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Short Report
Section: Food Technology

Effect of Microbial Transglutaminase on Ice Cream Heat Resistance Properties – a Short Report

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The objective of this study was to investigate the effect of the addition of transglutaminase (TG) preparation Saprovia L ® (PMT TRADING Co. Ltd, Lodz, Poland) on the properties of ice cream with 40 g/kg and 70 g/kg fat content. TG was added at a concentration of 2 U/g protein. We studied the effect of transglutaminase on fresh and 3-month-stored at -25°C ice cream. Ice cream mixes were prepared with 5 g/kg stabilizer. Melting test was performed after thermal shocks until the "1st drop" occurrence. The amount of effluent was measured within the 0–120 min time frame. We evaluated the appearance of the samples and carried out the TPA and compression analysis. The addition of the enzyme has increased the resistance of stored ice cream to repeated thermal shocks.

INTRODUCTION

Technology of ice cream production is one of the most sophisticated in the dairy industry. Consumers expect that new products with new materials and additives will have the same sensorial and rheological characteristics as "traditional" ice cream. This stems from the fact that customers are accustomed to good quality ice cream and technological status. Ice cream is a complex physicochemical system consisting of ingredients in different phases. Typical ice cream is composed of ice crystals (30%), air bubbles (50%), fat globules (*ca*. 5%) and matrix (15% - non-frozen phase containing sugar), stabilizers and milk proteins. The ingredients of ice cream are in three different states: steady – ice crystals and fat globules; liquid – sugar solution, and the gas state. Liquid and gas states are composed of small ice crystals, fat globules and air bubbles suspended in a matrix. It is important to obtain the smallest size of ice crystals, fat globules and air bubbles to retain the desired quality and stable structure during frozen storage, often lasting several months [Clarke, 2004; Cook & Hartel, 2005; Hartel, 1996; Wildmoser *et al*., 2004].

The quality of ice cream, except for the formulation, depends on a number of factors, such as the viscosity of ice cream mixes, freezing point temperature, air volume, resistance to melting and storage conditions [Clarke, 2004]. Resistance to temperature changes during storage and transport is a very important functional feature of ice cream. Selected procedures allow providing stable, low temperature of ice cream during storage by the producer, transport and retailer stock. Ice cream is a very unique product, that requires continuous cold chain to retain good quality. During storage and transport ice cream can be subject to unexpected thermal shocks due to power cuts, unsealed fridge doors or transport without thermal protection from the retailer to the consumer.

Thermal shocks can cause changes in the quality of ice cream, contributing to overrun loss and ice recrystallization, which lower organoleptic properties and may be dangerous to the health of consumers. It is highly important to find new technological solutions, that could increase resistance to this processes in order to avoid such problems. The application of transglutaminase is one of the solutions.

Transglutaminase, TG (EC 2.3.2.13) is an enzyme recognized as safe (GRAS) and used in various sectors of the food industry. This enzyme acts by modifying the functional properties of milk proteins [Nuernberg-Rossa *et al*., 2012]. Concentration of proteins in the substrate is one of the most important factors in TG gelation. When protein concentration is low, the enzyme only increases the viscosity, without gelation. Transglutaminase is used for cross-linking soy protein, casein and other milk proteins, gluten, fish and bovine proteins as well as some other food proteins [Ando *et al*., 1989; Ashie & Lanier, 2000; Bönisch *et al*., 2007; Collar *et al*., 2005; Gauche *et al*., 2008; Huang *et al*., 2008; Kołakowski & Sikorski, 2001; Lauber *et al*., 2000; Wang *et al*., 2011].

The aim of this study was to investigate the effect of transglutaminase addition on ice cream stability after repeated thermal shocks. Different fat content and the same amount of stabilizer was used in ice cream mixes in all samples. Samples with no enzyme served as control.

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TABLE 1. Experimental ice cream mixes composition

Ingredients	Mix composition (g/kg)			
	70 g/kg of fat		40 g/kg of fat	
	control sample	enzyme treated	control sample	enzyme treated
Skimmed milk powder	100	100	100	100
Sugar	130	130	130	130
Stabilizer	5	5	5	5
Starch syrup	40	40	40	40
TG – Saprovia L®	0	20	0	20
Coconut fat	70	70	40	40
Water	655	635	685	665

MATERIALS AND METHODS

Four different ice cream formulations were prepared according to the recipes in Table 1. Ice cream with TG were compared to control samples without the enzyme.

