Formation of Volatile Branched Chain Esters in Bananas (*Musa sapientum* L.)

S. Grant Wyllie*,† and John K Fellman‡

Centre for Biostructural and Biomolecular Research, University of Western Sydney, Hawkesbury, Richmond, New South Wales 2753, Australia, and School of Horticulture and Landscape Architecture, Washington State University, Pullman, Washington 99164

Substrates controlling the formation of branched chain volatile esters in ripening bananas were investigated by the application of alcohol and amino acid precursors to whole fruit and tissue samples. The resulting changes in the profile of the volatile esters were determined using SPME and GC. These changes revealed the selectivity characteristics of the esterification enzyme AAT, the availability of acyl CoA's for ester formation, and the role of substrate supply on volatile production. The results obtained suggest that substrate supply is a major determinant of the quantitative and qualitative composition of the resulting aroma profile.

Keywords: *Banana ripening; branched chain esters; alcohol acyltransferase; substrate supply; aroma profile; Musa sp.*

INTRODUCTION

The biosynthetic pathway for the formation of volatile esters in ripening climacteric fruits is well-established (Rowan et al., 1996; Sanz et al., 1997; Schreier, 1984). However, the factors that control the qualitative and quantitative composition of the ester profile, factors that in many cases determine the character and perceived quality of the fruits, are not yet fully understood. Fruit esters are formed by the reaction between alcohols and acyl CoA's derived from both fatty acid and amino acid metabolism. This reaction is catalyzed by the enzyme, acyl alcohol transferase (AAT) (Sanz et al., 1997; Perez et al., 1996; Ueda et al., 1992). The characteristic branched chain carbon skeletons found in many volatile fruit esters can be derived from either of the branched chain amino acids leucine, isoleucine, or valine. A typical biochemical scheme for the biosynthesis of esters containing such carbon skeletons is shown in Figure 1.

The first step is catalyzed by an aminotransferase and produces a branched chain α-keto acid that in turn is transformed into either a branched chain alcohol or an acyl CoA. These substrates then contribute to an acyl CoA and alcohol pool that is utilized to produce esters via the enzyme AAT. The actual composition of the resulting esters could be controlled either by the selectivity of the enzymes involved or by the availability of the necessary substrates in this pool. It has been suggested that the selectivity properties of the enzymes controlling the reduction (ADH) and ester formation steps (AAT) of ester biosynthesis do not provide the specificity required to explain the composition of the branched chain esters obtained from bananas (Wyllie et al., 1996; Ueda et al., 1992). The specificity must therefore be determined by the properties of the remaining enzymes of the pathway, aminotransferase and α-keto acid decarboxylase, or by the availability of the necessary substrates.

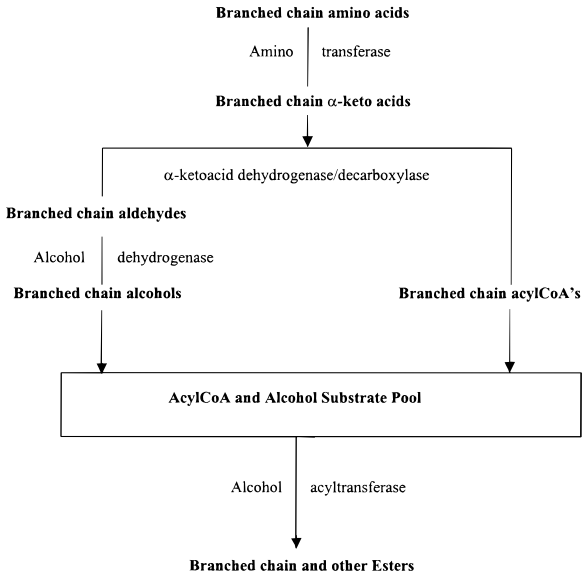

Figure 1. Scheme for the conversion of branched chain amino acid into esters containing branched chain carbon skeletons.

The qualitative and quantitative control of the volatile ester profile plays a major role in determining the characteristic aroma of the fruit and is presumably under genetic control (Wyllie et al., 1992). Rowan et al. (1996) have shown, for example, that there are significant differences in products and product distributions between two apple cultivars provided with the same substrate. This work investigates the role of substrate availability and enzyme selectivity in determining the composition of the volatile esters formed in ripening bananas.

