



ELSEVIER

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.journals.elsevier.com/food-chemistry-xActive food packaging through controlled *in situ* production and release of hexanalMari Lehtonen^{a,1}, Sonja Kekäläinen^a, Ida Nikkilä^a, Petri Kilpeläinen^b, Maija Tenkanen^{a,c},
Kirsi S. Mikkonen^{a,c,*}^a Department of Food and Nutrition, University of Helsinki, P.O. Box 66, Helsinki 00014, Finland^b Natural Resources Institute of Finland (Luke), P.O. Box 2, Helsinki 00791, Finland^c Helsinki Institute of Sustainability Science (HELSUS), P.O. Box 65, University of Helsinki, Finland

ARTICLE INFO

Chemical compounds studied in this article:

Hexanal (PubChem CID: 6184)

Hexanoic acid (PubChem CID: 8892)

Keywords:

Active packaging

Aerogels

Galactoglucomannans

Hexanal

Lipid oxidation

ABSTRACT

Transportation and storage of vegetables and fruits, including berries, is increasing to meet growing consumer demand for fresh foods. Ripening and softening of plant tissues may be slowed down by hexanal, a safe volatile compound that also has antimicrobial properties. Thus hexanal could be applied during the food distribution chain to slow down the spoilage of plant-based products and reduce food waste. Nonetheless, due to the rapid evaporation of hexanal, a constant supply is needed. Our aim was to develop a concept to incorporate food-grade sunflower oil in a polysaccharide aerogel matrix for controlled *in situ* production and release of hexanal. We compared enzyme- and light-catalyzed lipid oxidation reactions, determined the release of hexanal at different conditions, and performed storage stability tests of blueberries and cherry tomatoes. The lipid-loaded aerogels assessed here are a potential novel delivery matrix for controlled hexanal formation to extend the shelf life of plant-based products.

1. Introduction

To support a sustainable food system, the consumption of plant-based foods, including fruits and vegetables, should be doubled by year 2050 (Willett et al., 2019). Accordingly, worldwide trade of plant products is needed to respond to growing consumer demand for availability of a wide selection of fruits, berries and vegetables all year round. Currently, EU citizens consume on average 140 kg fruits and vegetables/year/person (EUFIC, 2012).

Long-distance transportation and extended storage expose these goods to various hazards, such as over ripening, softening, and microbial spoilage. Roughly one third of all produced food is lost during the distribution chain, including 45% of fruits and vegetables. Agriculture is one of the sectors that generate the highest amount of CO₂ emissions. The estimated contribution of agriculture to the emissions is 20–25%, and global food waste is estimated to contribute about 8% for total greenhouse gas emissions (FAO, 2019).

Fruit maturation and spoilage are generally slowed down by adjusting the storage and transportation conditions to optimize the product quality from harvesting throughout the food distribution chain.

This is achieved by controlling the temperature and gas composition of storage spaces. However, at the time when fruits, berries and vegetables reach the consumer, they often have a short shelf life. Tissue softening and browning due to over ripening and physical stress lead to unappealing appearance and rapid deterioration. When the cell walls of plants are wounded, for example by mechanical stress, by pathogen attack or by senescence, activation of lipid oxidizing enzymes occurs (Siedow, 1991; Feussner, Kühn, & Wasternack, 2001; Porta & Rocha-Sosa, 2002). Disruption of cell structure brings these enzymes in contact with their substrates. Lipoxygenases (LOX) catalyze the direct addition of molecular oxygen to the pentadiene double bond system in unsaturated fatty acids. As a result, hydroperoxides are formed (Andreou & Feussner, 2009; Schaich, Shahidi, Zhong, & Eskin, 2013). When these hydroperoxides decompose further by hydroperoxide lyases, volatile aldehydes, such as hexanal, and 3-hexenal are formed (Noordermeer, Veldink, & Vliegthart, 2001). Hexanal and 3-hexenal are six carbon containing aldehydes and are formed during the oxidation of linoleic acid and linolenic acid, respectively. Nine carbon aldehydes 3-nonenal and 3,6-nonadienal may also be formed.

In addition to enzyme-catalyzed reactions, lipid oxidation may

* Corresponding author.

E-mail addresses: mari.lehtonen@ymparisto.fi (M. Lehtonen), sonja.e.kekalainen@gmail.com (S. Kekäläinen), ida.nikkila@helsinki.fi (I. Nikkilä), petri.kilpelainen@luke.fi (P. Kilpeläinen), majja.tenkanen@helsinki.fi (M. Tenkanen), kirsi.s.mikkonen@helsinki.fi (K.S. Mikkonen).¹ Present address: Finnish Environment Institute (SYKE), Laboratory Centre, Mustialankatu 3, 00790 Helsinki, Finland.<https://doi.org/10.1016/j.fochx.2019.100074>

Received 19 May 2019; Received in revised form 6 November 2019; Accepted 11 December 2019

Available online 13 December 2019

2590-1575/ © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license

<http://creativecommons.org/licenses/by-nc-nd/4.0/>.

occur via non-enzymatic autoxidation or photoxidation. In autoxidation, the initiation of radical chain reaction occurs generally via elevated temperature (Schaich et al., 2013). Formed lipid radicals react with oxygen producing hydroperoxides. In photoxidation, photosensitizer absorbs low-level light energy and transforms it into chemical energy by producing either singlet oxygen or lipid radicals (Schaich et al., 2013). Hydroperoxides are formed via all these routes, and decompose further forming hexanal and other oxidation products.

Aldehydes and ketones having the chain lengths of six and nine carbons, e.g., hexanal, 3-hexanone, nonanal and 2-nonanone, function among others as inhibitors of phospholipase D activity, and of mycotoxins and ethylene syntheses (Siedow, 1991; Bleé, 1998; Tiwari & Paliyath, 2011; El Kayal, Paliyath, Sullivan, & Subramanian, 2017). Thus, they have been shown to reduce the necrosis caused by the maturation and growth of pathogens (Andersen et al., 1994; Bleé, 1998; Sharma, Jacob, Subramanian, & Paliyath, 2010; Sholberg & Randall, 2007; Song, Fan, Forney, Campbell-Palmer, & Fillmore, 2010; Tiwari & Paliyath, 2011). These compounds increase the shelf-life of fruits (Lanciotti, Corbo, Gardini, Sinigaglia, & Guerzoni, 1999; Sharma et al., 2010; Sholberg & Randall, 2007) and berries (Almenar, Auras, Rubino, & Harte, 2007; Song et al., 2010; Misran, Padmanabhan, Sullivan, Khanizadeh, & Paliyath, 2015). Volatile aldehydes and ketones can be applied industrially to reduce the growth of bacteria and fungi in blueberries and pome fruits, as reported by Song, Leepipattanawit, Deng, & Beaudry, 1996; Almenar et al., 2007; and Sholberg & Randall, 2007. Release of aldehydes and ketones also has a biological function to attract insect predators (Siedow, 1991; Feussner et al., 2001; Porta & Rocha-Sosa, 2002).

Hexanal is considered safe, and its use as a flavor substance is allowed in food products (EU No 872/2012). Fruits and vegetables may be treated with hexanal either prior to harvesting or immediately after. Pre-harvesting treatment involves spraying the plants with hexanal formulations (1–2%) (Cheema, Padmanabhan, Subramanian, Blom, & Paliyath, 2014; Misran et al., 2015). Post-harvesting treatment often involves either dipping the harvested fruits or vegetables into hexanal solutions or storing them under a hexanal-containing atmosphere. Vacuum infiltration was also studied (Pak Dek, Padmanabhan, Subramanian, & Paliyath, 2018). For storage, hexanal is generally applied as a batch treatment in chambers (Song et al., 1996; Almenar et al., 2007; Sholberg & Randall, 2007; Song et al., 2010; Misran et al., 2015). That is, fruits or berries are maintained in a chamber enriched with hexanal vapour for a certain time period prior to transportation and storage. The treatment may be repeated during the storage. In these batch treatments, a decrease in the vapour concentration of hexanal has been shown. This decrease may reduce the effectiveness of the treatment. An alternative approach would be continuous presence of hexanal in the storage space or packaging (Cheema et al., 2014).

To achieve controlled release and prolonged diffusion of hexanal, it was incorporated into cyclodextrin inclusion complexes (Almenar et al., 2007). Incorporation of 450–900 $\mu\text{mol/g}$ of hexanal in cyclodextrin enabled a final release of 2–15 $\mu\text{mol/L}$ air. This concentration reduced or inhibited the growth of various fungi. However, the concentration decreased gradually by 30–100% throughout 7 days of storage, leading to an expected affective lifetime of 1–2 weeks. In a recent work, hexanal was released from a synthetic stable precursor compound 1,3-dibenzylethane-2-pentylimidazolidine (Jash, Paliyath, & Lim, 2018). The precursor was encapsulated in electrospun poly(lactic acid) (PLA) and the release of hexanal via the hydrolysis of the C–N bond on the imidazolidine ring structure was activated by the addition of citric acid. The release of hexanal exhibited rapid initial release followed by slow release, which was followed up to six hours.