Ice cream preparation

Four mixes of ice cream were made according to the scheme in Table 1. The mixes were pasteurized for 15 min at 85°C with continuous stirring. Next, they were rapidly cooled to *ca*. 70°C and homogenized (Homogenizer MPW –120) at 3000 rpm for 15 min. In the next step, the mixes were cooled to 4°C and stored for 20 h at 4±1°C for aging process. Then, they were cooled in a freezer (Promag DS 40/50, Italy). Ice cream were dispensed into plastic boxes in a volume of 150 g per sample. Afterwards, they were frozen at -22°C and stored under this condition until analysis. Ice cream tests were carried out directly after their preparation and after three months of their storage.

Repeated thermal shocks

Ice cream after preparation and after 3 months of storage were subjected to repeated thermal shocks according to the scheme given below. The samples were placed in a refrigerator chamber at -25°C for 48 h. The samples were then transferred to -19°C for 4 h. Afterwards, ice cream samples were incubated for 1 h at 22°C and subsequently moved back -19°C for 4 h. Then the samples were incubated at 22°C for 1 h and moved back again to -19°C for 20 h. Following this procedure, ice cream were placed at 20°C and the melting test was carried out. Volume of the effluent was measured at 0÷120 min time interval and "the 1st drop" occurrence was recorded. The appearance of the samples was also evaluated.

Textural analysis

Ice cream texture measurements were carried out with a Brookfield texture analyzer CT3 (Brookfield Engineering Laboratories, Inc., Massachusetts, USA). Texture profile analysis (TPA) was conducted with TA17/1000 at 0.05 N and a test speed of 1 mm/s. Compression test was also performed with the TA25/1000 probe at 1 N, 50% deformation and a test speed of 1 mm/s.

Statistical analysis

Statistical analysis was carried out using one-way ANOVA and the Tukey's test ($p<0.05$) using Statistica® 10.0 PL software (StatSoft Poland Sp. z o. o., Cracow).

RESULTS AND DISCUSSION

The first stage of research was to investigate ice cream properties in the melting test (melting rate), in which the amount of effluent in the interval of 0÷120 min was measured and the time of the "1st drop" occurrence was recorded.

Figures 1 and 2 present ice cream samples after the preparation and after 3 months of storage, subjected to repeated thermal shocks and subsequent melting test at a temperature of 20°C. Several effects of TG addition were observed during the experiments. Transglutaminase increased sample stability and melting resistance, relative to control samples without the enzyme. Lower melting rate was caused by the polymerization of milk proteins by TG [Nuernberg-Rossa *et al*., 2011], which increased ice cream stability, particularly when the fat content was reduced. Similar effects were noticed by Koxholt *et al*. [2001], Karaca *et al*. [2009] and Nuernberg–Rossa *et al.* [2012].

Thermal shocks applied to 3-month-stored ice cream showed that the stability of samples with 40 g/kg fat, TG and 5 g/kg stabilizers was higher compared to the samples with 70 g/kg fat and TG as well as control samples without TG but with different fat content. Ice cream melting test after repeated thermal shocks showed that the addition of transglutaminase resulted in the extension of the time till the appearance of the "1st drop" compared to control samples. Ice cream with TG, 40 g/kg fat and 5 g/kg stabilizer, subjected to repeated thermal shocks directly after preparation were tested for melting resistance, similarly as ice cream with a higher fat content (Figure 3). In both cases, the "1st drop" was observed after 30 min of the test. For control samples, that time was shorter by about 6÷8 min. The "1st drop" in the 3-month--stored ice cream with 40 g/kg fat and TG, after repeated thermal shocks, was also recorded after 30 min, however, in the analogical sample but with 70 g/kg fat, it was only 23 min.

After 3 months of storage, ice cream with the enzyme, subjected to repeated thermal shocks, retained the shape for a longer period of time and signs of melting were less pronounced compared to the ice cream heat-shocked directly after preparation. Furthermore repeated thermal shocks resulted in a lower amount of effluent in the samples stored for 3 months (Figure 4).

