Impact of processing stresses on enzymatic activity of lysozyme

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Protein application as food supplement, vaccines, antibiotics and biopharmaceutical enzymes increased in the last two decades (Thomas, 2019; Miron, 2017). Taking into account that high quality product is required for the final customer, protein stability and efficiency is a key point in medical, pharmaceutical and food sectors. During manufacturing, protein processing involves multiple steps like purification, concentration, mixing, heating and pumping. All of them could induce changes in protein conformation and/or denaturation, interfering on its biological activity or wasting initial product. To reduce this loss during production, a better understanding of the stresses encountered in processing steps and their impact on protein stability are required. We propose to evaluate the impact of different production steps by evaluating the loss of enzymatic activity of lysozyme after different treatments.

Hen Egg White Lysozyme (LSZ) is a globular small protein (14kDa) that presents antibacterial action through cell wall destruction by polysaccharide hydrolysis (figure 1). This action is dependent of its conformation (Bhat, 2018). Stresses encountered during processing can modify lysozyme's structure resulting on antibacterial activity decrease. In the present investigation, the influence of stress factors (heating, chemical denaturation, ultrasound application and ultrafiltration) on the lysozyme antibacterial activity were evaluated to determine limiting conditions of industrial processes.

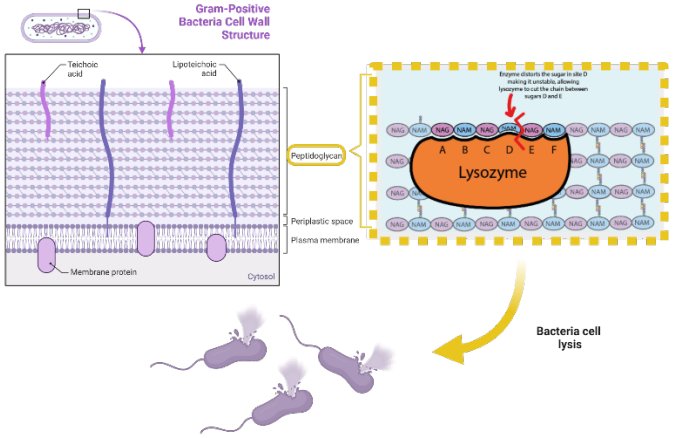

Figure 1: Figure 1: Lysozyme antibacterial action against gram-positive bacteria. Created with BioRender.com.

Treatments to evaluate different stress conditions were applied on a 0.025 mM LSZ aqueous solution. After each modification, final solutions were evaluated according to their antibacterial activity. The enzymatic assay was evaluated by an optimized turbidity test at 450 nm using the bacteria *Micrococcus Lysodeikticus* as

substrate. The whole assay was done 3 times in triplicate and the results are presented as an activity index (ratio between sample activity and the reference (untreated lysozyme).

The results show that the temperature of denaturation is higher than 70°C and a reduction of approximately 25% of activity was observed after a treatment at 90°C. Chemical denaturants (urea and guanidine hydrochloride) impact differently the LSZ conformation, and the concentration of denaturant is important only in the case of guanidine hydrochloride where the concentration of 10M reduced the activity by approximately 30%. During ultrasonic operation, non-aggregated LSZ was stable until 15 minutes. For ultrafiltration (treatment combining surface and mechanical stresses) antibacterial activity decrease was observed with transmembrane pressure increase.

Two successive treatments were also combined to understand the effect of the protein initial state. For that, LSZ thermally pre-treated at 90°C was ultrafiltered. No additional activity loss was observed for the filtration of lysozyme already denatured, showing that thermal denaturation at 90°C leads to a more irreversible modification than ultrafiltration. Taking all these results together, it is possible to understand that each stress factor can change differently the LSZ conformation and consequently, the antibacterial activity.

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