Trehalose Reduces Host Cell-Derived Impurities During Harvesting Process Using Chromatographic Clarifier Device

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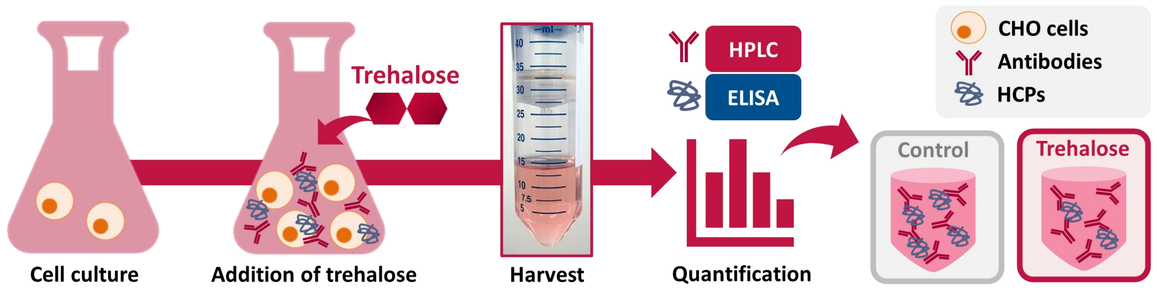
Abstract

Over the past decades, advancements in cell culture process have led to an increased carry-over of host cell-derived impurities[1]. Although most of these impurities are removed by Protein A purification, it has been reported that fouling due to feed material has the most impact on resin lifetime and performance of the purification process[2]. To remove the impurities during a harvesting process, various strategies have been developed[3-5]. Most of the strategies rely on interactions between the membrane and impurities, which are enhanced under low conductivity conditions[3]. Our previous study has demonstrated that addition of saccharides leads to a decrease in the conductivity of the culture supernatant (CS). In this steady, we investigated the effects of adding trehalose during the harvest process.

Chinese hamster ovary (CHO) cells (CHO-MK cells, Chitose) were cultured for antibody production. After cultivation, trehalose was added to the culture fluid (CF). Addition of trehalose significantly reduced host cell proteins (HCPs) in the filtrate clarified using a chromatographic clarifier device (3M^{TM} Harvest RC). This significant reduction in HCPs was maintained after Protein A purification. Furthermore, trehalose enhanced antibody recovery during the protein A purification. Additionally, trehalose reduced HCPs in harvested pools obtained through depth filtration after cell removal, and reduced HCPs and host cell DNA (hcDNA) in harvested pools obtained through tangential flow filtration (TFF). These findings propose a novel strategy for minimizing impurity carry-over into downstream purification steps.



