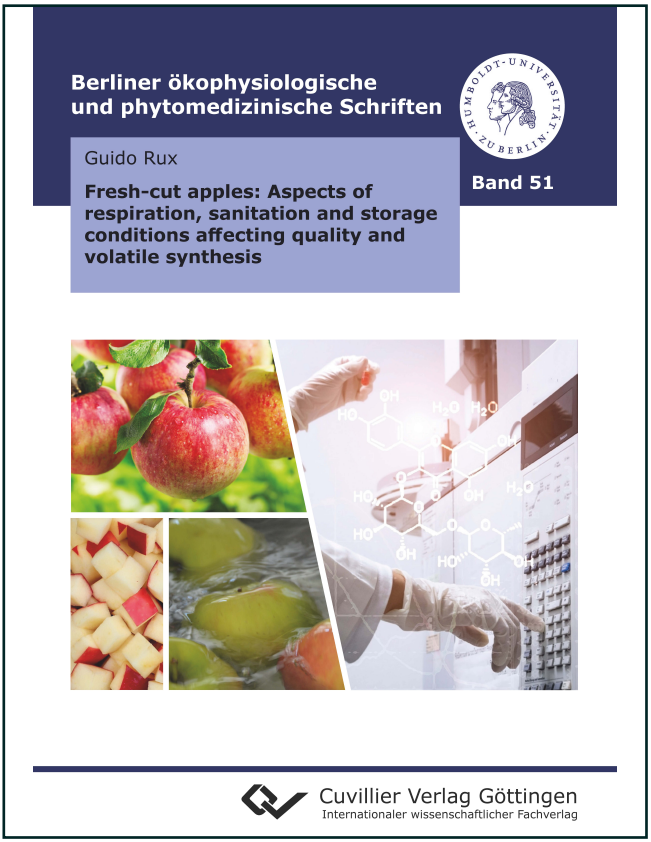
Guido Rux (Autor)

Fresh-cut apples: Aspects of respiration, sanitation and storage conditions affecting quality and volatile synthesis



https://cuvillier.de/de/shop/publications/8588

1 Introduction

The fresh-cut fruits sector has shown consistent growth in the last few years, due to changes in consumers' lifestyle and the growing demand for convenient, nutritional and safe "ready-to-eat" fruits (Rabobank, 2010; Baselice et al., 2015). The strongest incentive to buy fresh-cut products is convenience (Ragaert et al., 2004). Restaurants, fast-food outlets and institutional food-service operators seeking to reduce labour costs by buying minimally processed, ready-to-use or ready-to-eat fresh-cut fruits further boost this demand. According to Beaulieu (2010), the higher prices of fresh-cut produce will be accepted by customers, if the quality is equal to the uncut product. However, due to the inevitable wounding and tissue destructions during minimal processing, fresh-cut fruits become highly perishable and show a short shelf life. In fresh-cut apples, which are often a major component of fruit salads, microbial loads, rapid oxidative tissue browning and loss of texture and flavour are important factors limiting the customers' acceptability (Dixon and Hewett, 2000; Toivonen and DeEll, 2002).

To optimize the shelf life of fresh-cut fruit, various postharvest treatments have been investigated or applied over the past years to minimize the adverse impact of minimal processing. These include the use of modified atmosphere (MA) and humidity packaging (Rux et al., 2015; Caleb et al., 2016), edible coatings (Rojas-Graü et al., 2007; Rojas-Graü et al., 2008; Salvia-Trujillo et al., 2015), thermal (e.g. Koukounaras et al., 2008) and plasma (e.g. Tappi et al., 2014) treatment, UV-C combined with MA-packaging (López-Rubira et al., 2005) or the application of organic acid solutions and sugar (Verlinden and Garcia, 2004; Nishikawa et al., 2005). In particular, chemicals (Buta et al., 1999) and/or MA during storage (Gorny et al., 1998) are used to control oxidative browning and to delay undesirable biological and biochemical changes (Beaulieu, 2010). Postharvest sugar-syrup application or storing fresh-cuts in suitable fruit juice solutions as an additive are also general practices.

