VTT Technical Research Centre of Finland

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Comparison of low energy and high energy electron beam treatments on sensory and chemical properties of seeds

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ABSTRACT

Consumption of fresh and minimally processed foods such as seeds as a part of a healthy diet is a trend. Unfortunately, fat-rich seeds are often contaminated with pathogenic microorganisms and face frequent product recalls. Electron beams have been applied as a microbial decontamination measure for decades. Conventionally high energy electron beams (HEEB) are being used, whereas low energy electron beams (LEEB, <300 keV) have only recently been introduced to the food industry and more studies are needed. Electron beam treatment has several advantages over other decontamination technologies. The treatment is non-thermal, chemical-free, water-free, and does not use radioactive substances. The effect of electron beams on the sensory and chemical properties of seeds has not been widely studied. This study assessed LEEB and HEEB treated pumpkin and flax seeds immediately after treatments, and after three months of storage. The seeds' sensory profiles were altered after both treatments when compared with non-treated samples, with a higher dose leading to a greater level of alteration. However, the sensory profile of LEEB treated seeds was similar to the non-treated seeds whereas HEEB treated seeds differed from both. The storage period of three months further increased the observed differences between the samples. LEEB and HEEB treatments seemed to cause lipid degradation as the content of volatile aldehydes was increased. This effect was more profound in HEEB treated samples. The data presented in this study shows that LEEB as a microbial reduction solution has great potential to preserve the chemical and sensory properties of nutritious seeds.

1. Introduction

The consumption of seeds as a part of healthy diets has increased over the last decade. Unfortunately, fat-rich seeds such as pumpkin seeds or flaxseeds have been associated with product recalls due to contamination by pathogenic microorganisms (Willis, Little, Sagoo, de Pinna, & Threlfall, 2009). The European Rapid Alert System for Food and Feed (RASFF) (European Commission, 2020) includes several notifications on contaminated seeds, e.g. sesame seeds (RASFF references 2020.1377, 2020.1271, 2020.1149, 2020.1150, 2020.0791), pumpkin seeds (RASFF reference 2019.4135) and sunflower seeds (2019.2505). Furthermore, consumers increasingly demand more fresh or minimally processed foods, which poses an additional challenge to the food industry (Gould, 1996). Foodborne pathogens have a long history of leading to food safety outbreaks and not only do they pose a significant risk to public health, but also cause major financial losses to the involved food brands (World Health Organization, 2019). Therefore, it is crucial to implement measures to control and reduce microbial contamination in foods. Thermal processing, such as pasteurization or steam treatment, is most widely applied as a microbial control measure in the food industry. While a thermal treatment is effective to inactivate bacterial load, it also often alters the perceived quality of food and decreases its nutritional value. Therefore, non-thermal and less invasive decontamination technologies such as low energy electron beams are preferred by the food industry (De Corato, 2020). Fumigation with ethylene oxide is widely used to reduce microbial loads in seeds across the world. However, it has been banned in the European Union due to its carcinogenic properties (EU Council, 1986). Food irradiation has been researched and applied for decades and it is the most effective in reducing microbial load (U.S. Food and Drug Administration, 2017). However, irradiated foods are

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often associated with low consumer acceptance due to misinterpretations related to radioactivity (Balatsas-Lekkas, Arvola, Kotilainen, Meneses, & Pennanen, 2020; Eustice & Bruhn, 2012).

Irradiation with γ-irradiation requires non-renewable radioactive sources and has further technical, financial, and operational challenges, which creates a need for alternative irradiation technologies. Various novel food processing technologies such as high-pressure and pulsed electric fields are emerging with the aim to bridge the gap of food safety requirements and consumer acceptance (De Corato, 2020; Institute of Food Technologists, 2000). The electron beam technology applies accelerated electrons that carry ionizing potential and is therefore classified as ionizing radiation. When ionizing radiation interacts with biomolecules they will disrupt molecular bonds and damage the cells (Krieger, 2012; Tahergorabi, Matak, & Jaczynski, 2012). It has been suggested that both low energy electron beam (LEEB, $\leq$ 300 keV) and high energy electron beam (HEEB, $>$ 300 keV) treatments have similar mechanisms to inactivate microorganisms by causing DNA damage (Zhang et al., 2020). Upon sufficiently intensive treatment (sufficient dose) the microorganisms can no longer recover from the damage (Zhang et al., 2020). LEEB and HEEB have been shown to effectively reduce bacteria in experimental settings but also on food matrices such as meat, dried fruits, beans, grains, spices, and model surfaces (Arthur et al., 2005; Bouzarjomehri, Dad, Hajimohammadi, Shirmardi, & Yousefi-Ghaleh Salimi, 2020; Etter, Rupp, Prange, & Drissner, 2018; Fan et al., 2017; Hertwig, Meneses, & Mathys, 2018; Woldemariam et al., 2021; Zhang et al., 2018).

A major difference between LEEB and HEEB is that electrons applied by HEEB have enough energy to penetrate food products up to several centimeters. Therefore, HEEB is a volumetric treatment and the whole seed is treated with ionizing radiation. Due to significantly lower energy of the electrons, the penetration depth LEEB is up to few hundred micrometers in a food material (Krieger, 2012; Pillai & Shayanfar, 2015). This means that LEEB is a surface treatment and therefore, is not expected to affect the quality properties in the inner food matrix. Additional benefit to LEEB is that due to the significantly lower energy of the electrons in comparison to conventional electron beams, LEEB could be easily implemented to the processing line and operated on-site. However, LEEB has only recently been introduced to the food industry (TetraPak, 2017).

Both LEEB and HEEB treatments have demonstrated the ability to preserve food quality. For example, fresh watermelon cubes, grapes, cherry tomatoes, strawberries, and brick tea were shown to retain their appearance, odor, color, firmness, and flavor in sensory evaluations (Smith, Ortega, Shayanfar, & Pillai, 2017; Smith, Shayanfar, Walzem, Alvarado, & Pillai, 2020; Zhang, Zhang, Chambers, & Dai, 2020). However, the studied effects of electron beam treatments on the sensory profiles of fat-rich seeds are still scarce. There are a limited number of publications studying the effects of electron beam treatments on pumpkin, flax, or sesame seeds. Thus, descriptive sensory data related to seeds are lacking as most similar studies are done on almonds (Lanza et al., 2013; Sánchez-Bel, Egea, Romojaro, & Martínez-Madrid, 2008).

In this study, LEEB was selected as a promising surface microbial decontamination technology anticipated to have a minor impact on seed quality. Untreated and HEEB treated seeds were used as a reference to evaluate the difference between surface and volumetric treatments. To compare the quality changes of LEEB and HEEB treatments on seeds, a trained sensory panel evaluated the sensory attributes of pumpkin and flax seeds after the treatments. In addition, chemical analyses were performed to support and further explain the sensory observations. The outcome of this study supports the notion of LEEB as a microbial control treatment with minimal impact on sensory perception.

