapy, cheesy, cooked potato-like, malty, and caramel-like odor notes characterized both olfactory profiles. The olfactory profile analysis confirmed that all odorants contributing to the aroma of the jackfruit pulp had been correctly identified and quantitated.

Johanna Grimm designed and conducted the experiments including the volatiles isolation, the GC-O screenings, the structure assignments, the quantitations, the OAV calculations, and the reconstitution experiments. Furthermore, Johanna evaluated the resulting data and prepared the manuscript. Martin Steinhaus conceived and directed the study, supervised Johanna’s work and revised the manuscript. He also participated in the GC-O analyses and in the sensory tests.

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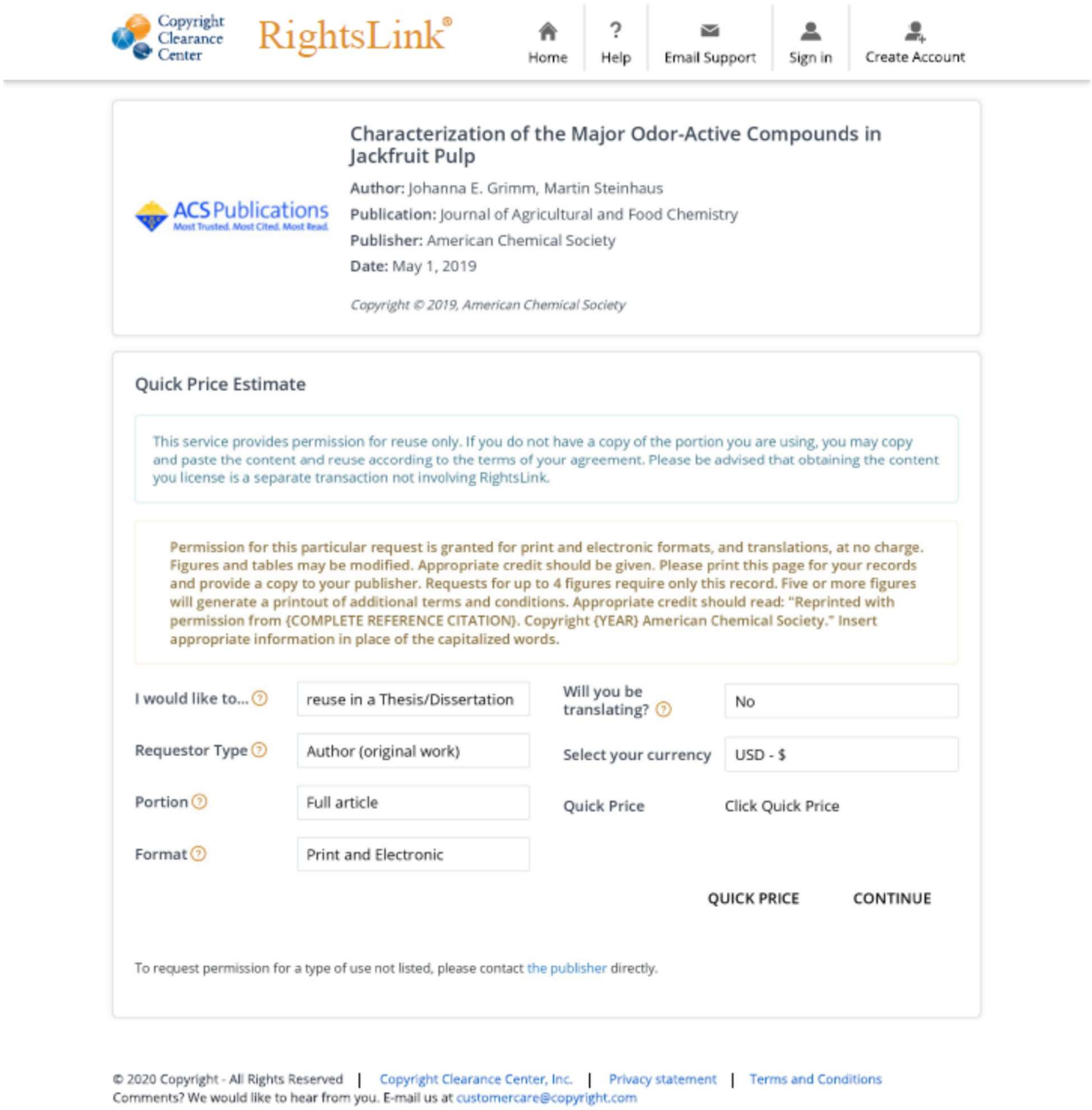

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Characterization of the Major Odor-Active Compounds in Jackfruit Pulp

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8.2 Publication 2: Characteristic Chiral Sulfur Compounds Aroma-Active in Cempedak (*Artocarpus integer* (Thunb.) Merr.) Fruits

8.2.1 Bibliographic Data

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8.2.2 Publication Reprint

For the reprint of publication 2, please turn to the next page.

Chapter 9

Characteristic Chiral Sulfur Compounds Aroma-Active in Cempedak (*Artocarpus integer* (Thunb.) Merr.) Fruits

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A comparative gas chromatography−olfactometry (GC-O) analysis of the volatiles isolated from tree-ripened fruits of cempedak (*Artocarpus integer* (Thunb.) Merr.) and jackfruit (*Artocarpus heterophyllus* Lam.) afforded 33 aroma-active compounds. Two of them were detected in the cempedak fruit sample, but not in the jackfruit sample and exhibited an odor corresponding to the characteristic sulfury, smear cheese-like note distinguishing cempedak fruit aroma from jackfruit aroma. These compounds were identified as 2-(methylthio)-butane and 2-(methylthio)pentane. Both compounds have rarely been reported from nature and were as yet unknown in any fruit. Enantio-GC analysis revealed an (*R*)/(*S*) ratio of 33/67 for 2-(methylthio)butane and 41/59 for 2-(methylthio)pentane in cempedak fruit. The odor qualities of the enantiomers were virtually identical, but slight differences were found in the odor threshold values.

Introduction

The cempedak tree (*Artocarpus integer* (Thunb.) Merr.) is an evergreen tropical tree in the mulberry family Moraceae. It is native to Southeast Asia and is nowadays particularly found in Indonesia, Malaysia, and New Guinea, but also some parts of Thailand, India, and Australia. Cempedak fruits are multiple fruits derived from the coalescence of the individual fruits of a whole inflorescence and

Engel and Takeoka; Importance of Chirality to Flavor Compounds
ACS Symposium Series; American Chemical Society: Washington, DC, 2015.

grow directly from the trunk of the tree. They are cylindrically shaped and reach a length of up to 40 cm. A leathery skin of yellow, green or brown color covers numerous seeds attached to the peduncle. Each seed is covered by a fleshy edible aril. The ripe arils are mainly eaten fresh. They are pale yellow to orange in color, soft, sweet, and fragrant.

Cempedak fruits in many respects resemble jackfruits, the fruits of *Artocarpus heterophyllus* Lam., a closely related, but more widely known species. Jackfruits are cultivated in the entire tropics today, but their natural origin is in Southern Asia. Compared to cempedak fruits, jackfruits are bigger in size and may reach a length of 1 m and a weight of 30 kg. The arils of major jackfruit varieties are less soft than cempedak arils and are often sold packaged and refrigerated as "seedless jackfruit flesh" in supermarkets, whereas cempedak is typically sold as fresh whole fruit.

The aroma properties of cempedak and jackfruit arils are similar, but differ in a specific characteristic: Both are dominated by sweet, fruity and malty notes, but cempedak arils exhibit an additional intensely sulfury, smear cheese-like aroma note, not perceived in jackfruit aroma. The molecular background of this specific note has yet been unclear.

In jackfruit, numerous volatile constituents have been identified so far. Major compounds include alcohols such as 1-propanol, 1-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol, as well as esters such as methyl 3-methylbutanoate, ethyl 3-methylbutanoate, propyl 3-methylbutanoate, butyl acetate, 3-methylbutyl acetate, 2-methylpropyl 3-methylbutanoate, butyl 3-methylbutanoate, and 3-methylbutyl 3-methylbutanoate (*1*–*8*). Application of gas chromatography−olfactometry (GC-O) and aroma extract dilution analysis (AEDA) (*9*) to an extract obtained from jackfruit arils revealed ethyl butanoate, ethyl 3-methylbutanoate, butyl butanoate, and butyl 3-methylbutanoate as major aroma-active compounds (*10*).

In cempedak fruits, 3-methylbutanoic acid, 3-methyl-1-butanol, 3-hydroxy-2-butanone, 2-phenylethanol, 2-methyl-1-propanol, ethyl 3-methylbutanoate, 2,3-pentanedione, and methyl 3-methylbutanoate were identified as dominating volatiles (*3*). An early GC-O analysis suggested 3-methylbutyl 3-methylbutanoate and butyl 3-methylbutanoate as major odorants (*11*). More recently, potent odorants in two cempedak cultivars were systematically characterized using AEDA, quantitation assays, calculation of odor activity values (OAV), aroma reconstitution, and omission tests (*12*). AEDA resulted in high flavor dilution (FD) factors for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, 3-methylbutanal, 3-(methylthio)propanal, 2-acetyl-1-pyrroline, and 3-methylbutanoic acid. Sulfury odor characteristics were attributed to 2-(methylthio)acetaldehyde (meaty), 3-(methylthio)-1-butanol (meaty), 3-(methylthio)propanal (potato-like), 3-(methylthio)butanal (potato-like), and dimethyl trisulfide (catty). The aroma of cempedak, cultivar Tongtapan, was reported to be successfully reconstituted using 12 odorants in their natural concentrations, namely 3-methylbutanal, ethyl 3-methylbutanoate, methyl 3-methylbutanoate, octanal, HDMF, 3-(methylthio)propanal, 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (MDMF), 3-methyl-1-butanol, 3-methylbutanoic acid, vanillin, benzaldehyde, and 2-phenylethanol.

However, when we repeated the reconstitution experiment detailed in (*12*), we observed that the aroma of the model was closely resembling the aroma of jackfruit, but lacking the typical sulfury, smear cheese-like aroma note characterizing cempedak fruits. Therefore, the aim of the present study was to reinvestigate the aroma-active compounds in cempedak in comparison to jackfruit by GC-O with special emphasis to compounds potentially contributing to this characteristic aroma difference.

Materials and Methods

Fruit Samples

Tree-ripened cempedak fruits and jackfruits organically grown in Thailand were handpicked by local farmers and sent to Germany by air freight within two days.

Chemicals

Reference samples of the following odorants were purchased from commercial vendors: **1**, **14** (Alfa Aesar, Karlsruhe, Germany); **2**, **3**, **5**–**7**, **10**–**13**, **17**–**21**, **22**–**25**, **27**, **28**, **30**–**33** (Sigma-Aldrich, Taufkirchen, Germany); **9** (Merck, Darmstadt, Germany). **15** was synthesized according to (*13*). **4**, 3-methyl-2-(methylthio)butane, (*R*)-**1**, and (*R*)-**4** were synthesized as detailed below.

(*S*)-2-Butanol, (*E*)-2-decenal, 3-methyl-2-butanol, *rac*-2-pentanol, (*S*)-2-pentanol, sodium methanethiolate, and tosyl chloride (4-methylbenzenesulfonyl chloride) were from Sigma-Aldrich. Dichloromethane, diethyl ether, and pentane (Merck) were freshly distilled before use.

Synthesis of 2-(Methylthio)alkanes

Starting from the corresponding 2-alcohols, **4**, 3-methyl-2-(methylthio)butane, (*R*)-**1**, and (*R*)-**4** were synthesized by tosylation and subsequent substitution of tosylate for methanethiolate.

Tosylation of the 2-alcohols was accomplished by reaction with tosyl chloride in pyridine as detailed in (*14*).

