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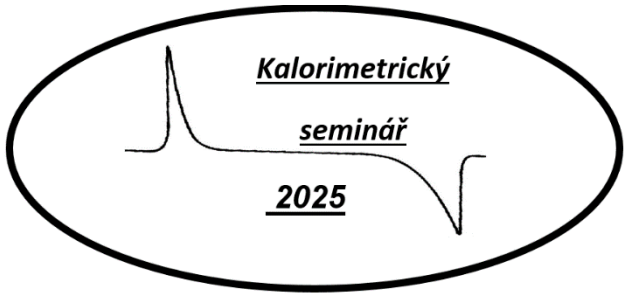
Cell Survival in Cryopreservation: The Critical Role of Water

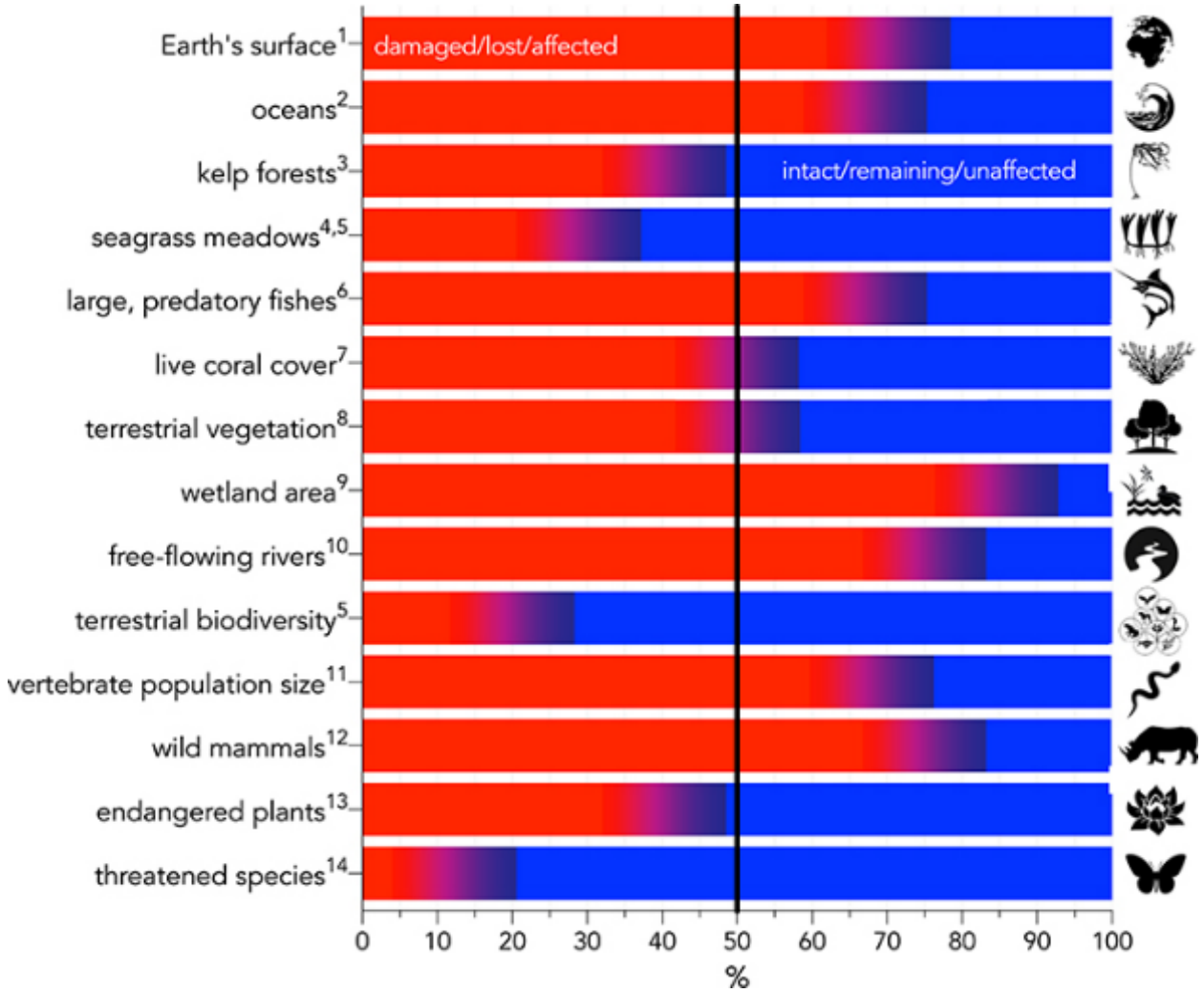
Olena Bobrova,

Miloš Faltus, Alois Bilavčík, Jiří Zámečník, Lesia Golosna, Anton Prystalov, Viktor Chizhevskiy

Institute for Problems of Cryobiology & Cryomedicine



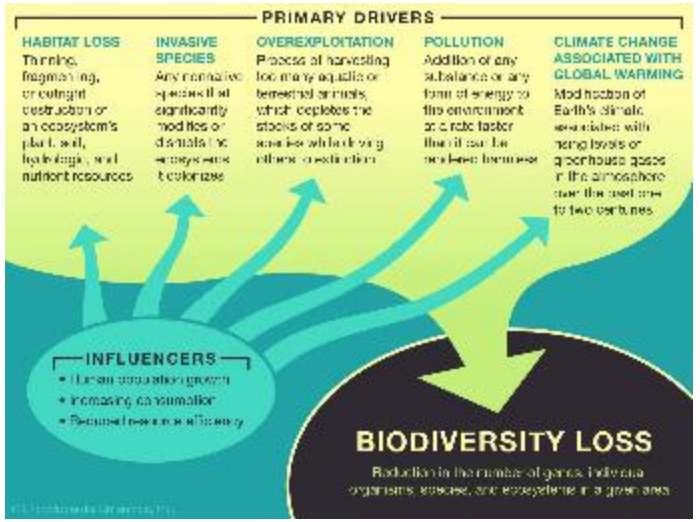
Czech Agrifood Research Center



Almost 600 plant species have been lost from the wild in the last 250 years [Humphreys A. M. et al., 2019].

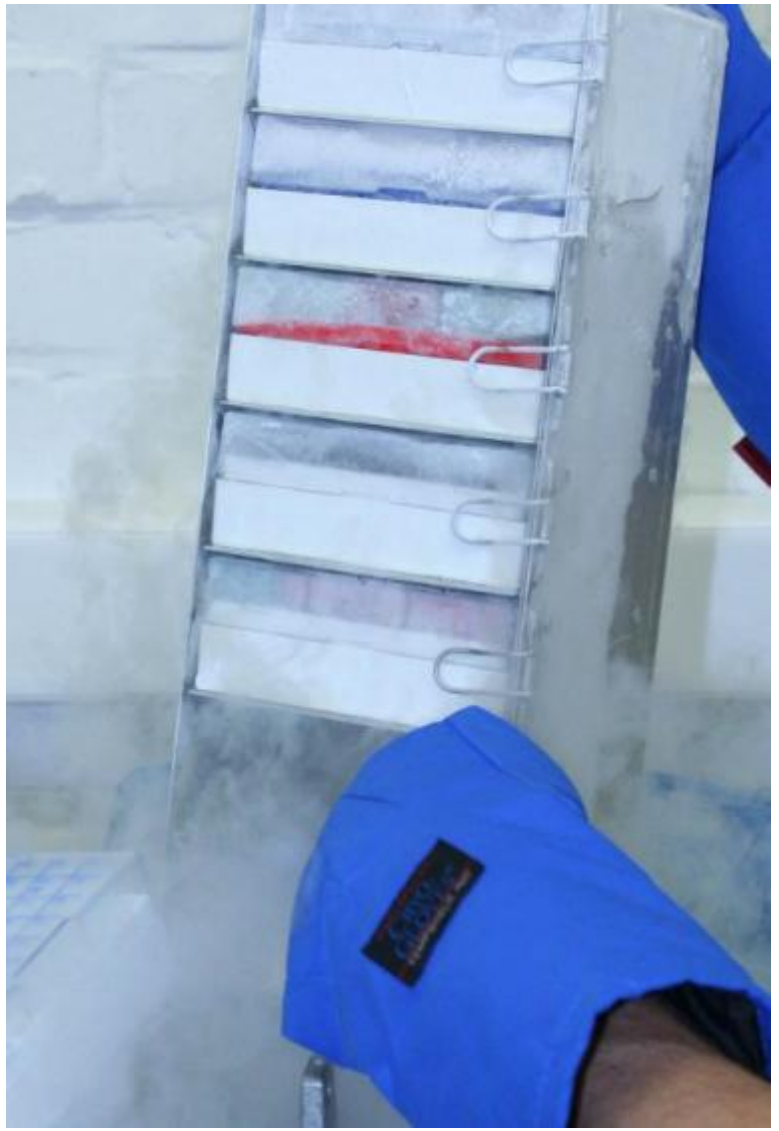
> *"Ecosystems, species, wild populations, local varieties and breeds of domesticated plants and animals are shrinking, deteriorating or vanishing. The essential, interconnected web of life on Earth is getting smaller and increasingly frayed," said Prof. Settele. "This loss is a direct result of human activity and constitutes a direct threat to human well-being in all regions of the world."*

PARIS, May 2019– report from the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)



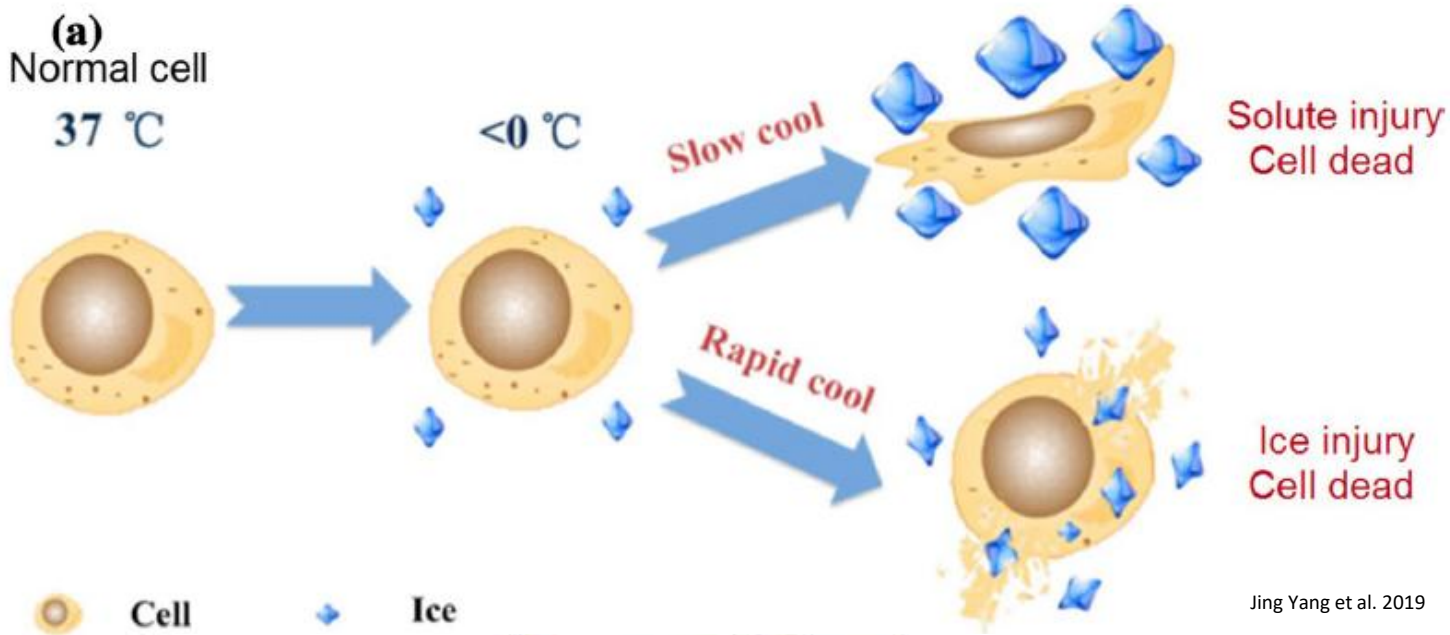
Cryopreservation

- Preservation of Genetic Diversity
- Ecosystem stability
- Agricultural resilience
- Conservation of species
- Human well-being
- Disease-Free Storage
- Support for Biotechnology and Research
- Sustainability and Cost-Effectiveness
- Supporting Global Genetic Resource Networks
- Minimizing Genetic Drift and Maintaining Traits
- Crisis Resilience

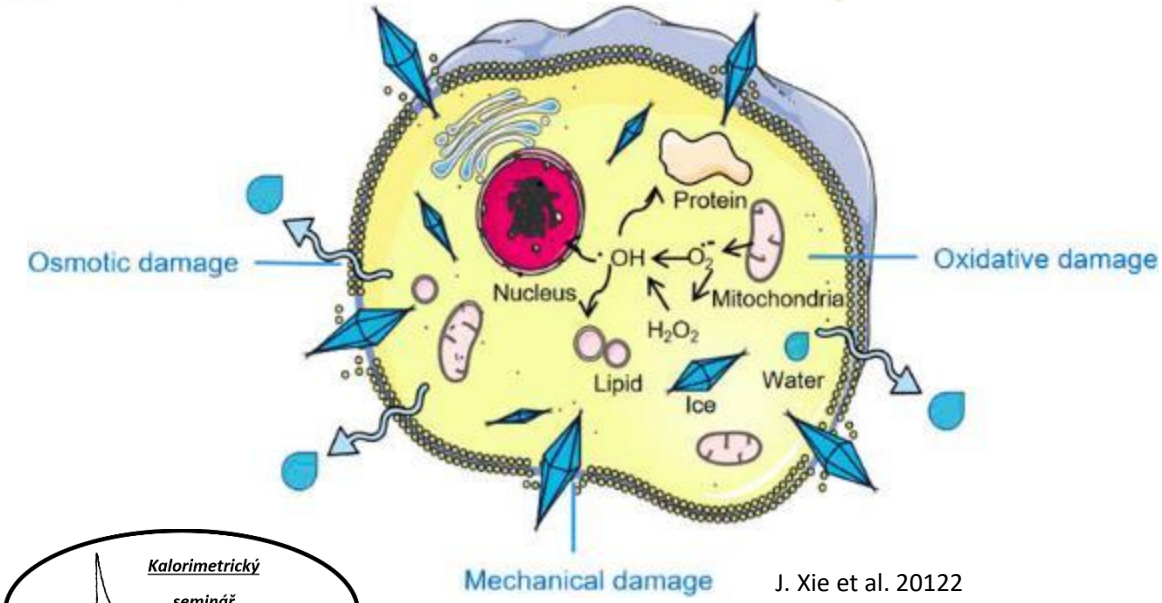


Mechanisms of Cryoinjury

- Ice Formation
- Dehydration
- Osmotic Stress
- Membrane Damage
- Thawing Injuries



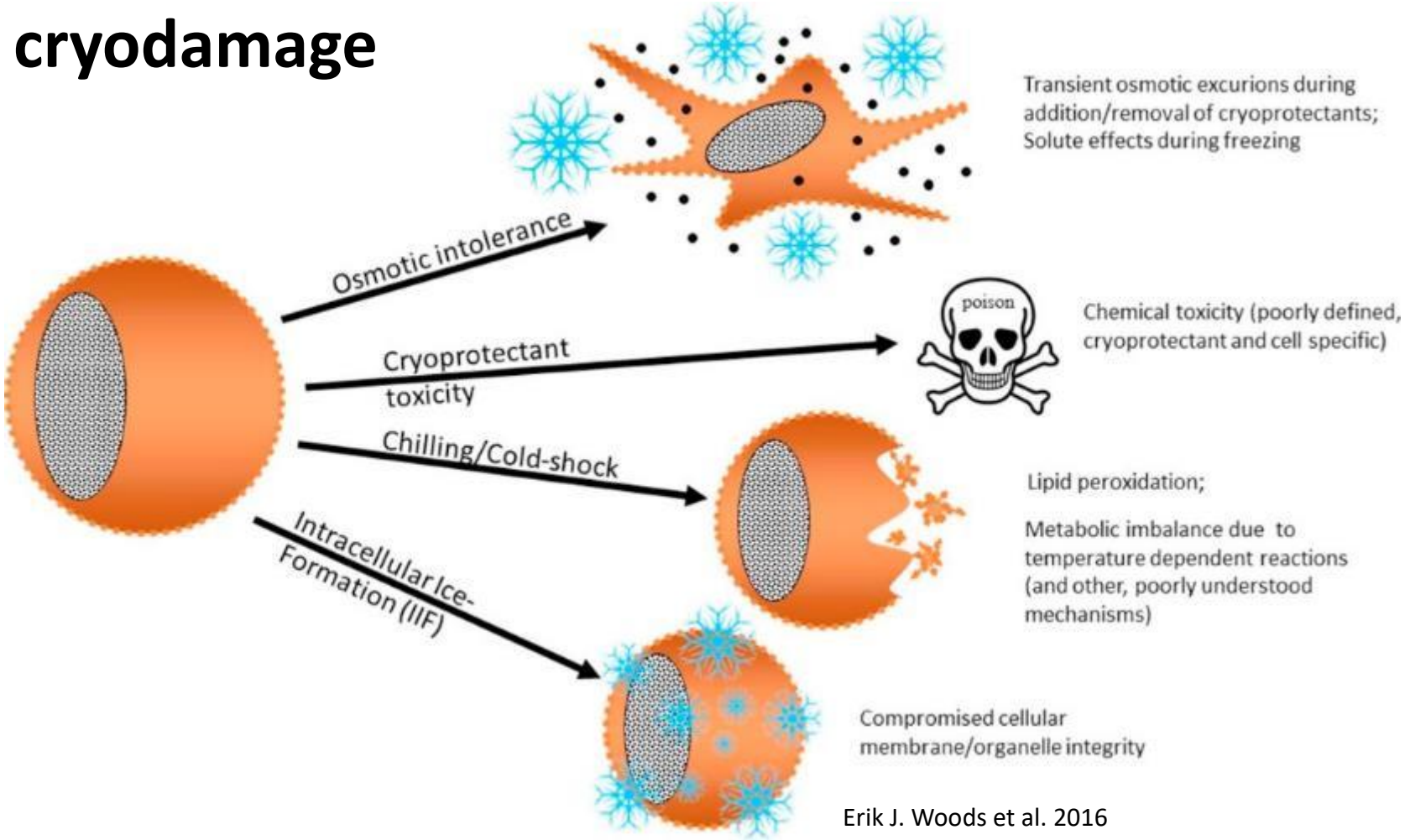
Jing Yang et al. 2019



J. Xie et al. 20122

Strategies to prevent cryodamage

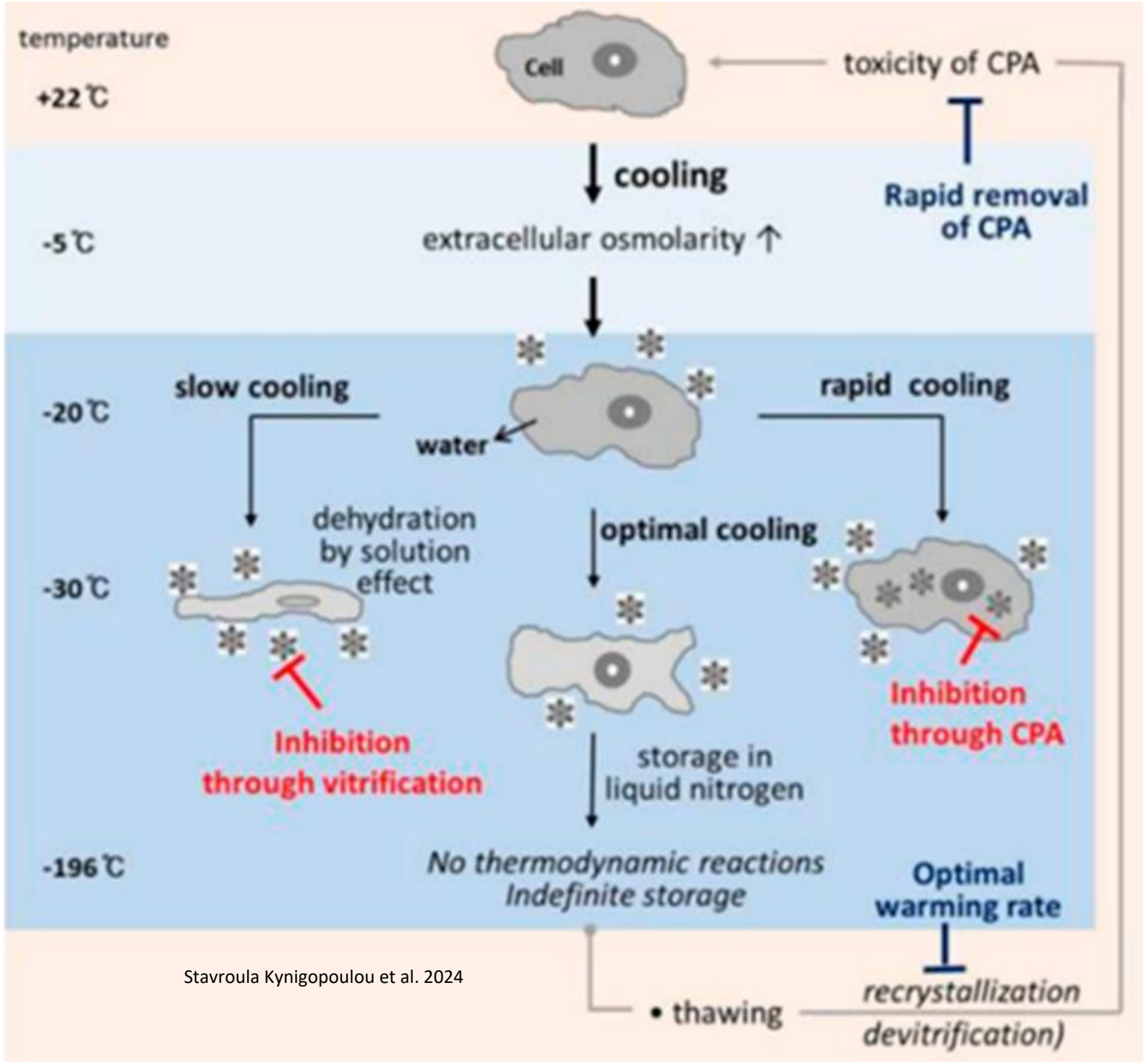
- **Use of Cryoprotectants**
- **Controlled Freezing Rates**
- **Minimizing Osmotic Stress**
- **Hydration Management**
- **Optimizing Cooling Protocols**
- **Antioxidant Protection**
- **Use of Protective Additives**
- **Post-Thaw Recovery Optimization**



Erik J. Woods et al. 2016

Kalorimetrický seminář 2025

Cryodamage Avoidance Strategies



Stavroula Kynigopoulou et al. 2024

Kalorimetrický seminář 2025

Cryobiology
Volume 118, March 2025, 105167

Nanocrystalline cerium dioxide reduces recrystallization in cryopreservation solutions

Olena Bobrova [a b], Oksana Falko [a], Anna Polyakova [a], Volodymyr Klochkov [c], Miloš Faltus [b], Viktor Chizhevskiy [a]

Table 1. The effect of CeO_2 NPs on the onset and enthalpy of crystallization during cooling and melting during warming at 10°C/min. Data are presented as mean±SD.

