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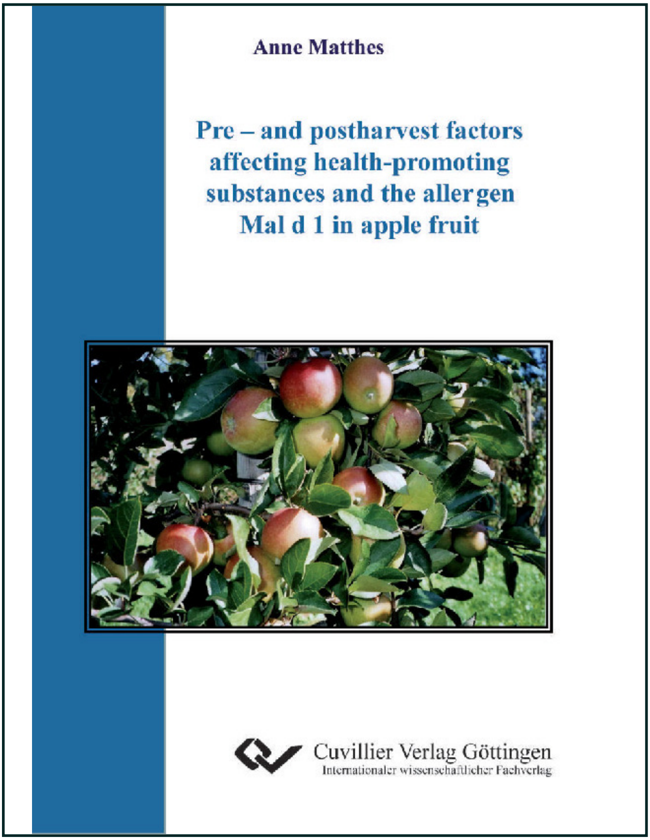
Pre – and postharvest factors affecting health-promoting substances and the allergen Mal d 1 in apple fruit



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A Introduction

1 Quality

Quality includes factors like appearance (size, shape, colour, freedom of defects, texture, firmness, crispness), flavour (sweetness, acidity, aroma, astringency) and nutritive value (vitamins, minerals, antioxidants) (Kader, 2001). The relative importance of each quality component depends on the commodity and possible use. It means different things to different handlers within the distribution chain (Shewfelt, 1999). Most producers and handlers are product-orientated and quality is defined by specific attributes of the product itself, that can be evaluated by different measurement methods e.g. sugar content, firmness, colour (Shewfelt, 1999; Kader, 2001). Contradictionary a consumer-orientated approach to quality is described by the consumers' wants and needs (Shewfelt, 1999). Consumer purchase fresh products on the basis of appearance and textural quality, the repeat purchase decision depends on satisfaction with flavour (Kader, 2001). In recent years health-promoting attributes and nutritional quality (intrinsic product factors) of fresh fruits and vegetables seem to be of rising interest (Kader, 2001; Hyskens-Keil and Schreiner, 2003). Recently quality focuses on the consumers' acceptability and is defined as "all characteristics of a food that lead a consumer to be satisfied with the product" (Harker et al., 2003). European consumer surveys revealed that for 38% flavour is the main decision criterion for purchasing food, while 32% of the consumers focus more on health aspects of fruits (Hyskens-Keil and Schreiner, 2003). Health aspects have an additional value for fruits and vegetables and are gaining more importance. Further decision factors are extrinsic factors like pricing and personal preferences. An overview about estimated quality aspects of fruits and vegetables is given in table 1.

Table 1: Components of quality of fresh fruits and vegetables (Schreiner et al., 2000; Hyskens-Keil and Schreiner, 2003).