2. Materials and methods

2.1. Samples and treatments

Pumpkin (*Cucurbita* ssp. L.) and flax (*Linum usitatissimum L.)* seeds (Kündig, Zürich, Switzerland) were used as research samples. The seeds were treated with either low-energy electron beam (LEEB) or high-energy electron beam (HEEB) technology. The LEEB treatments were performed at Bühler AG (Uzwil, Switzerland) with the Laatu equipment running at 200 keV. The Laatu equipment conveys the product towards the treatment zone, where the product free-falls between two LEEB lamps in a face-to-face configuration. 10 kGy and 8 kGy were selected as absorbed doses for the study. According to Codex General Standard for Irradiated foods, the maximum absorbed dose should not exceed 10 kGy except when necessary to achieve a technological purpose (Codex Alimentarius, 2003). The dose must also be sufficient to achieve a technological purpose, which here was microbial safety of the samples. Maximum absorbed dose of 8 kGy was selected as a reference dose to evaluate the impact on quality preservation, whilst still sufficient to control the microbial load. The absorbed dose depends on the beam current set point and the exposure time, which is given by the product speed in the treatment zone. The feeding parameters were chosen based on high-speed video footage of product in the treatment zone to ensure that the product falls with minimal rotation at the same speed and separated from each other to avoid shadowing effects. B3 radiochromic film was used for dosimetry on Laatu which has been calibrated and is traceable to international standards.

The LEEB beam has then been characterized according to ISO/ASTM 51818(2013), including depth dose measurements. The final dose on pumpkin seeds was assessed by treating pumpkin seeds with B3 film directly taped onto them. The doses are given as the dose received by the first micrometer water equivalent density (Dμ) and were set by varying the current and keeping the feeding parameters, and thus the exposure time, constant. The process was run at ambient conditions and delivered the final dose of 8.8 ± 0.7 kGy (n = 8) and 10.4 ± 0.9 kGy (n = 8) for the two selected treatments and are marked as LEEB 8 kGy and 10 kGy samples later in this study. The penetration depth of the electrons in pumpkin seeds is in the range of 295–340 μm and was assessed by correcting the depth dose measurements from the beam characterization with the true density of pumpkin seeds (1.06–1.22 g/cm^3, (Khodabakhshian, 2012)). For flax seeds, the high-speed video footage revealed the exposure time to be 3–6% less, which would correspond to 3–6% less dose. The true density of flax seed is between 1.0 and 1.1 g/cm^3 and leads to a penetration depth between 330 and 360 μm. The effects of the topology of the seeds on the true surface dose were not considered, as this would go beyond the purpose of this paper to investigate sensory properties of electron beam treated samples.

The HEEB treatments were performed at the facilities of German Institute of Food Technologies (DIL) e.V. at the Max Rubner-Institut (MRI) in Karlsruhe, Germany. The treatment of pumpkin and flax seeds was carried out at room temperature (20 ± 2 °C, normal atmosphere) with the linear electron accelerator (LINAC), type CIRCE III (Thomson-CSF-Linac Technologies S. A., Orsay, France). Electron beam is a non-thermal process. A dose of 10 kGy causes a temperature increase of a maximum of 3 °C. At 5 MeV acceleration energy and beam frequency of 145 Hz, the calculated beam power was 2100 ± 30 W. To control scanning uniformity, a pretest was performed with Dos'ASAP dosimetry equipment for a CTA dosimeter readout (Aérial, Illkirch-Graffenstaden, France). These experiments verified that all areas of the sample received the same e-beam dose, within the uncertainty of the dosimeter (6–8%). The working condition and process parameters of the linear accelerator used for seed treatment are described in detail by Woldemariam et al. (2021).

For carrying out these experiments, 500 g pumpkin or flax seeds per sample were packed in plastic bags (300 mm × 400 mm PA/PE). The packaging surface was pressed against the seeds to remove the air to great extent and bags were sealed. The thickness of the sample packaging was approximately 1.5 + 0.3 cm. Two bags were taped to each tray in a single flat layer to receive equal dosage. The doses to the sample were applied by regulating the conveyor speed. The conveyor was set at 13 mm/min, which provided 10 kGy and 16 mm/min to reach 8 kGy. The absorbed doses were measured using alanine pellets from Aérial (Illkirch-Graffenstaden, France) which were located above and below sample bags and treated together with the samples. The Alanine tablets were analyzed by Aérial. The following mean values and standard deviations were obtained: 8.2 ± 0.1 kGy and 9.9 ± 0.1 kGy. These are marked as HEEB 8 kGy and 10 kGy samples.

A non-treated sample was used as a control. After treatment, the samples were shipped to VTT (Espoo, Finland) for sensory and chemical analysis. The samples were analyzed immediately after shipping (0 months) and after a three-month storage at 17 °C in dark conditions (3 months).

2.2. Microbiological safety assessment

The untreated and treated samples were tested for the survival of microbiological organisms to ensure their safety prior to sensory evaluation. The microbiological quality of untreated flax seeds was safe and HEEB treatment substantially further decreased the microbiological load. Thus, the LEEB treated flax seeds were not analyzed (Supplementary Table S2). All analyses were performed according to the following normative methods: Total plate count: DIN EN ISO 4833-2:2014-05; Coagulase-positive *Staphylococci*: DIN EN ISO 6888-1/A2:2017-04; *Bacillus cereus*: DIN EN ISO 7932:2004-03; Coliforms DIN ISO 16649-2:2009-12; mesophilic sulphite-reducing *Clostridia*: DIN 10103:1993/08; *Listeria monocytogenes*: Afnor AES 10/3-09/00 Bio-Mérieux; *Salmonella* spp.: ISO 6579; yeasts and Moulds: ASU L 01.00-37:1997-12. Aerobic spore-forming bacteria were analyzed by heating the sample to 80 °C for 10 min to eliminate all vegetative bacteria. A complex culture medium (plate count agar) was then used to count all mesophilic spore-forming bacteria after cultivation for 3 days at 30 °C.

