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High hydrostatic pressure effects on spectral-optical variables of the chlorophyll pool in climacteric fruit

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ABSTRACT

High hydrostatic pressure (HHP) has been widely used for producing plant-based food of fresh-like quality. In this study, HHP-induced changes in the spectral-optical properties of the chlorophyll pool was approached in climacteric tomato fruit. The pericarp in pre- and post ethylene production stages was treated with 250 MPa and 400 MPa at room temperature. The chlorophyll pool, consisting of chlorophyll *a*, chlorophyll *b*, and pheophytin, was measured at the Q band of chlorophyll absorption considering the spectral intensity at peak maximum (I_{PP}) and the peak position (PP). The I_{PP} remained unchanged after HHP treatments. This finding confirms earlier studies on the stable, total chlorophyll pool. The single chlorophyll types, however, were affected with decline of chlorophyll *a* and increase of pheophytin content, suggesting that HHP is enhancing the chlorophyll breakdown process. When comparing fruit developmental stages, the relatively enhanced pheophytin caused the observed bathochromic (red) shift of PP of the Q band. After HHP treatment, however, this effect was outweighed by a strong hypsochromic (blue) shift of PP, assumingly due to enhanced electron state when pigments were allocated out of the stable matrix. The strong hypsochromic shift of the Q band PP may serve as a non-destructively measurable, general variable for optimizing HHP conditions in climacteric fruit.

1. Introduction

1.1. Subjecting fresh fruit to high hydrostatic pressure

For answering the demand on healthy, high quality food with attractive appearance and convenient shelf life, preservation technology is one valuable tool. Processing using high hydrostatic pressure (HHP) provides an alternative method to traditional food processing and preservation, potentially avoiding the damaging effects of heat (Hendrickx & Knorr, 2002). The application of HHP at mild temperatures has been shown to inactivate microorganism in vegetative form without tremendously changing sensory and nutritional properties of fruit and vegetables (Georget et al., 2015; Oey, Lille, Van Loey, & Hendrickx, 2008; Tao, Sun, Hogan, & Kelly, 2014). In particular, pressure strengths for microorganisms' inactivation range from 200 MPa to 800 MPa, considering keeping time of 1–15 min and temperature range of 20–25 °C, with extreme application from 2 °C to 45 °C. Conditions of preservation depend on species, strain, growth stage, and age of the culture, as well as composition and properties of the medium (Georget et al., 2015; San Martín, Barbosa-Cánovas & Swanson, 2002). illustrating, that conditions of HHP application are multifactorial, depending on the preservation purpose and the produce. Generalisation is also impossible when reviewing the impact of processing on produce having a wide range of attributes and physiological responses. Additionally, it is indeed challenging to assess processing conditions in non-destructive and potentially automated way when working on products having a wide range of physiological responses.

The impact of high hydrostatic pressure and combined pressure-temperature treatments were frequently studied in plant-based food considering the quality of produce that is related to sensory perception and consumer acceptance such as colour, texture, and

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aroma (Oey et al., 2008; Tao et al., 2014; Castro & Saraiva, 2014; Sanchez, Baranda, & de Maranon, 2014). The effects on colour are mainly described in CIE L*a*b colour space using a multi-spectral approach with three broad passbands (Krebbers, Matser, Koets, & Van den Berg, 2002; Medina-Meza, Barnaba, Villani, & Barbosa-Canovas, 2015; Weemaes et al., 1999; Woolf et al., 2013). Colour appearance of produce is used to potentially inform about acceptance by consumers and estimate the nutritional quality of the produce. Hyperspectral analysis in the entire visible wavelength range provides increased spectral resolution compared to 3-band colour analysis, and can be applied to measure pigment contents of samples non-destructively (Merzlyak, Solovchenko, & Gitelson, 2003; Pflanz & Zude, 2008). The interesting groups of pigments in this context are carotenoids, anthocyanins (Castro & Saraiva, 2014), and chlorophylls (Koca, Karadeniz, & Burdurlu, 2007), the latter intensively studied due to its universal importance for photosynthesis and consumer expectation on fresh-like produce. In climacteric fruits, the degradation of chlorophyll, and with it the loss of green colour, is temporally connected to the characteristic peak of ethylene production and respiration (Zude-Sasse, Truppel, & Herold, 2002). At this climacteric peak the fruit get mature and develop their typical aroma, while fruit flesh firmness decreases (Zude, Herold, Roger, Landahl, & Bellon-Maurel, 2006). Thus, changes in the chlorophyll pool, with main components being chlorophyll *a*, chlorophyll *b*, and pheophytin, may qualify as general fruit quality marker, potentially addressing enhanced consumer awareness towards “vital” products. Chlorophyll quantification due to absorption measurements in the red wavelength region, Q band, is readily accessible. Absorption in the blue range, Soret band, shows coinciding absorption of other molecules, which perturbates the analysis. Consequently, in this study, we aim to use existing knowledge of chlorophylls’ spectral-optical properties of Q band and its changes during fruit development for characterizing the impact of HHP on fruit and vegetables.