To the 2-alkyl tosylates (2-3 mmol) 2.5 equivalents of sodium methanethiolate in DMF (15 mL) were added and the mixture was heated (80 °C) for 2 h. After cooling to ambient temperature, brine (50 mL) was added. The mixture was extracted with diethyl ether (3 × 50 mL). The combined organic phases were washed with brine (5 × 50 mL). After drying over anhydrous sodium sulfate, the solvent was removed in vacuo and the residue was purified by flash chromatography (silica gel, pentane) to yield between 12% and 35% 2-(methylthio)alkane in ≥ 90% purity (GC).

GC-O, GC-MS, and Heart-Cut GC-GC-MS Analyses

Cempedak and jackfruit arils (100 g), respectively, were homogenized under addition of dichloromethane (300 mL) and anhydrous sodium sulfate (250 g) using a stainless steel blender. After further magnetic stirring (30 min), the mixture was filtered and the eluate was subjected to solvent-assisted flavor evaporation (SAFE) (*15*). The distillate was stepwise concentrated to 1 mL using a Vigreux column (50 cm × 1 cm) and a microdistillation device.

Aliquots (1 μL) of the concentrates were analyzed by GC-O, GC-MS, and heart-cut GC-GC-MS using the instruments, columns, and procedures detailed in (*16*). GC-O results of four trained panelists were combined.

Odor Threshold Values

An AEDA was applied to a mixture of racemic 2-(methylthio)butane, racemic 2-(methylthio)pentane, and (*E*)-2-decenal as internal standard using a chiral BGB-176 column (BGB Analytik, Rheinfelden, Germany). Odor thresholds in air were calculated from the FD factors obtained according to the approach detailed in (*17*) and a threshold of 2.7 ng/L for (*E*)-2-decenal (*18*). Results of three trained panelists were averaged.

Results and Discussion

Comparative GC-O Analysis

Arils obtained from a freshly opened cempedak fruit were homogenized together with dichloromethane as extraction solvent and anhydrous sodium sulfate as desiccant. After filtration, the volatile fraction was isolated by SAFE. The distillate was concentrated and subjected to GC-O analysis. In parallel, the same procedure was applied to jackfruit arils.

The comparative GC-O analysis of cempedak and jackfruit aril volatiles resulted in 33 aroma-active compounds in the retention index (RI) range of < 1000 to 2203 on an FFAP capillary (Table 1). Comparison of the RI data and odor qualities with in-house database entries allowed for the structural assignment of 26 compounds. Identifications were confirmed by parallel GC-O and GC-MS analyses of authentic reference compounds and the fruit volatile isolates using two GC columns of different polarity (FFAP and DB-5). The identities of seven compounds, however, remained unclear. Among these, compounds **1** and **4** (cf. Table 1) particularly attracted our attention, because their odor clearly represented the characteristic sulfury, smear cheese-like aroma note of cempedak fruits. Both were detected during GC-O of the cempedak fruit aroma isolate, but not in the jackfruit sample, indicating that they might play a major role for the aroma difference between both fruits.

Identification of Compounds 1 and 4

GC-MS analysis of the cempedak fruit volatile fraction resulted in the mass spectra displayed in Figure 1 for compounds **1** and **4**. The mass spectrum of **1** matched the MS database (*19*) entry of 2-(methylthio)butane. GC-O and GC-MS analysis of a purchased 2-(methylthio)butane reference using FFAP and DB-5 GC columns confirmed the structure assignment.

Database search for the mass spectrum of **4** was unsuccessful. However, comparison with the mass spectrum of **1** suggested that **4** was a homologue differing from **1** by an additional methylene group. Major peaks in the mass spectrum of **1** included *m*/*z* 104 corresponding to the molecular ion, *m*/*z* 89 indicating loss of a methyl group, the base peak *m*/*z* 75 indicating loss of an ethyl group, and *m*/*z* 56 indicating loss of methanethiol. Analogous interpretation of the mass spectrum of **4** suggested *m*/*z* 118 as molecular ion, *m*/*z* 103 as loss of methyl, *m*/*z* 89 as loss of ethyl, and *m*/*z* 70 as loss of methanethiol. The base peak *m*/*z* 75 would then correspond to the loss of propyl, suggesting that **4** was either 2-(methylthio)pentane or 3-methyl-2-(methylthio)butane.

Table 1. Aroma-Active Compounds in Cempedak Fruit and Jackfruit

No.	*Compound*[a]	*Odor*[b]	*RI*[c]	*Odor Intensity*[d]	
				Cempedak	*Jackfruit*
1	**unknown**	**cempedak**	**<1000**	++	−
2	methyl 3-methylbutanoate	fruity	1013	++	−
3	ethyl butanoate	fruity	1028	++	++
4	**unknown**	**cempedak**	**1041**	++	−
5	ethyl 2-methylbutanoate	fruity	1044	−	++
6	ethyl 3-methylbutanoate	fruity	1061	+++	+++
7	hexanal	grassy	1073	++	+
8	unknown	skunky	1103	++	+
9	1-butanol	malty	1140	++	++
10	methyl hexanoate	fruity	1180	++	+
11	2-/3-methyl-1-butanol	malty	1207	++	++
12	ethyl hexanoate	fruity	1225	++	−
13	octanal	citrusy	1279	++	+
14	1-octen-3-one	mushroom	1290	++	+
15	2-acetyl-1-pyrroline	popcorn	1332	+++	++

Continued on next page.

Table 1. (Continued). Aroma-Active Compounds in Cempedak Fruit and Jackfruit

No.	*Compound*[a]	*Odor*[b]	*RI*[c]	*Odor Intensity*[d]	
				Cempedak	*Jackfruit*
16	unknown	sulfury	1365	+++	+
17	acetic acid	vinegar	1454	+	++
18	3-(methylthio)propanal	potato	1457	+++	++
19	decanal	citrusy	1485	++	−
20	(*E*)-2-nonenal	fatty	1530	++	+
21	MDMF	caramel	1589	++	−
22	unknown	fruity	1610	++	+
23	butanoic acid	cheesy	1620	+	++
24	phenylacetaldehyde	flowery	1645	++	+
25	3-methylbutanoic acid	cheesy	1665	+++	++
26	unknown	meaty	1724	++	+++
27	hexanoic acid	cheesy	1844	++	++
28	2-methoxyphenol	smoky	1863	+	++
29	unknown	fruity	1887	++	+
30	2-phenylethanol	flowery	1916	++	++
31	HDMF	caramel	2047	+++	+++
32	ethyl cinnamate	fruity	2120	++	−
33	sotolon	seasoning	2203	++	++

[a] Compounds are listed in the order of increasing retention time observed during GC-O (FFAP column). [b] Odor quality as perceived at the sniffing port during GC-O. [c] Retention index (FFAP column). [d] Odor intensity as perceived at the sniffing port during GC-O: +++, intense; ++, moderate; +, weak; −, not detected.

As neither 2-(methylthio)pentane nor 3-methyl-2-(methylthio)butane was commercially available, both compounds were synthesized from the corresponding alcohols via tosylation followed by substitution of tosylate for methanethiolate as detailed in Figure 2. The structures of the reaction products were confirmed by NMR (data not shown) and they were then analyzed by GC-O and GC-MS in parallel to the cempedak fruit volatile isolate.

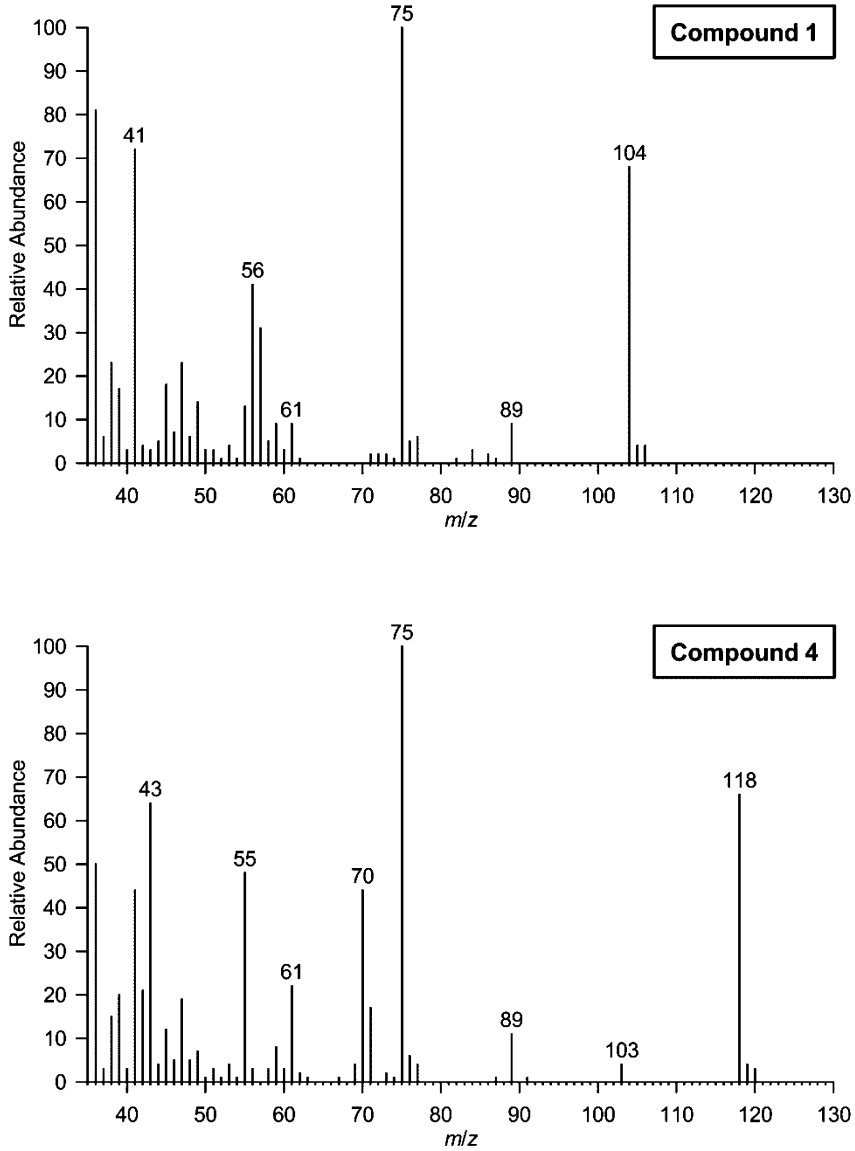

*Figure 1. Mass Spectra Obtained for Compounds **1** and **4** by GC-MS(EI) of the Cempedak Fruit Volatile Isolate.*

Results showed identical RIs for 2-(methylthio)pentane and **4** on the FFAP (1041) and DB-5 column (870), identical mass spectra, and identical odor properties. 3-Methyl-2-(methylthio)butane, on the other hand, although exhibiting the same cempedak-like odor, clearly differed in its RI data (FFAP: 1031; DB-5: 861) and the intensities of major fragments in the mass spectrum (data not shown). Thus, compound **4** was identified as 2-(methylthio)pentane.

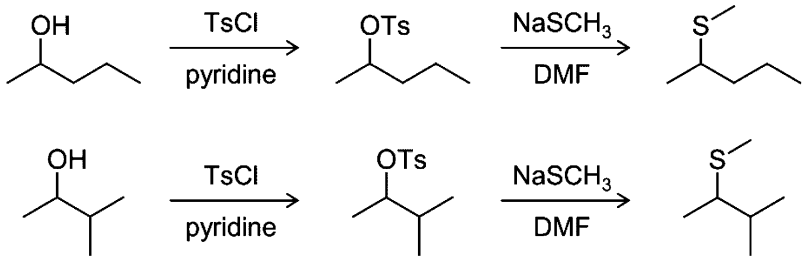

Figure 2. Synthetic Route to 2-(Methylthio)pentane and 3-Methyl-2-(methylthio)butane.