Sample	Crystallization		Melting	
	T, °C	Enthalpy, J/g	T, °C	Enthalpy, J/g
Water	−9.6±2.57	282.6±4.61	0.0±0.05	336.9±4.62
0.02 g/L CeO_2	−8.6±1.52	284.4±14.40	−0.4±0.06[a]	327.3±11.84
1 g/L CeO_2	−7.7±0.69[a]	290.7±8.62	−0.6±0.10[a]	329.0±3.20[a]
2.1 g/L CeO_2	−9.6±0.55	279.7±5.49	−0.5±0.49[a]	323.3±2.99[a]

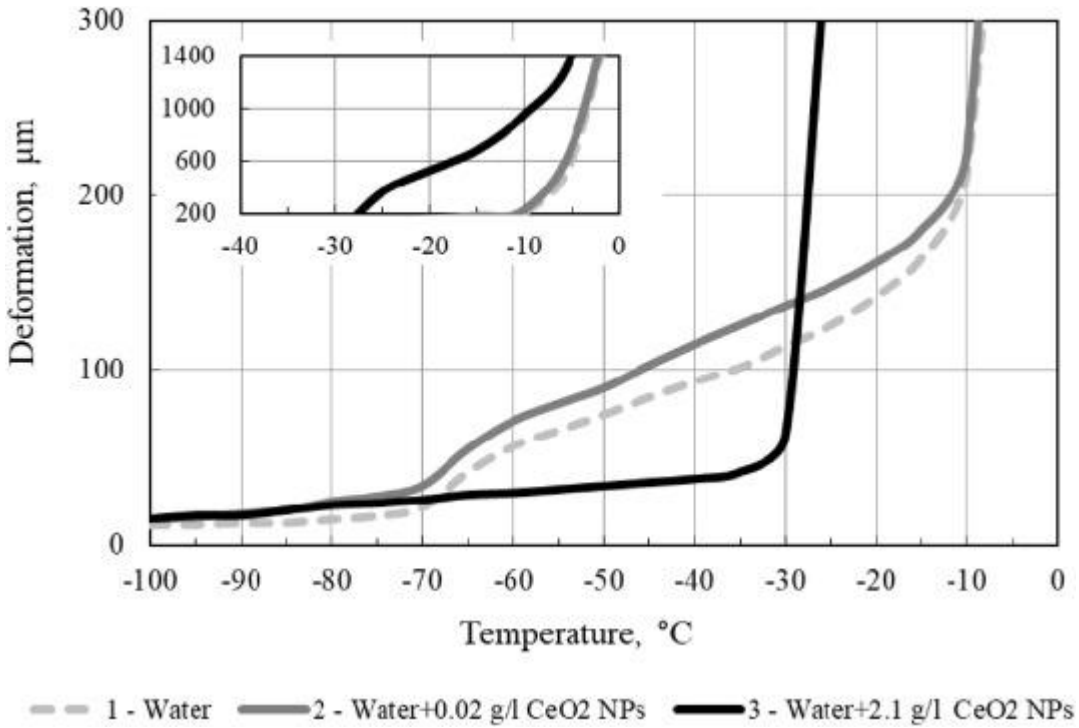


Fig. 1. TMA curves of distilled water and aqueous solutions of CeO_2 NPs in final concentrations of 0.02 g/L and 2.1 g/L under the same experimental conditions: 20°C/min cooling rate, 1°C/min heating rate, $\sigma=2\cdot 10^5$ kg/m^2.

20 °C/min cooling rate, 1 °C/min heating rate, σ = 2• 10^5 kg/m^2

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Table 2. The effect of CeO_2 NPs on the onset and enthalpy of crystallization during cooling and melting during warming at 10 °C/min in 1% Me_2SO solution. Data are presented as mean±SD.

Sample	Crystallization		Melting	
	T, °C	Enthalpy, J/g	T, °C	Enthalpy, J/g
1% Me_2SO	−8.4±0.10	280.0±10.17	−1.98±0.26	307.9±8.10
1% Me_2SO +0.02 g/L CeO_2	−11.1±1.12	274.2±4.07	−2.04±0.49	308.5±4.81
1% Me_2SO+1 g/L CeO_2	−8.4±1.23	269.1±2.12[a]	−1.9±0.28	297.8±7.39[a]
1% Me_2SO+2.1 g/L CeO_2	−9.2±0.80	272.7±7.12	−2.4±0.32[a]	296.4±6.57[a]

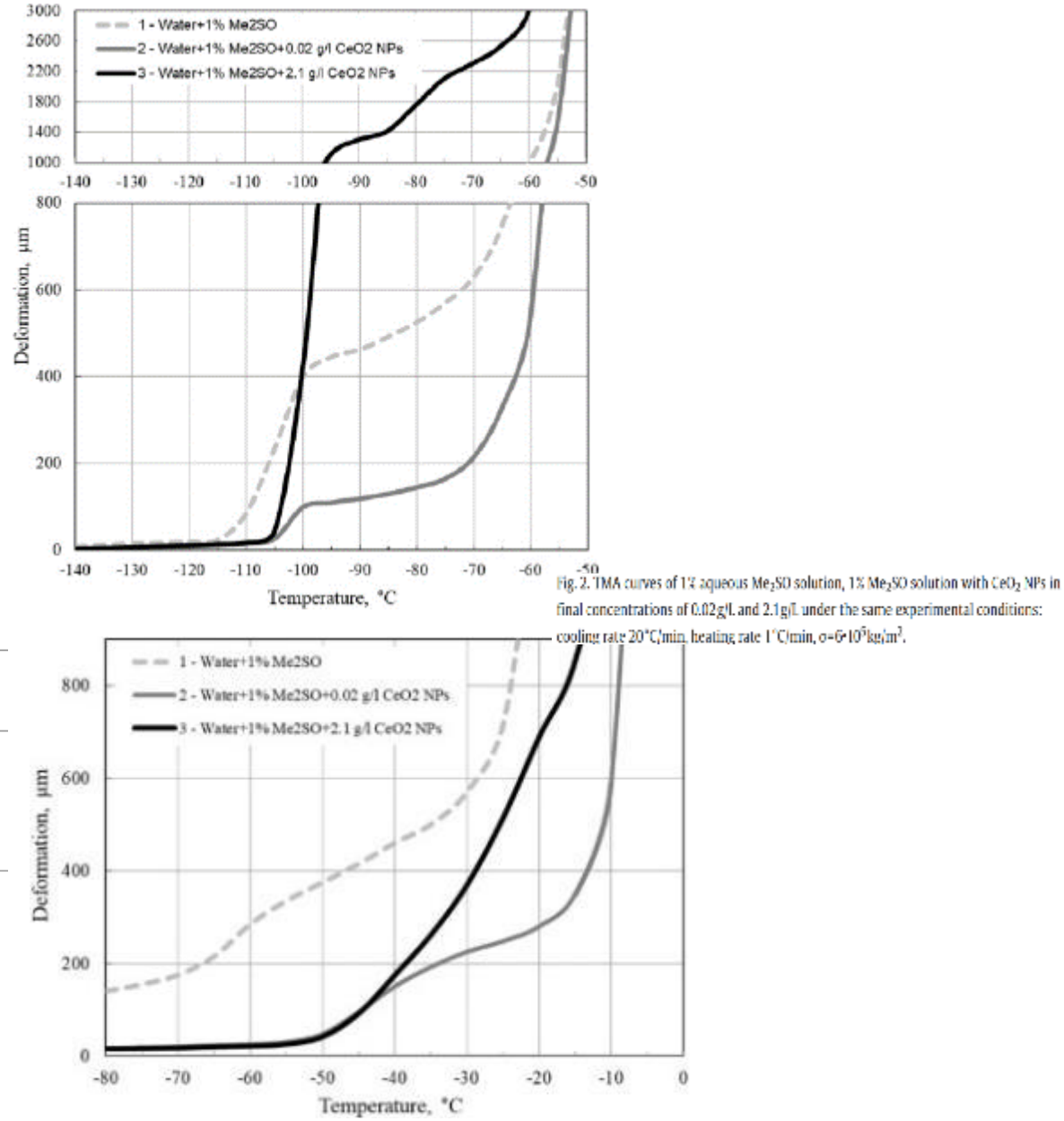

Fig. 2. TMA curves of 1% aqueous Me_2SO solution, 1% Me_2SO solution with CeO_2 NPs in final concentrations of 0.02 g/L and 2.1 g/L under the same experimental conditions: cooling rate 20°C/min, heating rate 1°C/min, σ=6•10⁵kg/m².

Fig. 3. TMA curves of an aqueous solution of Me_2SO with a concentration of 1% (1), aqueous solutions of Me_2SO with CeO_2 NPs in final concentrations of 0.02 g/L (2) and 2.1 g/L (3) under the same experimental conditions: 20°C/min cooling rate, 1°C/min heating rate, σ=2•10⁵kg/m.².

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Table 3. The effect of CeO_2 NPs on the onset and enthalpy of crystallization during cooling and melting during warming at 10°C/min in complex nutrient and cryoprotective medium. Data are presented as mean±SD.

Sample	Crystallization		Melting	
	T, °C	Enthalpy, J/g	T, °C	Enthalpy, J/g
Ham's F12	−12.0±2.28	265.9±15.05	−2.3±0.15	286.0±12.17
Ham's F12+FBS	−11.7±1.79	264.2±6.49	−2.5±0.56	287.7±8.07
Ham's F12+FBS +0.02 g/L CEO_2	−9.6±1.96[a]	275.8±8.75[a]	−2.4±0.44	293.0±6.53[a]
Ham's F12+FBS +1 g/L CEO_2	−7.4±1.46[a]	276.0±1.24[a]	−2.3±0.47	295.9±8.46[a]
Ham's F12+FBS +2.1 g/L CEO_2	−9.9±1.40	259.4±2.20	−2.5±0.29	281.9±5.76
Ham's F12+FBS +1%DMSO	−12.3±2.80	257.8±8.01	−3.9±0.25	287.9±7.06
Ham's F12+FBS +1%DMSO+ 0.02 g/L CEO_2	−10.5±1.33	260.7±13.05	−3.7±0.25	280.6±9.39[a]
Ham's F12+FBS+1%DMSO+ 1 g/L CEO_2	−10.3±3.65[a]	261.3±11.13	−3.6±0.07	280.9±8.27[a]
Ham's F12+FBS+1%DMSO+ 2.1 g/L CEO_2	−9.5±1.10	261.3±9.88	−3.4±0.29[a]	280.8±6.34[a]

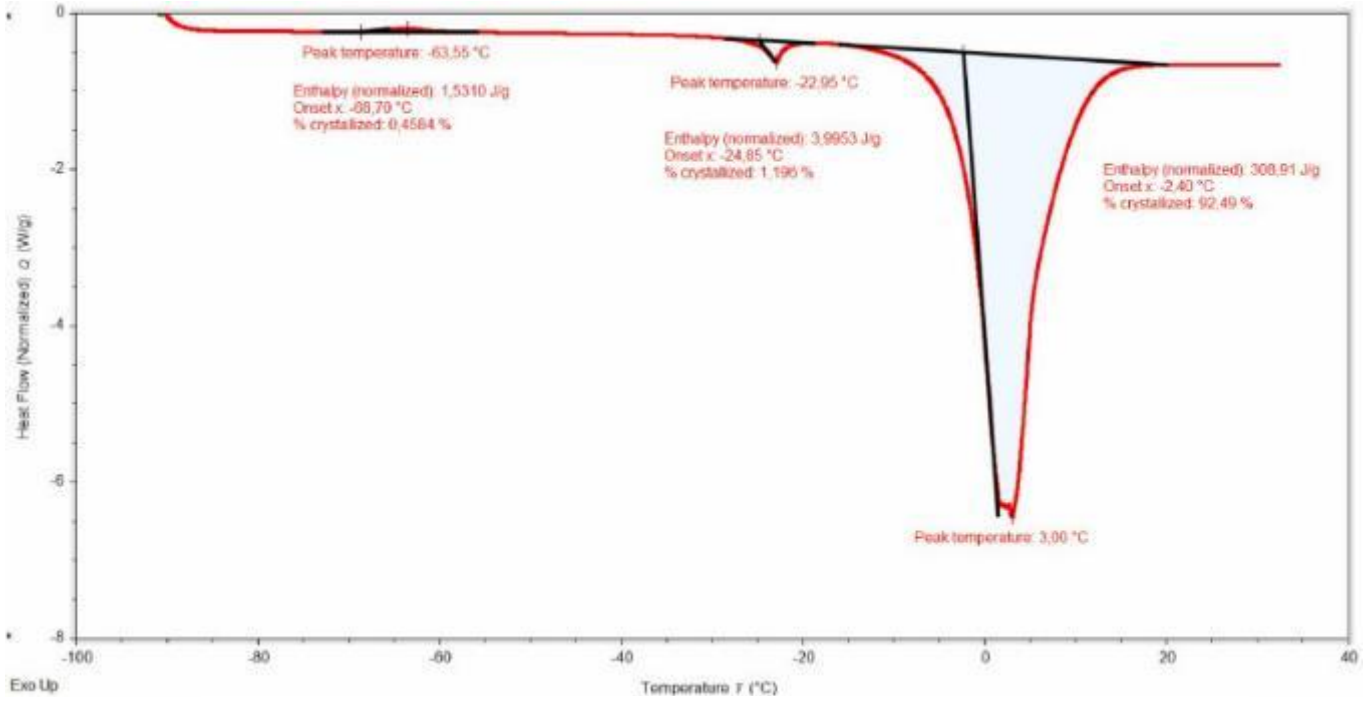

Fig. 4. Phase transition in Ham's F12 medium during warming (10°C/min).

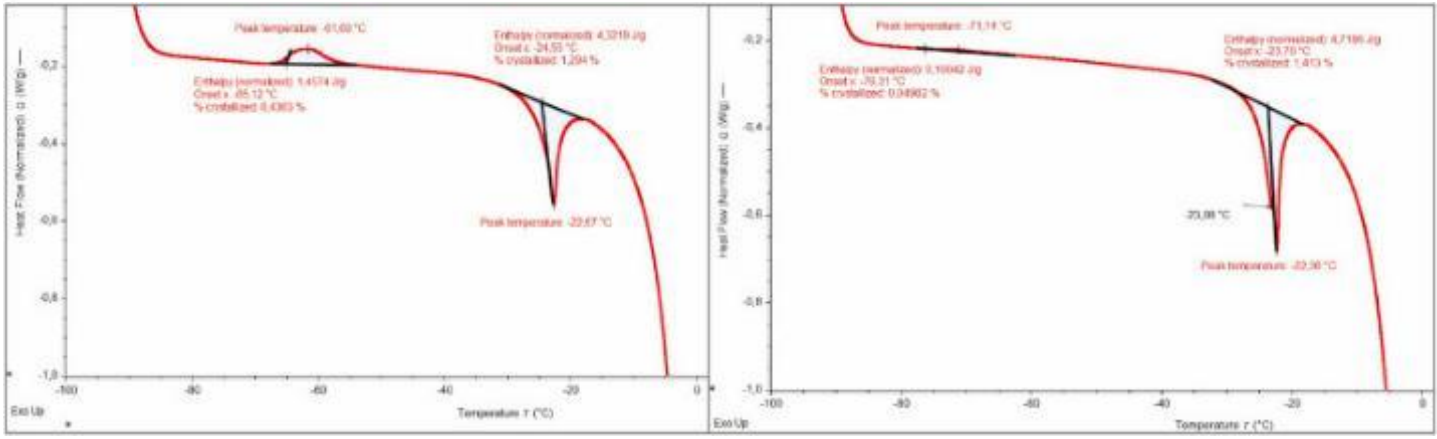

Fig. 5. Eutectic crystallization and melting during warming in complex nutrient medium: Ham's F12+FBS (in left side) and Ham's F12+FBS +0.02 g/L CEO_2 (in right side).

Cryobiology
Volume 118, March 2025, 105167

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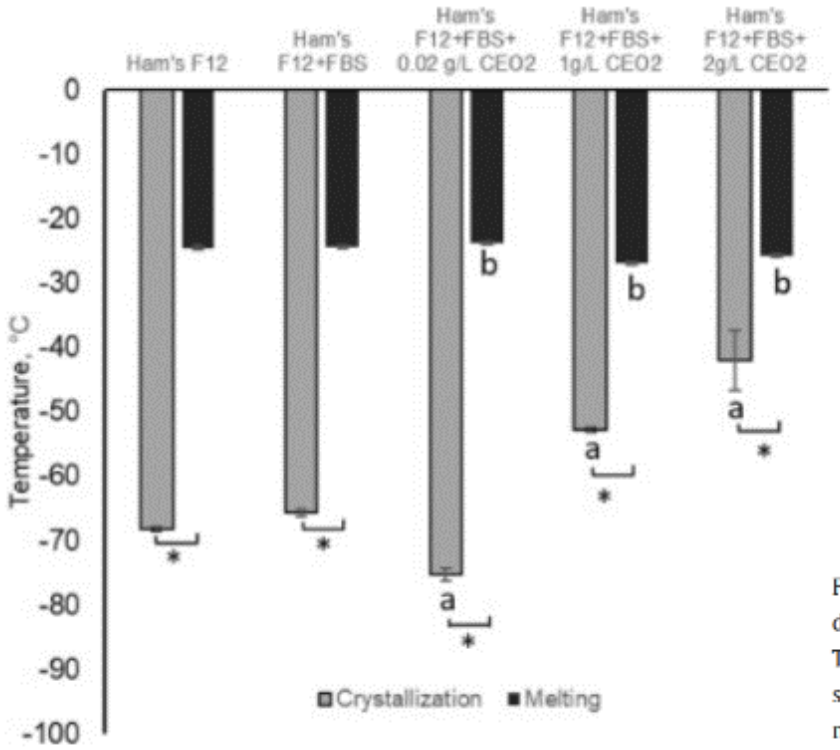

Fig. 6. The influence of CeO_2 NPs on the onset of eutectic crystallization and melting during warming at 10°C/min in complex nutrient medium.
The values of onset of crystallization (a) and melting (b) from nanoparticles supplemented solution were significantly different from those for the control (P<0.05, n=6).

* significant differences between the onset of crystallization and the onset of melting (p<0.05, n=6).

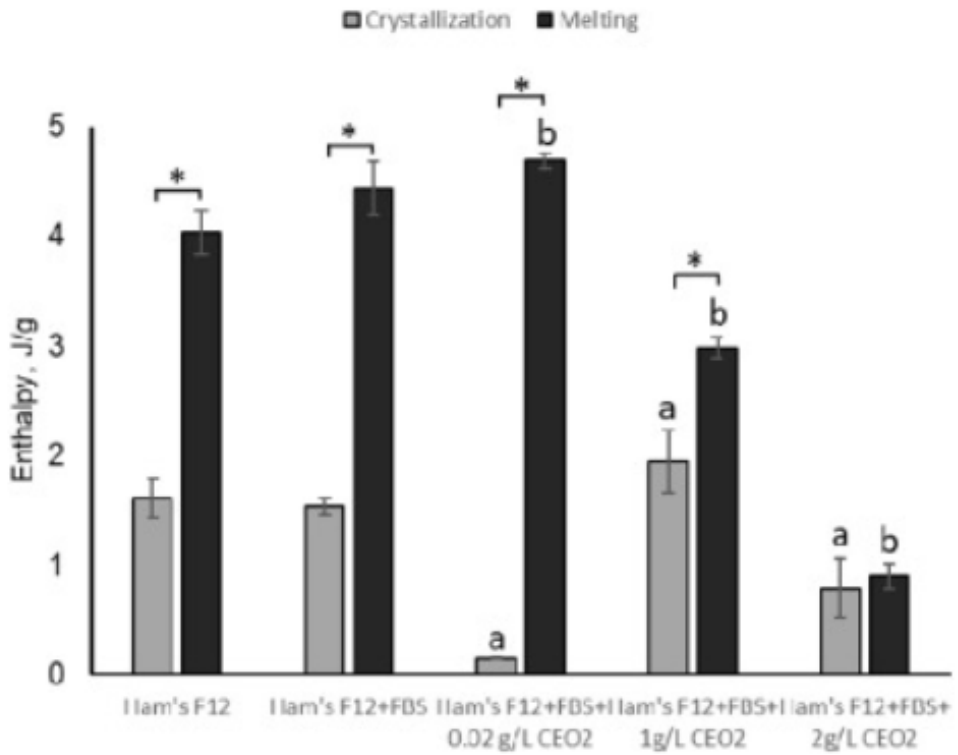

Fig. 7. The influence of CeO_2 NPs on the enthalpy of eutectic crystallization and melting during warming at 10°C/min in complex nutrient medium.
The values of onset of crystallization (a) and melting (b) from nanoparticles supplemented solution were significantly different from those for the control (P<0.05, n=6).

* significant differences between the onset of crystallization and the onset of melting (p<0.05, n=6).

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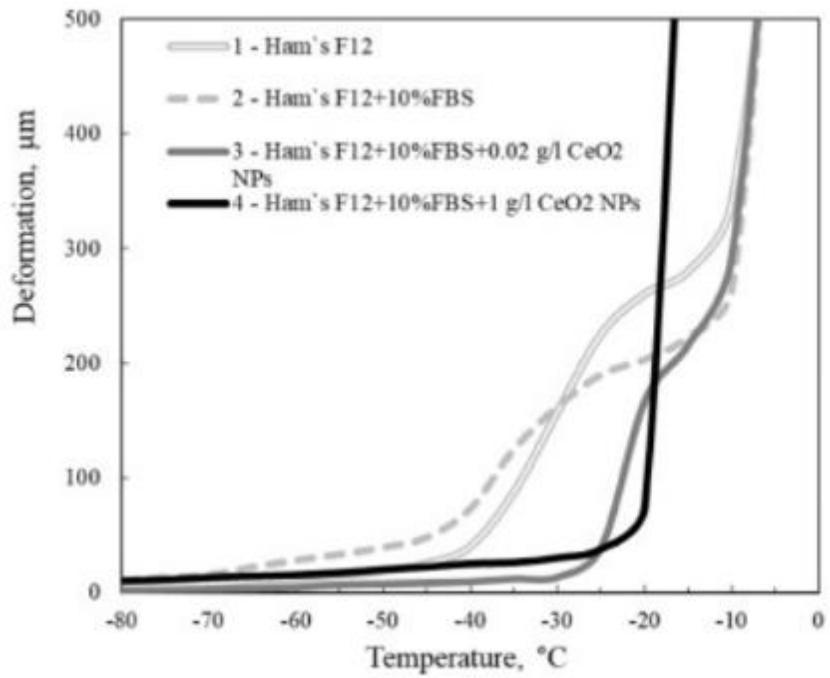


Fig. 8. TMA curves of Ham's F12 (1), Ham's F12 complemented with 10% FBS (2), Ham's F12 with the addition of 10% FBS and CeO_2 NPs in final concentrations of 0.02g/L (3) and 1g/L (4) under identical experimental conditions: cooling rate 20°C/min, heating rate 1°C/min, $\sigma=0.6\cdot10^5$kg/m.^{2}.