2.3. Sensory profiling

Sensory evaluation of the seed samples was carried out by a trained panel using generic descriptive analysis (Lawless & Heymann, 2010). The panel fulfilled the requirements of the ISO standards (ISO 8589:2007 and ISO 6658:2017). Panelists were VTT employees belonging to the company's food and beverage sensory panel. The Ethical Committee of VTT assessed the evaluation protocol and gave their recommendations. In accordance with these recommendations, prior informed consent was obtained from all panelists. The panelists also gave their consent for the collection of necessary individual information in accordance with the EU General Data Protection Regulation GDPR (2016/679). The sensory lexicons for both seeds were developed in a one-hour consensus session with a 5-member mini panel. The sensory descriptors were further refined in a one-hour ballot training session for all the 14 panelists from which 11 assessors participated in each main evaluation (both treated seeds at 0- and 3-month time points). The attributes for the pumpkin seeds were odor intensity, seed crispness, seed toughness, nutty flavor, bitterness, rancidity, total flavor intensity and total off-flavor intensity. The attributes for flax seeds were odor intensity, seed hardness, grain-like flavor, pea-like flavor, bitterness, rancidity, total flavor intensity and total off-flavor intensity. At the three-month evaluation timepoint, the "off-flavor intensity" was changed to "possible other flavor intensity" in the additional panel training session to limit redundancy with the rancidity attribute. Additionally, the term "sharp aftertaste" was added to the flax seed sensory lexicon. The attribute intensities were rated on 0–10 linear scales that were verbally anchored from both ends (0 = attribute not present, 10 = attribute perceived as very intense). The samples were coded with three-digit numbers and served in random order in two replicate sessions. The assessors were instructed to cleanse their palate between the samples by drinking water and by brushing their teeth with the provided toothbrush. They were instructed to spit the samples out after tasting them. The data was collected using Compusense Five version 5.6 (Compusense Inc., Guelph, Canada).

2.4. Chemical analyses

2.4.1. Fatty acid and polar component profiling by GC–MS

Lipid bound fatty acids, free fatty acids and sterols were determined from ground pumpkin and flax seeds by using successively transesterification and trimethylsilylation (Seppänen-Laakso, Nygren, & Rischer, 2020). The seed samples were ground with a Bamix mixer and three replicate 5 mg aliquots were weighed from each control or treated sample into Eppendorf tubes. The samples were spiked with triheptadecanoate and heptadecanoic acid (198 and 138 μg, respectively) and mixed with 200 μL 0.9% sodium chloride solution. Lipids were extracted with chloroform:methanol (ratio 2:1; 800 μL) by mixing with a Retsch homogenizator (5 min, 20 Hz). After 30 min extraction time the samples were centrifuged (5 min, 10,000 RPM). The lower organic phase was separated and evaporated into dryness under N_2 flow. The residue was dissolved into petroleum ether (bp. 40–60 °C; 700 μL) and transesterified with 0.5 M NaOMe solution in methanol by boiling at 45 °C for 5 min. The samples were acidified with 15% $NaHSO_4$ (500 μL) and the lipid compounds were extracted into petroleum ether. The ether was separated and evaporated to dryness and the residue was dissolved into 1000 μL hexane for fatty acid analyses.

For the analysis of free fatty acids, sterols, hydroxy fatty acids, cycloartenol and tocopherol, 100 μL aliquot was taken from the transesterified sample and spiked with 1 μg cholesterol (IS, Sigma-Aldrich). The sample was evaporated and dissolved into dichloromethane (75 μL) and the polar compounds were trimethylsilylated with 25 μL of MSTFA (MSTFA - N-Methyl-N-(trimethylsilyl) trifluoroacetamide; Sigma-Aldrich) at 80 °C for 20 min. The fatty acid methyl esters were analyzed on an FFAP column (25 m × 200 μm × 0.3 μm) and the trimethylsilylated polar compounds on an NB-5 (30 m × 250 μm × 0.25 μm) silica capillary column by using Agilent GC–MS.

2.4.2. Volatile compounds by Headspace-SPME-GC–MS

The ground pumpkin and flax seed samples were weighed (0.500 g; 3 replicates) into 20 mL headspace bottles, spiked with 1.1 μg internal standard (heptanal) and mixed with 4 mL of water saturated with NaCl. The samples were incubated at 60 °C for 1 min. The SPME fiber (Supelco DVB/Car/PDMS Stableflex 2 cm) was exposed in the sample headspace for extraction and desorption times of 30 min and 480 s, respectively. The runs were performed on Agilent GC–MS equipped with an FFAP (25 m × 200 μm × 0.3 μm) column and by using a mass range of 30–400 *m/z*.

Quantification was based on the use of an internal standard and calibration curves were run for selected compounds representing different compound groups. Identification of the volatile compounds was based on the NIST08 MS library.

2.4.3. Analysis of thiamine by UPLC-MS/MS

The samples (200 mg) were spiked with 2.5 μg of internal standard (Thiamine-(4-methyl)-13C-thiazol-5-yl-13C) hydrochloride; Sigma Aldrich) and boiled in a water bath with 5 mL of 0.1% HCl for 30 min. After hydrolysis and cooling the volumes of the samples were rechecked and the solutions were filtrated. The analyses were performed by a UPLC–MS/MS system consisting of an Acquity UPLC (Waters, Milford, MA) coupled to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters, Milford, MA). An analytical column, Waters HSS T3 column (1.8 μm, 2.1 × 100 mm) was kept at 30 °C. The mobile phases were 0.1% formic acid in water (A) and in methanol (B), and the gradient was from 99% A to 70% A in 1.47 min and back to 99% A from 1.61 to 4.00 min with a flow rate of 0.400 mL/min. The MS runs were performed in ESI positive ion mode and thiamine content was determined on UPLC-MS by

MRM technique following transition m/z 265.1 → 122.0 (m/z 269.3 → 122.0 for labelled thiamine). The capillary voltage was 2.5 kV and cone voltage 40 V. The desolvation gas flow was 750 L/h and MS source and desolvation temperatures were 150 and 500 °C, respectively.

2.4.4. Phenolic acids by UPLC-DAD-QTof-MS

Ground pumpkin and flax seed materials (25 mg; 3 replicates) were extracted with petroleum ether to remove fat. Total phenolic acid contents were determined then after basic hydrolysis with 2 M NaOH (1.1 mL; 16 h in dark). The samples were acidified with 5 M HCl (700 μL) and extracted 3 times with 2 mL ethyl acetate. The combined ethyl acetate extracts were evaporated into dryness under N_2 flow and the residue was dissolved into 0.5 mL 50% MeOH. The samples were filtrated (0.2 μm) and run in ESI negative ion mode on a UPLC-DAD-QTof-MS (Waters) equipped with an Acquity BEH C18 column (100 mm × 2.1 mm; 1.7 μm). The solvent system consisted of 0.1% formic acid (A) and acetonitrile (B) and the gradient was from 5% B to 90% B in 9 min (10% at 1.13 min, 40% 5.67 min) after which there was a 3 min re-equilibrium period. The flow rate was 0.43 mL/min, the column temperature 30 °C and the injection volume 2 μL. Identification and quantification of the compounds was based on the UV-absorption and mass spectral data of reference substances and on calibration curves for external standards, respectively.

2.5. Statistical analysis

Sensory data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics, Ver. 24 (IBM Corporation, New York, USA). A mixed assessor model was used with samples as the fixed factor and assessors and sessions as random factors. The model included all main effects and the sample × assessor interaction effect. Statistically significant differences ($p < 0.05$) between the samples were identified by Tukey's HSD test. The panel agreement, discrimination and repeatability were investigated with Panelcheck 1.4.2 (Nofima, Tromsø, Norway) following the suggested workflow (Naes, Brockhoff, & Tomic, 2010). A principal component analysis model of the consensus sensory data (averaged over duplicate sessions and assessors) combining the two time points of each seed was built with the Unscrambler Ver. 10.4 (Camo Analytics, Oslo, Norway).