Solid porous foams, such as aerogels, are lightweight materials which have a large surface area (Mehling, Smirnova, Guenther, & Neubert, 2009; García-González, Alnaief, & Smirnova, 2011; Mikkonen, Parikka, Ghafar, & Tenkanen, 2013). They may be used as delivery systems for active compounds for example in food packaging or in

pharmaceuticals. Aerogels are often prepared using silica or carbon, but polysaccharides are also suitable for aerogel matrices. Polysaccharides, such as cellulose, hemicelluloses and starch, are sustainable bio-based raw materials that are suitable for direct food contact and can even be edible (Mikkonen et al., 2013; Alakalhunmaa et al., 2016). Polysaccharide aerogels can be tailored for strong or flexible structures, enabling their wide applicability. In addition, polysaccharide-rich raw materials can be recovered from side streams of for example the paper and pulp industry.

In this study, we introduce a solution for controlled *in situ* production and continuous long-term release of hexanal from edible oil loaded in an active bio-based packaging material, prepared without using organic solvents. Our aim was to characterize hexanal production and release via various lipid oxidation pathways in polysaccharide aerogels under various storage conditions. We monitored the profile of the released volatile products during storage and evaluated the capacity of hexanal-releasing aerogels to extend the shelf life of blueberries and cherry tomatoes.

2. Materials and methods

2.1. Materials

Sunflower oil (SFO) (Bunge Finland Oy, Raisio, Finland) used as a substrate for hexanal production was purchased from a supermarket. Galactoglucomannans (GGM) were obtained from Norway spruce by pressured hot-water extraction and ethanol precipitation (Kilpeläinen et al., 2014). For aerogels used in storage tests, 2.7% suspension of anionic cellulose nanofibrils (CNF) (fiber width 4–10 nm, zeta potential – 25 mV) refined from birch kraft pulp was acquired from UPM, Finland. For aerogels used in shelf life studies, 1% neutral CNF from pine and spruce cellulose mixture (Domsjö Fabriker AB, Örnsköldsvik, Sweden) was fibrillated according to Berglund, Noël, Aitomäki, Öman, and Oksman (2016). Ammonium zirconium (IV) carbonate (AZC) used for crosslinking was purchased from Sigma-Aldrich (Steinheim, Germany). Tween20 (technical; VWR Prolabo) used as emulsifier in control samples, was acquired from WVR International. Hexanal (Sigma-Aldrich, St. Louis, MO, USA) was used for the incorporation of aerogels in preliminary studies and for preparation of a standard curve for quantification. Lipase (LIP) from *Candida rugosa* (Type VII, ≥ 700 unit/mg solid) and lipoxygenase (LOX) from soybean (*Glycine max*; Type I-B, lyophilized powder, $\geq 50,000$ units/mg solid), used for the enzyme catalyzed oxidation of SFO were purchased from Sigma-Aldrich. Methylene blue (Merck, Darmstadt, Germany), riboflavin (Merck), β -carotene (Merck), and chlorophyll (extracted from spinach or *Chlorella* by accelerated solvent extraction using acetone) were used as catalysts for the light-induced oxidation of SFO.

2.2. Preparation of aerogels

In preliminary experiments, hexanal (1–100 mg/g) incorporated SFO was directly mixed with hydrogels consisting of 2 wt-% of GGM and anionic CNF (60:40, w/w). In addition, the amount of SFO that could be incorporated into hydrogel without affecting the formed aerogel structure to a large extent was investigated. For active production and release of hexanal, hydrogels were prepared using 2 wt-% of GGM and CNF (70:30, w/w) in water with ammonium zirconium carbonate (AZC, 2.5–12.5 wt-% of polysaccharides) as cross-linker (Alakalhunmaa et al., 2016) (Fig. 1). In short, GGM was dissolved in water, CNF and AZC were added, and the suspensions were mixed with an Ultra-Turrax homogenizer (Ika-Werke, Staufen, Germany) at 9500 rpm for 15 min. The crosslinking reaction of the suspensions with AZC was carried out at 80 °C for 5 h. Sunflower oil (60 wt-%) was emulsified in water by Ultra-Turrax, using GGM (12 wt-%) as a stabilizer (Mikkonen et al., 2016). Tween20 (12 wt-%) was used for comparison in some of the experiments. For enzyme-catalyzed oxidation,

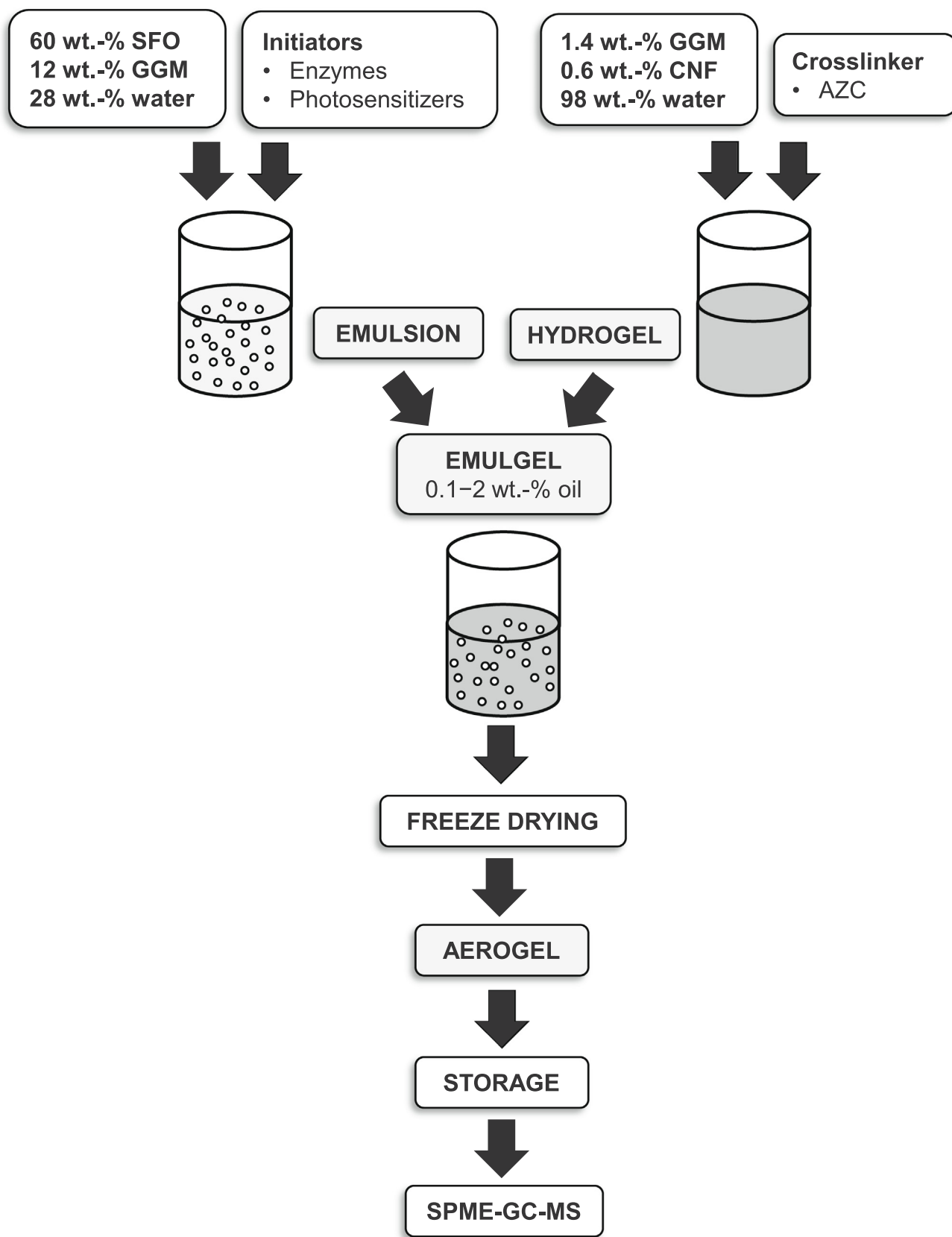


Fig. 1. Preparation of hexanal producing aerogels. SFO = sunflower oil, GGM = spruce galactoglucomannan, CNF = cellulose nanofibrils, AZC = ammonium zirconium carbonate.