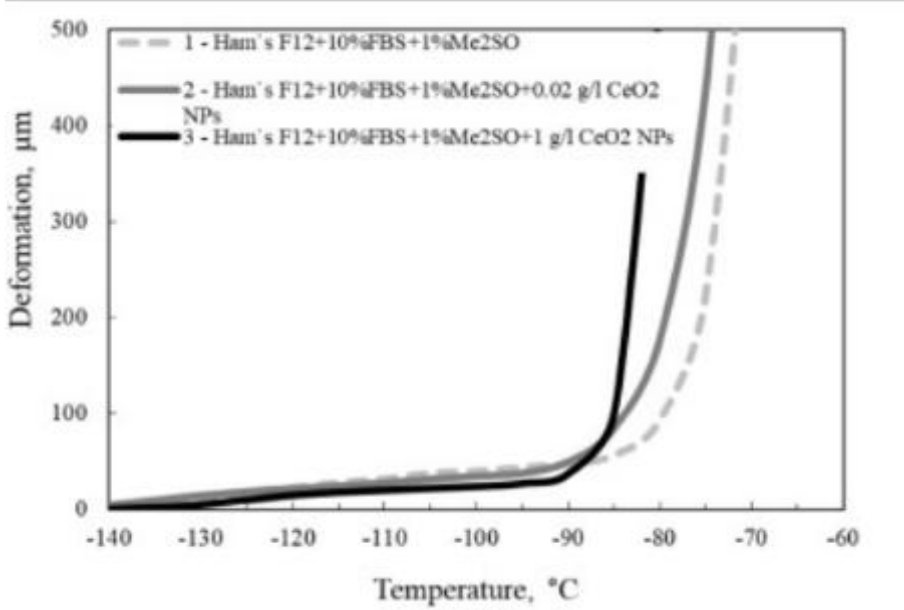
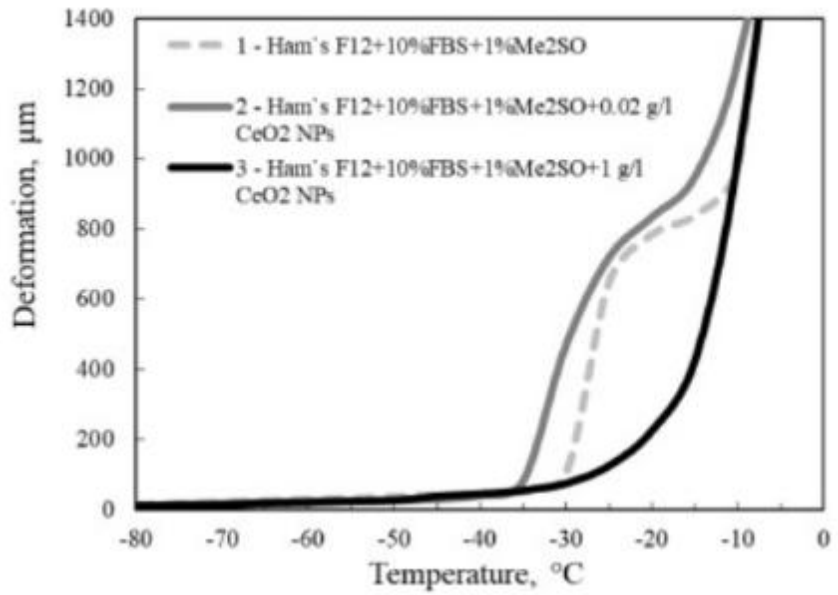


Fig. 9. TMA curves of Ham's F12 with 10% FBS and with 1% Me_2SO without (1) and with CeO_2 NPs in final concentrations of 0.02g/L (2) and 1g/L (3) under the same experimental conditions: 20°C/min cooling rate, 1°C/min heating rate, $\sigma=6\cdot10^5$kg/m.^{2}.

Fig. 10. TMA curves of Ham's F12 with 10% FBS and with 1% Me_2SO without (1) and with CeO_2 NPs in final concentrations of 0.02g/L (2) and 1g/L (3) under the same experimental conditions: 20°C/min cooling rate, 1°C/min heating rate, $\sigma=0.6\cdot10^5$kg/m.^{2}.



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Review Article

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Blood transfusion in veterinary medicine

ssue 4 - 2017

umar
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H, India

dence: Rahul kumar, Department of Veterinary
College of Veterinary Science & AH, DUVASU,
lia, Email:

February

Transfusion Medicine in Exotic Pets

Marla Lichtenberger, DVM, DACVECC

The decision to transfuse a patient should always be based on the packed cell volume and clinical status of the patient. This

ood substi-
n of blood
ge of blood

Indications fo

Peracute to acute blo
mammals, acute loss
shock; loss of greater

Veterinary
Anaesthesia and Analgesia

Formerly the Journal of Veterinary Anaesthesia

Veterinary Anaesthesia and Analgesia, 2014 doi:10.1111/vaa.12135

REVIEW ARTICLE

Canine and feline blood transfusions: controversies and recent advances in administration practices

Caroline Kisielewicz & Ian A Self
Queen Mother Hospital for Animals, Royal Veterinary College, Hatfield, Hertfordshire, UK

CLINICAL

Mymms,

The Veterinary Nurse | April 2019, Volume 10 No 3

Canine and feline blood transfusions

Despite the various blood products for dogs made available by Pet Blood Bank UK, there is currently no system available for cats in the UK. It is crucial that veterinary nurses and surgeons know how to collect blood in emergency situations and how to administer blood and blood products safely.
This article focuses on the importance of blood typing and cross matching to try and minimise potential reactions. It also highlights the criteria for appropriate canine and feline donors and how to collect blood safely.

Gemma Webb ISFMDipFN CertVNECC RVN, Area Account Manager for Woodley Equipment Ltd, Old Station Park Building, St John Street, Horwich, Bolton BL6 7NY

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"ST26733", International Conference "Agriculture for Life, Life for Agriculture"

Transfusion Triggers and Therapeutic Efficacy in a Group of Dogs That Underwent Whole Blood Therapy

Ognean L.[1]*, Viorica Chiurciu[2], Cristina Ştefănuţ[1], L. Oana[1], I. Morar[1], Ildikó Barabási[1]1

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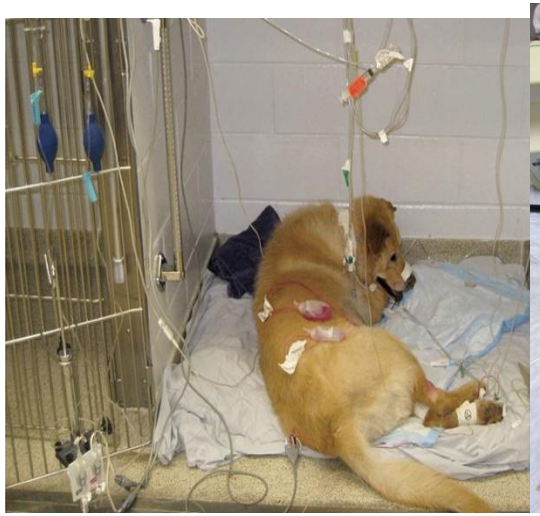
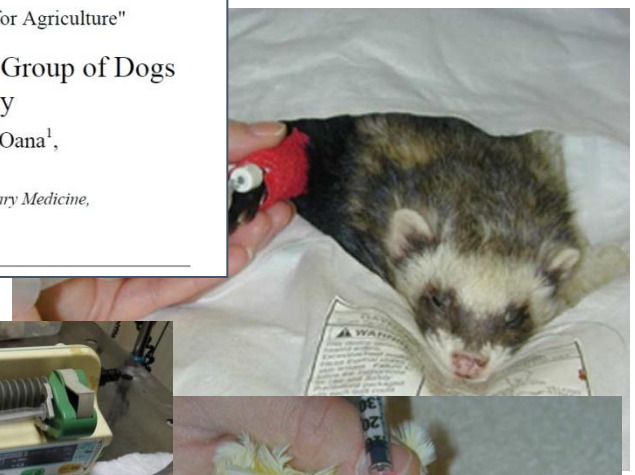
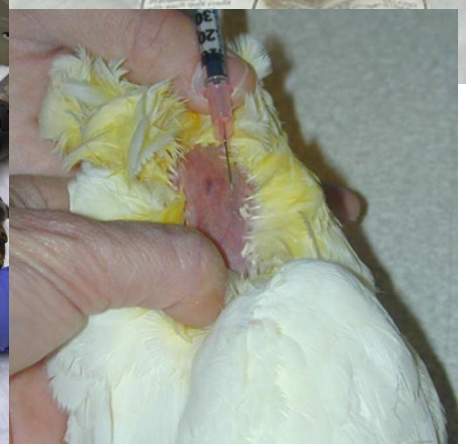
[2]*Romvac company, Romania*





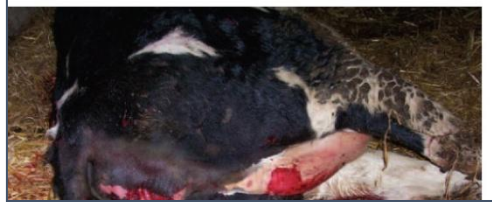


Blood transfusions in cattle

Gareth Bell BVM&S CertCHP DBR MRCVS
JUBILEE VET GROUP, JUBILEE ROAD, NEWTOWNARDS, CO. DOWN, N. IRELAND. BT23 4YH

INTRODUCTION

All farm veterinarians dread the midnight call to severe vaginal haemorrhage in the freshly calved cow. Many of us will have arrived half-dressed and breathless, only to find our patient dead or on the point of expiring (Fig. 1). Sometimes haemorrhage has been controlled, but the patient is in a state of haemorrhagic shock. Therefore, a simple, rapid and inexpensive blood transfusion technique is vital for the farm veterinarian in treating this and many other bovine conditions (Table 1).



may reduce losses sufficiently to preserve life or even make transfusion unnecessary. A severely shocked patient may be stabilised by administration of hypertonic saline while a donor can be identified. However, this effect is temporary, and after 30 minutes the crystalloid solution will have redistributed through the body and shock will once again set in.

Cattle have the most complex blood group system of domestic animals with 11 groups, but most individuals do not have antibodies against other blood group antigens unless sensitised by previous transfusion. Even where cross-matching is carried out, transfused erythrocytes only survive for 2-4 days compared to a normal cattle lifespan of 160 days. Nevertheless, this window is usually enough to allow endogenous haematopoesis to fill the deficit.

The anticoagulant of choice in bovine blood transfusions is sodium citrate, which is both cheap and readily available. Unlike heparin, it does not have

Hindawi Publishing Corporation
The Scientific World Journal
Volume 2014, Article ID 734397, 7 pages
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Research Article

Clinical Response and Transfusion Reacti to Single Homologous Blood Transfusion

Rejane Santos Sousa,[1] Antonio Humberto Hamad Minervino,[2]
Carolina Akiko Sato Cabral Araújo,[1] Frederico Augusto Mazzocca Lopes Rodrigues,[1]

Chapter 2

An introduction to blood groups and blood transfusion in domestic animals

Aasif Ahmad Sheikh[1]; Showkat Ahmad Bhat[2]; Ankita Kalyan[1]; Mohammad Rayees Dar[1]*

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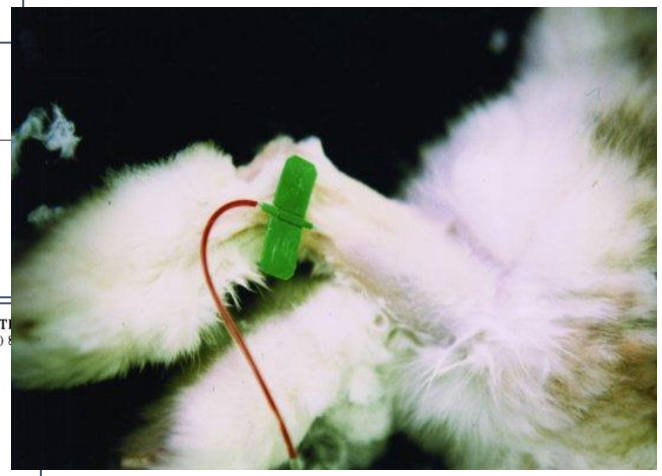
Blood Transfusion in a Calf with Life-Threatening Anemia

G.Saritha[1], G.S.Haritha[2], K.Nalini Kumari[3], N.Syaama Sundar[4]



22

Equine Transfusion Medicine

Margaret C. Mudge[1] and Olivia H. Williams[2]
[1] *The Ohio State University, Department of Veterinary Clinical Sciences, Columbus, Ohio, USA*
[2] *Piedmont Equine Associates, Madison, Georgia, USA*

Severely anaemic cow receiving a blood transfusion

Blood transfusions in cattle

EQUINE VETERINARY EDUCATI
Equine vet. Educ. (1996) **8** (1)

Satellite Article

Blood and plasma transfusion in the horse

A. E. DURHAM
The Equine Veterinary Hospital, Forest Mere, Liphook, Hampshire GU30 7JG, UK.

Introduction

are only weakly immunogenic and multiple transfusions are required to raise significant antibody titres against them

Dogs – 8 antigens
Horses – 7 internationally recognized blood groups
Cows - 11 major blood groups

COMPLEX CRYOPRESERVATION MEDIUM FOR MAMMALIAN ERYTHROCYTES

INTRODUCTION

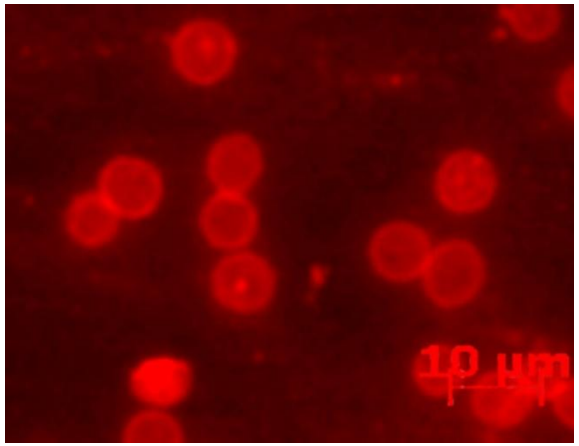
Successful cryopreservation of animal erythrocytes is hampered by the need for individual choice of the protocol due to species differences. The solution to this problem can be complex cryoprotective media (CCM). The aim of this work was to develop CCM that could become unified for mammalian erythrocytes.

MATERIALS AND METHODS

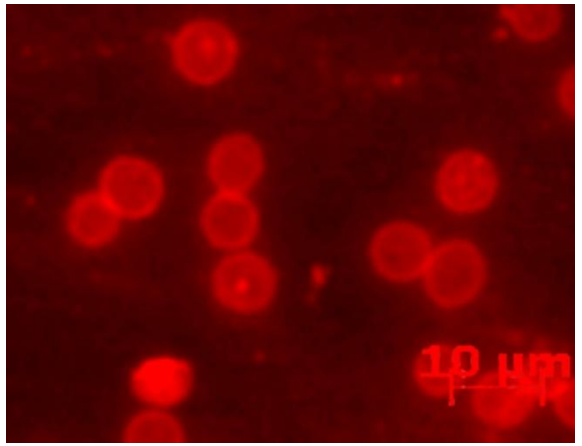
1. washed erythrocyte + cryopreservative medium
2. 15 min incubation
3. Freezing by immersion into liquid nitrogen (–196°C)
4. Thawing in a water bath at 40°C up to liquid phase appearance
5. Washing

Final cryoprotective concentration in cell suspension:

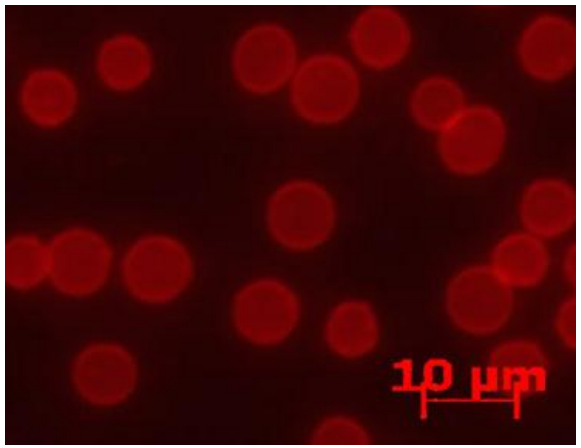
1. 2.5% PEO-1500 +7.5% Me_2SO
2. 5% PEO-1500 + 5% Me_2SO
3. 10% PEO-1500 +5% Me_2SO
4. 5% PEO-1500 + 5% Me_2SO + 5% 1,2-PD + 5% sucrose
5. 7.5% PEO-1500 + 5% Me_2SO + 2.5% 1,2-PD + 2.5% sucrose
6. 7.5% PEO-1500 + 7.5% Me_2SO + 2.5% 1,2-PD + 2.5% sucrose
7. 10% PEO-1500 + 5% Me_2SO + 2.5% 1,2-PD + 2.5% sucrose
8. 5% PEO-1500 + 5% Me_2SO + 5% 1,2-PD + 2.5% sucrose
9. 10% PEO-1500 +2.5% Me_2SO + 2.5% 1,2-PD +2.5% sucrose
10. 5% PEO-1500 + 5% Me_2SO + 5% 1,2-PD + 5% mannitol



equine



bovine



rabbit

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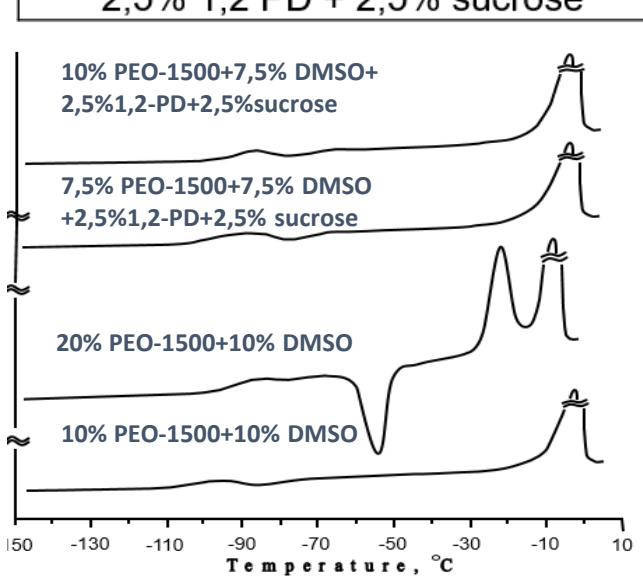
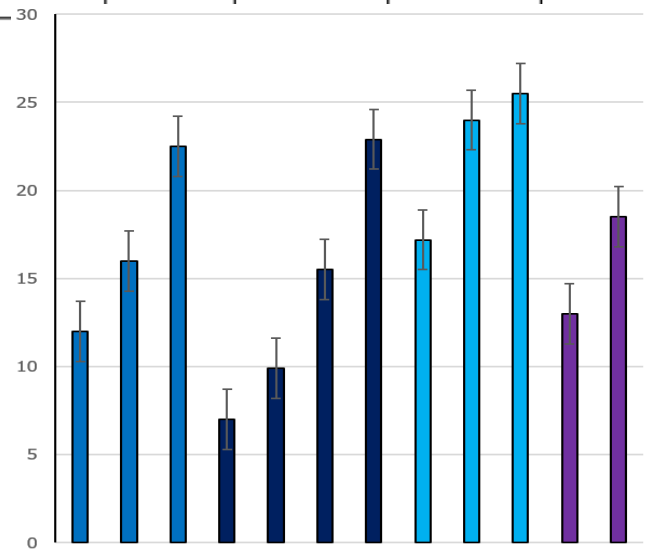
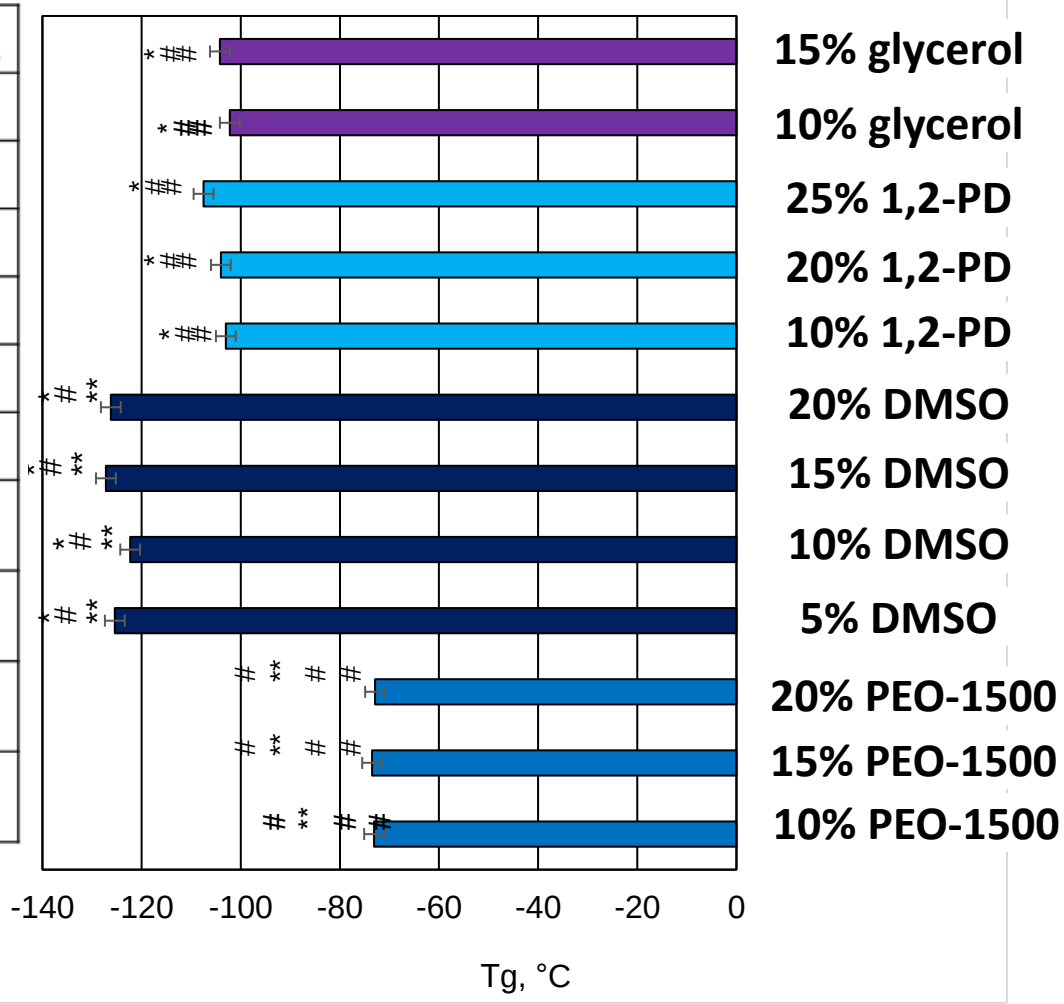
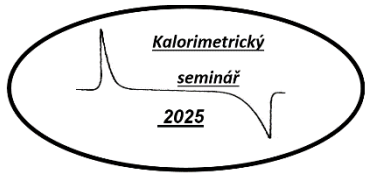
Solution	T_g, °C	T_c, °C	T_{ce}, °C	T_{me}, °C	T_m, °C
2,5% PEO-1500+7,5% DMSO	-101,1	-64,5	—	—	-0,5
5% PEO-1500 +5% DMSO	-97,5	-84,2	—	—	-3,9
5% PEO-1500+15% DMSO	-115.5	-92	—	—	-14.5
10% PEO-1500+5% DMSO	-92,2	—	-51,5	-28,7	-6,1
10% PEO-1500+10% DMSO	-104.5	-87,2	—	—	-10,2
20% PEO-1500+10% DMSO	-91,5	—	-60	-26	-16
5% PEO-1500 + 5% DMSO+ 5% 1,2 PD + 5% sucrose	-94,8	-74,9	—	—	-12,5
7,5% PEO-1500 + 5% DMSO+ 2,5% 1,2 ПД + 2,5% sucrose	-95,2	-77,2	—	—	-7,3
7,5% PEO-1500 + 7,5% DMSO+ 2,5% 1,2 - PD + 2,5% sucrose	-99	-79,2	—	—	-10
10% PEO-1500 + 5% DMSO+ 2,5% 1,2 PD + 2,5% sucrose	-92,8	-77,5	—	—	-9,8

