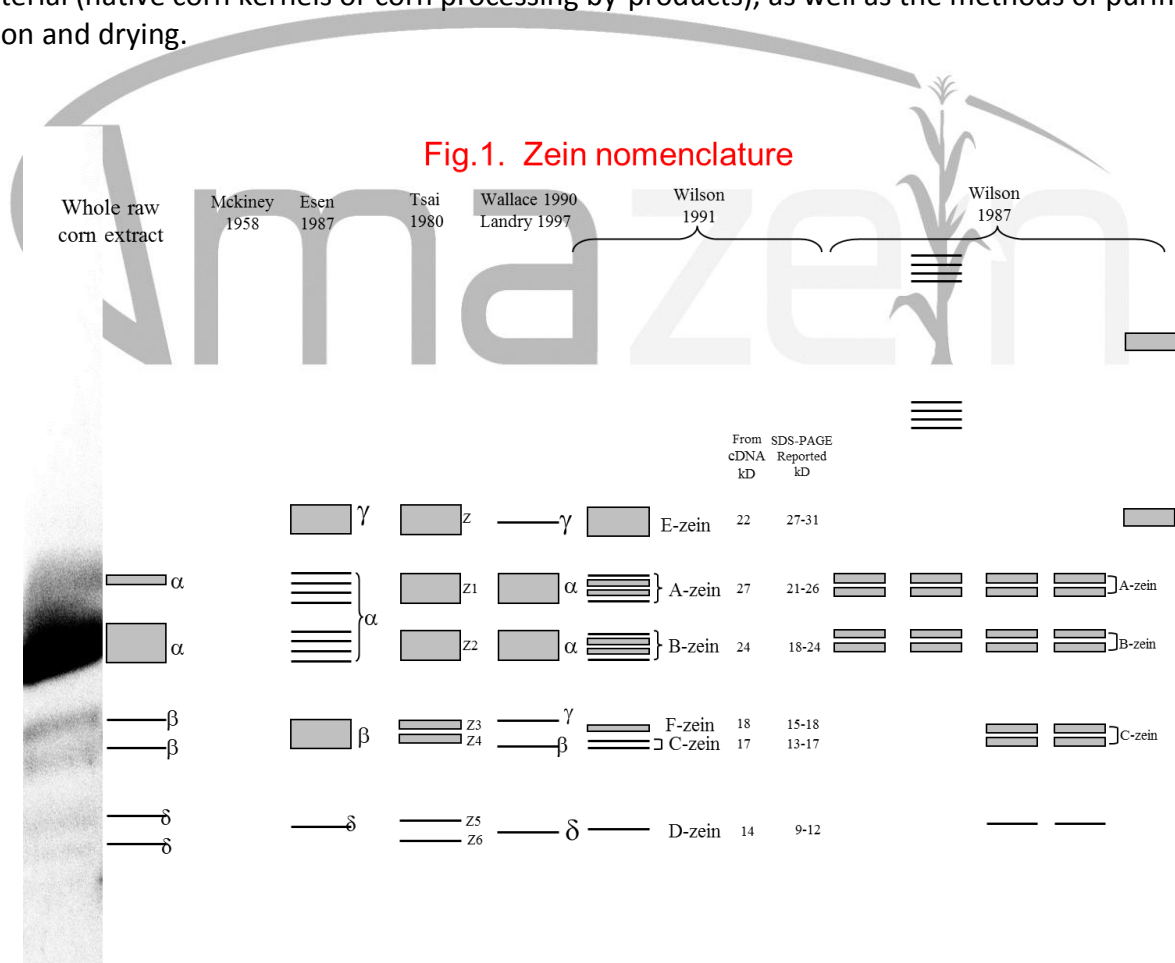


Amazein

Amazein™ is the trade name of a highly functional protein produced from raw whole corn (maize) by Prairie Gold Inc. The protein is “zein” (commonly pronounced “zayne” to rhyme with *rain* or *cane*), which is one of four different classes of proteins in corn. Zein refers to a family of alcohol-soluble proteins known historically as prolamines. Prolamines in corn (maize) are called zein, while they are known as hordein in barley, kafirin in sorghum and gliadin in wheat. Commercial zein is not a single protein, but a mixture of individual zein fractions. Individual zein fractions in a commercial product depend on the extraction conditions, such as the type of alcohol (ethanol vs. isopropanol), the concentration of alcohol (typically 50-95% v/v ethanol or isopropanol in water), temperature, reagents added (such as alkali or reducing agents) and the raw material (native corn kernels or corn processing by-products), as well as the methods of purification, concentration and drying.