MATERIALS AND METHODS

Fruit. Mature green bananas (*Musa sapientum* L. [AAA group, Cavendish subgroup] cv. Williams) were obtained from

* Corresponding author telephone: +61 2 4570 1632. Fax: +61 2 4570 1671. E-mail: g.wyllie@uws.edu.au.
† University of Western Sydney.
‡ Washington State University.

10.1021/jf0001841 CCC: $19.00
Published on Web 07/12/2000

a commercial source. None of the bananas used were exposed to ethylene to initiate ripening.

SPME Analysis. All volatile analysis was carried out using a solid-phase microextraction device (SPME) (Supelco Co.) equipped with a poly(dimethylsiloxane) (PDMS) fiber (100 μm). In all analyses the SPME needle was inserted into the headspace of the container, and the fiber was exposed for 15 min before injection.

GC/MS Analysis of Volatiles. GC/MS analysis was carried out on a HP 5970 GC/MS fitted with a DB-1 (J&W), fused silica capillary column (60 m × 0.32 mm i.d. × 0.25 μm film thickness). The injector, at a temperature of 200 °C, was maintained in the splitless mode for the first 2 min of the injection. The column was maintained at 35 °C for 3 min and then programmed at 8 °C min^{-1} to 225 °C. The transfer line was maintained at 250 °C. Data were collected using a Hewlett-Packard Chemstation G 1034C data processing package.

Treatment of Whole Bananas with Alcohol Precursors. A whole post-climacteric banana assessed as color stage 5 ripeness (CSIRO, 1971) was placed in a glass chamber (~4 L) and ventilated with purified air at a flow rate of ~10 mL min^{-1}. A silicone rubber septum in the lid permitted headspace sampling by the SPME. After the system had been at equilibrium for 1 h, a control headspace sample was taken. The alcohol precursor (5 μL) was then added via the septum, and the incubation continued for an additional hour at room temperture, after which time the headspace was again sampled.

Treatment of Banana Tissue Disks with Alcohol Precursors. Pulp tissue disks from the center section of a single fruit (color stage 5 fruit were selected) (~5 g; 3 mm thick) were placed in a conical flask (50 mL) and sealed with a rubber seal. The precursor alcohol (2 μL) was injected into the headspace, and the sample was incubated at room temperature for 1 h. The headspace was then sampled using SPME. A control flask was used for reference.

Incubation of Banana Tissue Disks with Amino Acids. A single banana (color stage 5) was sliced laterally into disks approximately 3 mm thick, and the resulting disks, consisting of both skin and pulp, were weighed into glass containers (~250 mL) such that each container contained ~10 g of banana. The skin was retained to help maintain the integrity of the pulp over the long incubation times used in these experiments. Sufficient phosphate buffer (0.8 M, pH 7) containing 1 mg/mL L-amino acid (Sigma) was added to each chamber to just cover the fruit disks (~10 mL). The chambers were sealed, and a flow of ~5 mL min^{-1} of purified air passed through each. The chambers were fitted with a septum to enable SPME headspace sampling while the air flow was maintained. Volatiles in the headspace of the chamber were sampled at approximately 24-h intervals for up to 72 h. Experiments with isoleucine as substrate were reproduced using three different bananas.

RESULTS AND DISCUSSION

Esterification Reaction. The relative reactivity of a range of alcohols and the availability of the acyl CoA's involved in the ester-forming reaction in bananas was ascertained in situ, by exposing post-climacteric whole fruit or fruit pulp disks to a range of alcohol precursors. The resulting volatile esters profile was analyzed using solid phase microextraction (SPME) and gas chromatography. The use of the alcohol 2-methylbutanol was particularly revealing because 2-methylbutyl carbon skeletons are not found in significant concentrations in banana esters (Macku and Jennings, 1987; Shioata, 1993). The use of this precursor coupled with an analytical method that separates 2-methyl and 3-methylbutyl esters provided a means of estimating the selectivity of AAT toward this particular substrate pair in situ and also measuring the extent of esterification without interference from endogenous compounds. Figure 2 shows a chromatogram of the aroma profile obtained from a whole banana (color stage 5, evenly yellow) after the application of 2-methylbutanol for 1 h. The incorporation of the 2-methylbutyl moieties into the ester profile is clearly shown by the production of large quantities of 2-methylbutyl acetate and 2-methylbutyl isovalerate. This demonstrates both that AAT is not specific for 3-methylbutanol and that the amount of ester produced in the ripening banana fruit appears to be limited by the supply of precursor alcohol.

