A peer-reviewed version of this preprint was published in PeerJ on 18 May 2017.

Lyu C, Nastase G, Ukpai G, Serban A, Rubinsky B. 2017. A comparison of freezing-damage during isochoric and isobaric freezing of the potato. PeerJ 5:e3322 https://doi.org/10.7717/peerj.3322

Isochoric Refrigeration of Food Products

Chenang Lyu [1, 2] , **Gabriel Nastase** [Corresp., 1, 3] , **Gideon Ukpai** [1] , **Alexandru Serban** [3] , **Boris Rubinsky** [1]

[1] Department of Mechanical Engineering, University of California, Berkeley, Berkeley, California, United States

[2] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, Hangzhou, China

[3] Department of Building Services, University Transilvany of Brasov, Brasov, Brasov, Romania

Corresponding Author: Gabriel Nastase
Email address: gabrielnastase@berkeley.edu

Background: Food preservation is essential to the growing world population, food economy. Freezing is a commonly used method for food preservation. While extending the life of the product, freezing has detrimental effects. It is causing loss of food weight and is causing changes in food quality, e.g. enzymatic browning.

Method: Freezing of food is usually done under constant atmospheric pressure (isobaric). We have developed a new technology in which biological materials are preserved at subfreezing temperatures in an isochoric (constant volume) system. Experiments were performed with a food product, potato, in a thermodynamic isochoric device designed by us, that is robust and has no moving parts.

Results: We have shown that under similar storage conditions, freezing to -5°C, the isochoric preserved potato experienced no weight loss and limited enzymatic browning. In contrast the -5°C isobaric frozen potato experienced substantial weight loss and substantial enzymatic browning. Microscopic analysis, shows that the mechanism responsible for the different results is related to the integrity of the cell and the cell membrane, which are maintain during freezing in the isochoric system and lost during freezing in the isobaric system.

Discussion: The main mechanism of cell damage during isobaric freezing are the increase in extracellular osmolality and the mechanical damage by ice crystals. In contrast, during isochoric freezing the cells in the preserved material are under conditions in which the intracellular osmolality is comparable to the extracellular osmolality and they are not affected by ice mechanical damage. The conditions during isochoric freezing result in improved quality of the preserved food products.

Conclusion: We have shown that the quality of food products preserved by isochoric freezing is better than the quality of food preserved to the same temperature in isobaric conditions. This is only a preliminary study on isochoric preservation of food. However, it illustrates the potential of the technology.

Isochoric Refrigeration of Food Products

Chenang Lyu[1,2], Gabriel Năstase[1,3], Gideon Ukpai[1], Alexandru Șerban[3], Boris Rubinsky[1]

[1] Department of Mechanical Engineering, University of California, Berkeley, California, USA

[2] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, Zhejiang, China

[3] Department of Building Services, University of Transilvania, Brasov, Brasov, Romania

Corresponding Author:

Gabriel Năstase[1,3]

737 Balra Dr., El Cerrito, CA, 94530, USA

Email address: gabrielnastase@berkeley.edu

Isochoric Refrigeration of Food Products

Chenang Lyu[1,2,+], Gabriel Năstase[1,3,*,+], Gideon Ukpai[1,+], Alexandru Şerban[3] and Boris Rubinsky[1]

[1]University of California Berkeley, Department of Mechanical Engineering, Berkeley, CA, 94720, USA
[2]Zhejiang University, College of Biosystems Engineering and Food Science, Hangzhou, 310058, China
[3]Transilvania University of Brasov, Department of Building Services, Brasov, 500036, Romania

*Corresponding author:
Gabriel Năstase[1,3]

Email Address: gabrielnastase@berkeley.edu
[+]these authors contributed equally to this work and are listed in alphabetical order

ABSTRACT

Background: Food preservation is essential to the growing world population, food economy. Freezing is a commonly used method for food preservation. While extending the life of the product, freezing has detrimental effects. It is causing loss of food weight and is causing changes in food quality, e.g. enzymatic browning.
Method: Freezing of food is usually done under constant atmospheric pressure (isobaric). We have developed a new technology in which biological materials are preserved at subfreezing temperatures in an isochoric (constant volume) system. Experiments were performed with a food product, potato, in a thermodynamic isochoric device designed by us, that is robust and has no moving parts.
Results: We have shown that under similar storage conditions, freezing to -5°C, the isochoric preserved potato experienced no weight loss and limited enzymatic browning. In contrast the -5°C isobaric frozen potato experienced substantial weight loss and substantial enzymatic browning. Microscopic analysis, shows that the mechanism responsible for the different results is related to the integrity of the cell and the cell membrane, which are maintain during freezing in the isochoric system and lost during freezing in the isobaric system.
Discussion: The main mechanism of cell damage during isobaric freezing are the increase in extracellular osmolality and the mechanical damage by ice crystals. In contrast, during isochoric freezing the cells in the preserved material are under conditions in which the intracellular osmolality is comparable to the extracellular osmolality and they are not affected by ice mechanical damage. The conditions during isochoric freezing result in improved quality of the preserved food products.
Conclusion: We have shown that the quality of food products preserved by isochoric freezing is better than the quality of food preserved to the same temperature in isobaric conditions. This is only a preliminary study on isochoric preservation of food. However, it illustrates the potential of the technology.

INTRODUCTION

Refrigeration, is indispensable in the modern food economy. Low temperatures aid preservation by reducing deleterious chemical reactions in food and inhibition of the growth of microorganisms and other pathogens. In theory, the lower the temperature, the further are chemical reactions rates reduced, and preservation is improved. However, biological matter is mostly water, and lowering the temperature to below the freezing temperature of water produces a marked change in the physical state of the food. The ice crystals that form intracellularly and extracellularly affect the texture of the thawed food and the quality of the preserved food.

Conventional freezing processes occur at constant pressure, isobaric, because this is the thermodynamic state on earth. The preservation is usually done under atmospheric pressure. Our group has recently developed the fundamental thermodynamics of phase transformation of aqueous solutions in an isochoric, constant volume, system (Rubinsky, Perez & Carlson, 2005; Szobota & Rubinsky 2006; Preciado & Rubinsky 2010; Perez et al. 2016; Mikus et al. 2016; Năstase et al. 2016). This study will expand on the previous, mostly theoretical work, and describe results from the first experimental study on a food product, the potato, exposed to isochoric refrigeration at subfreezing temperatures. The value of this technology for frozen-food preservation, will become evident from the analysis of the results.

