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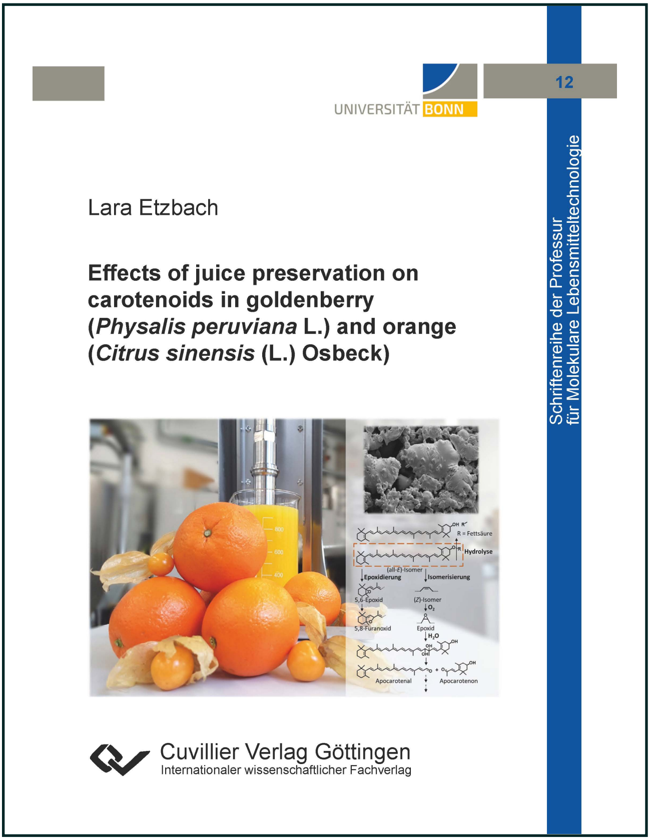

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Effects of juice preservation on carotenoids in goldenberry (*Physalis peruviana* L.) and orange (*Citrus sinensis* (L.) Osbeck)

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Chapter 1

General introduction

1 Fruits and fruit juices

Fruits are essential for human nutrition since they contribute greatly to the mineral, vitamin, and dietary fiber intake. Due to the presence of bioactive compounds and the associated health-promoting effects, the interest in fruits and fruit-based products has increased in recent decades. The most frequently studied groups of dietary phytochemicals are polyphenols and carotenoids. Numerous epidemiological studies show a positive correlation between plant-based diets and the prevention of chronic diseases including cardiovascular diseases and cancer (Hertog et al., 1995; Kris-Etherton et al., 2002; Liu, 2013).

The German Nutrition Society (DGE) and the World Cancer Research Fund/American Institute for Cancer Research recommend eating five servings of fruits and vegetables a day (Clinton et al., 2020; Deutsche Gesellschaft für Ernährung e.V., 2012). However, only a minority of the population reaches this recommended amount for the following reasons: practicability, the effort required, and convenience.

Moreover, the shelf life of fruits is limited due to microbiological, biochemical, and enzymatic reactions accompanied by softening of the fruits during ripening, leading to quality deteriorations and safety issues. Therefore, processing and preservation are necessary to extend the shelf life of fruits, making them available in the off-season and outside the region where they are grown, and to ease their handling and transportation (Escobedo-Avellaneda et al., 2017). Juice processing is one of the most frequently used methods to process fruits.

Thus, consuming fruit juices might be a simple and effective way to reach the goal of five servings a day of fruits and vegetables. However, there are controversial opinions on the contribution of juices to a healthy diet. Besides the health benefits of fruit juices due to bioactive substances such as vitamins, polyphenols, carotenoids, and dietary fibers, fruit juices are viewed as a rich source of sugar leading to obesity. Nevertheless, studies reported healthier lifestyle habits associated with the consumption of 100% fruit juice without the prevalence of overweight and metabolic syndrome (Pereira & Fulgoni, 2010; Sakaki et al., 2019). Yet, the majority of national bodies setting food policy hold the opinion that fruit juices can contribute to the German Nutrition Society and World Cancer Research Fund recommendation if they are consumed in moderate quantities since a moderate consumption of fruit juices is associated with a lower risk of cardiovascular disease and cognitive decline in the elderly (Scheffers et al., 2019; Yuan et al., 2019). However, altered gut microbiota owing to excessive sugar consumption in early life was associated with neurocognitive impairments (Noble et al., 2021). Therefore, the consumption of sugar-containing beverages should be limited, especially for children.