The chemical data were analyzed with a one-way ANOVA with Tukey's HSD test or with Tamhane T2 test in the cases the variances were not equal. The fatty acid data was likewise modeled with a principal component analysis model where all compound contents were normalized. The limit for statistical significance was set to $p < 0.05$ in all analyses.

For predicting the sensory perception of seeds from the contents of volatile compounds (VOC), sensory and VOC data were related by partial least squares (PLS) regression using the Unscrambler Ver. 10.4 (Camo Analytics, Oslo, Norway). The intensities of the sensory attributes with statistically significant differences between treatments (Y-data) were predicted from the contents of volatile compounds of the seeds that are linked to lipid oxidation (predictors, X-data) (Dunkel et al., 2014). Both Y- and X-data were normalized before the analysis. The model was validated by cross-validation.

3. Results and discussion

The aim of the study was to assess the changes in chemical and sensory properties after low energy (LEEB) and high energy electron beam (HEEB) treatments of pumpkin and flax seeds. The evaluation was performed immediately after the treatments, and after three months of storage.

3.1. Microbiological quality of the samples

The microbiological load in untreated control was within allowed limits set by the German Society for Hygiene and Microbiology (DGHM, Deutsche Gesellschaft für Hygiene und Mikrobiologie), and both LEEB and HEEB treatments in the applied doses reduced the microbiological load (Supplementary Tables S1 and S2). The reduction in the microbiological load was as expected based on the published literature (Gryczka, Migdał, & Bułka, 2018; Smith et al., 2017). Thus, the sensory evaluations could be performed. The more in-depth examination on the survivability of pathogens and viruses in the applied LEEB and HEEB treatments has been published elsewhere (Butot, Galbusera, Putallaz, & Zuber, 2021; Henz et al., 2020).

3.2. Sensory profiles for pumpkin seeds

The sensory properties of the pumpkin seeds differed in six attributes (Table 1, Figure S1). Overall, the LEEB treated samples had no statistically significant differences to non-treated seeds in most sensory attributes, while HEEB treated samples differed from both LEEB and non-treated samples in multiple sensory attributes. The degree of changes between treated samples seemed to be higher in samples receiving 10 kGy in comparison to samples receiving 8 kGy. The major difference was seen in the degree of rancidity: the observed rancidity in the HEEB treated samples was doubled in comparison to the control and LEEB treated samples. Additionally, the HEEB treated samples had a more intense overall odor and less nutty flavor. Further, the sensory panel described the HEEB samples as pungent and cardboard-like.

After a three-month storage period a similar trend was seen in observed differences. Rancidity increased in all samples. However, only HEEB treated samples were described to have a rancid odor and a sharp aftertaste in the open comments of the panelists. On the other hand, the untreated control and LEEB treated samples had no statistically significant differences in any of the eight sensory attributes.

3.3. Sensory profiles for flax seeds

The sensory properties of the flax seeds differed in four attributes (Table 2, Figure S2). Comparable to the pumpkin seeds, the HEEB treated flax seeds differed from the untreated control. The 10 kGy HEEB samples were more intense in odor and total flavor as well as rancidity. The HEEB samples were also described in open comments to be pungent and fish oil-like. The LEEB samples and the control sample had no statistically significant differences in any sensory attribute. The 10 kGy HEEB samples continued to differ the most from the four other treatments after a three-month storage period due to their more intense total odor and rancidity.

Overall, the magnitude of the differences between the control and LEEB samples were comparable to or even smaller than the differences between the two time points (Supplementary materials, Figs. S3 and S4). This indicates that LEEB treatment causes only small sensory differences to the samples.

3.4. Comparison of published irradiation effects on the sensory profiles of seeds

In this study the effects of HEEB and LEEB treatments on the sensory and chemical properties of pumpkin and flax seeds were compared. This was a novel approach as especially the effects of different irradiation treatments (γ, electron beam) on the sensory properties of seeds have been studied only in a few publications. Previous research has studied HEEB treatment of almonds at 10 MeV (Sánchez-Bel et al., 2008) and 5 MeV (Lanza et al., 2013) as well as γ-irradiated pecan kernels (Taipina, Lamardo, Rodas, & del Mastro, 2009), cashew nuts (Mexis & Kontominas, 2009b), hazelnuts (Koç Güler, Bostan, & Çon, 2017), almond kernels (Mexis, Badeka, Chouliara, Riganakos, & Kontominas, 2009) as well as shelled peanuts and pistachio nuts (Mexis & Kontominas, 2009a). The sensory methodology compared to the present study was different as, apart from Lanza et al. (2013), these studies did not use descriptive

Table 1

Sensory profiling of the pumpkin seeds (n = 2x11) at 0 month and 3 month time points. The table includes the mean intensities (and standard deviations) of the 8 evaluated attributes. Statistically significant differences between treatments ($p < 0.05$) are marked with different letters a-c on each row.

	Sample/attribute	F test p value (partial η^2)		Control			LEEB 8 kGy			LEEB 10 kGY			HEEB 8 kGy			HEEB 10 kGy		
0 months	Total odor intensity *	0.026	(0.24)	3.4	(1.9)	b	3.7	(1.9)	ab	4.1	(2.2)	ab	4.5	(2.5)	a	4.2	(2.0)	ab
	Crispness	0.386	(0.10)	5.8	(2.0)		5.6	(1.6)		5.4	(1.9)		5.4	(1.8)		5.6	(1.6)	
	Toughness	0.170	(0.14)	4.5	(2.2)		3.9	(2.0)		3.9	(2.1)		4.0	(2.1)		4.5	(2.3)	
	Nutty flavor intensity *	<0.001	(0.51)	6.3	(1.6)	a	5.5	(1.8)	ab	5.0	(1.8)	bc	4.2	(2.2)	cd	3.7	(1.8)	d
	Bitterness *	0.001	(0.36)	3.0	(1.7)	c	3.4	(1.8)	abc	3.1	(1.5)	bc	4.2	(2.0)	ab	4.3	(2.1)	a
	Rancidity *	<0.001	(0.54)	1.1	(1.4)	b	1.6	(1.2)	b	1.6	(1.5)	b	3.2	(2.2)	a	3.4	(2.5)	a
	Total flavor intensity *	0.006	(0.30)	5.5	(1.6)	ab	5.6	(1.5)	ab	5.2	(1.7)	b	6.0	(1.4)	a	6.3	(1.5)	a
	Total off-flavor intensity *	<0.001	(0.70)	1.2	(1.3)	b	1.8	(1.3)	b	1.4	(1.3)	b	4.1	(2.2)	a	4.3	(2.3)	a
3 months	Total odor intensity*	0.005	(0.33)	5.4	(1.5)	b	5.4	(1.6)	b	5.2	(1.5)	b	5.7	(1.6)	ab	6.3	(1.1)	a
	Crispness	0.331	(0.12)	5.9	(1.2)		5.9	(1.3)		5.9	(1.1)		6.1	(1.2)		6.5	(0.9)	
	Toughness	0.419	(0.10)	5.2	(1.8)		5.3	(1.7)		5.4	(1.7)		5.3	(1.5)		4.9	(1.8)	
	Nutty flavor intensity	0.081	(0.20)	5.8	(1.6)		5.1	(1.9)		5.3	(1.8)		4.6	(2.2)		4.9	(2.1)	
	Rancidity*	<0.001	(0.55)	2.2	(1.8)	b	2.4	(1.9)	b	2.7	(2.3)	b	4.2	(2.5)	a	5.0	(2.4)	a
	Bitterness*	0.005	(0.33)	3.5	(1.7)	ab	2.9	(1.0)	b	3.1	(1.5)	b	4.1	(1.9)	a	3.8	(1.8)	ab
	Total flavor intensity*	<0.001	(0.49)	5.9	(1.2)	bc	5.5	(1.7)	c	5.7	(1.5)	c	6.4	(1.6)	ab	6.7	(1.2)	a
	Other flavor intensity*	0.010	(0.30)	1.1	(1.0)	bcd	0.8	(1.1)	cd	1.1	(0.9)	c	1.8	(1.6)	ab	2.0	(1.9)	a

