SPECTROSCOPIC ASSESSMENT OF ETHYLENE HORMONE PRODUCTION AT STRAWBERRY FRUITS

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In this study, the experiments were focused on the analysis of the potential of laser spectroscopy in the assessment of ethylene phytohormone that is normally produced by strawberry fruits (Fragaria anannassa). Strawberries are considered to grow as non-climacteric fruits, the role of ethylene being currently unclear in fruit ripening process. Our results conflict this perception, because we found that ethylene phytohormon was released in higher concentrations from opening strawberry flowers to green and red mature strawberry fruits.

Keywords: Laser photoacoustic spectroscopy, Ethylene phytohormone, Strawberry fruit, climacteric, non-climacteric

1. Introduction

Ethylene is a colorless gas that is naturally produced by plants and acts as a plant phytohormone. The ethylene gaseous plant phytohormone not only plays an important role in various aspects of plant growth and development, including seed germination, organ senescence, and fruit development, but also regulates stress responses to environmental challenge [1, 2].

Fruit species are classified as either climacteric or non-climacteric, depending on their response to ethylene or based on physiological differences in their ripening patterns. Climacteric fruits produce ethylene as they ripen, and the harvested product is capable of ripening during the postharvest period. These fruits, such as bananas, tomatoes, apples, and peaches, tend to become sweeter and softer after harvest. All these changes in fruits act as a key signal for the initiation and coordination of ripening in all climacteric fruits, and have been shown to regulate key genes that control color change, fruits softening, cell breakdown, pathogen defence, and nutrient composition [2-4]. Non-climacteric species, such as leafy vegetables, citrus, grape, and strawberry do not continue to ripen after harvest; they will soften and rot, but this is due to moisture loss, decay, and tissue deterioration. By definition, there is no major peak in ethylene levels or respiration during ripening of non- climacteric fruits, although more recent evidence of a transient increase in ethylene concentrations prior to the veraison in

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grape does not rule out that the ethylene may play some role during grape berry development [5, 6]. In strawberries, auxin (the first plant hormone identified), acts as an inhibitor of ripening [7].

A climacteric fruit can be picked at full size or maturity, but before it is ripen. Generally, there is an increase in flavor quality, juice, sugars and other factors. Non-climacteric fruits tend to maintain what ever quality they had at harvest without many beneficial changes. More technically, in climacteric fruits, ripening is controlled by fruits production of ethylene and a significant increase in CO_2 production. Non climacteric fruits produce little or no ethylene and no large increase in CO_2 production [8].

Some fruits are picked full sized and green in color and held under refrigeration with ethylene gas added to make them suitable for sale. It seems that much of what we know about the ripening of non climacteric fruit remains poorly understood [8, 9].

The definition of a climacteric fruit has three distinct component parts: (a) an autocatalytic increase in C_2H_4 production; (b) an associated increase in respiration, which is referred to as the respiratory climacteric; and (c) that (a) and (b) are accompanied by phenotypic (and genetic) changes in the fruit that lead them to be identified as ripe [10-12].

The division of fruits into climacteric and non-climacteric types has been very useful for postharvest physiologists. However, some fruits, for example kiwi fruit and cucumber, appear to blur the distinction between the groups. Increase in respiration rate occurs during stress and other developmental stages, but a true climacteric only occurs coincident with fruit ripening [12-14].

We used a photoacoustic technique for the detection and monitoring of ethylene at low levels. The instrument, a CO_2 laser-based photoacoustic spectroscopy (LPAS), ensures high output power in a wavelength region (9-11 μm) where more than 200 molecular gases of environmental concern for atmospheric, industrial, medical, military, and scientific spheres exhibit strong absorption bands. This laser can be only stepwise tuned when operated in cw, and is an ideal source to push the sensitivity of gas detection into the concentration range of part-per-bilion-by volume (ppbV) or even lower [15].

LPAS was used to evaluate the ethylene that is produced by strawberry fruits while they developed in plants. Strawberry fruits for ethylene hormone analysis were selected at four developmental stages: flowers, green fruits, pink and red mature fruits, respectively. We investigated the physiological response of fruits only under aerobic conditions.

2. Fruit material and experimental method

To test the strawberry fruits, we analyzed the level of ethylene by using the LPAS technique (illustrated in Fig. 1 and detailed in [15-17]), because it is one of the mostly used approaches for sensitive trace gas detection that can routinely detect trace gas quantities down to 1 ppb. Here it is used to detect ethylene emission from fruits in relation to their application.

With LPAS, ethylene can be detected in nearly real time, with high sensitivity, high speed and very good selectivity.

The use of LPAS in combination with a flow through system is proven to be unbeatable in sensitivity and time response in comparison with traditional methods.

