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Characterization of high hydrostatic pressure effects on fresh produce cell turgor using pressure probe analyses

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ABSTRACT

To improve the safety of fresh high quality convenience products, high hydrostatic pressure treatments (HHP) may provide a gentle, quality retainion alternative to traditional chemical preservation. However, information about the potential impact of HHP on metabolic functionality of fresh produce is rare; the few published studies indicate HHP has effects on physiological activity. These effects were highly product specific due to pronounced variations in both pressure sensitivity and stress responsivity. Biomembranes generally seem to be major physiological targets of HHP treatments. Cell turgor inevitably requires fully intact cell membranes. Turgor has an important impact on the fresh appearance of fresh produce, especially texture. Up to now, there is no investigation available that has directly evaluated the effects of HHP treatments on turgor of fresh vegetable tissue. In this study, the pressure probe technique was applied to comprehensively analyse the turgor of red cabbage tissue by directly measuring the hydrostatic pressure of individual cells. The effects of HHP on the cell turgor were tested at pressures ranging between 150 MPa and 250 MPa, applied for 5, 10, 15 and 20 min. As pronounced changes in pressure could result in related temperature changes, the effects of temperature (35 °C to 55 °C) on turgor was characterised separately. At 35 °C and 45 °C, leaf turgor transiently declined after HHP treatment of 150 MPa (for up to 10 min) but recovered again within 24 h; at 55 °C, however, it irreversibly dropped to near zero. The same occurred when HHP of 175 MPa or above was applied. In general, HHP treatment and temperature increases as well as the duration of treatments interactively affected cell turgor of red cabbage leaves. Critical process parameters derived for gentle application of HHP were 150 MPa at 45 °C for 10 min treatment time.

1. Introduction

During recent years, the growing demand for healthy and safe high quality fresh convenience food intensified the development of new products, e.g. fresh-cut ready-to-eat salads (Verlinden, 1996; Martens et al., 1999). To improve the safety status of fresh convenience products while retaining a high quality, new non-thermal sanitation techniques need to be developed and evaluated (Ohlsson, 2000).

One of these advanced approaches is the application of high hydrostatic pressure (HHP). HHP is mostly applied as an alternative to traditional chemical preservation to increase the safety of processed food (Bermúdez-Aquirre and Barbosa-Cánovas, 2011; Krebbers et al., 2002; Schlüter et al., 2009). This technique (Knorr, 1993; Thakur and Nelson, 1998; Tewari et al., 1999; Palou et al., 1999) was shown to preserve quality related factors such as vitamins, pigments, and flavour components (Ludikhuyze and Hendrickx, 2002). It may effectively inactivate microorganisms (Patterson, 2005); it may, however, also change the functionality of proteins and quality related enzymes (Indrawati et al., 2000) and the structure of food systems (Knorr et al., 2006).

Information about the applicability of HHP for hygienisation of fresh fruits and vegetables and its potential impact on their metabolic functionality is rare. In this context, Knorr (1995) reported that applying hydrostatic pressures of up to 350 MPa to plant systems did not affect texture and tissue structure of various plant derived foods. According to Arroyo et al. (1997), however, 350 MPa effectively reduced the indigenous microflora on lettuce and tomato but also caused some changes in produce appearance and structure. No adverse effect of HHP at 150 MPa on photosynthetic activity of lamb's lettuce leaves was observed when it was applied for up to 5 min (Schlüter et al., 2009). Increasing pressure above this threshold or increasing the treatment duration progressively induced irreversible physiological damages (Schlüter et al., 2009). At high HHP above 250 MPa, these effects may include leakage of chlorophylls into the intercellular space and tissue yellowing due to oxidative degradation of chlorophylls during storage at room temperature (Krebbers et al., 2002; Seifert

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and Zude, 2016). In addition, carotenoids and/or anthocyanins may also rapidly decompose during storage after HHP treatment (> 200 MPa; Kouniaki et al., 2004). All these substances are, however, generally stable when under HHP (Oey et al., 2008). Additionally, enzymatic browning mediated by polyphenol oxidase reactions may occur (Wu et al., 2012).

In general, currently published studies indicated a great variation in the impacts of HHP treatment on intact fresh produce. Thus, high hydrostatic pressures may differentially affect the physiological activity of different fruits and vegetables due to large variations in their pressure sensitivity and stress responsivity (Marigheto et al., 2004; Trejo Araya et al., 2007; Schlüter et al., 2009; Vargas-Ortiz et al., 2013).

Texture is an important quality parameter of fruits and vegetables, and it influences consumer appreciation to a large extent. Dependent on pressure level and treatment duration, changes in texture during HHP treatment may arise from cell disintegration and turgor loss, disappearance of intercellular spaces and enzymatic transformations of cell walls (Basak and Ramaswamy, 1998). Turgor, the mechanical component of total water potential (Nilsson et al., 1958; von Willert et al., 1995), has important impacts on the fresh appearance of leafy vegetables and influences sensory aspects such as firmness and crispness (De Baerdemaeker et al., 1978; Verlinden, 1996; Harker et al., 1997, 1998; De Belie et al., 2000a).

In this context, treating fresh carrots with high pressures in the range of 100 MPa and 300 MPa reduced tissue hardness by 5 % to 50 %, assumed to be mainly because of turgor losses (Trejo Araya et al., 2007). Préstamo and Arroyo (1998) treated cauliflower and spinach with 400 MPa for 30 min and found the structure of parenchyma was disrupted. This seems to partially result from losses of symplastic water to the apoplast. Consequently, intracellular spaces were no longer filled with gas and, thus, occluded the vascular tissue.

