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Review

# The Impact of Harvesting, Storage and Processing Factors on Health-Promoting Phytochemicals in Berries and Fruits

Anna Kårlund<sup>1</sup>, Ulvi Moor<sup>2</sup>, Mari Sandell<sup>3</sup> and Reijo O Karjalainen<sup>1,\*</sup>

- <sup>1</sup> Department of Biology, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland; E-Mail: anna.karlund@uef.fi
- <sup>2</sup> Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1A, EE51014 Tartu, Estonia; E-Mail: ulvi.moor@emu.ee
- <sup>3</sup> Functional Foods Forum, University of Turku, FI-20014 Turku, Finland;
  E-Mail: mari.sandell@utu.fi
- \* Author to whom correspondence should be addressed; E-Mail: reijo.karjalainen@uef.fi; Tel.: +358-040-3553834.

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Abstract: Increasing epidemiological and experimental data now emphasize that a diet rich in vegetables and fruits confers many health benefits. Functional products containing elevated levels of bioactive compounds are attracting considerable attention due to their potential to lower the risk of chronic diseases and their associated huge healthcare costs. On a global scale, there is an increasing demand for berries and fruits, since they are natural polyphenol-rich raw material to be incorporated into functional foods, nutraceuticals and pharmaceuticals. This is a major challenge for both industry and horticultural experts, because the content of health-promoting compounds in plants varies widely not only in different plant species, but also between cultivars. The content is also significantly affected by harvesting, storage and processing factors. This review summarizes the recent data and clarifies the main contributors of harvesting time, various storage conditions and post-harvest procedures, such as temperature management, controlled atmosphere, 1-MCP, calcium and plant activators, as ways to influence health-promoting compounds in fruits. Furthermore, the ways processing factors, e.g., enzymatic treatment, pressing, clarification, temperature, pressure and fermentation, can influence the levels of polyphenols and vitamins in berries and soft fruits will be discussed. Finally, strategies for preventing the decline of health-promoting compounds in fruits during long-term storage will be assessed in light of recent scientific progress and modern methods, which preserve the levels of polyphenols, will be highlighted.

**Keywords:** fruits; berries; polyphenols; vitamins; harvesting; storage; processing; human health; food industry

# 1. Introduction

There is increasing epidemiological and experimental data indicating that the consumption of vegetables and fruits confers many health benefits. Functional products containing elevated levels of bioactive compounds are attracting considerable attention, as they are believed to lower the risk of major diseases and, thus, reduce their associated huge healthcare costs. Numerous studies have highlighted the key role of oxidative stress and inflammation in major chronic diseases, including cardiovascular and Alzheimer's disease (AD), several cancers, type 2 diabetes (T2D) and age-related macular degeneration. Nuclear factor-erythroid 2-related factor-2 (Nrf2) is a transcription factor playing an important role in the antioxidant response element (ARE)-mediated activation of protective enzymes [1]. Fruit/berry polyphenols may be able to activate the Nrf2-ARE signaling pathway and Nrf2 target genes and, hence, attenuate cellular oxidative stress; this represents an interesting therapeutic approach against inflammation, oxidative damage and cell death. Diverse groups of phenolic compounds are capable of reducing oxidative stress by activating Nrf2 and inducing protective enzymes, such as heme oxygenase-1 and glutathione S-transferase pi 1 [2]. Furthermore, polyphenols can attenuate oxidative stress by correcting the dysregulation of autophagy function. An impaired autophagy system has been associated with many chronic diseases [1], and for example, improved cardioprotection in rat was demonstrated when autophagy was induced by polyphenols [3].

Major chronic diseases, including cardiovascular disease, AD, T2D, obesity and several cancers, are inflammatory-related diseases. Bioactive compounds possessing anti-inflammatory properties have been isolated from a number of plants. Many well-known polyphenols have been demonstrated to inhibit the development of inflammatory cytokines *in vitro* and in animal models. In a recent human trial [4], long-term supplementation with polyphenol-rich grape extract downregulated the expression of key pro-inflammatory cytokines, suggesting that these polyphenol treatments may have a beneficial immunomodulatory impact on humans suffering from hypertension.

Polyphenols may also modulate specific molecular targets. For example, polyphenol treatment affects several genes important in lipid and energy metabolism, as well as key factors in adipocytes differentiation [5]. Polyphenols can also directly modulate glucose transport, insulin levels and 5' adenosine monophosphate-activated protein kinase phosphorylation and may, hence, offer new specific molecular targets for T2D management [6]. Polyphenols are also cardioprotective; they inhibit low-density lipoprotein oxidation and platelet aggregation, enhance vasodilatation of blood vessels by increasing nitric oxide production, inhibit the adherence of monocytes to vascular endothelium and promote fibrinolysis [7].

A recent work demonstrated [8] that anthocyanin-rich berries can directly affect key pathogenesis process in AD; this was found to be associated with the alleviation of behavioral abnormalities in a

mouse model of AD. Furthermore, it was found [9] that blueberry-fed animals exhibited a faster rate of learning and improved spatial memory performance compared to those on control diet; improved behavioral performance was associated with favorable changes in total cyclic adenosine monophosphate response element-binding protein and pro- and mature brain-derived neurotrophic factor in the hippocampus. This factor acts in brain cell development, growth and survival by promoting synaptic plasticity in the hippocampus.

The majority of recent progress is based on cell studies and animal models, and we have only few human data available from extensive trials [4]. Limited bioavailability, low target specificity and rapid metabolism of polyphenols in human body are gaps in scientific knowledge; the challenge of getting beneficial compounds to plasma and target tissues in high enough concentrations still needs to be overcome.

There is a wealth of data indicating that lifestyle factors, including healthy food, such as vegetables, berries, nuts and fish containing substantial amounts of polyphenols and other bioactive compounds, are effective means to improve the quality of life at older ages [10–12]. On a global scale, there is a growing potential to increase the use of berries and fruits as natural polyphenol-rich raw material in the areas of functional foods, nutraceuticals and pharmaceuticals. Each year, the food industry utilizes massive amounts of high-quality polyphenol-rich material, which need to be in stable conditions, and an inadequate supply of these materials, particularly fruits and berries, poses a major challenge to these industries. Pomace and other by-products of the juice processing industry are very rich sources of polyphenols, unsaturated fatty acids, phytosterols and fibers [13,14], and they are being increasingly exploited in food and health-related products.

It is known that the polyphenol concentration varies widely not only in different plant species, but even between cultivars, and it is influenced by numerous site-specific and environmental factors. In addition, polyphenol concentrations and health-promoting properties may be affected by harvesting and storage conditions [15], and many important bioactive compounds may suffer during inappropriate processing. Thus, these factors are crucial determinants of the quality of berry and fruit food materials used fresh and in various functional-type food products and supplements. Therefore, this review assesses the most recent data on the impact of harvesting, storage and processing on health-promoting compounds.