A fundamental study on the thermodynamics of freezing of aqueous solutions in an isochoric (constant volume) system, was published first in (Rubinsky, Perez & Carlson, 2005). The temperature-pressure phase diagram in the insert in Figure 1 illustrates the difference between the process of freezing in an isobaric (constant pressure) system and an isochoric system. An isobaric freezing process occurs along the vertical line on the temperature-pressure diagram. Freezing under constant atmospheric pressure, was studied extensively, because it is the most accessible thermodynamic system (Hobbs, 2010). Pure water freezes at 0 °C, at a pressure of one atmosphere. Hyperbaric freezing is also of interest for preservation. In hyperbaric freezing the system is at a constant pressure, albeit above the atmospheric pressure. Research was done on hyperbaric freezing, with particular application to electron microscopy (Riehle, 1968; Riehle, 1975; Möbius et al. 2016), food preservation (Tinneberg et al. 1980; Toepfl et al. 2006; Kalichevsky, Knorr, & Lillford, 1995; Rastogi et al. 2007) and living biological matter preservation (Persidsky, 1971; Ahlgren, Dorman & Blackshear, 1971; Huebinger, Han & Grabenbauer, 2016; Fahy, Macfarlane, & Angell, 1983). The hyperbaric process usually occurs in two steps; first the pressure is elevated to a constant value, above the atmospheric pressure, followed by decreasing the temperature at constant pressure. Figure 1 shows that in hyperbaric systems, freezing starts at lower temperatures than in atmosphere, at the point of intersection between the isobaric vertical line and the ice I – water thermodynamic equilibrium curve (liquidus).

In isochoric freezing, the volume is constant while the temperature is decreased. From basic principles of thermodynamic equilibrium, in a two-phase system, temperature and pressure are prescribed by the liquidus curve in Figure 1; until the triple point between ice I, ice III and liquid water. For pure water the pressure and temperature at the triple point are -21.985 °C and 209.9 MPa, respectively. Our thermodynamic analysis has shown, with both mathematical modeling and experiments, that, the process path during the cooling of an isochoric system in the presence of an

ice nucleating agent, is always along the liquidus curve (Rubinsky, Perez & Carlson, 2005). A simplistic intuitive explanation is as follows. The density of ice is less than that of water. Le Chatelier's principle tells us that as water expands upon freezing the high pressure that is generated in a constant volume system, will hinder the further creation of ice. Therefore, the system should minimize the pressure for a given subfreezing temperature. This minimum occurs along the liquidus curve and should follow the curve as the temperature is depressed. This process continues until the triple point is reached. Beyond this point, ice I cannot maintain equilibrium and other types of ice exist that do not expand upon phase change so they do not favor freezing inhibition.

The thermodynamic analysis (Rubinsky, Perez & Carlson, 2005), led to an interesting observation with relevance to, biological matter preservation at subzero Centigrade temperatures. The observation is also depicted in Figure 1. Our analysis and experiments have shown that when freezing is in an isochoric system, about 45% of the initial volume remains unfrozen, at the triple point. Various chemical additives can depress the freezing point temperature. However, at the triple point, about 45% of the volume always remains unfrozen, regardless of the initial composition (Rubinsky, Perez & Carlson, 2005). This has suggested the following concept for biological matter preservation. This concept is illustrated by the two left hand side panels of Figure 2. When a system is designed in such a way that the matter to be preserved occupies less than 45% of the total volume and nucleation is initiated outside the preserved volume, substantial amounts of biological matter can be preserved to the triple point temperature, without freezing.

Our original work was done with cryopreservation of living biological matter in mind (Rubinsky, Perez & Carlson, 2005; Szobota & Rubinsky 2006; Preciado & Rubinsky 2010; Perez et al. 2016; Mikus et al. 2016). We have built and tested several isochoric refrigeration systems. Using one of the isochoric systems, we found that antifreeze proteins behave in a different way under isochoric conditions from isobaric conditions (Preciado & Rubinsky 2010). Recently we have shown that the nematode C. elegans, can survive under isochoric conditions similar to those that may occur at the bottom of lake Vostok in the Antarctica (Mikus et al. 2016). However, our experimental and theoretical studies also suggested the potential of isochoric preservation for the food industry. A theoretical study on this aspect was published in (Năstase et al. 2016). This paper is the first experimental study on the effect of isochoric refrigeration on a food relevant biological material, the potato (*Solanum tuberosum L.*). For this study, we have used an isochoric refrigeration device and thermodynamic conditions similar to those used in (Mikus et al. 2016). This is a first study of its' kind. It is preliminary and no attempt was made to produce comprehensive results. Nevertheless, the study illustrates the potential of isochoric refrigeration for the food industry. Obviously, much more work remains to be done to evaluate the effect of isochoric systems on food products and to explore the value of isochoric preservation to the food industry.

MATERIALS AND METHODS

Isochoric system

The isochoric freezing systems are simple. They require only a constant volume chamber, capable of withstanding the pressures that develop in the system, with minimal deformation. For control, they require a pressure transducer. A photograph of the system is shown in Figure 2, the right-

hand side panel. The isochoric chamber is based on a modified stainless steel OC-1 pressure vessel, (O-ring 316 SS, inner volume 125 ml, working pressure 13,800 psi, test pressure 20,000 psi) custom designed by High Pressure Equipment Company (Erie, PA, USA). We used the standard O-ring made of BUNA-N, for sealing. The constant volume chamber is sealed with a screw and metal seal and is connected to an Ashcroft 4–20 mA Loop-Powered 20,000 psi Pressure gauge, connected through a NI myDAQ Connector (National Instruments, Austin TX) to a laptop and the data recorded and displayed with LabVIEW. For safety, a rupture disk limited the pressure to 60 MPa. The isochoric chamber was immersed in a water-ethylene glycol bath (50/50), cooled by means of a Nestlab RT-140 cooling system (Thermo Scientific, Waltham, MA).