LIP and LOX were added to the aqueous phase of emulsions at contents 10–1200 U/g oil and 15–12 000 U/g oil, respectively, prior to emulsification. For light-induced oxidation, either methylene blue or riboflavin were added to the aqueous phase prior to emulsification, or β -carotene or chlorophyll were dissolved in acetone and 1–5 mL of this solution was dispersed into SFO. Methylene blue and riboflavin dosages were 5–50 $\mu\text{g/g}$ oil, β -carotene was used at 2700 $\mu\text{g/g}$ oil, and chlorophyll was used at 15 $\mu\text{g/g}$ oil. To ensure thorough dissolvent of photosensitizers and evaporation of acetone, mixing was continued overnight. Emulsification was accomplished at 11,000 rpm for 5 min using UltraTurrax stirrer (T-18 basic, IKA, Staufen, Germany). Approximately 1.5 wt-% or 3.5 wt-% of the emulsion was added to hydrogel and mixed properly using UltraTurrax stirrer (9500 rpm for 5 min), resulting in oil/polysaccharide ratio of 0.41 or 0.87, respectively. After mixing, the hydrogel was divided into clear or amber glass vials (75.5 \times 22.5 mm) each containing 2 g. The sample vials were left to settle overnight at room temperature. The hydrogels were frozen at -20 °C and further at -70 °C, after which the hydrogels were freeze dried into aerogels at 1 mbar for 48 h. The final oil content in aerogels was 30 or 45 wt-%. Previously the density of similar aerogels, but without SFO addition, was determined to be about 0.02 g/cm³ (Alakalunmaa et al., 2016). The specific surface area of similar materials is typically 2–4 m²/g (Ghafar et al., 2015). The open sample vials were placed at different relative humidities and temperatures for storage.

2.3. Hexanal release from aerogels

For enzyme-catalyzed production of hexanal, aerogels containing 45% oil, 120 U LIP/g oil, and 1250 U LOX/g oil were placed in desiccator cabinets under adjusted relative humidities (RH) of 0% by dry phosphorous pentoxide, 54% by saturated calcium chloride solution, and 76% by saturated sodium chloride solution. The cabinets were placed at controlled temperatures of 10 °C and 22 °C. In addition, sealed vials containing lyophilized aerogels representing RH of 0–10% were placed at 4 °C. Samples were kept protected from light in amber vials.

For light-induced oxidation, aerogels containing 45% oil with added photosensitizer were placed into desiccator cabinets having adjusted RH of 0% by dry phosphorous pentoxide and RH of 76% by saturated sodium chloride solution. The cabinets were placed at controlled temperatures of 10 °C and 22 °C. Samples were kept in clear vials and they were exposed to continuous light (3.6–8.6 W/m²) in a climate chamber (BINDER KBF P 720, Binder GmbH, Tuttlingen, Germany) for 0–3 weeks.

For each sample type, three replicate vials were withdrawn every three days throughout a 3-week period for the headspace analysis of hexanal and other volatile compounds by headspace solid-phase microextraction combined with gas chromatography–mass spectrometry (HS-SPME-GC-MS).

2.4. Storage test of blueberries and cherry tomatoes

The effect of hexanal producing and releasing aerogels on the shelf life of blueberries (non-climacteric) and cherry tomatoes (climacteric) was investigated. Approximately 70 g of blueberries or cherry tomatoes were placed on one gram of aerogel, which was moulded on a Petri dish (92 mm diameter), and covered with a low-density polyethylene plastic bag (1 L, Lapin muovi). Plastic bags were sealed with heat and for every bag four small holes were punctured to enable cell respiration. For each sample type, ten replicate packages were prepared. The aerogel used in the shelf life experiments was reinforced with neutral CNF and hexanal production was catalyzed by 15 μg chlorophyll/g oil. In addition, aerogels without hexanal production (i.e., without addition of emulsion) were prepared for comparison. Packages were placed into desiccator cabinets under an adjusted relative humidity of 54% by saturated calcium chloride solution. Packages containing hexanal releasing aerogels and control aerogels were placed in separate cabinets. The

cabinets were stored under continuous lighting (3.6–8.6 W/m²) and at controlled temperatures of 22 °C in a climate chamber (BINDER KBF P 720, Binder GmbH, Tuttlingen, Germany) for four weeks. Changes occurring in the packed blueberries and cherry tomatoes were visually inspected. In addition, collapse force and compression slope were determined from cherry tomatoes. Production and release of hexanal from aerogels were monitored during the storage. For this purpose, part of the original hydrogel was dried into aerogels in vials which were stored under the same conditions as the packages. Released hexanal and other volatile products were determined by HS-SPME-GC-MS.

2.5. Determination of volatile compounds by HS-SPME-GC-MS

The formation of hexanal and other volatile products in aerogels was monitored by head space solid-phase microextraction combined with gas chromatography–mass spectrometry (HS-SPME-GC-MS) according to a previously described method (Lehtonen et al., 2016). At each sampling time, three replicate aerogel containing vials were withdrawn and sealed for the analysis. The sample vials were agitated at 40 °C and 250 rpm for 10 min prior to extraction with a DVB/CAR/PDMS fiber (10 mm, 50/30 μm film thickness; Supelco, Bellefonte, PA, USA) at 40 °C and 250 rpm for 30 min using an HS-SPME injector (CombiPAL, CTC Analytics AG, Zwingen, Switzerland). The extracted compounds were released in a splitless injector at 250 °C for 10 min and run with GC-MS (HP 6890 series coupled with an Agilent 5973 mass spectrometer; Agilent Technologies Inc., Santa Clara, CA, USA). Compounds were separated in a SPB-624 capillary column (30 m \times 0.25 mm, 1.4 μm film thickness; Supelco) using helium at a flow rate of 0.7 mL/min and a temperature program from 40 °C to 200 °C. The MS interface temperature was 280 °C, ion source temperature 230 °C, and electron impact (EI) energy 70 eV. The MS was run in full-scan mode (m/z 40–300). The volatile compounds were identified based on their mass spectra using Wiley 7n database (Wiley Registry™ of Mass Spectral Data, 7th ed., Hoboken, NJ, USA) and by comparing the retention times and mass spectra with those of known standards. The presence of hexanal was reported as peak area. The content of hexanal was further roughly estimated based on external standard curve. Hexanal was spiked into SFO at a range of 0.5–55,000 ng/g and 0.5 g of spiked oil was placed in headspace vials corresponding to a standard curve (Equation (1)) at a range of 0.25–27500 ng hexanal:

$$y = (-0.0439 \times x^2) + (5461.5 \times x) + 7 \times 10^6 \quad (1)$$

The determination coefficient r^2 of the standard curve was 0.999, limit of detection was about 25 ng, and limit of quantification was 250 ng. Averages and standard deviations of the three replicate samples were reported.

2.6. Determination of collapse force and compression slope by compression testing

Collapse force and compression slope were determined in order to estimate the effect of hexanal on senescence and cell wall eruption in cherry tomatoes. Measurements were performed after 0, 4, 9, 12, and 17 days of storage. At each sampling, two packages were withdrawn from the cabinets. Of these packages, eight replicate tomatoes were used for the measurements. Compression testing was performed with Texture Analyser TA-XT2i (Stable Micro Systems, Godalming, UK). The diameter of the aluminum compression plate was 100 mm (P/100, Stable Micro Systems, Godalming, UK). Pre-test speed was set to 1 mm/sec and both the test speed and the post-test speed were set to 2 mm/sec. Load cell was 30 kg and applied trigger force 10 g. Compression was performed until 60% of the initial height. The compression test consisted of two subsequent cycle compressions which resulted in stress–strain curves. Collapse force and compression slope were calculated from these curves. Averages and standard deviations of the eight

replicate samples were reported.