# 2. Major Health Beneficial Compounds in Berries and Fruits

Berries and fruits are excellent sources of a number of bioactive compounds believed to be beneficial for human health (Table 1). Flavanols (catechin and oligomeric procyanidins) are the major class of apple (*Malus domestica*) polyphenols [16,17], accounting for 70%–85% of polyphenols in apples, followed by hydroxycinnamic acids (3%–30%), flavonols (2%–10%) and dihydrochalcones (1%–5%); in red apples, there are minor amounts of anthocyanins (ACNs) (1%–3%). Interestingly, some flavonols, such as quercetin glucosides and cyanidin-3-galactoside, are almost exclusively present in apple peel, whereas hydroxycinnamic acids are found in the flesh, with procyanidins being present in high amounts in both flesh and peel [16]. Antioxidant activity varies between apple cultivars [18], and quercetin glycosides make the greatest contribution to overall antioxidant activity [19,20]; although, epicatechin and procyanidin B2 are also found to be responsible for antioxidant activity [21]. Generally, apples are not considered to contain high amounts of ascorbic acid

(AA), or vitamin C, in contrast to some berries, like strawberries or blackcurrants. Moreover, the contribution of AA to the total antioxidant activity of apples is minor as compared to some flavonoids, such as quercetin, epicatechin and procyanidin B2 [18].

The major polyphenols in pears (*Pyrus communis*) have been found to be procyanidins (96%); hydroxycinnamic acids (2%), arbutin (0.8%) and catechins (0.7%) are minor compounds [22]. In contrast, there is a recent report [23] that arbutin and catechin are the dominant polyphenol compounds in eight pear varieties grown in Asia, followed by chlorogenic acid, quercetin and rutin. Pomegranate (*Punica granatum*), which is cultivated throughout the Mediterranean region, in Southeast Asia and parts of the USA, contains a large number of bioactive compounds, but the predominant polyphenol is ellagic acid (EA) [24].

Dark-colored berries are important sources of ACNs. These compounds are attracting considerable interest for their potential health-promoting abilities, since they are claimed to modulate risk factors linked to AD [8], T2D [12,25] and obesity [26]. Blackcurrants (*Ribes nigrum*) contain four different ACNs; delphinidin-3-rutinoside seems to be the major ACN, followed by cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside [27,28]. Blackcurrants are recognized as a good source of AA; the AA content of seven blackcurrant cultivars (Bogatyr, Lentyai, Pamyati Vavilova, Seyanets Golubki, Titania, Varmas and Öjebyn) grown in Estonia ranged from 104 to 158 mg/100 g fresh weight (fw) [29]. In blueberry (Vaccinium corymbosum), 12-13 individual ACNs have been identified with delphinidin, malvidin and petunidin apparently being the major ACNs. Recently, it was revealed [30] that high concentrations of ACNs, as high as even 438 mg/100 g fw (i.e., 2762 mg/100 g dry weight (dw)), occur in blueberries grown in Germany. Bilberry (Vaccinium myrtillus), a wild relative of cultivated blueberry, is attracting considerable interest from the food industry, due to its potential health promoting properties. Müller et al. [30] determined the major ACNs in bilberry to be delphinidin-3-O-glucopyranoside, delphinidin-3-O-galactopyranoside and cyanidin-3-O-arabinopyranoside. The total mean content of ACNs was found to be 2878 mg/100 g dw in bilberries grown in Finnish forests [31], while those grown in German woods contained considerably higher amounts, a maximum of 1017 mg/100 g fw (7465 mg/100 g dw) [30].

Red-colored berries are particularly important sources of proanthocyanidins, ellagitannins (ETs), EA and some flavonols. ETs and EA are well-known bioactive compounds with putative protective activities against different types of cancers [32,33]. Strawberries (*Fragaria x ananassa*) are globally the most widely consumed berries; they are used fresh, frozen and in processed forms. In a recent analysis of 15 strawberry cultivars grown in Spain [34], proanthocyanidins were found to be the main phenolic compounds, and the content ranged from 54 to 163 mg/100 g fw. In 27 strawberry cultivars grown in Norway [35], the levels of flavanols ranged from 11 to 45 mg/100 g fw. The ACNs in strawberries are different from those in dark-blue berries, *i.e.*, pelargonidin-3-glucoside, cyanidin-3-glucoside, 3-rutinoside and 3-malonyl glucoside are the main ACNs present in strawberries; the concentrations have been found to vary between 20.2 and 47.4 mg/100 g of fw in cultivars grown in Spain [34] and between 8.5 to 65.9 mg/100 g of fw in the cultivars grown in Norway [35]. Strawberry is also a rich source of AA, but its content can be affected by several factors, including genotypic differences, cultural practices, climatic preharvest conditions and postharvest procedures. There are reports that the AA content varies between 48–62 mg/100 g fw [36] and 47–72 mg/100 g fw [37] in different strawberry cultivars grown in Northern Europe.

#### Processes 2014, 2

Raspberries (*Rubus idaeus*) are locally important in several countries and also used in different processed products. The raspberry is a versatile source of ETs and EA. In one report, the concentrations of EA varied between 38–118 mg/100 g fw [38], and in another from 120 to 323 mg/100 g fw [39], depending on the cultivar. The antioxidant capacity in raspberry cultivars is also clearly correlated with the total EA content [39,40]. The quercetin content was stated to vary from 0.32 to 1.55 mg/100 g fw [38], while the AA varied from 15.6 to 24.4 mg/100 g fw in different raspberry cultivars [39].

Plant species	Compounds	Plant species	Compounds
Apple [16,17]	anthocyanins	Pear [22,23]	arbutin
	dihydrochalcones		flavanols/procyanidins
	flavanols/procyanidins		flavonols
	flavonols		hydroxybenzoic acids
	hydroxycinnamic acids		hydroxycinnamic acids
Bilberry [31,41]	anthocyanins	Pomegranate [24]	anthocyanins
	flavanols/procyanidins		ellagic acid/ellagitannins
	flavonols		flavonols
	hydroxycinnamic acids		gallotannins
Blackcurrant [27,28]	anthocyanins		hydroxybenzoic acids
	flavonols		hydroxycinnamic acids
	hydroxycinnamic acids	Raspberry [38,39]	anthocyanins
Blueberry [42]	Anthocyanins		ellagic acid/ellagitannins
			flavonols
	flavanols	Strawberry [34,35]	anthocyanins
			ellagic acid/ellagitannins
	flavonols		flavanols/procyanidins
			flavonols
	hydroxycinnamic acids		hydroxycinnamic acids

Table 1. Phenolic compound groups determined in some fruits and berries.

In addition, cranberry (*Vaccinium, Oxycoccos* ssp.) has been extensively evaluated [43] and is recognized as a rich source of proanthocyanidins [44]; however, cranberries also contain numerous other bioactive compounds, including phenolic acids, benzoates, hydroxycinnamic acids, terpenes, as well as seven different flavonol glycosides [45]. Moreover, wild lingonberries (*Vaccinium vitis-idaea*) and sea buckthorn (*Hippophae*) are increasingly used in many different health-related and processed products, as these berries contain substantial amounts of polyphenols and other bioactive compounds. Chokeberry (*Aronia melanocarpa*) is a very rich source of polyphenols; it has among the highest total ACN content of all berries (1,480 mg/100 g fw) [46], as well as being a good source of proanthocyanidins (up to 664 mg/100 g of fw) and flavonols (267 mg/100 g fw) [47]. Plum (*Prunus domestica*) is an important fruit species consumed mainly fresh or dried, but also as juice and nectar [48]. The main phenolic compounds of the flesh and peel of different plum genotypes are flavanols (13.9–183.7 mg/ 100 g fw), flavonol glycosides (14.3–35.2 mg/100 g fw), neochlorogenic (1.6–34.2 mg/100 g fw) [49].