Sample preparation

Russian Banana Fingerling potatoes (*Solanum tuberosum* L.), weight between 12 and 20 g, purchased at a local store, were used in this study. The osmolality of the potatoes was determined in preliminary experiment by measuring the samples' weight loss in different sucrose solution. We found that a solution of 10% w/w sucrose was isotonic with the potatoes. In preparation for the experiments the samples were peeled, cut into cuboid, weighed and enclosed into cryogenic vials (standard 12 mm inner diameter, 1.2 ml, Corning Incorporated cryogenic vial, capped and self-standing) filled with the isotonic sucrose solution (10% w/w) in such a way to ensure there was no air in the vials. We made a small hole (0.5 mm) in the vial's wall to ensure thermodynamic and osmolality equilibrium between the interior of the vial and the interior of the isochoric chamber.

Experimental protocol

The samples were treated in three different procedures: untreated, isochoric treatment, isobaric treatment. The untreated samples were preserved in isotonic sucrose solution at room temperature for 120 min. The isochoric treatment was processed using the isochoric experimental system. A steel nut (the ice nucleating surface) was dropped to the bottom of the isochoric chamber to ensure that ice formation starts at the bottom of the chamber at a distance from the vials, which were on the top of the chamber. The isochoric chamber was filled with isotonic sucrose solution and sealed, with care to avoid the entrapment of air bubbles. It is important to emphasize that care must be exercised to eliminate air from the system. The presence of undissolved air can affect the results (Perez et al. 2016). Then, the chamber was completely immersed in the cooling bath and cooled to -5 °C. The pressure was monitored and recorder in real time, using LabVIEW. It took about 60 min to reach the desired pressure and the experiment was terminated after another 60 min. The isochoric chamber was warmed at room temperature until the pressure reached atmospheric. Then the chamber was opened for sample analysis. The isobaric treatment followed the same procedure as the isochoric treatment except that the chamber was open to atmospheric pressure. The samples were kept in the cooling bath at -5 °C for 120 min.

Sample analysis

Three methods were used to evaluate and compare the untreated samples with samples after isobaric refrigeration and isochoric refrigeration: weight loss, color change and microscopic appearance.

The weight of the sample was measured before and after the treatments by electric balance (ER-182A, A&D Company, Tokyo Japan) to obtain the weight loss. The surface water on the sample was absorbed by filter papers before weighing. This experiment was done in five repeats.

Colorimetric measurements were taken with a color meter (TES-135A, TES Electric electronic CORP. Taiwan) in Hunter L* a* b* color space values after 120 min when the treatments were terminated. Total color difference (ΔE) between the different treated samples was calculated as follows (Cserhalmi et al. 2006):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

The colorimetric experiments were done in three repeats.

The micro structure of potatoes were observed by stereomicroscopy (Lumar, V12 Stereo Zeiss) at a magnification of 45x and 80x immediately after the treatment. The samples were stained by Toluidine Blue O (Obrien, Feder & McCully, 1964). All the treated samples were examined under the microscope.

The statistical analysis was done with the statistical t-test.

RESULTS AND DISCUSSION

An important aspect of isochoric refrigeration relates to technology. An isochoric refrigeration system is essentially a closed container, inserted in a refrigerator. It is a simple and robust device, that does not require maintenance. The technology of an isochoric system is very simple relative to that of a comparable, high pressure freezing (hyperbaric) system. Unlike a hyperbaric freezing system, an isochoric system contains no moving parts and requires no power for continuous operation and there is no concern of sealing or deterioration of moving parts (Rubinsky, Perez & Carlson, 2005; Koch et al. 1996). Figure 2 shows a schematic of the device, a rigid closed container, designed to withstand the pressure, and a photograph of the device used. Figure 2, right panel, shows the isochoric device used in this study. It is a capped cylinder, made from a standard, commercial, stainless steel pressure vessel.

Control over the isochoric refrigeration process is also very simple. Figure 2 shows that we have used a pressure transducer connected to the vessel. In an isochoric refrigeration system, only either temperature or pressure, need to be controlled; because, a two-phase system in a closed fixed volume, is always at thermodynamic equilibrium – from the second law of thermodynamics. Therefore, either pressure or temperature, but not both, completely specify the system. In contrast, in a hyperbaric system there is the need to control both temperature and pressure (Koch et al. 1996).

Figure 3, shows a typical curve depicting the change in pressure with time during the isochoric refrigeration process in our experiments. The interesting aspect is that the pressure reaches steady state and stays at that value for over an hour, to the termination of the experiment. This demonstrates that the isochoric system has reached thermodynamic equilibrium. The time to reach steady state, obviously depends on the thermal mass the device and the heat transfer coefficient to the cooling bath. In all our experiments, the samples reached isochoric thermodynamics equilibrium, and our results represented the state of the treated material after it has reached thermodynamic equilibrium.

Figures 4 and 5 compare, respectively, the weight loss and color change after two hours of: freezing to -5 °C in an isochoric system, freezing to -5 °C in an isobaric weight and storage at room temperature. Figure 6, are microscope micrographs that provide an explanation for the mechanisms involved.

Weight loss during storage, is of concern to the food industry. It occurs during preservation of all foods, including potatoes (Wang, Brandt, Olsen, 2016). Frozen storage is particularly detrimental as it leads to substantial weight loss (Campañone, Salvadori, Mascheroni, 2001; Koch et al. 1996). Figure 4 shows a comparison between the change in weight of the potato samples after two hours of storage in a 10% w/w sucrose solution at: room temperature, -5 °C in isochoric conditions and -5 °C in isobaric condition. The figure shows that there is no statistically significant change in weight neither during storage at room temperature nor during storage at -5 °C in isochoric conditions. In contrast, storage at -5 °C in isobaric conditions resulted in a weight loss of 13.1 +/- 1.1 percent. The weight loss with isobaric freezing observed here is consistent with findings of many other studies (Koch et al. 1996). To the best of our knowledge, the fact that there was no weight loss after isochoric storage at temperatures lower than 0 °C, is unique to isochoric refrigeration.