Quality parameters	
Sensory attributes	Flavour (aroma, taste, texture)
	Extrinsic factors (colour, form, size)
Bioactive substances	Secondary plant metabolites
Essential nutritive compounds	Carbohydrates
	Vitamins
	Minerals, trace elements
Health-affecting substances	Mycotoxines
	Allergens
	Pesticide residues
	Other ingredients (nitrate, solanine…)
Other factors	Cultivation method
	Regional production

2 Health-promoting substances

Epidemiological studies indicate that health benefits from fruit and vegetable consumption, and it may prevent cardiovascular diseases and cancer (Eastwood, 1999). These protectible effects are attributed to several plant nutrients such as water-soluble ascorbate, glutathione and polyphenols and lipid-soluble tocopherols and carotenoids (Eastwood, 1999). As nutritional value and health aspects become more important factors for choosing fruits and vegetables health-promoting substances move into focus.

2.1 Secondary plant metabolites

Several health-promoting substances are products of the plant's secondary metabolism and are therefore called secondary plant metabolites. They arise from precursors of the primary metabolism through a sequence of reactions like methylation, hydroxylation and glycosylation pathways, catalysed by specific enzymes. Primary metabolites, like carbohydrates, amino acids and lipids cover all processes that are essential for growth and development. For a long time secondary plant metabolites were designated as waste or detoxification products of the plant metabolism, but now they are seen as components of the survival strategy of plants. They comprise all interactions of plants with their biotic and abiotic environment and are therefore indispensable for the survival of the plant (Hartmann, 2007; Korkina, 2007). As pigments they are attractors of pollinators and seed dispersers and they serve as signaling molecules and hormones (Hartmann, 2007). An overview is given in figure 1.

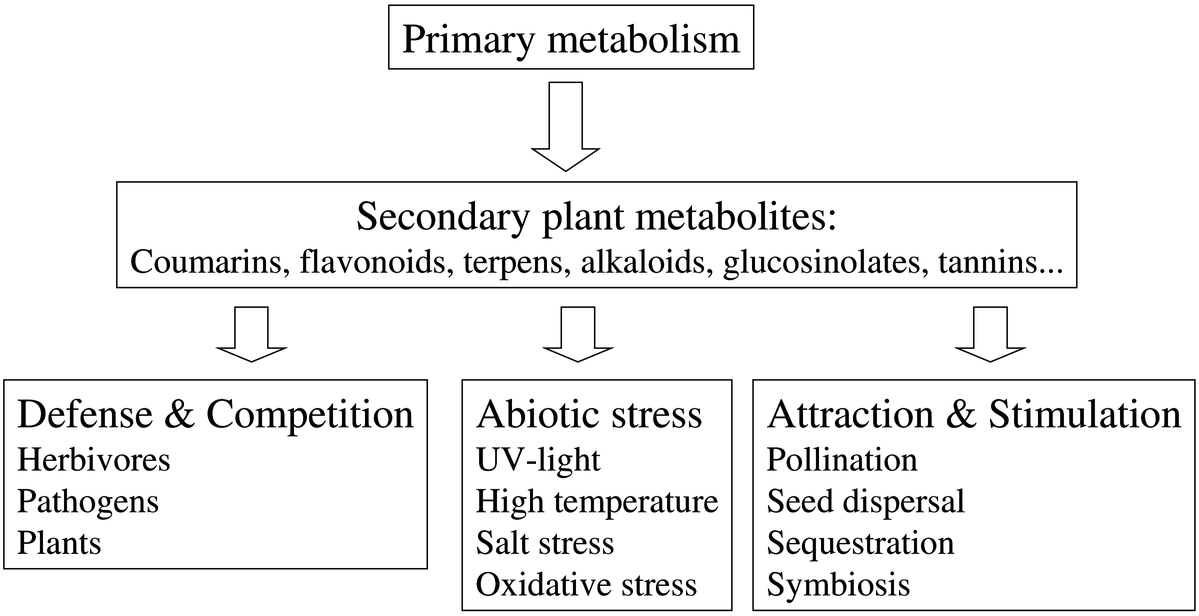


Figure 1: Secondary plant metabolites and their functions in plant (Hartmann, 2007).