We also conducted ice cream texture analysis following repeated thermal shocks. The results of the texture profile analysis (TPA) are presented in Table 2. Fresh-made ice cream, after repeated thermal shocks, had a higher T_1 hardness than the 3 month-stored samples after the same procedure. The control samples (40 g/kg and 70 g/kg fat in mixes, without enzyme) stored for 3 months showed a decrease in DT_1 (deformation at hardness) after repeated thermal shocks, compared to the samples heat-shocked directly after preparation. This value was higher in the sample with TG and 40 g/kg fat in the ice cream mix. Freshly made samples with-

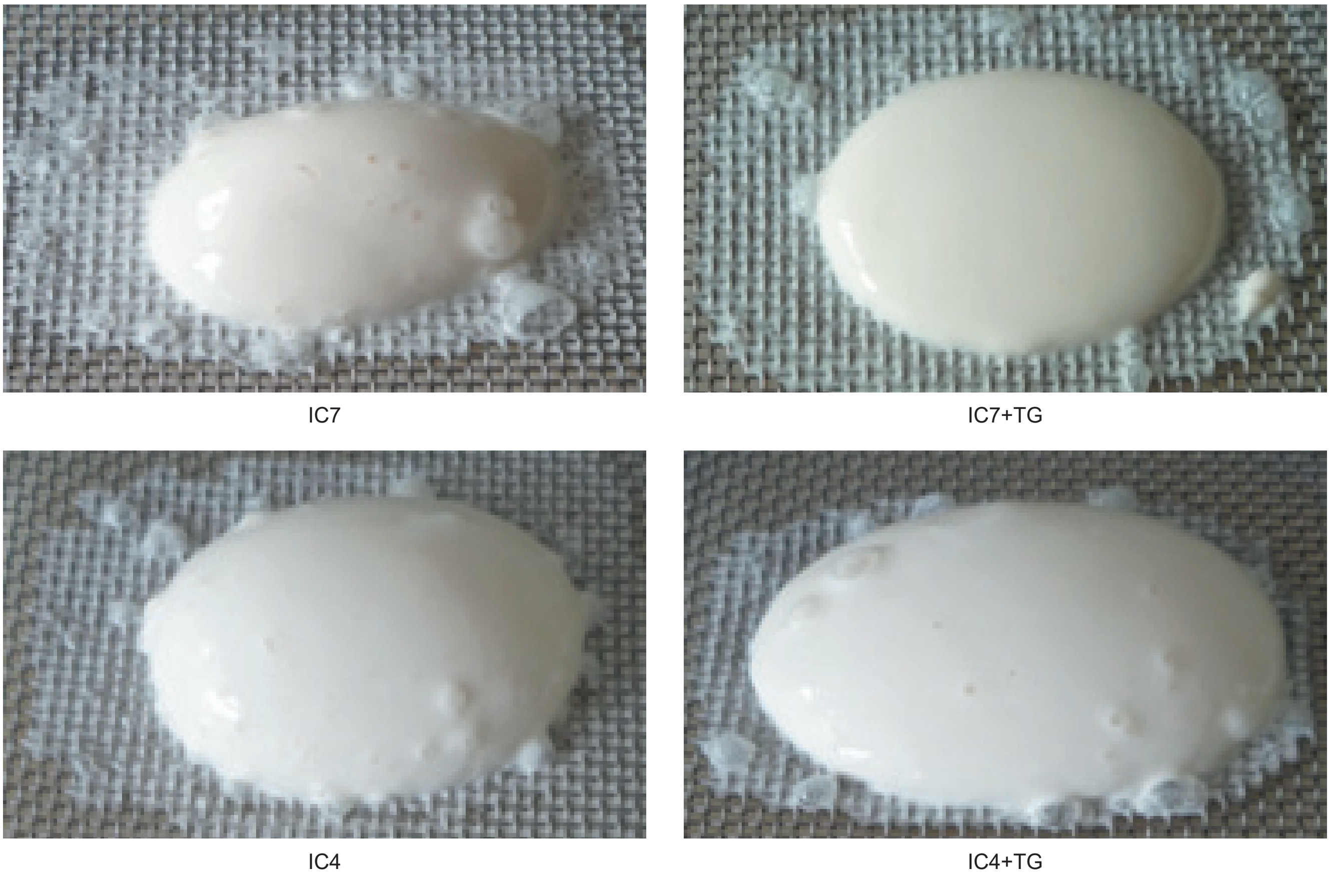

FIGURE 1. Ice cream melting test after repeated thermal shocks (after 120 min) – without storage.