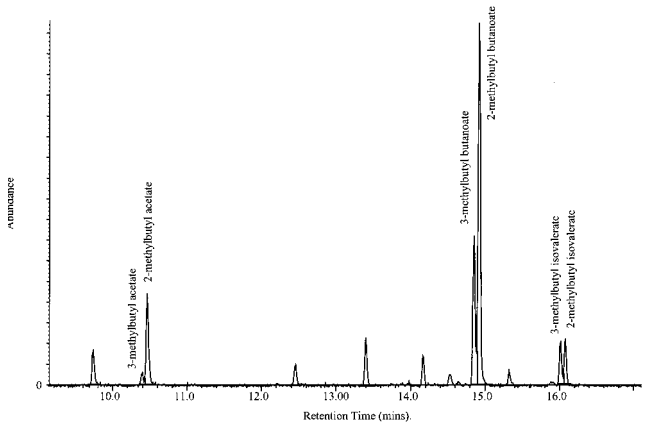

Figure 2. GC/MS aroma profile of a whole banana treated with 2-methylbutanol showing the formation of 2-methylbutyl esters not normally found in this fruit.

Similar experiments with the alcohols ethanol, 1-propanol, 1-butanol, 1-pentanol, 2-pentanol, and 1-hexanol showed that each of these alcohol precursors were capable of being utilized by banana fruit for volatile ester formation. Ethanol and propanol were clearly the least preferred substrates, and the five and six carbon alcohols were the most reactive of those tested (data not shown).

Analysis of all of the exogenous esters formed during treatment with the various alcohols revealed the range of acyl CoA's available in the banana acyl CoA pool at the time of the experiment. It contained predominantly acetyl CoA, butanoyl CoA, and 3-methylbutanoyl CoA. Indeed these three acyl CoA's together with the alcohols ethanol, 2-methylpropanol, 1-butanol, 3-methylbutanol, and 2-pentanol can be seen to provide all the precursors necessary for the formation of the major esters observed in the aroma profile of bananas of this cultivar (Golding et al., 1999). It seems highly likely that this group of compounds constitutes the principal components of the acyl CoA−alcohol pool in this fruit. However, it is possible that the ester profile observed over fruit or especially fruit tissue was influenced by the selective hydrolysis of biosynthesized esters by extracellular esterases during or after emergence from the tissue. However, comparison of the headspace volatiles from a whole banana and from disks taken from the same banana showed only minor quantitative changes in composition, suggesting that esterase activity is not high in ripe bananas.

The results obtained however are consistent with previous observations (Wyllie et al., 1996) that banana AAT is not highly selective for specific alcohol substrates. The control of the composition of the banana volatile ester profile must therefore lie either with the availability of suitable substrates or with the selectivity properties of the aminotransferase and α-keto acid decarboxylase enzymes at the beginning of the biosynthetic pathway.

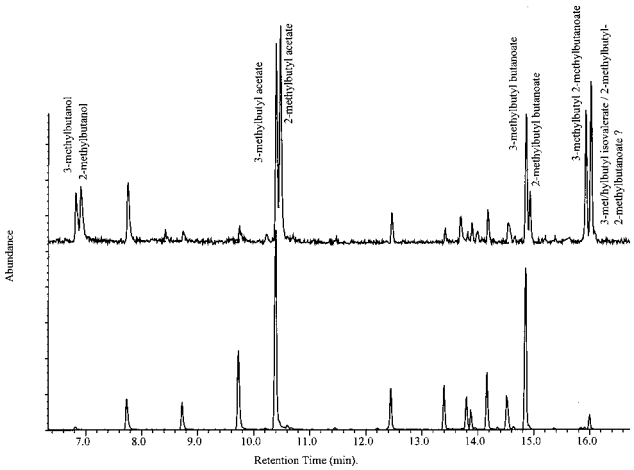

Figure 3. GC/MS aroma profile from banana disks incubated with L-isoleucine for 48 h (upper trace). Lower trace is from control banana with no added L-isoleucine.