The immersion of fresh-cut fruits in sugar syrup is a common practice especially used for bulk purchasers (Alzamora et al., 1993) to extend the common maximum shelf life for fresh-cut fruit salads of 5 d (Seide et al., 2017) to up to 10 d. According to practical experience, storage of fruits in sugar syrup (ca. 20 %) extend product shelf life by preventing enzymatic and oxidative browning and transpiration and slows down respiration, ethylene metabolism and other physiological processes. Despite reports on the effects of storage in sugar syrup on ethylene metabolism (Nishikawa et al., 2005) or chlorophyll degradation of fresh-cut fruit (Coupe et al., 2003), the knowledge on the

impact of sugar solution on the physiological properties of immersed fresh-cuts is still limited. However, information on the physiological responses and changes in quality of fresh-cuts immersed in sugar solution or fruit juice is lacking. The current industrial practices are based on "trial-and-error approach" without an adequate evaluation of the sugar content or juice concentrations. This may result in non-optimal shelf life and quality, which cumulates in economic and postharvest losses. For instance, low O_2 atmospheres, as they are found after immersion, can negatively affect flavour and aroma (El Hadi et al., 2013). The aroma is comprised by a high number of volatile organic compounds (VOCs) (DeRovira, 1996; Baldwin et al., 2007) and is important for the consumers' sensorial acceptance (Beaulieu, 2010).

According to the fresh-cut producer, microbial spoilage is the main factor limiting the shelf life of apple slices immersed in sugar syrup. Consequently, careful removal of microorganisms, adherent to fruit skin (Pietrysiak and Ganjyal, 2018) is essential to avoid microbiological contamination and cross-contaminations (Kabelitz and Hassenberg, 2018). In practice, apple slices are often dipped in a mixture of citric and ascorbic acid solutions before immersion to prevent browning and for sanitation purposes. To prevent the consumption of these chemicals especially in organic production, gentle physical sanitation methods are demanded.

Hot-water treatment (HWT) in the temperature range of 40 – 80 °C may effectively reduce microbial contaminations, is relatively inexpensive and is easy to use (Lurie, 1998). In addition, HWT maintains storage quality of fruits (Shao et al., 2007; Spadoni et al., 2015; Kabelitz and Hassenberg, 2018). Being chemical-free, particularly short-term (15 to 60 s) hot-water treatment (sHWT) is suitable for organic production (Fallik, 2004; Maxin et al., 2014). Consequently, the application of short-term hot-water treatments (sHWT) of apples before processing could represent an effective but gentle sanitation technique to additional prolong their shelf life. Besides earlier studies on the impacts of HWT on structure and function of fruit epidermal tissue (Roy et al., 1994; Lurie et al., 1996), only recently, the effects of sHWT on surface tissue, heat transfer dynamics and suitability for pre-processing of intact apples for fresh-cut salads were investigated in detail (Kabelitz and Hassenberg, 2018; Kabelitz et al., 2019). For fruit salad production, however, sHWT needs to be optimised to prevent fruit injury and reduction of produce quality, and to guarantee the commercially required shelf life of 10 d.

This study aims to evaluate systematically the impacts of postharvest storage in sugar-syrup and sHWT on important quality parameters and quality relevant biosynthetic pathways, and microbial loads of fresh-cut apples. However, both methods are not investigated adequately and the knowledge on their functional mechanisms, their effectiveness and their potential effects on fresh-cut fruits' quality and shelf life is sketchy. A special emphasis is put on the potential synergistic interactive effects caused by sugar syrup immersion of apple slices and restricted O_2 availability on the synthesis and the emission of VOCs.