A vast array of compounds are designated as secondary plant metabolites, a sum of more than 200.000 components have been defined so far (Hartmann, 2007). According to their chemical structure they are divided into several major classes (Korkina, 2007):

- terpens (isoprenoids, terpenoids)
- phenylpropanoids and their derivates (flavonoids, tannins, lignins)
- nitrogen containing components (alkaloids, heterocyclic aromatics).

In the 1990s it became evident that secondary plant metabolites may possess health benefits in humans (Scalbert et al., 2005). Epidemiological studies showed an inverse relationship between the intake of fruits and vegetables and the mortality from coronary heart diseases

(Hertog et al., 1993). A low incidence for coronary heart diseases in some French cities despite a high fat intake, has been explained by high consumption of red wine (‘French Paradoxon’). The antioxidant effect of the polyphenols may be responsible for that effect (Gordon, 1996). The role of free radicals and reactive oxygen species (ROS) in processes leading to degenerative diseases like cancer, atherosclerosis and Alzheimer became appearent in recent years (Gordon, 1996). Based on their antioxidant properties secondary plant metabolites are involved into prevention of these degenerative diseases as they are able to scavenge free radicals and ROS and prevent formation of such molecules *in vitro*. Recent knowledge suggests that in particular polyphenols exert health promoting activities (Scalbert et al., 2005).

2.1.1 Polyphenols

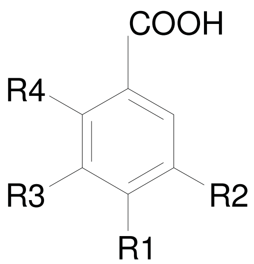
2.1.1.1 Structure

Polyphenols are widely distributed throughout the plant kingdom and are therefore the largest group of secondary plant metabolites (Kroll et al., 2003; Lattanzio, 2003) and the major antioxidant component in the human diet (Kondratyuk and Pezzuto, 2004). They are structurally characterized by one or more six-carbon aromatic rings and one or more hydroxy substituents. In plants they are usually bound to sugars as glycosides (Kroll et al., 2003). Biosynthesis of polyphenols is divided into two major steps. In the shikimate pathway the amino acid phenylalanine is built. Via the action of phenylalanine ammonium lyase (PAL) cinnamic acid is formed (phenylpropanoid pathway), which is then modified to coumaric acid and further derivates. So PAL represents the branch point enzyme between primary and secondary metabolism. All major groups of plant phenolics are derived by further metabolism of phenylpropanoids (Lattanzio, 2003). Diversity in structure is achieved by hydroxylations, carboxylation, methylations and linkage with amines, lipids and other phenols (Stevenson and Hurst, 2007). Up to now thousands of phenolic structures have been described (Lattanzio, 2003).

Based on their chemical structure polyphenols can be divided into at least ten subgroups. In the following part only the two main types phenolic acids and flavonoids are described (Kondratyuk and Pezzuto, 2004).

2.1.1.2 Phenolic acids

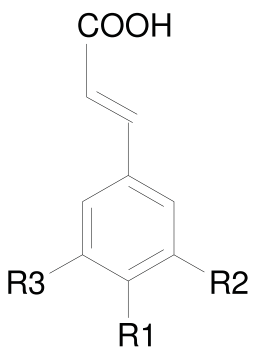
Phenolic acids can be divided into **hydroxybenzoic acids** (C_6-C_1) and **hydroxycinnamic acids** (C_6-C_3). Hydroxybenzoic acids appear in small amounts in fruit, especially in berries (Herrmann, 1992). Structures of the most abundant hydroxybenzoic acids are given in figure 2.



Hydroxybenzoic acid	R1	R2	R3	R4
Benzoic acid	H	H	H	H
Para-hydroxybenzoic acid	OH	H	H	H
Gallic acid	OH	OH	OH	H

Figure 2: Structures of hydroxybenzoic acids with basic C_6-C_1 skeleton.