Table 2

Sensory profiling of the flax seeds (n = 2x11) at 0 month and 3 month time points. The table includes the mean intensities (and standard deviations) of the evaluated attributes. Statistically significant differences between treatments ($p < 0.05$) are marked with different letters a-c on each row.

	Sample/attribute	F test p value (partial η^2)		Control			LEEB 8 kGy			LEEB 10 kGY			HEEB 8 kGy			HEEB 10 kGy		
0 months	Total odor intensity *	0.016	(0.26)	2.3	(1.7)	b	2.9	(2.0)	b	2.8	(1.7)	b	2.9	(2.2)	b	3.9	(2.3)	a
	Hardness	0.23	(0.13)	6.6	(1.3)		7.0	(1.1)		6.7	(1.3)		7.1	(1.4)		6.8	(1.3)	
	Grain-like flavor	0.14	(0.16)	3.8	(1.9)		3.1	(2.1)		3.6	(1.8)		3.2	(2.5)		2.7	(2.2)	
	Pea-like flavor	0.09	(0.18)	4.3	(2.0)		4.7	(2.5)		4.6	(1.9)		3.9	(2.1)		3.5	(2.4)	
	Bitterness	0.14	(0.16)	2.8	(1.7)		3.3	(1.8)		3.6	(1.8)		3.7	(1.9)		4.0	(2.1)	
	Rancidity *	<0.001	(0.54)	1.2	(1.2)	b	1.5	(1.3)	b	1.6	(1.4)	b	2.9	(2.2)	a	3.8	(2.5)	a
	Total flavor intensity *	0.004	(0.31)	5.1	(1.6)	c	5.3	(1.8)	bc	5.4	(1.5)	bc	5.9	(1.8)	ab	6.1	(2.0)	a
	Total off-flavor intensity *	<0.001	(0.57)	1.1	(1.1)	b	1.5	(1.3)	b	1.5	(1.3)	b	3.0	(2.4)	a	3.7	(2.4)	a
3 months	Total odor intensity*	<0.001	(0.48)	3.5	(1.4)	b	4.2	(1.8)	ab	3.7	(1.2)	b	4.5	(1.6)	a	4.9	(1.6)	a
	Hardness	0.88	(0.03)	6.8	(1.2)		6.9	(0.9)		7.0	(1.2)		6.8	(0.7)		6.8	(0.7)	
	Grain-like flavor	0.35	(0.11)	5.1	(1.7)		4.9	(1.8)		4.5	(1.8)		4.4	(2.1)		4.3	(2.1)	
	Pea-like flavor	0.64	(0.66)	4.6	(2.0)		4.6	(1.7)		4.5	(2.0)		4.1	(2.1)		4.0	(1.9)	
	Rancidity*	0.000	(0.55)	1.9	(1.8)	c	2.2	(1.8)	bc	3.3	(2.3)	b	3.2	(2.5)	b	4.9	(2.6)	a
	Bitterness	0.30	(0.13)	2.6	(1.7)		2.4	(1.1)		2.8	(1.4)		2.9	(1.4)		3.4	(1.7)	
	Total flavor intensity*	0.006	(0.32)	4.8	(1.5)	b	5.0	(1.6)	ab	5.3	(1.6)	ab	5.5	(1.8)	ab	6.0	(2.0)	a
	Sharp aftertaste*	0.044	(0.23)	1.8	(1.4)	a	1.8	(1.0)	a	2.2	(1.5)	a	2.5	(2.1)	a	2.8	(1.8)	a
	Other flavor intensity	0.062	(0.22)	0.7	(1.0)		1.0	(1.2)		1.0	(1.2)		1.1	(1.2)		1.4	(2.1)	

intensity scales but a 5-point quality scale (Sánchez-Bel et al., 2008), a 9-point difference from control scale (Taipina et al., 2009), or a 9-point hedonic scale.

In almonds, the untreated, 3 kGy and 7 kGy dose 10 MeV HEEB-treated samples had no statistically significant differences. In contrast, the 10 kGy samples had a lower quality in sweetness and color but especially in rancidity and overall quality (Sánchez-Bel et al., 2008). With 1.5 kGy dose at 5 MeV the sensory profiles of irradiated almonds were otherwise similar to control, but for peeled almond flour the irradiated samples had a more intense off-odor (Lanza et al., 2013). These observations are in line with the present study with pumpkin seeds and flax seeds that the 10 kGy samples differed from untreated samples. However, it seems that whole almonds were less susceptible to changes than pumpkin and flax seeds as the 7 kGy samples still had only minor differences to control compared to increased rancidity in the 8 kGy HEEB samples in the present study despite the two-fold difference in electron beam energy level (10 MeV and 5 MeV).

In γ-irradiated pecan kernels the untreated and 1 kGy samples were similar; 3 kGy dose was statistically significantly different with the largest difference of 2.2 units from untreated samples in flavor (Taipina et al., 2009). The difference in doses was more drastic in cashew nuts where up to 1.5 kGy there were minimal changes but the odor and taste in the 3–7 kGy doses were below the acceptable limit (Mexis & Kontominas, 2009b). For almonds, peanuts and pistachio nuts, the taste acceptability was still good at 3 kGy but below the allowed limit at 5 kGy and 7 kGy (Mexis & Kontominas, 2009a; Mexis et al., 2009).

Altogether, these reports indicate that HEEB and γ-irradiation of lipid-rich seeds and nuts often cause moderate changes in sensory quality already at ≤5 kGy doses, which is still a considerably milder treatment than the chosen 8 kGy and 10 kGy doses in the present study. Thus, it is interesting that LEEB treated seeds were statistically similar in their sensory profiles to the control especially after the 3-month storage. Lower doses than studied here are most probably used in the future treatment applications. Consequently, the differences between the non-treated and LEEB-treated samples will most likely be minor.

