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ABSTRACT

Freezing process is usually used to extend the shelf life of raspberries, but these fruits are highly sensitive and encountered freeze damages. Furthermore, frozen raspberries are known to be responsible of foodborne diseases through the transmission of enteric viruses like Hepatitis A virus (HAV). Although the resistance of HAV to freezing is recognized, the impact of the freezing process on its persistence remains unclear. This research aims to provide a comprehensive understanding of the relationships between microscopic, macroscopic, and viral risk developments after freezing and during storage. Results show that the texture of raspberries, as well as drip loss, was altered during freezing and storage, likely due to cell perforation resulting from the formation and growth of ice crystals, as evidenced by X-ray microtomography images. Infectious HAV titers on raspberries were assessed over time using the Real-Time Cell Analysis assay, revealing a decrease in HAV persistence as storage temperatures increased.

Keywords: Freezing, Microstructure, X-Ray computed Microtomography, Raspberries, Hepatitis A virus, Persistence.

1. INTRODUCTION

Freezing is widely used for food preservation. Even if frozen food are handy for day-to-day life and to extend shelf life, foods can permanently be damaged by freezing with crystallization and recrystallization phenomena. Crystallization occurs with water phase transition, from liquid to solid, when the food temperature is lowered and multiple ice crystals are formed into the food matrix. The size distribution and location of ice crystals in frozen foods is called crystalline microstructure. This microstructure depends on the freezing rate so that fast freezing promotes small ice crystals formation evenly distributed into the food matrix whereas slow freezing leads to bigger ice crystals mainly in the extracellular spaces (Li et al., 2018). The crystalline microstructure initially defined during the freezing process is likely to evolve during frozen storage mainly due to recrystallization phenomena. Recrystallization is characterized by the size growth and shape changing of ice crystals in frozen foods. Small ice crystals having high surface energy are unstable, recrystallization enables to reduce this surface energy in order to reach a thermodynamic equilibrium. This phenomenon happens naturally in stored frozen food but is greatly enhanced by temperature fluctuations (Hartel, 2013).

Frozen food quality is highly related to the crystalline microstructure (Petzold & Aguilera, 2009). In plant based materials, ice crystal formation and growth can severely injured cell membranes and constituents

leading to a loss of turgescence, content, nutrients and texture (Li et al., 2018). Several studies have examined the effect of storage temperature and duration on quality attributes (texture, drip loss, etc.) of frozen fruits and vegetables (Bulut et al., 2018; Dawson et al., 2020; De Ancos et al., 2000; González et al., 2002; Häkkinen et al., 2000; Mullen et al., 2002; Sousa et al., 2007; Zhao & Takhar, 2017). Among plant based food, raspberries are commonly found in the frozen food industry. They are broadly used for culinary preparation such as sorbet, jam, cakes or yogurts but they are very perishable and difficult to preserve fresh. On one hand, freezing raspberries allows to delay their processing by increasing their shelf life (González et al., 2002). However, freezing can have negative impacts such as loss of texture, juiciness, redness and taste changes (Sousa et al., 2007) or loss in anthocyanin content along the storage (Lo Piccolo et al., 2020). Despite the large quantities of raspberries frozen throughout the world and the high sensitivity of the fruit to freezing damages, very few studies have been carried out on the quality of frozen raspberries. Moreover, to the best of our knowledge, no work has been reported on the impact of freezing and frozen storage temperatures on the microstructure of these products. However, relating quality changes to microstructure evolving requires characterizing both within the framework of the same study. 3D imaging techniques such as X-ray micro-computed tomography (X-ray µCT) are well suited to provide reliable data on the microstructure of frozen products. X-ray µCT has been recently used to study the microstructure of several frozen foods (Latil et al., 2022; Masselot et al., 2021a, 2021b; Mulot et al., 2019; Vicent et al., 2017, 2019; Zennoune et al., 2022). However, to the best of our knowledge, no study has been reported yet regarding the microstructure of frozen raspberries.

On a second hand, the freezing of raspberries allows long distance importation (González et al., 2002) but is also associated with safety concerns as they are known to be responsible of foodborne diseases with the transmission of enteric viruses such as Hepatitis A virus (HAV) (Bozkurt et al., 2021; Di Cola et al., 2021). Cultivated, harvested and frozen in developing countries where the HAV is endemic, contaminated raspberries are imported in developed countries. In these countries, the HAV is an emerging concern for food safety because the seroprevalence of the population is low (Hu et al., 2020). HAV infections occur mainly by the faecal-oral route by the consumption of contaminated food or water (Hu et al., 2020) and 2 to 7% of worldwide HAV outbreaks are due to HAV contaminated food ingestion (Randazzo & Sánchez, 2020). Even though HAV is known to resist to freezing process (Di Cola et al., 2021), their survival capacity on frozen berries is poorly documented despite they are frequently associated to food-borne outbreaks related to such foodstuff. Moreover, the impacts of the freezing process and the storage conditions on its persistence are not established yet.

This work tends to get a deep insight of the links between microscopic, macroscopic and viral risk evolutions after freezing and during storage by studying microstructural changes using X-Ray µCT, quality changes and infectious HAV persistence of frozen raspberries submitted to different storage conditions.

2. MATERIALS AND METHODS

2.1. Raspberries sampling

Class I raspberries were purchased at a local store in one kilogram packages. For quality measurements, 25 ± 2.5 g of raspberries, corresponding to 4 - 7 fruits depending on their size and mass, were placed in zipped polyethylene bags. They were stored at 4°C for 24 h prior to freezing. Three replicates have been prepared to be analyzed at each storage duration after freezing.

For microstructure characterization, slices of raspberries were cutted carefully to excise several intact drupes and were placed in plastic straws (d = 6mm and h = 2.5cm) for further X-ray µCT image acquisition. For each condition, five replicates were prepared and put in zipped polyethylene bags before freezing.

2.2. Freezing and frozen storage conditions

Raspberry samples prepared for quality measurements were placed on an aluminium grid in a monolayer and frozen using an air blast freezer set at -30°C (Bonnet Biostore Turbo M, Bonnet Neve, EPTA, France)

corresponding to a freezing rate of 1°C/min. To achieve the same freezing rate for microstructure characterization, samples were frozen using a static horizontal freezer (Electrolux, ECM30132W, Stockholm, Sweden). For both samples types, calibrated type T thermocouples of about 0.2 mm diameter (0.01°C of precision) measured the ambient temperature and the core temperature of one representative sample for each grid. The temperatures were recorded as a function of time during the freezing process using a data logger system (34970A, Agilent HP, Santa Clara, USA) connected to a computer. The freezing process was considered accomplished when the sample core temperature reached -18°C.