Besides health properties, phenolic compounds contribute to the sensory attributes of fruits and berries and fruit/berry products [50–52]. Astringency and bitterness are the taste properties most often associated with phenolic compounds. For example, procyanidins are responsible for the astringent taste of apples and plums; instead, in pomegranate, raspberries and strawberries, ellagitannins are the main contributors of astringency [51]. In addition, some berry flavonols and hydroxycinnamic acids have astringent properties, and it has been suggested that some flavanols may partly elicit the bitter taste of black currant [52].

# 3. Impact of Harvesting on Quality and Health-Promoting Compounds

# 3.1. Accumulation of Polyphenols and Ascorbic Acid during Fruit Maturation: Gene Expression and Biosynthetic Pathways

Maturity stage is an important factor affecting the polyphenols and the overall quality of berries and fruits. It is important to understand the key pathways involved in polyphenol formation during the maturation process to permit the optimal production of polyphenols. Phenylalanine is the driving chemical in the synthesis of phenylpropanoids that are later channeled down the flavonoid pathway by chalcone synthase (CHS), chalcone isomerase (CHI), flavone 3 B hydrolase (FHT), dihydroflavonol 4-reductase (DFR) and anthocyanin synthase (ANS), which form the basis of the anthocyanin pigments. In this pathway, flavonol synthase (FLS) produces flavonols, but leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) synthesize flavanols, the key precursors of proanthocyanidin polymers (further details are provided in Hichri *et al.* 2011) [53].

It has been reported [54] that strawberries start to accumulate flavanols at high levels during early growth stages, whereas ACN synthesis starts later and is the most abundant when the berries are ripe. Accordingly, the amount of total phenolics (TPs), total EA, total flavonoids and total antioxidant activity were found to be higher at the pink stage than at the ripe stage, whereas ACNs and AA were highest when the berries were ripe [55–57]. This is in accordance with the data of Griesser et al. [58], who found that the glycosyltransferase sequence (FaGT1) gene, which encodes for strawberry ACN, was almost undetectable in green berries, but gene expression increased dramatically when berries turned color and ripened into red berries, corresponding closely to the increase concentrations of ACNs occurring during berry ripening. The regulation of structural gene expression appears to be tightly organized in a spatial and temporal manner during plant development, and the MYB transcription factors are the key regulators of the flavonoid pathway, including the enzymes involved in ACN biosynthesis [54]. In strawberry, the most important transcription factors also belong to the MYB-family, and FaMYB10 seems to play the key general regulatory role in the flavonoid/phenylpropanoid pathway during the ripening of strawberry [59]. In pear, there is data [60] that most of the structural genes are upregulated in the red-skinned cultivar during fruit development, but the CHI and UFGT genes are highly expressed only at the early stages. The ACN biosynthesis in the apple is developmentally regulated and also occurs in two phases. The first peak occurs during the early, fruitlet stage in both red and non-red cultivars, but the most important second stage occurs during the ripening stage only in red cultivars, and this determines to a great extent the consumer value of the apple [61]. At the gene level, ACN accumulation in apples is coordinated by the R2R3 MYB transcription factor [62] that has been shown to control fruit flesh and foliage ACN pigmentation (MYB10) and fruit skin color (MYB1).

Two genes, *FaGalUR* and *FaMIOX*, appear to play key roles in the regulation of L-ascorbic acid (L-AA) biosynthetic pathways during the ripening stages in strawberry. For example, it has been reported that the expression of *FaGalUR* increased by over 150-fold during the development of the fruit from the green to the ripe stage in strawberries, while at the same time, the L-AA content on a dry weight basis in strawberry (cv. Chandler) increased 12-fold from the green immature to the ripe stage [63].

#### 3.2. Harvesting Time

The harvesting time of fruits, such as apples, for the fresh market differs from those apples planned for long-term storage. Generally, apples intended directly for consumption as fresh products are harvested at a later stage when color, sweetness, acidity, juiciness and other quality attributes meet consumer preferences. However, when apples, pears or berries are harvested at a later stage, or the fully red-stage, when ACN and aroma biosynthesis are fully expressed, these fruits are no longer suitable for long-term storage, since postharvest decay and other losses will be high. For example, it has been postulated [64] that the optimal time for harvesting of apple cv. Jonagold for long-term storage should be close to their pre-climacteric respiration minimum, which coincides with the early onset of the climacteric ethylene production. However, optimal harvesting time is largely dependent on the prevailing temperature and genotype-environmental interaction.

In a recent North American study, strawberries (cv. Jewel) were harvested at the white tip stage or at the red ripe stage and stored for 12 days at 3 or 10 °C in 65% or 95% relative humidity (RH) [56]. It was observed that the overall quality and firmness declined more rapidly in berries harvested at the red ripe stage than in berries harvested at the white tip stage. Quality factors decreased more rapidly at 10 °C than at 3 °C in berries of both maturity stages. Furthermore, the initial ACN concentrations in red ripe berries were about five times greater than the concentrations in white tip stage berries and declined during the storage. The TP and total flavonoid concentrations, as well as the antioxidant activity of berries harvested at the white tip stage were greater in comparison to berries harvested at the red ripe stage. Interestingly, these differences were well maintained during the storage; in red ripe berries, however, compound concentrations and antioxidant activity decreased rapidly by the Day 12 in berries stored at 10 °C, especially at 95% RH. The total AA concentrations of white tip berries were lower than the concentrations of red ripe berries at harvest, but increased slightly over time, while concentrations in red ripe berries remained relatively stable until Day 12. However, strawberries harvested at the three-quarters colored stage developed the same pH, acidity, soluble solids, AA and TP content during storage as berries harvested at the full red stage [65]. Collectively, these data confirm the belief that harvesting strawberries at an early stage (white tip/three-quarters colored stage) helps to maintain berry quality during the storage period, whereas the quality of berries harvested at the red ripe stage will decline more rapidly. In most crops, the prevailing environmental conditions, particularly temperature and solar radiation, are critical determinants of the levels of health-promoting compounds at harvesting time and should be considered when planning optimal harvesting dates for a specific area and specific crops.

# 4. Impact of Storage on the Health-Promoting Compounds

Many horticultural crops, such as berries and fruits, are highly perishable and vulnerable to a number of factors, worsening quality during storage. Softening of berries and fruits associated with increasing respiration and ethylene production predisposes fruits/berries to decay and microbial contamination. Importantly, it has been claimed [66] that a loss of flesh firmness is correlated with an initial increase in the transcript accumulation of two expansin-encoding genes and pectate lyase genes in strawberry, followed by increases in the transcript accumulation of pectin methylesterase, polygalacturonase and  $\beta$ -galactosidase. It seems likely that the high transcript accumulation of the expansin genes at an early stage of maturation may act as precursors in the softening process, and the significant expression of pectin methylesterase and polygalacturonase eventually may be responsible for the rapid decline of firmness in the strawberry [67]. Furthermore, the levels of free amino acids declined gradually before the red-ripening stage, but increased significantly in the over-ripening stage [68], suggesting that several metabolites may be involved in the softening process in strawberries. We will first summarize the recent work about the impact of storage conditions on the major health-promoting compounds and subsequently describe potential ways to prevent the deterioration of quality factors during storage.