The objective of the first scientific part of this thesis, presented in chapter 3, was a comprehensive scientific evaluation of postharvest storage in sugar-syrup. In this chapter, the impact of the complete immersion of fresh-cut apples in sugar syrup and orange juice solutions on the respiratory behaviour and on commonly used quality attributes (colour, texture, acidity and sugar content) was investigated. Special focus was laid on the optimisation of the respective concentrations to increase quality or extend the shelf life. Next to the quality factors, chapter 4 focuses on the potential synergistic interactive effects caused by the sugar syrup immersion of apple slices and the O_2 availability on development and emission of VOCs. Chapter 5 and 6 are focusing on the potential implications of sHWT on the physiological quality parameters (5) and fruit aroma development (6) of immersed fresh-cut 'Braeburn' apple slices. The results will allow assessing whether sHWT could potentially supplement or replace post-processing chemical treatments. Additional, it will enable comprehension of the respective quality-related physiological processes to effectively select the optimal process conditions in processing of ecologically produced fresh-cut fruit salads. In the following chapter 7, important aspects of fresh-cut apple metabolism and investigated sanitation methods are summarized. In addition, the results of the different investigations are comprehensively discussed.

2 Scientific background

The fresh-cut produce is different from intact fruit in terms of physiology and postharvest handling requirements. Processing of fresh-cut fruit salads involves sorting, cleaning, washing, peeling, deseeding/coring and cutting. The initial responses of fresh-cut produces to wounding include a strong temporary increase in both respiration and ethylene production (Mahajan et al., 2014) mainly due to the initial stress response (Finnegan et al., 2013). The inevitable wounding and tissue destructions during minimal processing render fresh-cut fruits highly perishable and reduce their shelf life. Peeling or slicing enlarges the surface and leads to the release of cell contents (Beaulieu, 2010). This facilitates the spread and growth of naturally occurring microorganisms, normally only located on the fruit skin (Nicoli et al., 1994). Mixing of enzymes and the cytoplasmic and nucleic substrates, normally sequestered in different cell compartments, also leads to enzymatic browning of cut surfaces. The release of secondary fermentative metabolites also may negatively affect fruit aroma (Artés et al., 2007). Furthermore, peeling or slicing intensifies water losses, thus, increasing the risk of wilting and dehydration (Toivonen and DeEll, 2002). This effect results from both artificially enhanced surface and reduced diffusion resistance due to the removal of the natural protecting epidermal layer (Beaudry, 2000; Watkins, 2000).

Additional to this, fresh produce remain physiologically active and, thus, mechanical injury of plant tissues, induces a catena of physiological responses (Watada and Qi, 1999; Beaulieu, 2010), including the increase in respiration and in the biosynthesis of ethylene and secondary metabolites, which finally results in the loss of quality and aroma (Toivonen and DeEll, 2002). Additional, the activation of enzymatic systems may accelerate cell membrane degradation and shorten the shelf life of fresh-cut produces (Toivonen and DeEll, 2002; Nicoli et al., 1994). In fresh-cut apples, which are often a major component of fruit salads, also cultivar-specific properties, stage of maturity at cutting, and storage atmosphere and temperature (Gorny et al., 1998) further affect the shelf life.

2.1 PPO-mediated apple tissue browning

One of the most important quality parameters for fresh-cut apples is the colour of the cut tissue, which essentially affects the appearance and thus has a strong impact on the consumer's buying decision (Toivonen and Brummel, 2008). Therefore, preservation of normal tissue colour and control of discolouration or surface browning is an important consideration during fresh-cut fruit processing and storage. Tissue browning due to enzymatic action mainly changes the lightness (L*) and the greenness-redness (a*) of the pulp. The enzymatic action is induced by tissue wounding through the slicing of apples and associated destruction of cell membranes inside plant tissues, which leads to the mixing of phenolic compounds with the endogenous polyphenol oxidase (PPO) (Gil et al., 1998; Toivonen, 2004). Therefore, enzymatic browning is mainly determined by the PPO activity and the phenolic compound concentrations (Martinez and Whitaker, 1995). Further factors are the pH, the temperature and the oxygen availability of the tissue (Martinez and Whitaker, 1995). The oxygen availability is considerably increased by cutting due to the reduced gas diffusion resistance (Gil et al., 1998).

