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Characterisation of morphological and chemical traits of Costa Rican papaya (*Carica papaya* L.) fruit genotypes with special reference to their carotenoid bioavailability

Ralf Martin Schweiggert

Universität Hohenheim
Institut für Lebensmittelwissenschaft
und Biotechnologie

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Universität Hohenheim
Prof. Dr. habil. Dr. h.c. Reinhold Carle

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zur Erlangung des Grades eines Doktors der Naturwissenschaften

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der Universität Hohenheim

von

Ralf Martin Schweiggert

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2013

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I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale.

Marie Curie (1867 – 1934)

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PRELIMINARY REMARKS

The work presented in this thesis is a collection of papers published in international peer reviewed journals, which are listed below.

Full Papers

1. SCHWEIGGERT, R.M., KOPEC, R.E., VILLALOBOS-GUTIERREZ, M.G., HÖGEL, J., QUESADA, S., ESQUIVEL, P., SCHWARTZ, S.J., CARLE, R. (2013). Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised crossover study. *British Journal of Nutrition, accepted for publication.* DOI: http://dx.doi.org/10.1017/S0007114513002596.

2. KOPEC, R.E.*, SCHWEIGGERT, R.M.*, RIEDL, K.M., CARLE, R., SCHWARTZ, S.J. (2013). Comparison of HPLC-MS/MS and HPLC-PDA for the quantitation of carotenoids, retinyl esters, α-tocopherol, and phylloquinone in chylomicron-rich fractions of human plasma. *Rapid communications in mass spectrometry*, 27(12), 1393-1402. (* both authors contributed equally).

3. SCHWEIGGERT, R.M., MEZGER, D., SCHIMPF, F., STEINGASS, C.B., CARLE, R. (2012). Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato. *Food Chemistry*, 135(4), 2736-2742.

4. SCHWEIGGERT, R.M., STEINGASS, C.B., ESQUIVEL, P., CARLE R. (2012). Chemical and morphological characterization of Costa Rican papaya (*Carica papaya* L.) hybrids and lines with particular focus on their genuine carotenoid profiles. *Journal of Agricultural and Food Chemistry*, 60(10), 2577-2585.

5. SCHWEIGGERT, R.M., STEINGASS, C.B., HELLER, A., ESQUIVEL, P., CARLE, R. (2011). Characterization of chromoplasts and carotenoids of red- and yellow-fleshed papaya (*Carica papaya* L.). *Planta*, 234(5), 1031-1044.

6. SCHWEIGGERT, R.M., STEINGASS, C.B., MORA, E., ESQUIVEL, P., CARLE, R. (2011) Carotenogenesis and physico-chemical characteristics during maturation of red fleshed papaya fruit (*Carica papaya* L.). *Food Research International,* 44(5), 1373-1380.

Further scientific contributions resulted from the same period:

Oral communications

1. SCHWEIGGERT, R.M., KOPEC, R.E., COOPERSTONE, J.L., VILLALOBOS-GUTIERREZ, M.G., HÖGEL, J., YOUNG, G.S., FRANCIS, D.M., QUESADA, S., ESQUIVEL, P., SCHWARTZ, S.J., CARLE, R. (2013). Enhanced bioavailability of carotenoids: The influence of chromoplast morphology, dietary lipid, and thermal processing. *Plenary lecture at Pigments in Food 2013, Novara, Italy.*

2. SCHWEIGGERT, R.M., STEINGASS, C.B., HELLER, A., RIEDL, K., KOPEC, R.E., QUESADA, S., ESQUIVEL, P., CARLE, R., SCHWARTZ, S.J. (2013). Chromoplasten-Morphologie als entscheidender Einflussfaktor auf die Bioverfügbarkeit von Carotinoiden aus Papaya, Tomate und Karotte. 50. DGE-Congress 2013 in Bonn, *written abstract available in Proceedings of the German Nutrition Society 2013*, 18, p. 42.