# 4.1. Changes in the Total Phenolic Compounds during Storage

Polyphenols are known to be rather unstable during storage at ambient temperatures [69]. TP and total flavonoid concentrations of red ripe strawberries were less well retained at 10 °C than at 3 °C after a 12-day storage period, and the effect of RH on TPs and flavonoids was also clearer at the higher temperature with 65% RH being less detrimental than 95% [54]. The TP content of pomegranate cv. Bhagwa displayed relatively good stability during a four-week storage period at 5, 7 and 10 °C in 92% RH, but the TP content, which increased at 7 and 10 °C, had started to decline at 5 °C after eight weeks of storage; in contrast, the TP content of pomegranate cv. Ruby decreased within four weeks if the fruit was stored at 10 °C [70]. In fact, after eight weeks, the TP content in Ruby started to decline at all storage temperatures tested (5, 7 or 10 °C) [70]. There is another report that the levels of TPs in pomegranate cv. Mollar de Elche kept at 2 °C in 90% RH were reduced after 84 days of storage [71].

The levels of TPs in raspberry cultivars generally seemed to decline as a result of freezing, but the extent of reduction was dependent on the cultivar (cv. Aksu KIrmIzIsI, Rubin, Newburgh, Hollanda Boduru or Heritage); for example, the EA contents of raspberries fell during a six-month storage period at -22 °C, but the reduction was even greater in just-frozen (air blast freezing at -35 °C for 5 h) samples [72]. The decrease in raspberry EA content during frozen storage at -22 °C was associated not only with the freezing procedure, cultivar and environment-dependent aspects, but also with the formation of chelates between free EA and metallic cations, reactions with free radicals and the release of polyphenol oxidase from the raspberry matrix [72]. The radical scavenging activity (RSA) of raspberry cv. Glen Ample increased with four days of storage independently of the storage temperature (1 or 4 °C) or packaging material (lidded punnets, 30- $\mu$ m polypropylene film, 25- $\mu$ m oriented polypropylene film or Xtend<sup>®</sup> film (StePac, Western Galilee, Israel)) [73].

Changes in apple phenolics have been shown to be altered in a cultivar-dependent manner during cold storage in air or under a controlled atmosphere (CA). During 120 days of storage at 0 °C in a

normal atmosphere (NA, 85%–90% RH) or in a CA (2% CO<sub>2</sub>, 2% O, 95% RH), TPs of apple cv. Jonagold were found to increase [74]. The TP content of apple cv. Champion stored in a CA (2% CO<sub>2</sub>, 2% O, 95% RH) at 0 °C became enhanced during 120 days [74]. Even though the TP content of Champion apples was not altered during the initial 120-day cold storage period in a regular cold chamber (0 °C, 85%–90% RH), their TP content increased with an additional seven days at 16 °C [74]. In an extensive study, apple phenolics (19 cultivars) in general were found to be rather stable with a storage period of 4.5 months in a regular cold chamber at 1 °C or under CA (1.2% O<sub>2</sub>, 2.5 CO<sub>2</sub>) at 1.5 °C, as well as during a two-week shelf life at room temperature (20 °C) after the cold storage [75]. It has been claimed that the RSA of the peels of apple cv. Jonagold previously stored at 0 °C in a regular cold chamber or in CA became enhanced when the apples were transferred to 16 °C for an additional seven days [74]. The increase of RSA in apple peel during long-term cold storage at 0°C with an additional short period at 16 °C paralleled the increase in the TP content [74]. Long-term cold storage at 0 °C has also increased the levels of RSA in the peels and flesh of Cripps Pink apples [76].

A significant increase in the TP content was observed to take place in a harvest date and cultivar dependent manner in blueberries stored at 5 °C for three weeks [77]. During the 35-day storage period at 5 °C in air, the TP content of highbush blueberries (cv. Duke) at first was increased within the first week of the experiment, but then reached a steady-state level, although under high-oxygen conditions (40%–100%  $O_2$ ), the TP content steadily increased during the whole storage period [78]. No significant differences in the levels of phenolic compounds in relation to storage atmosphere were found in strawberry cultivars Aromas, Diamante and Selva [79].

By choosing optimal storage methods and conditions, such as temperature, RH and the gas composition of storage atmosphere, for each berry or fruit species and cultivar, it is possible to minimize the phenolic degradation and harmful chemical interactions during storage.

# 4.2. Changes in the Concentrations of Anthocyanins

The biosynthesis of ACNs continues after harvest and is clearly affected by the storage temperature. The ACN content in several fruits increases during short-term storage. For example, ACN content in Sonata, Polka and Honeoye strawberries increased with a five-day storage at  $3 \pm 1$  °C [37], while the ACN content of raspberry cultivars Resa, Rumiloba, Schönemann and Tulameen fruits stored one day at 20 °C or three days at 2–4 °C followed by one day at 20 °C contained higher ACN levels than the fresh berries [80]. Moreover, blueberry cultivars stored at 5 °C exhibited a significant increase (5%, on average) in the ACN content even after three weeks, although the impact was both harvest date and cultivar dependent [77].

Lower temperatures generally inhibit the synthesis of ACNs. The concentrations of ACN of pomegranate cultivars Bhagwa and Ruby increased during an eight-week storage period at 5, 7 or 10 °C, and this increase occurred in parallel with the increasing temperature [70]. In addition, the ACN content of Glen Ample raspberries increased significantly (26%–61%) during storage, with the effect being more pronounced at 4 °C than at 1 °C [73]. The extent of increase of ACNs in Glen Ample raspberries was also affected by the packaging material [73]. However, exposure to higher temperatures during a longer period might accelerate the degradation of ACNs. The ACN content of Jewel

strawberries suffered a decline at 10 °C if storage was longer than nine days, whereas at 3 °C, the ACN content in strawberries remain rather stable [56].

There is recent information indicating that during long-term storage, the ACNs are easily degraded [81]. However, the ACN synthesis and degradation patterns are cultivar dependent: the ACN content in the pomegranate cv. Ruby increased at 7 and 10 °C up to Week 12, only declining until the end of Week 16, whereas the ACN content of cv. Bhagwa started to fall with eight weeks of storage at 5, 7 and 10 °C, a trend that continued until the end of the 16-week experiment [70]. Nonetheless, there is one report that the ACN content in pomegranate cv. Mollar de Elche increased significantly (86%) during 84 days of cold storage at 2 °C [71].

In addition to storage temperature, the metabolism of ACNs can be regulated by changing the gas composition in the storage facility or package by using CA. The storage atmosphere is a factor affecting the degradation rate of ACNs. After 24 days of CA storage, blueberries exhibited higher firmness, better color and higher ACN and acidity levels as compared with controls [82]. High-oxygen levels at 60%–100% O<sub>2</sub> were found to maintain or even increase the total ACN content and concentrations of individual ACNs of highbush blueberry cv. Duke during 35 days of storage at 5 °C [78]. In raspberries (cv. Malling Orion, Malling Admiral, Glen Lyon, Glen Ample and Lyon), the average ACN pigment level increased during one week of storage in a normal atmosphere (NA), but not in the CA (10% O + 15% CO<sub>2</sub> and 10% O + 31% CO<sub>2</sub>) [83]. In contrast, the ACN contents of Jonagold and Champion apples did not change during 120 days of CA storage (2% CO<sub>2</sub>, 2% O, 95% RH), whereas in NA conditions (85%–90% RH at 0 °C), the concentrations of ACNs did decline [74]. An additional seven days of storage at 16 °C was found to further decrease the ACN contents in apples stored in NA, but this trend was observed in cv. Jonagold only when fruits were stored in CA [74].