Browning in raw fruits, vegetables and their processed products is a major problem in the food industry and is believed to be one of the main causes of quality loss during post-harvest handling and processing. The browning reaction in the potato is an important area of research in the food industry and was studied for well over half a century (e.g. Makower & Schwimmer 1954). It results from the oxidation of phenolic compounds under the action of an enzyme called polyphenol oxidase (PPO, phenolese). In the presence of oxygen from air, the enzyme catalyzes the first steps in the biochemical conversion of iron-containing phenolics, that are also found in the potato, to produce quinones, which undergo further polymerization to yield dark, insoluble polymers referred to as "melanins". Browning and the formation of melanins occur in the potato when the PPO enzyme is released through damaged cell membranes. Figure 5 shows the color difference between the samples treated with storage at room temperature, at -5 °C with isochoric refrigeration and at -5 °C with isobaric freezing. For each storage modality, the figure shows a typical photograph of the sample, the total color change $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$, and the changed in lightness, L*. Obviously browning is substantially reduced in isochoric preservation relative to isobaric freezing to the same temperature; which is another potential important attribute of isochoric refrigeration.

Figure 6, shows microscopic images of the treated samples and explains the effects of isochoric refrigeration. The micrographs show the appearance of the samples after staining with the Toluidine stain, at two magnifications, x45 (top row) and x80 (bottom row). In analyzing the micrographs it is important to realize that Toluidine stains the cell membrane in plants as well as the starch (Obrien, Feder & McCully, 1964). The arrow points to the cell membrane. It is obvious that the cell membrane in the room temperature storage and the isochoric -5 °C storage is intact and encircle the cell. In contrast in the isobaric frozen sample at -5 °C, most of the cell membranes are deteriorated and they do not surround an intact cell. Furthermore, in the isobaric frozen sample the Toluidine has stained the entire volume. This suggests that the cell membrane has broken and the intracellular starch has become accessible to the stain throughout the sample. In contrast, there is no staining of starch neither in the room temperature stored sample nor in the isochoric stored sample. The fact that the cell membrane integrity has deteriorated after isobaric freezing and the

intracellular content was released explains both, the changes in weight and the browning of the isobaric preserved samples in relation to the room temperature preserved samples and the isochoric preserved samples in Figures 4 and 5.

An additional mechanism for weight loss during isobaric freezing relates to the composition of the extracellular solution. The major mechanisms of damage to biological materials during slow rate freezing, like those in the isobaric system of this experiment, are the breach in the cell membrane and the increase in extracellular solute osmolality. The cell membrane damage is due to the mechanical effect of ice crystals (Chaw & Rubinsky 1985; Ishiguro & Rubinsky 1994). The increase in extracellular concentration is because ice has a tight crystallographic structure that cannot incorporate solutes (Rubinsky 1983). Therefore, during freezing of biological materials, the concentration of solutes increases when water is removed from the solution as ice. This high extracellular concentration plays a major role in the process of cell death during freezing and in the deterioration of frozen biological materials (Mazur 1970). It also leads to water loss from the intracellular volume to the extracellular space to equilibrate the difference in osmolality. Obviously in isobaric refrigeration, there is no ice in the preserved biological material and, therefore, the mechanism of cell damage by freezing is eliminated. With respect to solute concentration, our analysis and experiments show that when a physiological saline solution is frozen under isobaric, atmospheric conditions, to the triple point, the concentration of saline in the unfrozen volume, at -5 °C is about 1.8 M (Rubinsky, Perez & Carlson, 2005). In contrast, when the physiological saline is frozen to -5 °C under isochoric conditions, the unfrozen milieu composition is almost undistinguishable from isotonic concentration (Rubinsky, Perez & Carlson, 2005). In fact, our analysis and experiments show that when a physiological saline solution is frozen under isobaric, atmospheric conditions, to the triple point, the concentration of saline in the unfrozen volume, at – 20 °C, is about 5 M (Rubinsky, Perez & Carlson, 2005). In contrast, when the physiological saline is frozen to – 20 °C under isochoric conditions, the unfrozen milieu composition is 0. 75 M (Rubinsky, Perez & Carlson, 2005). This has significance for biological matter preservation in the field of cryobiology and food preservation. It should be noticed, though, that in isochoric refrigeration the pressure increases, while in an isobaric system the pressure remains constant. Obviously, this is a potential mechanism of cell damage during isochoric freezing, that does not exist in isobaric freezing. However, the increase in pressure is hydrostatic and mild. Experiments have shown that even whole livers can survive the pressures in our isochoric experiments conditions (Takahashi et al. 2001). This should explain why the cell membrane is intact and the intracellular content is maintained in isochoric refrigeration. The integrity of the cell membrane and the isosmotic composition of the intracellular milieu and the extracellular milieu during isochoric refrigeration is the reason why there is no weight loss or substantial browning during isochoric refrigeration to -5 °C; as shown in Figures 4 and 5. In contrast the breaching of the cell membrane and the hyperosmotic extracellular concentration in isobaric freezing to -5 °C, results in weight loss to the extracellular milieu and browning of the intracellular and membrane enzymes.

In summary, this is a first experimental study on the feasibility of isochoric refrigeration of a food product. While obviously, much more research must be done on this technology, it is evident that a food product, the potato, can be preserved at -5 °C in isochoric conditions without the deleterious effects of freezing to -5 °C, i.e. weight loss and browning.

We would like to add that at first sight it may appear that isochoric systems are similar to high pressure isobaric systems (Tinneberg et al. 1980; Toepfl et al. 2006; Kalichevsky, Knorr, Lillford, 1995; Rastogi et al. 2007; Persidsky 1971). However, there is a fundamental difference in the thermodynamics. In isochoric systems conditions for thermodynamic equilibrium are derived from minimization of the Helmholtz free energy. In contrast, in isobaric (constant pressure) systems, thermodynamic equilibrium conditions are derived from minimization of the Gibbs free energy. The de Chatelier's principle will lead to different outcomes in an isochoric system from an isobaric system. For example, in isochoric systems, the critical radius for ice nucleation in pure water can be formed only at temperatures lower than - 100 °C (Szobota & Rubinsky 2006). In isobaric systems, this critical radius can occur from temperatures lower than 0 °C. In fact, our theoretical work predicts that the homogeneous nucleation temperature in an isochoric system, will be lower than the glass transition temperature (Szobota & Rubinsky 2006), suggesting that isochoric refrigeration can be conducive to vitrification. This is an interesting topic of further studies.

ACKNOWLEDGEMENTS

The assistance of Dr. Steven Ruzin to the microscope study is gratefully acknowledged.

FINANCIAL SUPPORT

This study was supported by the discretionary funds of the Mechanical Engineering Department at UC Berkeley to B. Rubinsky. Chenang Lyu was supported by Zhejiang University, Hangzhou, China. Gabriel Nastase was supported by CRIOMEC SA, 63, Drumul de Centura Street 800248 Galati, Romania

CONFLICT OF INTEREST

No conflict of interest exists.