3.5. Chemical analyses for pumpkin and flax seeds

The major fatty acids in pumpkin seeds (fat content 34–36%, Table S3) were linoleic (C18:2n-6, 42%), oleic (C18:1n9, 32%) and palmitic acids (C16:0, 14%). Due to the high linoleic acid content the fat is susceptible to oxidation, which can further deteriorate the sensory and nutritional quality of the oil in seeds. This can be, for example, perceived as rancidity. Here the relative amounts of saturated fatty acids in pumpkin seeds were stable regardless of different treatments or 3 months of storage. LEEB treatment at 10 kGy and HEEB treatment at 8 and 10 kGy slightly decreased the proportion of linoleic acid (n-6 PUFA, Fig. 1A, Table S3) and caused a simultaneous increase in oleic acid (MUFA). However, the proportion of linoleic acid remained stable during 3 months' storage time despite the different treatments. Thus, the

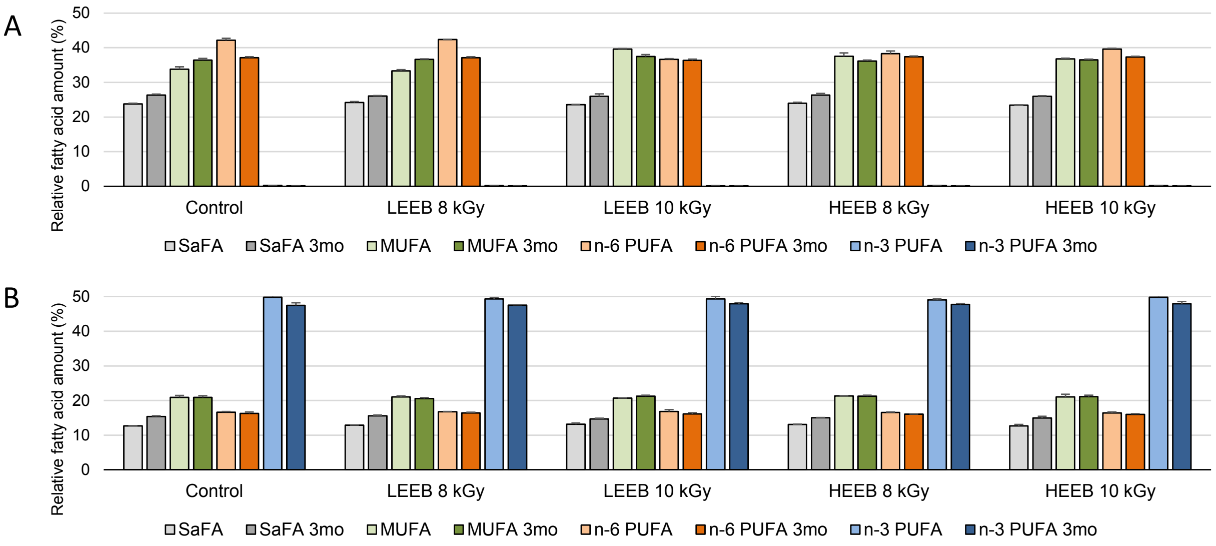

Fig. 1. Relative amounts of fatty acids in pumpkin (A) and flax seeds (B) at the baseline and after 3 months' storage. The errors bars are standard deviations of 3 replicates. In pumpkin seed there were slight significant decrease ($p < 0.05$) in n-6 polyunsaturated fatty acids in LEEB 10 kGY and HEEB 8 kGy groups compared to control group. However, no significant differences between treatments were anymore observed in pumpkin seeds after 3 months' storage. In flax seeds, there were no statistically significant ($p < 0.05$) differences in fatty acids between treatments either in the beginning or at 3 months' follow-up. Abbreviations: SaFa: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

storage of seeds had overall higher impact on fatty acid contents than the electron beam treatment.

The flax seeds (fat content 28–31%, Table S3) were especially rich (50%) in α-linolenic acid (C18:3n3), the plant-based n-3 essential fatty acid, while the level of linoleic acid (C18:2n6) was 17%. Thus, the total proportion of polyunsaturated fatty acids from all fatty acids was nearly 70% giving rise to possible oxidative degradation. In case of edible oils, the fatty acid composition is considered a strong indicative factor predicting oxidation stability, especially at the early oxidation stage (Yun & Surh, 2012). In the present study, the changes in the fatty acid composition of ground flax and pumpkin seeds were marginal. However, there was a slight trend towards higher saturated and lower polyunsaturated fatty acid content as well as a higher level of free fatty acids. In *Canavalia* seeds, the electron beam irradiation increased the proportion of saturated fatty acids and decreased that of unsaturated fatty acids when increasing the dose of irradiation from 2.5 to 15 kGy. However, the changes were dependent also on the extraction method and plant species (Supriya, Sridhar, Nareshkumar, & Ganesh, 2012).

Vitamin E is a natural antioxidant present in vegetable oils, nuts and seeds. For soybeans, δ-tocopherol was most sensitive to HEEB (10 MeV) treatment, however all tocopherol contents decreased at 9.2 kGy and higher doses in all three soybean genotypes (Kumar et al., 2017). γ-irradiation with doses of 5 to 10 kGy have shown to induce oxidation and loss of tocopherols in peanuts and γ-tocopherol was affected at the greatest extent (de Camargo & de Vieira, 2012). In this study, GC–MS analyses showed that γ-tocopherol (and γ-tocopherol) was predominant over α-tocopherol in both pumpkin and flax seeds (Supplementary Table S4), which has been found also earlier in selected seeds (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). Other minor components included sterols and their triterpenoid precursors: squalene in pumpkin seeds and cycloartenol in flax seeds. In our analysis, no decrease in tocopherol content or changes in the levels of other minor components were observed in the treated seeds.

Phenolic acids have been associated with sensory qualities of food, like flavor, astringency, and hardness. Majority of phenolic acids are bound through ester, ether or acetal bonds to other plant constituents and a basic hydrolysis is needed to free them for further analysis (Robbins, 2003). In this study, the level of total phenolic acids was approximately 39–50 μg/g in pumpkin seeds and 578–744 μg/g in flax seed (Supplementary Figure S5). Caffeic and p-coumaric acids as their hexosides were the major components in pumpkin seeds, while ferulic acid was the predominant phenolic acid in flax seeds. In baby carrots, γ-irradiation at doses of 0.5 and 1 kGy was decreasing the levels of phenolics by 10% and 20%, respectively (Hirashima, Fabbri, Sagretti, Nunes, Galvao, Lanfer-marquez, & Sabato, 2013). However, several papers have reported opposite results. An increase in total phenolics after γ-irradiation was found in *Nigella sativa* seeds (Khattak & Simpson, 2008) and an increase in polyphenols was reported in Fuzhuan brick-tea after electron beam irradiation (Zhang et al., 2020). On the other hand, no changes in total phenolics were observed for HEEB treated (5 MeV) red pepper powder up to a received dose of 10 kGy (Woldemariam et al., 2021).

