Optimizing radiofrequency assisted cryogenic freezing to improve meat microstructure and quality

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Corresponding Author:	Marilisa Alongi, Dr. ITALY
First Author:	Lara Manzocco, Professor
Order of Authors:	Lara Manzocco, Professor
	Marilisa Alongi, Dr.
	Giovanni Cortella, Professor
	Monica Anese, Professor
Abstract:	A radiofrequency-assisted cryogenic freezing approach previously proposed was optimized to further increase the quality of frozen meat. Nitrogen was delivered in a pulsed instead of a continuous mode to reduce tissue damage, while radiofrequency pulses of different duration and time were tested to guarantee complete and homogeneous freezing. The developed processes were compared to conventional freezing (i.e. , slow and blast) by assessing microstructure of frozen meat and selected quality indicators (i.e. , drip loss, color, firmness) of thawed meat. Pulsing nitrogen instead of continuously delivering it limited tissue damages. In addition, combination of nitrogen pulsing with radiofrequency was crucial to preserve tissue integrity, thus improving meat firmness and reducing exudate loss upon thawing. Besides guaranteeing an optimal retention of meat quality, the optimized radiofrequency-assisted cryogenic freezing could be easily adapted to different matrices thanks to the possibility to finely tune processing conditions.

Dear Editor,

I would like to submit the manuscript entitled *Optimizing radiofrequency assisted cryogenic freezing to improve meat microstructure and quality* by Lara Manzocco, Marilisa Alongi, Giovanni Cortella, and Monica Anese for consideration for publication in *Journal of Food Engineering*.

This study represents the progression of a previous work in which low voltage radiofrequency was used as assisting technology for cryogenic freezing to obtain frozen meat with increased quality [Anese, M., Manzocco, L., Panozzo, A., Beraldo, P., Foschia, M., & Nicoli, M. C. (2012). Effect of radiofrequency assisted freezing on meat microstructure and quality. Food Research International, 46, 50–54]. To improve the efficiency of cryogenic freezing, a more homogeneous nitrogen delivery was first achieved by properly modifying the equipment. Secondly, pulsing both nitrogen and radiofrequency instead of continuously delivering them resulted to be a key factor to limit tissue damages, thus improving meat firmness and reducing exudate loss upon thawing. The freezing approach set up in the present study, besides guaranteeing an optimal retention of tissue integrity, could be easily adapted to different food matrices thanks to the possibility to finely tune processing conditions.

Best regards,
Marilisa Alongi

Author Conflict of Interest Declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author (marilisa.alongi@uniud.it)

Udine, October 28th, 2021

Signed by the Corresponding Author on behalf of all authors:

Marilisa Alongi

Highlights

Radiofrequency (RF) cryogenic freezing of meat was optimized by a pulsing approach

Nitrogen pulsing during meat freezing reduced tissue damage

RF pulsing was modulated to guarantee homogeneous cryogenic freezing of meat

Combining nitrogen pulses with RF was crucial to preserve tissue integrity

Optimizing radiofrequency assisted cryogenic freezing to improve meat microstructure and quality

Lara Manzocco[a], Marilisa Alongi[a]*, Giovanni Cortella[b], Monica Anese[a]

[a] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Italy

[b] Polytechnic Department of Engineering and Architecture, University of Udine, Italy

*corresponding author: marilisa.alongi@uniud.it

Abstract

A radiofrequency-assisted cryogenic freezing approach previously proposed was optimized to further increase the quality of frozen meat. Nitrogen was delivered in a pulsed instead of a continuous mode to reduce tissue damage, while radiofrequency pulses of different duration and time were tested to guarantee complete and homogeneous freezing. The developed processes were compared to conventional freezing (*i.e.*, slow and blast) by assessing microstructure of frozen meat and selected quality indicators (*i.e.*, drip loss, color, firmness) of thawed meat. Pulsing nitrogen instead of continuously delivering it limited tissue damages. In addition, combination of nitrogen pulsing with radiofrequency was crucial to preserve tissue integrity, thus improving meat firmness and reducing exudate loss upon thawing. Besides guaranteeing an optimal retention of meat quality, the optimized radiofrequency-assisted cryogenic freezing could be easily adapted to different matrices thanks to the possibility to finely tune processing conditions.

Keywords

Cryogenic freezing; Radiofrequency; Meat quality; Microstructure

1 Introduction

Freezing has been extensively used for decades to preserve a wide variety of food products. Traditional freezing methods, including air blast, plate contact, fluidized bed, and cryogenic freezing (Reid, 1997), lead to low freezing rates due to the low thermal conductivity of food (Singh & Heldman, 2009), which may jeopardize food quality to an extent that varies depending on food features (Sun, 2011). Quality loss is particularly serious in the case of meat, due to the presence of a high fraction of water immobilized within the myofibrils. Slow freezing can induce their denaturation, triggering the release of water in the intercellular space (Li, Zhu, & Sun, 2018). As a result, there is a high risk of producing large and irregular ice crystals especially located in the intercellular space (Kaale & Eikevik, 2013). Being muscle cells smaller than plant cells, the formation of such large ice crystals during freezing compromises their microstructure, leading to severe damages (Kaale & Eikevik, 2013). From a macroscopic standpoint, such a loss of tissue integrity turns into an increased thawing drip loss together with textural changes, overall impairing the quality of frozen meat (Leygonie, Britz, & Hoffman, 2012; You, Kang, & Jun, 2021).

Researchers explored different strategies to overcome these issues. Different thawing conditions that allow minimizing meat tissue damages have been extensively studied and include radiofrequency (RF), infrared radiation, and microwaves (Choi *et al.*, 2017; Hong, Shim, Choi, & Min, 2009; Sales *et al.*, 2020). Although optimizing thawing conditions helps to avert the worst, it cannot restore the damages produced by freezing on meat tissue. Indeed, its integrity should be preserved by applying high freezing rates leading to small ice crystals evenly distributed both in the intra- and intercellular space (Evans, 2008). In this regard, efforts to preserve tissue integrity have been made by applying novel freezing methods driving the formation of small and homogeneously distributed ice crystals. Until now, high-pressure freezing, electrically assisted freezing, magnetically assisted freezing, electromagnetic assisted freezing, ultrasound-assisted freezing, as well as the use of antifreeze proteins have been proposed (Zhan,

Sun, Zhu, & Wang, 2019). However, most of these technologies are in their early stages of development, as the control of ice crystal formation, in particular of their size and location, as well as the deriving tissue damages, still need to be thoroughly addressed (Li *et al.*, 2018). Moreover, such novel freezing methods have been mainly applied to plant-based food, whereas only a few studies have investigated their effect on animal derivatives (Li *et al.*, 2018).