The frozen samples were then immediately and equally dispatched in two horizontal freezers (Electrolux, ECM30132W, Stockholm, Sweden) set at −18°C and −5°C, respectively, and equipped with electronic temperature controllers (Eliwell WM961, Villeneuve La Garenne, France) as described by Ndoye & Alvarez, (2015). Within each freezer, samples were stored in a polystyrene box enclosed by cardboard to minimize temperature fluctuations. Calibrated type T thermocouples were positioned inside the polystyrene boxes to monitor both air and product temperatures over the entire storage duration. All samples, for both quality and microstructure analysis, were stored for a duration of 4 months at −18°C or −5°C. The analyses were made before and just after freezing as well as after 7, 14, 28, 56, 84 and 112 days of storage as shown in Fig. 1.

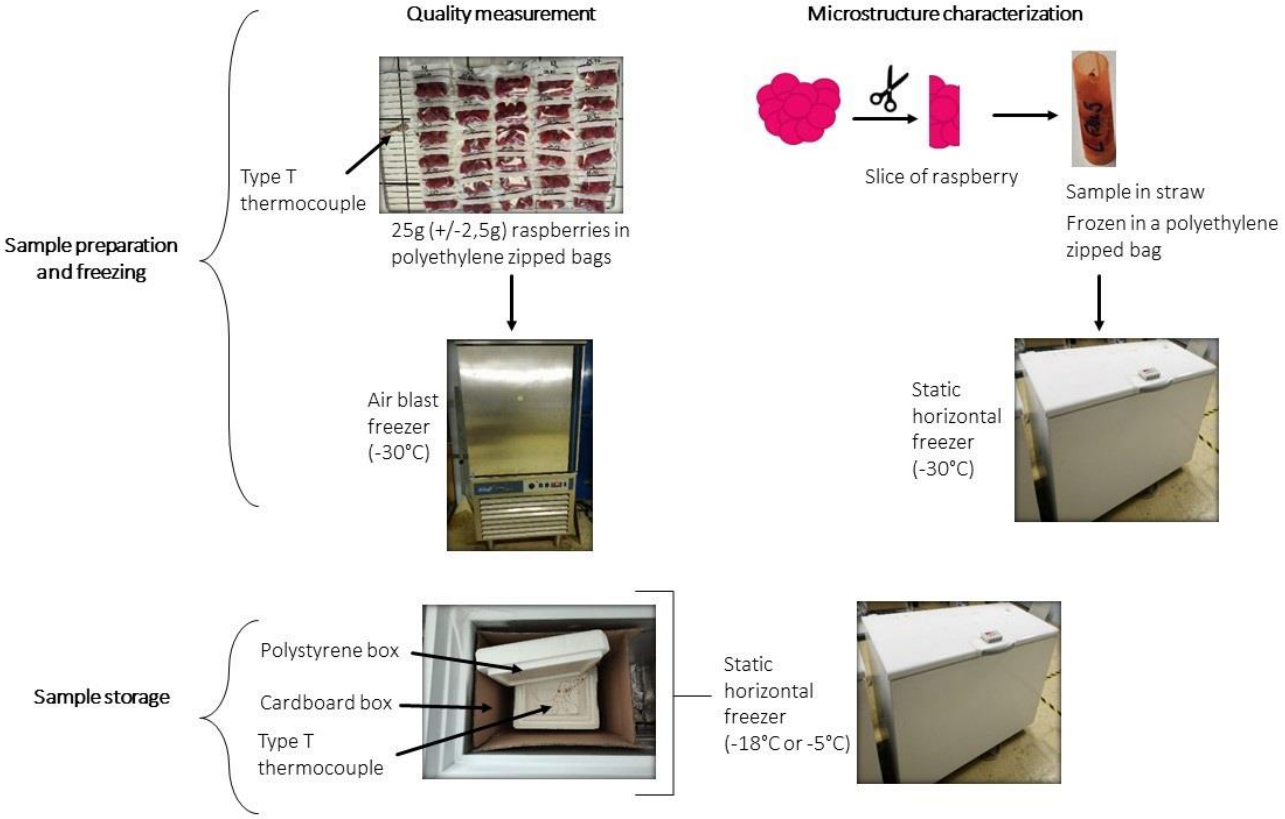

Figure 1: Freezing and frozen storage conditions workflow of raspberries samples for quality measurement and microstructure characterization.

These storage conditions and temperatures reproduce two thermal history scenarios. At –18 °C, the raspberries are stored at the regulatory temperature, whereas at –5 °C poor practices that can be encountered through the cold chain for temperature storage are reproduced. Indeed, at −5 °C, the temperature of the raspberries are close to the phase change plateau as illustrated in the freezing curve depicted in Fig. 2 and is likely to melt partially. Consequently, the storage temperature set at −5 °C may allow to accelerate moisture migration and ice redistribution (Vicent et al., 2019). In Fig.2, the temperature fluctuations observed after the freezing plateau could be explained by the heterogeneous structure (cell types and distribution) of the raspberry, that induce non-uniform heat transfers locally.