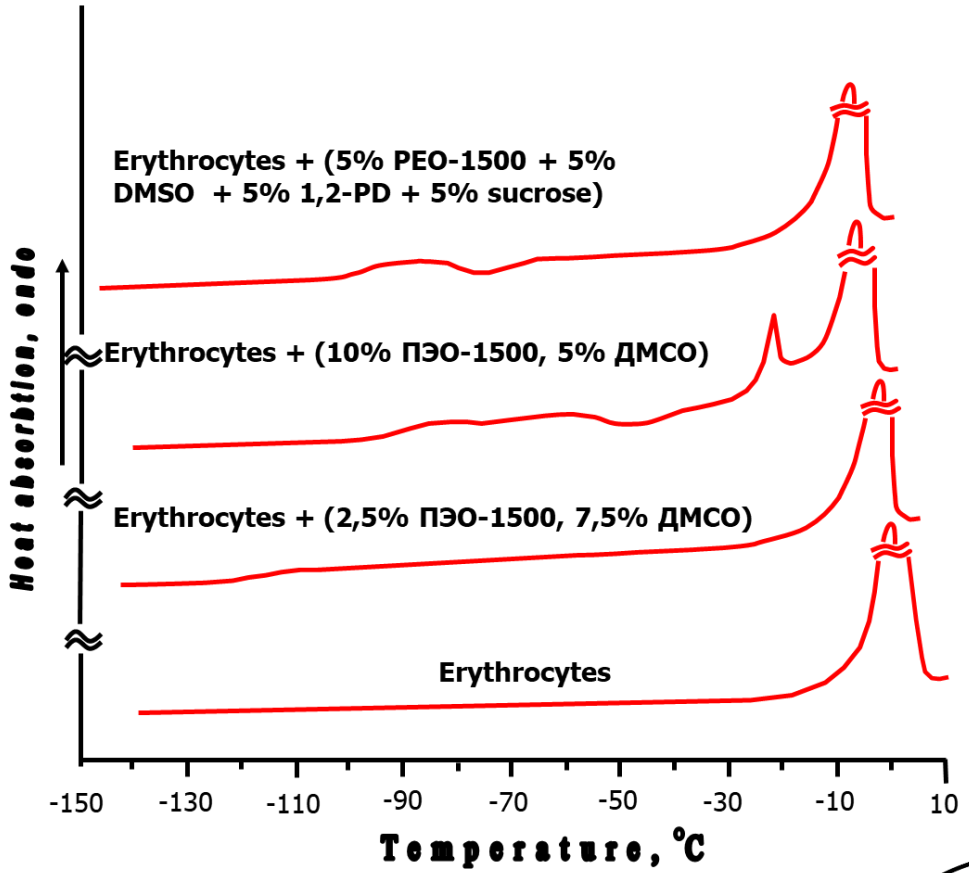








COMPLEX CRYOPRESERVATION MEDIUM FOR MAMMALIAN ERYTHROCYTES



Samples	T_g, °C	T_c, °C	T_{ce}, °C	T_{me}, °C	T_m, °C
Control	—	—	—	—	-0,5
5% PEO-1500 +15% DMSO	-114.5	—	—	—	-5
10% PEO-1500 +10% DMSO	-104	-61,5	—	—	-4.5
20% PEO-1500 +10% DMSO	-88	-77,9	-49,5	-26	-8,5
10% PEO-1500 + 10% DMSO + 10% 1,2-PD + 10% sucrose	-95,1	-74,9	—	—	-12
15% PEO-1500 + 10% DMSO + 5% 1,2-PD + 5% sucrose	-92	-75	—	—	-9
15% PEO-1500 + 15% DMSO + 5% 1,2-PD + 5% sucrose	-97	-75,9	—	—	-13
20% PEO-1500 + 10% DMSO + 5% 1,2-PD + 5% sucrose	-91,5	-75,3	—	—	-13,2

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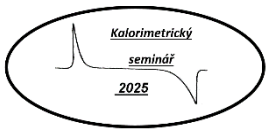
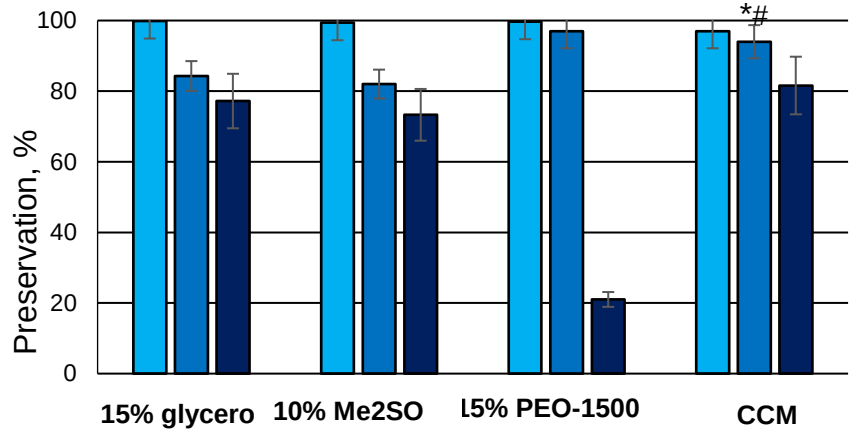
COMPLEX CRYOPRESERVATION MEDIUM FOR MAMMALIAN ERYTHROCYTES

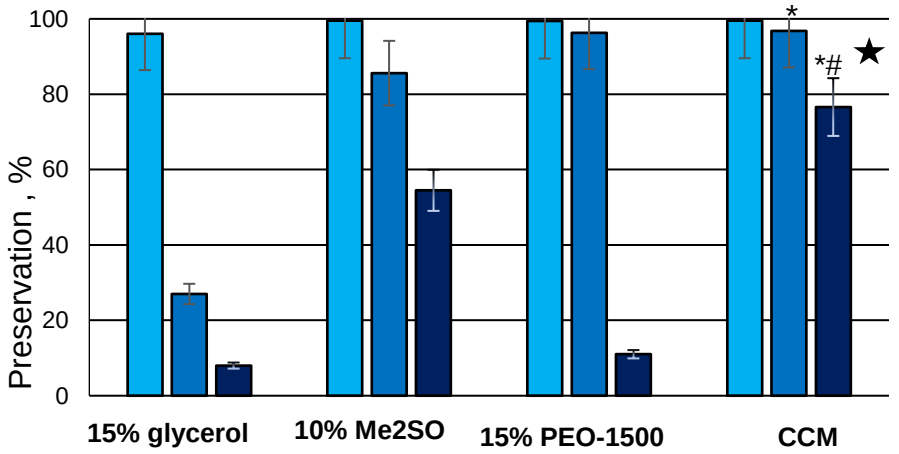
Medium	Hemolysis of bovine erythrocytes			Hemolysis of eguine erythrocytes			Hemolysis of rabbit erythrocytes		
	After 15 min incubation	After freeze-thawing	After freeze-thawing and washing	After 15 min incubation	After freeze-thawing	After freeze-thawing and washing	After 15 min incubation	After freeze-thawing	After freeze-thawing and washing
2.5% PEO-1500 +7.5% Me_2SO	0,8±0,2	18,4±1,3	38,2±2,5	**0,8±0,2**	**16,7±1,5**	**45,8±3,7**	**0,8±0,2**	**16,4±1,4**	**46,9±3,9**
2. 5% PEO-1500 + 5% Me_2SO	0,7±0,2	13,8±1,2	46,2±4,1	**0,3±0,1**	**16,2±0,9**	**51,2±5,1**	**0,7±0,2**	**15,8±1,6**	**48,5±4,2**
10% PEO-1500 +5% Me_2SO	0,5±0,2	13,2±1,1	35,5±2,4	**0,7±0,2**	**16,5±1,2**	**39,5±2,7**	**0,4±0,3**	**15,4±1,3**	**36,5±2,5**
5% PEO-1500 + 5% Me_2SO + 5% 1,2-PD + 5% sucrose	0,6±0,2	10,4±1,0	38,4±2,7	**0,5±0,2**	**12,5±1,2**	**44,3±3,2**	**0,5±0,1**	**11,4±1,1**	**40,4±3,5**
7.5% PEO-1500 + 5% Me_2SO + 2.5% 1,2-PD + 2.5% sucrose	**0,5±0,2**	**5,2±0,4**	**23,4±2,0**	**0,7±0,2**	**7,2±0,6**	**25,4±2,0**	**0,8±0,2**	**6,4±0,5**	**24,5±2,1**
7.5% PEO-1500 + 7.5% Me_2SO + 2.5% 1,2-PD + 2.5% sucrose	0,8±0,1	7,2±0,8	27,5±2,3	**0,9±0,3**	**7,6±0,5**	**27,8±2,7**	**0,9±0,1**	**7,8±0,2**	**28,9±2,8**
10% PEO-1500 + 5% Me_2SO + 2.5% 1,2-PD + 2.5% sucrose	0,5±0,2	5,2±0,6	41,1±4,0	**0,5±0,2**	**6,1±0,5**	**42,2±3,8**	**0,6±0,2**	**6,7±0,6**	**43,8±4,2**
5% PEO-1500 + 5% Me_2SO + 5% 1,2-PD + 2.5% sucrose	0,5±0,1	13,8±1,1	47,3±4,1	**0,6±0,2**	**15,8±1,4**	**54,5±3,5**	**0,6±0,1**	**15,5±1,3**	**49,5±3,4**
10% PEO-1500 +2.5% Me_2SO + 2.5% 1,2-PD +2.5% sucrose	0,3±0,2	6,6±0,5	30,9±2,3	**0,3±0,2**	**7,7±0,7**	**34,9±2,8**	**0,4±0,2**	**7,3±0,8**	**32,7±2,2**
5% PEO-1500 + 5% Me_2SO + 5% 1,2-PD + 5% mannitol	0,6±0,1	10,0±0,5	38,5±3,4	**0,6±0,1**	**8,1±0,5**	**44,8±3,9**	**0,7±0,2**	**9,2±0,5**	**40,7±3,8**



COMPLEX CRYOPRESERVATION MEDIUM FOR MAMMALIAN ERYTHROCYTES



Human

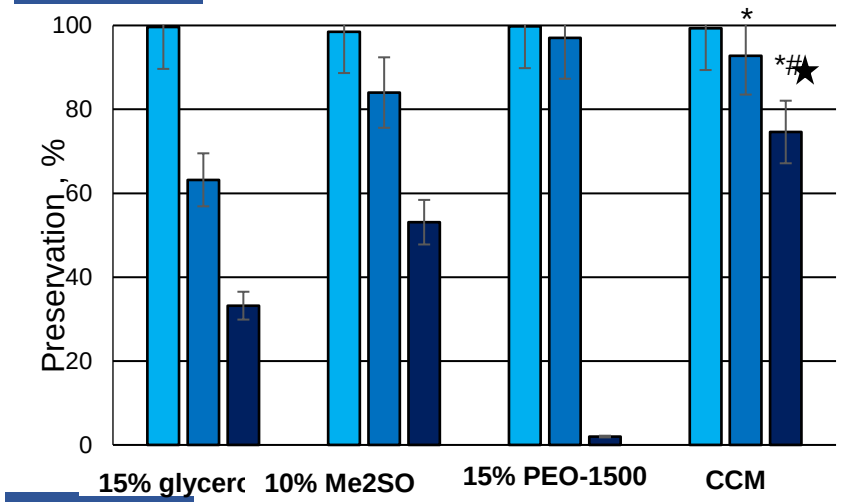


Bovine

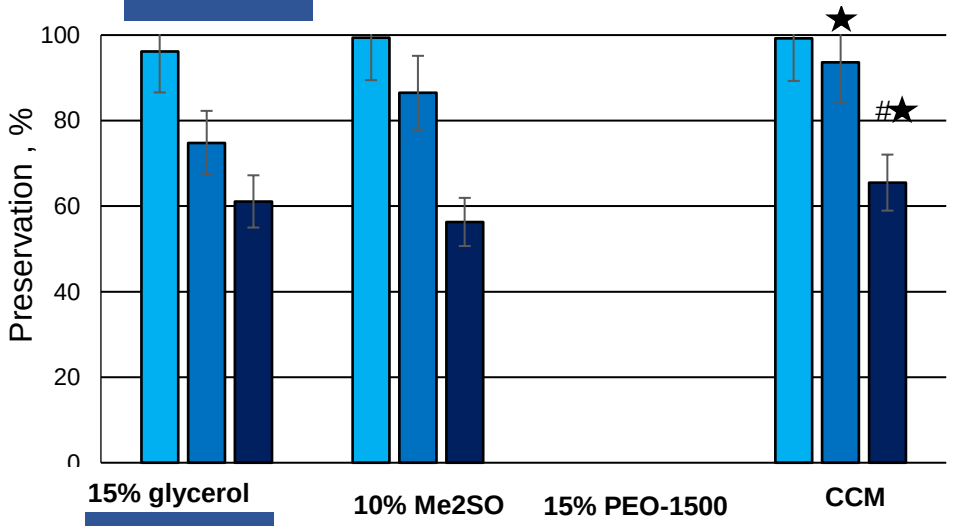
- after incubation

- after freeze-thawing

- after freeze-thawing and washing



Equine



Rabbit

* - statistically significant difference relative to 15% glycerol; # - statistically significant difference relative to 10% Me2SO; ★- statistically significant difference relative to 15% PEO-1500 ($p < 0.05$), $n = 5$.

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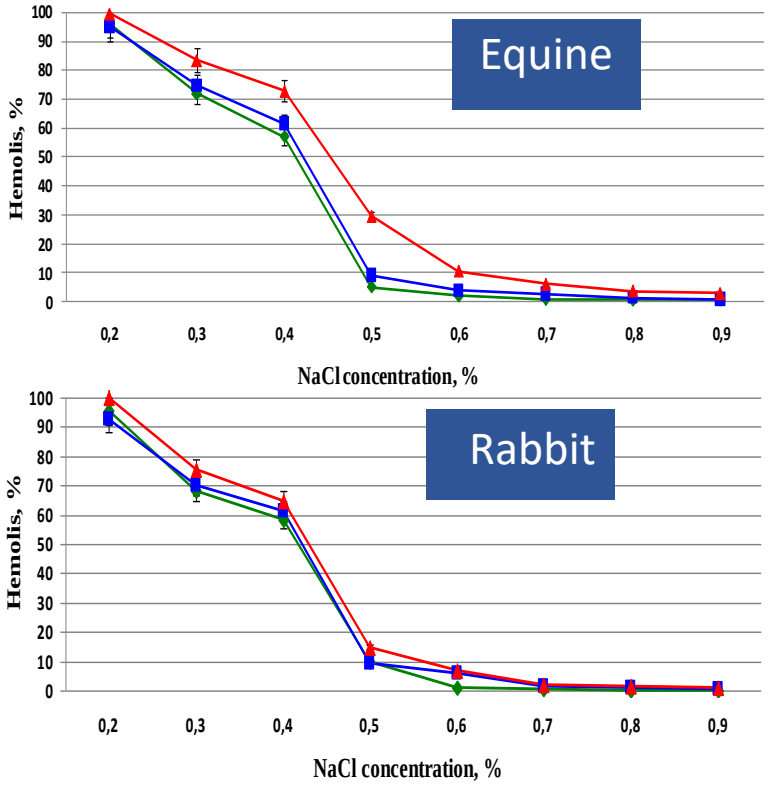
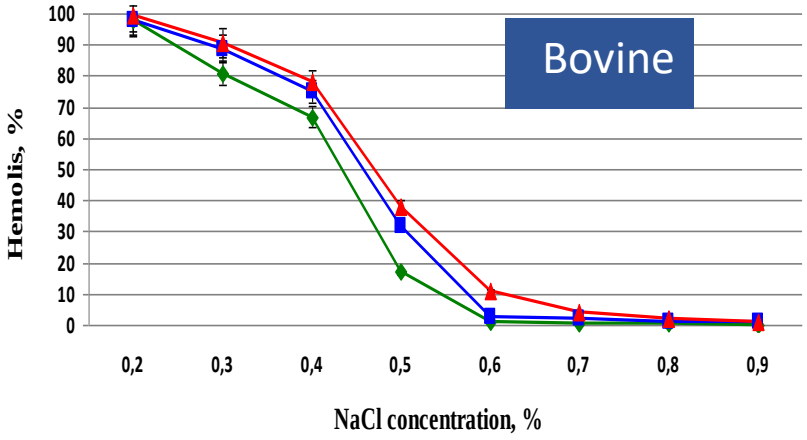
COMPLEX CRYOPRESERVATION MEDIUM FOR MAMMALIAN ERYTHROCYTES

The osmotic fragility curves of erythrocytes

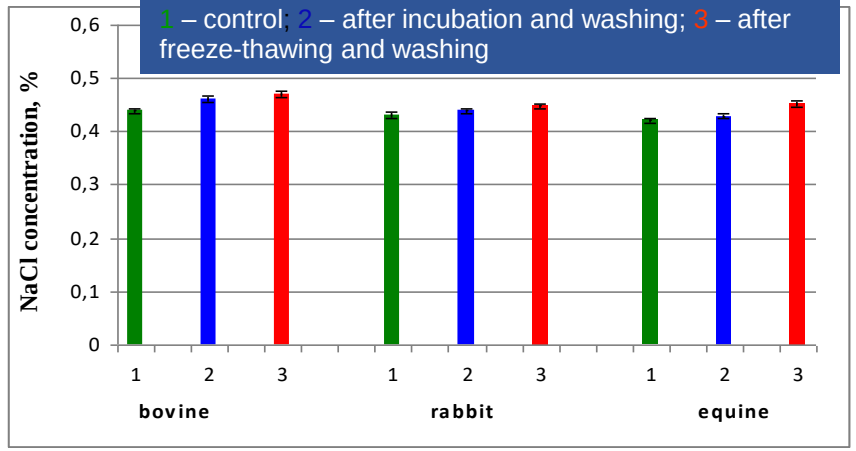




◆ – control
■ – after incubation and washing
▲ – after freeze-thawing and washing

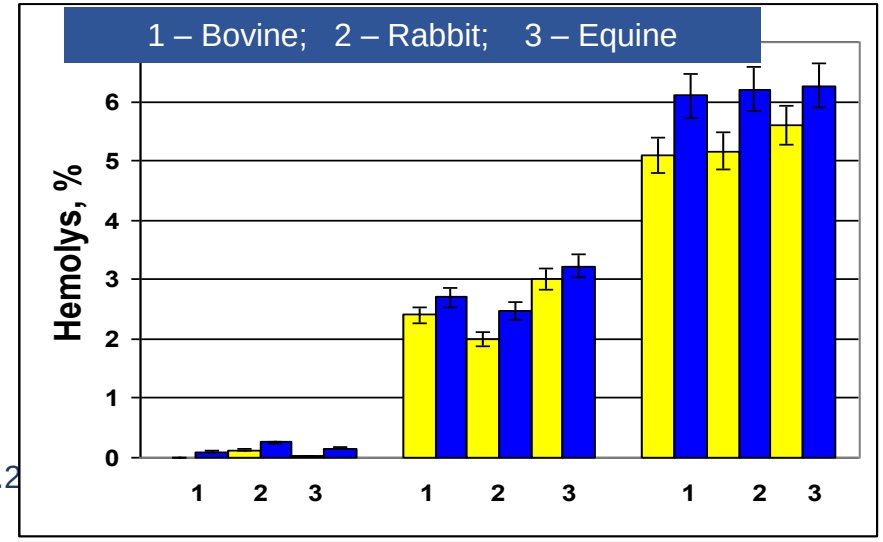
The osmotic fragility index

1 – control; 2 – after incubation and washing; 3 – after freeze-thawing and washing



The transfusion simulation

1 – Bovine; 2 – Rabbit; 3 – Equine



CONCLUSIONS

Thus, the developed complex cryoprotective media containing polyethylene glycol 1500 (7.5%), Me2SO (5%), 1.2 propanediol (2.5%), sucrose (2.5%) is potentially unified for erythrocytes of different mammals.

PHASE BEHAVIOR OF SUCROSE-CONTAINING CRYOPROTECTIVE SOLUTIONS AT TEMPERATURES BELOW 0 °C

Introduction

Sucrose is often used both as a primary cryoprotectant and in complex cryoprotective media. In particular, sucrose is a component of many media for cryopreservation of mammalian embryos by vitrification.

Purpose

to study low-temperature phase transitions in sucrose-containing solutions of glycerol (Gl), 1,2-PD, 1,3-PD, ethylene glycol (EG), and Me_2SO.

Materials and methods

Preparing of cryoprotectant solutions

Cryoprotectant solutions (30% concentration) were prepared with Dulbecco's nutrient medium supplemented with sucrose.

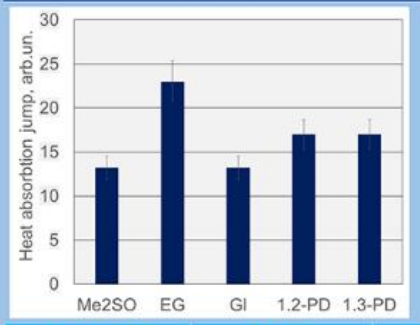
Low temperature differential scanning calorimetry (DSC)

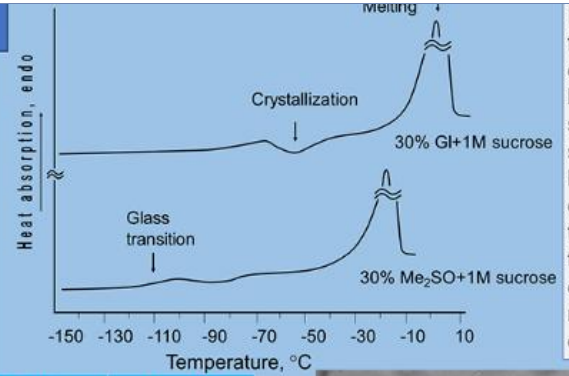
Cryoprotectant solutions (30% concentration) were prepared with Dulbecco's nutrient medium supplemented with sucrose. DSC thermograms were recorded at the heating stage (0.5 grad/min) after rapid cooling of the solutions by immersion into liquid nitrogen (200°/min). For all investigated solutions, the temperatures of phase and glass transitions were determined.

Cryopreservation of mouse embryos

2-cell and 8-cell mouse embryos in cryoprotective medium were vitrified by immersion into liquid nitrogen using plastic straws and preserved 3-7 days. Thawing was performed in water bath (38°C). To remove the cryoprotectant there was used a 10-min equilibration in 0.5M sucrose solution. Then the embryos were three times washed-out with physiological medium, transferred into CO_2 -incubator for culturing. Rate of embryo viability was estimated by their developmental capacity to the stage of extended blastocyst.