A first attempt to exploit RF as assisting technology during cryogenic freezing of meat was made by Anese *et al.* (2012), who demonstrated that a low voltage RF was able to displace water molecules from their equilibrium by dipole rotation, without inducing thermal effects but producing more nucleation sites (Hanyu, Ichikawa, & Matsumoto, 1992; Jackson, Ungan, Critser, & Gao, 1997). RF might thus interfere with ice crystal growth, leading to an effective reduction of their size, and allowing to control their distribution in meat tissue, ultimately resulting in successfully frozen meat cubes. Despite these promising results, several criticisms arose. The designed process was a two-step procedure, in which the sample was first exposed to the combination of nitrogen and RF for a short time until a frozen crust was formed, and then moved to a conventional thermostatic cell set at -18 °C to allow temperature equilibration and finalize freezing. Moreover, a high nitrogen consumption was required to lower the RF chamber temperature before the first step of the process. This also resulted in the formation of an ice layer on the equipment parts in contact with the nitrogen flow due to water moisture crystallization. Based on these technical issues, the feasibility of such a process was still lacking and RF-assisted cryogenic freezing remained in its early stage of research (Anese *et al.*, 2012; Hafezparast-Moadab, Hamdami, Dalvi-Isfahan, & Farahnaky, 2018).

The present work aimed to further investigate the potential of low voltage RF combined with cryogenic freezing, to increase process feasibility while guaranteeing the highest retention of frozen meat quality and of its fresh-like features upon thawing. To this purpose, a fine tuning of process conditions was

performed to improve the RF-assisted cryogenic freezing approach previously proposed (Anese *et al.*, 2012).

In particular, a more even distribution of nitrogen was pursued by properly modifying the equipment, to gradually deliver nitrogen onto the product surface, while possibly reducing cryogenic fluid consumption and ice formation on the equipment. Nitrogen delivery was further tuned by applying it both in a continuous or a pulsed mode. RF pulses of different duration and time frequency were then tested in combination with nitrogen to reduce tissue damage while guaranteeing complete and homogeneous meat freezing in a single step. The quality of meat frozen by the processes here developed was compared to that of meat obtained by conventional, *i.e.*, slow and blast, freezing.

2 Materials and methods

2.1 Meat

Pork loin was purchased in a local market and stored at 2 °C. Immediately before experiments, the meat was manually cut with a sharp knife to obtain 4 cm-edge cubes with an average weight of 70 g. Meat cubes were wrapped in polyethylene (PE) film to prevent surface dehydration during freezing.

2.2 Equipment

The source of RF energy was represented by a pilot-scale RF equipment (3.5 kW, 27.12 MHz, Stalam Spa, Nove, Vicenza, Italy) with plate applicators, modified to allow the precise delivery of low voltage RF pulses (Figure 1a). The cryogenic fluid (nitrogen, N_2) was delivered to a polytetrafluoroethylene (PTFE) treatment chamber through a 70 mm inner diameter PTFE pipe or a 12 mm inner diameter polycarbonate (PC) pipe depending on the configuration. The pipe was connected by a flexible steel hose to a set of valves for flow rate control, i.e. an electrovalve (ASCO SCE222E002LT, ASCO Emerson) and a needle valve (Dinafluid, Padova, Italy) both for cryogenic fluids. Finally, a further flexible steel hose connected a pressurized liquid nitrogen reservoir (Medicair, Pogliano Milanese, Italy).

N_2 flowed into the treatment chamber through nozzles set according to two different configurations. In one case (Figure 1b), three nozzles (Mod. H1/8VV-6503, 6502, 6501, Spray Systems Co., Wheaton, IL, USA) were placed on the PTFE pipe at 3 cm distance from each other and oriented to continuously deliver N_2 perpendicularly to the surface of the sample. In the other case (Figure 1c), the PC pipe was bent by 90 ° and supplied with a 10-vent PC nozzle. Vents (1 mm holes) were arranged to prevent N_2 from being directly delivered to the sample surface, being rather sprayed into the chamber. In this case, spraying was performed in a pulsed mode, guaranteed by the above mentioned electrovalve adequately controlled.

2.3 Freezing

Meat freezing was carried out according to 6 different combinations of processing parameters as detailed below. Frozen samples were stored at -18 °C for 24 h before further analyses.

2.3.1 Slow freezing

Slow freezing (SF) was carried out in a freezer (Electrolux Professional S.p.A., mod. REX71FF, Pordenone, Italy) set at -20 °C. Meat cubes were located on a rack in the center of the freezer. Freezing was stopped when the temperature in the center of the meat cubes reached -18 °C.

2.3.2 Blast freezing

Blast freezing (BF) was carried out in a blast freezer (Electrolux Professional S.p.A., mod. AOFPS061C, Pordenone, Italy). Meat cubes were located on a rack in the center of the freezer. The airflow and temperature were fixed at 3 m/s and -40 °C, respectively. Freezing was stopped when the temperature in the center of the meat cubes reached -18 °C.

2.3.3 Radiofrequency-assisted continuous cryogenic freezing

Radiofrequency-assisted continuous cryogenic freezing (RF-CCF) was carried out in the RF equipment (Figure 1b). N_2 was flowed for 20 min to cool the treatment chamber at -80 °C and achieve a constant flow. A meat cube was then placed in the PTFE treatment chamber and maintained for 2.5 min under continuous N_2 flow while applying 2 kV RF pulses (*i.e.*, 10 s pulse with 20 s interval). This voltage allowed to induce water dipole rotation while limiting sample temperature increase (Anese *et al.*, 2012). A schematic representation of the application of RF and N_2 during RF-CCF is reported in Figure 2a. Samples were immediately stored at -18 °C to allow thermal equilibration.

2.3.4 Continuous cryogenic freezing

Continuous cryogenic freezing (CCF) was carried out as described for RF-CCF, without applying RF.