3. Results

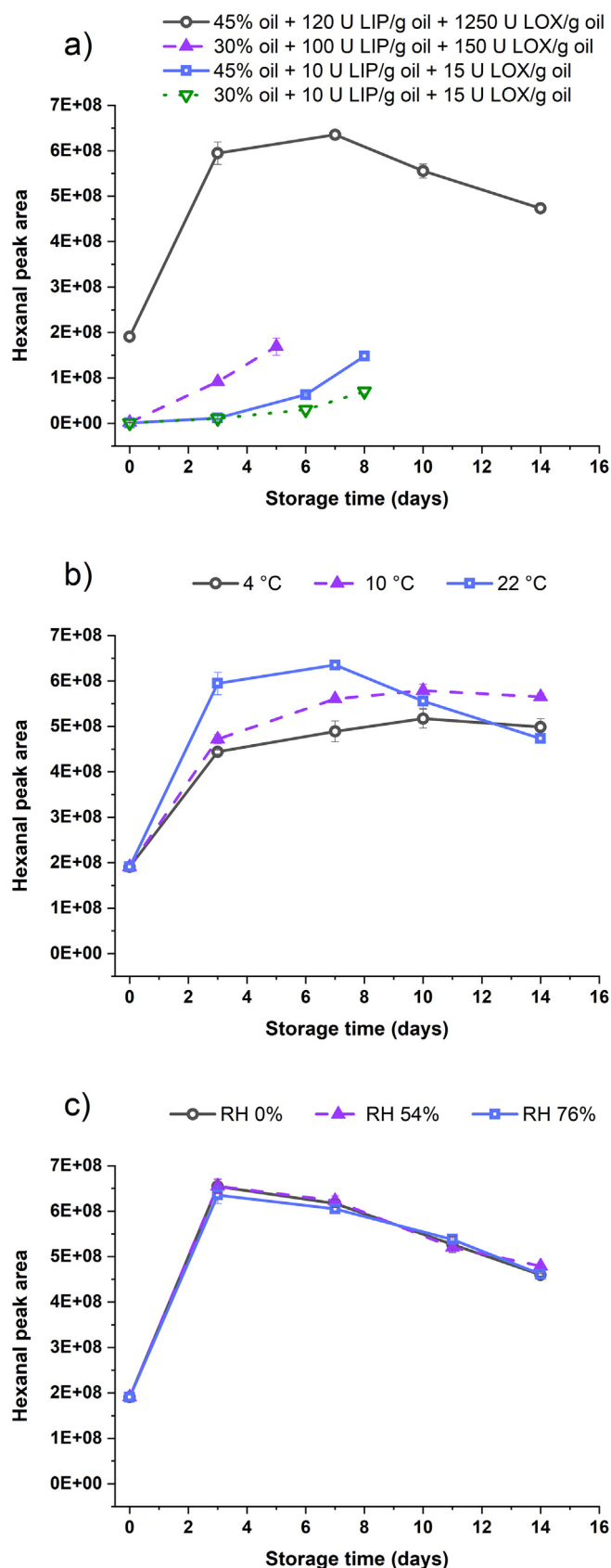
Aerogels consisting of GGM and CNF can be formed by crosslinking the polysaccharides with AZC and lyophilizing the formed hydrogel (Alakalhunmaa et al., 2016). For the release of hexanal from aerogels, lipids had to be incorporated in the polysaccharide hydrogel matrix prior to drying into aerogels. Additional ingredients could possibly affect the formation of polysaccharide network, but more importantly, the release of hexanal could be influenced by the aerogel matrix. Therefore, aerogels containing SFO and added hexanal were first tested. Aerogels consisting of 30–45 wt-% oil, 28–35 wt-% GGM, and 28–35 wt-% CNF maintained their structure during the drying process. According to the preliminary experiments, this high oil content did not affect the volume nor the compression strength of aerogels (data not shown). Direct incorporation of hexanal spiked SFO into the hydrogel led to 60–80% decrease in hexanal content during the homogenization, and further 15% decrease during the freeze-drying steps. These losses led to the final contents being approximately 5% of the original added hexanal. In addition, hexanal-incorporated aerogels cannot be stored for a long time before their use, as hexanal will be exhausted. Therefore, the next step was to investigate whether *in situ* production and prolonged release of hexanal could be achieved in aerogels. For this purpose, vegetable oil and various catalysts were introduced into hydrogel prior to their drying into aerogels. Initiation of the reaction was assisted by either enzymes, heat, or light.

3.1. Enzyme-catalyzed production of hexanal

For enzyme-catalyzed lipid oxidation and hexanal production, the amount of required substrate and catalysts were investigated. Production of hydroperoxides and further hexanal was not achieved if only LOX was incorporated to the emulsion, but LIP was also needed to release free fatty acids as substrates for LOX. Production of hexanal was apparent already during hydrogel preparation and the production continued throughout aerogel preparation. Even though the method is not quantitative, to get an estimation of the amount of released hexanal, we made a rough standard curve. Peak areas (specific to the HS-SPME-GC-MS method used) as high as 2×10^8 , corresponding to hexanal levels of $7 \mu\text{mol/g}$ aerogel, were measured immediately after freeze-drying the hydrogels into aerogels (Fig. 2a). The amount of hexanal increased up to 17–23 $\mu\text{mol/g}$ aerogel during the first three days and the proportions were maintained at 15–72% of all formed volatile oxidation products throughout two weeks of storage. The reaction rates, and thus the proportions of hexanal, were highly dependent on the substrate and catalyst contents. Hexanal remained the predominant compound for 3–8 days, after which hexanoic acid, a reaction product of hexanal, comprised 10–28% of the volatile products. Other detected volatile compounds included 2-heptenal, heptanal, octanal, pentanoic acid, and 3-ethyl-2-methyl-1,3-hexadiene (results not shown).

The production of hexanal could be controlled by altering the amount of SFO as substrate and LOX and LIP as catalysts. With low amount of substrate and catalyst (30% SFO and 15 U LOX/g oil), the level of released hexanal was approximately $1.2 \mu\text{mol/g}$ aerogel after 8 days. At low enzyme activity of 15 U LOX/g oil, hexanal was generally the main volatile product (19–72% of the total volatile compounds) and only low levels of its reaction products, mainly hexanoic acid, were detected (0–19% of the total). The main side product was octanal, which comprised 50% of volatile compounds at day 0, 10% of volatile compounds after three days, and minor proportions after that (results not shown).

Increased content of SFO and enzymes increased the production of hexanal. When the activity of LOX was increased to 150 U LOX/g oil, the level of released hexanal increased. However, the level of other oxidation products increased even more, so the proportion of hexanal of



(caption on next page)

all volatile compounds decreased 4-fold. At this LOX activity, further reaction products of hexanal were already detected after three days. When the activity of LOX was further increased to 1250 U LOX/g oil,

Fig. 2. a) Effect of substrate (30 or 45 wt-%) and catalyst contents (10, 100 or 120 U LIP/g oil and 15, 150 or 1250 U LOX/g oil) at 22 °C and 0–10% RH; b) storage temperature (T) (4, 10 or 22 °C) at 0–10% RH, 45 wt-% substrate, 120 U LIP/g oil, and 1250 U LOX/g oil; and c) relative humidity (0–10, 54, or 76% RH) at 22 °C, 45 wt-% substrate, 120 U LIP/g oil, and 1250 U LOX/g oil on enzyme-catalyzed production and release of hexanal in CNF supported GGM aerogels. LIP = lipase, LOX = lipoxygenase. Data points represent averages and standard deviations of three replicate samples. Hexanal content is expressed as peak area, specific for the HS-SPME-GC-MS method used, due to the semi-quantitative nature of the method.

the production of hexanal was in a desirable range of 17–23 $\mu\text{mol/g}$ of aerogel for at least two weeks. The proportion of hexanoic acid also increased up to 29% of all volatile compounds, but the proportion of other individual volatile lipid oxidation products remained low, that is, 0–4% of the total products (results not shown).

The production and release of hexanal was feasible at the studied temperature range 4–22 °C (Fig. 2b). The formation of hexanal was increased by increased temperature: After three days of storage, the content of hexanal was 26–32% greater when aerogels were stored at 22 °C compared to those stored at 10 °C or at 4 °C. However, side reactions were favored at elevated temperatures. Further reactions of hexanal occurred rapidly in aerogels stored at 22 °C, which was detected as a significant decrease in its content. In addition, after one week of storage at 22 °C, the proportion of hexanoic acid became greater (29% of all volatile compounds) than that of hexanal (18% of all volatile compounds).

Production and release of hexanal was unaffected by the relative humidity during storage (Fig. 2c). The levels of released hexanal were similar at RH 0–10%, 54% and 76% throughout two weeks of storage. Moreover, the proportion of hexanal compared to other volatile oxidation products remained similar at each of the studied RHs. Further reactions of hexanal occurred in a similar manner at each of the studied RHs.

3.2. Light-induced production of hexanal

To store hexanal producing aerogels before their intended use in packaging, it is desirable to control the initiation of hexanal release after the aerogel preparation. For this purpose, either autoxidation or photo-oxidation of lipids could be utilized. Autoxidation, being a free radical chain reaction, produces a wide variety of reaction products and is highly dependent on the surrounding compounds varying from one matrix to another. This render specific and controlled production impossible. In our preliminary studies, the production of hexanal (and other volatile products) could be launched by short-term heat treatment, but the amount and proportion remained below 12 $\mu\text{mol/g}$ aerogel and 14%, respectively. Therefore, only photo-oxidation was studied in more detail. The hexanal formation in the presence of GGM was also compared to that of Tween20 as emulsifier. The oxidation mechanisms were altered by trialing various photosensitizers and their contents.

The light-induced lipid oxidation progressed slowly with low amounts of catalysts (5–50 $\mu\text{g/g}$ oil) (Fig. 3). The slower the progress, the higher the proportion of hexanal: 30–71% of formed volatile products throughout two weeks of storage.