3. SCHWEIGGERT, R.M., MEZGER, D., SCHIMPF, F., STEINGASS, C.B., HELLER, A., RIEDL, K., KOPEC, R.E., QUESADA, S., ESQUIVEL, P., CARLE, R., SCHWARTZ, S.J. (2012). Bioavailability and bioaccessibility of carotenoids from papaya, tomato, and carrot are modulated by chromoplast morphology. *Experimental Biology, San Diego, CA, USA.*

4. KOPEC, R.E., COOPERSTONE, J.L., SCHWEIGGERT, R.M., RIEDL, K.M., HARRISON, E.H., FRANCIS, D.M., CLINTON, S.K., SCHWARTZ, S.J. (2012). Provitamin A absorption and conversion from a unique high β-carotene tomato is higher when consumed with avocado. *9th Annual Russell Klein Nutrition Symposium*, Columbus, OH, USA.

5. RACHEL E. KOPEC, JESSICA L. COOPERSTONE, RALF M. SCHWEIGGERT, KENNETH M. RIEDL, EARL H. HARRISON, DAVID M. FRANCIS, STEVEN K. CLINTON, STEVEN J. SCHWARTZ (2012). Provitamin A absorption and conversion from a unique high β-carotene tomato is higher when consumed with avocado. *Experimental Biology, San Diego, CA, USA.*

6. SCHWEIGGERT, R.M., STEINGASS, C.B., HELLER, A., RIEDL, K., KOPEC, R.E., QUESADA, S., ESQUIVEL, P., CARLE, R., SCHWARTZ, S.J. (2012). Comparison of *β*-carotene and lycopene bioavailability from papaya, carrot and tomato. *16th World Congress of Food Science and Techonology – IUFoST, Foz Iguazu, Brasil.*

7. SCHWEIGGERT, R.M. (2010/2011). Trends in der Obstverarbeitung – Säfte – Smoothies – Fruchtaufstriche. *Fortbildungsseminare an der Landesanstalt für Entwicklung der Landwirtschaft und der ländlichen Räume, Schwäbisch Gmünd, Germany.*

Poster presentations

1. SCHWEIGGERT, R.M., STEINGASS, C.B., HELLER, A., ESQUIVEL, P., CARLE, R. (2013). Deposition of lycopene, β-carotene, and β-cryptoxanthin in different chromoplast substructures in papaya fruits. *Pigments in Food 2013, Novara, Italy.*

2. COOPERSTONE, J.L., SCHWEIGGERT, R.M., MEULIA, T., FRANCIS, D.M., SCHWARTZ, S.J., (2012). Ultrastructural characterization of chromoplasts from tomatoes with unique carotenoid profiles. *IFT Annual Meeting, Las Vegas, NV, USA.*

3. SCHWEIGGERT, R.M, KOPEC, R.E., RIEDL, K., SCHWARTZ, S.J., CARLE, R. (2012). Vergleich von HPLC-MS/MS und HPLC-DAD zur Quantifizierung von Carotinoiden, Retinylestern, Phyllochinon und α-Tocopherol in der triglyzerid-reichen Lipoprotein-Fraktion aus humanem Plasma. *Deutscher Lebensmittelchemikertag 2012, Münster, Germany.*

The co-authors´ contributions to the papers presented in CHAPTERS 1 – 6 are specified as follows:

The work presented in this thesis was carried out under the supervision of Prof. Dr. habil. Dr. h. c. **Reinhold Carle** at the Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, who substantially contributed to the conception of this work. He made essential contributions to the interpretation of the results as well as to proof-reading of all manuscripts (CHAPTER 1 – 6).

Prof. Dr. **Patricia Esquivel** supervised the work carried out at the University of Costa Rica. She contributed to designing the respective research and interpreting the results (CHAPTER 1, 2, 3, and 6). Patricia Esquivel enabled the conduction of the human study at the University of Costa Rica (CHAPTER 6). During the conduction of the human study, Prof. Dr. **Silvia Quesada** was responsible for all clinical procedures (CHAPTER 6).

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The HPLC-PDA-MS/MS method development (CHAPTER 5) and the analysis of blood plasma samples during the human study (CHAPTER 6) were conducted under the supervision of Prof. Dr. **Steven J. Schwartz,** who also contributed to the design of experiments and the interpretation of results.