2.3.5 Radiofrequency-assisted pulsed cryogenic freezing

Radiofrequency-assisted pulsed cryogenic freezing (RF-PCF) was carried out in the RF equipment (Figure 1c). N_2 was pulsed (3 s pulse with 10 s interval) until the temperature chamber reached -80 °C (*i.e.*, circa 10 min). A meat cube was then placed in the PTFE treatment chamber and maintained for 10 min under pulsed N_2 flow while applying 2 kV RF pulses (*i.e.*, 30 s pulse with 60 s interval). A schematic representation of the application of RF and N_2 during RF-PCF is reported in Figure 2b.

2.3.6 Pulsed cryogenic freezing

Pulsed cryogenic freezing (PCF) was carried out as described for RF-PCF, without applying RF.

2.4 Temperature measurement

Before freezing, a copper-constantan thermocouple probe (Ellab, Denmark), connected to a portable data logger (mod. 502A1, Tersid, Milano, Italy), was placed in the slowest cooling point of the meat cube, corresponding to the meat cube center. Temperature was not measured during RF-assisted freezing

because of technical constraints, *i.e.*, metals could not be inserted into the chamber while RF pulses were applied.

2.5 Thaw drip loss

Meat samples were unwrapped from the PE film and placed on a PTFE rack at 3 cm distance from the bottom of a plastic box (15×15×15 cm) closed with a pressure lid. The lid was holed to allow the connection of the thermocouple probe to the temperature data logger. The plastic box was introduced in a thermostatically controlled chamber (Ignis, Comerio, Varese, Italy) at 10 °C to allow thermal equilibrium, which was reached within 10 h. Drip loss was measured by weighing the meat cube before and after thawing. The drip loss (DL) was calculated as:

$$DL\ (\%) = \frac{w_0 - w_{10\,h}}{w_0} \times 100 \qquad \text{Equation 1}$$

where w_0 and $w_{10\,h}$ are the weights of the meat cube before and after 10 h thawing, respectively.

2.6 Color

Color analysis was carried out on fresh and thawed meat using a tristimulus colorimeter equipped with a CR-300 measuring head (Chromameter-2 Reflectance, Minolta, Osaka, Japan) and standardized against a white tile before measurements. Color was expressed as L*, a* and b* scale parameters (Clydesdale, 1978), and color differences (ΔE*) were calculated (Equation 2):

$$\Delta E* = \sqrt{[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]} \qquad \text{Equation 2}$$

where L_0*, a_0*, and b_0* were the color parameters of fresh meat.

2.7 Firmness

A Warner–Bratzler (V-notch blade) attached to an Instron 4301 (Instron Ltd, High Wycombe, UK) equipped with the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, UK) was used. Firmness was measured on meat sticks (2×2×4 cm) obtained by manually

cutting fresh and thawed meat cubes with a sharp knife. The test speed was 4 mm/s. Firmness was defined as the force (N) required to cut the meat stick.

2.8 Histology

Sections of 1.0×1.0×0.5 cm were cut from frozen meat cubes by a sharp knife preliminary cooled at -20 °C. The samples were fixed in -20 °C cooled Carnoy's fixative (30% v/v chloroform, 60% ethanol, and 10% acetic acid) for 24 h at -20 °C (frozen meat) or 4 °C (fresh meat). After fixation, samples were equilibrated at room temperature and processed by an automatic histoprocessor (TISBE tissue processor, Diapath, Martinengo, Italy) to embed the tissue in paraffin (ParaplastPlus, Diapath, Martinengo, Italy). Serial 5 μm sections were cut to obtain transversal specimen to the fiber direction by a programmable microtome (Reichert-Jung 2050, Nussloch, Germany). For histological evaluation, the paraffin sections were stained with hematoxylin-eosin (Sigma Aldrich, St. Louis, MO, USA). The specimens were examined by a light microscope (DM 2000, Leica Microsystems, Heerbrugg, Switzerland) at 40× magnification, and images were taken using a Leica EC3 digital camera and elaborated by the Leica Suite Las EZ software (Leica Microsystems, Heerbrugg, Switzerland).

2.9 Statistical analysis

Results were reported as mean value ± standard deviation of at least three measurements on two replicated samples. Analysis of variance (ANOVA) was performed with significance level set to $p < 0.05$ and the Tukey procedure was used to test for differences among means (R, version 3.2.3, The R Foundation for Statistical Computing, Vienna, Austria).

3 Results and discussion

3.1 Set up of pulsed cryogenic freezing

The research was performed by using a RF pilot equipment modified to deliver nitrogen inside the operating chamber. Initially, meat cubes were frozen according to the procedure reported in the literature

177 by Anese *et al.* (2012), hereafter named continuous cryogenic freezing (CCF). Complete freezing of meat
178 cubes was achieved by a two-step procedure. Firstly, the meat cubes equilibrated at 2 °C were maintained
179 in the RF chamber for 2.5 min under continuous N_2 flow until a frozen crust was formed. Following, the
180 sample was moved to a thermostatic cell at -18 °C to allow temperature equilibration. Figure 3 shows the
181 temperature profile of the central part of meat cubes frozen according to the CCF procedure. It can be
182 noted that the freezing front reached the sample core within 15 min. It is noteworthy that nitrogen was
183 continuously delivered on the meat surface by the three-vent nozzle configuration (Figure 1b). The
184 continuous flowing of nitrogen was critical: not only was nitrogen consumption particularly high, but
185 water moisture also crystallized on the equipment parts in contact with the nitrogen flow (pipe, door,
186 chamber). To reduce these drawbacks, the equipment was further modified to allow nitrogen flowing in
187 the chamber in pulsed mode through a time-controlled valve, while the flow rate was significantly
188 reduced and calibrated by means of a needle valve. In addition, nozzle configuration was rearranged to
189 prevent N_2 from being directly delivered onto the sample surface, being rather sprayed into the chamber
190 (Figure 1c). Nitrogen delivery pipes were reduced in diameter and length, and vacuum insulated flexible
191 hoses were used. All these modifications resulted efficacious, allowing a gradual delivery of nitrogen to
192 the product surface while reducing by more than 15-fold the cryogenic fluid consumption, thanks to the
193 decreased heat gains of the piping and the accurate spreading of nitrogen. The lower flow rate of gas
194 prevented its massive release in the RF equipment, thus substantially reducing ice formation onto its
195 metallic surfaces. Furthermore, a better distribution of nitrogen in the sample chamber allowed faster and
196 more effective pre-cooling of the apparatus. Some preliminary trials were thus performed to identify
197 nitrogen pulsing conditions that allowed sample freezing in a single step. A treatment with the same
198 duration of CCF (*i.e.*, 2.5 min) but under pulsed N_2 flow (*i.e.*, 5 s pulse with 10 s interval) was initially
199 tested. However, the resulting meat cube was partially unfrozen (Figure 4a). Under this condition, the
200 overall N_2 flow lasted 50 s and this exposure was insufficient to freeze the sample. Treatment time was