Structures of common hydroxycinnamic acids are displayed in figure 3. The most frequently found hydroxycinnamic acid in apples is chlorogenic acid, an ester between certain trans-cinnamic acids and (L)-quinic acid (Herrmann, 1992). Cinnamic acid is precursor for lignins, stilbenes and flavonoids (Kondratyuk and Pezzuto, 2004).



Hydroxycinnamic acid	R1	R2	R3
Cinnamic acid	H	H	H
Para-coumaric acid	OH	H	H
Caffeic acid	OH	OH	H
Ferulic acid	OH	OCH_3	H

Figure 3: Structures of hydroxycinnamic acids with basic C_6-C_3 skeleton.

2.1.1.3 Flavonoids

Flavonoids are the largest group of polyphenols (Lattanzio, 2003) and can be divided into six subclasses. All share a three-ring structure of two aromatic centers (rings A and B) and ring

C, an oxygenated heterocycle (Fig. 4). According to their substituents in the heterocyclic C-ring they are divided into six subclasses (Roos and Kasum, 2002):

- Flavones
- Flavonols
- Flavanones
- Catechins
- Anthocyanidins
- Isoflavones

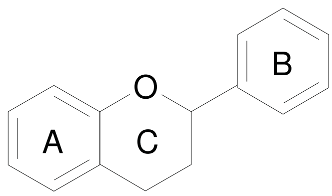


Figure 4: Basic flavonoid structure

The flavonol quercetin and the flavan-3-ols catechin and procyanidin are the most abundant flavonoids in apple fruit (Herrmann, 1992).

2.1.1.4 Function in plants and antioxidant properties

Due to their diversity polyphenols appear to have a wide range of functions in plants including defense against herbivores and pathogens and attraction of animals for pollination and seed disposal. They are known to enhance tolerance to various abiotic stressors e.g. UV, high temperature, heavy metals and oxidative stress (Kondratyuk and Pezzuto, 2004; Korkina, 2007; Stevenson and Hurst, 2007). These properties are based on their ability to act as antioxidants, metal chelators and free radical scavengers (Kondratyuk and Pezzuto, 2004) and chain-breaking antioxidants due to their hydrogen donating ability (Es-Safi et al., 2007). The antioxidant activity is influenced by the number and position of hydroxylic groups. The greater the number of hydroxylic groups, the higher the antioxidant capacity (Es-Safi et al., 2007). Polyphenols with catechol groups have greater antioxidant activity than compounds with simple phenol groups (Scalbert et al., 2005). Substituents on the aromatic ring and side chain structures affect antioxidant properties (Scalbert et al., 2005). The presence of a hydroxyl group on C_3 and a 2-3 double bound in conjugation with a C_4-ketone function is important for the antioxidant effects of flavonoids (Es-Safi et al., 2007). These antioxidant properties may be responsible for any health-promoting effects of polyphenols. The risk of various degenerative diseases is associated with oxidative stress. Reactive oxygen species are formed *in vivo* during normal aerobic processes, and can cause damage to DNA, lipids and proteins (Ross and Kasum, 2002). As antioxidants polyphenols may protect cell constituents against oxidative damage and limit the risk of several degenerative diseases associated with oxidative stress. In various animal, *in vitro* and epidemiological studies it became evident that polyphenols show anti-carcinogenic effects and may prevent cardiovascular and cerebrovascular diseases (Manach et al., 2005; Scalbert et al., 2005). More human studies have to be conducted to get definitive proofs of the protective roles of polyphenols.