Analysis of Monomer Ratio after Protein A Chromatography

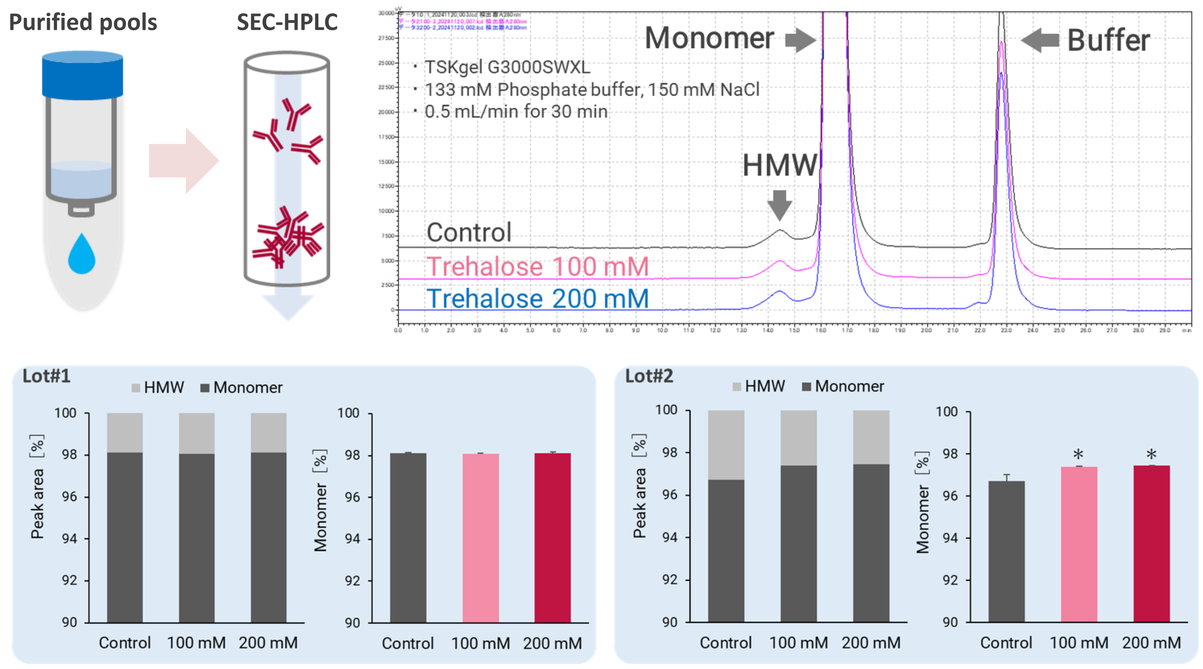


Figure 3. Trehalose maintained / improved the monomeric fraction of IgG.

To evaluate the impact of trehalose on aggregation, the purified pools were analyzed using size exclusion chromatography (SEC). Since no degradation products were detected, the monomer ratio was calculated as the proportion of the monomer peak relative to the sum of the monomer and high molecular weight (HMW) peaks. (*$p < 0.05$ vs. Control, n=3-4)

Decrease in Conductivity by Trehalose Addition

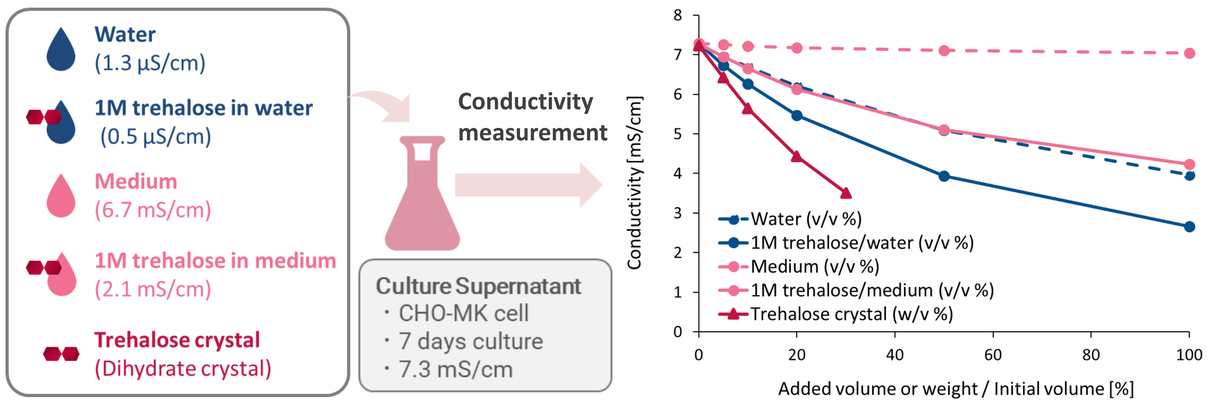


Figure 1. Addition of trehalose dose-dependently decreased conductivity of CS.

5-100 v/v % of water or fresh medium (with or without 1 M trehalose), or 5-30 w/v % of trehalose crystals were added to 4 mL of the CS, and the conductivity was measured using CM-40S (DKK-TOA). Each sample was measured three times, and average of them was shown.

Impact of Conductivity on HCPs Reduction

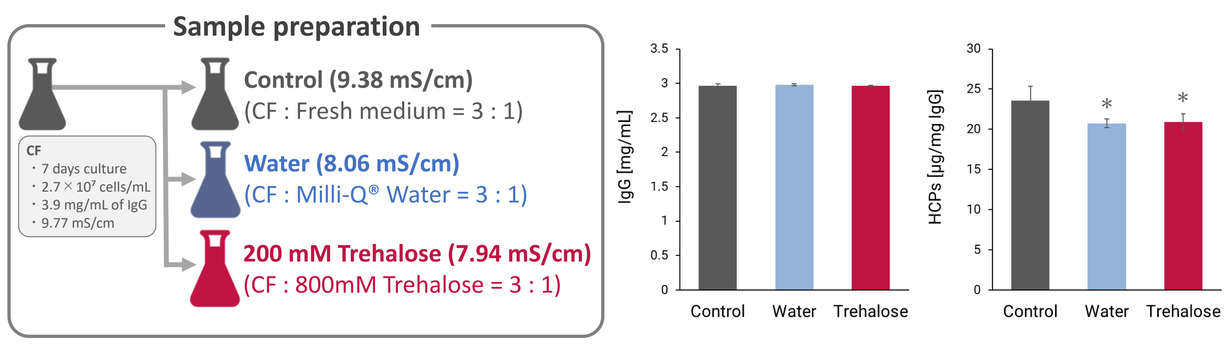


Figure 4. Adjustment of conductivity with ultrapure water to a level equivalent to that obtained by trehalose addition resulted in a comparable reduction in HCPs.