thus increased to 10 min while keeping constant N_2 pulse, but the sample thereof obtained was cracked (Figures 4b and 4c). In this case, N_2 was actually delivered for 3 min 20 s, resulting in excessive exposure to the cryogenic fluid. Another trial considering an intermediate treatment, *i.e.*, accounting for 7.5 min overall treatment, was thus carried out. During this treatment, N_2 pulse was kept constant and the overall N_2 flow was the same as for the CCF, *i.e.*, 2.5 min. Although the resulting meat cube was completely frozen and not cracked, it still presented cold burns (Figure 4d) due to the intense contact of meat with the cryogenic fluid. To reduce such a thermal shock, the pulse was modified to 3 s with 10 s interval, while prolonging treatment duration to 10 min to keep the overall nitrogen delivery constant. These conditions produced a frozen meat cube without defects (Figures 4e and 4f) and were chosen to carry out the pulsed cryogenic freezing (PCF). The temperature profiles of meat cubes frozen under these conditions were thus recorded and compared to those of CCF meat and of meat submitted to slow (SF) and blast (BF) freezing (Figure 3). The curves confirmed that PCF allowed complete freezing of meat in less than 10 min, with no need for a second equilibration step (CCF). By contrast, SF and BF control samples reached -18 °C in the core after 8 h and 45 min, respectively. PCF was thus selected to be further combined with RF.

3.2 Set up of radiofrequency-assisted pulsed cryogenic freezing

To combine PCF with RF, different trials were performed by applying RF pulses at different time intervals during PCF. Temperature monitoring during the RF-assisted freezing could not be performed due to the lack of efficacious instruments allowing temperature measurement during RF treatments. As known, thermocouple probes cannot be used due to the presence of metals that can modify the electric field applied and cause discharges. Similarly, optical fibers are hardly applicable due to their fragility at low temperatures (Yang, Zhao, & Wells, 2003). In the light of those constraints, meat cube sections were visually assessed. Although this procedure is clearly a compromise, it allowed to approximately evaluate the occurrence of complete freezing, since unfrozen meat portions are distinctly redder than frozen ones.

Initially, meat cubes were exposed to 1 min RF pulse with 1 min interval during 10 min treatment. As shown in Figure 5a, these conditions led to incomplete and uneven freezing. Based on this result, a second sample was prepared by keeping constant the overall application of RF but reducing pulse duration (30 s pulse with 1 min interval during 15 min treatment). As a result, the sample obtained was particularly hard (Figure 5b), probably due to temperature dropping below -55 °C, which accounts for the second transition affecting structural proteins (Hansen, Andersen, & Skibsted, 2003). To prevent sample overcooling, an intermediate treatment was carried out, by applying 30 s pulse with 1 min interval during 10 min treatment. These conditions, which are schematically summarized in Figure 2b, led to a frozen meat cube without defects (Figure 5c) and were chosen to carry out the radiofrequency-assisted pulsed cryogenic freezing (RF-PCF).

3.3 Effect of different freezing conditions on meat quality

Pork meat quality frozen under the application of different radiofrequency pulses and/or nitrogen delivery conditions was assessed. To this aim, meat cubes frozen in the RF equipment by continuous cryogenic freezing (CCF), pulsed cryogenic freezing (PCF), radiofrequency-assisted continuous cryogenic freezing (RF-CCF), and radiofrequency-assisted pulsed cryogenic freezing (RF-PCF) were thawed and analyzed for color, firmness, drip loss, and microscopic appearance. Slow (SF) and blast (BF) frozen meat were also considered as control samples. The CIE L*a*b* color parameters of thawed meat as affected by different freezing conditions is reported in Table 1. Being color the most important and direct attributes for evaluating product visual quality, color determination is often addressed in meat studies. The color parameters of the unfrozen (NF) pork meat were in line with those reported in the literature (Botinestean, Hossain, Mullen, Kerry, & Hamill, 2021; Choi *et al.*, 2017; Hong, Ko, Choi, & Min, 2007; Teuteberg, Kluth, Ploetz, & Krischek, 2021). None of the freezing conditions affected lightness (L*) (Table 1). Slow and blast freezing did not affect meat redness (a*), while cryofreezing induced a slight decrease in this parameter, which was more pronounced when nitrogen was pulsed (PCF) instead of being continuously

(CCF) delivered. The application of RF (RF-CCF and RF-PCF) did not further affect meat redness. On the contrary, yellowness (b*) did not change when nitrogen (CCF and PCF) or its combination with radiofrequency (RF-CCF and RF-PCF) were applied, whereas it decreased upon slow (SF) and blast (BF) freezing, as compared to the fresh sample. Since protein and lipid oxidation are generally associated with an increase in yellowness, results shown in Table 1 indicate that these alterative phenomena are not specifically triggered by the tested freezing conditions (Muela, Monge, Sañudo, Campo, & Beltrán, 2015). Moreover, in all cases, the total color difference from the fresh product varied in a very small range ($\Delta E^* = 3.8 - 4.5$) being thus hardly expected to impair the perceived quality of thawed meat.