Neither 2-(methylthio)butane nor 2-(methylthio)pentane has been reported in cempedak fruit before. 2-(Methylthio)butane, however, has been found among the volatiles of asafoetida, a gum exuded from *Ferula assa-foetida* L. that is used as spice in South Asian cuisines (*20*), and 2-(methylthio)pentane has previously been found in fermented fish sauce (*21*).

Enantiomeric Distributions of the 2-(Methylthio)alkanes in Cempedak

2-(Methylthio)butane and 2-(methylthio)pentane are chiral compounds with one stereogenic center each and therefore exist as pairs of enantiomers. As enantiomers may fundamentally differ in their odor properties, we assessed the enantiomeric distribution of both compounds in cempedak and evaluated odor qualities and odor thresholds of the individual isomers.

Analytical enantiomer separation was achieved by heart-cut GC-GC-MS using a chiral cyclodextrin phase in the second dimension. The elution order was determined by analysis of enantiopure samples of (*R*)-2-(methylthio)butane and (*R*)-2-(methylthio)pentane, which were obtained from the corresponding enantiopure (*S*)-alcohols using the above mentioned tosylation/substitution approach (Figure 3).

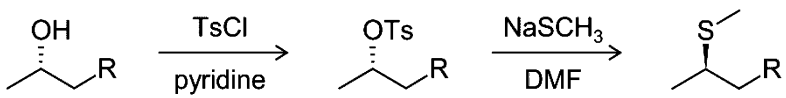

Figure 3. Synthetic Route to Enantiopure (R)-2-(Methylthio)butane (R = CH_3) and (R)-2-(Methylthio)pentane (R = CH_2CH_3).

Results (Table 2) showed that all four compounds exhibited virtually the same sulfury, cempedak-like odor quality, but slightly differed in their odor threshold values. In 2-(methylthio)butane as well as in 2-(methylthio)pentane the less odor-active (*S*)-isomer predominated with 67% and 59%, respectively.

Table 2. (Methylthio)butane and 2-(Methylthio)pentane Enantiomers: Chromatographic/Sensory Properties and Distribution in Cempedak Fruits

Compound	*RI*[a]	*Odor*[b]	*OTV*[c] *(ng/L)*	*Enantiomeric Ratio*[d]
(*R*)-2-(methylthio)butane	718	sulfury, cempedak	1	33%
(*S*)-2-(methylthio)butane	725	sulfury, cempedak	2	67%
(*R*)-2-(methylthio)pentane	802	sulfury, cempedak	2	41%
(*S*)-2-(methylthio)pentane	804	sulfury, cempedak	5	59%

[a] Retention index on the chiral, 2,3-dimethyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin-based BGB-176 column used for enantio-GC analysis. [b] Odor quality as perceived at the sniffing port during GC-O. [c] Odor threshold value in air. [d] Enantiomeric ratio determined in the cempedak fruit arils.

Conclusions

In summary, the results detailed above suggest that 2-(methylthio)butane and 2-(methylthio)pentane play a major role for the characteristic aroma of cempedak fruit and particularly account for the aroma difference to jackfruit. Nevertheless, further studies are needed to confirm this assumption. These will have to include exact quantitation of 2-(methylthio)butane, 2-(methylthio)pentane, and other potent odor-active compounds in cempedak fruit and jackfruit by application of stable isotope dilution assays, aroma reconstitution experiments, and omission tests.

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8.2.3 Summary and Individual Contributions

The cempedak tree (*Artocarpus integer* (Thunb.) Merr.) is a tropical tree in the mulberry family Moraceae and has its origin in Southeast Asia. The jackfruit tree (*Artocarpus heterophyllus* Lam.) is closely related to the cempedak tree, but it is better known as it is spread throughout the entire tropics. The aroma of cempedak pulp and jackfruit pulp is dominated by sweet, fruity, and malty odor notes. However, cempedak pulp aroma includes an additional sulfury, smear cheese-like odor note, which differentiates the aroma of cempedak pulp from the aroma of jackfruit pulp. The aim of the study was to analyze the odorants in cempedak and jackfruit by GC-O with emphasis on the compounds contributing to the sulfury, smear cheese-like odor note.

A comparative GC-O analysis of cempedak pulp and jackfruit pulp revealed a total of 33 odorants with different odor intensities. 26 odorants could be structurally identified. However, the identification of seven odorants remained unknown. Among them two compounds attracted special attention, because their odor represented the sulfury, smear cheese-like odor note of cempedak pulp, which is not present in jackfruit pulp. The GC-MS analysis revealed mass spectral data for both unknown compounds. The comparison of the mass spectra with MS database spectra suggested one of these two compounds to be 2-(methylsulfanyl)butane. The GC-O and GC-MS analyses of the reference compound confirmed the structural assignment. The database search for the mass spectrum of the second compound was not successful. However, the comparison with the mass spectrum of 2-(methylsulfanyl)butane suggested that the second compound was a homologue. The fragmentation pattern suggested that the second compound was either 2-(methylsulfanyl)pentane or 3-methyl-2-(methylsulfanyl)butane. Both compounds were synthesized from the corresponding alcohols by tosylation followed by the substitution of the tosylate with methanethiolate. The comparison of the RI data and the odor of the synthesized reference compounds identified the second sulfury, smear cheese-like smelling compound as 2-(methylsulfanyl)pentane. 2-(Methylsulfanyl)butane and 2-(methylsulfanyl)pentane are chiral compounds with one stereogenic center. Therefore, the enantiomeric distribution in cempedak pulp, as well as the OTVs in air were determined by using a chiral GC column. The enantiomers exhibited the same sulfury, cempedak-like odor, but slightly differed in their OTVs. The respective enantiomers all showed the same odor. In cempedak pulp, the (*S*)-isomers of 2-(methylsulfanyl)butane (67%) and 2-(methylsulfanyl)pentane (59%) dominated. The results suggested that 2-(methylsulfanyl)-butane and 2-(methylsulfanyl)pentane play a crucial role for the characteristic sulfury, cempedak-like odor note of cempedak pulp and the aroma difference between cempedak pulp and jackfruit pulp.

Johanna Grimm designed and conducted the experiments including the GC-O screenings, structure assignments, the syntheses, the GC-O-enantioGC-O/MS analyses, and the OTV determinations in air. Johanna evaluated the resulting data. Martin Steinhaus conceived and directed the study, supervised Johanna’s work and prepared the manuscript. He also participated in the GC-O analyses.

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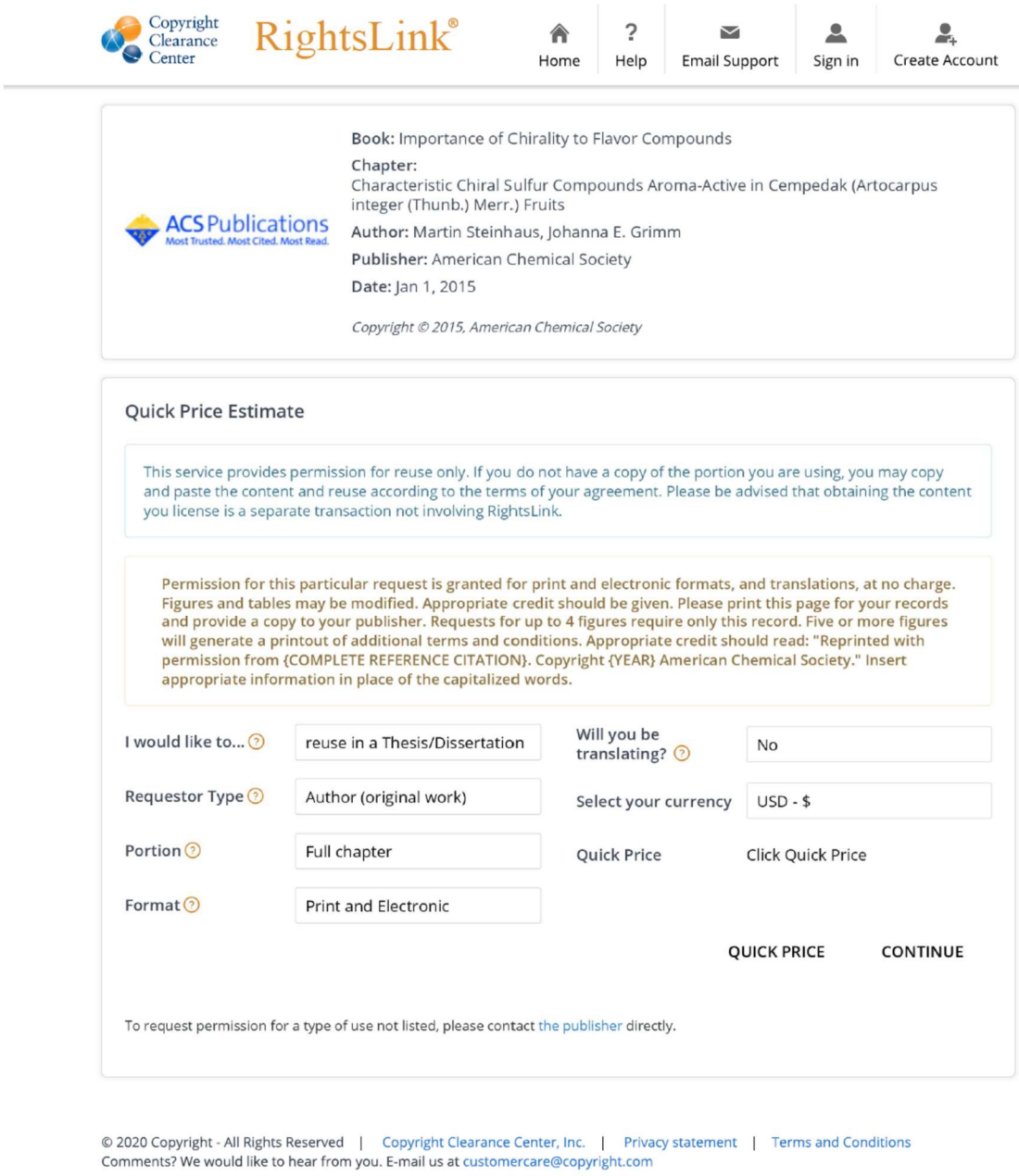

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Characterization of the Major Odorants in Cempedak—Differences to Jackfruit

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Supporting Information

ABSTRACT: Screening the volatiles of cempedak [*Artocarpus integer* (Thunb.) Merr.] pulp for odor-active compounds by aroma extract dilution analysis and gas chromatography (GC)−olfactometry of static headspace samples revealed a total of 55 odorants, among which 47 were identified. Using stable isotopically substituted odorants as internal standards, these compounds were quantitated by GC−mass spectrometry, and odor activity values (OAVs) were calculated as ratios of the natural concentrations in cempedak pulp to the orthonasal odor detection thresholds. An aroma reconstitution model based on the 41 compounds with OAVs >1 in their natural concentrations successfully mimicked the characteristic aroma of cempedak pulp including the pronounced sulfury, oniony note which is intense in cempedak pulp but absent in jackfruit pulp. Further sensory tests finally showed that 2-(methylsulfanyl)propane, 2-(methylsulfanyl)butane, and 2-(methylsulfanyl)pentane are the key compounds responsible for this unique aroma note in cempedak pulp and vitally contribute to the aroma difference between cempedak pulp and jackfruit pulp.