Lipid oxidation via singlet oxygen pathway using methylene blue as photosensitizer produced approximately 7–10 $\mu\text{mol/g}$ aerogel of hexanal (Fig. 3a and 3b), that is, half of the amount achieved by enzyme-catalyzed reaction. Light treatment was applied for 7 days to reach this level, and it was maintained throughout the 2–3-week storage test. Equal contents were obtained in systems emulsified with GGM and Tween20. The oxidation proceeded faster at 22 °C than at 10 °C, that is, the higher level of hexanal was measured earlier (Fig. 3a and 3b). The maximum levels of approximately 9 $\mu\text{mol/g}$ aerogel were reached after 7 days of storage at 22 °C and after 11 days at 10 °C. RH also affected

the proceeding of the oxidation. With GGM as emulsifier, the oxidation proceeded faster at RH 76% than at RH 0%, as indicated by higher hexanal release in the beginning of the test and lower amount of released hexanal towards the end of the experiment. The reaction of hexanal into hexanoic acid and production of other volatile products also increased (results not shown). However, RH did not significantly affect the reached maximum levels of released hexanal with GGM as the emulsifier. Interestingly, the opposite behaviour was observed in aerogels incorporated with Tween20-stabilized emulsion: Initial oxidation rates were greater at RH 0% than at RH 76%.

When lipid oxidation was initiated by radical forming photosensitizer, riboflavin, hexanal levels of 7–10 $\mu\text{mol/g}$ were released after 11–18 days of storage under continuous light (Fig. 3c and 3d). At RH 0%, hexanal remained as the main volatile product for 7 days (34–58%) after which the proportion of hexanoic acid became greater (35–43%) (results not shown). At RH 76%, on the other hand, hexanal remained the main volatile product (36–49%) throughout the 18-day experiment. Thus, oxidation in GGM-emulsified systems proceeded significantly faster at RH 0% than at RH 76%. This was especially evident when there was a high proportion of hexanoic acid. Formation of hexanal and hexanoic acid was similar in samples containing 10 $\mu\text{g/g}$ oil and 50 $\mu\text{g/g}$ oil of riboflavin. In addition, emulsifier did not influence the hexanal production, and similar contents were measured in systems emulsified with GGM and Tween20.

Lipid-soluble sensitizers were also investigated to produce hexanal. They were dissolved in the oil phase before emulsification. Excess β -carotene, that is, 2700 $\mu\text{g/g}$ oil, was able to initiate lipid oxidation and thus hexanal formation (Fig. 3e). The oxidation initiated relatively slowly compared to other studied systems, but the final hexanal levels were higher. Levels of approximately 3 $\mu\text{mol/g}$ were reached in one week and further contents of 10–14 $\mu\text{mol/g}$ were reached in two weeks. After reaching this level, it was maintained at least for additional week. While the content of hexanal was still increasing, it was the main constituent (61–68%) of the formed volatile products. After two weeks, the proportion of hexanal was 20–30% at RH 0% and 40–45% at RH 76%. Correspondingly, the proportion of hexanoic acid increased, being 30–36% at RH 0% and 10–16% at RH 76% (not shown). The obtained results were similar both in GGM- and Tween20-emulsified lipid systems.

Chlorophyll is a lipid-soluble photosensitizer which may act both via singlet oxygen and via radical reactions. This could potentially ensure hexanal production over a wide temperature range and accelerate the formation of hexanal compared to the purely singlet oxygen pathway. At the initial stage, hexanal production with chlorophyll was similar as in aerogels containing methylene blue. As the storage time was prolonged, contents of 11–15 $\mu\text{mol/g}$ hexanal were reached (Fig. 3f). Both in GGM- and in Tween20-emulsified systems, the lipid oxidation proceeded faster at RH 0% than at RH 76% (Fig. 3f). Thus, lower levels of hexanal were detected at RH 0% (11 $\mu\text{mol/g}$) than at RH 76% (15 $\mu\text{mol/g}$) and the levels started to decrease earlier due to the reaction of hexanal into hexanoic acid. At RH 0%, the proportion of hexanal was 24–28% of all volatile compounds during the first week of storage. After three weeks, the proportion reduced to 13%. After 10 days of storage, the content of hexanal levelled and, at the same time, the proportion of hexanoic acid (35%) became larger than that of hexanal. In comparison, at RH 76%, the proportion of hexanal was 24–30% for the whole 3-week storage period. The proportion of hexanoic acid remained below 20% throughout 3 weeks.

Control samples that were not exposed to light treatment, but were covered with aluminum foil and otherwise maintained at similar conditions, did not release any hexanal (data not shown).

3.3. Storage stability of cherry tomatoes and blueberries

To investigate whether the developed hexanal releasing aerogel would prevent deterioration of climacteric (i.e., ethylene producing)

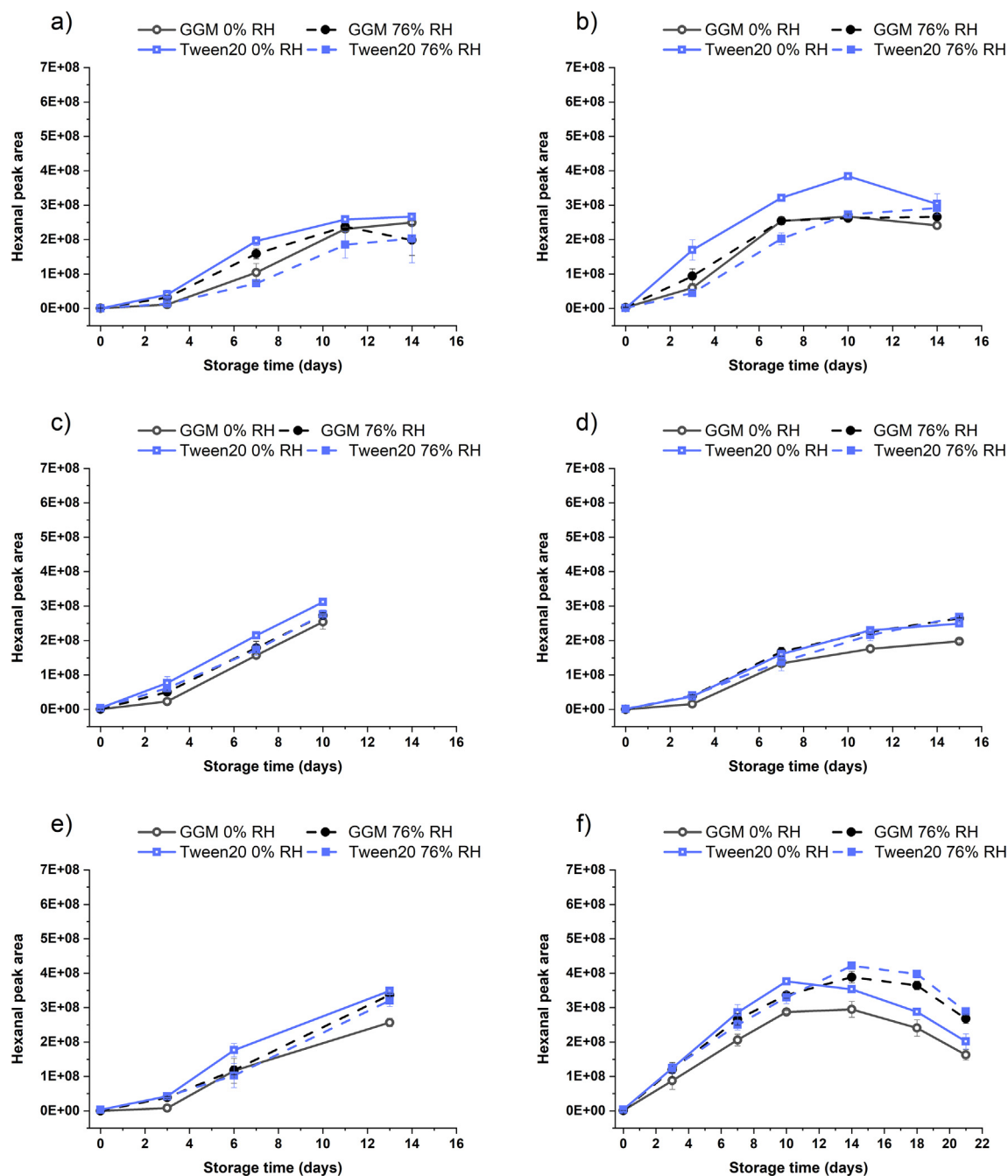


Fig. 3. Effect of photosensitizer, emulsifier, and storage conditions on the light induced production and release of hexanal in CNF supported GGM aerogels. a) 5 $\mu\text{g/g}$ oil methylene blue at 10 °C, b) 5 $\mu\text{g/g}$ oil methylene blue at 22 °C, c) 10 $\mu\text{g/g}$ oil riboflavin at 22 °C, d) 50 $\mu\text{g/g}$ oil riboflavin at 22 °C, e) 2700 $\mu\text{g/g}$ oil β -carotene at 22 °C, and f) 15 $\mu\text{g/g}$ oil chlorophyll at 22 °C. Data points represent averages and standard deviations of three replicate samples. In the figure labels, GGM and Tween20 refer to the emulsifiers used. Hexanal content is expressed as peak area, specific for the HS-SPME-GC-MS method used, due to the semi-quantitative nature of the method.

and non-climacteric berries and vegetables, storage stabilities of cherry tomatoes and blueberries, respectively, were studied. Compression testing was applied on cherry tomatoes, and physical changes occurring in the blueberries and cherry tomatoes were visually inspected.