Despite the stress and hectic pace of everyday life, the demand for a balanced diet that does not require much time and is ready to consume is increasing. However, fruit juices do not replace intact fruits but offer a good source of vitamins, minerals, fiber, and phytochemicals. Thus, juices may be an important source of fruits for several parts of the population.

2 Fruit juice preservation

The shelf life of fruit juices at ambient temperatures without preservation is limited to a couple of days until incipient fermentation modifies the character of the juice. Among the spoilage microflora in fruit juices, lactic acid bacteria (*Lactobacillus* and *Leuconostoc* species), fermentative yeasts (*Saccharomyces cerevisiae*), and spore-forming molds are the most common microorganisms since they can grow even at low pH values (<4.0) (Fellows, 2009). In addition to the prevention of microbial growth, preservation is used to inactivate fruit juice enzymes such as polyphenol oxidase (PPO), lipoxygenase (LOX), peroxidase (POD), and pectinmethylesterase (PME), which lead to undesirable quality changes (Escobedo-Avellaneda et al., 2017). Browning is one of the major quality deterioration caused by polyphenol oxidases and peroxidases due to the oxidation of phenolic compounds to *o*-quinones, which may subsequently polymerize to yield dark pigments (Chisari et al., 2007). Especially for orange juice quality, the inactivation of PME is of high importance since its cloud reduction capability leads to high quality losses in flavor, color, and mouthfeel characteristics. Cloud is the suspension of protein, pectin, lipids, hemicellulose, cellulose, and other minor components (Tiwari et al., 2009), responsible for the turbidity, viscosity, and body of the juice. During juice extraction, PME is released and cleaves the methyl esters of the pectin molecules, resulting in methanol and free pectic acid. The latter is involved in the formation of insoluble calcium pectate gels resulting in cloud destabilization and clarification (Ellerbee & Wicker, 2011).

For fruit products, fermentation, natural chilling, and freezing were the predominant preservation methods for a long time. Nowadays, the common technique for preservation of fruit juices is thermal pasteurization, which was first applied in the nineteenth century (Pina-Pérez et al., 2017).

2.1 *Thermal pasteurization*

The heat transfer mechanism of thermal pasteurization is based on conduction and convection. Energy in form of heat is transmitted from the surface to the interior of the food by means of hot water, vapor, or hot air via temperature gradient (Fellows, 2009; Pina-Pérez et al., 2017). Conventional thermal pasteurization processes can be distinguished into low-temperature/long-time (LTLT, 63 °C, 30 min), high-temperature/short-time (HTST, 75 °C, 15 s), and ultra-high-temperature pasteurization (UHT, >121 °C, 1–2 s) (Escobedo-Avellaneda et al., 2017). Besides low costs and easy handling, thermal pasteurization has several advantages since heat is chemical-free and safe, most spoilage agents are sensitive to heat, and thermally processed foods have a long shelf life when stored in sterile containers.

The pH of the food strongly determines the required heat exposure and shelf life extension since microbial growth in fruit products is pH dependent. Microorganism inhibition and death is caused by cell membrane damage, DNA damage, and enzyme inactivation. Enzyme inactivation is caused by protein denaturation leading to conformational changes in the three-dimensional configuration of the molecules and, thus, to the loss of enzyme activity (Ağçam et al., 2018).

2.2 *Alternative pasteurization techniques*

Conventional thermal pasteurization has been reported to adversely affect texture, flavor, nutritional value, color, and bioactive properties of fruit compounds. Since the 1990s, the concepts of minimally processed and fresh-like food products have become common in the food industry (Mertens & Knorr, 1992). Therefore, there is growing interest in more gentle alternative pasteurization techniques maintaining the fresh-like characteristics while guaranteeing the inactivation of microorganisms and enzymes. These technologies include alternative thermal strategies like microwave and ohmic heating and so called “non-thermal” technologies such as high pressure processing (HPP), pulsed electric fields (PEF), UV-C irradiation, and ultrasound (Jayawardena et al., 2019; Pina-Pérez et al., 2017). In **Chapter 2** and **3**, conventional thermal and alternative pasteurization techniques were assessed for the treatment of goldenberry puree and orange juice, respectively. Due to their relevance for this thesis, high pressure processing, pulsed electric fields, and ultrasound technology will be discussed in more detail in the following.