There has been some confusion in the scientific literature about the nomenclature for individual zeins since McKinney (1958) first defined zein components based on solubility: α-zein is soluble in 95% ethanol or

85% isopropanol, while β -zein is insoluble in 95% ethanol but soluble in 60% ethanol. Figure 1 compares several nomenclatures mentioned in the literature.

Esen's method is now widely accepted and is used by Prairie Gold with Amazein™. In the Esen system, there are four major fractions:

- (1) Alpha-zein (α -zein) with a molecular weight of 21-25 kD, which is the most abundant fraction,
- (2) Beta (β -zein), molecular weight of 17-18 kD,
- (3) Delta (δ -zein), molecular weight of 9-12 kD, which is often coextracted with α -zein because of its immunological relationship to α -zein
- (4) Gamma (γ -zein), 26-29 kD.

In Figure 1, the “whole raw corn extract” is a gel scan of an extract obtained with yellow dent #2 corn prior to purification by the Amazein™ process. Alpha-zein is the most prominent band and is easily visible, while the beta and delta zeins show less prominent bands reflecting their lower concentrations.

WHAT MAKES AMAZEIN™ DIFFERENT?

Amazein™ is manufactured using the patented *COPE* process developed at the University of Illinois and licensed exclusively by Prairie Gold. The main factors that make Amazein™ unique and superior are:

- It is produced from whole raw corn, and not from corn processing by-products. Zein has traditionally been isolated and manufactured from corn gluten meal, a coproduct of the corn wet milling industry which has been exposed to steeping chemicals such as sulfur dioxide and lactic acid, among others, and high temperatures during drying. Another potential raw material is DDG (distillers dried grains) or DDGS (with solubles) which is a by-product of the dry-grind ethanol industry. The dry-grind ethanol process uses high temperatures during jet cooking and during drying, and the corn is also exposed to enzymes and microorganisms (yeast). The properties of the zein products from these raw materials could be affected by these processing treatments and by batch-to-batch variations in the quality and properties of the raw materials.

In contrast, Amazein™ is produced at the “front-end”, i.e., from whole raw corn *before* any processing. Amazein™ will thus reflect the natural protein profile and functional attributes of corn better than zein produced from corn processing by-products.

- No extreme processing conditions are used. Temperatures are typically less than 50°C during the extraction and purification and the final product is efficiently and rapidly dried so as to minimize thermal exposure and degradation of the product.
- No chemicals are used, except alcohol and water. Other methods sometimes add alkali or other chemicals.
- Amazein™ is devoid of the off-flavors that come from rancid oils, the wet milling process or by addition of sodium hydroxide or other chemicals
- Zein that has been available until now is mostly alpha-zein; Amazein™ also contains beta-zein and gamma-zein and perhaps others, which seem to broaden its functionality.

There are a number of other zein fractions that could exist in native corn. For example, dimers and possible trimers of alpha-zein have been identified in commercial products and in Amazein™ by electrophoresis. Figure 2 shows electrophoretic patterns of Amazein™ of various protein contents and

commercial samples of zein from Freeman Company (USA) and Showa Sangyo (Japan). The protein contents are expressed as N x 6.25 on a dry basis. The major fractions (alpha-zein, dimers and trimers) are visible but the beta- and gamma-zeins are not easy to see in gels, although they are sometimes visible in the scans of the gels. Figures 3 and 4 show scans of typical gels of Amazein™ and commercial samples.

Figures 5-7 show additional evidence for the presence of multiple fractions of zein in corn extract, Amazein™ and a Freeman zein by MALDI-MS (matrix-assisted laser desorption ionization – mass spectroscopy). The lower molecular weight fractions of zein are easier to identify by this technique. Interestingly, the β -zein (17-19K) appears to be missing in Freeman products (Figure 7).