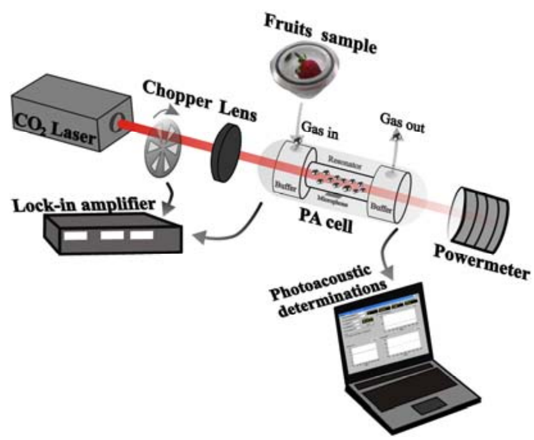

Fig. 1 Configuration of the LPAS system with the sample glass cuvette.

Traces of ethylene released by various fruit samples absorb laser radiation inside the cell and the ethylene concentration is calculated from a comparison of the photoacoustic signals on various laser emission frequencies. The assessment of ethylene was performed for one specific CO_2 laser line 10P (14) - 949.479 cm^{-1}, where we have the maximum absorption coefficient (30.4 cm^{-1} atm^{-1}) [15].

The CO_2 laser based instrument allows detection of gas emission in a continuous-flow system down to 1 ppb and the sensitivity of the technique is such that absorptions of $<10^{-7}$ cm^{-1} can be measured over path lengths of a few tens of centimeters [16, 17].

3. Results and discussion

The strawberries were sorted and evaluated at four developmental stages and expressed in grams (g): opening flowers (0.8 g), green fruits (33 g), pink fruits (33 g) and red fruits (33 g). The organic cultivation area for harvested strawberry fruits was treated only with compost of animal manure (natural fertilizers), the weeds were controlled with the help of hand weeding, crop rotation, mulching and tilling, while insects are controlled using only natural methods like birds or traps. The strawberry fruits were obtained from local farmers and transported immediately to the Laboratory for analysis of ethylene hormone using LPAS technique.

All fruits were stored at 4^0C for subsequent use. Before starting the ethylene analysis, all fruits were acclimatized over 1 h at room temperature (23 - 25^0 C) and then introduced into a small glass cuvette (with a volume of 150 cm^3) for measurements.

The fruit samples were flushed with syntethic air flow (20% oxygen and 80% nitrogen with impurities: hydrocarbons max. 0.1 ppmV and nitrogen oxides max. 0.1 ppmV) at atmospheric pressure (1024 mbar) and the resulting gas from the glass samples was transferred in the cell and analyzed.

Fig. 2 presents the production of ethylene measured experimentally for strawberry opened flowers, green strawberry fruits, pink strawberry fruits and red strawberry fruits.

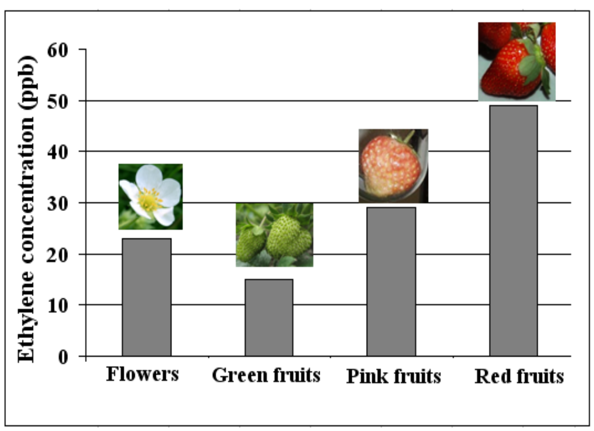

Fig. 2 Ethylene gas detection at for different developmental stages of strawberry fruits.

From Fig. 2 we can observe that ethylene release is elevated only at opened flower stage and at red fruit stage, while at green and pink fruit stage the ethylene production is relatively low. We found the highest rates of ethylene production in red strawberry fruits.

The peer-reviewed literature conflicts regarding the role of ethylene during ripening and the research has proven so far that strawberry fruit grow as a non-climacteric fruit [2, 18, 19], but our results (Fig. 2) conflict with this perception, because ethylene was released in higher concentrations from opening strawberry flowers to red strawberry fruits.

Over the past decades, the effect of ethylene on non-climacteric fruit, particularly strawberry, has been studied extensively and although a role for this hormone in strawberry fruit was suggested by pharmacological and transcriptional tests, new measurements about the effect of ethylene in non-climacteric fruit ripening were needed [20-28].

4. Conclusion

Our measurements demonstrated that a non-climacteric considered fruit determines a greater increase of the ethylene concentration in the respiration of strawberries from opening flowers to mature red fruits.

These important results imply the complexity of ethylene regulation for strawberry fruits ripening, but a causal role for ethylene has still to be done.

LPAS has allowed the accurate determination of ethylene concentrations from a range of whole ripening stages. It is a powerful spectroscopic technology for quantitative and qualitative trace gas analysis, measuring extremely low concentrations, offering a degree of confidence that cannot be attained by other methods.