1.2. HHP studies on chlorophyll in vivo

At elevated temperatures (>50 °C), HHP affects stability of chlorophylls with stronger effects on chlorophyll *a* compared to chlorophyll *b* (Oey et al., 2008; Sanchez et al., 2014; Van Loey et al., 1998). However, at the present state of art, HHP is applied as mild preservation treatment, with temperature controlled avoiding negative effects on the quality of produce. Both, carotenoids and chlorophylls, are said to be not or not strongly influenced by HHP at room temperature, even at pressure up to 800 MPa (Medina-Meza et al., 2015; Sanchez et al., 2014; Van Loey et al., 1998; Wang et al., 2012; Weemaes et al., 1999). In some studies, enhanced greenness - suggesting increased chlorophyll content - was measured after HHP treatment (Krebbers et al., 2002; Sanchez et al., 2014). Mainly in spinach, enhanced greenness has indeed been measured after HHP treatment, what is discussed as higher pigment extractability, while no hints to increased chlorophyll content, but to slight chlorophyll decrease was found (Arnold, Schwarzenbolz, & Böhm, 2014; Medina-Meza et al., 2015; Sanchez et al., 2014). However, it may be expected that such findings result from changes of scattering coefficient due to ruptures of membranes, cell wall, and leakage of solution into intercellular space with varying refractive index (Krebbers et al., 2002).

The unfavourable degradation of chlorophyll due to HHP is not expected at low temperature and will not necessarily follow the same pathways as in enzymatic degradation process in living tissue (Hortensteiner & Krautler, 2011). However, chlorophyll degradation would equally lead to formation of its catabolite pheophytin. In pheophytin, magnesium central atom is replaced by hydrogen, exhibiting bathochromic shift of Q band compared to chlorophyll, what is also reflected by colour change from green to yellow-olive or brown (Krebbers et al., 2002; Weemaes et al., 1999). This change in appearance is undesirable for consumer acceptability (Steet & Tong, 1996).

However, we assume that physiological changes of produce due to HHP are undetectable with analysis of colour alone. HHP effects on absorption spectrum of chlorophyll have been studied in depth on solvated chlorophyll and isolated membrane-embedded, in wild-type and mutagen chlorophyll-binding light harvesting complexes, both from higher plants and purple photosynthetic bacteria (Ellervee, Linnanto, & Freiberg, 2004; Gall, Ellervee, Tars, Scheer, & Freiberg, 1997; Puusepp, Kangur, & Freiberg, 2015). In solution, response to HHP was characterized most often by red shift, while hypsochromic, blue shift occurred in chlorophyll due to hydrogen bonding as pointed out by Renge and Maurig (2013). Blue shift was described for gas pressure, while responses to hydrostatic pressure has been rarely studied (Renge, 2000). In vivo, the HHP effects can potentially vary even more. Thus, empirical studies appear necessary to evaluate chlorophyll-related spectral-optical changes as an appropriate assessment method for optimizing the preservation process of fresh fruit. In apple fruit, Kurenda, Zdunek, Schlüter, and Herppich (2014) tested optical methods pointing to functional changes in chlorophyll showing increased effect at 200 MPa. Particularly, enhanced response to increased pressure was seen in the analysis of chlorophyll fluorescence kinetic, pointing to decreased efficiency of photosystem II. In lettuce, efficiency of photosystem II was reduced with enhanced keeping time under lower pressure of 150 MPa, while increasing pressure to 200 MPa resulted in immediate response (Schlüter, Foerster, Geyer, Knorr, & Herppich, 2009). Consequently, high pressures of 400–600 MPa (Sanchez et al., 2014) or up to 1000 MPa (Tao et al., 2014) as applied in the industry may exceed the physiological threshold of fresh plant material. However, better insight in plant responses would be necessary. In our study, we aimed to address relative mild conditions with room temperature and short keeping time at 250 MPa and 400 MPa. Thus, we address the range between low pressure used in modern approaches for mild treatment and industrial application targeting microbial safety.

1.3. Tomato as model system

Tomato fruit has been extensively studied as genetic-to-phenotype model system for climacteric fruit development (Giovannoni, 2004; Pesaresi, Mizzotti, Colombo, & Masiero, 2014). As typical climacteric fruit, Tomato shows strong respiratory increase and burst in ethylene production at the transition from final-sized mature green (MG) stage to first stage of fruit maturation: the breaker (BR) stage (Gillaspy, Ben-David, & Gruissem, 1993; Giovannoni, 2004; Handa, Tiznado-Hernández, & Mattoo, 2012). These stages capture the timeframe of commercial tomato harvest. During transition, chloroplasts develop into chromoplasts as reflected by colour change due to carotenoid accumulation and chlorophyll degradation (Egea et al., 2011; Pesaresi et al., 2014). These maturity-related physiological changes are discussed as important factor (Castro & Saraiva, 2014; Sanchez et al., 2014), the influence of physiological variability in fruits on the outcome of HHP applications has to our knowledge only been approached in one study on persimmon fruit considering astringency and carotenoids content (Plaza, Colina, de Acons, Sánchez-Moreno, & Cano, 2012).

For spectral-optical quantification of total chlorophyll content, commonly the intensity of absorbance at fixed wavelength in the Q band range is analysed. The peak position (PP) of maximal absorption of the chlorophyll pool bound to thylakoid membrane appears at 680 nm in tomato. It has been shown that this PP changes slightly during fruit development (Seifert, Pflanz, & Zude,