After cultivation of CHO cells for 7 days, CF was mixed with fresh medium containing trehalose or Milli-Q® water to adjust the conductivity to 8.0 mS/cm. Then, these samples were clarified using 3M^{TM} Harvest RC CT15. 3M^{TM} Harvest RC was filled with 15 mL of the samples and centrifuged (400 g, 10 min). IgG and HCPs were quantified by HPLC and CHO HCP ELISA Kit, 3G F550-1 (Cygnus), respectively. (*$p < 0.05$ vs. Control, n=4)

Reduction of HCPs by Trehalose Addition

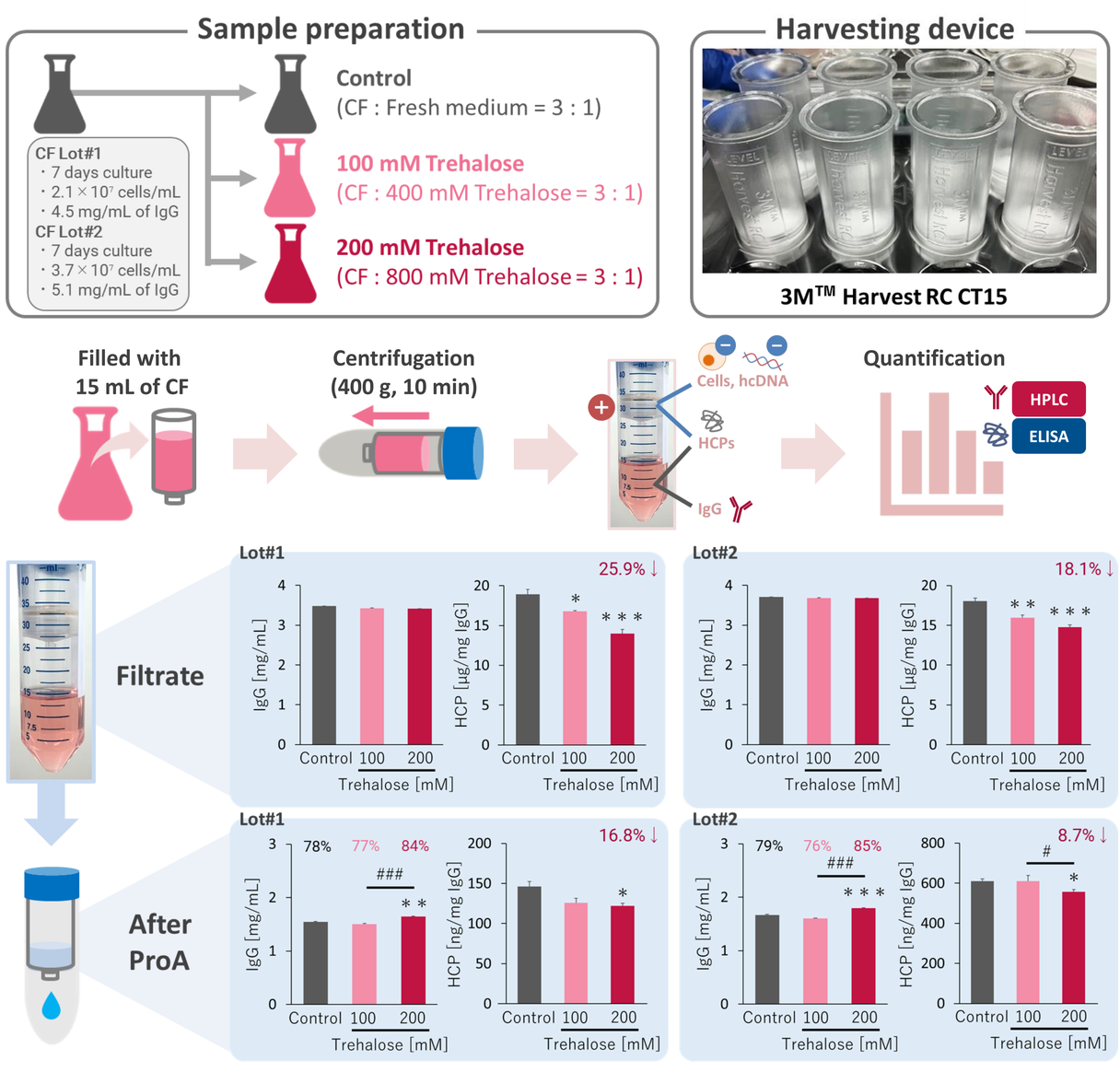


Figure 2. Addition of trehalose reduced HCPs in the filtrate clarified by 3 M^{TM} Harvest RC, and the significant reduction was maintained after protein A chromatography.