A clear trend was observed in the softening of cherry tomatoes: the collapse force and compression slope decreased at a slower rate in hexanal-treated cherry tomatoes than in the control tomatoes stored without hexanal release (Fig. 4).

Levels of hexanal as low as 12–17 $\mu\text{mol/L}$ in air were able to prevent softening of the studied cherry tomatoes. Mould growth was visually inspected for blueberries, which were purchased from a local market

and placed on hexanal releasing aerogel. The blueberries stored with hexanal-releasing aerogel remained visually unaltered for five days at room temperature (Fig. 5a). Meanwhile, mould growth was evident in blueberries which were stored on the control aerogel (Fig. 5b).

4. Discussion

Hexanal is a natural preservative and fungicide that can be utilized to prolong the storage time of various fruits and berries (Almenar et al., 2007; Lanciotti et al., 1999; Sharma et al., 2010; Sholberg & Randall, 2007; Song et al., 2010; Misran et al., 2015). Hexanal research has

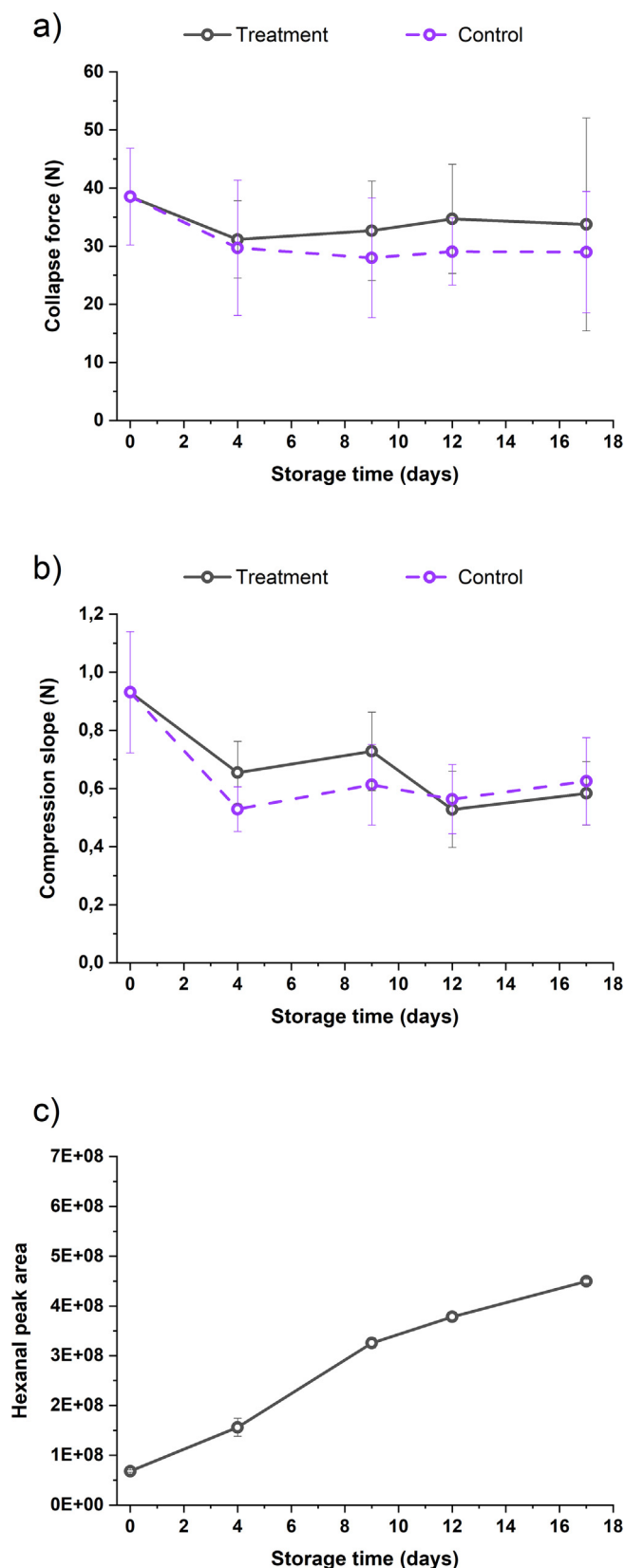


Fig. 4. Changes in a) collapse force and b) compression slope of cherry tomatoes during c) hexanal release. For collapse force and compression slope, the data points represent averages and standard deviations of eight replicate samples. For hexanal, the data points represent averages and standard deviations of three replicate samples. Hexanal content is expressed as peak area, specific for the HS-SPME-GC-MS method used, due to the semi-quantitative nature of the method.

mostly focused on the possibilities to utilize it in spraying solutions for preharvest treatment of fruits, or in batch-wise chamber treatments of fruits and berries after harvesting. The effects of such treatments are rather short-term. Continuous storage under a hexanal atmosphere has not been feasible, as hexanal is rapidly exhausted. To maintain its concentration, additional hexanal should be incorporated to replace the consumed portion. This could be possible under industrial conditions, but after the goods are packed and delivered to retailers, shelf life is limited. In this paper we introduced a potential active packaging material in which continuous *in situ* production and release of hexanal is possible. This type of packaging material is feasible to be used in consumer packaging to prolong the shelf-life of various fresh plant-based goods.

In this study, SFO was used as a substrate and as a delivery medium for hexanal. The release of these lipid-soluble volatile products is slower from lipid medium than from water, due to lower vapour pressure (Haahr, Bredie, Stahnke, Jensen & Refsgaard, 2010). This enables long-term steady release rather than intensive short-term release. To prevent uncontrolled autooxidation of unsaturated lipids, SFO was emulsified and stabilized by GGM. We have previously shown that GGM is able to inhibit lipid oxidation in emulsions up to months, even in accelerated conditions (Lehtonen et al., 2016; Lehtonen et al., 2018). However, the present results showed that GGM did not inhibit the controlled oxidation of SFO catalyzed by LOX and LIP or photosensitizers.

Lipid-loaded aerogels containing up to 45 wt-% of substrate for hexanal production could be successfully prepared by lyophilization. In addition, catalysts for the lipid oxidation could be incorporated during production. Aerogels are lightweight porous materials which have a large surface area (Mehling et al., 2009; García-González et al., 2011; Mikkonen et al., 2013). High porosity makes aerogels beneficial materials for the release and delivery of compounds into the surroundings. In addition, its low density is a desirable feature for packaging materials. Aerogels are prepared from hydrogels by replacing liquid with air, for example by lyophilization or by supercritical carbon dioxide. In lyophilization, polysaccharide hydrogel retains its dimensions, and thus leads to high volume aerogels. In supercritical carbon dioxide drying, on the other hand, part of the structure of polysaccharide hydrogel is lost in the solvent exchange step and further in the drying step. In addition, lipid-soluble compounds, such as hexanal, are easily extracted by the used solvent and carbon dioxide.

The production and release of hexanal could be controlled by substrate and catalyst contents. Low oil content would be desirable, as high oil content might compromise the physical properties of packaging materials. However, by the reduction of SFO, hexanal production may be exhausted leading to less-efficient shelf-life enhancement. High catalyst content would increase the rate of formation, but at the same time it would also increase the formation of side products, such as further oxidation products of hexanal. Thus, by maintaining as high a substrate content as feasible, and as low a catalyst content as possible, enables steady and long-term production and release of hexanal in aerogels.