Fig. 2. Characterizing zein by PAGE

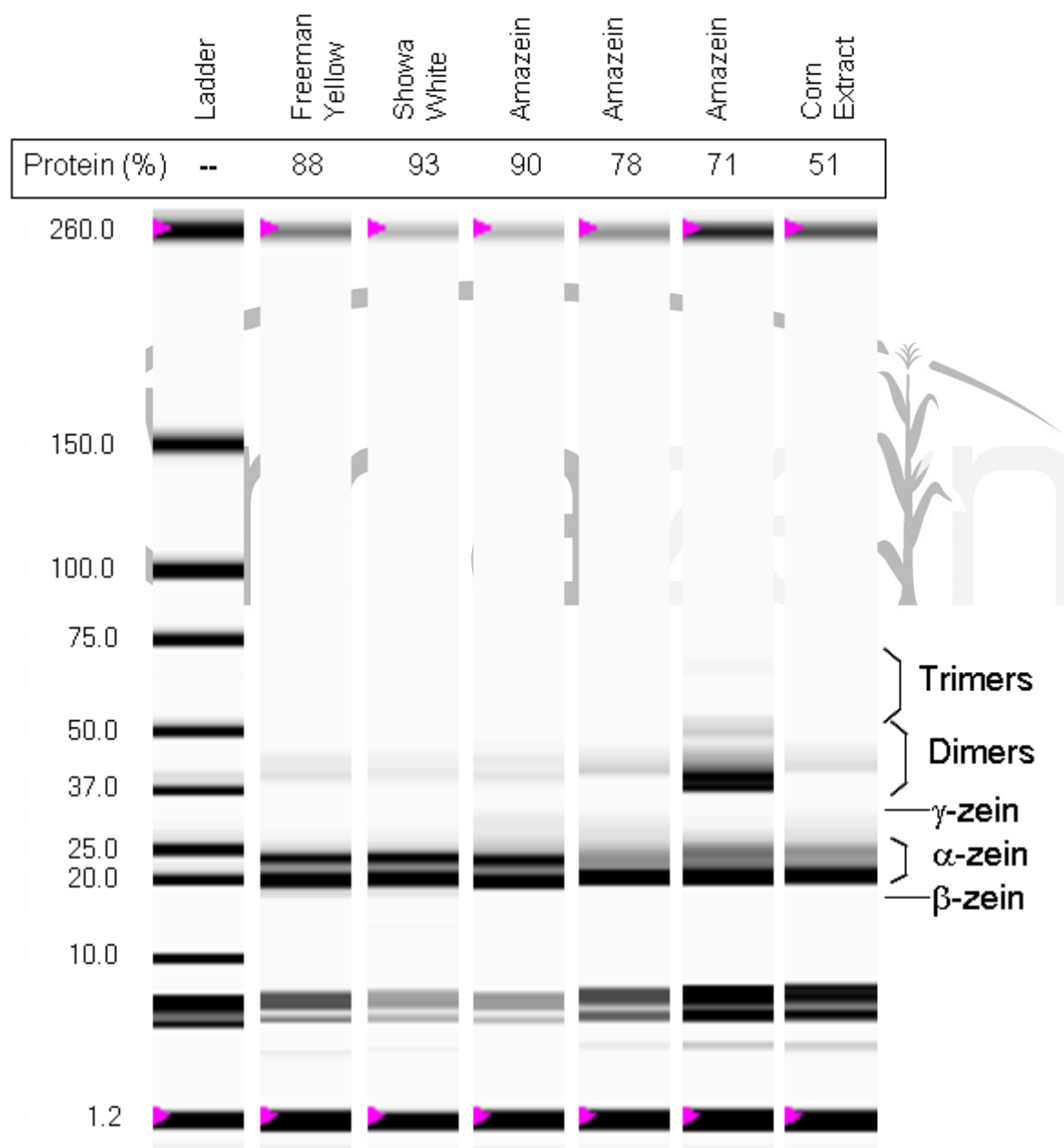


Fig.3. Electrophoresis of zein products

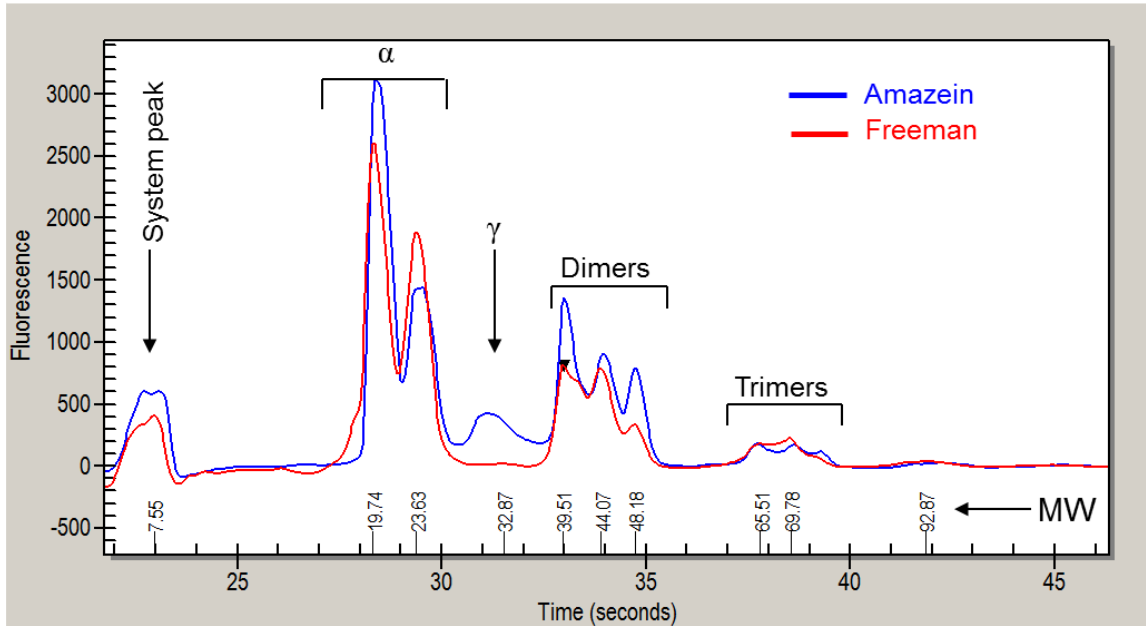


Fig.4. Characterizing zein by PAGE