KEYWORDS: *cempedak, Artocarpus integer (Thunb.) Merr., jackfruit, Artocarpus heterophyllus Lam., 2-(methylsulfanyl)propane, 2-(methylsulfanyl)butane, 2-(methylsulfanyl)pentane, aroma extract dilution analysis (AEDA), stable isotopically substituted odorants, aroma reconstitution*

INTRODUCTION

Like the closely related jackfruit tree, *Artocarpus heterophyllus* Lam., the cempedak tree, *Artocarpus integer* (Thunb.) Merr., is an evergreen tropical tree in the mulberry family Moraceae and originates in Southeast Asia. While jackfruit trees are cultivated in the entire tropics today, cempedak is of only local importance and can be found in Indonesia, Malaysia, and New Guinea, as well as parts of Thailand, India, and Australia.[1] The cempedak tree is cauliflorous, that is, the fruits grow directly on the trunk or on old and thick branches. Like jackfruit, the cempedak fruit is a multiple fruit derived from the coalescence of the individual fruits of a whole inflorescence. Cempedak fruits are clearly smaller than jackfruits and rather cylindrically shaped. The length is typically ∼35 cm and the diameter is ∼15 cm, whereas jackfruits can reach dimensions of 90 cm × 50 cm.[1] The leathery and warty skin does not show the green color of jackfruits but is rather yellow or brownish. Similar to jackfruit, the inside of the fruit is filled with slim and tough ribbons and numerous seeds attached to a central peduncle. Each seed is covered by an edible bulblike pulp which is much softer than that in jackfruits, shows a pale yellow to deep orange color, and is intensely fragrant. The aroma of the cempedak pulp is similar to the aroma of jackfruit pulp. However, in addition to the sweet, fruity, malty, and cheesy notes, which constitute the typical jackfruit pulp aroma,[2] cempedak pulp aroma includes a very unique odor note, which is described as sulfury and somewhat oniony.

The compounds contributing to jackfruit pulp aroma have recently been revealed.[2] Application of aroma extract dilution analysis (AEDA)[3] to fresh jackfruit pulp in combination with gas chromatography−olfactometry (GC−O) of static headspace samples resulted in a total of 53 odor-active compounds, among which 44 were identified and subsequently quantitated. Thirty-five odorants showed concentrations exceeding their respective odor threshold values in water. Among them, ethyl 3-methylbutanoate (fruity), ethyl butanoate (fruity), 3-methylbutanal (malty), 2-methylpropanal (malty), butanal (malty), octanal (citrusy, soapy), hexanal (green, grassy), methyl 3-methylbutanoate (fruity), and 3-methylbutanoic acid (cheesy, sweaty) exhibited the highest odor activity values (OAVs = concentration/odor threshold value).[2]

Cempedak pulp aroma has also been the subject of previous studies.[4−7] Wong et al. identified 3-methylbutanoic acid, 3-methylbutan-1-ol, 3-hydroxybutan-2-one, 2-phenylethanol, 2-methylpropan-1-ol, ethyl 3-methylbutanoate, pentane-2,3-dione, and methyl 3-methylbutanoate as major volatiles of cempedak pulp but did not assess their odor contributions.[4] Moreover, GC−O analysis is essential to avoid overlooking important odor-active trace compounds.[8] GC−O was first applied to cempedak volatiles by Wijaya et al.[5] They suggested 3-methylbutyl 3-methylbutanoate and butyl 3-methylbutanoate as character impact compounds but did not substantiate their suggestions by further experiments such as OAV calculation and aroma reconstitution.[8] A comprehensive study on cempedak aroma was more recently published by Buttara et al.[6] The odorants in two cempedak cultivars were characterized by using AEDA, stable isotope dilution quantitation, calculation of OAVs, aroma reconstitution, and omission tests. 3-Methylbutanal, octanal, ethyl 3-methylbutanoate,

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methyl 3-methylbutanoate, and 2-acetyl-1-pyrroline were suggested as key odorants of cempedak. However, none of these compounds were able to plausibly explain the sulfury, oniony note distinguishing cempedak aroma from jackfruit aroma. Nevertheless, some odor-active organosulfur compounds were reported in cempedak pulp in this study. Among them, 3-(methylsulfanyl)propanal, 3-(methylsulfanyl)butanal, and 3-(methylsulfanyl)butan-1-ol did not show a significant aroma contribution in the reconstitution experiments, and 2-(methylsulfanyl)ethanal and dimethyl trisulfide were only tentatively identified and not quantitated. Our own preliminary GC−O data suggested that the characteristic sulfury, oniony note in cempedak pulp aroma is caused by two compounds that were identified as 2-(methylsulfanyl)butane and 2-(methylsulfanyl)pentane.[7] Both compounds exhibit a sulfury, oniony odor clearly reminiscent of cempedak pulp. Furthermore, they were clearly detected by GC−O in a volatile isolate obtained from cempedak pulp, but not in a volatile isolate in parallel obtained from jackfruit pulp.

The objectives of the current study thus were the reinvestigation of the odor-active compounds in cempedak pulp by application of AEDA and static headspace dilution analysis, quantitation of potent odorants, calculation of OAVs, aroma reconstitution, and discussion of the results in relation to data obtained from jackfruit pulp. Omission testing should finally be used to clarify the role of 2-(methylsulfanyl)alkanes in the aroma of cempedak pulp.

MATERIALS AND METHODS

Fruit. Cempedak fruits and jackfruits grown in Thailand were purchased from a local Internet shop in Germany. Fruits were handpicked and sent to Germany by airfreight within 2 days. If necessary, fruits were stored for 1−2 days at room temperature to reach full ripeness indicated by softening and release of the characteristic odor.

Reference Odorants. The following compounds were purchased from commercial sources: **2**, **3**, **5**, **7**−**9**, **12**−**16**, **18**, **22**−**29**, **31**−**33**, **38**−**43**, **45**−**48**, **50**−**52**, **56**, and **57** (Sigma-Aldrich; Taufkirchen, Germany); **1**, **17**, **53**, and **54** (Alfa Aesar; Karlsruhe, Germany); **11** and **49** (Merck; Darmstadt, Germany); **4** (Lancaster; Mühlheim, Germany); **21** (Acros Organics; Geel, Belgium); and **55** (abcr; Karlsruhe, Germany). **32** was freshly distilled before use. **6**[7] and **19**[9] were prepared according to literature procedures.

Stable Isotopically Substituted Odorants. The following compounds were purchased from commercial sources: ($^{13}C_4$)-**17** (Toronto Research Chemicals; Toronto, Canada); ($^{2}H_3$)-**22**, ($^{2}H_8$)-**45**, ($^{13}C_2$)-**48**, and ($^{2}H_6$)-**50** (Sigma-Aldrich); ($^{2}H_5$)-**41** (CDN Isotopes; Quebec, Canada); and ($^{13}C_2$)-**43** (aromaLAB; Planegg, Germany). The following compounds were synthesized as detailed in the literature: ($^{2}H_3$)-**2**,[2] ($^{13}C_4$)-**3**,[10] ($^{2}H_3$)-**4**,[2] ($^{13}C_4$)-**5**,[11] ($^{2}H_5$)-**7**,[2] ($^{2}H_3$)-**8**,[2] ($^{2}H_4$)-**9**,[11] ($^{2}H_4$)-**11**,[2] ($^{2}H_3$)-**12**,[2] ($^{2}H_2$)-**13**,[12] ($^{2}H_3$)-**15**,[2] ($^{2}H_4$)-**16**,[2] ($^{2}H_4$)-**18**,[2] ($^{2}H_{2\text{-}5}$)-**19**,[13] ($^{2}H_4$)-**21**,[2] ($^{2}H_3$)-**23**,[2] ($^{2}H_4$)-**24**,[14] ($^{2}H_3$)-**25**,[15] ($^{2}H_2$)-**26**,[2] ($^{2}H_2$)-**27**,[16] ($^{2}H_2$)-**28**,[17] ($^{2}H_3$)-**29**,[10] ($^{2}H_2$)-**31**,[2] ($^{13}C_2$)-**32**,[18] ($^{2}H_2$)-**33**,[12] ($^{2}H_2$)-**38**,[19] ($^{2}H_3$)-**39**,[20] ($^{2}H_5$)-**40**,[2] ($^{2}H_2$)-**42**,[21] ($^{2}H_5$)-**46**,[2] ($^{13}C_2$)-**47**,[22] ($^{2}H_3$)-**49**,[15] ($^{2}H_6$)-**51**,[2] ($^{2}H_4$)-**52**,[2] ($^{2}H_2$)-**53**,[21] ($^{2}H_2$)-**54**,[21] ($^{2}H_{11}$)-**56**,[23] and ($^{13}C_2$)-**57**.[24] ($^{2}H_3$)-**1**, ($^{2}H_3$)-**6**, and ($^{2}H_3$)-**55** were synthesized from the corresponding 2-alcohols butan-2-ol (Sigma-Aldrich), pentan-2-ol (Sigma-Aldrich), and propan-2-ol (Roth; Karlsruhe, Germany) by tosylation, thioacetylation, reduction, and subsequent trideuteromethylation with ($^{2}H_3$)methyl iodide (Sigma-Aldrich) using the approach detailed by Polster and Schieberle.[25]

Miscellaneous Chemicals and Reagents. Glucose, fructose, sucrose, and malic acid were purchased from Merck. Succinic acid and oxalic acid were from Sigma-Aldrich. Dichloromethane, diethyl ether, and pentane (VWR; Darmstadt, Germany) were freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings. Silica gel 60 (0.040−0.63 mm; VWR) was washed with hydrochloric acid before use.[26] Mercurated agarose gel was prepared from Affi-Gel 10 (Bio-Rad; Munich, Germany).[27]

GC Systems. GC analyses were accomplished by using the GC−O/flame ionization detection (FID) system, the one-dimensional GC−mass spectrometry (GC−MS) system, the two-dimensional heart-cut GC−GC−MS system, the comprehensive two-dimensional GC × GC−MS system, and the static headspace GC system described in detail in our previous paper.[2]

Isolation of Fruit Volatiles. Fruits were opened and the seeds were removed from the pulp. The representativeness of the pulp odor was assured. Using liquid nitrogen, the pulp was flash-frozen and then ground into a powder.[2] Under ice-cooling and continuous homogenization with a stainless steel blender, dichloromethane (300 mL) was added to the powder (100 g) followed by anhydrous sodium sulfate (250 g). The mixture was stirred at ambient temperature for 1 h and then filtered through a plug of sea sand and defatted cotton wool placed at the bottom of a glass column [30 cm × 5 cm inner diameter (ID)]. The solid residue was washed with dichloromethane (3 × 100 mL). The combined organic phases were subjected to solvent-assisted flavor evaporation (SAFE)[28] at 40 °C, and the distillate was concentrated at a 50 °C water bath temperature to a final volume of 1 mL by using a Vigreux column (50 × 1 cm) and a Bemelmans microdistillation device.[29]

Aroma Extract Dilution Analysis. A cempedak volatile isolate (1 mL) prepared as described above was repeatedly subjected to GC−O analysis (FFAP column; 40 min total analysis time) by four trained and experienced sniffers. This was continued until for each panelist run-to-run variations disappeared and results became reproducible. Then, the cempedak volatile isolate was stepwise diluted with the solvent at a ratio of 1:2, and samples representing dilutions of 1:2, 1:4, 1:8, 1:16, and so forth of the initial solution were analyzed by GC−O (three sniffers). Each odorant was assigned a flavor dilution (FD) factor, which was the dilution factor of the highest diluted sample in which the odorant was detected during GC−O analysis by any of the three panelists.[8]

Fractionation of Cempedak Volatiles. Using the procedure recently detailed for the fractionation of jackfruit volatiles,[2] a cempedak volatile isolate (1 mL) prepared as described above was fractionated into five fractions by liquid chromatography using silica gel as stationary phase and pentane/diethyl ether mixtures of 100 + 0, 90 + 10, 70 + 30, 50 + 50, and 0 + 100 (v + v) as eluents. Another cempedak volatile isolate was used to obtain a fraction of volatile cempedak thiols by using mercurated agarose gel.[26]

Static Headspace Dilution Analysis. Cempedak powder (5 g) prepared from flash-frozen pulp as detailed above was placed in a 120 mL septum-sealed glass vial. After equilibration for 1 h under continuous magnetic stirring at room temperature (22 ± 1 °C), portions of the headspace (10, 5, 2.5, 1.25, 0.63, 0.31, and 0.16 mL) were withdrawn by a gastight syringe and subjected to static headspace GC analysis by three sniffers. Each odorant was assigned an FD factor representing the ratio of the initial injection volume (10 mL) to the lowest injection volume in which the odorant was detected during static headspace GC−O by any of the three panelists.