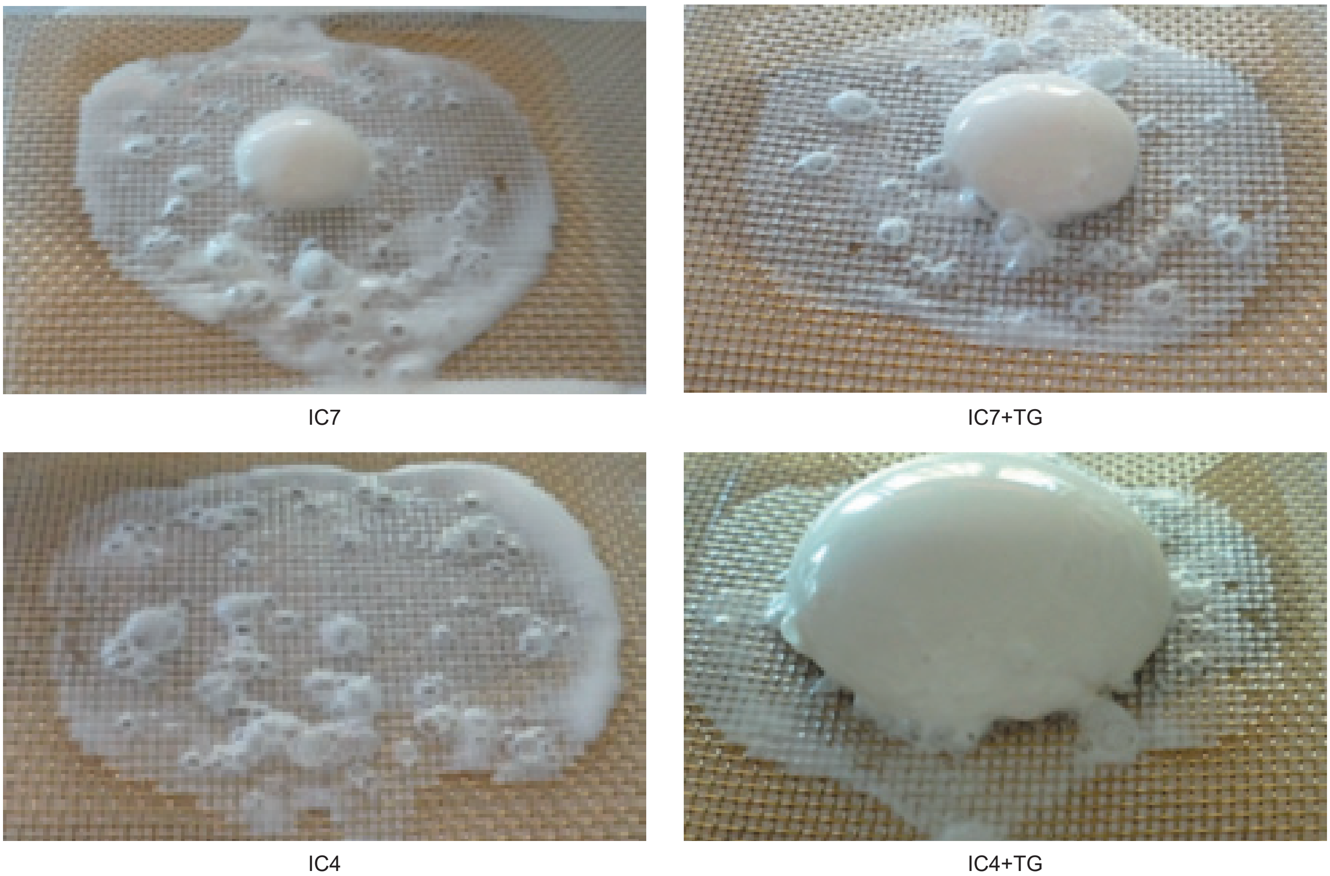

FIGURE 2. Ice cream melting test after repeated thermal shocks (after 120 min) – after 3-momth storage.