Seeds also contain several water-soluble vitamins, and they are a source of B-group vitamins such as thiamine (B1). Thiamine has a high sensitivity to radiation, and it has often been used as an indicator to evaluate the irradiation effects on vitamin content (Graham, Stevenson, & Stewart, 1998). On the other hand, a low dose γ-irradiation has reported to cause only a small decrease in thiamine content in two varieties of Brazilian beans (Villavicencio, Mancini-Filho, Delincée, & Bognár, 2000). The thiamine loss can be minimized by using low temperatures and oxygen-free atmosphere during irradiation (Duodu, Minnaar, & Taylor, 1999). Furthermore, thermal treatment is reported to cause a more substantial loss of thiamine than a low dose irradiation (Kilcast, 1994).

The thiamine content in the control flax seeds was comparable to previously reported values (Katare, Saxena, Agrawal, & Prasad, 2012). The thiamine levels of treated flax seeds showed a significant decrease in LEEB treated samples and the decrease was more prominent in HEEB treated samples (Supplementary Fig. S5). Thiamine content of the pumpkin seeds was generally lower, and there were no statistically significant differences between samples. Due to the relatively low thiamine content the possible changes were not studied after 3 months of storage. However, further studies on the possible effects of the treatments on other radiation-sensitive vitamins, such as C, A, and E (Kilcast, 1994), would be needed.

The majority of volatile compounds identified in pumpkin seeds were alcohols and aldehydes. Only a few aldehydes were detected: 3-methyl-2-butenal, hexanal and benzaldehyde being the main compounds (Tables S6 and S7; Fig. 2A). In the post-treatment analysis, a significantly higher aldehyde content was observed only in LEEB treated

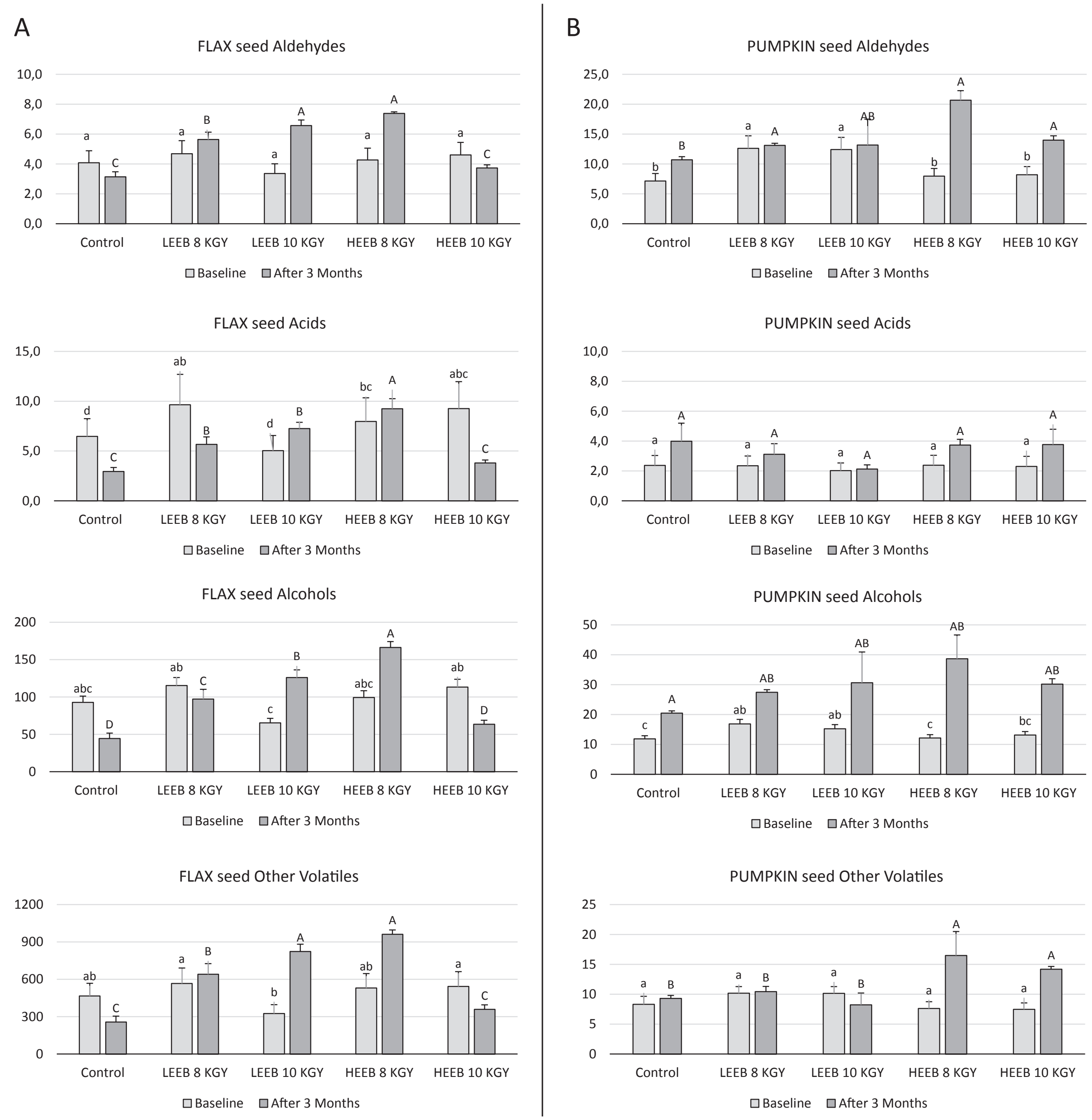

Fig. 2. Volatile compounds in pumpkin (A) and flax seeds (B) analysed by SPME-GC–MS in the beginning and after 3 months' storage. Contents are shown as mean (ug/g) with error bars showing the standard deviation (n = 3). Different letters above the columns indicate statistically significant differences ($p < 0.05$).

samples, whilst after 3 months of storage the aldehyde content compared to the control group was significantly higher in all treatment groups except in LEEB 10 kGy. The increasing trend was also clearly seen in the group of alcohols, where 3-methyl-1-butanol and 1-hexanol were the most abundant (Fig. 2A). Aldehydes, ketones, and alcohols are typical oxidation products of lipids, mainly PUFAs, indicating that the electron beam treatments have an enhancing effect on lipid peroxidation. Increased amounts of volatile aldehydes, ketones and alcohols have been shown earlier in γ-irradiated cashew nuts (Mexis & Kontominas, 2009b) as well as in peanut and pistachio nuts (Mexis & Kontominas, 2009a). Most aldehydes, alcohols and volatile acids were associated with treatment-related sensory changes, such as rancidity and bitterness (Fig. 3A).