Contrarily to color, which was only slightly affected by the different freezing conditions, meat firmness and drip loss significantly changed upon thawing, as shown in Figure 6. The meat thawed upon slow and blast freezing presented a firmness comparable to that of the fresh sample. Even so, during thawing these samples lost between 2 and 3% of their weight, in agreement with our previous findings (Anese *et al.*, 2012). The exposure to continuous nitrogen flow (CCF) further increased the firmness of thawed meat, which was associated with the highest drip loss (Figure 6b). Despite no macroscopic fractures on the surface of meat cubes were observed upon CCF (Figure 4), these results were probably determined by cell damages and microfractures occurring due to the high temperature difference between the sample and the cryogenic fluid (Diamante & Tran, 2016). When the meat was frozen under CCF assisted by radiofrequency (RF-CCF), its firmness was comparable to that of the fresh sample (NF), and drip loss accounted for only 1%, suggesting that radiofrequency was effective as an assisting technology to prevent the cell damages induced by the nitrogen flow alone (Fowler & Toner, 2005). Changing nitrogen delivery from the continuous to the pulsed mode appeared even more effective in preventing firmness changes. PCF actually presented firmness comparable to that of the fresh sample (NF) and resulted in an additional drip loss reduction (<1%), accounting for the lowest average value. The combination of PCF with radiofrequency (RF-PCF) produced analogous results to those observed for PCF. Overall, the correlation

between firmness and drip loss of thawed meat samples was very strong (r = 0.955), and further substantiated that the firmness of thawed meat mainly depends on the extent of exudates lost during thawing. As both firmness and drip loss are expected to rely on tissue integrity (Sun, 2011), micrographs were acquired to shade light on the effect of the different freezing techniques on meat tissue. Microstructural changes produced by the different treatments are shown in Figure 7. Upon slow freezing (SF), ice crystals were formed especially in the extracellular space (Figure 7), as previously observed (Anese *et al.*, 2012). This led to intense tissue damage that gave reason for the considerable drip loss and the firmness increase observed upon thawing (Figure 6). Blast freezing reduced such damages. Although ice crystals were preeminently formed outside the cells, their size was smaller than those induced by SF, thanks to the faster process (Figure 3) (Li *et al.*, 2018). On the contrary, under the microscope, the meat subjected to CCF appeared similar to that resulting from SF, with extensive tissue damages and large extracellular crystals. Even though nitrogen flow allowed to considerably reduce the freezing time, meat tissue was severely damaged probably due to the quick temperature drop suffered by the sample encountering the cryogenic fluid, as previously observed (Anese *et al.*, 2012). When this treatment was assisted by radiofrequency (RF-CCF), tissue integrity was better preserved, in agreement with firmness and drip loss results (Figure 6), thus confirming that the application of RF effectively counterbalanced the thermal shock suffered by the sample in the presence of nitrogen flow alone (Fowler & Toner, 2005). Nitrogen pulse (PCF) further protected meat microstructure. In this case, intercellular ice crystals were considerably smaller as compared to those produced by the previously described freezing conditions, and intracellular ones were also formed, resulting in fewer intercellular voids and limited cell disruption (Li *et al.*, 2018). Moreover, the transversal section of muscle bundles could be observed, further indicating good tissue integrity (Zhan *et al.*, 2019). Finally, RF-PCF was the most effective treatment in preserving muscle microstructure, as intercellular ice crystals were even smaller and more evenly distributed. Moreover, being intracellular ice crystals more abundant, it can be inferred that RF protected myofibrils

297 from denaturation, probably induced by PCF alone, making them able to retain the immobilized water
298 (Li *et al.*, 2018).

299 **Conclusion**

300 This study represents the progression of a previous work in which low voltage radiofrequency was used
301 as assisting technology for cryogenic freezing to obtain frozen meat with increased quality. To improve
302 the efficiency of cryogenic freezing, a more homogeneous nitrogen delivery was first achieved by
303 properly modifying the equipment. Secondly, pulsing both nitrogen and radiofrequency instead of
304 continuously delivering them resulted to be a key factor to limit tissue damages, thus improving meat
305 firmness and reducing exudate loss upon thawing. The application of radiofrequency resulted particularly
306 effective in maintaining tissue integrity, probably by limiting myofibril denaturation and thus favoring
307 the intracellular retention of immobilized water.
308 The freezing approach set up in the present study, besides guaranteeing optimal retention of tissue
309 integrity, could be easily adapted to different food matrices thanks to the possibility to finely tune
310 processing conditions (*i.e.*, nitrogen delivery mode, and nitrogen and radiofrequency pulses). In this
311 regard, future research should address other meat derivatives, as well as plant-based foods, with particular
312 attention to those more prone to tissue damages (*e.g.*, berries). The quality of products frozen by applying
313 these conditions should also be investigated during storage to verify whether tissue integrity is retained
314 to a satisfactory level until shelf life. Lastly, since radiofrequency assisted cryogenic freezing can be
315 regarded as a versatile novel freezing technology on a lab scale, further efforts should address its scaling
316 up, pursuing sample size increase, working toward continuous processing, and exploring the possibility
317 to use different and less expensive cryogenic fluids.

CRediT authorship contribution statement

Lara Manzocco: Conceptualization, Writing - original draft; Writing-review & editing; Marilisa Alongi: Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing; Giovanni Cortella: Conceptualization, Resources, Writing - review & editing; Monica Anese: Conceptualization, Supervision, Funding acquisition, 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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Captions for Figures

Figure 1. Schematic representation of the radiofrequency (RF) chamber for cryogenic freezing (a) and insight into nozzle configurations (b and c) for nitrogen delivery.

Figure 2. Radiofrequency (RF) pulse applied during nitrogen (N_2) delivery according to radiofrequency-assisted continuous cryogenic freezing (a, RF-CCF) or radiofrequency-assisted pulsed cryogenic freezing (b, RF-PCF, 3 s pulse with 10 s interval).

Figure 3. Temperature profile of meat during slow (SF), blast (BF), continuous cryogenic (CCF), and pulsed cryogenic (PCF) freezing.

Figure 4. Macroscopic appearance of meat cubes: (a) partially unfrozen upon PCF for 2.5 min with a 5 s nitrogen pulse every 10 s; (b and c) cracked upon PCF for 10 min with a 5 s nitrogen pulse every 10 s interval; (d) cold burned upon PCF for 10 min with 5 s nitrogen pulse every 10 s; (e and f) frozen without defects upon PCF for 10 min with 3 s nitrogen pulse every 10 s.

Figure 5. Macroscopic appearance of meat cubes partially unfrozen (a), overprocessed (b), and frozen without defects (c) upon different cryogenic + radiofrequency freezing conditions.