Levels of 7–23 μmol hexanal per gram of aerogel could be produced and maintained up to three weeks at room temperature under relative humidities of 0–76%. The production was most efficient in the enzyme-catalyzed system while being lowest during heat-catalyzed auto-oxidation and singlet oxygen photooxidation. Compared to literature (Almenar et al., 2007), the achieved hexanal levels were high enough in all systems to extend the shelf life of fruits, vegetables, and berries. Mold growth was reduced significantly in blueberries (70 g) stored under 12–17 $\mu\text{mol/L}$ of hexanal. Continuous exposure to hexanal at a concentration as low as 0.54 nmol/L has been shown to lead to a 50% reduction in the fungal growth (Andersen et al., 1994). In our systems, continuous production of this content would be reached with much less than 1 mg of aerogel. Conversely, other authors have proposed that for fungicidal effects up to 9–20 $\mu\text{mol/L}$ hexanal is needed (Almenar et al., 2007). These concentrations were shown to reduce fungal growth up to

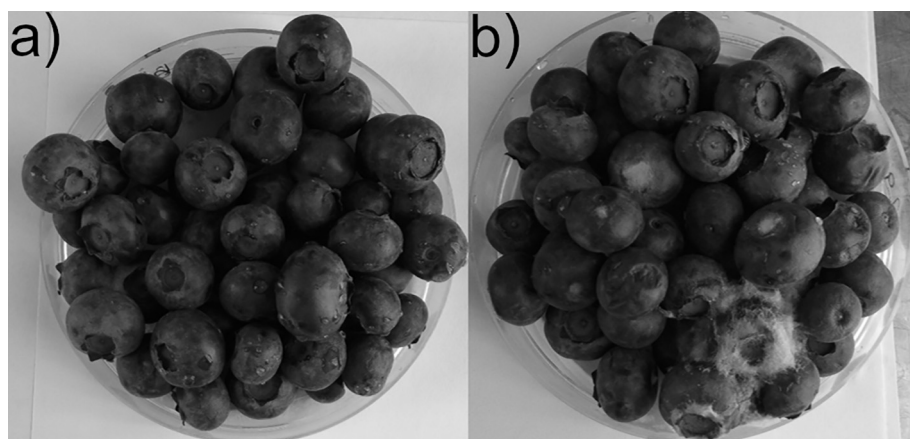


Fig. 5. Blueberries during 5 days of storage at RH 56% and 22 °C placed on a) hexanal releasing aerogel and b) non-releasing control aerogel.

57%. To produce such levels, 0.07–0.7 g of enzyme containing aerogel would be needed for a 1–2 L package. For light-induced production, 0.2–2 g would be sufficient.

According to present results, contents of 12–17 μmol or above for 70 g of product are likely needed to slow down tissue softening due to ripening and senescence. According to earlier studies, 150 μmol of hexanal applied as batch-wise treatment per 100 g sliced apples, extended the shelf life both at 4 °C and 15 °C (Lanciotti et al., 1999). Multiple treatments of blueberries with hexanal at concentrations as high as 37 mmol/kg blueberries for 24 h inhibited decay up to 70% (Song et al., 2010). Effective hexanal levels on ripening and senescence during continuous exposure are yet to be studied.

In an enzyme-catalyzed system, the production of hexanal begins already during the manufacture of the material. Initiation of hexanal production could be controlled by utilizing light-induced lipid oxidation—photooxidation. The levels of produced hexanal and side reaction could be controlled in an enzyme-catalyzed production, while in photooxidation this was not straight forward. Plant lipoxygenase catalyzes merely the formation of hydroperoxides in free fatty acids (Siedow, 1991; Andreou & Feussner, 2009; Schaich et al., 2013). As SFO consists ideally solely of triacylglycerols (TAG), formation of hydroperoxides and further production of hexanal is not achieved without prior hydrolysis of TAGs. Lipases are enzymes that catalyze hydrolysis of TAGs into free fatty acids. Using LIP and LOX together, formation of hydroperoxides and further production of hexanal was achieved. Breakdown of hydroperoxides into hexanal occurred spontaneously and therefore hydroperoxide lyase, which catalyzes the breakdown of hydroperoxides in plants, was not needed. Plant type-I lipoxygenases (13S-lipoxygenases, EC 1.13.11.12) have high regioselectivity, producing almost solely 13-OOH of linoleic acid [(9Z,11E, 13S)-13-hydroperoxyoctadeca-9,11-dienoic acid] (Siedow, 1991; Schaich et al., 2013). This ability leads to high proportion of hexanal and low production of other volatile oxidation products such as 3-nonenal, which is a 9-OOH product of linoleic acid.

Photosensitizers absorb low-level light energy and transform it into chemical energy. Photo-oxidation occurs mainly in two ways: via singlet oxygen or via radical-intermediated reactions. Singlet oxygen, which reacts directly with double bonds, may be selectively produced, for example by methylene blue ($\lambda_{\text{max}} = 600\text{--}700\text{ nm}$) (Schaich et al., 2013). Hexanal production via the singlet oxygen route using methylene blue was slow. The production of hydroperoxides in the singlet oxygen route is not temperature dependent, which is an advantage, but the breakdown of hydroperoxides into hexanal is. If the content of radicals in the lipid environment is low and the temperature is not elevated, the formation of hexanal remains slow. In addition, equal contents of four different hydroperoxide products of linoleic acid, that is, 9-OOH, 10-OOH, 12-OOH, and 13-OOH, are expected in photooxidation.

Since hexanal is a breakdown product of only 13-OOH, competitive formation of other hydroperoxides is likely to lead to lower production of hexanal and to greater production of side products, than for example in plant type I lipoxygenase-catalyzed lipid oxidation.

When oxidation of lipids was initiated by radical forming sensitizer, riboflavin, the formation rate of hexanal was greater than by singlet oxygen forming methylene blue. However, further reactions of hexanal were also greater, leading to low hexanal contents and elevated hexanoic acid contents. In radical intermediated oxidation of linoleic acid, two hydroperoxides, 9-OOH and 13-OOH, are formed. Formation of only two hydroperoxide isomers would be expected to lead to greater content of hexanal. However, in free radical chain reaction, formed lipid radicals are rapidly reacting with other lipid molecules and oxidized lipids producing a variety of new radicals and breakdown products (Schaich et al., 2013).

When chlorophyll was applied to initiate hexanal production, greater contents of hexanal could be obtained and the reaction of hexanal into hexanoic acid could be controlled. Chlorophyll ($\lambda_{\text{max}} = 400\text{--}500\text{ nm}$ and $650\text{--}700\text{ nm}$) has the ability to act via both of the above-described routes, enabling production of hydroperoxides even at low temperatures and concomitantly the production of radicals to induce the breakdown of hydroperoxides into hexanal.

β -carotene is usually considered as an antioxidant. Interestingly, β -carotene at excess concentrations was able to initiate lipid oxidation in aerogels. This may be because β -carotene itself is able to form reactive radical species (Schaich et al., 2013).

Formation of other volatile aldehydes and ketones during lipid oxidation is expected to assist the extension of shelf life, and these are therefore not directly detrimental. Aldehydes and ketones, having the chain lengths of six and nine carbons, e.g., 3-hexanone, nonanal, and 2-nonanone, have been shown to reduce the growth of fungi (Andersen et al., 1994). In addition, these products are considered safe and many of them are allowed as flavoring agents in food products (EU No 872/2012). The possible downside in their formation is that they compete with hexanal formation and they may catalyze further breakdown of hexanal. In addition, these side products may cause unwanted odors when present at significant levels.

Both enzyme activity and lipid oxidation are influenced by RH. In this study, an excess amount of water was available for enzyme-catalyzed lipid oxidation at the hydrogel stage. This enabled the production of hydroperoxides already prior to drying the hydrogels into aerogels. Storage humidity had no further influence on the production and release. When lipid oxidation was initiated in aerogels by photooxidation, RH influenced the production. The effect of RH varied between the studied systems: Both the emulsifier and oxidation mechanism influenced the rate of hexanal formation. In the singlet oxygen route, reaction rates in the GGM-emulsified system were greater at RH 76% than

at RH 0%, while in the Tween20-emulsified system, the opposite occurred. Interestingly, in radical-mediated oxidation, reaction rates in the GGM-emulsified system were greater at RH 0% than at 76%, while the opposite occurred in the Tween20-emulsified system. High oxidation rates are expected in very dry systems as oxygen is free to flow in a dry matrix and has direct contact with lipids (Schaich et al., 2013). When RH increases, the rate of oxidation is expected to decrease as water is bound as a monolayer providing protection to lipids. When RH is further increased, the rate of oxidation increases again due to the fact that catalysts are dissolved, and their mobility is increased leading to increased interaction with lipid molecules.