Results

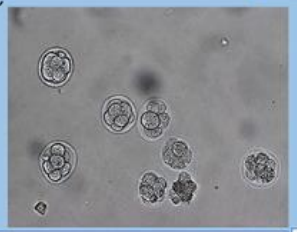




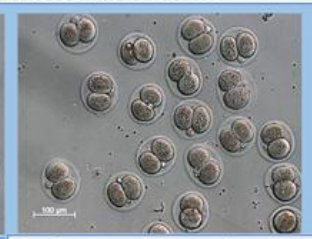
Immediately after glass transition, for all investigated solutions, crystallization completed at the heating stage, which indicated a low stability of the amorphous state. In solutions of Me2SO and EG, the lowest glass transition, crystallization and melting temperatures were recorded. After the addition of sucrose no crystallization of eutectic was recorded in any of the studied cryoprotectant solutions.

Samples	T_g, °C	T_c, °C	T_m, °C
Me_2SO	-108.2±0.5	-85.3±0.5	-34.6±0.5
EG	-109.1±0.5	-86.9±0.5	-25.8±0.5
Gl	-76±0.5	-54±0.5	-11.7±0.5
1.2-PD	-72.2±0.5	-51.6±0.5	-13.9±0.5
1.3-PD	-83±0.5	-62.1±0.5	-15.1±0.5

[a] -25 min of 2- cell embryo exposure in Dulbecco's physiological medium at room temperature; [b] – statistically significant difference compared to the control, $p<0.05$



8-cell mouse embryos after vitrification using Gl-sucrose medium



2-cell mouse embryos after vitrification using EG-sucrose medium

Developmental stage	Groups	Cryopreservation medium	Number	Exposure time in cryoprotectants	Number of embryos, approaching blastocyst stage,(n)%
2-cell	control	30% EG + 0.7 M of sucrose	52[a]	–	(47) 90,4±4,1
8-cell		30% Gl + 0.7 M of sucrose			(39) 90,2±2,2
2-cell	I	30% EG + 0.7 M of sucrose	58	1,5 min	(52) 89,7±4,0
8-cell		30% Gl + 0.7 M of sucrose			(31) 84,9±4,3
2-cell	II	30% EG + 0.7 M of sucrose	53	3 min	(38) 75,5±6,3
8-cell		30% Gl + 0.7 M of sucrose			(23) 56,8±6,7[b]
2-cell	III	30% EG + 0.7 M of sucrose	74	1,5 min	(31) 55,4±7,6[b]
8-cell		30% Gl + 0.7 M of sucrose			(65) 80,2±6,2

Conclusions

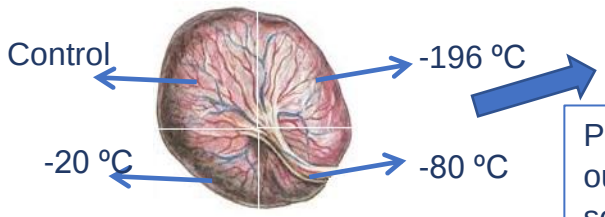
the addition of sucrose to cryoprotective solutions leads to an increase in the glass transition temperature and the disappearance of crystallization and melting of eutectic compositions.

EFFECT OF LOW TEMPERATURE PLACENTA STORAGE ON BIOLOGICAL ACTIVITY OF ITS EXTRACTS

Introduction

Low temperature storage of placenta is widely used. We have recently shown that at high cooling rates there is a small amount of liquid phase that remains uncrystallized and goes into to a glassy state at temperatures from -77°C to -81.5°C. The aim of this work was to study the effect of placental storage temperature on biological activity of its extracts.

Materials and methods



Obtaining of human placenta extracts (HPEs)

Placental fragments were washed out with 0.15 M sodiium chloride solution until the visual removal of mucus and blood; then fetal membranes (amniotic and chorion) were removed. Placenta fragments were homogenized in physiological solution (1:1, w/v) with a following incubation for 12h at 4 ºC.

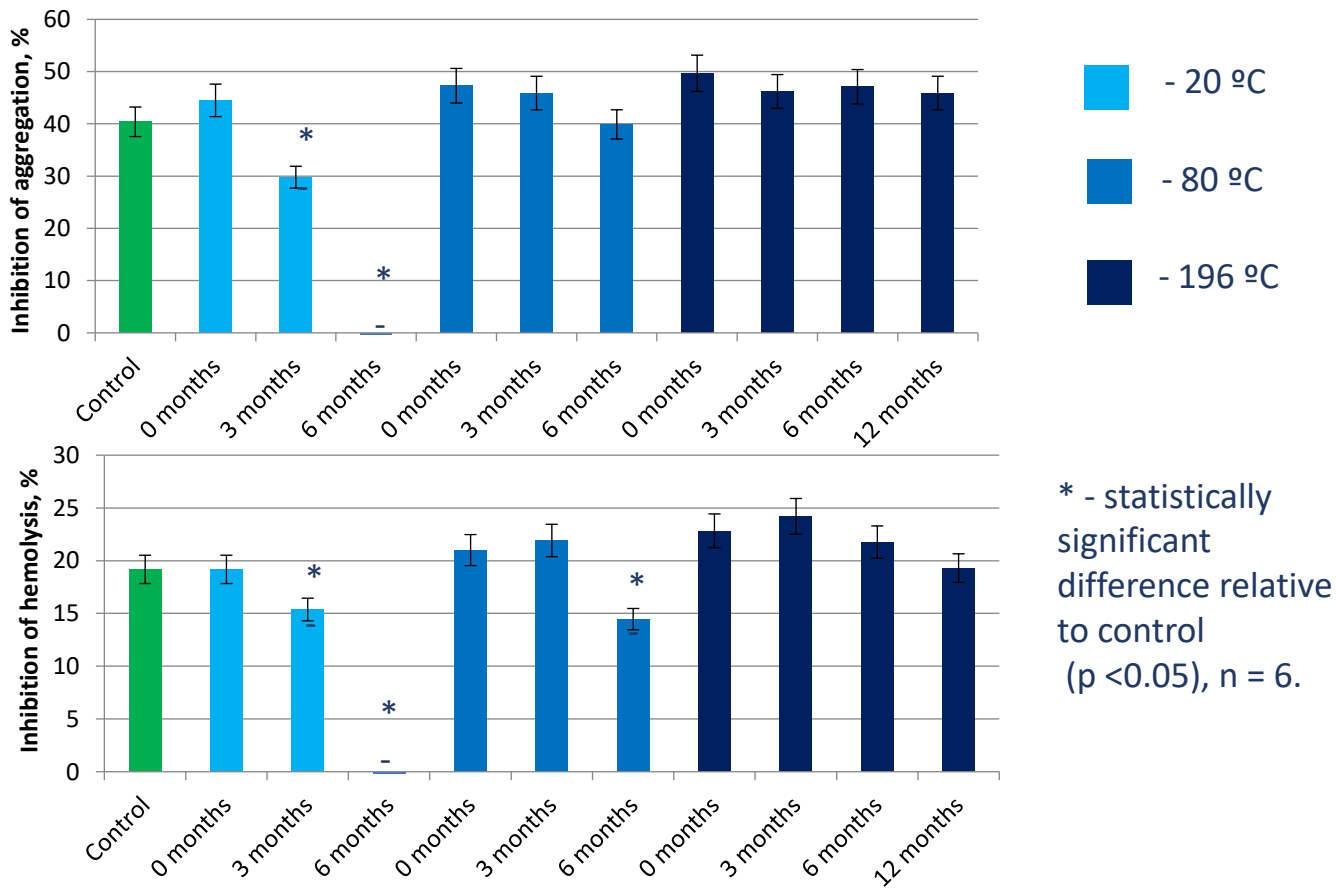
Ability of HPEs to reduce erythrocytes' thermohemolysis (20 min in thermostat at 55 ° C)

% inhibition of hemolysis = (hemolysis of control) - (hemolysis of sample)) / (hemolysis of control) * 100

Anti-aggregation activity of HPEs was determined by spectrophotometric method. Platelet-enriched plasma was incubated for 15 minutes with HPEs at +37 °C. 5'-ADP at a concentration of 10^{-5} mol/l was used as an inducer of platelet aggregation.

% inhibition of aggregation = (1- (aggregation of sample)/(aggregation of control)) * 100

Results

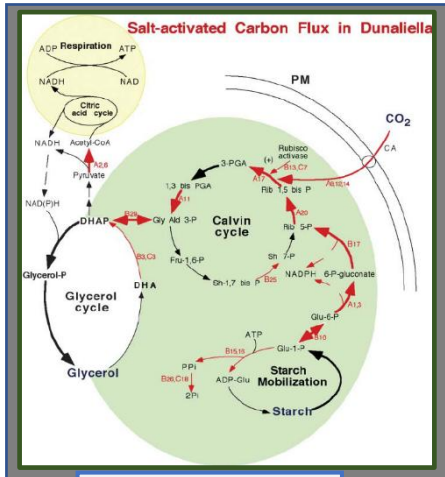


* - statistically significant difference relative to control ($p < 0.05$), n = 6.

Conclusions

Thus, placenta biological activity was maintained at -196˚C at least during 1 year, at -80°C it was kept for 6 months, and for 3 month at -20°C.

LOW TEMPERATURE PHASE TRANSITIONS IN GREEN MICROALGAE SUSPENSIONS



Liska A. et al., 2004

Introduction

Green microalgae *Dunaliella salina* is a promising source of carotene for the biotechnology industry. Cryopreservation is one of the best ways to preserve important strains of microalgae for a long time without losing their characteristic features. It is known that with a high degree of salinity, Dunaliella salina are capable of glycerol hypersynthesis. Glycerol, which accumulates in *Dunaliella salina* cells under stress, can act as a natural cryoprotectant.

Purpose

to study low-temperature phase transitions in suspensions of green microalgae *Dunaliella salina*, which were cultivated on Ramaraj media with different content of sodium chloride.

Qualitative and quantitative composition of Ramaraj growth medium

Substances	Concentration, g / l
H_3BO_3	0,00928
$CoCl_2 \cdot 6H_2O$	0,00005
$ZnCl_2$	0,00011
$MnCl_2 \cdot 4H_2O$	0,00198
Na_2MoO_4	0,00049
$NaVO_3$	0,00024
$CuCl_2 \cdot 6H_2O$	0,00005
$MgSO_4 \cdot 7H_2O$	1,23
KCl	0,2
$CaCl_2 \cdot 2H_2O$	0,044
KNO_3	0,5
KH_2PO_4	0,014
$FeCl_3 \cdot 6H_2O$	0,0005
Na_2EDTA	0,074
$NaHCO_3$	2,1
NaCl	87,7 (1,5 M)
	175,4 (3 M)
	233,76 (4 M)

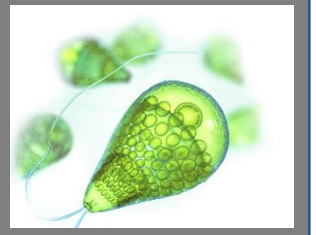
Materials and methods

Suspensions of green microalgae

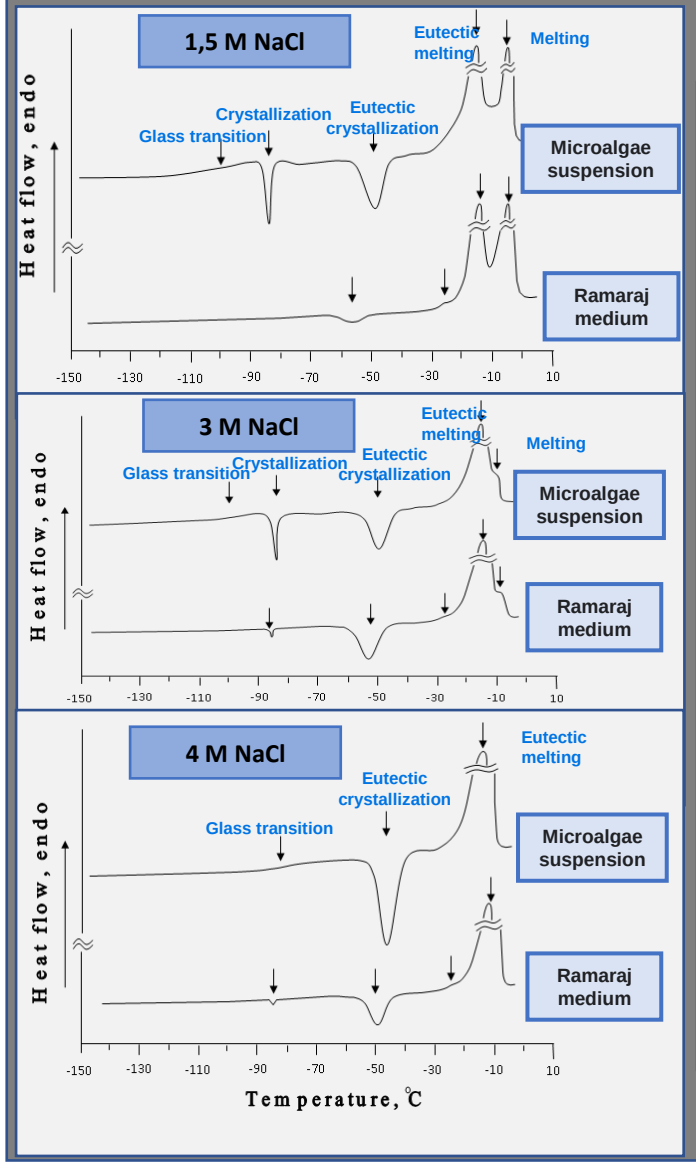
↓

Snap freezing in liquid nitrogen (~ 200°/min)

↓



Low temperature differential scanning calorimetry
Thermograms of frozen samples
were recorded while rewarming at 0.5 °/min.



Results

Glass transition was registered in microalgae suspensions that were cultured in Ramaraj medium with 1.5 M, 3M and 4M of NaCl. This indicates an effective hypersynthesis of glycerol by *Dunaliella salina* microalgae. An intense exothermic peak was recorded at the heating stage at NaCl concentrations of 1.5 M and 3M almost immediately after completion of glass transition. This indicates low stability of the amorphous state in studied cell suspensions and the need for high heating rates to prevent crystallization processes from the amorphous state. The crystallization peak was not recorded in microalgae suspension with 4M of NaCl. Two separate melting peaks were registered at 1.5 M of NaCl. They probably correspond to the melting of eutectic compositions and ice melting. An increase in NaCl concentration leads to a decrease in the melting point of ice and a lack of separation of the two melting peaks.

Many critical food and nutrition security crops cannot be conserved in perpetuity by seeds!

- Seedless crops
- Crops that do not breed true from seeds
- Crops with recalcitrant or short-lived seeds

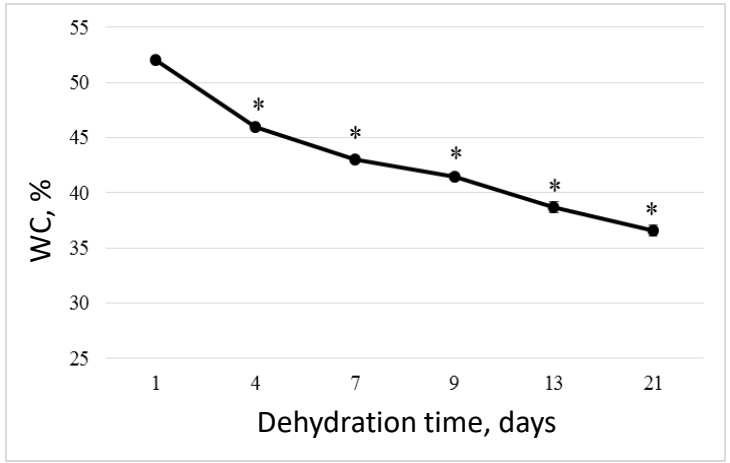
Methods of conservation

- Field collection
- *In vitro* collection
  - Normal growth
  - Slow growth (temp↘, O_2 ↘, H_2O ↘, medium ~)
- Cryopreservation (-196°C)

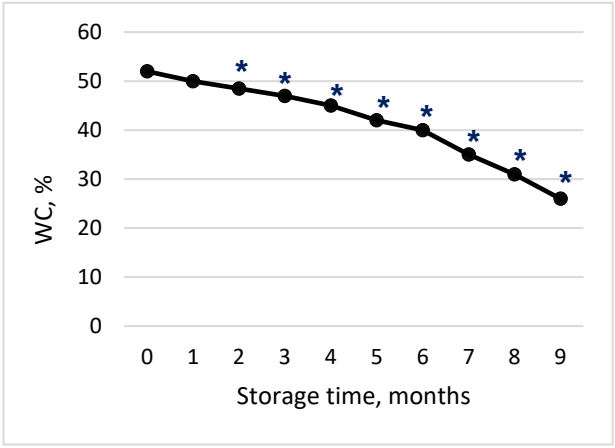
Field collection = vulnerable to pests, diseases, and climate

Grape dormant buds cryopreservation

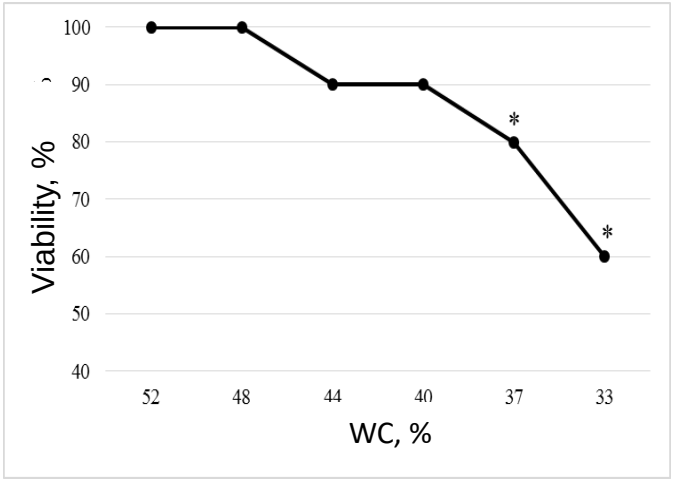
Changing the water content (WC) of grape cuttings during dehydration



Changing the water content during storage plant material in plastic bag in a fridge

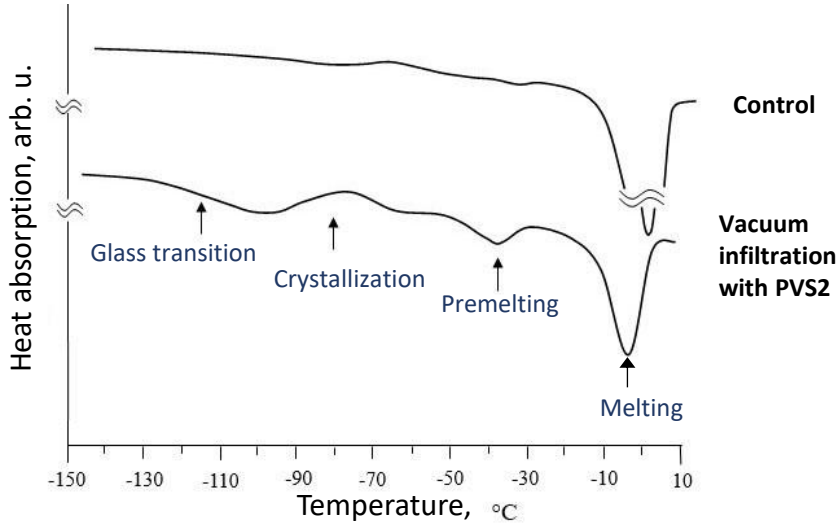


Influence of dehydration of grape cuttings on their viability



– statistically significant differences compared to control (p<0,05), n=10.

Phase transitions in grape dormant buds



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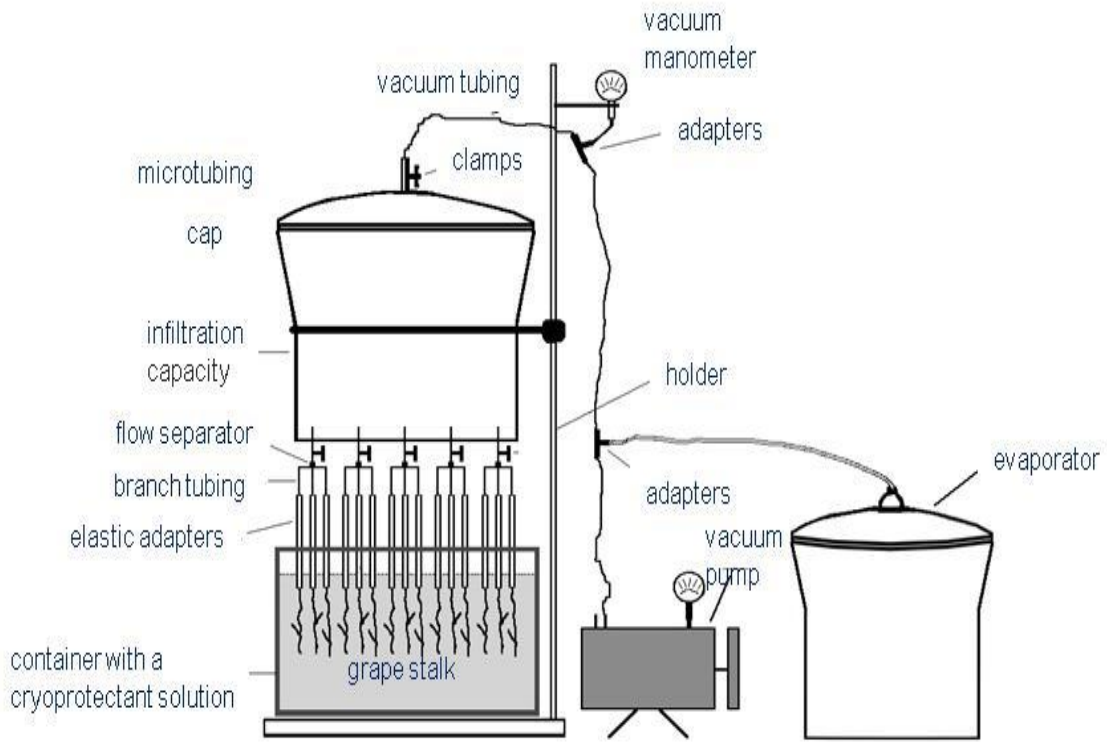
Experimental workflow for vine cuttings drying

Vine
1. Russian Concord
2.Riparia X Rupestris
3. Zagadka
Single-bud cuttings were collected in autumn and winter



Saturation with sucrose
by vacuum infiltration method (0.5 M sucrose).

Drying
by refrigerating (+4°C) until final humidity 37-42%. Final sucrose concentration ~0.7 M.

Experimental workflow for dormant grapes buds saturation with PVS

PVS 2 solution

30% glycerol
15% ethylene glycol
15% DMSO
0.4M sucrose
in Murasige-Skuga medium

Dormant grapes buds

Standard vitrification

Passive saturation

- incubation in PVS 2
- normal atmospheric pressure;
- saturation time in PVS2 - 60 minutes.

Vacuum-infiltration- vitrification (VIV)

Active vacuum-infiltration saturation

- Degassing time: 5 minutes;
- Pressure: 20-40 kPa;
- Saturation time in PVS2: 15-20 minutes.

