TABLE 9.5

Preventive Actions Against Proteolysis

Factor	Comment
pH	There is no specific pH range in which all enzymes are considered inactive; at slightly alkaline pH, the nonspecific enzymes of the vacuoles and lysosomes are minimally active. Use strong buffers for extraction to prevent unintended shift in pH as a result of cell disruption. Some yeast enzymes are least active in the pH range 4–5 but active in the pH range 7–9. Phosphate may exhibit a stabilizing effect on proteins.
Temperature	Low temperature decreases the proteolytic activity. It is recommended to store harvest at 4°C–8°C or frozen.
Time	Enzymatic protein degradation is a function of time. Lengthy procedures and long storage times should be avoided during harvest, capture, and initial purification steps.
Conductivity	Noncritical.
Redox potential	Reducing and oxidizing conditions may alter the disulfide bridge arrangement and state of free cysteine residues, thus influencing the secondary and tertiary structures of protein.
Cosolvents	Presence of other proteins in excess (e.g., albumin) will reduce the proteolytic damage. Cosolvents, such as glycerol or dimethylsulfoxide, may have a stabilizing effect but will probably be too expensive for large-scale operations.
Low-MW compounds	Substrates, substrate analogs, and cofactors can help in stabilizing the protein. Potential proteinase activators (e.g., divalent metal ions) are excluded from the extraction buffer.
Techniques	Careful cell disruption and specific extraction procedures may lower the enzymatic cleavage.
Denaturation	The proteolytic enzymes lose their biological activity upon denaturation. However, some enzymes are stable under mild denaturing conditions that lead to increased activity if the target protein is partly denatured under the same conditions.

Source: *Handbook of Biogeneric Therapeutic Proteins: Regulatory, Manufacturing, Testing and Intellectual Property Issues*, Taylor & Francis Group, Boca Raton, FL, 2005.
Abbreviation: MW, molecular weight.

hydrolyzed at either the α- or β-carbonyl group to generate a mixture of normal- and iso-residues. Under strongly acidic conditions, asparagyl or glutamyl residues are hydrolyzed to the corresponding carboxyl residues. The indicators of deamidation include extra bands in electrophoresis and extra peaks in chromatographic recordings. Table 9.6 lists preventive actions against deamidation.

9.9.4.3 Oxidation

The amino acid residues histidyl, methionyl, cysteinyl, tryptophanyl, and tyrosinyl are potential oxidation sites at neutral or at slightly alkaline conditions. Oxidation of the said residues often results in a loss of immunological and/or biological activity. The list of proteins that have been oxidized is comprehensive and includes biopharmaceutical products, such as albumin, growth hormone, glucagons, and interleukin-1*b* and −2.