Odorant Quantitation. For the quantitation of compounds **1**−**9**, **11**−**19**, **21**−**29**, **31**−**33**, **38**−**43**, **45**−**49**, **51**−**54**, **56**, and **57** in cempedak pulp, solvent (20−150 mL) was added to cryomilled fruit pulp (0.5−50 g) under ice-cooling. The solvent was diethyl ether in the quantitation assays for **1**−**4** and **51**−**54** and dichloromethane in the case of the other compounds. Under continuous cooling and stirring, stable isotopically substituted odorants (cf. Supporting Information, Table S1; 0.1−125 μg) in solvent (10 μL to 3 mL) were added as internal standards followed by anhydrous sodium sulfate (10−125 g). The mixture was stirred at ambient temperature for 1 h and then filtered through sea sand and cotton wool as detailed above. Extracts were subjected to SAFE. Distillates were concentrated (0.2 μL to 5 mL) and analyzed by GC × GC−MS or heart-cut GC−GC−MS. Quantitation of **1**, **6**, **27**, and **29** in jackfruit pulp was done accordingly.

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J. Agric. Food Chem. 2020, 68, 258−266

Table 1. Odorants in the Volatile Isolate Obtained from Cempedak Pulp

no.	odorant[a]	odor[b]	RI[c] FFAP	RI[c] DB-5	FD factor[d]	previously reported[e]
1	2-(methylsulfanyl)butane	oniony, sulfury, cempedak	<1000	786	32	
2	ethyl 2-methylpropanoate[f]	fruity	<1000	759	1	
3	butane-2,3-dione	buttery	<1000	<700	8	4
4	methyl 3-methylbutanoate	fruity	1013	931	64	4, 5, 6
5	ethyl butanoate	fruity	1028	809	32	
6	2-(methylsulfanyl)pentane	oniony, sulfury, cempedak	1041	870	32	
7	ethyl 2-methylbutanoate	fruity	1044	849	32	6
8	ethyl 3-methylbutanoate	fruity	1061	859	≥8192	4, 6
9	hexanal	green, grassy	1073	800	256	4, 6
10	unknown[g]	skunky	1103	839	64	
11	butan-1-ol	malty	1140	<700	128	4
12	methyl hexanoate	fruity	1180	928	512	6
13	3-methylbutan-1-ol[h]	malty	1207	742	256	4, 5, 6
14	2-methylbutan-1-ol[h]	malty	1207	742	256	
15	ethyl hexanoate	fruity	1225	1002	32	4
16	octanal	citrusy, soapy	1279	1005	256	5, 6
17	3-hydroxybutan-2-one	buttery	1280	716	4	4
18	1-octen-3-one[f]	mushroom	1290	979	32	
19	2-acetyl-1-pyrroline	cooked rice	1332	921	4096	4, 6
20	unknown[g]	sulfury	1365		1024	
21	nonanal	citrusy, soapy	1390	1100	4	
22	acetic acid	vinegar	1454	<700	64	4, 5
23	3-(methylsulfanyl)propanal	cooked potato	1457	910	128	6
24	decanal	citrusy, soapy	1485	1208	32	
25	3-isobutyl-2-methoxypyrazine[f]	bell pepper	1512	1181	4	
26	(2*E*)-non-2-enal[f]	fatty	1530	1163	32	
27	linalool	citrusy, floral	1544	1102	8	
28	(2*E*,6*Z*)-nona-2,6-dienal[f]	cucumber	1580	1159	4	
29	MDMF[i]	caramel	1589	1060	64	5, 6
30	unknown[g]	fruity	1610	1224	128	
31	butanoic acid	cheesy, sweaty	1620	815	16	
32	phenylacetaldehyde	floral, honey	1645	1046	32	4
33	3-methylbutanoic acid	cheesy, sweaty	1665	871	512	4, 5, 6
34	unknown[j]	citrusy, soapy, green	1690	1550	16	
35	unknown[k]	meaty	1724	1025	128	
36	unknown[g]	green	1759		32	
37	unknown[g]	cooked potato	1769	1170	32	
38	hexanoic acid	sweaty	1844	1010	256	4
39	2-methoxyphenol	smoky	1863	1091	16	6
40	ethyl 3-phenylpropanoate	fruity	1887	1352	16	
41	2-phenylethanol	floral	1916	1116	128	4, 5, 6
42	γ-octalactone	coconut	1920	1260	4	
43	HDMF[l]	caramel	2047	1068	4096	4, 6
44	unknown[g]	seasoning	2068		16	
45	4-methylphenol	fecal, horse stable	2095	1098	8	
46	ethyl (2*E*)-3-phenylprop-2-enoate	fruity	2120	1469	128	
47	sotolon[f]	seasoning	2203	1109	32	6
48	2-phenylacetic acid	floral, honey	2589	1264	2	6
49	vanillin	vanilla	2600	1409	32	6

[a]Each odorant was identified by comparing its RIs on two GC capillaries of different polarities (DB-FFAP and DB-5), its mass spectrum obtained by GC−MS, as well as its odor as perceived at the sniffing port during GC−O analysis to data obtained from authentic reference compounds analyzed in parallel. [b]Odor as perceived at the sniffing port during GC−O analysis. [c]Retention index, calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [d]FD factor; dilution factor of the highest diluted cempedak volatile isolate in which the odorant was detected during GC−O analysis by any of the three panelists. [e]References reporting the compound as cempedak pulp volatile; the minus sign (−) indicates compounds that have not been reported before. [f]An unequivocal mass spectrum of the compound could not be obtained in the cempedak volatile isolates, identification was based on the remaining criteria detailed in footnote a. [g]Neither comparison of retention index and odor nor mass spectral data allowed for a conclusive structure proposal. [h]3-Methylbutan-1-ol and 2-methylbutan-1-ol were not separated on the GC column used for AEDA; an FD factor of 256 was determined for the mixture of both compounds. [i]MDMF, 4-methoxy-2,5-dimethylfuran-3(2*H*)-one. [j]Mass spectral data allowed for the tentative identification of the compound as an octenol; however, positions of the double bond and the hydroxy group have not been elucidated. [k]The compound could be enriched by mercurated agarose gel, thus was a thiol, but no further information on its structure was gained. [l]HDMF, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one.

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Compounds **50** and **55** were quantitated by static headspace GC−MS. For the quantitation of **55** in cempedak and jackfruit pulp, a portion of cryomilled fruit pulp (0.5 g) was placed together with a magnetic stir bar into a 20 mL septum-sealed glass vial. ($^2H_3$)-**55** (0.8 μg) in water (0.5 mL) was added and the mixture was allowed to equilibrate for 1 h under continuous magnetic stirring at room temperature before headspace GC−MS analysis. Quantitation of **50** in cempedak pulp was done as recently reported for jackfruit pulp.[2]

In all quantitation assays, the odorant concentrations were finally calculated from the peak areas corresponding to the analyte and internal standard as obtained from the extracted ion chromatograms of characteristic quantifier ions, the amount of fruit pulp used, and the amount of standard added, by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in five different concentration ratios (5:1, 2:1, 1:1, 1:2, and 1:5). Quantifier ions and calibration line equations are available in the Supporting Information, Table S1.

Odor Thresholds. These were determined orthonasally in pure water according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced choice ascending concentration series method of limits.[30] Details on the experimental setup have been reported earlier.[31]

Cempedak Aroma Reconstitution. Aliquots (0.01−1 mL) of aqueous or ethanolic stock solutions of the reference odorants were combined and made up to a defined volume (10 mL) with water. An aliquot of the mixture (100 μL) was added to a buffered (pH 5.5) aqueous solution (100 g) of sugars and organic acids. As no quantitative data on sugars and organic acids in cempedak were available, these were approximated by data available for jackfruit (glucose, 9.8 g/kg; fructose, 12.5 g/kg; sucrose 46.6 g/kg; L-malic acid, 12.0 g/kg; citric acid 3.6 g/kg; succinic acid, 2.2 g/kg; and oxalic acid, 0.04 g/kg).[32] The concentrations of the stock solutions of the reference odorants and the volume of the aliquots were adjusted to obtain final odorant concentrations in the aroma model solution that represented the concentrations previously determined in the pulp. As (2*S*)-2-(methylsulfanyl)butane and (2*S*)-2-(methylsulfanyl)pentane were not available as reference compounds, their odor contribution in the aroma model solution was approximated by racemic 2-(methylsulfanyl)butane and (2*R*)-2-(methylsulfanyl)pentane, respectively, in amounts that corresponded to the same OAVs. The final ethanol concentration in the aroma model solution was below its odor threshold in water (1 g/L). Besides the complete cempedak aroma model with 41 odorants, an incomplete cempedak aroma model was prepared from which the 2-(methylsulfanyl)alkanes **1**, **6**, and **55** were omitted.

Quantitative Olfactory Profiles. Samples (10 g), either freshly homogenized fruit pulp or aroma model solutions, were placed in cylindrical ground neck glasses (height 7 cm, ID 3.5 cm) with lids (VWR). Seventeen trained panelists evaluated the olfactory profile of the samples orthonasally in a single session by rating the intensities of nine predefined descriptors on a scale from 0 to 3 and 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Each descriptor was defined by the odor of a reference compound dissolved in water in a concentration 100 times above its respective odor threshold value. The nine descriptors and the corresponding reference compounds were "oniony, sulfury, cempedak-like" (2-(methylsulfanyl)butane), "cooked potato" (3-(methylsulfanyl)-propanal), "malty" (3-methylbutan-1-ol), "caramel" (4-hydroxy-2,5-dimethylfuran-3(2*H*)-one), "cheesy" (3-methylbutanoic acid), "fruity" (ethyl 3-methylbutanoate), "banana" (3-methylbutyl acetate), "cooked rice" (2-acetyl-1-pyrroline), and "soapy" (octanal). Ratings of all panelists were averaged by calculating the arithmetic means (cf. Supporting Information, Table S4).

RESULTS AND DISCUSSION

Screening for Odorants in Cempedak by AEDA. Cempedak pulp volatiles were isolated from fresh fruit by solvent extraction and SAFE. The distillate was concentrated. Throughout the isolation procedure, the overall odor was monitored using fragrance testing papers. Results showed that the characteristic odor notes present in cempedak pulp could well be retained in the extract, the distillate, as well as in the concentrated volatile isolate. Application of AEDA to this cempedak volatile isolate resulted in 49 odorants with FD factors from 1 to ≥8192 (Table 1; Supporting Information, Figure S1).