Ester Substrate Supply. Within the fruit, the supply of substrate branched-chain alcohols and acyl CoA's will be dependent on their biosynthesis from the appropriate branched chain amino acids. The banana ester profile has many 3-methylbutyl esters arising from transformations beginning with leucine and a smaller number of 2-methylpropyl esters arising from valine. The concentration of these particular amino acids together with that of alanine has been shown to increase markedly during the climacteric of bananas (Yoshioka et al., 1982; Tressl and Drawert, 1973). Increases in the concentration of specific amino acids has also been observed during the ripening of other fruits (Wang et al., 1996; Perez et al., 1992). This suggests that the availability of branched chain skeletons may be controlled by the supply of amino acids that become available during ripening, either by amino acid biosynthesis or possibly from protein degradation. Thus, a major determinant of the branched chain volatile ester profile of a ripening banana will be the composition of the amino acid pool available. This conclusion was confirmed by the incubation of postclimacteric banana disks with a range of L-amino acids. After 48 h of incubation with L-isoleucine, a number of esters containing the 2-methylbutyl carbon skeleton (for example, 2-methylbutanol, 2-methylbutyl acetate, 2-methylbutyl butanoate, and 3-methylbutyl 2-methylbutanoate) were apparent in the aroma profile. (Figure 3). The appearance of 3-methylbutyl 2-methylbutanoate shows that the isoleucine is being utilized as a source for both alcohol and acyl CoA production. There is also a significant increase in the concentration of the final peak in the chromatogram identified as either 3-methylbutyl isovalerate or 2-methylbutyl 2-methylbutanoate. Since the analysis system used was unable to effectively distinguish these two isomers, it is not possible to attribute this increase definitively to 2-methylbutyl 2-methylbutanoate formation, although based on analogy this is the most likely explanation. The demonstration that isoleucine can be readily utilized by post-climacteric banana tissue shows that the transaminase/α-keto acid dehydrogenase/decarboxylase enzymes are not highly selective, at least between the leucine−isoleucine pair, and suggests that there is a direct link between substrate availability and the branched chain ester profile. These results are consistent with those of Hansen and Poll (1993), who demonstrated increased production of volatiles after the administration of isoleucine to intact Granny Smith apples. They were however unable to distinguish between the production of 2- or 3-methylbutyl isomers and thus validate the conversion of isoleucine directly.

The ready production of additional volatile esters after exposure of post-climacteric bananas to exogenous alcohols shows that there is an ample supply of acyl CoA's available from both amino acid and fatty acid metabolism. The even-carbon C2, C4, and to a lesser extent C6 straight chain acyl CoA's, derived from, for example, fatty acid β-oxidation are among the most important sources of the acid moiety of the esters. However, the presence of butyl acetate and butyl butanoate show that these precursors can also be converted to alcohols before being esterified.

CONCLUSION

The qualitative and quantitative composition of the volatile branched chain esters formed during ripening of banana fruit appears to be determined by two factors. One is the selectivity of the enzymes involved in the transformation of amino acids to either alcohols or acyl CoA's. The selectivity of the final enzyme, AAT, has been studied for a small number of fruit types using partially purified enzyme preparations, and it has been suggested that this property determines the ester profile of strawberries (Perez et al., 1996). However, in other fruit (Ueda et al., 1992; Wyllie et al., 1996) the observed selectivity of the AAT has not been sufficient to explain the specificity of the ester profiles obtained.

The other important factor determining the composition of branched chain esters is the supply of specific suitably functionalized substrates from the fatty acid and amino acid metabolic pathways that are active during ripening. The results obtained indicate that in bananas this control of substrate supply dominates the various processes that contribute to the formation of branched chain esters and hence determines their qualitative and quantitative composition. The composition of the substrate pool will be dependent on many factors including genetic control, the physiological state of the fruit, and any other influences that determine the balance of the metabolic pathways contributing to the pool. For example, it has been suggested that the change in aroma profile found in 1-MCP-treated bananas could be caused by a suppression of fatty acid metabolism associated with the decreased respiration brought on by this treatment (Golding et al., 1998). Similarly Macku et al. (1987) showed that the ratio of total acetates to total butanoates in the aroma profile of bananas varies linearly with ripening time. This implies that the acetyl CoA and the butanoyl CoA originate from the same metabolic source, possibly via fatty acid β-oxidation. This in turn suggests that this pathway is the main provider of straight chain acyl CoA's for volatiles production.

Thus, any agronomic, environmental, or post-harvest changes that influence the fundamental physiological and biochemical processes occurring in ripening bananas will inevitably be reflected in the volatiles profile. A greater understanding of how the supply of ester-forming substrates is influenced by these factors should provide valuable insight into the determinants of banana flavor quality.

ACKNOWLEDGMENT

The technical assistance of D. S. Mattinson and D. Rudell is gratefully acknowledged. S.G.W. thanks the University of Western Sydney, Hawkesbury, for sabbatical leave support.