TABLE 2. Ice cream texture after repeated thermal shocks – TPA test.

Samples[a]	T_1 (N)[bc]	DT_1 (%)[bc]	A_p (mJ)[bc]	S (mm)[bc]	I_s[bc]
Ice cream after production					
IC4	$10.35^{a}\pm0.05$	$82.30^{a}\pm0.09$	$8.70^{a}\pm0.06$	$5.35^{a}\pm0.15$	$0.23^{a}\pm0.02$
IC7	$24.63^{b}\pm0.05$	$77.70^{b}\pm0.05$	$9.90^{b}\pm0.06$	$6.64^{b}\pm0.05$	$0.27^{b}\pm0.03$
IC4+TG	$17.79^{c}\pm1.25$	$98.30^{c}\pm0.08$	$25.70^{c}\pm0.89$	$3.29^{c}\pm0.08$	$0.13^{c}\pm0.03$
IC7+TG	$21.51^{d}\pm1.20$	$97.50^{d}\pm0.05$	$24.50^{d}\pm0.75$	$3.07^{c}\pm0.04$	$0.12^{c}\pm0.04$
Ice cream after 3 months of storage					
IC4	$4.30^{e}\pm0.01$	$79.40^{e}\pm1.23$	$5.20^{e}\pm0.25$	$3.84^{d}\pm0.18$	$0.14^{c}\pm0.01$
IC7	$5.69^{f}\pm0.08$	$60.00^{f}\pm0.08$	$4.30^{f}\pm0.04$	$13.07^{e}\pm0.02$	$0.52^{d}\pm0.05$
IC4+TG	$4.67^{g}\pm0.56$	$99.60^{g}\pm1.05$	$14.10^{g}\pm1.00$	$12.51^{f}\pm0.89$	$0.50^{d}\pm0.08$
IC7+TG	$6.72^{h}\pm0.55$	$96.40^{h}\pm0.05$	$12.70^{h}\pm1.00$	$13.47^{g}\pm0.75$	$0.53^{d}\pm0.08$

[a]Ice cream with 40 g/1000 g fat without TG (IC4) and with TG (IC4+TG); ice cream with 70 g/1000 g without TG (IC7) and with TG (IC7+TG). [b]Mean values ± standard deviation, n = 5. Values with different letters in the same column are significantly different ($p < 0.05$) (Tukey test). [c]Explanatory notes: T1 (N) - hardness I, DT1 (%) - % deformation at hardness , Ap (mJ) - adhesiveness, Is - springiness index, S (mm) – stringiness length.

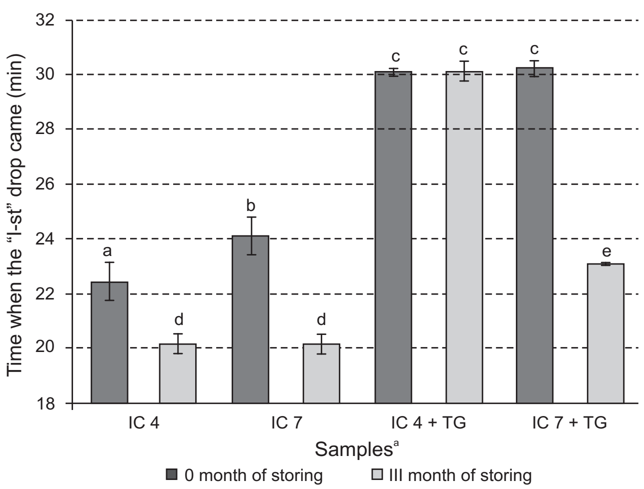

FIGURE 3. Time of the „1-st drop".
[a]Ice cream with 40 g/1000 g fat without TG (IC4) and with TG (IC4+TG); ice cream with 70 g/1000 g without TG (IC7) and with TG (IC7+TG). Different letters indicate a significant difference between samples ($p<0.05$) (Tukey test).

out the enzyme, after repeated thermal shocks, cracked under the pressure applied during the TPA test, while the samples with the enzyme retained the shape. The adhesion force that is needed to detach the probe from the sample was the highest for the sample mix with 40 g/kg fat and the TG enzyme. This value was decreased for all samples treated with repeated thermal shocks and stored for 3 months.