According to Harel et al. (1964), catechol oxidases are the most common polyphenol oxidases in apple fruit. Generally, PPO catalyses the oxidation of phenolic compounds to the corresponding o-quinone (Gacche et al., 2006), for which oxygen is needed. According to Cortellino et al. (2015), PPO hydroxylates monophenols to colourless diphenols and oxidates diphenols to coloured quinones. The hydroxylation is relatively slow, whereas the oxidation is relatively rapid. The brown or black melanin pigments, which are associated with “browning” in plant tissues, are accumulating through subsequent reactions of the quinones (Toivonen and Brummel, 2008). In these processes, the specific structure of present polyphenolic substrates determines the formation and sequence of brown or black-coloured reaction products (Toivonen and Brummel, 2008).

In the past, several commercial and research strategies were described to reduce PPO-mediated discolouration (Gorny, 2001; Garcia and Barrett, 2002; Hodges and Toivonen, 2008). According to Gorny (2001), reduced oxygen and/or elevated carbon dioxide concentrations may prevent enzymatic browning of fresh-cut fruits and vegetables. Soliva-Fortuny et al. (2002) compared ‘Golden Delicious’ apple slices stored (60 d) in air or under 100 % N_2 and reported slightly lower L* values of the samples in MA storage. Prevention of browning the modified gas atmosphere was much more effective when combined with a preceding ascorbic acid dip. In this context, Cortellino et al.

(2015) also confirmed a colour preservative effect of MA only for the samples dipped in anti-browning solutions before storage.

The application of reducing agents such as ascorbic acid (and its derivatives) and citric acid can inhibit the browning effects of fresh-cut fruits (Nicoli et al., 1994; Soliva et al., 2001). Ascorbic acid is commercial often used as an anti-browning agent due to its versatile action (Luo and Barbosa-Canovas, 1996; Buta et al., 1999; Cocci et al., 2006; Tortoe et al., 2007). It chelates copper ions, reduces o-quinones to their original polyphenolic compounds and acts as a competitive PPO inhibitor (Lozano-de-Gonzales et al., 1993). For example, Gil et al. (1998) showed the effectiveness of 2 % ascorbic acid as a reducing agent, which prevented the decrease of total polyphenol content during storage of 'Fuji' apple slices.

According to Rojas- Graü et al. (2006), the maturity stage of the processed apples affected the effectiveness of ascorbic acid as a browning inhibitor. It was pointed out that ascorbic acid, as a reducing agent, is consumed during anti-browning reactions (Luo and Barbosa-Canovas, 1997). However, according to Chiabrando and Giacalone (2012), the effectiveness of ascorbic acid in the inhibition of browning is lower than that of citric acid.

Citric acid is also known to prevent browning and to maintain the quality of fresh-cut samples (Jiang et al., 2004; Queiroz et al., 2011). The inhibitory effect of this agent may be related to the phenolase Cu-chelating power (Tortoe et al., 2007). To get a more effective browning preservation of fresh-cut fruits, different substances can be combined (Ahvenainen, 2000). In this context, ascorbic acid (antioxidant agent) has been long applied in combination with citric acid (acidulant) to prevent enzymatic browning of sliced apples (Ponting, 1972; Sapers and Douglas, 1987; Pizzoccaro, 1993; Laurila et al., 1998; Soliva-Fortuny and Martín-Belloso, 2003a).

In this context, it has been shown that solutions of acidic pH or the addition of acidulates may effectively inhibit or control enzymatic browning (Nicolas et al., 1994; Whitaker, 1994; Chiabrando and Giacalone 2012). Biegańska-Marecik (2007) suggests using 0.8 % citric acid in the dipping solution in combination with sucrose, to inhibit effectively colour changes in apple slices.