2.2.1 High pressure processing

The technology of high pressure processing is based on two fundamental principles: the principle of Le Chatelier and the isostatic rule. Le Chatelier's principle states that any phenomenon such as chemical reaction, phase transition, or change in molecular configuration that involves a decrease in volume ($\Delta V < 0$) is enhanced by pressure, while processes accompanied by an increase in volume are inhibited (Medina-Meza et al., 2014; Rahman, 2007). According to the isostatic rule, the pressure is uniformly transmitted throughout the sample independently of the size and shape of the food.

High pressure processing is mainly applied as a post-packaging pasteurization for the inactivation of vegetative cells, yeasts, and molds. The food products are placed into a vessel filled with a non-compressible fluid, usually water, and subjected to isostatic pressure (400–650 MPa at commercial level). Once a constant pressure is reached, typical treatment times at commercial level are a few minutes (about 3–7 min). Therefore, food is not crushed during the processing. Compression during high pressure processing lead to a slight increase in temperature of about 3 K per 100 MPa, whereas in case of high-fat food, the temperature increase is about 8–9 K per 100 MPa (Medina-Meza et al., 2014; Muntean et al., 2016).

Several studies showed that high pressure processing (600 MPa, 1 min) is suitable to reduce the population level of yeasts, molds, aerobic bacteria, Enterobacteriaceae, *Escherichia coli*, and lactic acid bacteria in orange juice below the detection limit (Bull et al., 2004; Timmermans et al., 2011). However, spore-forming microorganisms in their spore form show high resistance to high pressure processing and are only inactivated by high pressure-high temperature sterilization (Mathys et al., 2008). Pressure effects on microorganisms are complex and different mechanisms are leading simultaneously to cell death. Smelt et al. (2012) defined four factors causing pressure induced cell death of vegetative cells: (1) unfolding of proteins and enzymes leading to partial or complete denaturation, (2) phase transition of cell membranes inducing a change in fluidity, (3) disintegration of ribosomes, and (4) intracellular pH changes.

Enzymes such as PPO, PME, and POD showed high resistance to high pressure processing, whereby enzyme susceptibility toward pressure depends on enzyme type, origin, pH, medium composition, and temperature. Often, only a partial inactivation of PPO (Kim et al., 2001; Terefe et al., 2010), PME (Goodner et al., 1998; Guiavarc'h et al., 2005; van den Broeck et al., 2000), and POD (Cano et al., 1997; Terefe et al., 2010) is achieved by high pressure processing under commercially feasible conditions without additional temperature exposure. Covalent bonds are usually not affected during high pressure treatments, since the compression energy is too low. The inactivation is based on breaking weak bonds in which bond energy is distance-dependent such as van der Vaals forces, electrostatic forces, hydrogen bonding, and hydrophobic interactions (Medina-Meza et al., 2014). Enzyme denaturation by pressure is caused by an alteration in the tertiary and quaternary structure. In aqueous media, conformational changes are initiated by the penetration of water into the protein molecule and by the loss of non-covalent bonds (Terefe et al., 2014).

2.2.2 Pulsed electric fields

During pulsed electric field treatments, products are exposed to short pulses of high electric field intensity (10–40 kV/cm) of several microseconds to milliseconds in a treatment chamber confined between electrodes where high voltage pulses are applied. For pumpable fluids, typical systems consist of a PEF generation unit equipped with a high voltage generator and a pulse generator, a treatment chamber, a product handling system, and monitoring and controlling devices.

Studies reported sufficient reduction in the naturally occurring microbiota (yeasts, molds, and aerobic bacteria) in various juices such as orange (Yeom et al., 2000), pomegranate (Guo et al., 2014), carrot (Xiang et al., 2014), and apple juices (Noci et al., 2008) comparable with thermal pasteurization. In addition, low pH, as in citrus juices, enhances the effectiveness of the PEF treatment. Microbial and enzymatic inactivation is increased by the combination of PEF technology with mild temperature owing to the temperature-related phase transition of the membrane phospholipids from gel to liquid crystalline facilitating cell disruption. Inactivation of microorganisms due to PEF is caused by electrical breakdown of cell membranes, known as electroporation, DNA damage, and generation of toxic compounds (Escobedo-Avellaneda et al., 2017).