Snap freezing and storage in liquid nitrogen

Saturation efficiency

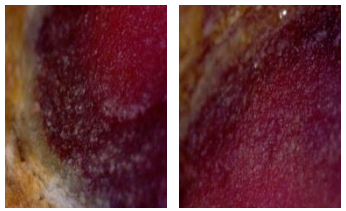
Low temperature differential scanning calorimetry (DSC)

Thermograms of frozen samples (-196°C, 200°/min) were recorded while rewarming at 0.5 °/min.
Following parameters were analyzed:

- glass transition intensity,
- ice melting temperature
- enthalpy of water crystallization

Grape buds' integrity assessment

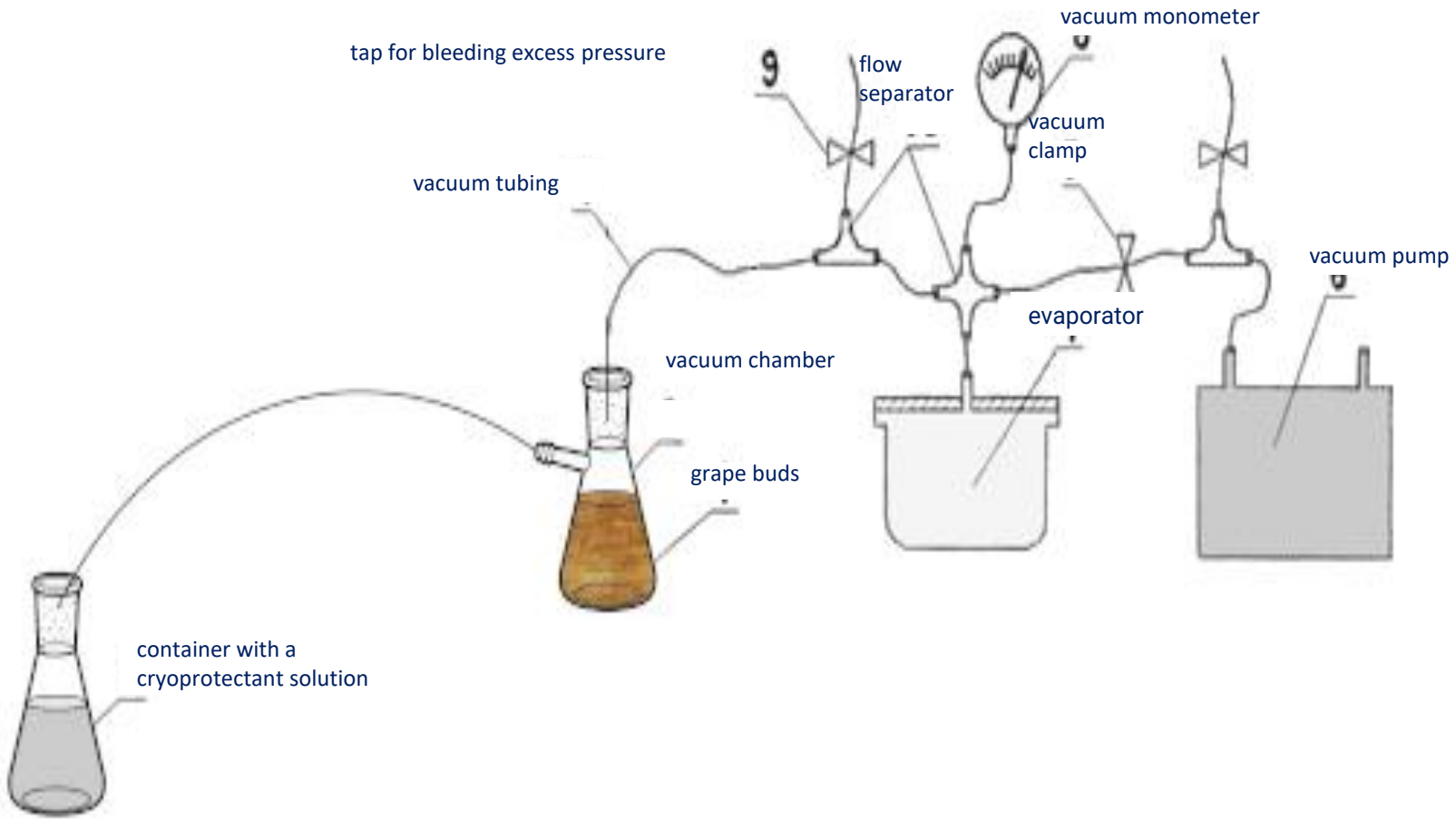
Staining with 2,3,5-triphenyltetrazolium chloride (1% solution).

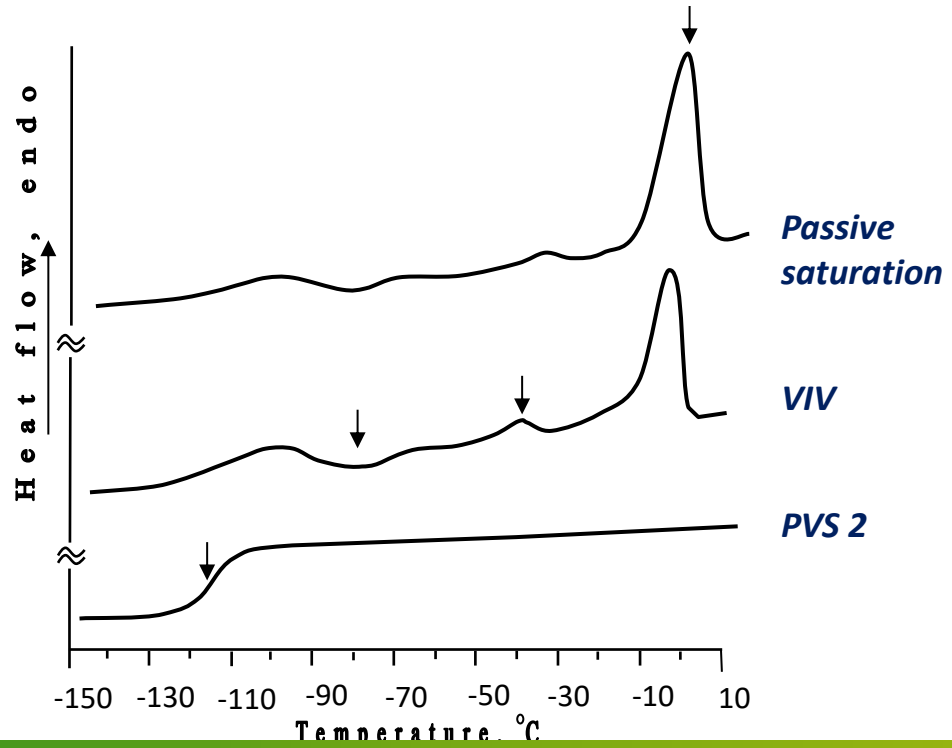


Grape buds viability assessment

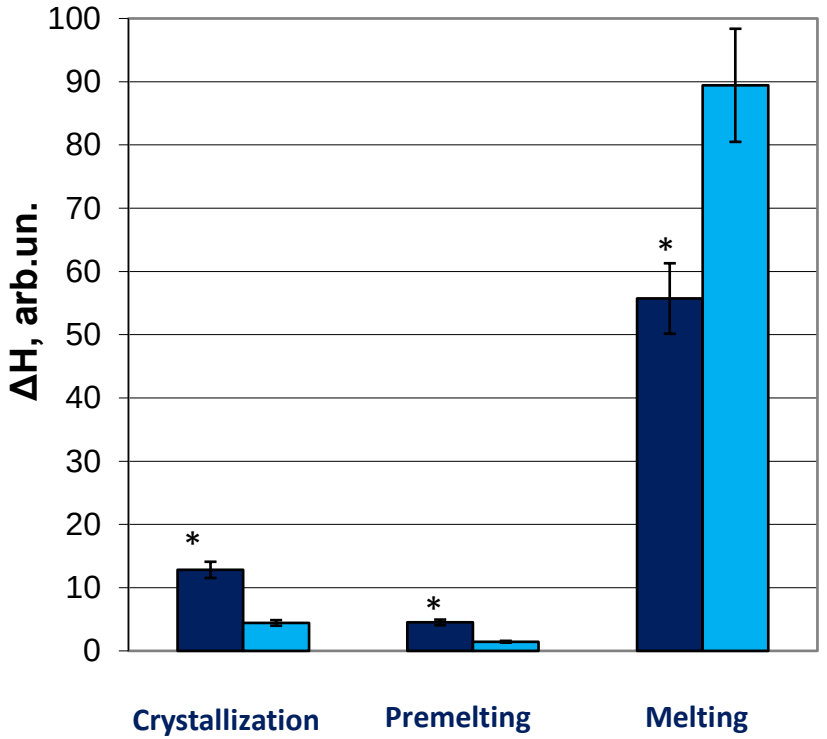
Grape buds viability was assessed by their swelling during cultivation under phytotron conditions with 5500 lux lighting at a temperature of 18-20°C.

Schematic diagram of laboratory vacuum infiltration unit for saturation of dormant grape buds

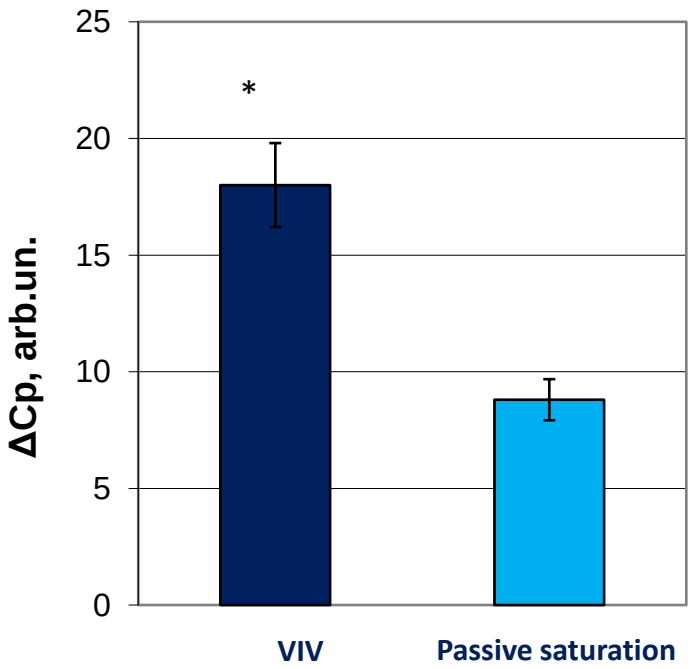




Enthalpy of phase transitions



Glass transition in grape buds



* Values are statistically different compared to the values for the passive saturation (P<0.05)

Grape dormant buds cryopreservation

Saturated buds	T_g, °C	T_c, °C	T_{pm}, °C	T_m, °C
Control	-87.9±1.6	-64.8±1.4	-37±0.5	-2.4±0.5
PVS1	-107.1±0.5*	-68.5±0.5	-37.2±0.5	-7.2±0.5*
PVS2	-115.1±0.5	-77.6±0.5	-37.1±0.5	-15.1±0.5*#
PVS3	-105±0.5*	-62.5±0.5	-36.4±0.5	-7.9±0.5*
PVS4	-107.2±0.5*	-77.4±0.5	-37.5±0.5	-9±0.5*
PVSN	-106.9±0.5	-85.2±0.5	-48.9±0.5	-4.1±0.5

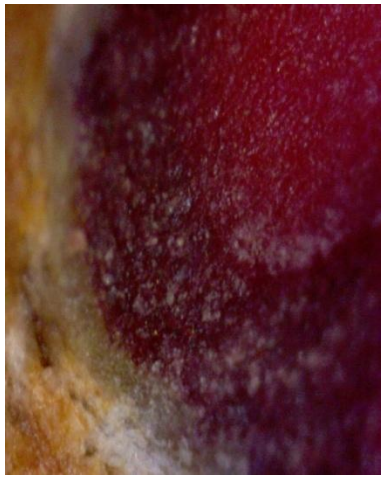
When saturated with PVSs, significant changes in heat absorption jump during glass transition, temperatures of phase transitions and their enthalpies were found. After saturation with PVS2, the temperature and enthalpy of melting were significantly lower than for PVS1, PVS3 and PVS4, which indicated a better saturation with PVS2. This may be due to the rather high content of Me_2SO.

* - statistically significant difference relative to control (p = 0.05), n = 5;
# –statistically significant difference relative to other PVS (p = 0.05), n = 5 .

Saturated buds	Δc_p	ΔH_c	ΔH_{pm}	ΔH_m
Control	9.1±1.0	0.83±0.21	0.38±0.16	174.1±29.8
PVS1	20.5±3.7*	9.22±1.15*	2.96±2.48*	68.3±9.9*
PVS2	18.0±0.9*	12.81±1.56*	4.5±0.51*#	55.72±6.34*#
PVS3	32.9±2.6*	1.98±0.56*	1.07±0.32*	116.7±17.3*
PVS4	34.8±3.9*	26.5±2.31*	0.42±0.13	73.6±11.5*
PVSN	12.3±2.1	1.33±0.17	0.79±0.13	123.5±11.5

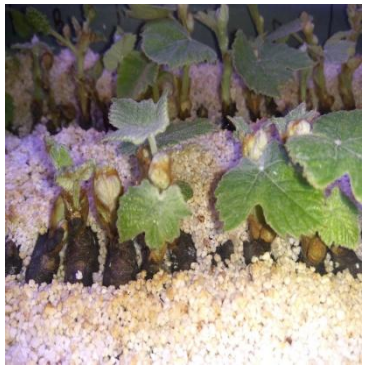
Grape dormant buds cryopreservation

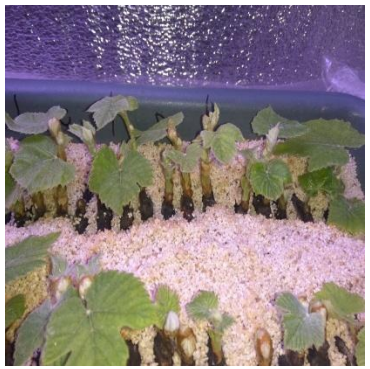
Viability of grape buds



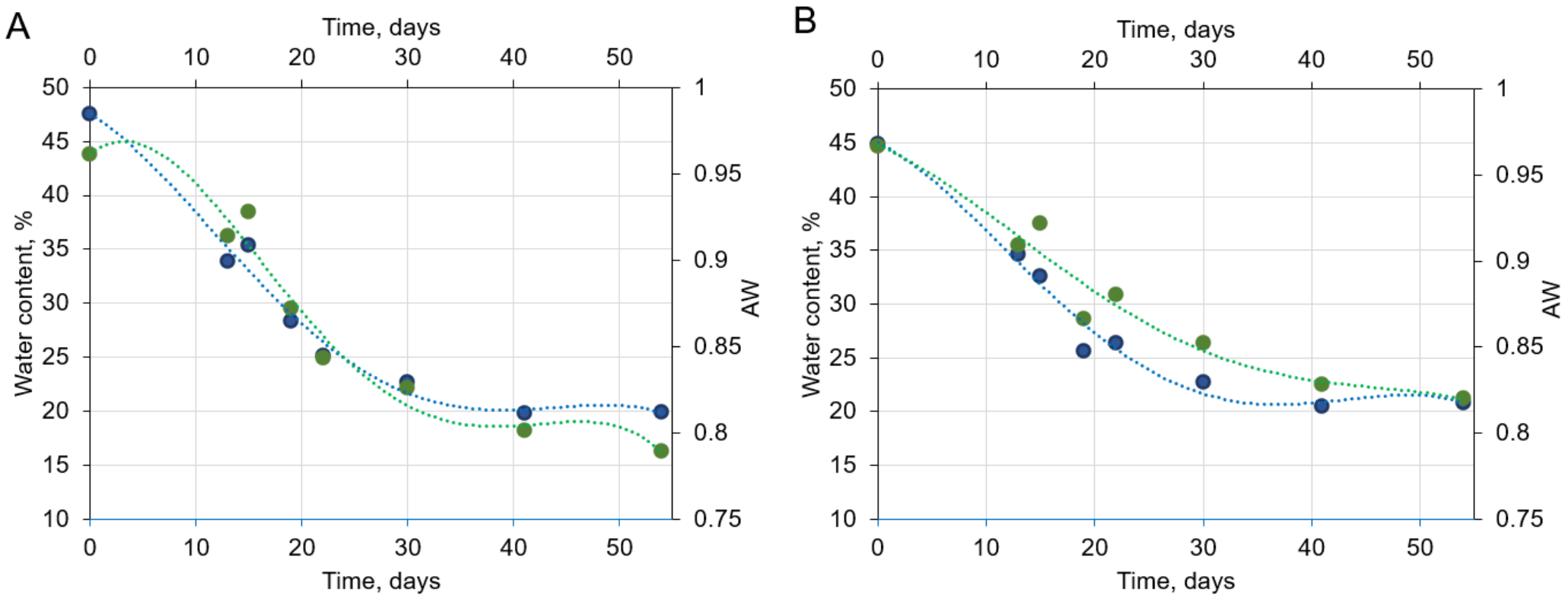
Regrowth of grape buds

VIV enabled 60% level of grape buds' viability for Russian Concord and Riparia X Rupestris varieties and 80% level of viability for Zagadka variety after cryopreservation. Regrowth of grape buds was 30% for Russian Concord, 40% for Zagadka , 0% for Riparia X Rupestris.
There were no integral and viable grape buds while using passive saturation approach.



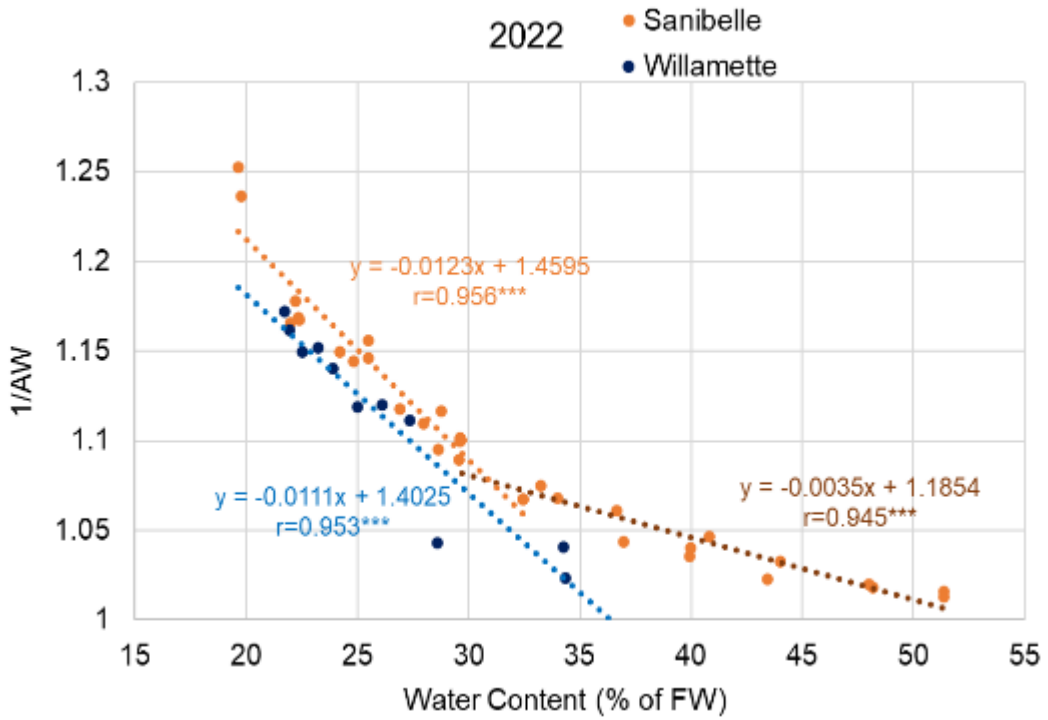


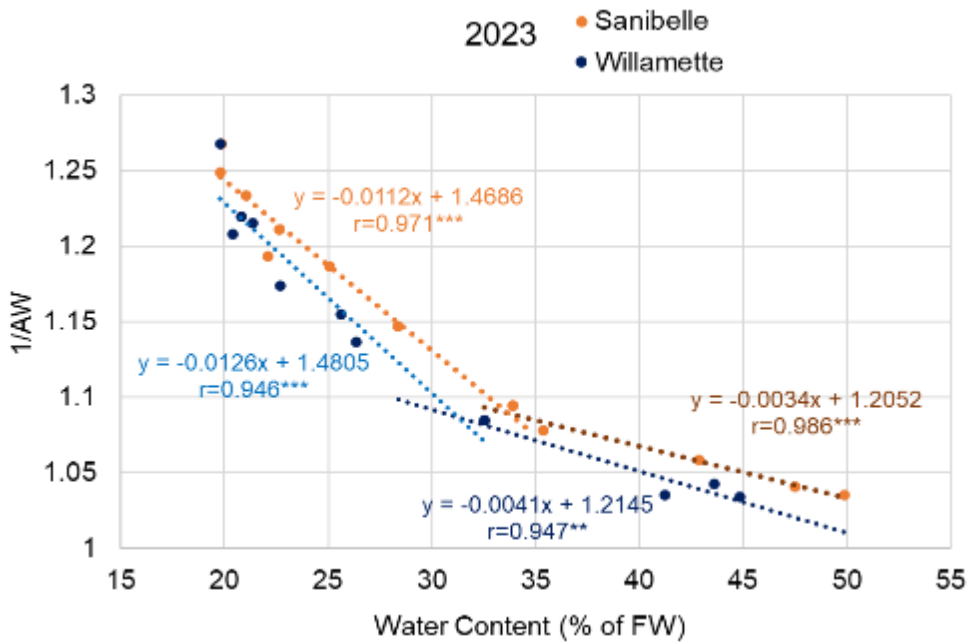
Raspberry dormant buds cryopreservation



Changes in water content (blue markers) and water activity (green markers) of nodal segments for Sanibelle (A) and Wilamette (B) varieties during dehydration

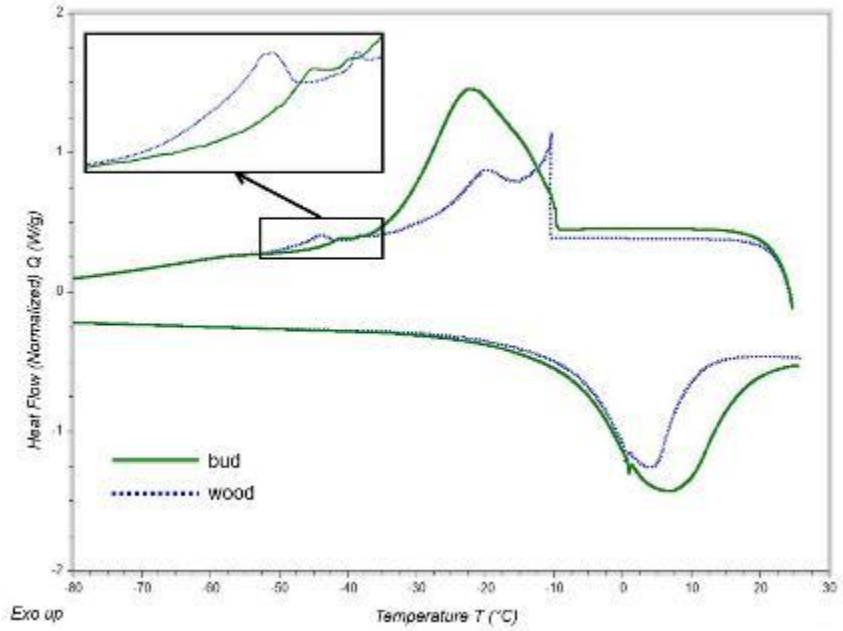
Raspberry dormant buds cryopreservation

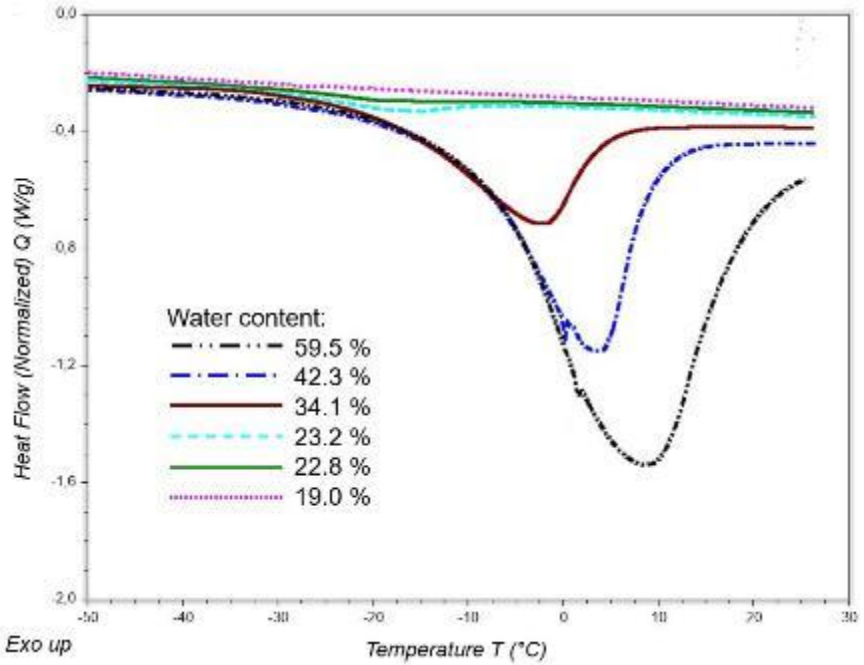




Changes in water activity (AW) of nodal raspberry segments with decreasing water content (WC) in two calendar years (2022 and 2023) for `Sanibelle` and `Willamette` varieties. The correlation between experimental points and the trendline is significant: ** $p<0.01$; *** $p<0.001$