As a conclusion, optimizing the storage temperature is crucial for the increase or preservation of ACN content during the short- or long-term storage of fruits and berries harvested at different maturity stages. ACN stability may also be supported by controlling RH and atmosphere during the storage period.

# 4.3. Changes in Ascorbic Acid Content

One of the most reactive compounds in fruits and berries is L-AA; thus, it is particularly vulnerable to storage conditions. In general, there is a progressive loss of L-AA with time, and the extent of this loss is profoundly affected by temperature [84]. However, the rate of loss varies widely between species and cultivars. Strawberries (cv. Chandler, Oso Grande and Sweet Charlie) stored at 20 °C suffered a significant loss of AA already after four days, whereas the loss was minor at 1 or 10 °C over eight days [85]. The AA content of Sonata strawberries increased, but that of Polka strawberries decreased during 12-days of storage at  $3 \pm 1$  °C [37]. It has been reported that the AA content in five Israeli strawberry cultivars stored in CA at relatively low O<sub>2</sub> and CO<sub>2</sub> levels (5 and 0.5 kPa, respectively) for 12 days was higher than that present in strawberries stored in NA [86]. Honeoye strawberries stored in modified atmosphere packages (low-density polyethylene or Xtend<sup>®</sup> film) for 12 days at  $3 \pm 1$  °C, had a higher AA content than the control berries (stored in NA) [37]. Strawberries (cv. Camarosa) packed in polypropylene baskets and stored in ozone (35 ppm) for 72 h maintained their L-AA content better than the fruit stored in air [63]. The average AA content of raspberries (cv. Malling Orion, Malling Admiral, Glen Lyon, Glen Ample and Lyon) did not change during one

week of storage at 1.7 °C and RH of 95% in either NA or different CA regimes (10% O + 15% CO<sub>2</sub> and 10% O + 31% CO<sub>2</sub>) [83].

The AA content of different raspberry cultivars (cv. Resa, Rumiloba, Schönemann and Tulameen) stored at 20 °C for one day or three days at 2–4 °C followed by a one-day period at 20 °C were not altered by the storage conditions [80]. In another study, a storage temperature of 1 or 4 °C exerted no significant effect on AA contents in raspberries Glen Ample packed in different kinds of materials [73]. The content and stability of AA in apples being stored depends greatly on the cultivar. Early maturing cultivars generally have a lower AA concentration and lack the ability to maintain that concentration over an extended period of storage in comparison with cultivars that mature later in the season [87]. Moreover, changes in AA also may depend on the light conditions [88], as the amount of AA in the apple flesh was nearly the same during storage, but the AA concentration increased in the skin [89].

In conclusion, available data suggest that storage conditions must be evaluated individually for each fruit or berry cultivar to preserve AA. In those species sensitive to AA degradation, modifying the storage atmosphere in the storage facility or by customized packaging may help to diminish losses.

# 4.4. Postharvest Technologies and Their Impact on Quality and Phytochemicals

The short postharvest life of fruits and berries demands that efficient technologies have to be exploited in order to control decay. The rapid cooling of berries and some fruits after harvesting and subsequent storage at a low temperature are effective means to increase the shelf life of fruits by reducing the respiration rate, ethylene production, disease development and the overall decay process. In general, CA storage appears to reduce fruit softening and extend the shelf life of fruits, but the benefits tend to be genotype-dependent. Storage in CA also influences the ethylene production rate, but the overall impacts seem to depend on both the date of harvesting and genotype [90,91]. The timing of harvesting may also be important in the management of ethylene production. For example, Bulens *et al.* [91] found that the increase in the ethylene production rate in early harvested apples did not occur until 14 days of shelf life, while apples harvested later possessed a higher initial ethylene production rate, then their ethylene levels increased exponentially after seven and 14 days of the shelf life. Accordingly, it was showed that spraying apples with a commercial ethylene inhibitor, Harvista<sup>TM</sup>, led to better postharvest firmness and reduced the ethylene production [92]. Treated apples exhibited differential induction of ethylene biosynthesis and receptor genes in apple, but the timing of the Harvista<sup>TM</sup> treatment was found to be critical.

1-methylcyclopropene (1-MPC), a commercial blocker of ethylene synthesis, is widely used to slow softening and to reduce the incidence of physiological changes [93]. 1-MPC treated highbush blueberries (cv. Lateblue) stored at 0 °C in regular cold storage conditions (0 °C, 90%–95% RH) or under CA (3 kPa O<sub>2</sub>, 11 kPa CO<sub>2</sub>, 90%–95% RH) were able to maintain their ACN and TP content similarly to control berries for 35–60 days [94]. Furthermore, 1-MPC treatment only slightly affected the TP content and individual phenolic compounds present in apples (cv. Cripps Pink) during long-term cold storage in plastic mesh bags at 0 °C in air or in CA (2% O<sub>2</sub> + 2% CO<sub>2</sub>) [76]. Moreover, 1-MCP-treated pears exhibited higher superoxide dismutase (SOD) and catalase (CAT) activities together with higher AA levels during cold storage; evidence that 1-MCP treatment can be used to modulate the antioxidant

properties of pears during storage and thereby inhibit harmful impact of reactive oxygen species production [95]. A reduced oxygen content in the storage atmosphere and ethylene inhibitors have sometimes been found to help in preserving the AA content in fruits and berries, but the effect again depends on species and cultivar. However, there is data [96] indicating that the AA concentrations were lower in 1-MCP treated Empire apple tissues towards the end of storage. Moreover, the AA content was better retained in 1-MCP-treated apples (cv. Talvenauding) in one experimental year, but not in a second year [97], suggesting that the impact of preharvest factors or harvest maturity on AA content might have a greater influence than any effects on the ethylene inhibitor.

The activation of plant defense pathways by external applications of elicitors is becoming a viable means to protect or limit microbial spread in storage. Benzothiadiazole (BTH), a salicylic acid analog, has been widely used to control plant diseases and to enhance polyphenol levels in berries and fruits [98,99]. For example, 0.2 g/L BTH treatment increased the activity of several antioxidant enzymes, including SOD, ascorbate peroxidase and glutathione reductase, and elevated the expression levels of many genes (phenylalanine ammonia lyase, cinnamate-4-hydroxylase and DFR) involved in the flavonoid pathway, with increased amounts of ACNs and overall antioxidant activity being detected in the fruits [100]. Moreover, BTH treated strawberries exhibited higher levels of ACNs during 10 days of storage at 1 °C [101]. In apples, BTH treatment was reported to increase significantly the levels of glutathione transferase and glutathione peroxidase [102].

The extent of decay in strawberries stored either 5 °C or 10 °C was significantly reduced, and the shelf life was extended by immersing fruits in chitosan solutions of 0.5, 1.0 and 1.5 g/100 mL for 5 min at 20 °C [103]. Moreover, it was demonstrated that chitosan treatment maintained better fruit quality with higher levels of many polyphenols, *i.e.*, ACNs, flavonoids (quercetin-3-glucoside, quercetin-3-glucuronide, kaempferol-3-glucoside, kaempferol-3-glucuronide, cyaniding-3-glucoside, pelargonidin-3-glucoside, cyaniding-3-glucoside-succinate and pelargonidin-3-glucoside-succinate), EA, EA glucoside and *p*-coumaroyl glucose than berries without chitosan treatment.