The rapid development of LPAS method and its use for gas analysis shows that this technique is promising for studying the control mechanisms in plant physiology such as those responsible for germination, blossoming, senescence, ripening, healing effects after wounding or post anaerobic injury.

As future work, more tests may provide more useful data to decipher the role of ethylene in the development of strawberry as a non-climacteric fruit.

REFERENCES

[1]. *R. Fluhr, A.K Matto*, Crit. Rev. Plant Sci. **15**, 5 (1996).
[2]. *G.M. Symons, Y-J. Chua, J.J. Ross, L.J. Quittenden, N.W. Davies, J.B. Reid,* J. of Exp. Botany doi: 10.1093/jxb/ers147 (2012).
[3]. *L. Alexander, D. Grierson,* J. of Exp. Botany **53**, 2039 (2002).
[4]. J. *J. Giovani*, The plant Cell **16**, S170 (2004).
[5]. *G. Hobson, D. Grierson*, Biochemistry of fruit ripening, 405 (1993).
[6]. *G.B. Seymour, J.E Taylor, G.A. Tucker*, Biochemistry of fruit ripening (1993)
[7]. *N.K. Given, M.A. Venis, D. Gierson*, Planta **174,** 402 (1988).
[8]. *C.B. Watkins, J.F. Nock*, NYS IPM Publication **10**, Department of Horticulture, Cornell University (2012).
[9]. http://www.quisqualis.com

[10]. *P.P.M. Iannetta, L.-J. Laarhoven, N. Medina-Escobar, E.K. James, M.T. McManus, H.V. Davies, F.J. M. Harren,* Physiologia Plantarum **127**, 247 (2006).
[11]. *L. Trainotti, A. Pavanello, G. Casadoro* , J. of Exp. Botany **56**, 2037 (2005).
[12]. *Z. Lin, S. Zhong, D. Grierson*, J.of Exp.Botany **60**, 3311 (2009).
[13]. *A.B. Bleecker, H. Kende*, Annu. Rev. Cell Dev. Biol. **16**, 1 (2000).
[14]. www.postharvest.com.au
[15]. *D.C. Dumitras D. C. Dutu, C. Matei, A. M. Magureanu, M. Petrus, C. Popa,* J. Optoelectron. Adv. Mater. **9**, 3655 (2007).
[16]. *D. C. Dumitras, S. Banita, A.M. Bratu, R. Cernat, D.C.A. Dutu, C. Matei, M. Patachia, M. Petrus, C. Popa*, Infrared Phys. Technol. **53**, 308 (2010).
[17]. *C. Popa A. M. Bratu, R. Cernat, Doru C. A. Dutu, S. Banita, Dan C. Dumitras*, Laser Phys. **21**, 1336 (2011).
[18]. *K.Ji, P.Chen, L. Sun, Y. Wang, S. Dai, Q.Li, P. Li, Y. Sun, Y. Wu, C. Duan and P. Leng*, Functional Plant Biology **39,** 351 (2012).
[19]. *M.C.N. Nunes, J. K. Brecht, A. MMB Morais, S. A Sargent*, Journal of the Science of Food and Agriculture **86**, 180 (2006).
[20]. *N.M.Villarreal, C.A.Bustamante, C.M. Civello,* J Sci Food Agric **90**, 683 (2010).
[21]. *C. Chervin, A. Tira-Umphon , N. Terrier , M. Zouine, D. Severac , JP. Roustan,* Physiol Planta **134**, 534 (2008)
[22]. *A. El-Kereamy , C. Chervin , JP. Roustan*, Physiol Planta **119**, 175 (2003).
[23]. *Y. Jiang , DC. Joyce , LA. Terry*, Postharvest Biol Technol **23**, 227 (2001).
[24]. *E. Katz, PM. Lagunes, J. Riov , D. Weiss*, *E. Goldschmidt* , Planta **219**, 243 (2004).
[25]. *M.S. Tian , S.Prakash , H.J. Elgar , H. Young , D.M. Burmeister , G.S. Ross*, Plant Growth Regul **32**, 83 (2000).
[26]. *L. Trainotti , A. Pavanello , G. Casadoro*, J. Exp. Bot. **56**, 2037 (2005).
[27]. *W.P. Wechter , A. Levi , K.R. Harris, A.R. Davis , Z. Fei, N. Katzir, J.J. Giovannoni , A.S.-Minkov A, A. Hernandez, J. Thimmapuram, Y. Tadmor , V. Portnoy, T. Trebitsh*, BMC Genomics **9**, 275 (2008).
[28]. *J.H. Sun , J.-J. Luo, L. Tian, C.-Li Li, Yu Xing,Y.-Y. Shen,* J. Plant Growth Regul **32**, 461 (2003).