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(54) Title: IMPROVED METHODS FOR THE SEPARATION OF STREPTOCOCCUS PNEUMONIAE TYPE 3 POLYSACCHARIDES

(57) Abstract: The present invention provides improved methods for the reduction or removal of protein impurities from a complex cellular Streptococcus pneumoniae lysate or centrate comprising serotype 3 polysaccharides involving steps relating to post-lysis heating or pH adjustment. In certain methods, the lysate is heated for a time and at a temperature sufficient to denature proteins present in the lysate and cause their aggregation and precipitation. In one embodiment, the lysate is heated to at least 60°C for at least 30 minutes to cause protein aggregation and precipitation, more particularly about 60°C to about 70°C for about 30 to about 50 minutes, and even more particularly about 65°C for about 40 minutes. In other methods, the pH of the lysate or centrate is increased to at least 8.0 to improve filterability, more particularly about 8.0 to 8.4, and even more particularly about 8.2. In further methods, heating and pH adjustment steps are combined to cause the aggregation and precipitation of proteins as well as to improve filterability of the lysates or centrates. In other methods, the pH of the lysate or centrate is lowered to about 3.0 to about 5.0 to cause protein aggregation and precipitation. Such methods allow for the production of substantially purified serotype 3 polysaccharide-containing lysates or centrates.