Mild ethylene or methyl jasmonate (MJ) application can also enhance the synthesis of some health-promoting secondary metabolites. For example, it was found [104] that strawberries exposed to ethylene displayed a 20% increase in antioxidant activity after four days of storage at 20 °C. Furthermore, MJ treatment (10 and 100  $\mu$ M) accelerated strawberry ripening and enhanced ACN accumulation in berries, but MJ also altered the expression of cell wall modifying enzymes (endoglucanase-1 and xyloglucan endo-transglycosylase/hydrolase-1), as well as increasing the expression of lysyl oxidase, allene oxide synthase and 12-oxophytodienoate reductase genes involved in the biosynthesis of jasmonic acids, and these changes correlated with a transient activation of fruit ripening [105].

Oxalic acid, a natural organic acid, has been also used for the postharvest preservation of fruits. Increased levels of AA and ACNs in pomegranates have been detected after oxalic acid treatment, but often in a dose-dependent manner [71]. A major increase in the concentrations of ACNs in pomegranate has been found to occur in fruits coated with polyethene wax emulsion (Brillaqua) after one month of storage [106]. The next highest levels were found in non-treated fruits, followed by fruits covered with polyethylene film and fruits coated with the combination of CaCl<sub>2</sub> and polyethene wax [106]. ACN concentrations in pomegranates coated with plain CaCl<sub>2</sub> were also elevated and reached their maximum level after two months of storage at 5 °C [106].

In the future progress, one can predict that there will be the development and fine-tuning of genotype-specific, intelligent monitoring software, perhaps even electronic evaluation of fruit quality during storage. Target specific activation of molecular defense pathway by elicitors may become an important component in the integrated tool box for improving postharvest quality and preventing the decline of health-promoting compounds in stored fruits and berries. Clarification of the signaling cascades involved in the defense pathway holds great promise for the development of specific elicitors to elevate the levels of polyphenols in berries and fruits. Moreover, another exciting possibility may be coating the elicitor compounds onto fruit or berry surfaces with different nanoparticles so that the active compounds will be released in a controlled manner.

# 5. Impact of Processing on Health-Promoting Compounds

Fruits and berries are typically processed into different kinds of jams, jellies juices, nectars, purees, syrups and wines. The refining of fruit/berry raw-material into processed products usually comprises several stages, e.g., crushing, pressing, heat and/or cold treatments, filtration and enzymatic treatments. Yeast fermentation is a crucial step in wine production. It needs to be remembered that fruit/berry processing may alter the chemical characteristics of raw material and subsequently impact the bioactivities of health promoting compounds.

# 5.1. Crushing

Manufacturing procedures of many fruit/berry products require some kind of crushing of the raw material. Crushing results in the disruption of the fruit/berry skins, which promotes the extraction of fruit/berry constituents.

Crushing has been found to significantly decrease the TP content, for example, in highbush blueberries (cv. Rubel) [107]. In contrast, the levels of ACNs of blackcurrants (cv. Ben Hope and Ben Gairn) were not significantly reduced after the milling process [108]. The amounts of total ETs and EA-rhamnose were found to be lower in micro-crushed strawberries (cv. Camarosa) as compared to fresh strawberries, but the total content of free EA was found to be higher in a pureed product [109]. In addition, methyl EA appeared to be present in a strawberry puree even though the compound was not found in fresh berries [109]. The changes in the strawberry polyphenol profile during puree preparation by micro-crushing were claimed to result from the crushing of berry achenes and the mild thermal treatment (80 °C, 5 min) employed during the process [109].

Strawberry puree (cv. Senga Sengana) prepared by hand with a masher was found to be more susceptible to losses of AA than pressed strawberry juice [110]. The AA degradation in pureed strawberry was postulated to have been caused by the higher endogenous enzyme activity in the puree [110]. In fact, AA may exert a protective antioxidant effect with respect to polyphenolic compounds when it is used as an exogenous additive in the crushing step; for example, the addition of AA to apple pulp during apple crushing helped to retain the concentrations of many polyphenols in cloudy apple juices (cv. Champion) [111].

It can be concluded that the effects of the crushing phase on bioactive compounds depend not only on the mechanical degradation of different plant tissues as a result of different processing methods and steps, but on the presence and activity of endogenous enzymes and different antioxidants in the raw material, as well.

### 5.2. Enzymatic Treatments

Pectolytic enzymes are generally used to enhance juice flavor, stability and yields [112], since they are able to digest the plant cell wall, lower the viscosity and reduce the water binding capacity of enzyme-treated mashes. Commercial enzyme preparations may differ in their ability to degrade cell walls and vacuolar membranes of the plant matrix and to release aglycones from flavonol glycosides [52,113]. Mixing different enzyme products may be one way to increase the levels of different phenolic compounds in the final products [112].

Enzymatic mash treatment may be a good way to release polyphenols, for example, from strawberry and blackcurrant matrices [110,114]. Enzymatically-treated strawberry puree (cv. Polka) [115] and blackcurrant mash (cv. Ben Tron and Ben Nare) [114] have been found to have an even higher ACN content than fresh berries. There is a report that pectinase treatment can also increase the TP content in pomegranate juices (cv. Wonderful) [116]. However, pectinase treatment may also be detrimental to some ACNs as reported with strawberry (cv. Elsanta) and raspberry (cv. Sugana) juice production [117]. An enzymatic mash treatment step has been found to be beneficial in maintaining the RSA in pressed strawberry juice [110].

Enzymatic treatments of preheated (45 °C) blackcurrant mashes (cv. Mortti, Mikael, Marski, Ola and Breed15) during juice processing increased the yields of TPs, ACNs, hydroxycinnamic acid derivatives and flavonol glycosides in the final products [52]. Increasing the incubation temperature from 40 °C to 50 °C during the enzymatic treatment was found to help in the release of phenolic compounds into blackcurrant juices [112]. The contents of flavonol glycosides, dihydrochalcone and polymeric procyanidins in cloudy apple juices produced from pectinase-treated mashes, however, have been observed to be less than those in fresh fruit [111]. A one-hour depectinization treatment of highbush blueberry material (cv. Bluecrop) executed at 40 °C was the most likely cause of the ACN degradation and polymerization found in the monomeric ACNs occurring during the production of different blueberry products [118]. Treatments with cell wall-degrading enzymes have also been observed to significantly reduce the AA content in strawberries (cv. Senga Sengana) [110]; in particular, a long standing time (90 min) during the strawberry mash depectinization appeared to be rather detrimental to the final product.

The reduction in the levels of strawberry ACNs during juice production has been reported to be associated with the pectinase enzyme incubation step if it is conducted at 50 °C for 4 h [117]. The degradation and polymerization of ACNs in highbush blueberries during pectinase treatment was thought to be partially caused by endogenous enzymes in the blueberries not being inactivated by the blanching of the berry material [118].

Enzymatic treatments and their pretreatments can be largely customized to meet the requirements of specific raw material, and the chemical composition of final products can be somewhat tailored by modifying this processing step. Especially the treatment temperatures should be carefully tested to minimize the phenolic losses and harmful endogenous enzyme activities.

# 5.3. Pressing

Pressing is a basic step in juice production leading to the formation of a juice and fruit/berry press cake, or a pomace, as the residue. The food industry utilizes several kinds of pressing procedures, *i.e.*, altering mechanisms, pressures, pressing times and filters. Press-cakes hold a significant portion of the phenolic compounds [107,108,114]; hence, pressing may decrease TPs, for example, in blueberry [107] and blackcurrant [114] juices. Pressing with a membrane press has been found to be one of the most destructive steps leading to a loss of AA in strawberry juice and nectar (cv. Polka) [115]. However, the AA content of strawberries (cv. Elsanta) was not affected by juice extraction in a domestic juice extractor [117]. The total ACN contents in pressed, non-clarified strawberry juice (cv. Elsanta) and paste made of thawed berries were found to be similar [117].