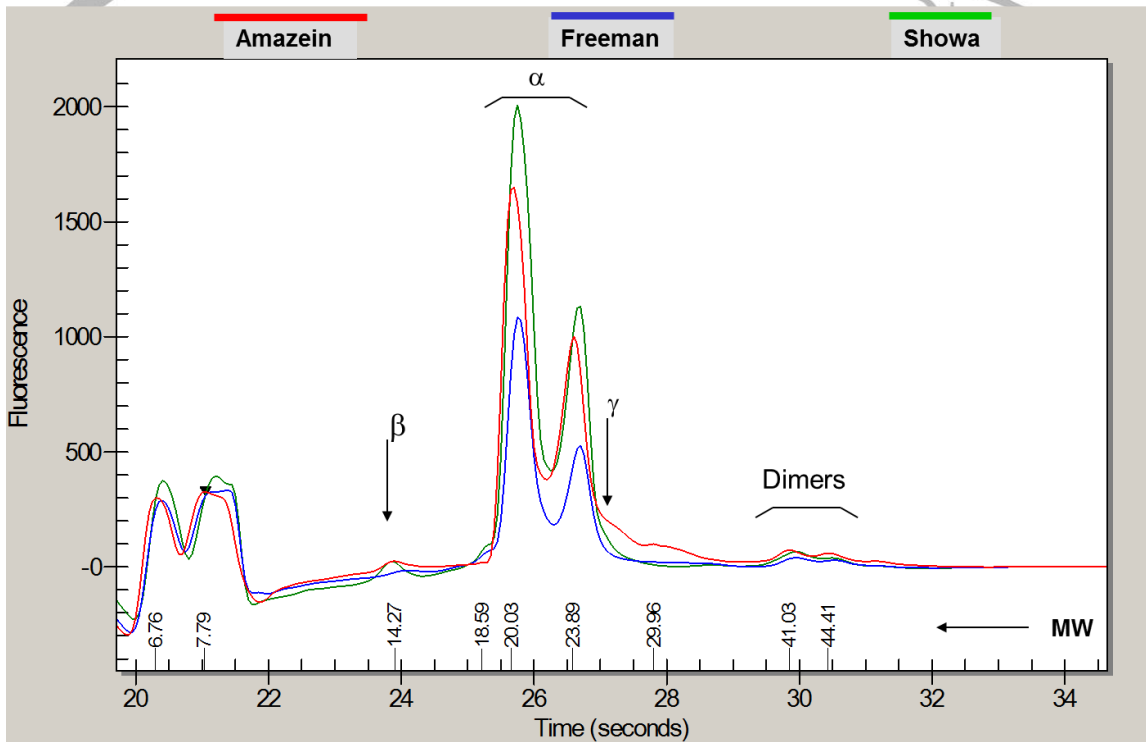


Fig.5. MALDI-MS of corn extract. The prominent peaks correspond to δ -zein (11-12K), β -zein (17K), α -zein (23-24K) and γ -zein (27K)

