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The influence of vacuum impregnation on the fortification of apple parenchyma with quercetin derivatives in combination with pore structures X-ray analysis

Beate Schulze [a,*], Stephan Peth [b], Eva Maria Hubbermann [a], Karin Schwarz [a]

[a] Department of Food Technology, Institute of Human Nutrition and Food Science, University of Kiel, 24118 Kiel, Germany
[b] Department of Soil Science, Institute of Plant Nutrition and Soil Science, University of Kiel, 24118 Kiel, Germany

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A B S T R A C T

The study aim was to investigate the vacuum impregnation (VI) technique for apple enrichment with quercetin glycosides from apple peel. Beside the determination of the quercetin content, structural effects on the apple parenchyma were analyzed by computerized microtomography (µCT). VI was an efficient method to enrich apples with quercetin derivatives which was affected by the apple cultivar, the vacuum pressure, the soluble solid concentration (SSC) and the viscosity of VI solution. After VI of 13 apple cultivars the quercetin content varied between 368 and 604 µg/g dry mass and correlated with the firmness of the native apple and the increased apple weight. The use of low SSC solution resulted in increased quercetin enrichment in contrast to apple pectin solutions with elevated viscosity. The µCT analyses demonstrate that VI was more effective in the inner apple sections than in the outer parts. The study indicates that differences of pore size and microstructure within the apple cortex substantially affected the enrichment process.

1. Introduction

Apples (*Malus domestica* Borkh.) feature an important role in the worldwide fruit market and in the daily flavonoid intake after tea and onions (Sampson et al., 2002). Flavonoids are secondary plant products which act as antioxidants and may be important determinants in protecting against oxidative stress-induced diseases, coronary heart disease (Hertog et al., 1993), stroke (Knekt et al., 2000) and cancer (Gerhauser, 2008). In apples, quercetin occurs in the form of 3-glycosidic bonded sugars, which are located predominantly in apple peel. The apple parenchyma contains only minor concentration. The quercetin content depends on several factors like the UV-B-visible light radiation, growing conditions, cultivar and post-harvest measures (Hagen et al., 2007; Reay and Lancaster, 2001).

In food industry processes, apples are often used in peeled form or the peel remains as by-products e.g. during apple juice production. However, apple peel rich in quercetin glycosides is an interesting source for a functional ingredient used for food fortification (Boyer and Liu, 2004).

In recent years vacuum impregnation (VI) has become a popular method to enrich food products, such as fruits and vegetables, with beneficial food ingredients for human health. Fruit tissues were enriched with probiotics, vitamins and minerals to reach beneficial effects (Betoret et al., 2003; Park et al., 2005; Xie and Zhao, 2003) by the VI technique. VI is employed in a wide range of fruit processes. It has been used for shelf life extension, for improving sensory properties such as taste (Chiralt et al., 2001; Guamis et al., 1997), color (Jeon and Zhao, 2005), as well as for structural changes and cell wall stabilization (Martinez-Monzo et al., 1998).

During VI, the porous fraction of food is penetrated by external liquids under controlled conditions. In apples, the porous fraction is characterized by the intercellular spaces. The VI process depends on the capillary pressure, the system pressure and the effective porosity of the food material. VI is parted in the vacuum phase when subatmospheric pressure is applied and the atmospheric phase is restored. The vacuum phase is characterized by duration and vacuum level. The gas of the porous tissue expands and flows out in combination with the deformation relaxation phenomena (DRP) of the solid matrix. After restoring the atmospheric pressure, the tissue contracts and the VI solution penetrates into the previously air-filled pores (Fito et al., 1996; Guillemin et al., 2008). The volume of externally penetrating liquid can account for almost the total volume of the intercellular space that was initially filled with gas (Fito, 1994). Even immersion of porous plant tissues in a liquid results in an influx of liquid due to capillary forces which are limited to the pores close of the surface. The mass transfer phenomena are described as hydrodynamic mechanisms (HDM).

* Corresponding author. Address: Department of Food Technology, Institute of Human Nutrition and Food Science, University of Kiel, Heinrich-Hecht-Platz 10, 24118 Kiel, Germany. Tel.: +49 431 8805034; fax: +49 431 8805544.
E-mail address: info@foodtech.uni-kiel.de (B. Schulze).

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The VI mass transfer process is affected by several factors, including the process duration, the process temperature, the subatmospheric pressure level, the form and shape, the samples' mechanical response, the porosity, the size of the pores and the composition of the applied VI solution (Zhao and Xie, 2004).

Important parameters of the VI impregnation fluid are the resulting osmotic pressure (i.e. hyper-, iso- and hypotonic) and its viscosity. Apple juice is a common external solution for an isotonic VI liquid for apple parenchyma enrichment (Contreras et al., 2005; Martin-Esparza et al., 2006). The isotonic properties of the solution ascribed to the low shrinkage and deformation reactions of the VI product (Zhao and Xie, 2004). VI solutions with a lower viscosity can be incorporated to a higher extent in the porous tissue, compared to solutions with higher viscosities and a lowered transfer rate (Zhao and Xie, 2004). For a similar penetration result the duration of the atmospheric pressure phase must be extended. Also, a hypoton basis as external liquid can enhance the incorporation of the solution (Guillemin et al., 2008).