Toward a preliminary structure assignment, the retention indices (RIs) and the odor of the individual odorants were compared to published data compiled in different databases.[33,34] Preliminary assignments were confirmed by GC−O of authentic reference compounds in appropriate dilution in parallel with the cempedak volatile isolate. These GC−O analyses were carried out by using two columns of different polarity, namely, DB-FFAP and DB-5. This approach allowed for the structure assignment of 39 out of the 49 odorants (**2**−**5**, **7**−**9**, **11**−**19**, **21**−**29**, **31**−**33**, **38**−**43**, and **45**−**49**). Final confirmation of the structure assignments was achieved by comparison of mass spectral data obtained from the reference compounds and the cempedak volatile isolate by GC−MS analyses in electron ionization and chemical ionization mode. To avoid coelution of the target compounds with matrix components, the cempedak volatile isolate was fractionated by silica gel chromatography according to compound polarity into five fractions. In a separate experiment, a fraction containing only the thiols was isolated from a cempedak volatile isolate by using mercurated agarose gel. Each fraction was separately analyzed by GC−O to localize the odorants before GC−MS analysis. With this approach, unequivocal structure assignment was achieved for 33 compounds, namely, odorants **3**−**5**, **7**−**9**, **11**−**17**, **19**, **21**−**24**, **27**, **29**, **31**−**33**, **38**−**43**, **45**, **46**, **48**, and **49**. Because of low concentrations, mass spectral confirmation was unsuccessful for compounds **2**, **18**, **25**, **26**, **28**, and **47**. Among the 10 compounds for which a preliminary structure assignment via RIs and odor properties failed were compounds **1** and **6**. These two compounds attracted our attention because of their oniony and sulfury odor that clearly represented the unique note in the overall aroma of cempedak pulp. As detailed previously,[7] mass spectral data finally led to their identification as 2-(methylsulfanyl)butane (**1**) and 2-(methylsulfanyl)-pentane (**6**). Enantio-GC analyses revealed the R/S ratios of 33/67 for 2-(methylsulfanyl)butane and 41/59 for 2-(methylsulfanyl)pentane. The odor qualities of the enantiomers were virtually identical, but they differed in their odor thresholds.[7] Compounds **10**, **20**, **30**, **34**−**37**, and **44** remained unknown.

As a result of AEDA and structural identification, high FD factors were determined for fruity smelling ethyl 3-methylbutanoate (**8**), cooked rice-like smelling 2-acetyl-1-pyrroline (**19**), and caramellike smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**43**), followed by unknown compound **20** (sulfury), methyl hexanoate (**12**; fruity), 3-methylbutanoic acid (**33**; cheesy, sweaty), hexanal (**9**; green, grassy), 3- and 2-methylbutan-1-ol (**13**, **14**; malty), octanal (**16**; citrusy, soapy), hexanoic acid (**38**; sweaty), butan-1-ol (**11**; malty), 3-(methylsulfanyl)propanal (**23**; cooked potato), unknown compounds **30** (fruity) and **35** (meaty), 2-phenylethanol (**41**; floral), and ethyl (2*E*)-3-phenylprop-2-enoate (**46**; fruity).

Ethyl 3-methylbutanoate (**8**), 2-acetyl-1-pyrroline (**19**), and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**43**) had been reported with high FD factors in cempedak pulp before.[6] The

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Table 2. Additional Odorants Detected in the Headspace above Cempedak Pulp

no.	odorant[a]	odor[b]	RI[b] DB-5	FD factor[c]	previously reported[d]
50	dimethyl sulfide	asparagus, putrid	518	32	
51	2-methylpropanal	malty	559	8	
52	butanal	malty	594	16	
53	2-methylbutanal[e]	malty	655	32	6
54	3-methylbutanal[e]	malty	655	32	6
55	2-(methylsulfanyl)propane	oniony, sulfury, cempedak	674	16	

[a]Each odorant was identified by comparing its retention index on the DB-5 capillary, its mass spectrum obtained by GC−MS, as well as its odor as perceived at the sniffing port during GC−O analysis to data obtained from authentic reference compounds analyzed in parallel. [b]cf. Table 1. [c]FD factor; calculated as the ratio of the initial injection volume (10 mL) to the lowest injection volume in which the odorant was detected during GC−O analysis by any of the three panelists. [d]cf. Table 1. [e]2-Methylbutanal and 3-methylbutanal were not separated on the GC column used for static headspace GC; an FD factor of 32 was determined for the mixture of both compounds.

same applied for 3-(methylsulfanyl)propanal (**23**) and 3-methylbutanoic acid (**33**). Despite their high odor potency, 2-methylbutan-1-ol (**14**) and ethyl (2*E*)-3-phenylprop-2-enoate (ethyl cinnamate; **46**) had not been reported as cempedak odorants prior to our investigations. Further odor-active compounds previously unknown in cempedak included 2-(methylsulfanyl)butane (**1**), ethyl 2-methylpropanoate (**2**), ethyl butanoate (**5**), 2-(methylsulfanyl)pentane (**6**), 1-octen-3-one (**18**), nonanal (**21**), decanal (**24**), 3-isobutyl-2-methoxypyrazine (**25**), (2*E*)-non-2-enal (**26**), linalool (**27**), (2*E*,6*Z*)-nona-2,6-dienal (**28**), butanoic acid (**31**), ethyl 3-phenylpropanoate (**40**), γ-octalactone (**42**), and 4-methylphenol (**45**). Among them, 2-(methylsulfanyl)butane (**1**) and 2-(methylsulfanyl)pentane (**6**) were the most interesting ones because their characteristic odor suggested that they could play a major role in the overall aroma of cempedak pulp.[7] With a value of 32, however, their FD factors were comparatively low. In the overall ranking according to FD factors, 21 odorants in the cempedak volatile isolate appeared to be more potent. To finally assess their impact on the aroma of cempedak pulp, further experiments were thus essential.

Screening for Highly Volatile Odorants in Cempedak by Static Headspace GC−O. GC−O in combination with a static headspace dilution analysis was applied as a complementary technique to AEDA. This approach additionally covers highly volatile compounds with boiling points below that of the extraction solvent used in AEDA. These compounds are typically not detected by AEDA as they are lost during concentration of the SAFE distillate. In the headspace above cempedak pulp, six additional odorants were detected with FD factors from 8 to 32 (Table 2). Among them, the highest FD factor was determined for asparagus, putrid smelling dimethyl sulfide (**50**), and malty smelling compounds 2-methylbutanal (**53**) and 3-methylbutanal (**54**). The other three odorants were malty smelling compounds 2-methylpropanal (**51**), butanal (**52**), and oniony, sulfury smelling 2-(methylsulfanyl)propane (**55**). Compounds **50**, **51**, and **53** are common key food odorants,[35] but such a role has not been reported for butanal and 2-(methylsulfanyl)propane. 2-(Methylsulfanyl)propane, however, has been found among the volatiles in a Taiwanese fish sauce.[36] In cempedak, only two out of the six compounds, namely, 2- and 3-methylbutanal, had been reported previously.[6]

In combination, the screenings by AEDA and by static headspace dilution analysis resulted in 55 odor-active compounds, among which 47 odorants were identified and 21 had not been reported in cempedak pulp before. Although our data showed some agreement with the data reported by Buttara et al.[6] regarding the compounds with high FD factors, there was a clear difference regarding organosulfur odorants. Our data suggested 2-(methylsulfanyl)propane, 2-(methylsulfanyl)butane, and 2-(methylsulfanyl)pentane as major contributors to the characteristic sulfury, oniony note in the overall aroma of cempedak. However, none of these compounds had been detected by Buttara et al.[6] To elucidate the key odorants of cempedak pulp and particularly clarify the role of 2-(methylsulfanyl)alkanes, we proceeded with quantitation of the odorants, OAV calculation, aroma reconstitution, and omission testing.

Odorant Quantitation and OAV Calculation. The 47 volatiles identified as odor-active compounds in the screening experiments were quantitated in cempedak pulp by GC−MS using stable isotopically substituted odorants as internal standards (cf. Supporting Information, Table S1) in order to compensate for workup losses. Two compounds, namely, 3-methylbutyl acetate (**56**) and butyl acetate (**57**), were additionally included in the quantitation experiments because they were recently found in odor-active amounts in jackfruit pulp.[2] All other important jackfruit pulp odorants were included anyway because they were also detected during odorant screening in cempedak pulp. Concentrations of the individual enantiomers of 2-(methylsulfanyl)butane (**1a** and **1b**) and 2-(methylsulfanyl)pentane (**6a** and **6b**) in cempedak pulp were calculated from the sum of both enantiomers as obtained by GC−MS and the enantiomeric ratios determined previously.[7]

Results of the quantitations revealed odorant concentrations in cempedak between 0.0391 and 40 700 μg/kg (Table 3). High values were found for 3-methylbutanoic acid (**33**; 40 700 μg/kg), acetic acid (**22**; 23 300 μg/kg), 3-hydroxybutan-2-one (**17**; 21 200 μg/kg), ethyl 3-methylbutanoate (**8**; 9400 μg/kg), 3-methylbutan-1-ol (**13**; 6950 μg/kg), butan-1-ol (**11**; 6060 μg/kg), hexanoic acid (**38**; 5770 μg/kg), 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**43**; 2920 μg/kg), 2-methylbutan-1-ol (**14**; 2300 μg/kg), ethyl hexanoate (**15**; 1450 μg/kg), and octanal (**16**; 1270 μg/kg).

To assess the odor potency of the individual cempedak odorants, OAVs were calculated from their concentrations and their orthonasal odor detection thresholds in water (Table 3). Among the 49 odorants with OAV >1, fruity smelling ethyl 3-methylbutanoate (**8**) showed by far the highest OAV, namely, 410 000. This corresponded very well to its high FD factor in the AEDA and the strong fruity note present in the aroma of cempedak pulp. The second highest OAV was calculated for malty smelling 3-methylbutanal (**54**; OAV 1700), suggesting this compound to predominantly account for the malty note in

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Table 3. Odor Threshold Values, Concentrations, and OAVs of Major Cempedak Pulp Odorants and Comparison to Jackfruit Pulp Data

			cempedak		jackfruit	
no.[a]	odorant	OT[b] (μg/kg)	conc.[c] (μg/kg)	OAV[d]	conc.[c] (μg/kg)	OAV[d]
8	ethyl 3-methylbutanoate	0.023	9400	410 000	1710[e]	74 000
54	3-methylbutanal	0.50	862	1700	759[e]	1500
15	ethyl hexanoate	1.2	1450	1200	98.3[e]	82
52	butanal	0.87	541	620	482[e]	550
28	(2E,6Z)-nona-2,6-dienal	0.0045	2.39	530	0.488[e]	110
1b	(2S)-2-(methylsulfanyl)butane	1.4[f]	666[g]	480	≤0.05[h]	<1
1a	(2R)-2-(methylsulfanyl)butane	0.7	329[g]	470	≤0.05[h]	<1
19	2-acetyl-1-pyrroline	0.053	23.8	450	5.41[e]	100
5	ethyl butanoate	0.75	293	390	1370[e]	1800
16	octanal	3.4	1270	370	1130[e]	330
50	dimethyl sulfide	0.30	111	370	55.0[e]	180
53	2-methylbutanal	1.5	532	350	248[e]	170
7	ethyl 2-methylbutanoate	0.13	21.7	170	25.1[e]	190
51	2-methylpropanal	0.49	80.6	160	689[e]	1400
9	hexanal	2.4	368	150	700[e]	290
55	2-(methylsulfanyl)propane	1.9	210	110	≤0.5[h]	<1
33	3-methylbutanoic acid	490	40 700	83	101 000[e]	210
3	butane-2,3-dione	0.96	67.3	70	61.7[e]	64
4	methyl 3-methylbutanoate	2.2	101	46	613[e]	280
17	3-hydroxybutan-2-one	590	21 200	36	44 600[e]	75
43	HDMF[i]	87	2920	34	3100[e]	36
13	3-methylbutan-1-ol	220	6950	32	5460[e]	25
21	nonanal	2.8	48.7	17	29.3[e]	10
23	3-(methylsulfanyl)propanal	0.43	5.61	13	12.1[e]	28
11	butan-1-ol	590	6060	10	478[e]	<1
24	decanal	9.3	78.3	8.4	15.8[e]	1.7
18	1-octen-3-one	0.016	0.126	7.9	0.547[e]	34
25	3-isobutyl-2-methoxypyrazine	0.0062	0.0391	6.3	0.0160[e]	2.6
32	phenylacetaldehyde	5.2	25.9	5.0	9.53[e]	1.8
6a	(2R)-2-(methylsulfanyl)pentane	1.3	5.65[g]	4.3	≤0.1[h]	<1
27	linalool	0.58	2.45	4.2	0.569	<1
22	acetic acid	5600	23 300	4.2	9940[e]	1.8
26	(2E)-non-2-enal	0.19	0.780	4.1	0.521[e]	2.8
2	ethyl 2-methylpropanoate	0.089	0.291	3.3	6.14[e]	69
46	ethyl (2E)-3-phenylprop-2-enoate	1.7	5.69	3.3	7.53[e]	4.4
39	2-methoxyphenol	0.84	2.69	3.2	0.338[e]	<1
40	ethyl 3-phenylpropanoate	2.1	5.66	2.7	66.1[e]	31
6b	(2S)-2-(methylsulfanyl)pentane	3.3[f]	8.14[g]	2.5	≤0.1[h]	<1
14	2-methylbutan-1-ol	1200	2300	1.9	2190[e]	1.8
41	2-phenylethanol	140	227	1.6	103[e]	<1
38	hexanoic acid	4800	5770	1.2	1560[e]	<1
49	vanillin	53	49.1	<1	1.87[e]	<1
47	sotolon	1.7	1.37	<1	5.64[e]	3.3
48	2-phenylacetic acid	68	49.9	<1	291[e]	4.3
29	MDMF[j]	56	29.9	<1	0.143	<1
42	γ-octalactone	6.5	1.75	<1	1.04[e]	<1
56	3-methylbutyl acetate	7.2	1.78	<1	111[e]	15
31	butanoic acid	2400	548	<1	1110[e]	<1
57	butyl acetate	27	4.97	<1	153[e]	5.6
45	4-methylphenol	3.9	0.633	<1	0.427[e]	<1
12	methyl hexanoate	90	10.4	<1	0.414[e]	<1

