Chapter 1

Introduction

1.1 Background

Recent advancements in cellular and molecular fields have allowed the development of novel stem-cell derived cosmeceutical bioactive ingredients in the beauty industry (Tan et al., 2022). An emerging class of these bioactive ingredients are mesenchymal stem cell (MSC) derived secretomes, which refer to the MSC produced substances that regulate resident cell responses. These substances include insoluble nano/microstructured extracellular vesicles (such as microvesicles and exosomes) and free-soluble factors such as cytokines and growth factors which have shown ability to trigger biological mechanisms (Mocchi et al., 2021).

Studies have shown that these components improve cell migration and proliferation to aid skin regeneration (Damayanti et al., 2021). Other studies have reported the use of MSC-secretome to prevent aging factors, increase collagen synthesis and reduce aging by improving skin elasticity (Kim et al., 2019). However, applications of MSC-secretome in the beauty industry could only be accomplished if their regenerative potential is preserved (Tan et al., 2022). The preservation of these regenerative properties proves to be a challenge as MSC-secretomes are susceptible to damage caused by many outside factors, including heat. Conventional methods of preservation include cryopreservation at -80^{o}C, utilizing ultra-freezers. However, this technique presents several challenges, mainly difficulties in logistics, the risk of cross-contamination with liquid nitrogen, and high costs of maintaining the cold chain system (Merivaara et al., 2020; Mensink et al., 2017).

Freeze drying, or also known as lyophilization, is a solution to these problems, as it dehydrates labile liquid formulations in order to increase their long term storage stability (Assegehegn et al., 2020). Reduction of water content stabilizes the product during distribution while also preventing thermal

denaturation (Adams et al., 2014). It consists of 3 stages, namely freezing, primary drying, and secondary drying. The product would first be frozen at very low temperatures to create ice crystals which would sublimate at a higher temperature in vacuum condition (the primary drying stage), where most of the water would be removed. Then, the secondary drying at a higher temperature is conducted, where unfrozen water would be desorbed (Nial et al., 2002).

Even though freeze drying is an attractive method to improve long-term storage stability of MSC-secretomes, the process is expensive, time consuming, and energy intensive. Furthermore, many parameters in each drying stage could impact the quality of the end product (Assegehegn et al., 2020). Therefore, a robustly designed product and process is needed to increase production effectiveness and prevent product defect, rejection, and recalls. This could be done through Quality by Design (QbD), where risk assessment is conducted prior to development studies in order to identify potentially high risk process variables that could impact the quality of the drug product (quality target product profile). The quality target product profile (QTPP) that could cause harm to patients if certain characteristics fail to fall in a certain range are termed critical quality attributes (CQA) (Yu et al., 2014). One of the process parameters that impacts the CQA of a product is the shelf temperature during primary drying. Since primary drying is the most energy intensive and longest freeze-drying step, many process optimization often aims to shorten its duration. However, careful consideration needs to be taken as insufficient drying leads to product damage. Therefore, this study aims to evaluate the effect of primary drying temperature towards the physicochemical characteristics of MSC-secretomes. The CQA examined in this study are cake appearance, residual moisture level, glass transition temperature (Tg), particle size, zeta potential, reconstitution time, pH, and solution appearance of reconstituted MSC-secretomes.

1.2 Objective

The objectives of this study are to:

1. Produce freeze-dried MSC-secretomes with acceptable physicochemical characteristics using the Huaihai Freeze-dryer
2. Compare the effect of different primary drying temperatures towards the cake appearance and moisture content freeze-dried MSC-secretomes
3. Compare the effect of different primary drying temperatures towards the physicochemical properties of reconstituted MSC-secretomes by comparing pH, and SDS-PAGE profile of freeze-dried MSC-secretomes to non-freeze-dried product, testing for reconstitution time of under 15 minutes, and a clear solution appearance where all solid dissolves completely.

1.3 Hypothesis

The hypothesis of this experiment include:

1. Freeze-dried MSC-secretomes with acceptable physicochemical characteristics can be produced using the Huaihai Freeze-dryer
2. Different primary drying temperatures would impact the physicochemical characteristics of the freeze-dried cake and reconstituted product.