Raspberry dormant buds cryopreservation

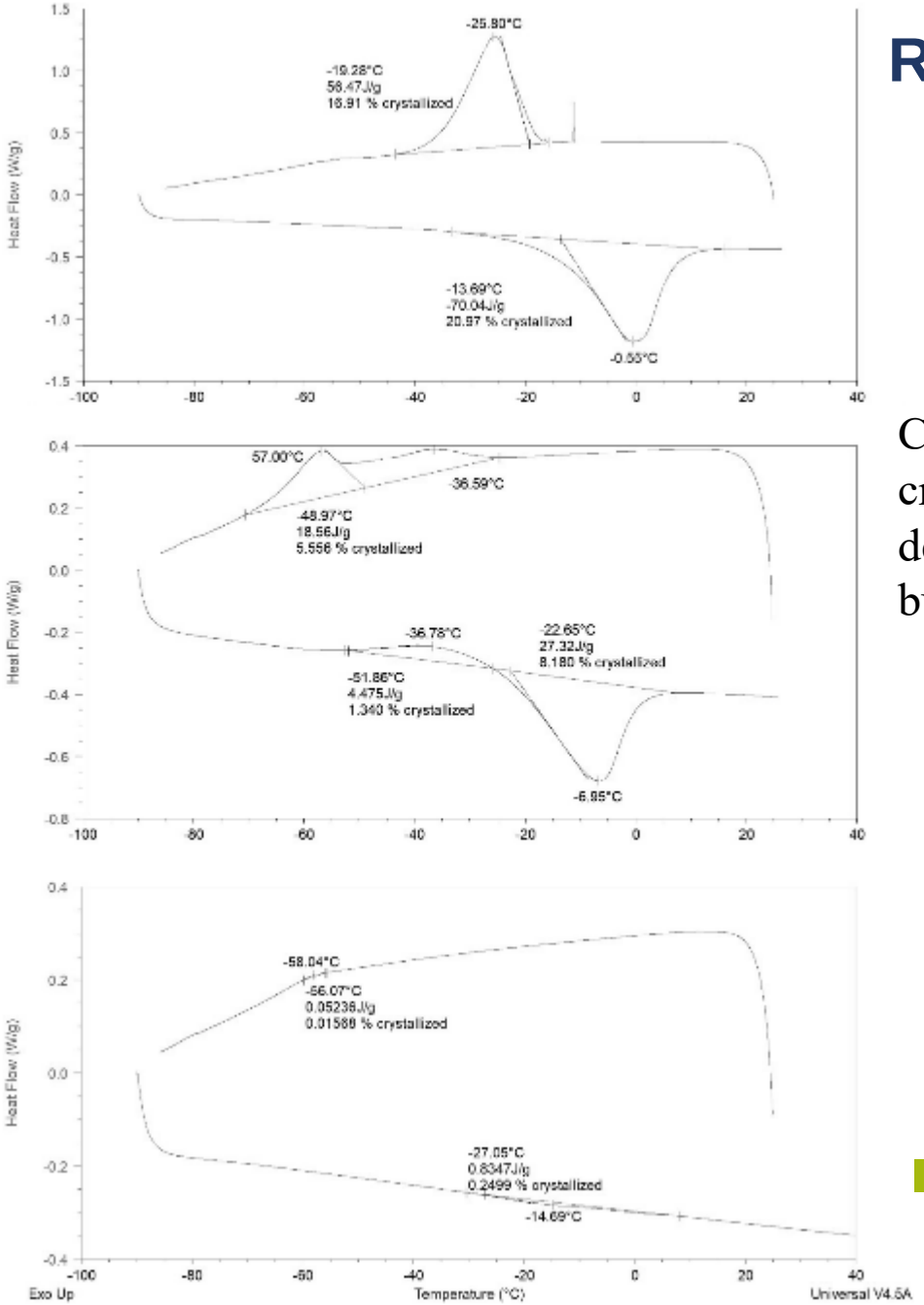




Differences in the state of water in the bud and wooden part of non-dehydrated nodal cane segments (`Willamette`)

Decrease in melting peak area and melting temperature of raspberry buds with decreasing water content (`Sanibelle`)

Raspberry dormant buds cryopreservation



Changes in temperatures of phase transitions and percentage of crystallized water in raspberry buds (Sanibelle variety) during dehydration (control at the top and the more dehydrated the buds, the lower in the graph)

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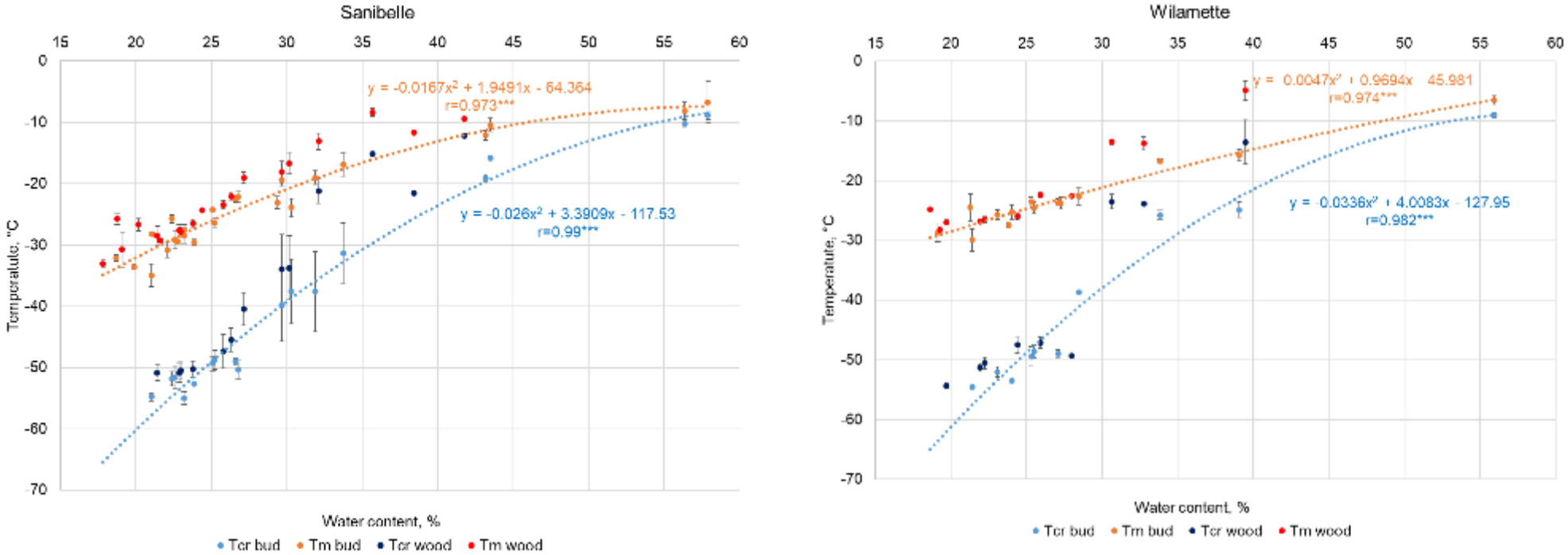
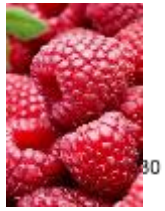
Raspberry dormant buds cryopreservation

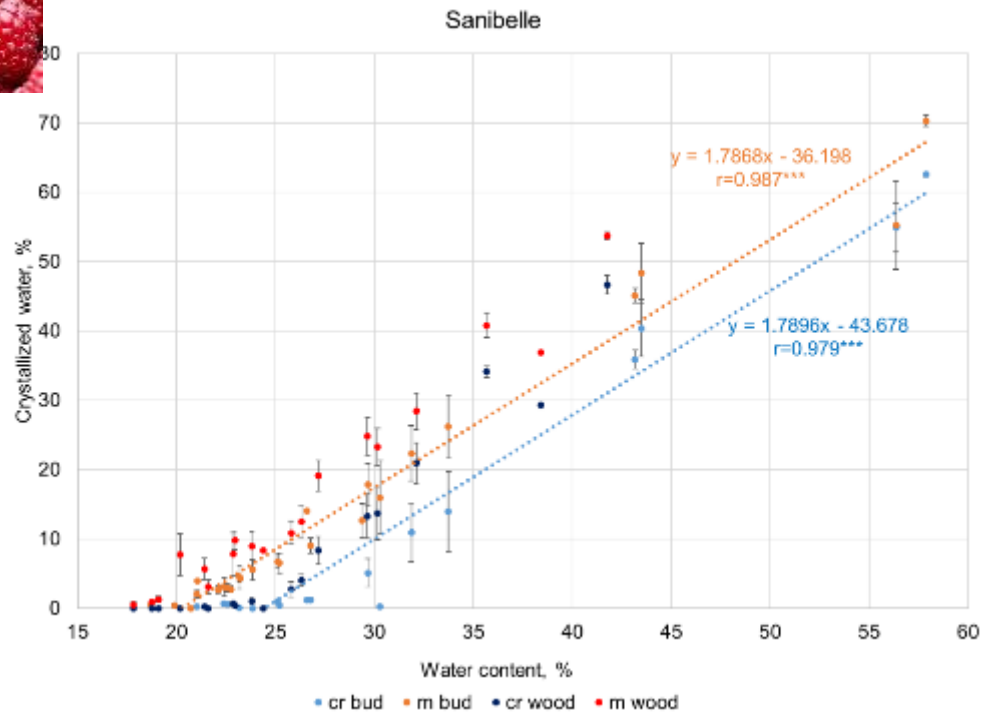


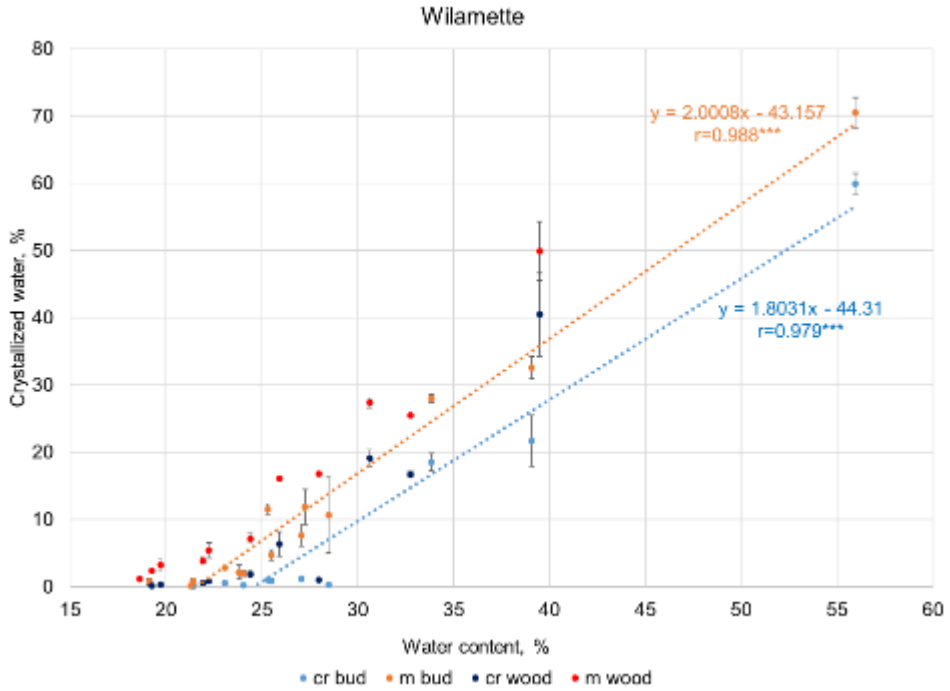
Decreasing of crystallization and melting peak temperature (onset) of raspberry buds and woody part with decreasing water content in `Sanibelle` and `Willamette` varieties. Data are presented as mean ± SE, n = 3-5. Designations in the figure: Tcr bud – crystallization temperature of buds; Tm bud – melting temperature of buds; Tcr wood – crystallization temperature of the woody part; Tm wood – melting temperature of the woody part. The trendlines are shown only for buds' crystallisation (blue) and melting (orange) temperatures.

***Correlation between experimental points and the trendline is significant at $p < 0.001$



Raspberry dormant buds cryopreservation

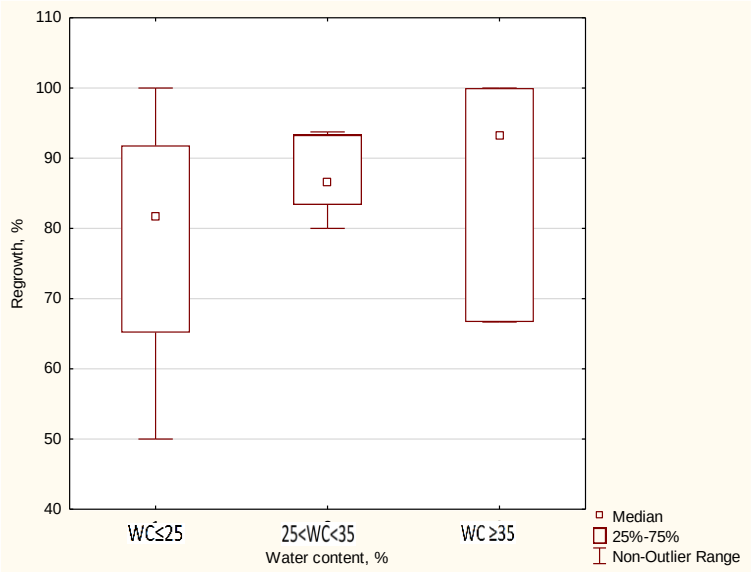


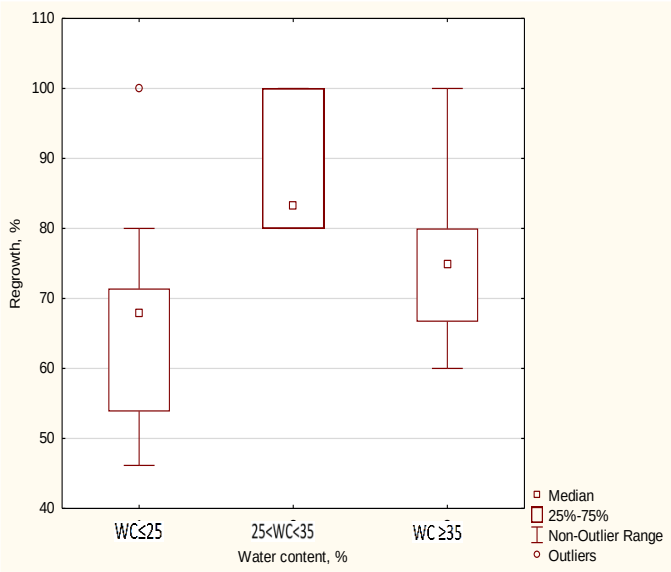


Crystallized water Crystallized water during cooling and warming of raspberry buds and woody part depending on water content. in `Sanibelle` and `Willamette`. Data are presented as mean ± SE, n = 3-5. Designations in the figure: cr bud – crystallization of buds; m bud – melting of buds; cr wood – crystallization of the woody part; m wood – melting of woody part; The trendlines are shown only for the percent of crystallized water during buds' crystallisation (blue) and melting (orange).

***Correlation between experimental points and the trendline is significant at $p < 0.001$

Raspberry dormant buds cryopreservation





54 days of dehydration

Willamette WC=20.8%

Sanibelle WC=19,9%

Regrowth of dehydrated '`Sanibelle`' (left) and `Willamette` (right) raspberry buds. The statistics signs are a small box for the median, a big box for the range 25% to 75% of data, whiskers for the non-outer range and a point for outliers.

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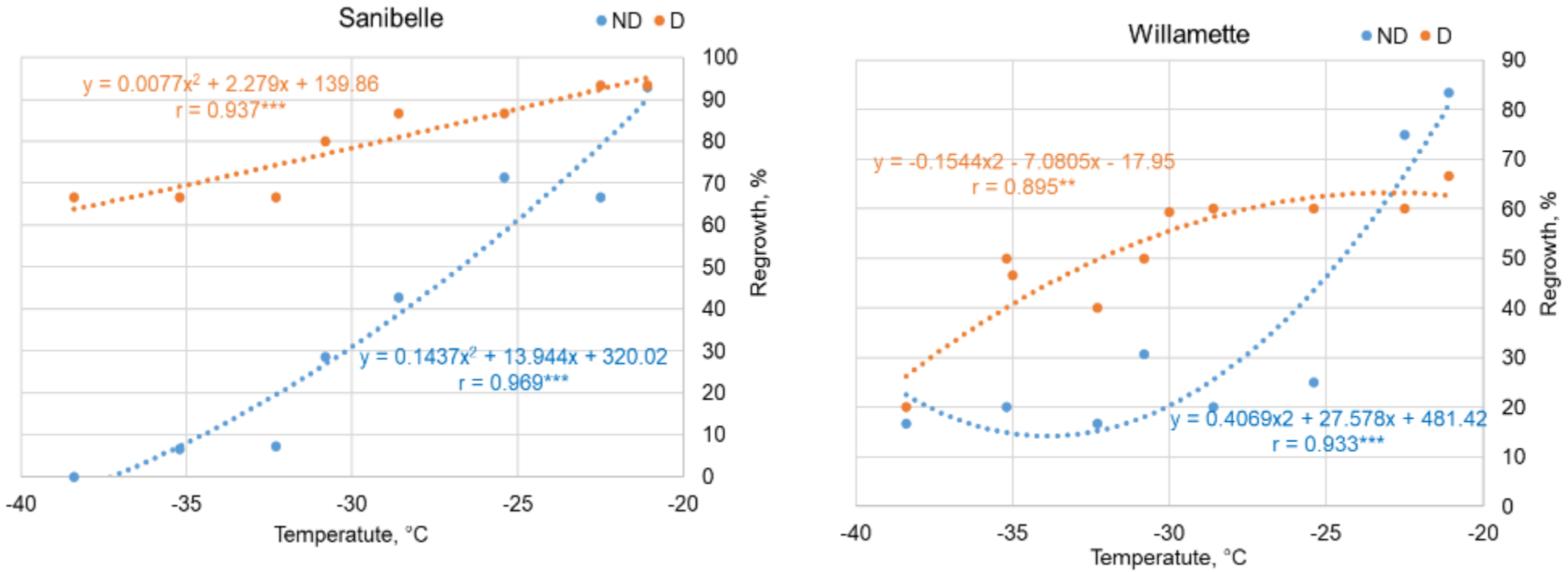
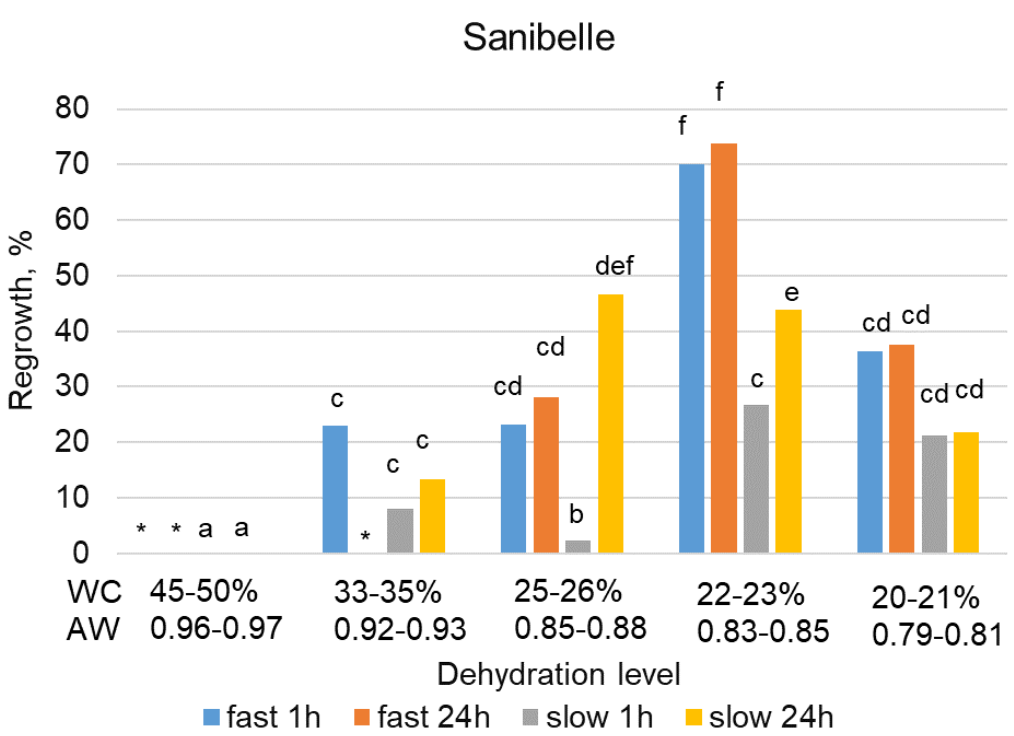
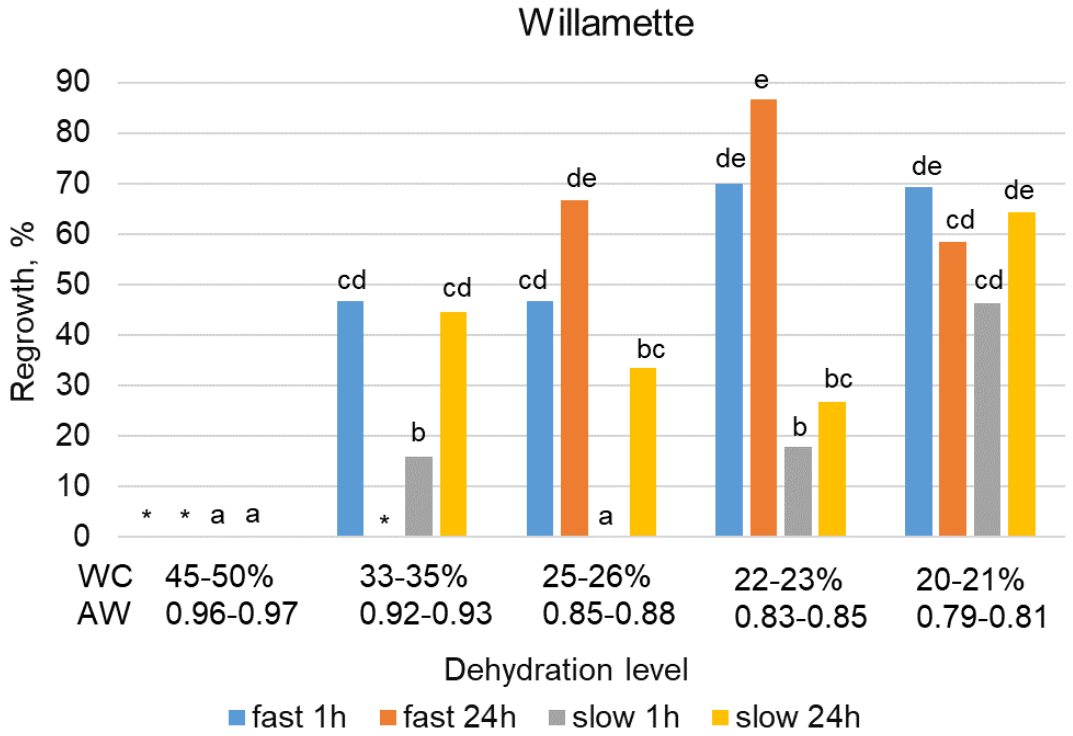
Raspberry dormant buds cryopreservation