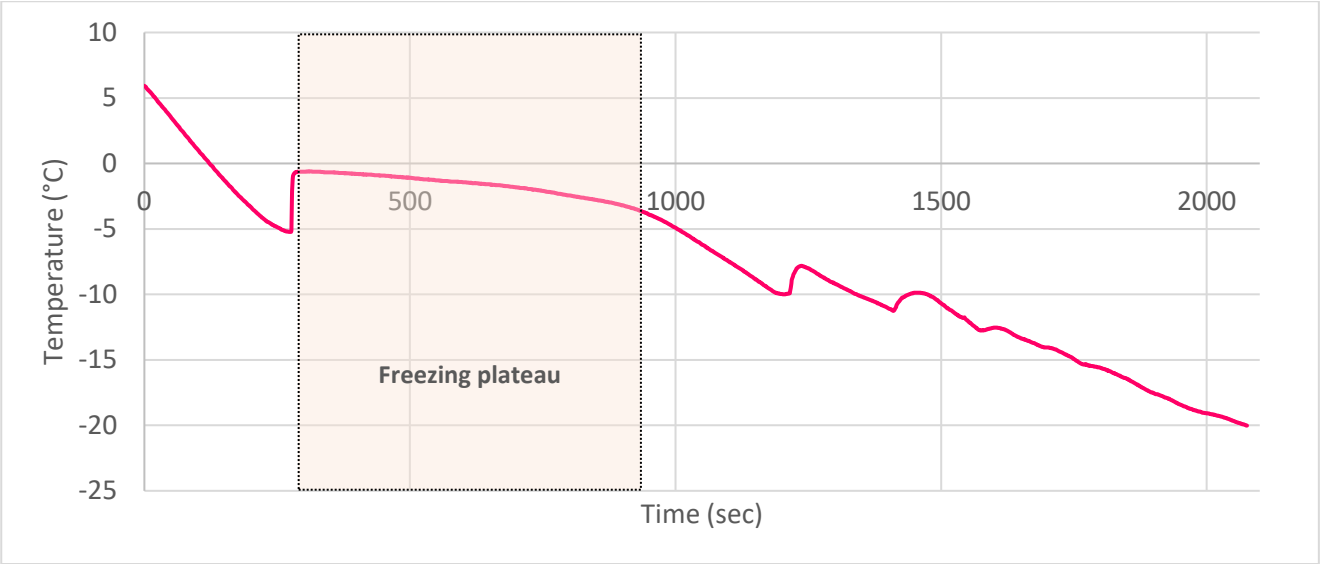

Figure 2: Freezing curve of 25g of raspberries in a zipped polyethylene bag.

2.3. Microstructure analysis by X-ray µCT

2.3.1. Image acquisition

Frozen raspberries samples were imaged using a high resolution X-ray µCT (DeskTom RX 130, RX Solution, Chavanod, France). Right before scanning, the frozen samples were inserted in a cooling stage consisting in a cylindrical double jacket box (d = 2cm and h = 3.5cm) containing a phase change material (PCM) at -20 °C, in order to maintain the sample temperature at frozen state during the entire scanning time. The PCM is a 25% w/w NaCl solution gelled with a 5% commercial gum blend (Germantown Premium IC Blend, Danisco). The cylindrical box was surrounded by 1 cm thickness polystyrene foam for insulation. The X-ray tube voltage was set at 53 kV. 896 projection images were captured with 0.2 s exposure time per projection and a voxel resolution of 8.4 µm. The required time for scanning the projection images over a 360° rotation with a step size of 0.4° was 14 min for each sample. After each scan, the frozen samples were immediately placed back in their storage freezer. Using this protocol, the same raspberry sample has been scanned all along the storage to follow up the microstructural changes.

2.3.2. Volume reconstruction

XAct 2 software (RX Solution, Chavanod, France) was used for 3D image reconstruction starting from a series of X-ray radiographs. The filtered back-projection algorithm (Feldkamp et al., 1984) was used with a high pass filter to reduce image blur in a projection along the acquisition direction. Image quality was improved thanks to noise filtering and phase contrast correction. Then the reconstructed images were converted to 16-bits resolution. Well-separated grey levels allow to clearly identify three phases (air, ice and unfrozen phase) in the frozen samples as shown by the µCT horizontal slice in Fig. 3. The black phase represents airspaces around the raspberry sample into the straw and air voids created during the storage. The light grey region represents the unfrozen phase (cryo-concentrated solutes and cell walls) while the dark grey voxels show the ice crystals.

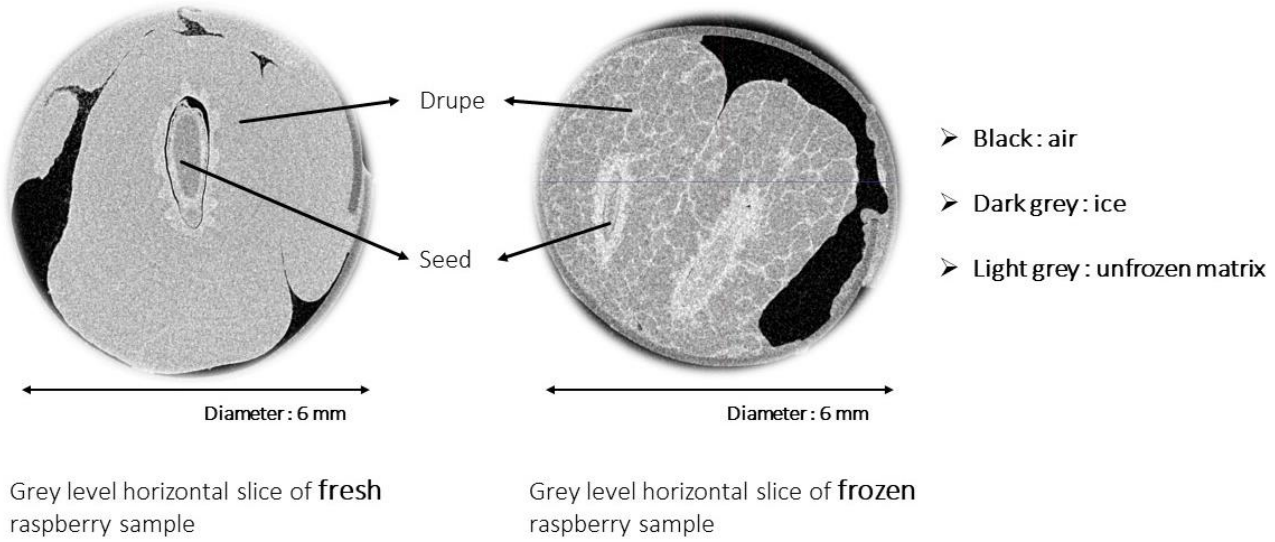

Figure 3: Grey scale level images of X-ray µCT scans of raspberries.

2.4. Quality analysis

2.4.1. Texture analysis

The analysis was performed after thawing the samples overnight at 4°C. The texture of the raspberry samples was studied by performing a compression test using the TA.XT. Plus texture analyzer (Stable Micro Systems, United Kingdom) equipped with the plate probe P100 (100 mm diameter stainless steel) and the software Exponent (Stable Micro Systems, United Kingdom). The Probe P100 was placed 30 mm above the texture analyser plate where the raspberries samples were placed in opened bags. The compression test was achieved by moving down the Probe P100, with a speed of 2 mm/sec by 27 mm to crush the raspberries. The results are expressed as the required force to crush 25 g of raspberries, in N.