Figure 6. Firmness (a) and drip loss (b) of fresh (NF) and/or thawed meat upon slow (SF), blast (BF), continuous cryogenic (CCF), pulsed cryogenic (PCF), radiofrequency-assisted continuous cryogenic (RF-CCF), and radiofrequency-assisted pulsed cryogenic (RF-PCF) freezing.

Figure 7. Microscopic appearance of meat frozen by slow (SF), blast (BF), continuous cryogenic (CCF), pulsed cryogenic (PCF), radiofrequency-assisted continuous cryogenic (RF-CCF), and radiofrequency-assisted pulsed cryogenic (RF-PCF) freezing.

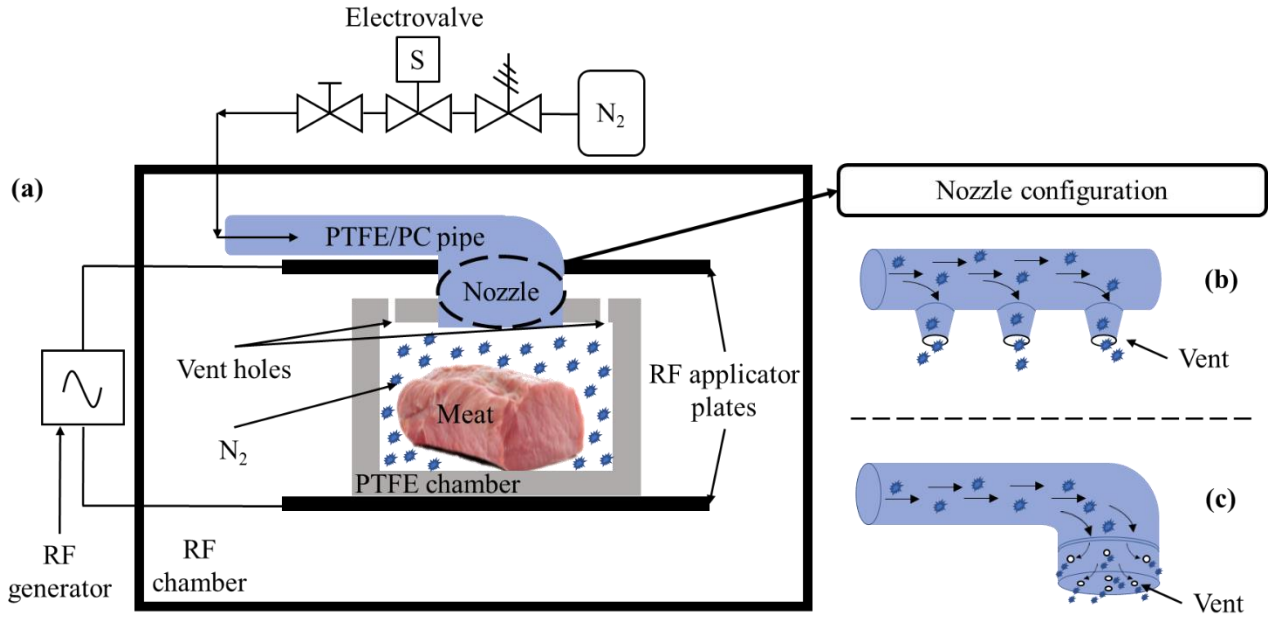

Figure 1.

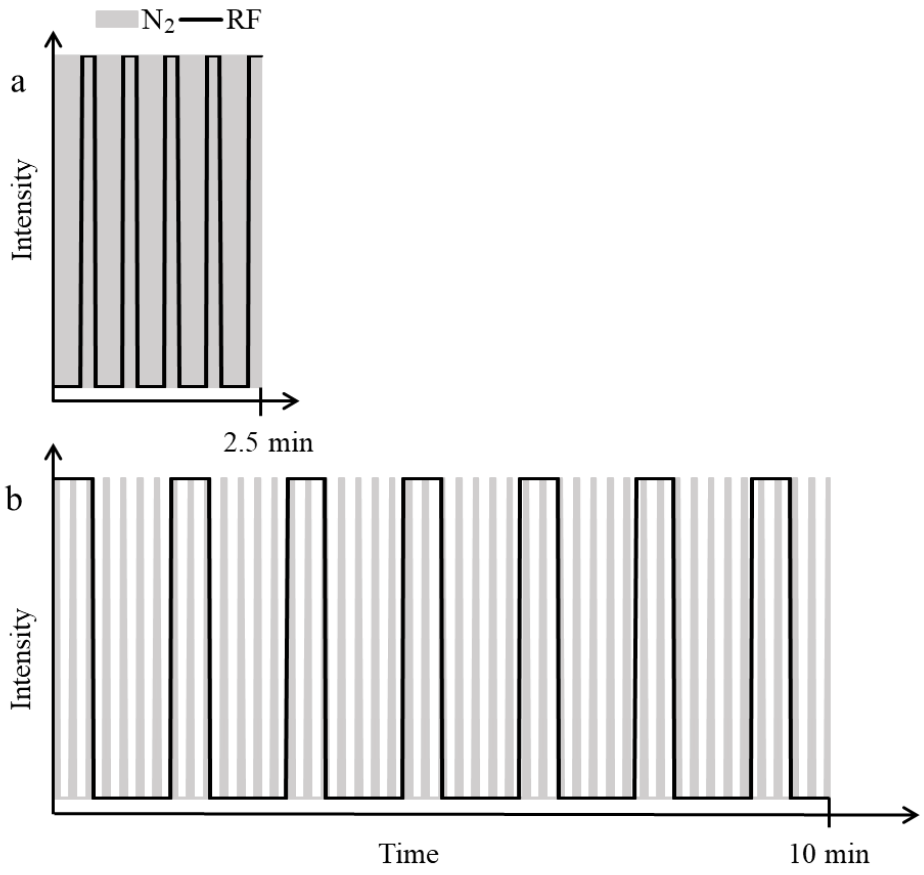

Figure 2.