LIST OF FIGURES

Figure 1. Pressure/temperature phase diagram of an isochoric system.

Figure 2. Schematic of an isochoric system (two left panels). Photograph of the isochoric system, right panel.

Figure 3. Change in pressure with time in the isochoric system.

Figure 4. Weight loss after room temperature preservation, isochoric refrigeration and isobaric freezing.

Figure 5. Colorimetric measurements - after room temperature preservation, isochoric refrigeration and isobaric freezing. ΔE (dark) and L* light data columns. The values on left are for both, ΔE and L*. Inserts, macroscopic photographs of the potato samples.

Figure 6. Microscopic photographs of the potato after room temperature preservation, isochoric refrigeration and isobaric freezing. Top row, x45, bottom row x 80. The arrow points to a typical cell membrane. Note the colors in the micrographs.

REFERENCES

391 Ahlgren, F., Dorman, F. & Blackshear, P.L., 1971. Hyperbaric blood freezing. *Cryobiology*, 8(4),
392 p.380. Available at: http://linkinghub.elsevier.com/retrieve/pii/0011224071901490.
393 Campañone, L.A., Salvadori, V.O. & Mascheroni, R.H., 2001. Weight loss during freezing and
394 storage of unpackage foods. *Journal of Food Engineering*, 47, pp.69–79.
395 Chaw, M.W. & Rubinsky, B., 1985. Cryomicroscopic observations on directional solidification in
396 onion cells. *Cryobiology*, 22(4), pp.392–399.
397 Cserhalmi, Z. et al., 2006. Study of pulsed electric field treated citrus juices. *Innovative Food*
398 *Science and Emerging Technologies*, 7(1–2), pp.49–54.
399 Fahy, G.M., Macfarlane, D.R. & Angell, C.A., 1983. Vitrification as an approach to
400 cryopreservation. *Cryobiology*, 20, pp.699–699.
401 Hobbs, P. V., 2010. Ice Physics. , p.856. Available at:
402 http://books.google.com/books?id=wnnkQwAACAAJ&pgis=1.
403 Huebinger, J., Han, H.M. & Grabenbauer, M., 2016. Reversible cryopreservation of living cells
404 using an electron microscopy cryo-fixation method. *PLoS ONE*, 11(10).
405 Ishiguro, H. & Rubinsky, B., 1994. Mechanical interactions between ice crystals and red blood
406 cells during directional solidification. *Cryobiology*, 31(5), pp.483–500. Available at:
407 http://www.sciencedirect.com/science/article/pii/S0011224084710595.
408 Kalichevsky, M.T., Knorr, D. & Lillford, P.J., 1995. Potential food applications of high-pressure
409 effect on ice-water transitions. *Trends in Food Science & Technology*, 6, pp.253–259.
410 Koch, H. et al., 1996. Pressure-shift freezing and its influence on texture, colour, microstructure
411 and rehydration behaviour of potato cubes. *Nahrung-Food*, 40, pp.125–131.
412 Makower, R. U. & Schwimmer, S., 1954. Inhibition of enzymic color formation in potato by
413 adenosine triphosphate. *Biochimica Et Biophysica Acta*, 14, pp.156–157.
414 Mazur, P., 1970. Freezing of Biological Systems. *Science*, 168, p.939-.
415 Mikus, H. et al., 2016. The nematode Caenorhabditis elegans survives subfreezing temperatures
416 in an isochoric system. *Biochemical and Biophysical Research Communications*, 477(3),
417 pp.401–405.
418 Möbius, W., Nave, K.A. & Werner, H.B., 2016. Electron microscopy of myelin: Structure
419 preservation by high-pressure freezing. *Brain Research*, 1641, pp.92–100.
420 Năstase, G. et al., 2016. Advantages of isochoric freezing for food preservation: A preliminary
421 analysis. *International Communications in Heat and Mass Transfer*, 78, pp.95–100.
422 Obrien, T.P., Feder, N. & McCully, M.E., 1964. Polychromatic staining of plant cell walls by
423 toluidine blue. *Protoplasma*, 59, p.368-.
424 Perez, P.A. et al., 2016. The effect of undissolved air on isochoric freezing. *Cryobiology*, 72(3),
425 pp.225–231.
426 Persidsky, M., 1971. Cryopreservation under high hydrostatic pressure. *Cryobiology*, 8, p.380.
427 Preciado, J.A. & Rubinsky, B., 2010. Isochoric preservation: A novel characterization method.
428 *Cryobiology*, 60(1), pp.23–29.
429 Rastogi, N.K. et al., 2007. Opportunities and challenges in high pressure processing of foods. *Crit*
430 *Rev Food Sci Nutr*, 47(1), pp.69–112. Available at:
431 http://www.tandfonline.com/doi/abs/10.1080/10408390600626420%5Cnhttp://www.ncbi.nl
432 m.nih.gov/pubmed/17364696.
433 Riehle, U., 1975. High pressure freezing - present outlook. *Arzneimittel-Forschung/Drug*
434 *Research*, 25, pp.453–453.
435 Riehle, U., 1968. Schnellgefrieren organischer Präparate für die Elektronen-Mikroskopie. *Chemie*
436 *Ingenieur Technik*, 40(5), pp.213–218. Available at:

http://reichling.physik.uos.de/download_paper.php?paper=ChemIngTech40p213(1968)_Riehle.pdf.

Rubinsky, B., 1983. Solidification processes in saline solutions. *Journal of Crystal Growth*, 62(3), pp.513–522.

Rubinsky, B., Perez, P.A. & Carlson, M.E., 2005. The thermodynamic principles of isochoric cryopreservation. *Cryobiology*, 50(2), pp.121–138.

Szobota, S.A. & Rubinsky, B., 2006. Analysis of isochoric subcooling. *Cryobiology*, 53(1), pp.139–142.

Takahashi, T. et al., 2001. Preservation of rat livers by supercooling under high pressure. *Transplantation Proceedings*, 33(1–2), pp.916–919.

Tinneberg, H.R. et al., 1980. High hydrostatic pressure as an improvement for sperm-preservation. *Archives of Andrology*, 5, pp.42–43.