2.4.2. Drip loss measurement

The samples were placed on absorbent paper at 4°C overnight for thawing and then weighed (Mettler AE 163, precision 10^{-4} g) the following day. The drip loss was calculated using Eq. (1):

$$DL\ (\%) = \frac{m_0 - m_f}{m_0} \times 100 \qquad \text{Eq. (1)}$$

where *DL* is the drip loss of the raspberries in %, m_0 is the mass of fresh raspberries in g and m_f is the mass of thawed raspberries in g.

2.5. Viral persistence analysis

2.5.1. Raspberry contamination

25 ± 2.5 g of raspberries were artificially inoculated with the culture adapted HM175/18f strain of HAV (VR-1402, ATCC™) by spreading 100 µL of viral stock in droplets. The raspberries were dried for an hour in a biosafety cabinet and then placed in zipped polyethylene bags to be frozen and stored as described in section 2.2.

2.5.2. Virus recovery

Raspberries samples were thawed overnight at 4°C before analysis. 15 mL of culture medium DMEM-Glutamax (Dulbecco's Modified Eagle's Medium) with 1% non essential amino-acids (NEAA), sodium pyruvate (PYR), 0.5% Penicillin-Streptomycin and 0.1M HEPES buffer (all reagents were from Gibco™) were added directly in the zipped polyethylene bags containing the samples and placed on an agitation plate at 160 rpm

for 15 min. Raspberries eluates were recovered and placed in Falcon tubes to be clarified by centrifugation at 1721 g for 10 min, then 4 mL of supernatants were collected and aliquoted for infectious HAV titration.

2.5.3. Infectious virus titration

Infectious HAV titration was realized using the Real Time Cell Analysis (RTCA) xCelligence MP System (Ozyme, montigny le Bretonneux, France) and ACEA Biosciences (San Diego, CA, USA) as described by Lebourgeois et al., (2018). Briefly, the RTCA system measures changes in electrical impedance of cell monolayers (cell index, CI) in specialized microplate (E-plate). After seeding and adhesion, the cell impedance increases as cells proliferate. When the cell monolayer is infected by the HM175/18f strain of HAV, the virus replication induces cell mortality and thus the impedance decreases. The time needed for the cell index to reach 25% of decrease is correlated to the initial quantity of infectious HAV, making RTCA a quantitative method to determine HAV titer in unknown samples.

48h before the titration, FRhK-4 cells (Fetal Rhesus monkey Kidney, CRL-1688, ATCC™) were seeded in 96 wells E-plate at a density of 10^4 cells per well and cultivated in growth medium containing DMEM-Glutamax with 1% NEAA, PYR and 10% foetal calf serum (FCS) (Gibco™) at 37°C with 5% CO2.

FRhK-4 cells were washed once with FCS-free medium and infected with 100 µL per well of known titer of HAV suspensions (standard range) or raspberries eluates diluted at 1:10 in FCS-free medium to avoid cytotoxicity. After 1h of viral adsorption at 37°C, 100µL of 4% FCS culture medium were added to each well (2% FCS final concentration). The cellular impedance was then measured for twelve consecutive days.

2.6. Statistical analysis

A statistical analysis based on the analysis of variance (ANOVA) method was performed to study the significance of the effect of the frozen storage conditions and duration on quality parameter evolution using XLSTAT software. The Tukey Kramer test for multiple range comparisons ($p < 0.05$) was used to identify the difference between the measured mean values.

3. RESULTS AND DISCUSSION

3.1. Qualitative analysis of the microstructure

Fig. 4 represents grey scale level images of X-ray µCT scans of frozen raspberries stored at −18°C or −5°C for 4 months (112 days). The images exhibit three distinguishable grey levels: the black phase represents air, the dark grey indicates ice crystals, and the light grey signifies the unfrozen phase. The major part of the X-ray µCT scans is dark grey, corresponding to ice crystals. This result indicates that after freezing the drupes are full of ice homogeneously distributed in the fruit (Fig. 4, 0 day for −18°C or −5°C storage). Light grey phase in X-ray µCT scans can be attributed to raspberry cell walls. Storing raspberry samples at -18°C reveals no discernible distinctions in the X-ray µCT scans, except for the emergence of pores within the drupes (Fig. 4, green arrows). The minimal alterations in microstructure observed at -18°C suggest that this storage temperature effectively maintains the fruit's structure during long-term frozen storage. In contrast, more changes were obtained at −5°C as can be seen in Fig. 4. Stripes appeared in the drupes and became larger and bigger over time (Fig. 4, orange arrows). At the same time, ice crystals seem to become larger with an extended storage duration. These stripes are probably broken cell walls and pushed back due to ice crystal growth in the drupes because of recrystallization phenomena. These images indicate that raspberries undergo several microstructural damages when they are stored at −5°C and that this temperature have to be avoided throughout the cold chain.