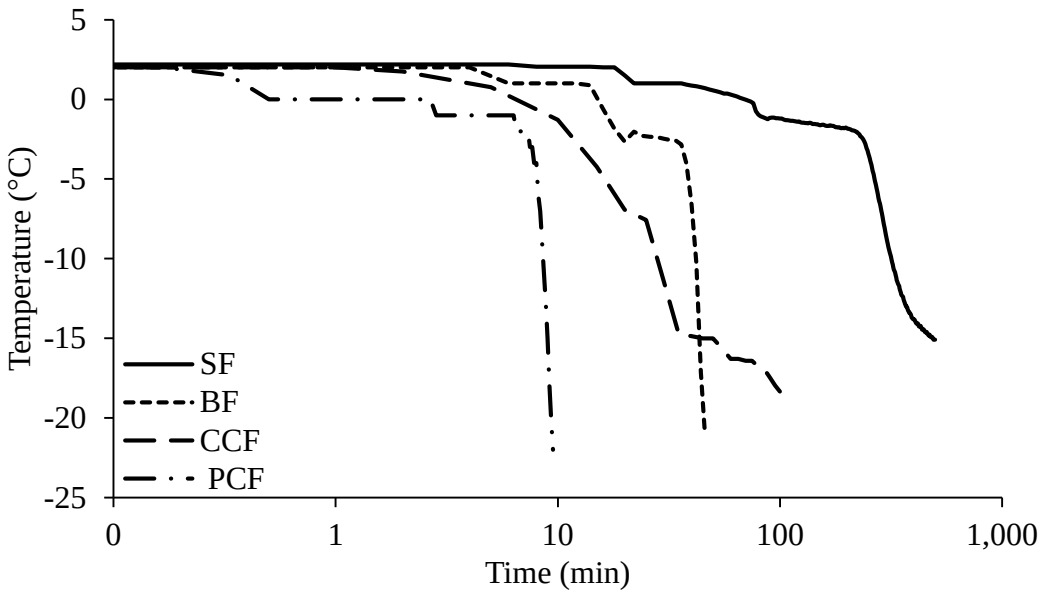

Figure 3.

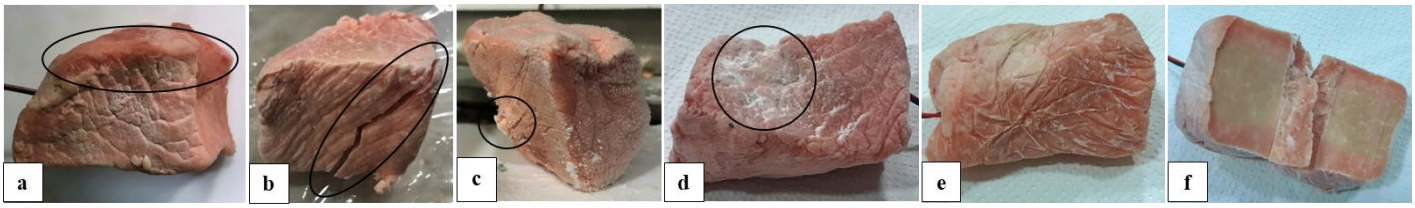

Figure 4.

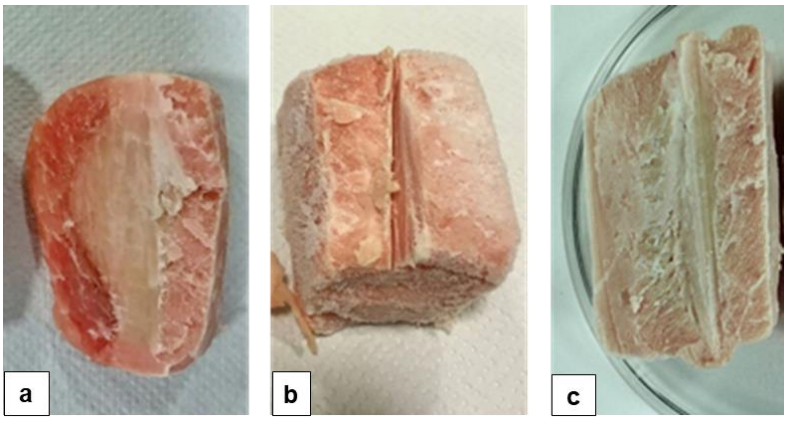

Figure 5.