Mr. **Christof B. Steingaß** performed investigations into the characterisation of several papaya genotypes during his Diploma thesis, which was combined with data on further genotypes in CHAPTER 1. He also conducted parts of the analytical work in CHAPTER 2. Furthermore, he significantly contributed to preliminary results regarding the morphology and ultrastructure of papaya chromoplasts by light- and electron microscopy at the University of Costa Rica (CHAPTER 3 and CHAPTER 4). Mr. **Dominik Mezger** and Ms. **Franziska Schimpf** carried out *in vitro* digestion experiments with papaya, tomato, carrot, and mango during their Diploma thesis (CHAPTER 4).

Mr. **Eric Mora** provided access to numerous papaya genotypes. He helped harvesting fruits and assisted in the exact assignment of papaya ripening stages. Furthermore, he substantially contributed to the discussion of results in CHAPTER 2.

Dr. **Annerose Heller** performed electron microscopic investigations on red- and yellow papaya fruits at Hohenheim University. She contributed to the interpretation of the light and electron micrographs (CHAPTER 3).

Ms. **Rachel E. Kopec** performed parts of the analytical method development, assisted in the analysis of human blood samples, and contributed to the interpretation and discussion of the respective results (CHAPTER 5 and 6). Dr. **Kenneth M. Riedl** contributed to the conception of the analytical method development and the interpretation of the data obtained (CHAPTER 5).

Ms. **Maria-Gabriela Villalobos-Gutierrez** contributed to designing and conduction of the human study at the University of Costa Rica. Her responsibility was enrolling participants, checking eligibility of the participants, test meal preparation, and the scientific supervision of the participants during the clinical part of the study (CHAPTER 6).

Prof. Dr. **Josef Högel** provided valuable advice for the experimental design of the human study and assisted during statistical data analysis in CHAPTER 6.

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GENERAL INTRODUCTION

In the year 2013, vitamin A deficiency still represents a major nutritional problem worldwide. The severity of this so-called "hidden hunger" was recently highlighted by the World Health Organization of the United Nations, ranking vitamin A deficiency as a moderate to severe public health issue in 122 countries. Besides several preventable diseases like xerophthalmia, anemia, and night blindness, an increased morbidity and mortality from infections is observed among vitamin A-deficient populations, mostly affecting pregnant women, infants, and young children (1).

Vitamin A deficiency most frequently occurs among the poorer populations of developing countries with a low per capita income. In such countries, provitamin A carotenoid-rich fruits and vegetables represent a most important dietary component, since such provitamin A carotenoids are rapidly metabolized to vitamin A by humans. At the same time, animal based food rich in preformed vitamin A is too expensive or simply unavailable for large parts of the population. In developed nations, vitamin A deficiencies are nearly absent. Nevertheless, the consumption of carotenoid-rich fruits and vegetables was also recommended due to numerous associated health benefits beyond vitamin A supply (1-3).

This doctoral thesis aimed at investigating the nutritional potential of carotenoid-rich papaya fruits "from the farm to the fork". Importantly, papaya fruits can be easily grown in many tropical and subtropical regions suffering from vitamin A deficiency. However, prior to describing the aims and scope of this thesis in detail (See Section 3, page 24), the following sections will provide a state-of-the-art summary on the interdisciplinary research fields involved.

1 Papaya (*Carica papaya* L.)

1.1 Origin and botany

The first documentation on papaya fruits published by Gonzalo Fernández de Oviedo y Valdés dates back to the 16th century. Being the director of the mines on Hispaniola (today's Haiti and Dominican Republic) from 1513 to 1525, he reported Alphonso de Valverde bringing papaya seeds from Panama to Santo Domingo on Hispaniola. From there, Valverde spread papaya seeds to other islands of the West Indies. Adapted from an indigenous name, the Spaniards called the fruit "papaya" and took it to the Philippines, from where it subsequently dispersed to South East Asia at the end of the 16th century (4, 5). Papaya (*Carica papaya* L.) represents the only member of the genus *Carica*, which belongs to the small dicotyledonous family Caricaceae of the order Brassicales (6). Although the biogeographic history of the Caricaceae involved long distance dispersal from

Africa to Central America about 35 Million years ago (7), the species *Carica papaya* L. is currently believed to have evolved subsequently between the Southern regions of Mexico and Costa Rica (4, 7). The papaya plant is classified as herbaceous perennial or "tree-like" shrub, since the stem remains widely non-lignified except for the tracheae. Deeply indented leaves are spirally arranged at the upper end of the stem and are characterized by a short life time. After leaf detachment, broad triangle-shaped scars mark the sites of abscission on the trunk (6, 8, 9).