5. Conclusions

Lipid-loaded aerogels were developed as a delivery system for substrates and catalysts for *in situ* production and release of hexanal. Controlled hexanal release was catalysed by the use of enzymes or photoinitiators. Such aerogels are envisioned as food packaging materials or parts of primary packaging, and they can be formed from bio-based polysaccharide, such as CNF- and GGM-containing matrix. GGM enabled the delivery of lipids in the matrix without adverse effects on the proceeding of oxidation. Hexanal was produced and released at levels of 7–23 $\mu\text{mol/g}$ of aerogel for at least three weeks. In a future bio-based active packaging concept, less than one gram of aerogel in one liter package could preserve fresh plant products against softening and mould growth. This novel active packaging could improve the economy and sustainability of the food chain and prevent food waste.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Linn Berglund and Kristiina Oksman for the neutral CNF used for aerogels in the shelf life studies, and Karlijn Remmers, Tapio Väänänen, Satu Kirjoranta, and Daniel Richterich for technical assistance. We gratefully acknowledge the Department of Food and Nutrition (University of Helsinki), the Academy of Finland (project numbers 268144, 281628, and 305517), Jane and Aatos Erkko Foundation, and Nordic Forest Research for funding.

References

Alakalunmaa, S., Parikka, K., Penttilä, P., Cuberes, M. T., Willför, S., Salmén, L., & Mikkonen, K. S. (2016). Softwood-based sponge gels. *Cellulose*, *23*, 3221–3238.

Almenar, E., Auras, R., Rubino, M., & Harte, B. (2007). A new technique to prevent the main post harvest diseases in berries during storage: Inclusion complexes β -cyclodextrin-hexanal. *International Journal of Food Microbiology*, *118*, 164–172.

Andersen, R. A., Hamilton-Kemp, T. R., Hildebrand, D. F., McCracken, C. T., Jr., Collins, R. W., & Fleming, P. D. (1994). Structure-antifungal activity relationships among volatile C6 and C9 aliphatic aldehydes, ketones, and alcohols. *Journal of Agricultural and Food Chemistry*, *42*, 1563–1568.

Androu, A., & Feussner, I. (2009). Lipoxygenases – Structure and reaction mechanism. *Phytochemistry*, *70*, 1504–1510.

Berglund, L., Noël, M., Aitomäki, A., Öman, T., & Oksman, K. (2016). Production potential of cellulose nanofibers from industrial residues: Efficiency and nanofiber characteristics. *Industrial Crops and Products*, *92*, 84–92.

Bleé, E. (1998). Phytooxylipins and plant defense reactions. *Progress in Lipid Research*, *37*, 33–72.

Cheema, A., Padmanabhan, P., Subramanian, J., Blom, T., & Paliyath, G. (2014). Improving quality of greenhouse tomato (*Solanum lycopersicum* L.) by pre- and postharvest applications of hexanal-containing formulations. *Postharvest Biology and*

Technology, *95*, 13–19.

EUFG. (2012). Fruit and vegetable consumption in Europe – Do Europeans get enough?

El Kayal, W., Paliyath, G., Sullivan, J. A., & Subramanian, J. (2017). Phospholipase D inhibition by hexanal is associated with calcium signal transduction events in raspberry. *Horticulture Research*, *4*, 17042.

FAO. <http://www.fao.org/food-loss-and-food-waste/en/> (Accessed 17th February 2019).

Feussner, I., Kühn, H., & Wasternack, C. (2001). Lipoxygenase-dependent degradation of storage lipids. *Trends in Plant Science*, *6*, 268–273.

García-González, C. A., Alnaief, M., & Smirnova, I. (2011). Polysaccharide-based aerogels - promising biodegradable carriers for drug delivery systems. *Carbohydrate Polymers*, *86*, 1425–1438.

Ghafar, A., Parikka, K., Sontag-Strohm, T., Österberg, M., Tenkanen, M., & Mikkonen, K. S. (2015). Strengthening effect of nanofibrillated cellulose is dependent on enzymatically oxidized polysaccharide gel matrices. *European Polymer Journal*, *71*, 171–184.

Haahr, A.-M., Bredie, W. L. P., Stahnke, L. H., Jensen, B., & Refsgaard, H. H. F. (2000). Flavour release of aldehydes and diacetyl in oil/water systems. *Food Chemistry*, *71*, 355–362.

Jash, A., Paliyath, G., & Lim, L.-T. (2018). Activated release of bioactive aldehydes from their precursors embedded in electrospun poly(lactic acid) nonwovens. *RSC Advances*, *8*, 19930–19938.

Kilpeläinen, P., Hautala, S., Byman, O., Tanner, J., Korpinen, R., Lillandt, M., ... Ilvesniemi, H. (2014). Pressurized hot water flow-through extraction system scale up from the laboratory to the pilot scale. *Green Chemistry*, *12*, 3186–3194.

Lanciotti, R., Corbo, M. R., Gardini, F., Sinigaglia, M., & Guerzoni, M. E. (1999). Effect of hexanal on the shelf life of fresh apple slices. *Journal of Agricultural and Food Chemistry*, *47*, 4769–4776.

Lehtonen, M. I., Teräslähti, S., Xu, C., Yadav, M. P., Lampi, A.-M., & Mikkonen, K. S. (2016). Spruce galactoglucomannans inhibit lipid oxidation in rapeseed oil-in-water emulsions. *Food Hydrocolloids*, *58*, 255–266.

Lehtonen, M., Merinen, M., Kilpeläinen, P. O., Xu, C., Willför, S. M., & Mikkonen, K. S. (2018). Phenolic residues in spruce galactoglucomannans improve stabilization of oil-in-water emulsions. *Journal of Colloid and Interface Science*, *512*, 536–547.

Mehling, T., Smirnova, I., Guenther, U., & Neubert, R. H. H. (2009). Polysaccharide-based aerogels as drug carriers. *Journal of Non-Crystalline Solids*, *355*, 2472–2479.

Mikkonen, K. S., Parikka, K., Ghafar, A., & Tenkanen, M. (2013). Prospects of polysaccharide aerogels as modern advanced food materials. *Trends in Food Science & Technology*, *34*, 124–136.

Mikkonen, K. S., Merger, D., Kilpeläinen, P., Murtomäki, L., Schmidt, U., & Wilhelm, M. (2016). Determination of physical emulsion stabilization mechanisms of wood hemicelluloses via rheological and interfacial characterization. *Soft Matter*, *12*, 8690–8700.

Misran, A., Padmanabhan, P., Sullivan, J. A., Khanzadeh, S., & Paliyath, G. (2015). Composition of phenolics and volatiles in strawberry cultivars and influence of pre-harvest hexanal treatment on their profiles. *Canadian Journal of Plant Science*, *95*, 115–126.

Noordermeer, M. A., Veldink, G. A., & Vliegthart, J. F. G. (2001). Fatty acid hydroperoxide lyase: A plant cytochrome P450 enzyme involved in wound healing and pest resistance. *ChemBioChem*, *2*, 494–504.

Pak Dek, M. S., Padmanabhan, P., Subramanian, J., & Paliyath, G. (2018). Inhibition of tomato fruit ripening by 1-MCP, wortmannin and hexanal is associated with a decrease in transcript levels of phospholipase D and other ripening related genes. *Postharvest Biology and Technology*, *140*, 50–59.

Porta, H., & Rocha-Sosa, M. (2002). Plant lipoxygenases. Physiological and molecular features. *Plant Physiology*, *130*, 15–21.

Schaich, K. M., Shahidi, F., Zhong, Y., & Eskin, N. A. M. (2013). Lipid oxidation. In N. A. M. Eskin, & F. Shahidi (Eds.). *Biochemistry of foods* (pp. 419–478). (Third edition). Cambridge: Academic Press.

Sharma, M., Jacob, J. K., Subramanian, J., & Paliyath, G. (2010). Hexanal and 1-MCP treatments for enhancing the shelf life and quality of sweet cherry (*Prunus avium* L.). *Scientia Horticulturae*, *125*, 239–247.

Sholberg, P. L., & Randall, P. (2007). Fumigation of stored pome fruit with hexanal reduces blue and gray mold decay. *HortScience*, *42*, 611–616.

Siedow, J. N. (1991). Plant lipoxygenase: Structure and function. *Annual Review of Plant Physiology and Plant Molecular Biology*, *42*, 145–188.

Song, J., Leepipattanawit, R., Deng, W., & Beaudry, R. M. (1996). Hexanal vapor is a natural, metabolizable fungicide: Inhibition of fungal activity and enhancement of aroma biosynthesis in apple slices. *Journal of the American Society for Horticultural Science*, *121*, 937–942.

Song, J., Fan, L., Forney, C., Campbell-Palmer, L., & Fillmore, S. (2010). Effect of hexanal vapor to control postharvest decay and extend shelf-life of highbush blueberry fruit during controlled atmosphere storage. *Canadian Journal of Plant Science*, *90*, 359–366.

Tiwari, K., & Paliyath, G. (2011). Microarray analysis of ripening-regulated gene expression and its modulation by 1-MCP and hexanal. *Plant Physiology and Biochemistry*, *49*, 329–340.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., ... Murray, C. J. L. (2019). Food in the Anthropocene: The EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet*, *393*, 447–492.