The combination of anti-browning dippings and storage in modified atmospheres were investigated in numerous studies. Ascorbic acid/citric acid-treated apple slices retained colour attributes better when additional MA-stored at 4 °C for 8 d (Cocci et al., 2006) or 11 d (Cortellino et al., 2013) than when stored in air. Similar results were reported by Gunes et al. (2001; 0 – 1 % O_2), Soliva-Fortuny et al. (2002; 100 % N_2) and

Rocculi et al. (2004; 5 % O_2 + 5 % CO_2 + 90 % N_2). Furthermore, Soliva-Fortuny et al. (2001) described a decrease in the PPO activity at additionally enhanced CO_2 concentration (2.5 % O_2 + 7 % CO_2 + 90.5 % N_2).

2.2 Changes in the texture of apple slices

The term texture summarizes the structural and mechanical properties of a product, as well as its sensory properties, which can be perceived by hand and mouth (Bourne, 2002; Abbott and Harker, 2004; Barrett et al., 2010; Beaulieu, 2010). Softening is an undesirable consequence of cutting resulting from changes in the physical and the mechanical properties, and in the chemical structure of the tissue. On the other hand, firmness is a sensorial property, which generally describes the impression and feeling of texture in sensory testing (Bourne, 2002; ASABE, 2008), but often misinterpreted as stiffness or tissue strength (Vincent, 1994). The latter is determined by cell size, biochemical and biophysical cell wall properties, cell-to-cell adhesion and tissue turgor (Toivonen and Brummel, 2008). The loss of tissue strength is associated with changes of the cell wall pectins to water-soluble pectins by pectinolytic hydrolysis via polygalacturonases and/or pectin methyl esterases, decreasing cellulose content crystallinity of cellulose fibrils, thinning of cell walls, diffusion of sugar to the intercellular spaces and ion movement from the cell wall (Toivonen and Brummel, 2008; Qi et al., 2011). This may be due to highly controlled developmental processes during maturation or due to degradation reactions e.g. following mechanical stresses of plant tissue (Varoquaux et al., 1990). The changes in structure and biochemistry of pectins play a key role in fruit softening (Fischer and Bennett, 1991). Furthermore, wound-induced ethylene synthesis may also promote development and also induce senescence processes resulting in the alterations of cell wall metabolism especially for climacteric fruits (Gorny et al., 2002). In addition, water losses intact but particularly in fresh-cut apples due to the removed cuticle and sub-epidermal layers may lead to a decrease in turgor and results in loss of stiffness (Toivonen and Brummel, 2008).

According to Varoquaux and Wiley (1997), MA conditions reduce tissue destruction. Mainly dependent on the availability of O_2, this inhibits the loss of compartmentation within cells and diminishes the interaction between enzymes and their substrates (Soliva-Fortuny et al., 2002, 2003b and 2005). For example, Cortellino et al. (2015) showed a better preservation of the firmness of ‘Golden Delicious’ apple slices during storage at different MA conditions (1 % O_2 + 99 % N_2; 5 % O_2 + 5 % CO_2 + 90 % N_2) than during air-storage.

Results of Cocci et al. (2006) indicated a very minor lower firmness of 'Golden Delicious' apple slices stored in air compared to MA-stored samples (1 % O_2 + 2 % CO_2). Storage of apple slices at low O_2 concentrations may prevent softening for up to 3 weeks (Soliva-Fortuny et al., 2005). In this context, Soliva-Fortuny et al. (2003b) evaluated the microstructural modification of fresh-cut apples stored under different atmospheric conditions using Cryo-Scanning Electron Microscopy. They detected progressed cell deterioration for samples stored under MA conditions (2.5 % O_2 + 7 % CO_2) and indicated by a great number of exudates formed as droplet-shape on the external surface of the cell walls. Samples stored under LOL conditions (100 % N_2), however, showed additionally smoother and more integer cell walls, ranked between fresh apples and MA samples. The authors attributed this to the maintenance of the aerobic metabolism, which may finally degrade the fruit tissue.