[a]Numbering according to Table 1 and 2. [b]Orthonasal odor threshold values in water. [c]Mean of duplicates, triplicates, or quadruplicates; individual data and SDs are supplied in the Supporting Information, Tables S2 and S3. [d]OAV, odor activity value; calculated as the ratio of concentration to odor threshold value. [e]Data were taken from our previous paper on jackfruit odorants.[2] [f]The (2*S*)-isomer was not available in sufficient purity for direct odor threshold value determination; the odor threshold value in water was approximated from the odor threshold of the (2*R*)-isomer in water and the ratio of the odor thresholds of both enantiomers in air.[7] [g]The concentrations of the (*R*)- and (*S*)- enantiomers were calculated from the concentration of the sum of both enantiomers as obtained from the quantitation experiments and the enantiomeric distribution in cempedak pulp reported previously.[7] [h]The presence of the compound could not be verified by GC−MS quantitation; the limit provided was obtained by integrating the background noise. [i]HDMF; 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one. [j]MDMF; 4-methoxy-2,5-dimethylfuran-3(2*H*)-one.

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J. Agric. Food Chem. 2020, 68, 258−266

cempedak aroma. Ethyl 3-methylbutanoate and 3-methylbutanal had also been reported with high OAVs in the cempedak fruits investigated by Buttara et al.[6] Our study additionally revealed high OAVs for fruity smelling ethyl hexanoate (**15**; OAV 1200), malty smelling butanal (**52**; OAV 620), cucumber-like smelling (2*E*,6*Z*)-nona-2,6-dienal (**28**; OAV 530), the two oniony, sulfury, and cempedak-like smelling enantiomers (2*S*)-2-(methylsulfanyl)butane (**1b**; OAV 480) and (2*R*)-2-(methylsulfanyl)butane (**1a**; OAV 470), cooked rice-like smelling 2-acetyl-1-pyrroline (**19**; OAV 450), fruity smelling ethyl butanoate (**5**; OAV 390), citrusy smelling octanal (**16**; OAV 370), asparagus-like smelling dimethyl sulfide (**50**; OAV 370), malty smelling 2-methylbutanal (**53**; OAV 350), fruity smelling ethyl 2-methylbutanoate (**7**; OAV 170), malty smelling 2-methylpropanal (**51**; OAV 160), green grassy smelling hexanal (**9**; OAV 150), and oniony, sulfury, and cempedak-like smelling 2-(methylsulfanyl)propane (**55**; OAV 110) (Figure 2). Besides these 16 compounds with the highest OAVs, 9 odorants showed OAVs below 100, but higher than 10, 16 more compounds showed OAVs between 1 and 10, and 10 compounds were present in concentrations below their odor threshold values (OAV < 1).

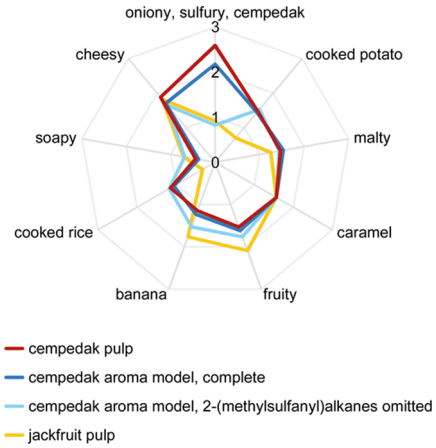

Figure 1. Olfactory profile of the cempedak aroma model in comparison to the olfactory profiles of cempedak pulp, jackfruit pulp, and the cempedak aroma model from which 2-(methylsulfanyl)-alkanes were omitted. Panelists rated the intensity of each descriptor on a scale from 0 to 3 and 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

In summary, the OAV data suggested that the fruity note in the aroma of cempedak is predominantly caused by ethyl 3-methylbutanoate (**8**; OAV 410 000), whereas the malty note is mainly due to 3-methylbutanal (**54**; OAV 1700), butanal (**52**; OAV 620), and 2-methylbutanal (**53**; OAV 350). 3-Methylbutanoic acid (**33**; OAV 83) might contribute to the cheesy note. The characteristic oniony and sulfury note, however, could be predominantly assigned to (2*S*)-2-(methylsulfanyl)butane (**1b**; OAV 480), (2*R*)-2-(methylsulfanyl)butane (**1a**; OAV 470), and 2-(methylsulfanyl)propane (**55**; OAV 110), whereas (2*R*)-2-(methylsulfanyl)pentane (**6a**; OAV 4.3) and (2*S*)-2-(methylsulfanyl)pentane (**6b**; OAV 2.5) were found to be much less potent.

The crucial role of the 2-(methylsulfanyl)alkanes could further be confirmed when the OAV data obtained for cempedak pulp odorants were compared to the data obtained

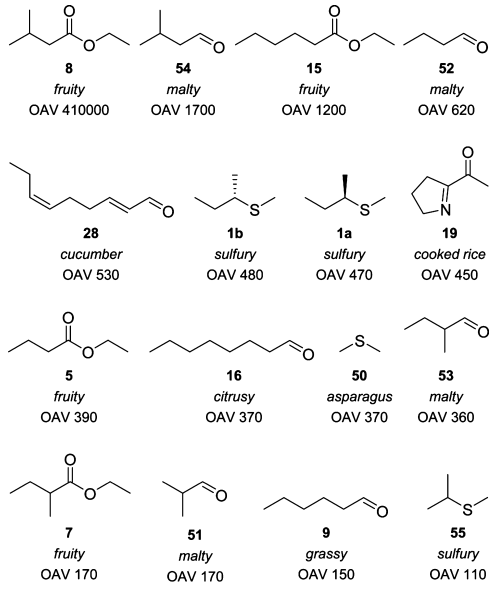

Figure 2. Most potent odorants (OAV 110−410 000) in cempedak pulp.

for jackfruit pulp odorants. For a comprehensive comparison, the cempedak pulp odorants 2-(methylsulfanyl)butane (**1**), 2-(methylsulfanyl)pentane (**6**), linalool (**27**), 4-methoxy-2,5-dimethylfuran-3(2H)-one (**29**), and 2-(methylsulfanyl)-propane (**55**), which had not been detected in jackfruit recently,[2] were additionally quantitated in jackfruit pulp. Results (Table 3) revealed higher OAVs in cempedak pulp for a majority of the compounds, namely, 27 out of 51, whereas 17 compounds showed higher OAVs in jackfruit pulp and 7 compounds did not show a difference between cempedak and jackfruit. Moreover, among the compounds with clearly higher OAVs in cempedak pulp than in jackfruit pulp were major odor-active compounds such as ethyl 3-methylbutanoate (**8**; OAVs 410 000 vs 74 000), ethyl hexanoate (**15**; OAVs 1200 vs 82), (2*E*,6*Z*)-nona-2,6-dienal (**28**; OAVs 530 vs 110), and 2-acetyl-1-pyrroline (**19**; OAVs 450 vs 100). This corresponded well with the higher overall aroma intensity of cempedak pulp. On the other hand, some important compounds also showed higher OAVs in jackfruit pulp than in cempedak pulp. These compounds included ethyl butanoate (**5**; OAVs 1800 vs 390), 2-methylpropanal (**51**; OAVs 1400 vs 160), and methyl 3-methylbutanoate (**4**; OAVs 280 vs 46). The jackfruit pulp odorants 3-methylbutyl acetate (**56**; OAV 15) and butyl acetate (**57**; OAV 5.6)[2] appeared in the cempedak pulp in concentrations below their odor threshold values. This well agreed with the fact that these compounds were not detected during odorant screening in the cempedak pulp. As expected, the most pronounced difference in the OAVs between cempedak pulp and jackfruit pulp was obtained for the 2-(methylsulfanyl)alkanes. Neither of the five compounds (**1a**, **1b**, **6a**, **6b**, and **55**) was present in odor-active amounts in jackfruit.

Aroma Reconstitution and Omission Testing. To verify the analytical data, an aroma reconstitution experiment was performed. An aroma model solution was prepared based on a buffered (pH 5.5) aqueous matrix containing sugars and organic acids. The model included all 41 odorants for which OAVs ≥1 had been determined in cempedak pulp in their natural concentrations (cf. Table 3). The aroma model

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J. Agric. Food Chem. 2020, 68, 258−266

solution was orthonasally compared to fresh cempedak pulp in a quantitative olfactory profile analysis by using a trained sensory panel and nine descriptors characterizing cempedak pulp aroma. An incomplete cempedak aroma model solution from which the 2-(methylsulfanyl)alkanes were omitted was additionally included in the sensory test. A fourth sample consisted of fresh jackfruit pulp.

Results (Figure 1) showed good agreement between the olfactory profile of the complete cempedak aroma model solution and the olfactory profile of fresh cempedak pulp. This provided final evidence that all major compounds contributing to cempedak pulp aroma were correctly identified and quantitated, even though some compounds detected during AEDA remained unidentified and thus were not included in the model solution. The olfactory profile of the incomplete aroma model solution without the 2-(methylsulfanyl)alkanes was clearly lower in the oniony, sulfury odor note considered to be typical in the aroma of cempedak. The intensity of this note in the olfactory profile of the incomplete cempedak pulp aroma model was close to its intensity in the olfactory profile of jackfruit pulp.

In summary, application of AEDA and static headspace dilution analysis to cempedak pulp volatiles, quantitation of potent odorants, and calculation of OAVs provided a comprehensive insight into the compounds contributing to cempedak pulp aroma. Data suggested 2-(methylsulfanyl)-propane and 2-(methylsulfanyl)butane to be responsible for the characteristic sulfury, oniony note in the aroma of cempedak. Finally, aroma reconstitution in combination with an omission test confirmed that the 2-(methylsulfanyl)alkanes are key odorants in cempedak pulp and vitally contribute to the aroma difference between cempedak pulp and jackfruit pulp.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.9b06564.