The application of an electrical field induces processes on a cellular level leading to the disruption of cell membranes (animal, plant, or microbial), however, there is still no clear evidence of the mode of action. The theory of Zimmermann et al. (1974) of the electromechanical model is well accepted. According to their theory, there is a naturally occurring transmembrane potential of cell membranes due to free charges of opposite polarities at the internal and external side of membranes. Exposure to an electric field results in an increase in the transmembrane potential leading to an increasing compression pressure owing to the attraction between opposite charges at the cell membrane. This may cause membrane thinning and, finally, locale ruptures with the formation of pores. The formation of pores is initiated when the potential difference across the membrane reaches a critical magnitude of voltage (Stewart et al., 2018). Depending on the field strength and treatment time, the formation of pores can be reversible due to pore resealing or irreversible owing to complete damage of the cell membrane (Soliva-Fortuny et al., 2009; Yeom et al., 2000; Zimmermann et al., 1974). For vegetative microorganisms, the critical field intensity for irreversible damages is in the range of 20–40 kV/cm, depending on the physicochemical properties of the food product, the target microorganisms, and the processing temperature (Terefe et al., 2014).

For enzyme inactivation by pulsed electric fields, more intensive treatment conditions are required compared to microbial inactivation (Terefe et al., 2015). It is assumed that both electrochemical and thermal effects associated with PEF lead to conformational changes in enzymes. The application of an external electric field may modify the three-dimensional configuration of the proteins due to the disruption of electrostatic interactions of peptide chains and may also modify functional groups owing to association and dissociation processes altering the functionality and activity of enzymes (Zhao & Yang, 2012). In several studies, a significant PME inactivation of up to 91% and good cloud stability was reached by PEF compared with thermal pasteurized juices (Elez-Martínez et al., 2007; Sentandreu et al., 2006; Yeom et al., 2000; Yeom et al., 2002). In addition, residual activities of POD (Riener et al., 2008; Schilling et al., 2008) and PPO (Giner et al., 2001; Giner et al., 2002; Riener et al., 2008; Schilling et al., 2008) were still observed after the application of PEF, whereas almost complete inactivation could be achieved with increasing field intensities and processing temperatures (Riener et al., 2008; Schilling et al., 2008).

2.2.3 Ultrasonication

Sound waves whose frequency exceeds the hearing limit of the human ear are called ultrasound (~ 20 kHz). Ultrasound technology was implemented in food production to minimize processing, to maximize food quality, to enhance the nutritional value, and to ensure safe and stable food products. Ultrasound technology is versatile in its use for extraction, microbial inactivation, enzyme inactivation or activation, removal of bacterial biofilms, emulsification, defoaming, and sonocrystallization (Awad et al., 2012). Based on the frequency range, ultrasound applications can be divided into low and high power ultrasound used as sensors or modifiers, respectively. In case of low power ultrasound, frequencies higher than 100 kHz at intensities below 1 W/cm² are applied. Low power ultrasound is used as a non-destructive analytical method for the monitoring of food materials during processing and storage, e.g., for the evaluation of the composition of meat products or for the pre- and postharvest quality control of vegetables and fruits. In high energy ultrasonication, intensities are higher than 1 W/cm² and frequencies are ranging between 20 and 500 kHz, inducting disruptive effects on biochemical, physical, and mechanical features of the food product by means of a sonication bath or ultrasonic immersion probes (Majid et al., 2015). Therefore, for food preservation and food modification purposes, high power ultrasonication is applied.

Ultrasonication can generate shear forces and fluid mixing. The mechanism is based on the phenomenon of acoustic cavitation owing to the formation, growth, and implosive collapse of cavitation gas bubbles in liquids causing thermal, mechanical (e.g., shear stress), and chemical effects. When sound waves of high frequencies are released into the medium, cycles of compression (formation of gas bubbles) and expansion (possible implosion) are provoked, leading to spots of high pressure (1000 atm) and temperature (5000 K) (Escobedo-Avellaneda et al., 2017; Kentish & Feng, 2014; Majid et al., 2015). The collapse of cavitation bubbles is the major mechanism for the generation of shear forces and may lead to particle size reduction and the formation of microjets, generating local turbulences und micro-circulation and, thus, facilitating emulsification and mass transfer (**Figure 1.1**). Moreover, free radicals may be generated by the dissociation of water molecules in aqueous medium, which cause chemical reactions (Carail et al., 2015).