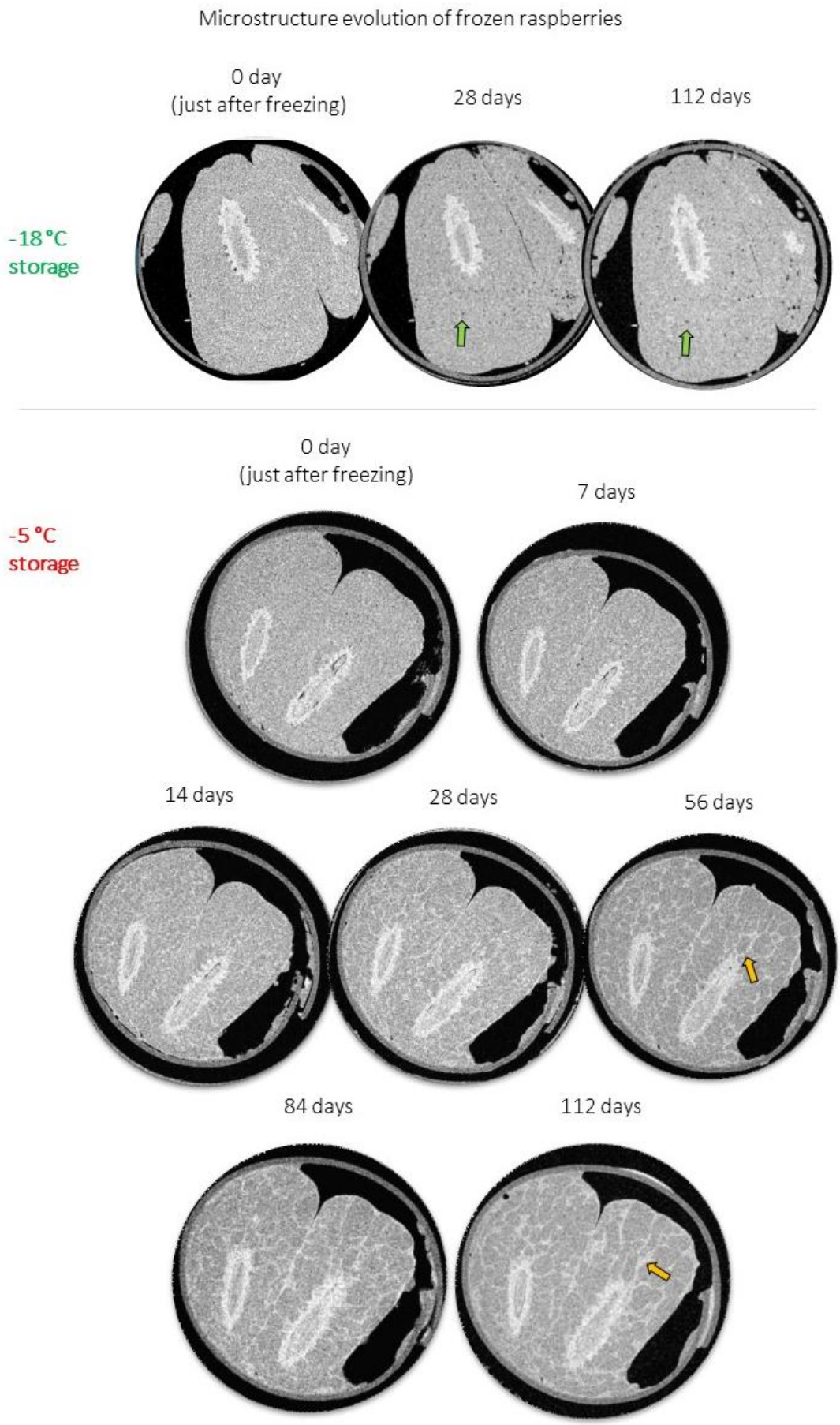

Figure 4: Grey scale level images of X-ray µCT scans of frozen raspberries stored at −18°C or −5°C for 4 months (112 days).

3.2. Quality analysis

3.2.1 Texture

Texture is one of the most important quality parameter for the consumer acceptability, especially for soft fruits such as raspberry. Fig. 5 represents the raspberry texture evolution (a) before and just after freezing and (b) during 4 months of storage at -18°C and -5°C.

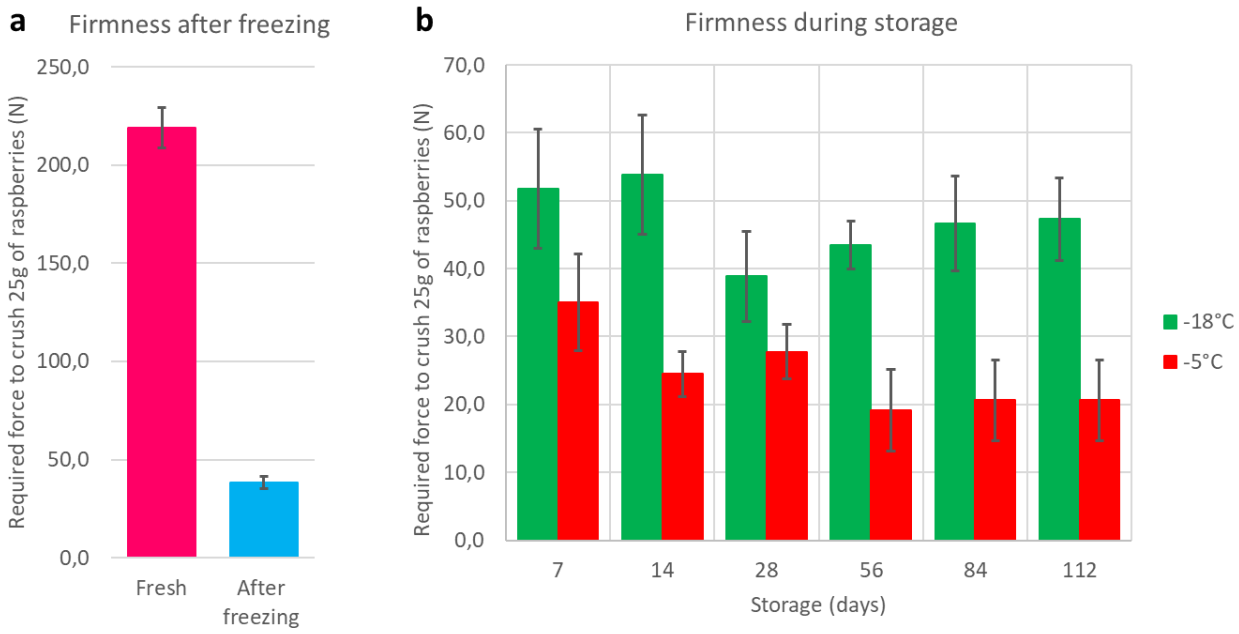

Figure 5: Raspberry texture evolution (a) before and just after freezing and (b) during 4 months (112 days) of storage at -18°C and -5°C. The results are presented as the required force in N to crush 25 g of raspberries. Mean ± SEM, n=3

Fig. 5a shows that the required force to crush 25 g of raspberries decreased significantly ($p < 0.05$) just after the freezing process, from 218.9 ± 10.3 N for the fresh raspberries to 38.2 ± 3.2 g for the frozen raspberries. This important loss of firmness (more than 80%) indicates the high sensitivity of raspberries to the freezing process. After freezing, the raspberry samples were stored at two different temperatures (−18°C and −5°C) for almost four months (112 days). When the raspberries were stored at −18°C, the firmness remained quite stable ($p < 0.05$) over time meaning that the texture obtained just after freezing is preserved during the four-month storage time. In contrast, when storing the frozen raspberries at −5°C, the required force to crush the raspberries dropped significantly ($p < 0.05$) from 31.1 ± 2.0 N to 19.1 ± 6.1 N between day 0 and day 56 followed by a stable trend until the fourth month of storage (day 112). The large decrease of the firmness of raspberry samples just after freezing highlights alterations in the cell membranes integrity that may result from either the formation of extracellular crystals or cell wall composition modification (Alabi et al., 2020; Chassagne-Berces et al., 2009; Delgado & Rubiolo, 2005). The additional reduction in firmness observed at −5°C may be explained by the growth of ice crystals by recrystallization phenomena during the storage as evidenced in Fig. 4. Ice crystal growth can lead to cell disruption and tissue damages resulting in undesirable changes in the texture of the product. On the other hand, the preservation of the structure during the storage at −18°C may be related to a limited evolution of the microstructure as shown in Fig. 4. Sousa et al., (2007) also reported a drastic lowering of the firmness of raspberry after freezing but, to the best of our knowledge, no work reported the effect of storage conditions and duration on the texture of frozen raspberries.