The pressing method of apple material (cv. Champion and Idared) during the wine making process may influence the polyphenol profile of musts [119]. The contents of epicatechin and procyanidin B2 in apple musts were many times higher in musts extracted with a juice extractor than in musts from a vertical basket pressing; it is thought that the apple material was more profoundly homogenized by the juice extractor, and hence, the phenolic compounds were more effectively released [119]. In pomegranate juices produced from whole fruits (cv. Hicaznar) with different pressing times (5.5–25 min) and pressure conditions (1.2–4.8 bar), TP contents were higher when higher pressures were used [120]. However, the total amounts of condensed tannins and monomeric ACNs in the juices decreased when higher pressures were used accompanied by longer handling times; the reduction of the monomeric ACN compounds may be attributable to the formation of copigmented and polymeric pigments during the processing [120].

# 5.4. Clarification

The solids (e.g., proteins, pectins and also some phenolic compounds) of juices are separated from the liquid in a clarification step in order to obtain a non-cloudy, haze-free product. Centrifugation is a common, mechanical way to remove cloud from juices, but also spontaneous sedimentation with flocculants, as well as pectinase and protease treatments are utilized. Micro- and ultra-filtrations are one-step procedures for both juice clarification and sterilization [121].

The total ACN and AA contents measured in strawberries (cv. Elsanta) were retained in the juice after the centrifugation step; however, a large proportion of the TPs measured in berries were found to be concentrated in the pellet instead of the juice fraction [117]. When highbush blueberry juice (cv. Bluecrop) was subjected to centrifugation, the ACNs were demonstrated to be extensively concentrated in the sediment [118]. Blanching was found to be beneficial for retaining ACNs in highbush blueberry (cv. Rubel) juices after the clarification step [107]. Ultra-filtration has been reported to decrease both ACN and flavonol levels of pasteurized blackcurrant juices (cv. Öjebyn) prepared with a hydraulic press and pre-subjected to pectinase treatment and centrifugation [121]. Clarification of blackcurrant juice (cv. Ben Tron and Ben Nare) by enzymatic treatments has been shown to increase the level of TPs in the product in comparison with raw, non-clarified juice [114].

### 5.5. Temperature-Related Treatments

The food industry often employs procedures that involve temperature associated steps, such as freezing, thawing, many kinds of heat treatments and cooling. Freezing generally enhances preservation of raw materials and products, but then thawing is required to enable subsequent processing of frozen fruits and berries. Heat treatments may be applied to help to dissolve product components and/or to destroy harmful enzymes and microorganisms. Cooling is often used between the manufacturing steps or prior to packing and storage of the product.

The different kinds of temperature-related steps involved in the production of juices, purees, jams, nectars and wines can reduce the levels of TPs and total ACNs, at least in strawberry products [110,115,117,122]. The reduction of TP levels in strawberries during jam production has been postulated to result from enzymatic and auto-oxidation of the compounds and to some extent from the breakdown of some ACNs and other flavonoids by high temperatures [122]. The amounts of ACN compounds in raspberry pastes (cv. Sugana) have also been found to significantly decrease during long-term heat treatment (100-140 °C, 20 min), and even at temperatures of 20-100 °C, a slight reduction has been recorded [117]. During raspberry jam processing (cv. Heritage), an increase of free EA has been detected, and heat and cooling have suggested to have only a minor effect on the levels of raspberry EA glycosides and flavonol glycosides (e.g., quercetin-3-glucoside and kaempferol-3-glucoside), in general [123]. The total ACN contents have been shown to be a result of a pasteurization step during the production of pomegranate juices (cv. Hicaznar) [124]. The canning process of strawberries (cv. Totem) has been reported to reduce the TP concentration in the processed berries as compared to frozen fruit as a result of heating at 100 °C for 15 min [122]. Moreover, canned highbush blueberries (cv. Bluecrop) prepared utilizing several heat treatments resulted in losses of monomeric ACNs [118]; the losses of TPs and monomeric ACNs in canned berry products occurred via ACN degradation and polymerization [118,122]. While antioxidative Maillard reaction products form during the pasteurization, nonetheless, the RSAs of pasteurized strawberry juice and puree (cv. Senga Sengana) do not necessarily undergo any consistent reduction upon heating [110]. The RSA of strawberries (cv. Senga Sengana) is only slightly affected by a thawing step [110], probably as a consequence of the antioxidant action of AA protecting the other phenolic compounds against oxidative stress.

Pasteurized highbush blueberry juices (cv. Rubel) have been found to contain higher amounts of ACNs than the non-pasteurized control product, but in comparison to the fresh berries, the levels of ACNs are clearly diminished after pasteurization [107]. After a process involving several heat treatments, the total ACN content in blueberry jam has been observed to be lower than that in fresh blueberries [125]. Blanching has been reported to either diminish or exert no significant effect on the ACN content in highbush blueberry juice [107,118]. Furthermore, a reduction in the TP content in frozen highbush blueberries (cv. Rubel) occurring after a thawing step of juice production has been observed [107]. In juice production, the addition of SO<sub>2</sub> during thawing and after crushing of highbush blueberry raw material has enhanced the retention of ACN compounds before and after the pasteurization step and also during clarification by centrifugation before pasteurization [107]. Pasteurized highbush blueberry juices, which had not been treated with SO<sub>2</sub> had higher amounts of polymeric ACNs than non-treated products [107]. There is a report that ACN polymers are formed, and the level of chlorogenic acid is reduced during blueberry jam processing, while flavonoids show good stability [125].

As compared to fresh blueberries and sugar-containing blueberry jams, processing of sugar-free jams has been found to lead to a higher concentration of procyanidin dimers in jams [125]. Generally procyanidins have been found to be better retained in sugar-free blueberry jams than in sugar-containing products probably, because these compounds were more tightly bound to the cell wall components in sugary jams [125]. It remains to be determined whether it is the aglycon structure or the sugar residue of the ACN that has a more significant impact on the hydrolysis rate of these compounds [107,124,125].

During thawing, the antioxidative properties of AA might be able to protect strawberry phenolics from oxidative stress [110]. AA itself may undergo significant degradation during long-term thermal treatment (100–140 °C, 20 min) in strawberry and raspberry pastes [117], and pasteurization of strawberry nectar and wine was reported to significantly decrease their concentrations of AA [105]. Strawberry juice and puree (cv. Senga Sengana) production [110] and the raspberry marmalade (cv. Aksu Kırmızısı, Rubin, Hollanda Boduru and Heritage) manufacturing procedure [126] have also been observed to reduce the AA contents in the berries. The decrease of AA concentrations during raspberry marmalade processing was probably a result of oxidation occurring during thermal processing and mixing [126].

There are many parameters in temperature-related treatment steps that can be refined and combined in various ways to ensure the best possible quality and safety of fruit and berry products. Some endogenous and exogenous chemical components should be considered when developing processes for products rich in polyphenols and vitamins.