LITERATURE CITED

Commonwealth Scientific and Industrial Research Organisation. *Banana Ripening Guide*; Banana Research Advisory Committee Technical Bulletin 3; CSIRO: Melbourne, Australia, 1971.

Golding, J. B.; Shearer, D.; Wyllie, S. G.; McGlasson, W. B. Application of 1-MCP and Propylene to Identify Ethylene Dependent Ripening Processes in Mature Banana Fruit. *Postharvest Biol. Technol.* **1998**, *14*, 87−98.

Golding, J. B.; Shearer, D.; Wyllie, S. G.; McGlasson, W. B. Relationships between Respiration, Ethylene, and Aroma Production in Ripening Banana. *J. Agric. Food Chem*. **1999**, *47*, 1646−1651.

Hansen, K.; Poll, L. Conversion of L-Isoleucine into 2-Methylbut-2-enyl Esters in Apples. *Lebensm.-Wiss. Technol*. **1993**, *26*, 178−180.

Macku, C.; Jennings, W. G. Production of Volatiles by Ripening Bananas. *J. Agric. Food Chem*. **1987**, *35*, 845−848.

Perez, A. G.; Sanz, C.; Rios, J. J.; Olias, J. M. Aroma Components and Free Amino Acids in Strawberry Variety Chandler during Ripening. *J. Agric. Food Chem*. **1992**, *40*, 2232−2235.

Perez, A. G.; Sanz, C.; Olias, R.; Rios, J. J.; Olias, J. M. Evolution of Strawberry Alcohol Acyltransferase Activity during Fruit Development and Storage. *J. Agric. Food Chem*. **1996**, *44*, 3286−3290.

Rowan, D. D.; Lane, H. P.; Allen, J. M.; Fielder, S.; Hunt, M. B. Biosynthesis of 2-Methylbutyl, 2-Methyl-2-butenyl, and 2-Methylbutanoate Esters in Red Delicious and Granny Smith Apples using Deuterium-Labeled Substrates. *J. Agric. Food Chem*. **1996**, *44*, 3276−3285.

Sanz, C.; Olias, J. M.; Perez, A. G. Aroma. Biochemistry of Fruits and Vegetables. In *Phytochemistry of Fruit and Vegetables*; Tomas-Barberan, F. A., Robins, R. J., Eds.; Clarendon Press: Oxford, 1997.

Schreier, P. *Chromatographic Studies of Biosynthesis of Plant Volatiles*; Dr Alfred Huthig Verlag: Heidelberg, 1984.

Shiota, H. New Esteric Compounds in the Volatiles of Banana Fruit (*Musa sapientum* L.). *J. Agric. Food Chem*. **1993**, *41*, 2056−2062.

Tressl, R.; Drawert, F. Biogenesis of Banana Volatiles. *J. Agric. Food Chem*. **1973**, *21*, 560−565.

Ueda, Y.; Tsuda, A.; Bai, J. H.; Fujishita, N.; Chachin, K. Characteristic Pattern of Aroma Ester Formation from Banana, Melon, and Strawberry with Reference to the Substrate Specificity of Ester Synthetase and Alcohol Contents in Pulp. *Nippon Shokuhin Kogyo Gakkaishi* **1992**, *39*, 183−187.

Wang, Y.; Wyllie, S. G.; Leach, D. N. Chemical changes during the development and ripening of the fruit of *Cucumis melo* (cv. Makdimon*), J. Agric. Food Chem*. **1996**, *44*, 210−216.

Wyllie, S. G.; Leach, D. N. The Role of Sulfur Volatiles in Melon Aroma. *J. Agric. Food Chem*. **1992**, *40*, 253−256.

Wyllie, S. G.; Leach, D. N.; Nonhebel, H.; Lusunzi, I. Biochemical Pathways for the Formation of Esters in Ripening Fruit. In *Flavour Science, Recent Developments*; Taylor, A. J., Mottram, D. S., Eds.; Royal Society of Chemistry: Cambridge, 1996; pp 52−57.

Yoshioka, H.; Ueda, Y.; Iwata, T. Development of Isoamyl Acetate Biosynthetic Pathway in Banana Fruit during Ripening and Suppression in Its Development at High Temperature. Physiological Studies of Fruit Ripening in Relation to Heat Injury. Part 4. *Nippon Shokuhin Kogyo Gakkaishi* **1982**, *29*, 333−339.

Received for review February 11, 2000. Revised manuscript received May 9, 2000. Accepted May 10, 2000.

JF0001841