3.2.2 Drip Loss

Fig. 6 represents the raspberry drip loss evolution during four months of storage at −18°C and −5°C. At a storage temperature of −18°C, the drip loss exhibited a notable increase ($p < 0.05$) in the initial days, rising from 1.7 ± 0.1% immediately after freezing to 2.9 ± 0.2% by day 7. After day 7, the drip loss did not vary

significantly ($p > 0.05$) until the end of the four-month storage period. Similar trend was observed for samples stored at −5°C with a significant increase ($p < 0.05$) between day 0 and day 7 followed by a stabilisation during the remaining storage period. It is worth noting that the drip losses measured for raspberries stored at −5°C (around 4.6%) were significantly ($p < 0.05$) higher than those obtained at −18°C (around 2.5%). These results suggest that the drip loss is enhanced by higher storage temperatures. Drip loss results from the release of the intracellular content, which may exude during thawing following the degradation of the parenchymatous tissue cells and ultimately cell wall disruption. As it was stated in the previous section, storage at -5°C may enhance ice crystal growth, which can increase cell wall fracture and consequently drip loss during the storage. The relationship between microstructure degradation and drip loss can be evidenced in Fig. 4. This effect was earlier reported by Chassagne-Berces et al. (2009) for frozen apples.

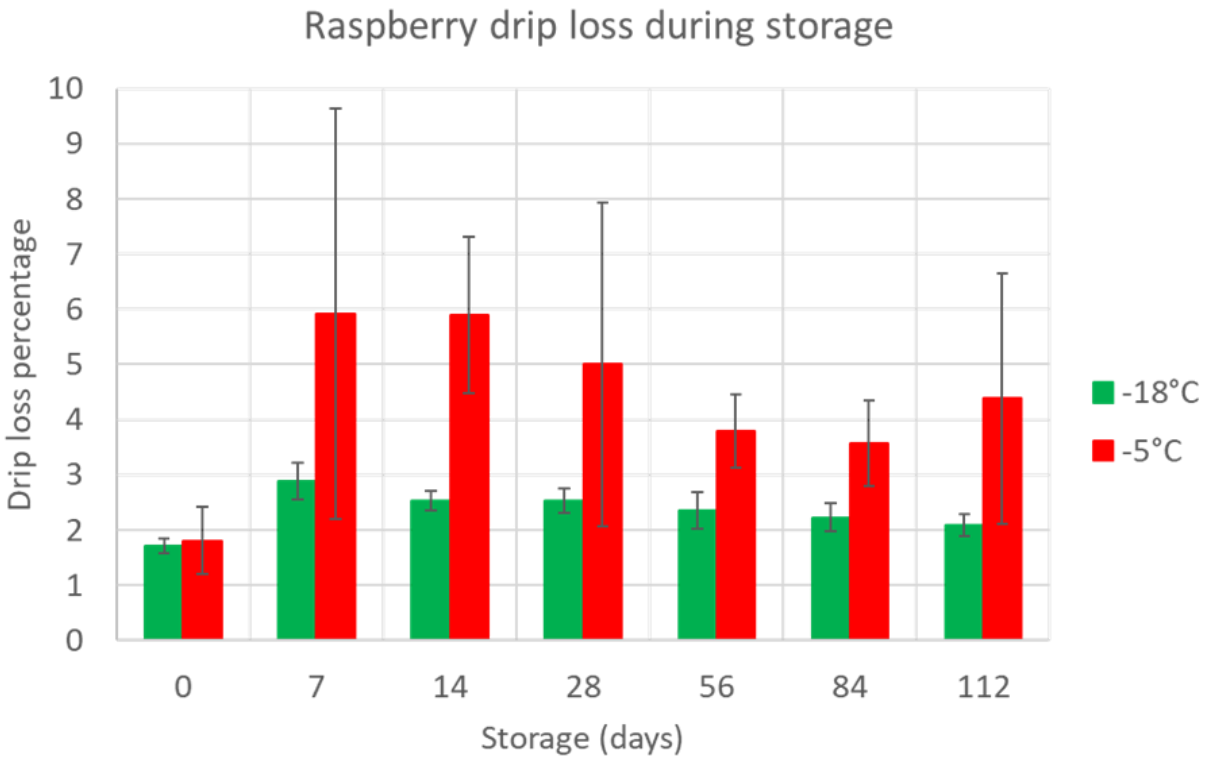

Figure 6: Raspberry drip loss evolution during 4 months (112 days) of storage at -18°C or -5°C. The results are presented as the drip loss percentage of 25g of raspberries. Day 0 indicate drip loss just after freezing. Mean ± SEM, n=3

3.3. Persistence of infectious viruses

Fig. 7 illustrates the quantity of HAV per 25g of raspberries (a) before and just after freezing and (b) during 4 months of storage at −18°C or −5°C.