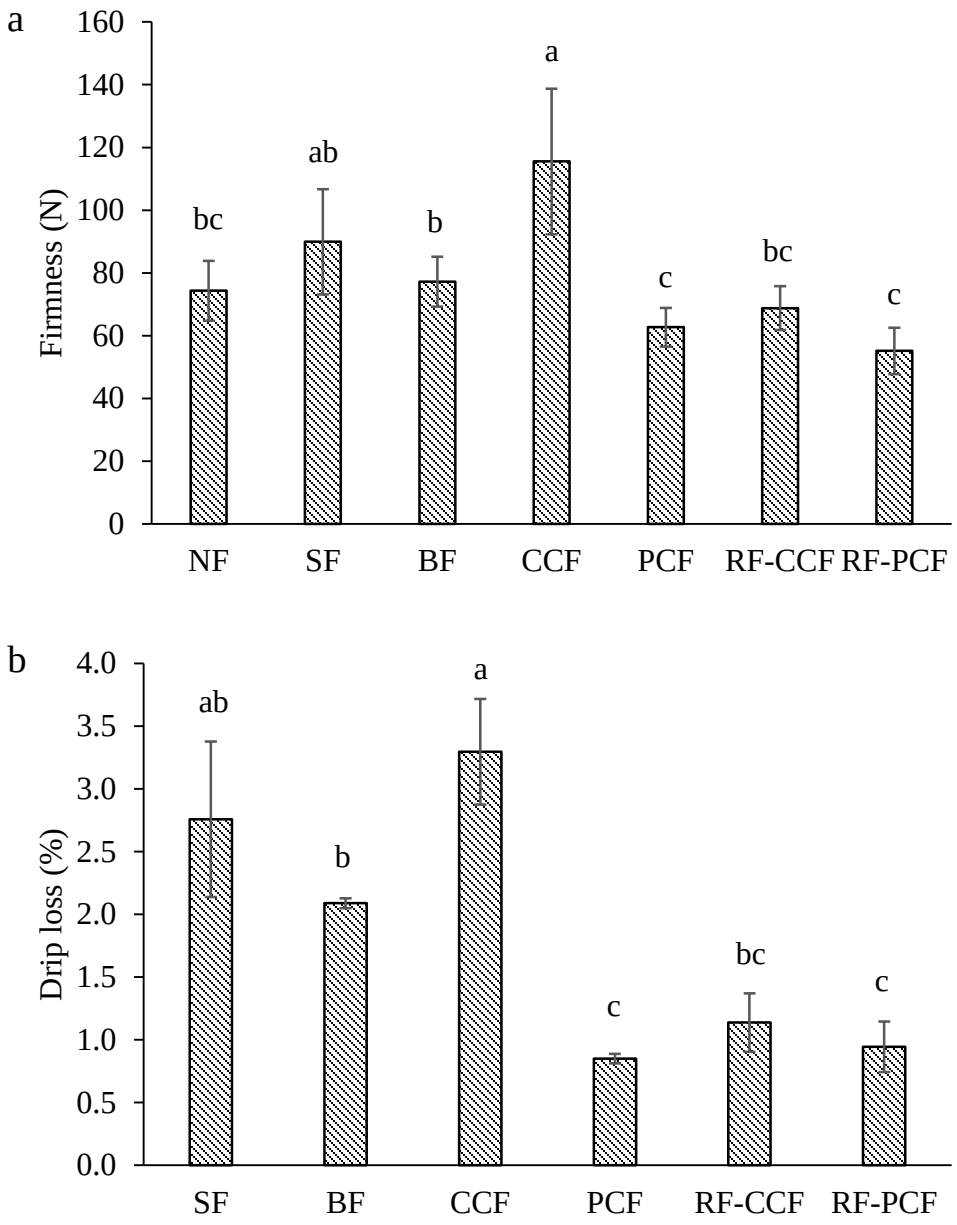

Figure 6.

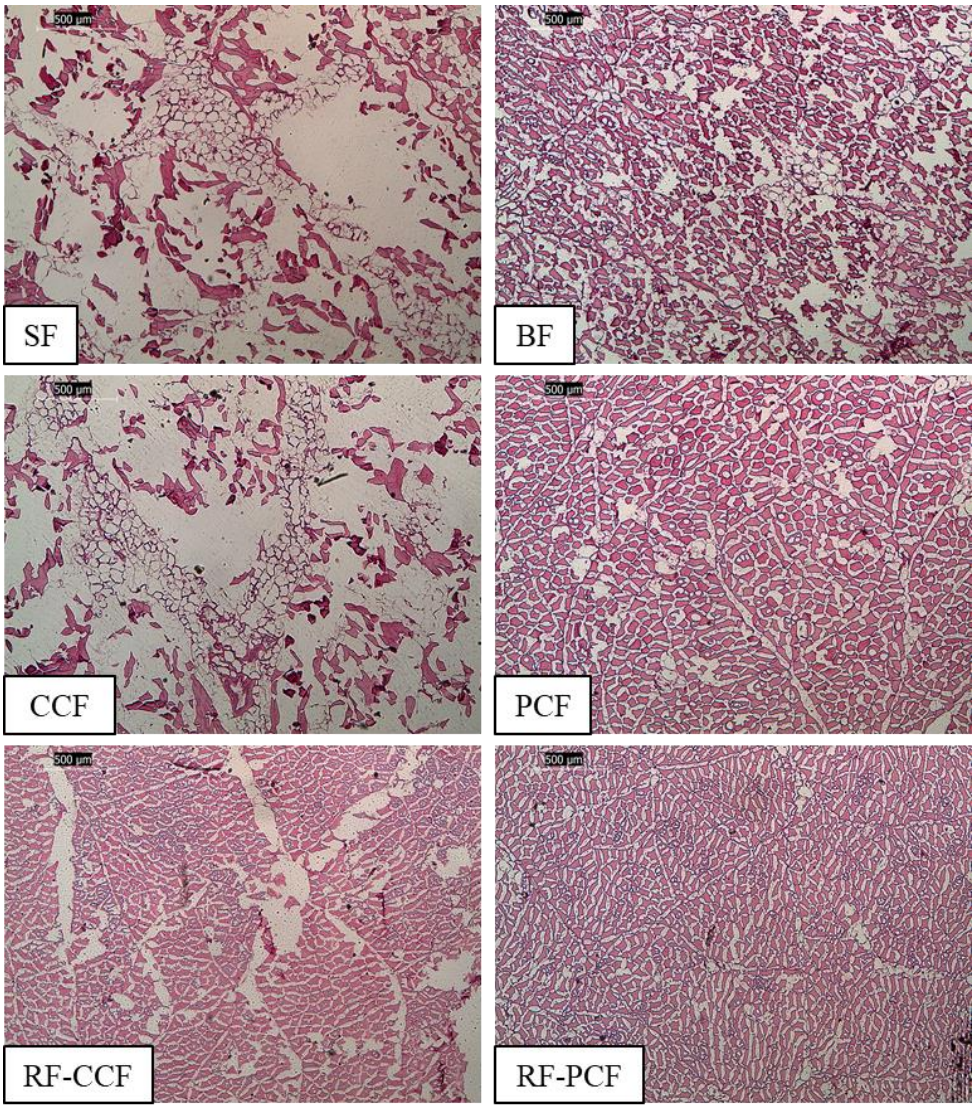

Figure 7.

Table 1

Color (CIE L*a*b* parameters) of fresh (NF) and thawed meat upon slow (SF), blast (BF), continuous cryogenic (CCF), pulsed cryogenic (PCF), radiofrequency-assisted continuous cryogenic (RF-CCF), and radiofrequency-assisted pulsed cryogenic (RF-PCF) freezing.

Sample	Color		
	L*	a*	b*
NF	48.9 ± 2.5 [a]	11.2 ± 0.7 [a]	3.88 ± 0.95 [a]
SF	49.6 ± 2.0 [a]	11.3 ± 0.6 [a]	0.36 ± 1.02 [b]
BF	48.8 ± 3.0 [a]	11.2 ± 1.0 [a]	1.07 ± 0.78 [b]
CCF	47.9 ± 4.1 [a]	9.7 ± 0.3 [b]	3.25 ± 1.55 [a]
PCF	49.5 ± 1.3 [a]	7.2 ± 0.9 [c]	4.39 ± 0.50 [a]
RF-CCF	48.9 ± 4.1 [a]	9.6 ± 0.3 [b]	4.25 ± 1.55 [a]
RF-PCF	49.0 ± 1.6 [a]	7.3 ± 1.0 [c]	4.30 ± 0.39 [a]

n.d. not determined.