The polygamous *Carica papaya* L. plants exist in three basic sexual forms with pistillate (female), staminate (male), and hermaphrodite (both genders) flowers (10). Hermaphrodite plants are widely cultivated due to the customer's preference of their pear-shaped fruits (Compare Figure 1), containing more edible flesh and less seeds than fruits from female plants (6, 8, 9). Progenies from hermaphrodite plants segregate into hermaphrodite and female plants at a ratio of approximately 2:1. Therefore, farmers plant three to five seedlings in one location. After 4-6 months, the sex types can be identified by inspection of the first flowers and the undesired female plants are removed, enabling orchards with only hermaphrodite plants (10). At about 150-164 days after anthesis, a fleshy berry from 200 g to as much as 10 kg develops. As shown in Figure 1, female plants produce fruits of a spherical, slightly ovoid shape, whereas hermaphrodite plants bear longer, cylindrical to pear-shaped fruits (8).

Irrespective of the plant's gender, the fruit is composed of five carpels which form a central ovarian cavity, resulting in a star-shaped cavity in the transverse cut. In hermaphrodite fruits, the cavity is smaller than in female ones. The placenta coats the cavity and carries up to 1000 black seeds. The skin of the fruit is thin, soft, smooth, and green when immature. A skin color change from green to yellow (color-break) indicates fruit maturity. Mesocarp of immature fruit is white, turning into a pale orange-yellow, salmon pink or red color depending on cultivar (8). As early as in 1964, the presence and absence of the carotenoid lycopene was found to determine the rather red and yellow appearance, respectively (11), as illustrated by the red- and yellow-fleshed fruits in Figure 1. Immature fruits as well as other green plant parts contain milky latex with a high activity of the protease papain. A network of lacticifers is present throughout the fruit and, as the fruit ripens, the lacticifers collapse resulting in only small or no amounts of latex at the fully ripe stage (8).

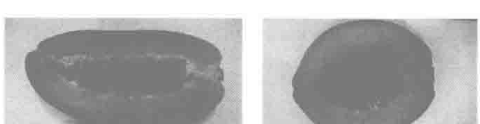

Figure 1 A) Papaya fruits from hermaphrodite (left, red-fleshed) and B) female (right, yellow-fleshed) plants. [Modified photograph from Schweiggert *et al.* (8)].

1.2 Horticulture and post-harvest treatment

In commercial cultivation, papaya is widely propagated from seeds, although propagation by cuttings or *in vitro* techniques has been reported (12). After about 6-8 weeks in the nursery, two or three seedlings are planted in one location spaced about 30 cm apart. As mentioned above, female plants are removed as soon as flowering allows identification of sex, yielding one hermaphrodite plant per location. Due to the fast growth of the plant, an abundant supply of nutrients is required to sustain sound plant development and high-yield production. Frequently, irrigation is applied to ensure adequate moisture and to avoid the retardation of plant growth and plant sex changes. In contrast to the general belief of a genetically fixed sex type, a gender shift by environmental factors such as high or low moisture, high temperature, and inadequate fertilization has been frequently observed in papaya (8, 13-17).

To ensure high fruit quality, harvesting at the proper stage of maturity is of utmost importance. Since immature, green fruit will not fully ripen, fruits are harvested at the 'color-break' stage, i.e. just when a first yellow spot appears on the skin. At this stage, fruits are mostly in a good condition for transportation and storage due to commonly little damage by anthracnose or fruit flies. If harvested at more than 40% yellow, the risk of browning and other transportation damage is high. During the first year of production, harvesting can be conveniently done by hand. Since plant height continuously increases, harvesters need to use poles in the second year in order to increase their reach. However, harvest time doubles if poles are used (8).