> Quantifier ions and calibration line data used in the quantitations; individual concentration data used for mean calculations and standard deviations (SDs); mean intensity ratings and SDs in the quantitative olfactory profile analyses; and GC−FID chromatogram and FD chromatogram of the cempedak volatile isolate (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AEDA, aroma extract dilution analysis; FD factor, flavor dilution factor; FID, flame ionization detector; GC−O, gas chromatography−olfactometry; GC−MS, gas chromatography−mass spectrometry; GC × GC−MS, comprehensive two-dimensional gas chromatography; HDMF, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one; MDMF, 4-methoxy-2,5-dimethylfuran-3(2*H*)-one; ID, inner diameter; OAV, odor activity value; RI, retention index; SAFE, solvent-assisted flavor evaporation

■ NOMENCLATURE

2-acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethanone; linalool, 3,7-dimethylocta-1,6-dien-3-ol; γ-octalactone, 5-butyldihydro-2(3*H*)-furanone; sotolon, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one; vanillin, 4-hydroxy-3-methoxybenzaldehyde

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8.3.3 Summary and Individual Contributions

The cempedak tree, *Artocarpus integer* (Thunb.) Merr., is an evergreen tree in the mulberry family Moraceae and is closely related to the jackfruit tree, *Artocarpus heterophyllus* Lam. The aroma of cempedak pulp is similar to the aroma of jackfruit pulp. However, in addition to the sweet, fruity, malty, and cheesy odor notes of jackfruit pulp, a very unique sulfury, oniony odor note is present in the aroma of cempedak pulp. 2-(Methylsulfanyl)butane and 2-(methylsulfanyl)pentane were suggested to cause this unique odor note. The aim of the study was to reinvestigate the odor-active compounds in cempedak pulp and to clarify the role of 2-(methylsulfanyl)alkanes for the aroma difference between cempedak pulp and jackfruit pulp.

AEDA of a cempedak pulp volatile isolate obtained by solvent extraction and SAFE revealed 49 odorants with FD factors ranging from 1 to ≥8192. 39 of the odorants could be assigned a structure. The odor of two compounds represented the characteristic oniony, sulfury odor note of cempedak pulp. According to mass spectral data, they were identified as 2-(methylsulfanyl)butane and 2-(methylsulfanyl)pentane. High FD factors were determined for ethyl 3-methylbutanoate, 2-acetyl-1-pyrroline, HDMF, a sulfury smelling unknown compound, methyl hexanoate, and 3-methylbutanoic acid. Additional six compounds were identified by static headspace dilution analysis. Among them, the highest FD factors were obtained for dimethyl sulfide and 2-/3-methylbutanal. Moreover, 2-(methylsulfanyl)propane was detected during headspace GC-O analysis. This odorant also exhibited the characteristic sulfury, oniony odor note of cempedak pulp and was identified for the first time in cempedak pulp. To substantiate the results of the AEDA, the structurally identified odorants in the cempedak pulp and the jackfruit pulp were quantitated in cempedak pulp using stable isotopically substituted odorants as internal standards and OAVs were calculated. The concentrations of 41 odorants exceeded their respective OTVs, whereas OAVs <1 were calculated for ten odorants. OAV data suggested that the fruity odor note in the aroma of cempedak pulp was mainly caused by ethyl 3-methylbutanoate, the malty odor note was associated with 3-methylbutanal, butanal, and 2-methylbutanal, while 3-methylbutanoic acid accounts for the cheesy odor note. The characteristic oniony, sulfury odor note of cempedak pulp could predominately be caused by 2-(methylsulfanyl)propane, 2-(methylsulfanyl)-butane, and 2-(methylsulfanyl)pentane. The comparison of the OAV data of cempedak pulp and jackfruit pulp revealed higher OAVs in cempedak pulp for the majority of the compounds, namely 27 compounds, whereas 17 compounds showed higher OAVs in jackfruit pulp. OAVs at the same level were obtained for seven compounds. The most pronounced differences in the OAVs between cempedak pulp and jackfruit pulp were obtained for the 2-(methylsulfanyl)alkanes. In contrast to cempedak pulp, none of these compounds occurred in odor-active amounts in jackfruit pulp. The olfactory profile analysis of a cempedak pulp aroma model solution and fresh cempedak pulp confirmed that all odorants contributing to the aroma of cempedak pulp had been correctly identified and quantitated. Finally, an omission experiment proved that the 2-(methylsulfanyl)alkanes were key odorants in cempedak pulp and contributed to the difference between cempedak pulp and jackfruit pulp.

Johanna Grimm designed and conducted the experiments including the volatiles isolation, the GC-O screenings, the structure assignments, the quantitations, the OAV calculations, the reconstitution experiments, and the omission experiments. Furthermore, Johanna evaluated the resulting data and prepared the manuscript. Martin Steinhaus conceived and directed the study, supervised Johanna's work and revised the manuscript. He also participated in the GC-O analyses and the sensory tests.

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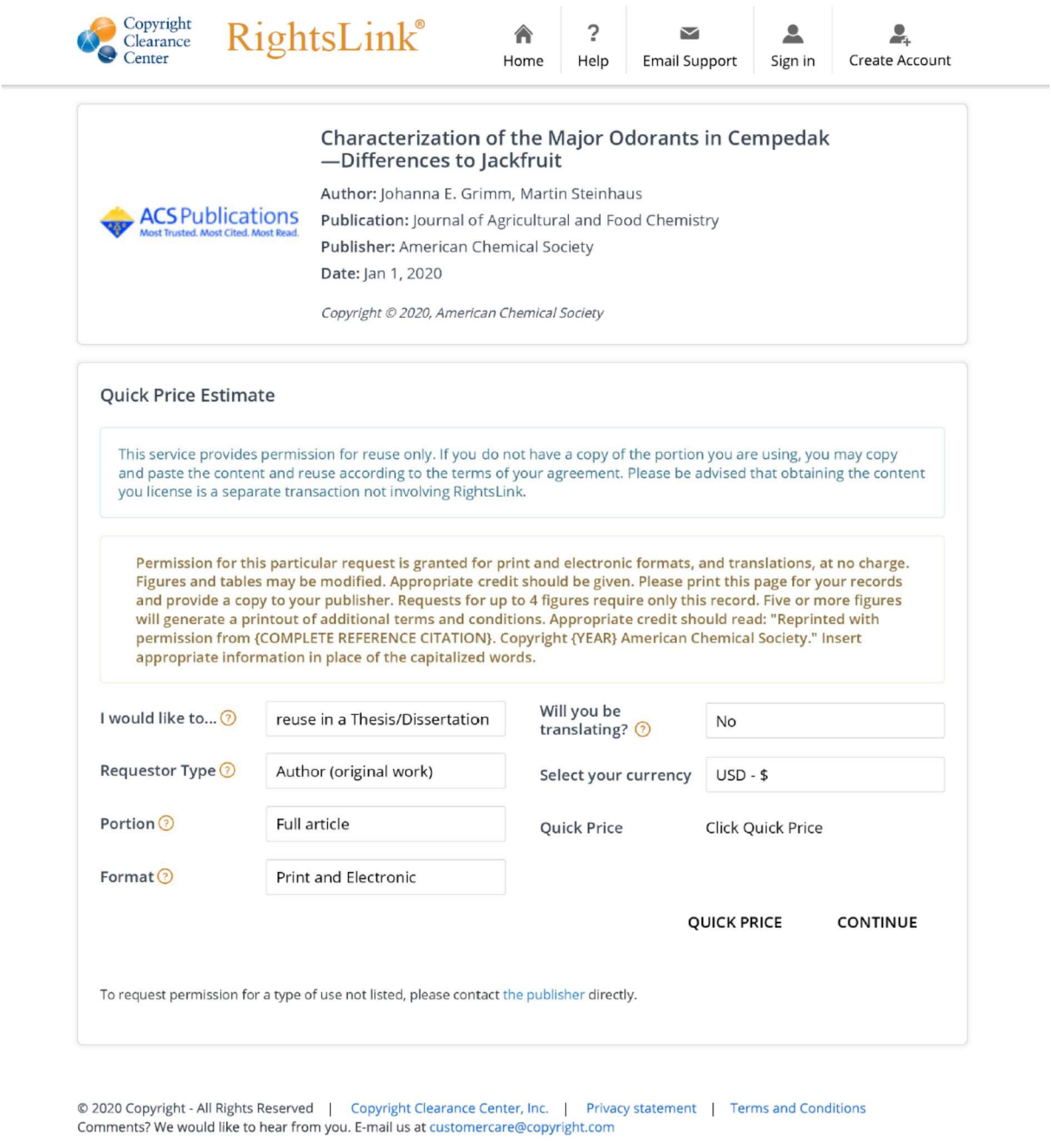

Characterization of the Major Odorants in Cempedak —Differences to Jackfruit

Author: Johanna E. Grimm, Martin Steinhaus
Publication: Journal of Agricultural and Food Chemistry
Publisher: American Chemical Society
Date: Jan 1, 2020

8.4 List of Publications, Poster Presentations, and Oral Presentations

Publications

Publications in Peer Reviewed Journals:

Grimm, J. E.; Steinhaus, M. Characterization of the major odor-active compounds in jackfruit pulp. *J. Agric. Food Chem.* **2019**, *67*, 20, 5838–5846. DOI: 10.1021/acs.jafc.9b01445

Grimm, J. E.; Steinhaus, M. Characterization of the major odorants in cempedak – differences to jackfruit. *J. Agric. Food Chem.* **2020**, *68*, 1, 256–266. DOI: 10.1021/acs.jafc.9b06564

Annual Reports and Conference Proceedings:

Steinhaus, M.; Grimm, J. E. Characteristic chiral sulfur compounds aroma-active in cempedak (*Artocarpus integer* (Thunb.) Merr.) fruits. In: Importance of Chirality to Flavor Compounds; ACS Symposium Series; American Chemical Society, 2015; Vol. 1212, pp 123–133. DOI: 10.1021/bk-2015-1212.ch009

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Grimm, J. E.; Li J.-X.; Steinhaus, M. Structure-odor relation in homologous series of dithio(hemi)acetals. In: Flavour Science, Proceedings of the XV Weurman Flavour Research Symposium, 18.–22. September 2017, Graz University of Technology, Austria. Siegmund, B.; Leitner, E., Eds.; Verlag der Technischen Universität Graz: Graz, 2018, pp 213–216. ISBN: 978-3-85125-593-5

Further Journal Contribution:

Grimm, J. E., Steinhaus, M. Aromaaktive Verbindungen in Cempedak – Unterschied zu Jackfrucht (aroma-active compounds in cempedak – difference to jackfruit). *Lebensmittelchemie* **2016**, *70*, 139. DOI: 10.1002/lemi.201690037

Scholarly Work:

Grimm, J. E. Aromaaktive Verbindungen in Cempedak (*Artocarpus integer* (Thunb.) Merr.) und Jackfruit (*Artocarpus heterophyllus* Lam.) (aroma-active compounds in cempedak (*Artocarpus integer* (Thunb.) Merr.) and jackfruit (*Artocarpus heterophyllus* Lam.). Abschlussarbeit, Technische Universität München, Freising, Germany, 2014.

Poster Presentation:

Grimm, J. E.; Li J.-X.; Steinhaus, M. Structure-odor relation in homologous series of dithio(hemi)acetals. Graz University of Technology, 15th Weurman Flavour Research Symposium. Graz, Austria, September 18–22, 2017.

Oral Presentation:

Aromaaktive Verbindungen in Cempedak – Unterschied zu Jackfrucht (aroma-active compounds in cempedak – difference to jackfruit). Lebensmittelchemische Gesellschaft (LChG), Fachgruppe in der Gesellschaft Deutscher Chemiker (GDCh), 67. Arbeitstagung des Regionalverbands Bayern (German Society of Food Chemistry, a division of German Chemical Society, 67th Bavarian Regional Meeting). Erlangen, Germany, March 10, 2016.