# 5.6. Pressure Treatments

High pressure (HP) treatments can be utilized as an alternative to high temperature protocols to destroy micro-organisms. These treatments may also affect the enzyme activities in products and thereby maintain the quality and extend the shelf-life. High pressures accelerate plant cell rupture and, hence, allow interactions to occur between cell components in enzymatic and non-enzymatic reactions [127]. In many cases, however, HP treatments seem to be gentler to bioactive compounds than heat treatments, and therefore, processes utilizing high pressures at ambient temperatures may represent a good option for preservation methods for berry products [117,128]. In general, the TP contents in strawberry (cv. Elsanta) purees [128] and blueberry juices [129] have exhibited relatively good stability during HP treatments, and some process parameter combinations have even been able to elevate the TP levels in the final products [128]. The ACN contents of blueberry juices [129,130] and strawberry purees [127] (cv. Camarosa) were well maintained during HP treatments. Blueberry juices treated with HP of 200-600 MPa for 5-15 min at room temperature had similar or increased ACN levels as compared to fresh control samples [130]. The total ACN contents were preserved in HP (100–400 MPa) pasteurized strawberry purees in both temperatures tested (20 and 50 °C) during the 15 min treatment [127], whereas HPs had a decreasing impact on the ACN contents in the production of a raspberry paste (cv. Sugana) [131]. Both temperature and pressure accelerated in a synergistic manner the ACN degradation in raspberry paste during preservation treatment combining high temperature (90-115 °C) and high pressure (200-700 MPa); temperature seemed to have a more pronounced effect than the pressure procedure [131]. In pomegranate juices processed with high

hydrostatic pressure at 25 °C, the ACN contents decreased as a function of an increasing pressure level (400–600 MPa) and treatment time (5 and 10min); at 45 °C, phenolic degradation was not dependent on time or pressure [132]. The TPs in pomegranate juices were diminished in a pressure-dependent manner during 10 min treatment at 25 °C [132].

It has been reported that HP treatment at 200 MPa did not affect the AA concentrations in blueberry juices during 5–15 min treatment [130], and no significant degradation was observed at 100–400 MPa at 20 °C in strawberry purees (cv. Camarosa) [127]. At higher pressures, e.g., 400–600 MPa, there was degradation of AA in strawberry puree (cv. Elsanta) [128]. Fifteen-minute HP treatments with 400–600 MPa have been found to decrease the RSAs in strawberries purees [128]. Despite the RSA reduction in the strawberry purees treated at 500 and 600 MPa, the HP-treated purees have shown higher anti-radical capacity than puree boiled in a vacuum package [128]. The AA content of HP-treated blueberry juices pressurized for 5–15 min was slightly, although significantly, reduced as compared to untreated fresh samples, and the decline was also time dependent [130]. AA degradation was reported to be less extensive in strawberry purees treated at 600 MPa than at 400 or 500 MPa [128]. When the temperature was increased to 50 °C, then the AA contents of HP pasteurized strawberry purees were found to decline already at 100–400 MPa [127].

# 5.7. Fermentation

Fermentation refers to ethanol and CO<sub>2</sub> production as a result of sugar metabolism by yeast cells. Yeast strain-dependent differences have been observed in the polyphenol profiles of apple wines made using cultured *Saccharomyces cerevisiae* yeast cultivars Johannisberg-Riesling and Steinberg; furthermore, the spontaneous fermentation of apple musts has resulted in higher polyphenol levels than fermentation using cultured wine yeasts [119]. The polyphenol profiles of apple wines (cv. Champion and Idared) differed from the profiles of unfermented musts, due to the release of biologically important compounds from the apple matrix; for example, quercetin glycosides were reported to be more abundant in wines than in musts [119]. The presence of yeasts significantly influences the ACN profile in berry products; hence, fermentative yeast metabolism may produce stable ACN derivatives in the wine [133].

The fermentation process of strawberry mash (cv. Polka) was found to reduce the TP and AA content of mash as compared to that in fresh berries [115]. Oxygen and enzymes have been postulated to cause the loss of AA content in strawberry wine over time [115]. The TP and ACN contents in pomegranate juices (cv. sweet Qingpi, sour Qingpi and Mountain Tai) decreased with fermentation; the addition of SO<sub>2</sub>, however, slightly inhibited this reduction in wines produced from the cultivars, sweet Qingpi and Mountain Tai [134]. Instead, the TP content of juice produced from a highbush blueberry cultivar mixture (14 cultivars) increased with fermentation during the wine making process, and this increase was thought to have resulted from the formation of EA [135].

#### 5.8. The Prospects of Fruit and Berry Processing

There is a growing consumer demand for natural and tasty functional and low-calorie foods, and this will be an impetus guiding product and process development [136–139]. In the future, less energy-intensive methods evoking minimal effects on berry/fruit product quality parameters, such as

color and vitamin and polyphenol contents, may replace traditional thermal and dehydration treatments [138,140–142]. The ways that many novel methods are best applied still require further optimization [138,140]. For example, by fermentation, it is possible to reduce sourness and increase the levels of bioactive compounds in berry products [136] without the need to mask unpleasant taste properties with sugar. Future additives intended to improve product texture may also allow gentler processing and a reduction of sugar content [139]. Furthermore, the presence of endogenous high concentrations or the addition of antimicrobial polyphenols in the products may also reduce the need for sugar [139] and synthetic preservatives. Since they are astringent and bitter [137], polyphenols represent a challenge to any company developing a product with high polyphenol content. Scientists and food industry experts are cooperating to encourage more effective use of berry and fruit processing residues rich in valuable components [137,142]. For example, apple and blackcurrant residues contain substantial amounts of flavanols, flavonols, sterols and fibers and are ideal biomaterial for a variety of food and non-food applications [14,137,142]. Future progress will lie in the clarification of chemical interactions in different plant matrixes during processing and the selection of special "target-specific" cultivars optimized for processing [141–144].

# 6. Conclusions

Ensuring the supply of huge amounts of high-quality polyphenol-rich materials in a stable manner is an increasing challenge facing the food processing and horticultural industries all around the world. This trend has changed the criteria of plant breeding objectives. Modern breeding is largely based on the exploitation of molecular tools, which today means that cultivars are being marketed to consumers and the food industry, because they also have enhanced levels of health-promoting compounds. However, after the harvesting of fruits or berries, their concentrations of polyphenols and AA may be significantly affected by storage conditions, and furthermore, many compounds may be destroyed during processing. The optimal timing of the harvest of fruits and berries is not simple when trying to optimize the levels of bioactive compounds. The ideal time of fruit and berry harvesting occurs when fruits are not fully ripe and, hence, are also suitable for long-term storage; this stage is optimal with respect to the concentrations of flavanols and many flavonols. The maximum ACN levels are detected when fruits are ripe, tasty and suitable for fresh market consumption. Berries and fruits are highly perishable and vulnerable to a number of quality deteriorating factors during storage, but these losses depend on many factors, such as genotype, harvesting time and storage conditions. Several approaches are available for managing fruit/berry softening and the concentrations of polyphenols and AA during storage for horticultural plants. Finally, when high-quality raw material is processed to high-quality foodstuffs, the options are yet diverse: many process parameters can be adjusted for specific plant species and cultivars to ensure a high standard, healthiness and reasonable manufacturing costs of the final product. In the up-coming years, one can anticipate that there will be real breakthroughs in the effective management of storage and processing of horticultural products through the combination of sophisticated omics tools with automation-related software technologies.

# **Conflicts of Interest**

The authors declare no conflict of interest.

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