Regrowth of dehydrated raspberry buds (n=15) after frost test for `Sanibelle` and `Willamette` varieties. Designations in the figure: ND – non-dehydrated buds (WC 45-50%, AW 0.96-0.97); D – dehydrated buds (WC 33-35%, AW 0.92-0.93)

Raspberry dormant buds cryopreservation





Regrowth of raspberry buds of `Sanibelle` and `Willamette` varieties with different dehydration levels after cryopreservation using various procedures. Designations in the figure: rapid – rapid thawing, slow - slow thawing, 1h and 24 h - annealing times at -30°C. * - no experimental data. a–f averages with the same index do not differ significantly

Kalorimetrický seminář 2025

Cryobiology
Volume 119, June 2025, 105240

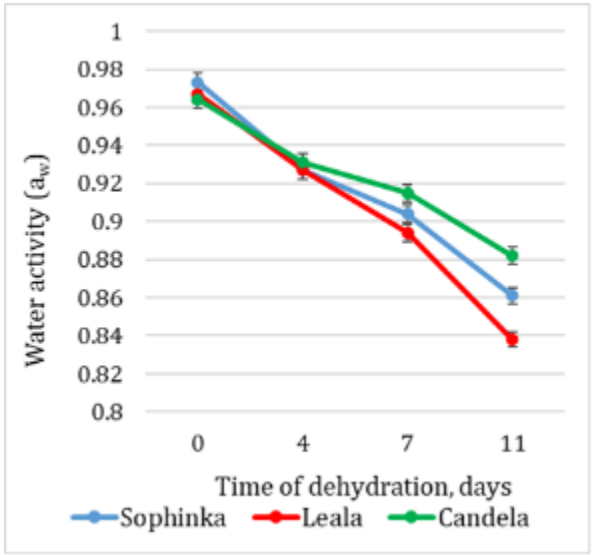
Dormant bud cryopreservation of Rubus idaeus L.

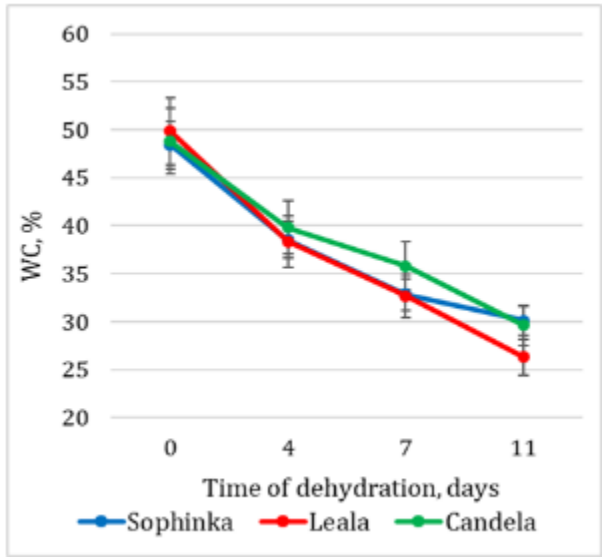
Olena Bobrova [a b], Jiri Zamecnik [a], Milos Faltus [a], Alois Bilavcik [a]

Conclusions

Cryopreservation of dormant buds has proven to be an effective method for preserving raspberry genetic resources. The two-step freezing process of dormant raspberry buds, following initial dehydration, achieved regeneration rate of up to 74–86 %. The highest regeneration rates were observed when the buds were dehydrated to a moisture level of 22–23 % and a water activity of 0.83–0.85. Optimal conditions for the two-step freezing process included an initial hardening temperature of −30 °C for 24 h, followed by rapid thawing after cryopreservation.

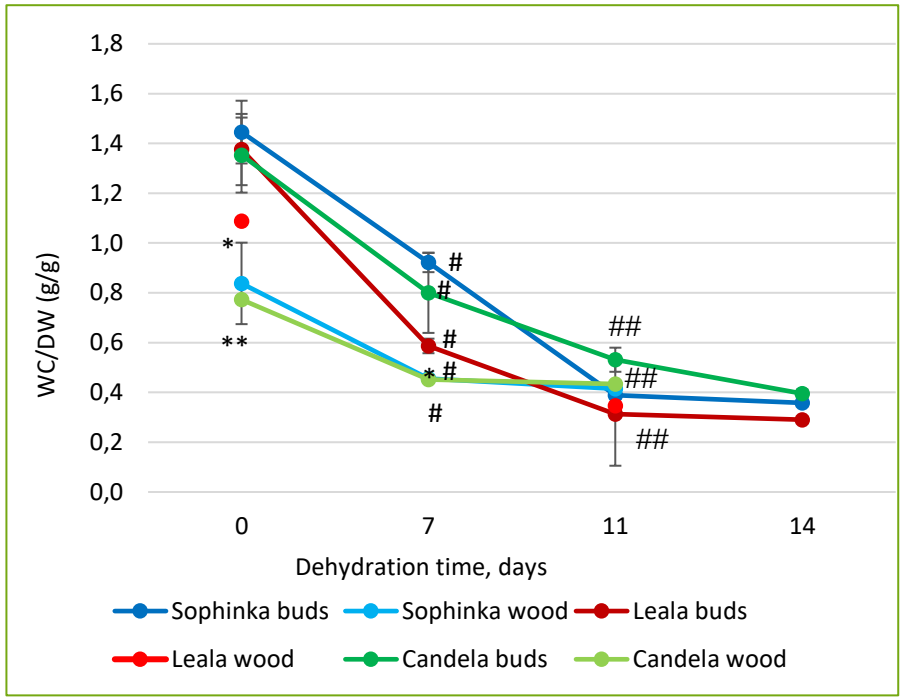
State of water in apricot dormant buds during dehydration





Water activity and water content (WC) changes in one-nodal segments of different varieties of apricot during dehydration time

Ratio of water content (WC) to dry weight (DW) of buds and twigs (wood) separately



*– differences are significant if compared with Sophinka buds; $p < 0.05$;
** – differences are significant if compared with Candela buds; $p < 0.05$;
# – differences are significant if compared with Control; $p < 0.05$;
– differences are significant if compared with 7 days; $p < 0.05$.

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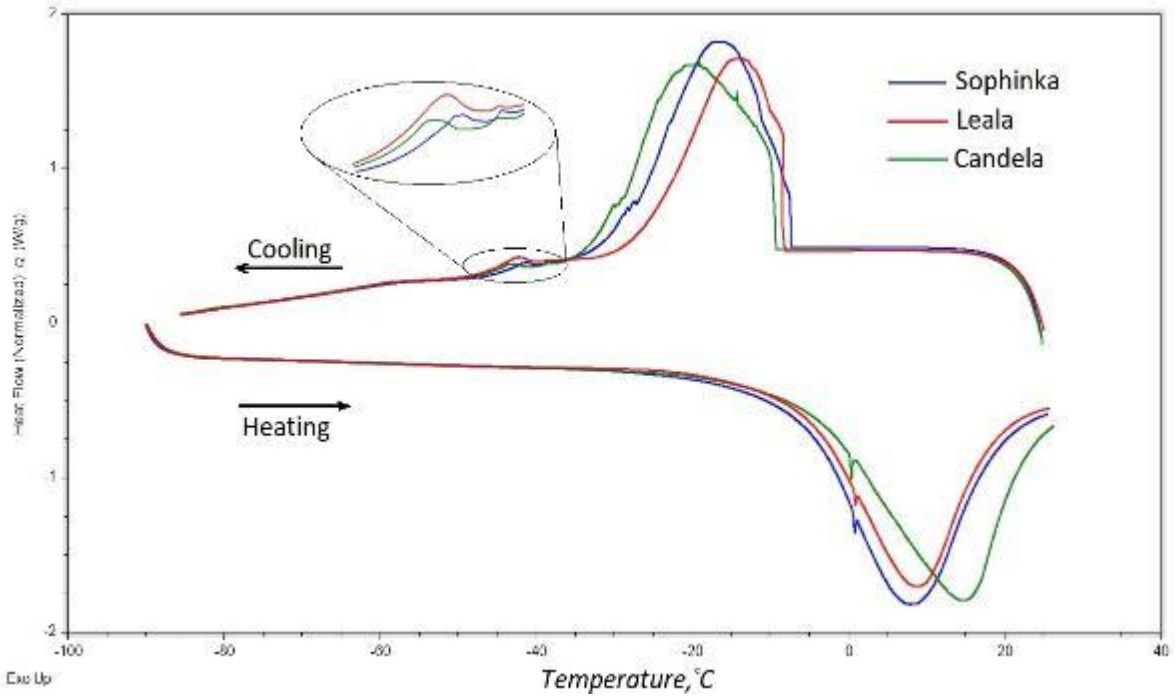
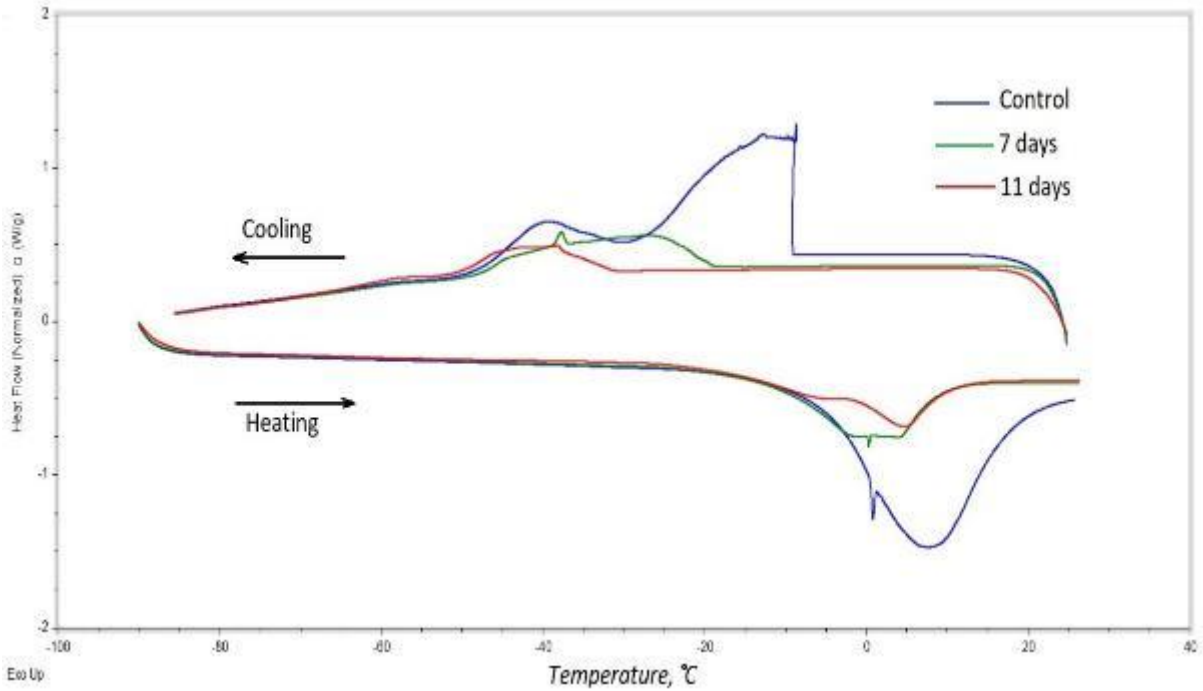
State of water in apricot dormant buds during dehydration and cooling (DSC)

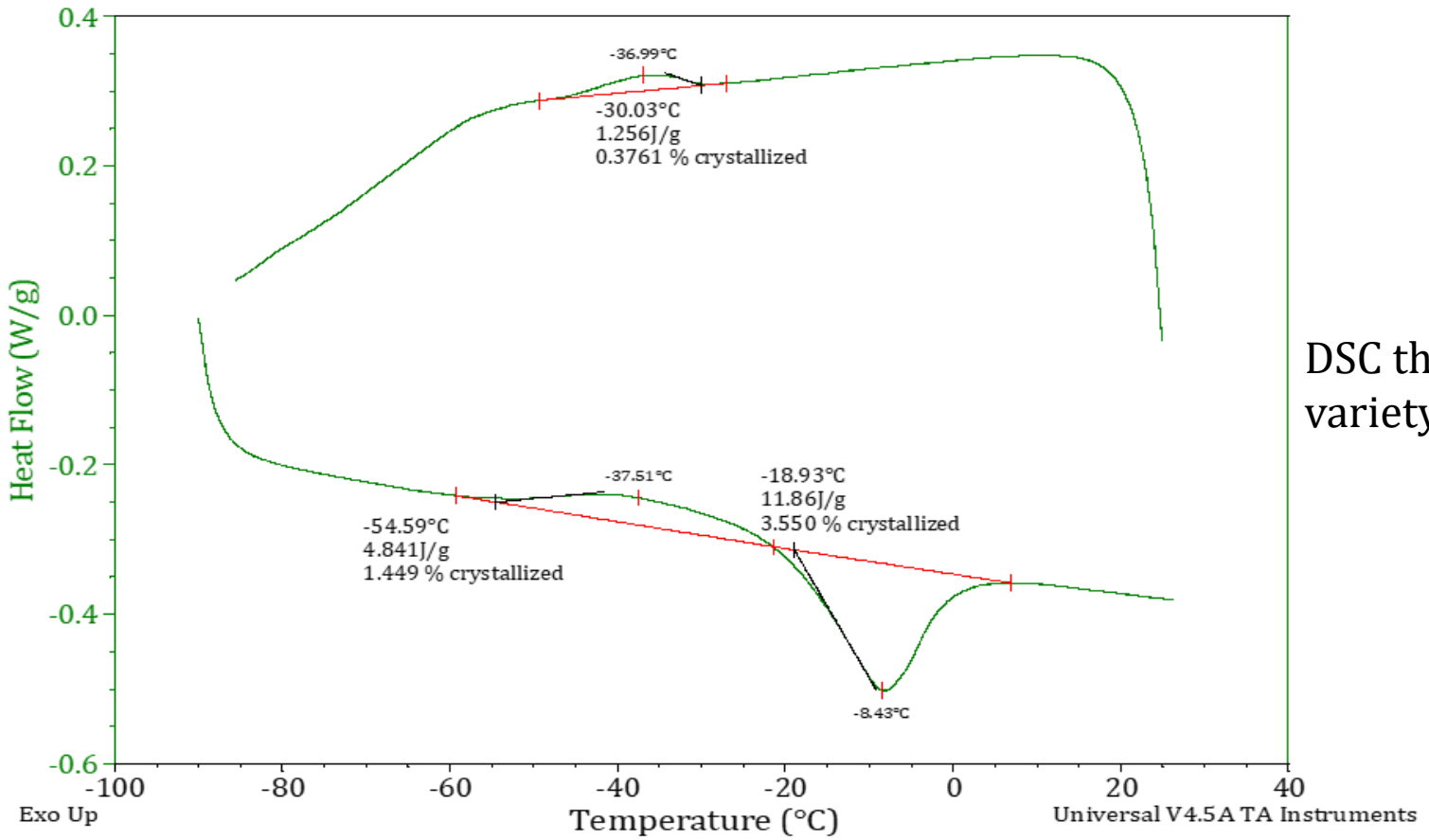


Control apricot dormant buds



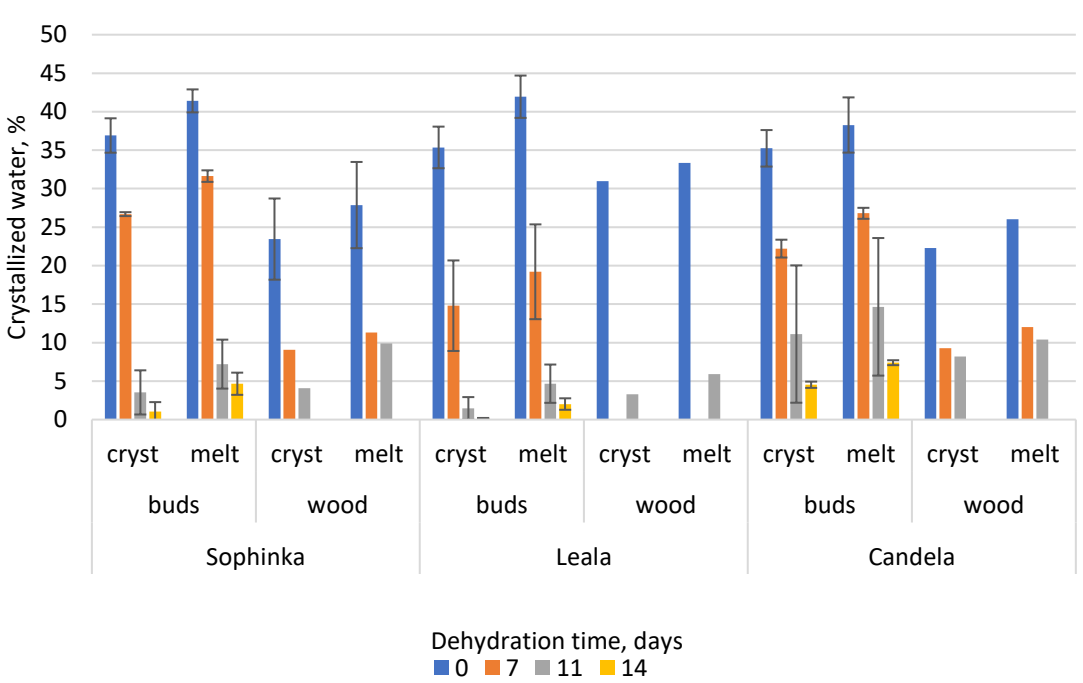
Wood of one-year old twigs during dehydration

State of water in apricot dormant buds during dehydration and cooling (DSC)

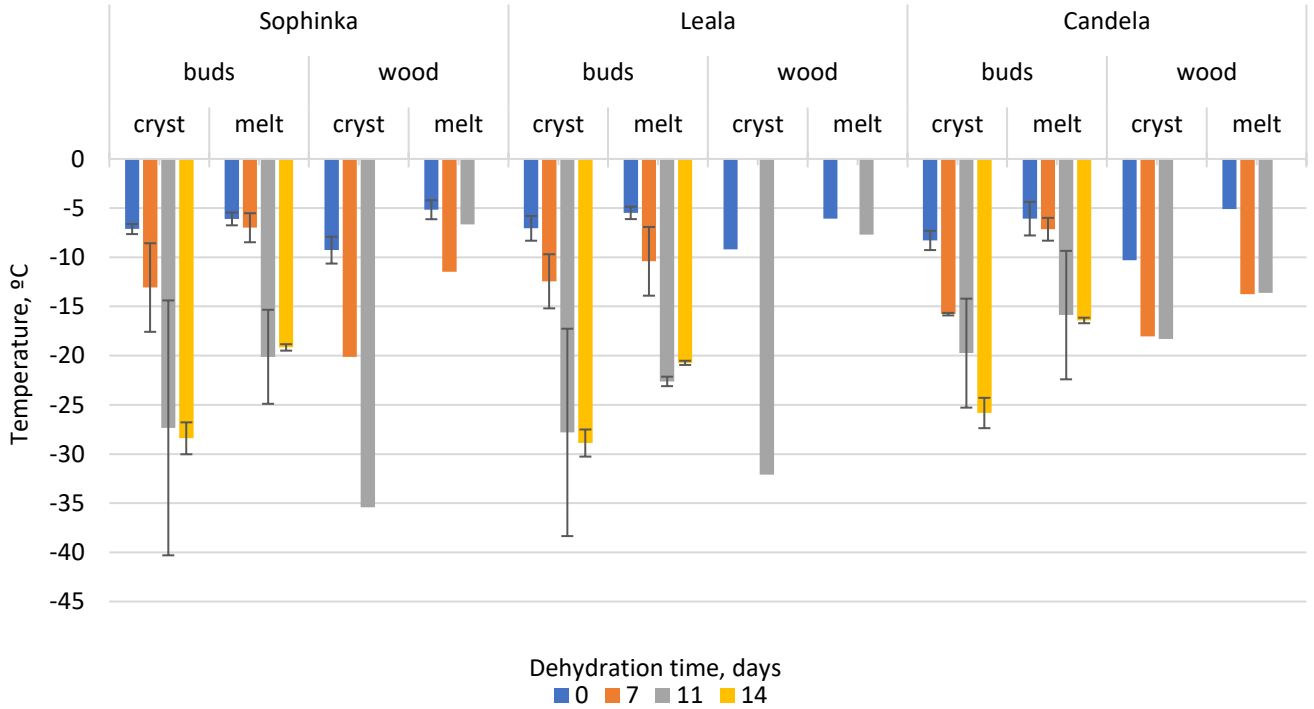


DSC thermogram of apricot dormant bud of variety Sophinka after 14 days of dehydration

State of water in apricot dormant buds during dehydration and cooling (DSC)



Changes in percentage of crystallized water at cooling and heating in apricot dormant buds and twigs (wood) during dehydration



Changes in onset temperatures of crystallization and melting in apricot dormant buds and twigs (wood) during dehydration

Water state of apricot stem cuttings during dehydration

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Conclusions

- Thermal characterization and analysis of water activity in twigs and buds provide important information about the water status of plant tissues, which is necessary for successful cryopreservation.
- Two isotherms during cooling are typical for apricot and raspberry tissues, however, for twig, the percentage of strongly supercooled water is many times greater than for buds.
- The moisture content of buds is higher than that of twig, therefore, the determination of the average moisture content of single-node segments for cryopreservation is incorrect and does not allow estimating the moisture content of the buds themselves.
- Crystallization in twig tissues occurs at lower temperatures and the percentage of crystallized water is lower than for buds.
- At moisture levels in plant tissues below 25-30%, the water is prone to supercool and may be in the glassy state at low temperatures. However, part of this glassy phase is metastable and can crystallize during heating.

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Thanks for your attention!

Physiology and Cryobiology of Plants Team

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