3 Health-affecting substances

Beside health-promoting substances fruits and vegetables can contain substances that might affect health of consumers. This could be substances such as mycotoxines, pesticide residues and nitrates that affect every consumer. But there might be substances like allergens that just affect sensitized persons. Due to cross- reactivities between allergens that can be inhaled and food proteins, a rising number of people show allergic reactions to food (Son and Lee, 2001). The immune system of allergy patients responds to normally harmless substances, called allergens. Most allergens are proteins, furthermore some glycoproteins can act as allergens (Bredehorst and David, 2001). Most frequently food allergy patients show adverse reactions to nuts, peanuts, milk and fruits (Schäfer et al., 2001). In recent years adverse reactions to fruits and vegetables become more important. About 2% of the European population suffer from apple allergy (Kerkhof et al., 1996).

3.1 The apple allergen Mal d 1

Up to now four major apple allergens have been characterized. Mal d 1, a Bet v 1 homologous protein (18 kDa, Vieths et al. 1994), Mal d 2, a thaumatin-like protein (31 kDa, Hsieh et al., 1995), Mal d 3, a lipid transfer protein (9 kDa, Sanchez-Monge et al., 1999) and Mal d 4, a profilin (14 kDa, Ebner et al., 1995). In Central and Northern Europe as well as in North America Mal d 1 represents the most important apple allergen due to cross-reactivities with Bet v 1, the major birch pollen allergen. Up to 90% of birch pollinosis patients develop food allergies, most frequently to apples (Fernandez-Rivas, 2003). Sensitization to Bet v 1 leads to reactions to other food proteins, due to structure homologies. Ebner et al. 1991 found homologous structures on nucleic acid level as well as on protein level. A 64.5% identity on the amino acid level and 55.6% on nucleic acid level was reported (Vanek-Krebitz et al., 1995). This sequence homology is essentiell for IgE-binding and cross-reactivity (Son et al., 1999). Furthermore all epitopes of the major apple allergen are present on Bet v 1 (Vieths et al., 1995).

Mal d 1 isoallergens have a molecular weight between 17.3 and 18 kDa and it is assumed that it consists of two isoproteins with nearly identical isoelectric points (5.1-6.2) (Vieths et al., 1994; Gao et al., 2005) The protein consists of 158-159 amino acids encoded by 480 – 483 nucleotides (Vieths et al., 1994; Schöning et al., 1996; Hoffmann-Sommergruber et al., 1997). The three-dimensional structure of Mal d 1 is likely similar to that of Bet v 1 (Uehara et al., 2001). Mal d 1 is a labile protein, which can easily be oxidized and has a low pepsin and heat resistance, so that it does not survive most processing steps (Hsieh et al., 1995; Asero et al., 2006). Therefore allergic symptoms are limited to the oral allergy syndrome, characterized by itching of lips, mouth and throat and swollen lips and tongue (Mari et al., 2005). Mal d 1 is equally distributed in peel and pulp of apple fruit (Marzban et al., 2005). Allergenic differences between apple cultivars are mainly related to expression levels of Mal d 1 (Son and Lee, 2001). Mal d 1 is located in the cytosol of the cell and a function as steroid carrier is discussed (Markovic-Housley et al., 2003). Bet v 1 and Mal d 1 belong to the so called pathogenesis related (PR)- proteins, a group of proteins related to stress response of plants (Hsieh et al., 1995, Pühringer et al., 2000).

4 Pathogenesis-related proteins

PR-proteins have been identified in numerous monocotyledonous and dicotyledonous plant across different genera, they can be considered as ubiquitously distributed in the plant kingdom (Edreva, 2005).

PR-proteins are involved into plant resistance; their genes are induced in response to various pathogens and to environmental stress (van Loon, 1997). Some PR-proteins are synthesized not only in response to pathogens, but they are also developmentally regulated in different plant tissues and organs, which has been shown for apples (Vanek-Krebitz et al., 1995; Pühringer et al., 2000). Gene expression is regulated by plant hormones and defense-related signaling molecules like jasmonic acid, abscisic acid and salicylic acid (Liu and Ekramodoullah, 2006).