Springiness index was increased for samples with 40 g/kg and 70 g/kg fat content, supplemented with TG, similarly as in the control sample with 70 g/kg fat. Statistical analysis showed that the obtained differences were statistically significant ($p<0.05$) (Table 2).

Compression test proved that the samples with 4% fat were the most stable during repeated thermal shocks (Figure 5). Hardness parameter in the samples stored for 3 months showed a 2–3% fluctuation range, while for the other samples it ranged from 10 to 25%. This test was also performed for the samples not subjected to the thermal shock treatment. Comparison of the results showed that the samples after repeated thermal shocks had a higher hardness value. Only

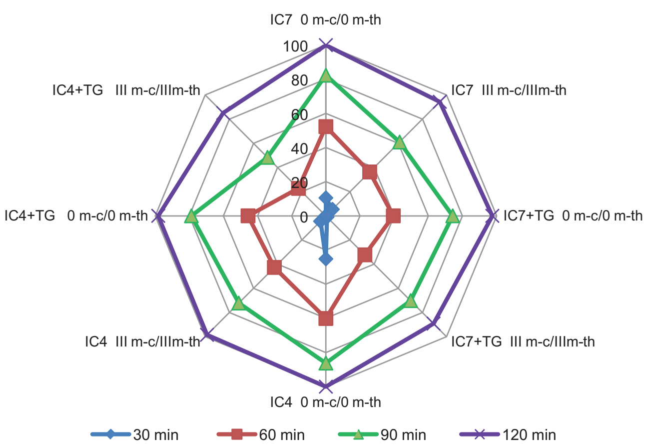

FIGURE 4. Ice cream melting test after repeated thermal shocks.

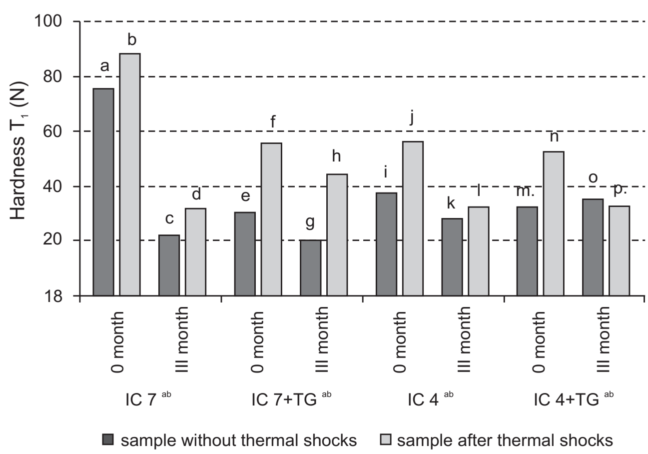

FIGURE 5. Ice cream hardness comparison after and without repeated thermal shocks in compression test.
[a]Ice cream with 40 g/1000 g fat without TG (IC4) and with TG (IC4+TG); ice cream with 70 g/1000 g without TG (IC7) and with TG (IC7+TG). [b]Values with different letters in columns are significantly different ($p<0.05$) (Tukey test).

the IC4+TG variant after 3 months of storage had a higher hardness.

The application of TG in the production of ice cream was also investigated by Nuerenberg–Rossa *et al*. [2012]. They have proved that transglutaminase could be used to replace a partial amount of fat. Owing to enzyme addition, ice cream with less amount of fat (4%) had properties similar to ice cream with 8% of fat.

Ice cream resistance to repeated thermal shocks is a very important and useful feature. Consumers expect high quality and unpredictable problems may expose manufacturers to heavy losses. By creating a product with high resistance to adverse temperature conditions, damage caused by this factor can be limited and manufacturers can gain a competitive advantage.

CONCLUSIONS

The results of the experiments demonstrated an advantageous effect of transglutaminase addition on the properties of ice cream. The addition of the enzyme slowed down the melting process.

TG addition caused an increase in ice cream resistance to repeated thermal shocks.

Experimental data proved that the addition of TG to the samples with a lower amount of fat, resulted in the properties similar to control ice cream with a higher fat content.

The addition of the enzyme allowed maintaining the shape of ice cream for a longer period of time.

The amount of effluent in the melting test was lower after 120 min than in the control samples.

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CONFLICT OF INTEREST

None declared.

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