However, studies also suggested softening of apple slice tissues due to the structural breakdown by the application of acid solutions (Ponting et al., 1971; Gil et al., 1998; Rojas-Graü et al., 2007; Cortellino et al., 2015). In this context, experiments of Cocci et al. (2006) showed that firmness of dipped apple slices decreased from 8.5 to 4.5 N within just one day of cold storage. Furthermore, the degree of softening was greatly affected by the mature stage of processed apples (Ponting et al., 1972; Rojas-Graü et al.; 2007). Apple slices dipped in ascorbic acid-solution rapidly lost firmness when they had been processed from ripe fruit, whereas this effect was less pronounced for apples in an advanced ripeness state and not observed for mature-green fruit (Rojas-Graü et al., 2007). This effect is related to the increased ethylene synthesis during ripening of these climacteric fruit, which is associated with the accelerated senescence leading to alterations in the cell wall metabolism (Gorny et al., 2002). In this context, low O_2 concentrations successfully preserved the firmness of apple slices by limiting the ethylene production (Cortellino et al., 2015). This positive effect, however, was almost completely neutralized by the acid-dipping, which caused structural breakdown. Biegańska-Marecik (2007) also found of the tissue of apple slices softened after the addition of 0.8 % citric acid to the dipping solution.

2.3 Quality relevant apple constituents

Flavour and nutritional value of fresh-cut apples are important quality factors for consumers (Beaulieu, 2010) and is mainly related to their natural ingredients. The sensory attribute flavour generally comprises aspects of both aroma and taste. According to Beaulieu (2010), flavour represents a very complex "trait" that is difficult to analyse because it is created by a large variety of very different compounds such as sugars, salts, acids, alkaloids, flavonoids and VOCs (DeRovira, 1996; Baldwin et al., 2007). Furthermore, sensory attributes such as flavour or the nutritional value of stored produce may be lost prior the deterioration of visible appearance (Beaulieu, 2010). As an example, the senescence of fresh-cut fruit may directly result in adverse changes of flavour that are concomitantly induced by distinct catabolic and/or metabolic mechanisms; it may also lead to changes in diffusional properties of tissues (Beaulieu, 2006, Forney, 2008). Therefore, the monitoring of flavour and nutritional relevant ingredients is general practice in fruit production to evaluate growing, postharvest treatments or storage conditions.

Apples consist of approx.. 850 g kg^{-1} water, as well as carbohydrates and fibres. In addition, apples contain numerous important micronutrients such as organic acids, vitamins, minerals (potassium, sodium, magnesium, calcium and iron), trace elements and secondary plant substances (e.g. phenols and carotenoids), 75% of which are found in or directly under the apple skin (Buchter-Weisbrodt, 1998). Most of these micronutrients have a positive effect on health (Hecke et al., 2006). As the flavour of an apple is strongly determined by the ratio of sugar to acidity (Hecke et al., 2006), both are relevant parameters in fruit industry. However, sour tasting cultivars are not necessarily low in sugar. This was shown, for example, by the regional 'Steirische Schafnase' apple, which was classified as sour by a taste-test only because the extremely high sugar concentration was masked by the high acid content (Hecke et al., 2006). The specific contents of sugars and acids are highly cultivar-specific, but can vary according to weather and soil (Hecke et al., 2006). The main controllable factor influencing flavour is the maturity stage at harvest (Kader, 2008), which also essentially determines the quality and shelf life of fresh-cut fruits. Thus, harvesting immature or overripe fruits may result in poor flavour (Kader, 2002). The contents of sugar and organic acids directly depend on the maturity stage. During ripening, sugar contents increase, whereas acidity decreases, while the synthesis of flavour compounds occurs relatively late in fruit maturation (Garcia and Barrett, 2005).