Toepfl, S. et al., 2006. Review: Potential of High Hydrostatic Pressure and Pulsed Electric Fields for Energy Efficient and Environmentally Friendly Food Processing. *Food Reviews International*, 22(4), pp.405–423.

Wang, Y., Brandt, T.L. & Olsen, N.L.A., 2016. A Historical Look at Russet Burbank Potato (Solanum tuberosum L.) Quality Under Different Storage Regimes. *American Journal of Potato Research*, 93, pp.474–484.

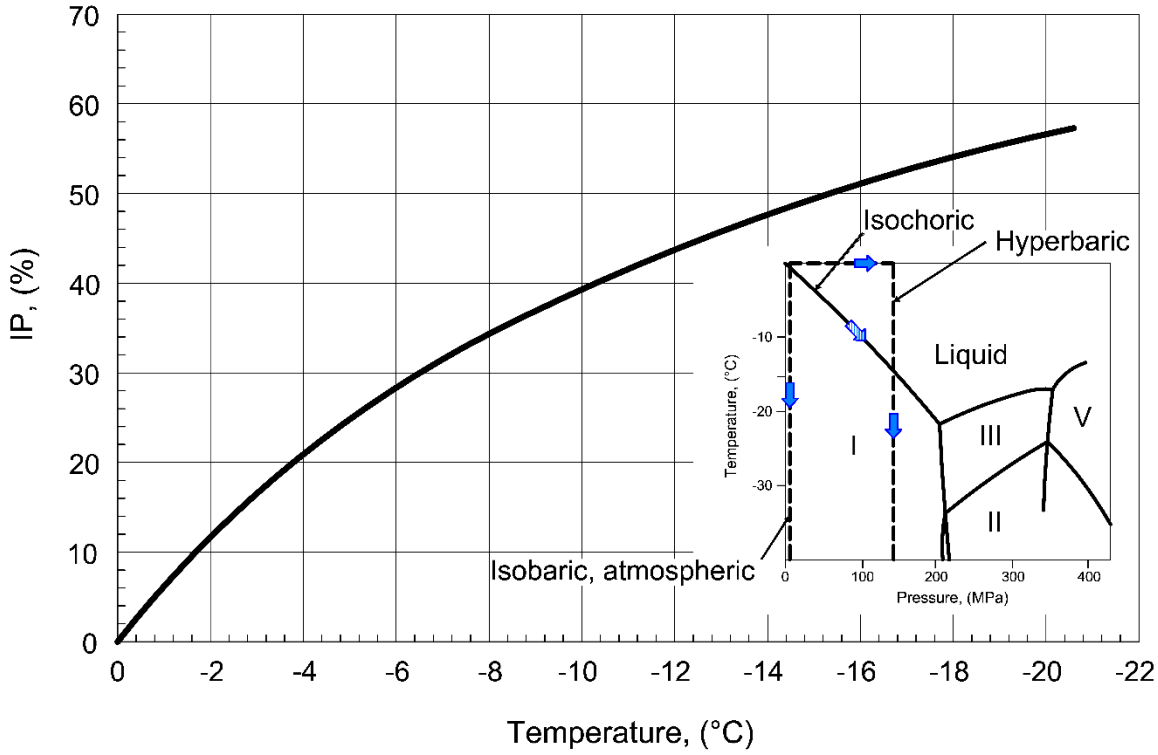


Figure 1. Pressure/temperature phase diagram of an isochoric system.

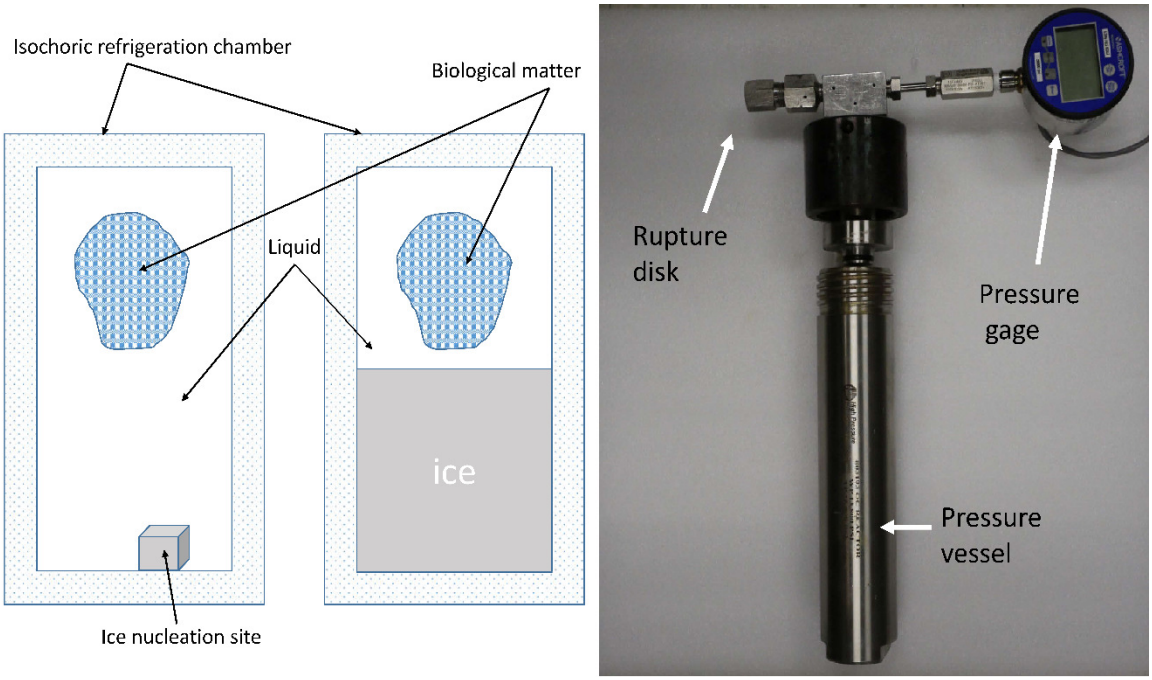


Figure 2. Schematic of an isochoric system (two left panels). Photograph of the isochoric system, right panel.