Because turgor can be built up only when cell membranes are fully intact (von Willert et al., 1995), disintegration of cell membranes by HHP treatment can cause turgor losses resulting in a loss of firmness. On the other hand, firmness losses were also associated with changes in pectin methyl esterification due to the HHP treatment (De Roeck et al., 2008). While both turgor and cell wall integrity are thought to be important components of elasticity or firmness of plant materials (Ilker and Szczesniak, 1990), only the latter has been focused in research on the effects of processing on texture.

Despite this importance of turgor, to the best of our knowledge, no previous reports have evaluated the effects of HHP treatments on cell membrane integrity and, thus, pressure potential of vegetable tissue. Therefore, a direct measurement of cell turgor is appropriate and may provide a sensitive indicator of membrane disintegration. Direct cell turgor measurements where, however, realized by only few experiments: red beet storage tissue (Tomos et al., 1984), tomato pericarp tissue (Shackel et al., 1991), carrot root tissue (Greve et al., 1994a, b) and apple fruit cells (Steudle and Wieneke, 1985). De Belie et al. (2000b) investigated the effect of a mild heat treatment on turgor in red cabbage cells.

Application of high hydrostatic pressure acts in a very similar though not identical manner as high temperature stress on the complex properties of biological materials (Lullien-Pellerin and Balny, 2002). Any pressurisation obtained within a time too short to ensure full isothermality induces some temperature increase. Even short-term application of high, sublethal temperatures of 45 °C can seriously inhibit metabolic activity of leaves (Schlüter et al., 2009). As indicated by the variation in photosynthetic competence, the inhibition largely increased with the duration of heat treatment. Furthermore, the general ability of metabolic activity to recover from heat treatment declined with the duration of heat exposure (De Belie et al., 2000b).

For a comprehensive analysis of HHP effects it is, hence, important to characterise and, if possible, to separate the effects of all three parameters on the physiological activity of fresh living plant organs. In this study, pressure probe technique (Hüsken et al., 1978) was applied to investigate the influence of HHP treatments and that of effects of short-term high temperatures on turgor kinetics of red cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* DC.). In the experiments, tissue discs were excised from leaves, and the turgor of individual cells measured directly. The effects of different HHP treatments (150 MPa, 175 MPa, 200 MPa and 250 MPa) on the turgor of epidermal cells were tested at three temperatures (35 °C, 45 °C and 55 °C) and at four treatment durations (5 min, 10 min, 15 min and 20 min) to closer evaluate the kinetics of HHP processes. Direct comparison of turgor losses dynamics at the different parameter combinations were expected to give further insight into high pressure related variations in membrane integrity.

2. Materials and methods

2.1. Material

Mature, previously cold-stored heads of red cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* DC.) were purchased at a wholesaler (Frucht Express GmbH, Groß Kreutz, Germany), transported to the laboratory and stored in water vapour-saturated atmosphere at 7 °C for the duration of the experiments. One day before the study, the two outermost covering leaves of three cabbage heads were detached and discarded, and then the respective next leaves removed and stored in water vapour-saturated atmosphere at room temperature in a box. Immediately before the high pressure treatment, up to 16 discs (d = 17.5 mm) were punched out of the intercostal area of leaves with a cork borer, carefully avoiding vein tissue. Subsequently, four discs were carefully inserted in one sample bag (Whirle Pak B00679, Nasco, Fort Atkinson, USA) and hermetically sealed after removing air. These samples were subjected to either thermal or high pressure treatments. After treatments, samples were placed into petri dishes lined with water-soaked (deionised water) paper tissue to prevent dehydration during the subsequent investigations. To differentiate short-term disturbances of plant physiology and biochemical and biophysical processes from irreversible damages, cell turgor were repeatedly measured over a period of 24 h. To evaluate natural variability of physiological properties, the measurements were repeated several times on untreated fresh plant material. Therewith, the influence of storage on overall plant performance was controlled, too.

2.2. High pressure processing

The high pressure experiments were carried out in a tailor-made high pressure apparatus (Uhde, Hagen, Germany), which had a maximum design pressure of 360 MPa. It consisted of a stainless steel vessel (volume: 600 mL; inner diameter: 56 mm; height: 250 mm) and an upper plug with the grommets for pressure release valve, two temperature sensors (Pt-100, thermal resolution better than 0.1 K in the range used) and a pressure transducer (HP28, Intersonde Ltd., Watford, England). The inner vessel temperature near the sample and the pressure were recorded for each pressure treatment. A high pressure reciprocating pump (DSXHW, Haskel Ltd., California, USA) pumped the pressure medium into the vessel from a reservoir. The vessel was filled with silicone oil (Type 6165, Huber, Offenburg, Germany) as pressure transmitting medium. The temperature in the vessel was externally controlled in the range of 10 °C–20 °C by flexible tubes coiled around the vessel and connected to a thermal bath (Haake, Karlsruhe, Germany) operating with deionised water.

Before the treatment, the sample bag was introduced in a deionized water-filled teflon cylinder. By pre-cooling the filled cylinder for about 45 min, the desired final temperature was adjusted during the high pressure treatment according to Knoerzer et al. (2010). The temperature and the pressure in the inner teflon cylinder were recorded near the sample for each pressure treatment.