The apple cortex is a plant tissue with high porosity. From the center to the periphery of the apple parenchyma, the porosity and intercellular spaces increase. The pore arrangement differs between the apple varieties and the pore volume elevates during apple growth (Mendoza et al., 2010). Studies in cell morphology of apple tissues showed cell anisotropy as well as heterogeneity of the porous structure, both in one variety and in different cultivars (Mendoza et al., 2010). The intercellular spaces are important for the gas exchange in the apple tissue (Verboven et al., 2008). In addition to dimension changes of the intercellular spaces, the apple cell morphology and form in the inner part differs, when compared to the outer part of the apple cortex (Khan and Vincent, 1990; Verboven et al., 2008). These structural findings may have an impact on the VI of the apple cortex.

The motivation of this study was to compensate the loss of secondary plant compounds, due to skin peeling by enrichment of the apple parenchyma with polyphenols, extracted from the peel. The aim was to investigate under which conditions and to which extent the enrichment in several apple varieties takes place. For detailed information the distribution of apple pore volume and structure were investigated by computerized microtomography (μCT) imaging.

2. Materials and methods

2.1. Chemicals

For high-performance liquid chromatography (HPLC) analysis, quercetin dihydrate, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-arabinopyranoside and quercetin-3-*O*-rhamnoside were purchased from Roth (Karlsruhe, Germany) as flavonoid standards. The high in flavonoids (hfv) apple peel extract for the vacuum impregnation (VI) solution was obtained from cider apples (Val de Vire Bioactives, Cond sur Vire, France).

2.2. Sample preparation

The apple cultivars Braeburn, Elstar, Fiesta, Fuji, Gloster, Golden Delicious, Granny Smith, Jonagold, Kanzi, Pink Lady, Red Delicious, Rubinstar and Topaz were purchased at commercial maturity from a local store. The fruits were stored in refrigerators maintained at 4 °C for a maximal period of 1 wk. Apples were washed with distilled water before analysis of selected physicochemical properties. Before VI the apples were peeled carefully and the apple cores were removed with a core borer. Then the apples were cut equatorially into 6 mm apple slices and were vacuum impregnated. Immediately after VI, the apple slices were frozen under liquid nitrogen and freeze-dried for 72 h (Christ Gamma 1-20, Osterode am Harz, Germany). For the cultivar analyses three apples of each cultivar were cut in six slices and treated by vacuum impregnation.

2.3. Physicochemical analysis

The soluble solid contents (SSC) of the apples and VI solutions were determined by a hand-held refractometer (HRT 32; Kruess, Germany) at 20 °C.

The apple cortex fruit firmness was investigated by a penetrometer, using an 11.1 mm tip (Effegi, Alfonsine, Italy) after the apple peel was removed.

The viscosity of the VI solutions was measured using a rotational viscometer (Haake Viscotester 7L, Thermo Electron Corporation, Dreieich, Germany).

2.4. Vacuum impregnation

For VI, the apple slices were immersed and fixed in the VI solution at room temperature. The VI was performed in a vacuum dryer (VDL 53, Binder, Tuttlingen, Germany) at a vacuum level of 100–800 mbar. When reaching a constant vacuum level the pressure was maintained for 5 min. After the vacuum step the atmospheric pressure was restored and the apple slices remained fixed in the VI solution for 10 min. After the incubation period the apple slices were removed and the remaining solution at the surface was allowed to drip for 10 min. During VI processes, the solution temperature was 25 °C. The time of vacuum treatment, the atmospheric pressure phase and for drip of the solution remained constant in all VI.

The standard solution consists of commercial apple juice (11.1 ± 0.1°Brix) enriched with 0.3% hfv apple peel extract. For the VI solutions of different viscosities, the apple pectin classic AU 701 from Herbstreith and Fox (Neuenbürg, Germany) was used. The viscous solutions were based on apple juice and water with 0%, 0.8% and 1.2% apple pectin. For the investigation of different osmotic solutions, either water or glucose was added to the commercial apple juice with 0.3% hfv apple peel extract.

2.5. Flavonoid analysis

Freeze-dried ground plant materials were extracted with 70% acetone and 30% 0.1% citric acid in water. Prior chromatographic analyses the apple extracts were filtered. To identify the quercetin glycosides an HPLC–ESI-MS^n Ion Trap (Agilent Technologies, Waldbronn, Germany) was used and compared with standard solutions. The apple quercetin glycosides quantification was carried out by external calibration using an HPLC HP 1100 system with a guard column protected Sphinx RP-C18 column (125×4 mm; 5 μm) at 30 °C. The mobile phase consisted of 0.5% formic acid (eluent A) and 100% acetonitrile (eluent B). The solvent gradient program was as follows: initial conditions 95% A, 5% B; 0–20 min, 5–20% B; 20–28 min, 20–35% B; 28–30 min, 35–80% B; 30–35 min, 80–5% B and an additional 5 min phase with 5% B. The flow rate was 1 ml min^{-1} and the injection volume was set to 10 μl. The DAD detection wavelengths were 280 nm and 365 nm.

2.6. X-ray analysis by μCT

The X-ray microtomography was performed by scanning parts of apple slices from the cultivar Elstar, before and after standard VI at 100 mbar for 5 min, with a phoenix nanotom® (GE Sensing and Inspection Technologies GmbH, Wunstorf, Germany) equipped with a molybdenum target. Because the temperature slightly increases during the μCT scan two apple slices of the same apple located one above the other were used for comparing native and impregnated tissue. The apple slices were cut equatorially and