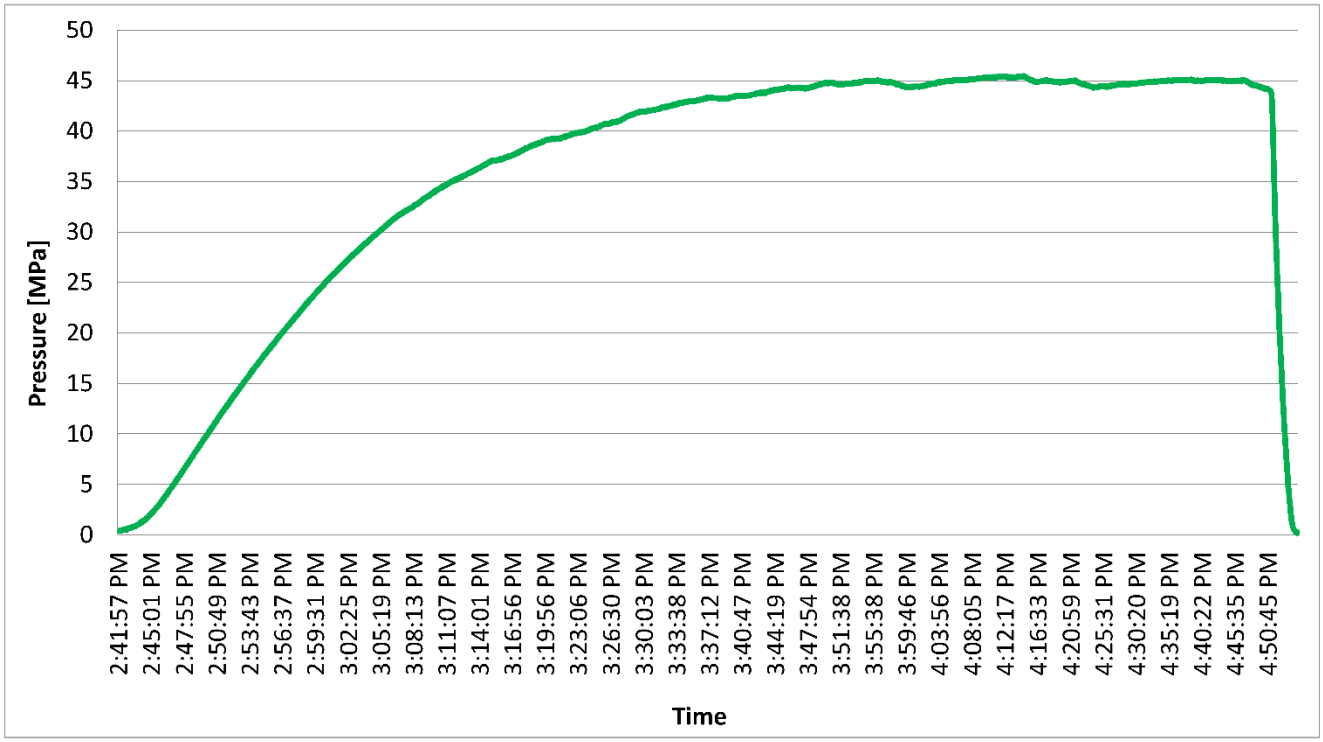
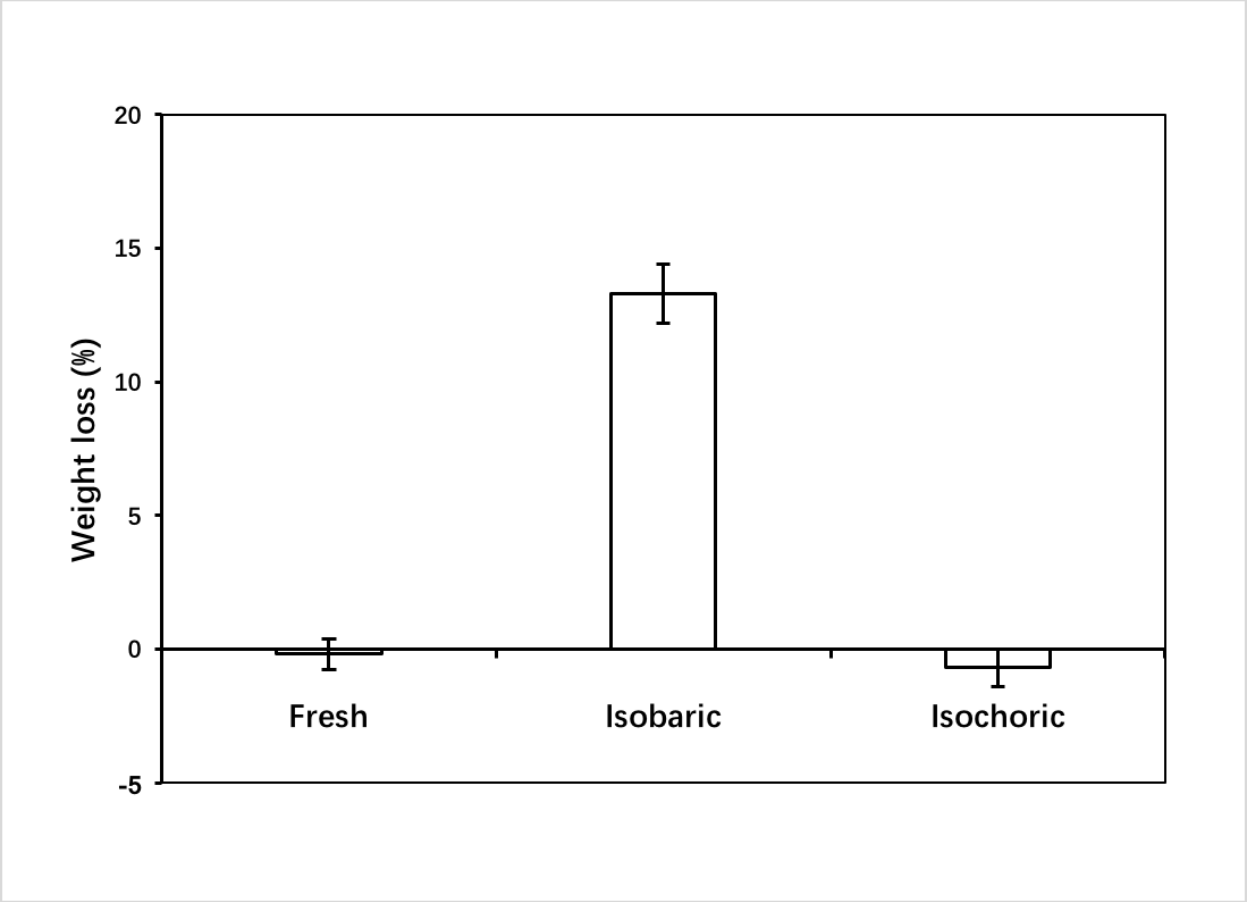


Figure 3. Change in pressure with time in the isochoric system.