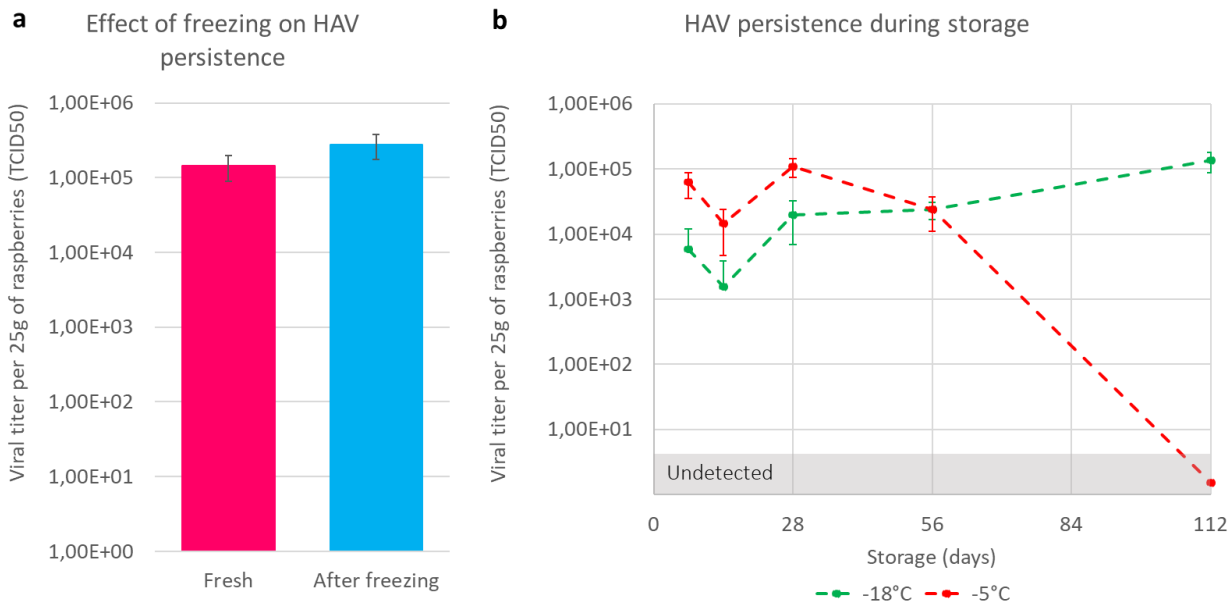

Figure 7: HAV titer per 25g of raspberries (a) before and just after freezing and (b) during 4 months (112 days) of storage at −18°C or −5°C. The results are presented as the virus quantity in TCID50 per 25g of raspberries. Mean ± SEM, n=6.

Since the quantity of HAV recovered from raspberries is similar before and just after freezing ($1.45\ 10^5 \pm 5.47\ 10^4$ and $2.77\ 10^5 \pm 1.02\ 10^5$ TCID50 respectively), it appears that the freezing process does not impact the infectious titer of HAV. For storage at −18°C, HAV titer remains quite stable over time indicating that this temperature of storage does not affect HAV persistence. In contrast, by storing the frozen raspberries at −5°C, HAV titer remains quite stable for 28 days and then decreases until no infectious viruses could be detected after 112 days of storage. These results suggest that higher storage temperatures can have an impact on HAV persistence. To the best of our knowledge, no work reported the effect of storage conditions on the HAV persistence on frozen raspberries. These experimental results might be explained by different phenomenon linking raspberries microstructure and HAV infectivity persistence. On the one hand, because of the growth of ice crystals during the storage as evidenced in Fig. 4, the viral particles could be directly altered by mechanical action (capsid damages for example), inducing a loss of infectivity. On the other hand, the growth of ice crystals, leading to textural damages, content loss, and drip loss in raspberries, might release certain constituents that could act as inhibitors (inhibiting a step in the virus cycle) or inactivate the viral particles.

4. CONCLUSIONS

The current work investigated the impact of temperature on microstructure evolving, quality changes and infectious HAV persistence during the storage of frozen raspberries. The results obtained show that the storage temperature that is potentially beneficial for reducing safety concerns, appears to be detrimental to the development of microstructure and quality changes in frozen raspberries.

Further work will focus on establishing quantitative indicators (quality indexes) that relate changes in ice crystals with quality attributes (texture and drip loss) as well as safety attributes (persistence of infectious viruses). Through this modelling approach, a deeper understanding of the relationships between mechanisms involved in the freezing process and frozen storage should be attained, ensuring the adequate preservation of microstructure, quality and safety.

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REFERENCES

Alabi, K. P., Zhu, Z., & Sun, D. W. (2020). Transport phenomena and their effect on microstructure of frozen fruits and vegetables. *Trends in Food Science & Technology*, *101*, 63–72. https://doi.org/10.1016/J.TIFS.2020.04.016

Bozkurt, H., Phan-Thien, K. Y., van Ogtrop, F., Bell, T., & McConchie, R. (2021). Outbreaks, occurrence, and control of norovirus and hepatitis a virus contamination in berries: A review. *Critical Reviews in Food Science and Nutrition*, *61*(1), 116–138. https://doi.org/10.1080/10408398.2020.1719383

Bulut, M., Bayer, Ö., Kırtıl, E., & Bayındırlı, A. (2018). Effect of freezing rate and storage on the texture and quality parameters of strawberry and green bean frozen in home type freezer. *International Journal of Refrigeration*, *88*, 360–369. https://doi.org/10.1016/j.ijrefrig.2018.02.030

Chassagne-Berces, S., Poirier, C., Devaux, M.-F., Fonseca, F., Lahaye, M., Pigorini, G., Girault, C., Marin, M., & Guillon, F. (2009). Changes in texture, cellular structure and cell wall composition in apple tissue as a result of freezing. *Food Research International*, *42*(7), 788–797. https://doi.org/10.1016/j.foodres.2009.03.001

Dawson, P., Al-Jeddawi, W., & Rieck, J. (2020). The Effect of Different Freezing Rates and Long-Term Storage Temperatures on the Stability of Sliced Peaches. *International Journal of Food Science*, *2020*. https://doi.org/10.1155/2020/9178583

De Ancos, B., Gonzalez, E. M., & Cano, M. P. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural and Food Chemistry*, *48*(10), 4565–4570. https://doi.org/10.1021/JF0001684

Delgado, A. E., & Rubiolo, A. C. (2005). Microstructural changes in strawberry after freezing and thawing processes. *LWT - Food Science and Technology*, *38*(2), 135–142. https://doi.org/10.1016/J.LWT.2004.04.015

Di Cola, G., Fantilli, A. C., Pisano, M. B., & Ré, V. E. (2021). Foodborne transmission of hepatitis A and hepatitis E viruses: A literature review. *International Journal of Food Microbiology*, *338*, 108986. https://doi.org/10.1016/j.ijfoodmicro.2020.108986

Feldkamp, L. A., Davis, L. C., Kress, J. W., Feldkamp, L. A., Davis, L. C., & Kress, J. W. (1984). Practical cone-beam algorithm. *JOSAA*, *1*(6), 612–619. https://doi.org/10.1364/JOSAA.1.000612