After cultivation of CHO cells for 7 days, CF was mixed with fresh medium containing trehalose and clarified using a chromatographic clarification device (3M^{TM} Harvest RC CT15, Solventum). 3M^{TM} Harvest RC was filled with 15 mL of the samples and centrifuged (400 g, 10 min). IgG and HCPs in the filtrate are shown for each of two independent harvesting tests (Lot#1 and #2). The filtrates were purified using Protein A spin columns (MonoSpin L ProA, GL Sciences). The spin columns loaded samples were washed with 20 mM sodium phosphate buffer (pH 7.2). Then, IgG was eluted with 50 mM citrate buffer (pH 3.0) and neutralized with 1M Tris-HCl (pH 7.5). IgG and HCPs were quantified by HPLC and CHO HCP ELISA Kit, 3G F550-1 (Cygnus), respectively. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Control, #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs. 100 mM trehalose, n=3-4)

Harvest Using Depth Filter or Hollow Fiber Membrane

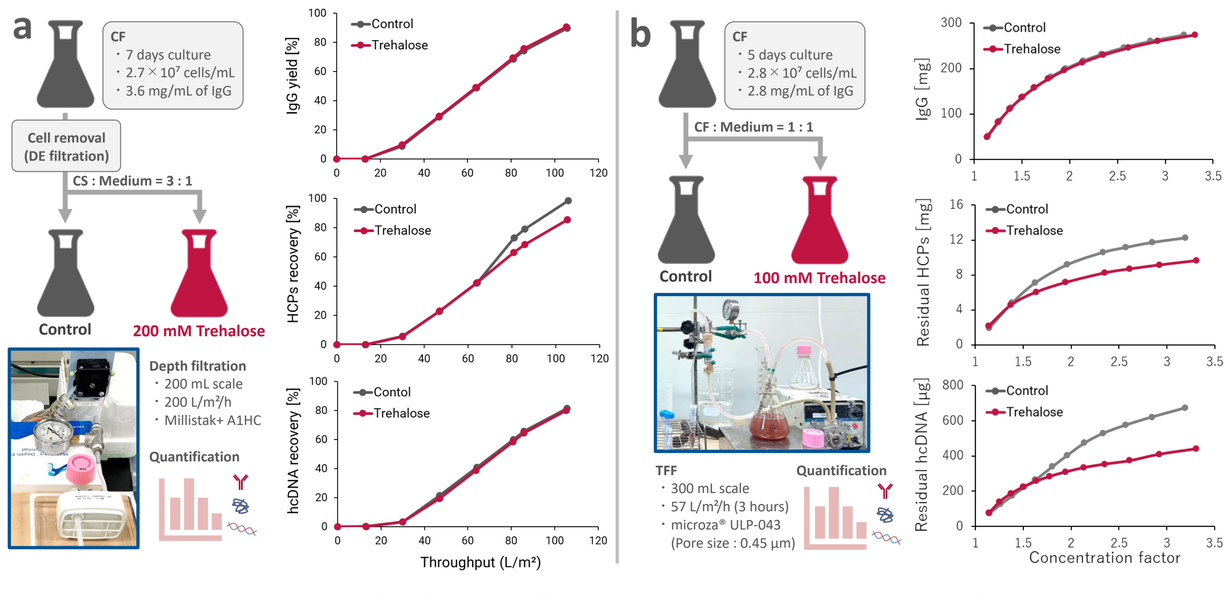


Figure 5. Trehalose reduces impurities in the clarified pools obtained through depth filtration or TFF.

(a) After cultivation for 7 days, CHO cells were removed by diatomaceous earth (DE) filtration. The filtrate was mixed with fresh medium containing trehalose. Then, the samples were clarified using Millistak+® A1HC (Millipore) (n=1). (b) After cultivation for 5 days, CF was mixed with fresh medium containing trehalose. Then, the samples were clarified using microza® ULP-043 (Asahikasei) (n=1). IgG, HCPs and hcDNA were quantified by HPLC, CHO HCP ELISA Kit, 3G F550-1 (Cygnus) and QuantiFluor® ONE dsDNA System (Promega) , respectively.

Key Takeaways

- Trehalose **decreases conductivity**.
- Trehalose **reduces HCPs** in clarified pools obtained through **Harvest RC clarification.**
- Trehalose **improves IgG yield** during Protein A purification.
- Trehalose **maintains or improves the monomeric fraction** of IgG.
- Trehalose **reduces HCPs** in the clarified pools obtained through **depth filtration or TFF**.

References

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