531

532 **Figure 4.** Weight loss after room temperature preservation, isochoric refrigeration and isobaric
533 freezing.

534

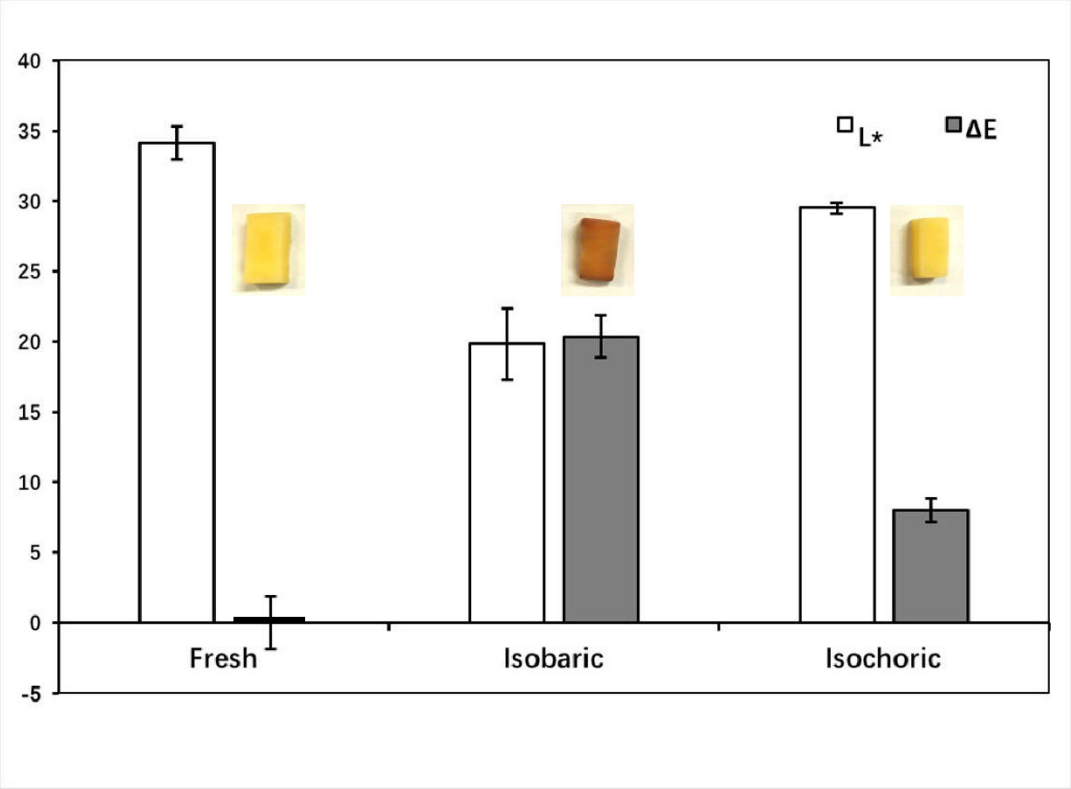
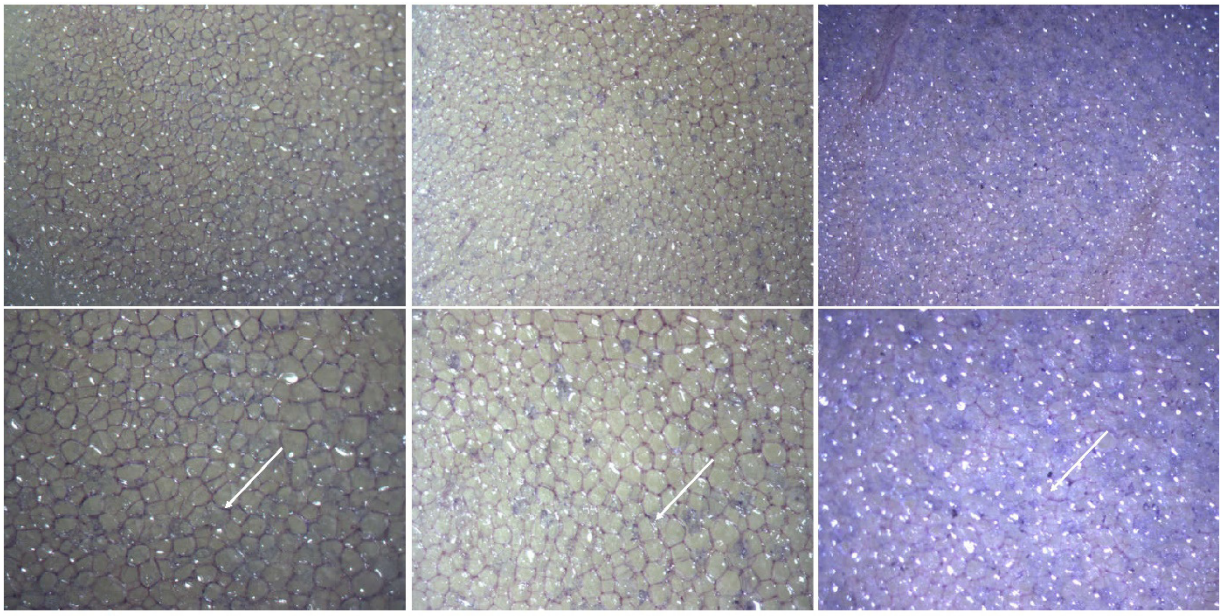


Figure 5. Colorimetric measurements - after room temperature preservation, isochoric refrigeration and isobaric freezing. ΔE (dark) and L* light data columns. The values on left are for both, ΔE and L*. Inserts, macroscopic photographs of the potato samples.

551



552

553 **Figure 6.** Microscopic photographs of the potato after room temperature preservation, isochoric
554 refrigeration and isobaric freezing. Top row, x45, bottom row x 80. The arrow points to a
555 typical cell membrane. Note the color in the micrographs.