González, E., Ancos, B. de, and, P. C.-E. F. R., & 2002, undefined. (2002). Preservation of raspberry fruits by freezing: physical, physico-chemical and sensory aspects. *Springer*, *215*(6), 497–503. https://doi.org/10.1007/s00217-002-0600-4

Häkkinen, S. H., Kärenlampi, S. O., Mykkänen, H. M., Heinonen, I. M., & Törrönen, A. R. (2000). Ellagic acid content in berries: Influence of domestic processing and storage. *European Food Research and Technology 2000 212:1*, *212*(1), 75–80. https://doi.org/10.1007/S002170000184

Hartel, R. W. (2013). Advances in food crystallization. *Annual Review of Food Science and Technology*, *4*(1), 277–292. https://doi.org/10.1146/annurev-food-030212-182530

Hu, X., Collier, M. G., & Xu, F. (2020). Hepatitis A Outbreaks in Developed Countries: Detection, Control, and Prevention. *Foodborne Pathogens and Disease*, *17*(3), 166–171. https://doi.org/10.1089/FPD.2019.2648/ASSET/IMAGES/LARGE/FPD.2019.2648_FIGURE1.JPEG

Latil, P., Zennoune, A., Ndoye, F. T., Flin, F., Geindreau, C., & Benkhelifa, H. (2022). X-ray microtomography of ice crystal formation and growth in a sponge cake during its freezing and storage. *Journal of Food Engineering*, *325*, 110989. https://doi.org/10.1016/J.JFOODENG.2022.110989

Lebourgeois, S., Fraisse, A., Hennechart-Collette, C., Guillier, L., Perelle, S., & Martin-Latil, S. (2018). Development of a Real-Time Cell Analysis (RTCA) Method as a Fast and Accurate Method for Detecting Infectious Particles of the Adapted Strain of Hepatitis A Virus. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/FCIMB.2018.00335

Li, D., Zhu, Z., & Sun, D. W. (2018). Effects of freezing on cell structure of fresh cellular food materials: A review. *Trends in Food Science & Technology*, *75*, 46–55. https://doi.org/10.1016/J.TIFS.2018.02.019

Lo Piccolo, E., Garcìa, L. M., Landi, M., Guidi, L., Massai, R., & Remorini, D. (2020). Influences of postharvest

storage and processing techniques on antioxidant and nutraceutical properties of rubus idaeus l.: A mini-review. *Horticulturae*, *6*(4), 1–13. https://doi.org/10.3390/horticulturae6040105

Masselot, V., Bosc, V., & Benkhelifa, H. (2021a). Analyzing the microstructure of a fresh sorbet with X-ray micro-computed tomography: Sampling, acquisition, and image processing. *Journal of Food Engineering*, *292*, 110347. https://doi.org/10.1016/J.JFOODENG.2020.110347

Masselot, V., Bosc, V., & Benkhelifa, H. (2021b). Influence of stabilizers on the microstructure of fresh sorbets: X-ray micro-computed tomography, cryo-SEM, and Focused Beam Reflectance Measurement analyses. *Journal of Food Engineering*, *300*, 110522. https://doi.org/10.1016/J.JFOODENG.2021.110522

Mullen, W., Stewart, A. J., Lean, M. E. J., Gardner, P., Duthie, G. G., & Crozier, A. (2002). Effect of Freezing and Storage on the Phenolics, Ellagitannins, Flavonoids, and Antioxidant Capacity of Red Raspberries. *Journal of Agricultural and Food Chemistry*, *50*(18), 5197–5201. https://doi.org/10.1021/jf020141f

Mulot, V., Benkhelifa, H., Pathier, D., Ndoye, F. T., & Flick, D. (2019). Measurement of food dehydration during freezing in mechanical and cryogenic freezing conditions. *International Journal of Refrigeration*, *103*, 329–338. https://doi.org/10.1016/J.IJREFRIG.2019.02.032

Ndoye, F. T., & Alvarez, G. (2015). Characterization of ice recrystallization in ice cream during storage using the focused beam reflectance measurement. *Journal of Food Engineering*, *148*, 24–34. https://doi.org/10.1016/J.JFOODENG.2014.09.014

Petzold, G., & Aguilera, J. M. (2009). Ice Morphology: Fundamentals and Technological Applications in Foods. *Food Biophysics 2009 4:4*, *4*(4), 378–396. https://doi.org/10.1007/S11483-009-9136-5

Randazzo, W., & Sánchez, G. (2020). Hepatitis A infections from food. *Journal of Applied Microbiology*, *129*(5), 1120–1132. https://doi.org/10.1111/JAM.14727

Sousa, M. B., Canet, W., Alvarez, M. D., & Fernández, C. (2007). Effect of processing on the texture and sensory attributes of raspberry (cv. Heritage) and blackberry (cv. Thornfree). *Journal of Food Engineering*, *78*(1), 9–21. https://doi.org/10.1016/J.JFOODENG.2005.08.047

Vicent, V., Ndoye, F. T., Verboven, P., Nicolaï, B., & Alvarez, G. (2019). Effect of dynamic storage temperatures on the microstructure of frozen carrot imaged using X-ray micro-CT. *Journal of Food Engineering*, *246*, 232–241. https://doi.org/10.1016/J.JFOODENG.2018.11.015

Vicent, V., Verboven, P., Ndoye, F. T., Alvarez, G., & Nicolaï, B. (2017). A new method developed to characterize the 3D microstructure of frozen apple using X-ray micro-CT. *Journal of Food Engineering*, *212*, 154–164. https://doi.org/10.1016/J.JFOODENG.2017.05.028

Zennoune, A., Latil, P., Flin, F., Perrin, J., Weitkamp, T., Scheel, M., Geindreau, C., Benkhelifa, H., & Ndoye, F. T. (2022). Investigating the influence of freezing rate and frozen storage conditions on a model sponge cake using synchrotron X-rays micro-computed tomography. *Food Research International*, *162*, 112116. https://doi.org/10.1016/J.FOODRES.2022.112116

Zhao, Y., & Takhar, P. S. (2017). Micro X-ray computed tomography and image analysis of frozen potatoes subjected to freeze-thaw cycles. *LWT - Food Science and Technology*, *79*, 278–286. https://doi.org/10.1016/j.lwt.2017.01.051