In flax seeds, the amount of aldehydes remained below 8 μg/g and hexanal was identified as the main component. After a 3-month storage, significantly increased levels of aldehydes, alcohols and acids were detected in LEEB and HEEB 8 kGy treated samples in comparison to the control (Tables S8 and S9, Fig. 3B). Similarly, increased levels of several other volatile compounds such as 2-butanone, the predominant species (200–700 μg/g) in "other volatiles" group, were observed in the LEEB and HEEB treated samples. Generally, the volatile compound content seemed to increase up to the HEEB 8 kGy dose, while the HEEB 10 kGy group deviated from other treated samples. Contrary to the pumpkin seeds, the increased content of volatiles of the treated flax seeds was inversely correlated with the sensory changes (Fig. 3B). This is likely due to the different volatile profile of the HEEB 10 kGy samples.

4. Conclusions

Food safety is of utmost importance, but to answer the consumer demand for fresh and minimally processed foods, the food industry is constantly looking for new microbial reduction technologies that have minimal impact on the characteristics of food and food ingredients. The aim of electron beam treatment is to preserve food quality whilst

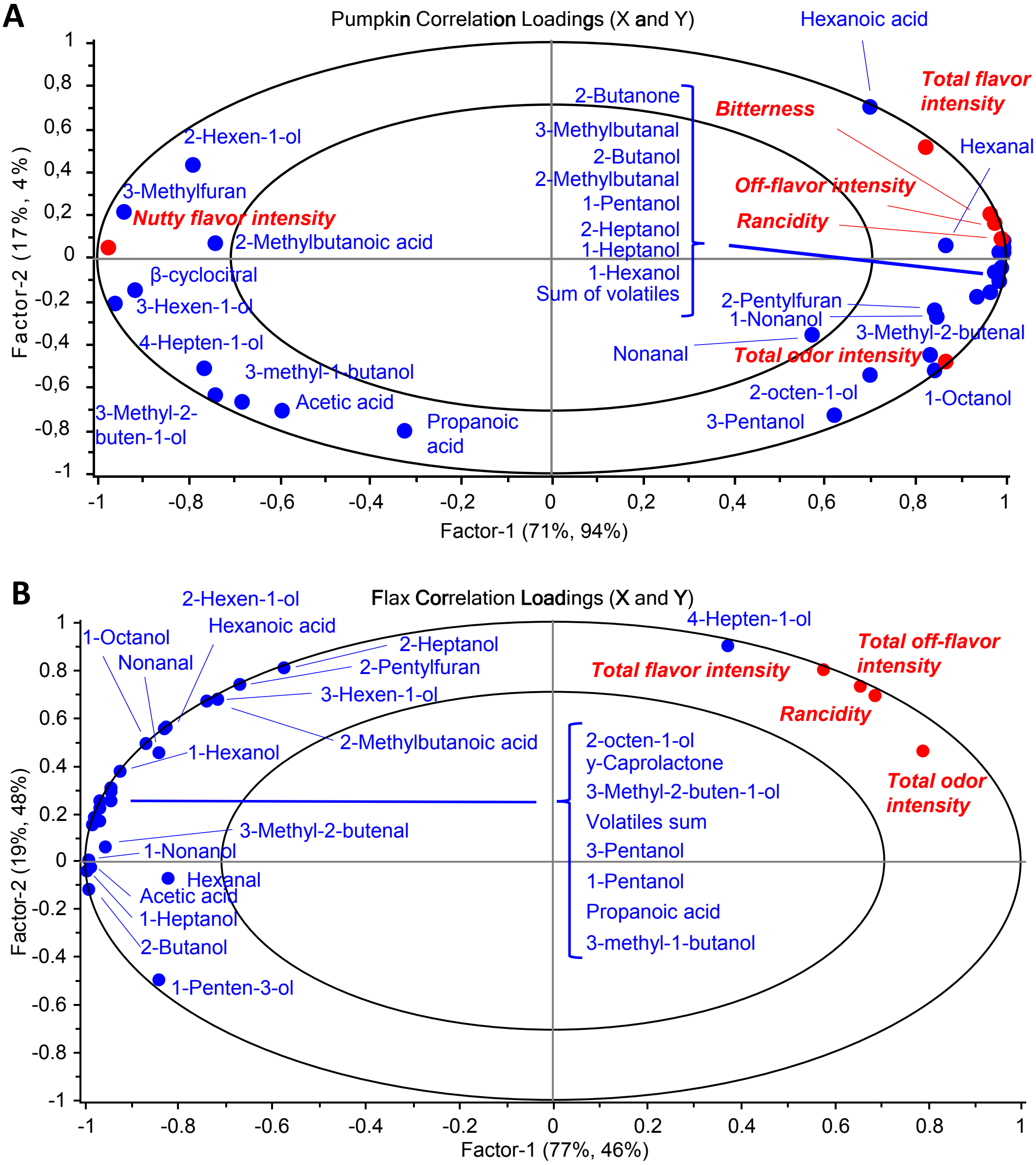

Fig. 3. Partial least squares regression model of (A) the pumpkin volatiles (blue) and (B) the flax volatiles (blue) at baseline (0 months after treatment) explaining the sensory attributes (in red and in italics) with statistically significant differences.

reducing food borne pathogens to safe levels. This study assessed the effect of HEEB and LEEB treatments on the quality of pumpkin and flax seeds. Both treatments caused changes in the sensory profiles of seeds when compared to the non-treated seeds. The LEEB treated seeds were more similar to the non-treated seeds whereas HEEB treated seeds differed from both. It was observed that the differences were more prominent in higher received doses in both treatments. In chemical measurements, both HEEB and LEEB treatments increased the amounts of volatile aldehydes in the studied seeds. This was likely due to lipid degradation, which was greater in HEEB treated samples. The observed differences in sensory and chemical properties of the treated samples persisted after three months of storage. Thus, the study findings support the preservation of sensory and chemical properties of foods and food ingredients using electron beam technology. In conclusion, LEEB treated samples resembled non-treated seeds even with the highest acceptable dose (10 kGy).

CRediT authorship contribution statement

H. Aisala: Investigation, Formal analysis, Writing - original draft. **H. Nygren:** Investigation, Conceptualization, Formal analysis, Writing - review & editing. **T. Seppänen-Laakso:** Investigation, Formal analysis, Writing - review & editing. **R.-L. Heiniö:** Funding acquisition, Supervision, Conceptualization, Investigation, Writing - review & editing. **M. Kießling:** Resources, Formal analysis, Writing - review & editing. **K. Aganovic:** Funding acquisition, Conceptualization, Writing - review & editing. **A. Waser:** Resources, Writing - original draft. **H. Kotilainen:** Resources, Funding acquisition, Writing - review & editing. **A. Ritala:** Funding acquisition, Project administration, Supervision, Conceptualization, Writing - review & editing.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110575.

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