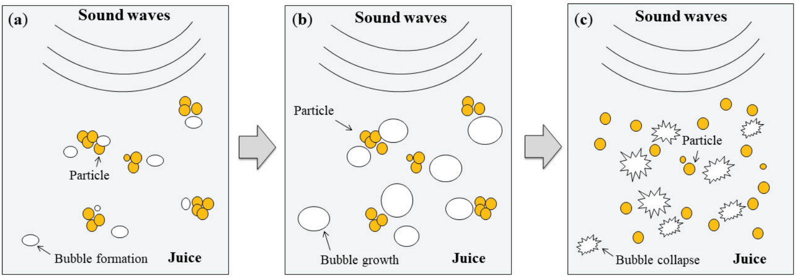

Figure 1.1. Principle of cavitation. (**a**) Formation of bubbles by ultrasound waves. (**b**) Growth of bubbles. (**c**) Collapse of bubbles when the maximum size is reached and particle dispersion. Modified according to Abdullah & Chin (2014).

Several studies showed that ultrasonication is suitable for the inactivation of microorganisms such as aerobic bacteria, coliforms, yeasts, *Escherichia coli*, and *Listeria monocytogenes* (Adekunte et al., 2010; Baumann et al., 2005; Gómez-López et al., 2017; Mohideen et al., 2015; Salleh-Mack & Roberts, 2007). However, temperature has been shown to significantly increase the efficiency of ultrasonication for microbial inactivation (Salleh-Mack & Roberts, 2007).

Physical forces due to cavitation may cause membrane thinning, erosion of cell walls, and an increase in membrane permeability (Zinoviadou et al., 2015). Moreover, intracellular cavitation may occur (Rojas et al., 2016).

Ultrasonication has been used to affect enzyme activity. Depending on the energy input through ultrasound and enzyme structure and properties, cavitation may lead to an increase in (Cruz et al., 2006; Larsen et al., 2021; O'Donnell et al., 2010) or a reduction of enzyme activity (Dars et al., 2019). Modifications in enzyme conformation and activity are caused by alterations in amino acid residues, the breakage of van der Waals interactions, hydrogen bonds, and disulfide bridges, and the destruction of functional groups by ultrasound induced radicals. Promotion of enzyme activity may be related to a higher enzyme release because of cell disintegration by cavitation or a conformation change toward the formation of more active binding sites. In addition, enzyme activity may be improved due to ultrasound induced spots of increased temperatures that are close to the enzymes' temperature optimum (Delgado-Povedano & Luque de Castro, 2015). At higher amplitudes, unfolding may result in enzyme denaturation or inactivation (Majid et al., 2015). Ultrasonication demonstrated synergistic effects both on substrates and enzymes and, thus, enhanced enzyme-substrate interactions (Larsen et al., 2021). Yet, complete enzyme inactivation through ultrasound is rarely achieved since temperature has a great effect on enzyme inactivation. Therefore, combined techniques such as thermosonication are recommended for the preservation of fruit juices (Aadil et al., 2020).

The potential of ultrasonication for the use in the fruit juice industry is demonstrated in **Chapters 2** and **3**. Ultrasonication is a promising technology to improve the extractability and bioaccessibility of bioactive compounds, since thermal and mechanical cavitation effects may lead to cell and organelle disintegration and, therefore, enhance the release of bioactive compounds such as carotenoids (Ordóñez-Santos et al., 2017), polyphenols (Aadil et al., 2020; Santhirasegaram et al., 2013; Yildiz & Feng, 2019; Zou & Hou, 2017), or vitamins (Santhirasegaram et al., 2013).

2.3 *Spray drying of fruit juices*

Besides pasteurization, drying is an alternative for the preservation of fruit juices. The high moisture content and, thus, the high water activity of fruits favor microbial growth and enzymatic activities. Drying of fruit juices is commonly applied to maintain the quality of fruit products, to increase the shelf life, and to handle the market demand for fruits throughout the year since juices are perishable and storage of fruit juices is accompanied by the loss of valuable compounds (Phisut, 2012). Compared to fruit juices, corresponding powders have many benefits, e.g., reduced volume and weight, reduced packaging, easier handling and transportation, and a longer shelf life of several months or even years (Goula & Adamopoulos, 2010). Among the different drying techniques, spray drying is a widely used method in the food and pharmaceutical industries to convert a liquid product to powder form. Owing to short processing times and the use of comparatively low temperatures, spray drying is suitable for heat-sensitive ingredients and is regarded as the most economic drying technique due to low operational expenditures.