Minimum requirements for commercialization of Hawaiian papaya fruits are a minimum of 11.5% total soluble solids. Therefore, fruits need to be harvested at or after the 'color break' stage (8). In some countries like Costa Rica, a fungicide treatment as well as the application of ethylene-releasing compounds to accelerate ripening is carried out after harvest. Subsequently, papayas are packaged in foam-mesh sleeves and placed in foam padded cartons to prevent abrasion injury. Storage temperatures of 7-13 °C at 90-95% relative humidity have been generally recommended. Typical chilling injury symptoms occurring at lower temperatures include skin scald, hard lumps in the pulp around the vascular bundles, and water soaking of the flesh. A variety of other storage parameters were described depending on the maturity stage (8, 18, 19).

1.3 Producing countries and utilization

In 2011, papaya world production exceeded 11.8 Mio. tons on over 420,000 ha. According to the Food and Agriculture Organization of the United Nations, India (4.2 Mio. tons), Brazil (1.9 Mio. tons), and Nigeria (0.9 Mio. tons) are the major producing countries (20).

Consumption of papaya is mostly limited to fresh fruit. Most commonly, papaya fruit is eaten at the fully ripe stage characterized by soft and succulent flesh. For instance, the flesh can be cut in wedges and served with a half or quarter of lime and lemon. The flesh of the ripe fruit is also used for cakes, desserts, ice cream, and jam. Also derived from the ripe fruit, fresh papaya nectars are sold in some countries. In Hawaii, fresh papaya puree is frozen for its later use (8, 19). During thermal processing of papaya, the occurrence of off-flavors and off-odors represents a major problem for the acceptance of processed papaya products by the consumer (21).

Besides ripe fruits, the use of immature green fruit as a vegetable or salad is common in various countries (8). Batch peeling of green papaya was reported in Puerto Rico to be carried out by immersion of the fruits in boiling lye solution and subsequent washing procedures using cold water. In the East Indies, young papaya leaves are cooked and consumed like spinach. Mature raw leaves are bitter due to the presence of bitter alkaloids (carpaine and pseudocarpaine), reportedly acting like cardiac glycosides from *Digitalis* sp. (foxglove) on the heart and respiratory system (19, 22). In contrast to leaves from *Digitalis*, about 18 kg (40 pounds) of papaya leaves would be needed to produce acute toxicity in man (23).

The proteolytic enzymes papain and chymopapain present in the latex of the papaya plant are of commercial importance. For their recovery, several incisions are made on the surface of a plant's green fruits. The leaking and rapidly coagulating latex is collected, spread on fabric for oven drying at low temperatures, and subsequently ground to a powder. Papain is used for tenderizing meat, clarifying beer, treating wool and silk before dyeing, and numerous other food, cosmetic, and even medical applications (19).

1.4 Nutritional relevance

Besides sweetness and its delicate aroma, high levels of provitamin A and vitamin C have been frequently described for papaya fruits. Franke *et al.* (24) reported high levels of 62.7-80.7 mg vitamin C per 100 g of fresh papaya. For comparison, vitamin C levels of apples, mangoes, and oranges varied from 1.4 to 3.5 mg/100 g of FW, from 9.1 to 18.6 mg/100 g of FW, and from 42.1 to 62.4 mg/100 g of FW, respectively (24). Therefore, papaya represents a rich source of vitamin C, although not reaching the high levels of acerola (*Malpighia glabra* L.) and rosehip (*Rosa* spp. L.) of up to 1300 and 1000 mg/100 g, respectively (25). The term vitamin C refers to L-ascorbic acid (L-AA) including several derivatives, while most biological and nutritional knowledge is available for L-AA. The biological activity of L-AA is based on its function as enzyme cofactor, radical scavenger, and electron donor/acceptor (25). Since humans are not able to biosynthesize vitamin C, a diet lacking vitamin C leads to a deficiency, ultimately resulting in scurvy. During the past

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