Spray drying of fruit juices needs the addition of carrier agents to prevent problems of product stickiness und clogging of the spray dryer nozzle due to the hygroscopicity and thermoplasticity of the products promoted by the high humidity and temperature of the drying air. The problem of stickiness is mainly caused by a low glass transition temperature of the low molecular weight sugars such as glucose, fructose, and sucrose present in fruits. The glass transition temperature is unique for each material and describes the intermediate from an amorphous glass to a crystalline state through a transitional rubbery state. It is presumed that spray dried powders begin to show adhesion or stickiness 10 °C or 20 °C above the glass transition temperature, respectively (Bhandari et al., 1997; Keshani et al., 2015). To increase the glass transition temperature of the feed, carrier agents are added for spray drying of fruit juices. Moreover, carrier agents might have protective effects on sensitive food ingredients such as carotenoids, preserve flavors, and reduce reactivity and volatility. For spray drying purposes, maltodextrin, gum arabic, inulin, alginate, and modified starch are the most commonly used carrier agents (Ferrari et al., 2012; Gharsallaoui et al., 2007; Saenz et al., 2009). However, there is an increasing demand for new potential carrier agents. As by-product upgrading is an ever-growing issue, the interest in side streams for spray drying purposes which exhibit good water solubility, good spray drying and economic properties, show a high retention of sensitive food ingredients during storage, and prevent stickiness increases. Since cellobiose is a water soluble by-product of the beet sugar production, available at an industrial scale, low in sweetness and calories, and shows a comparatively high glass transition temperature compared to other disaccharides (Thorat et al., 2018), it might be an interesting carrier agent and an alternative to widely utilized oligo- and polysaccharides. Therefore, it was screened for spray drying purposes for the first time (**Chapter 4**).

3 Effects of fruit juice preservation on carotenoids

While necessary for fruit juice preservation, processing might lead to organoleptic and nutritional quality deteriorations. Several fruit nutrients and bioactive compounds such as vitamins, polyphenols, and carotenoids are susceptible to heat, free radicals, oxygen, acidic environment, and light and will be degraded during processing. In the following, the stability of carotenoids during fruit processing is discussed in more detail.

Carotenoids are lipophilic pigments exhibiting yellow, orange, and red color. They are the most widely distributed pigments in nature, being biosynthesized by plants and some microorganisms but not by animals and humans, who need to ingest them with food. Carotenoids are divided into two groups of non-oxygenated carotenes (e.g., β-carotene, lycopene) and oxygen-containing xanthophylls (e.g., lutein, zeaxanthin). Due to their structural diversity, xanthophylls are the more complex group and can be found either in free form or esterified with fatty acids. In plants, xanthophylls are mainly esterified with saturated fatty acids such as lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), but esterifications with unsaturated fatty acids such as oleic acid (C18:1), linoleic acid (C18:2), and α-linolenic acid (C18:3) and with short-chain fatty acids such as butyric acid (C4:0) were also reported (Bunea et al., 2014).

One of the best documented functions of carotenoids is their provitamin A activity. Humans are not able to synthesize vitamin A *de novo*, which is essential for the vision process, skin, tissue, growth, and reproduction, and must, therefore, be ingested with food. Due to their wide distribution and their occurrence in high concentrations in fruits and vegetables, provitamin A active carotenoids are the most important source of vitamin A. The minimum requirement for a provitamin A activity is an unsubstituted β-ionone ring with a C_{11} polyene chain, rendering β-carotene as the carotenoid with the highest provitamin A activity of 100%. Thus, only approximately 10% of all carotenoids have the structural requirements for a provitamin A activity. Other biological functions of carotenoids have been attributed to their antioxidant activity, owing to their ability to quench singlet oxygen and to scavenge free radicals related to their conjugated double-bond system. The consumption of carotenoids is associated with numerous health benefits such as cancer chemoprotection and the prevention of degenerative, heart, vascular, and other chronic diseases, e.g., cataracts and age-related macular degeneration (Gammone et al., 2017; Koushan et al., 2013; Tanaka et al., 2012).

3.1 *Effects of fruit processing on carotenoid stability*

Although the conjugated double bond system of carotenoids is fundamental for their light absorbing and antioxidative properties, it also causes their susceptibility to oxidation, isomerization, and cleavage initiated by heat, free radicals, oxygen, acidic environment, and light. Possible degradation reactions of carotenoids that occur first are shown in **Figure 1.2**.