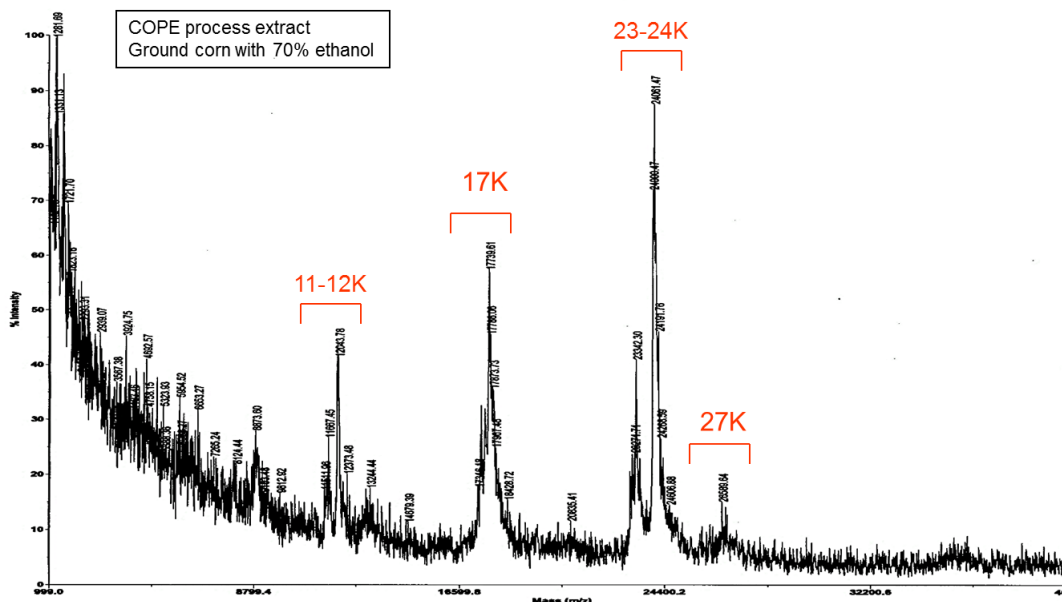


Fig.6. MALDI-MS of Amazein. The prominent peaks correspond to δ -zein (12-13K), β -zein (17K), α -zein (23-24K) and dimer of α -zein (48K)

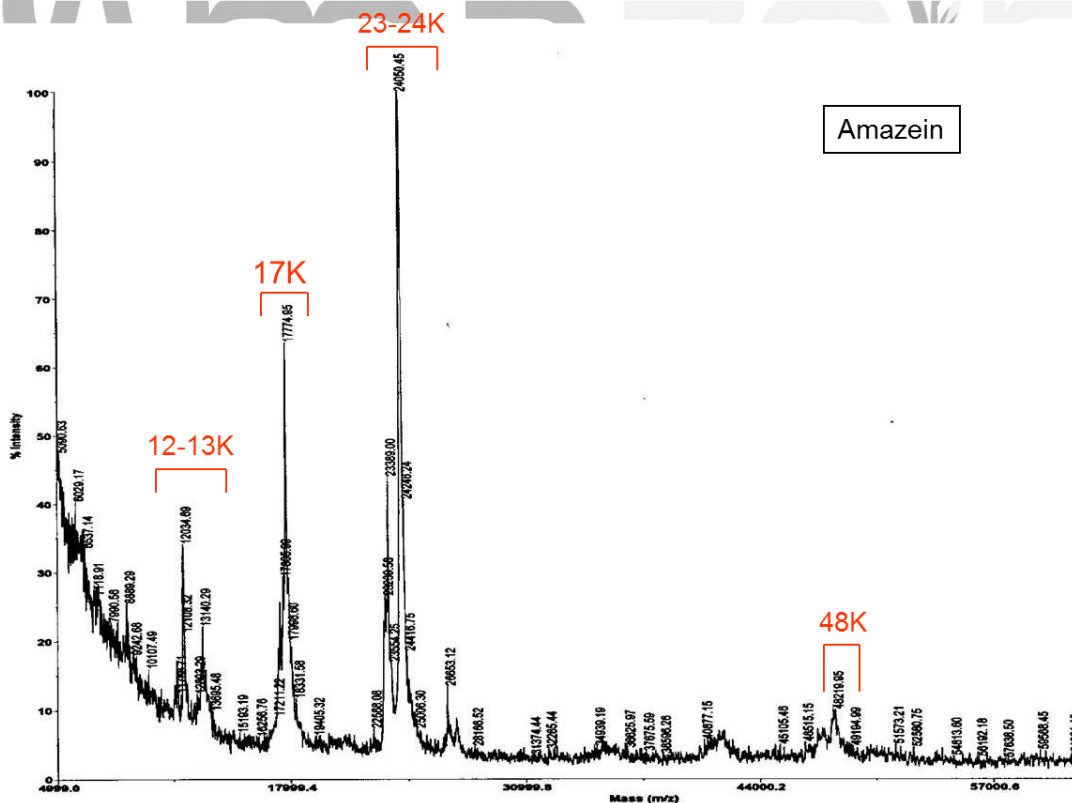
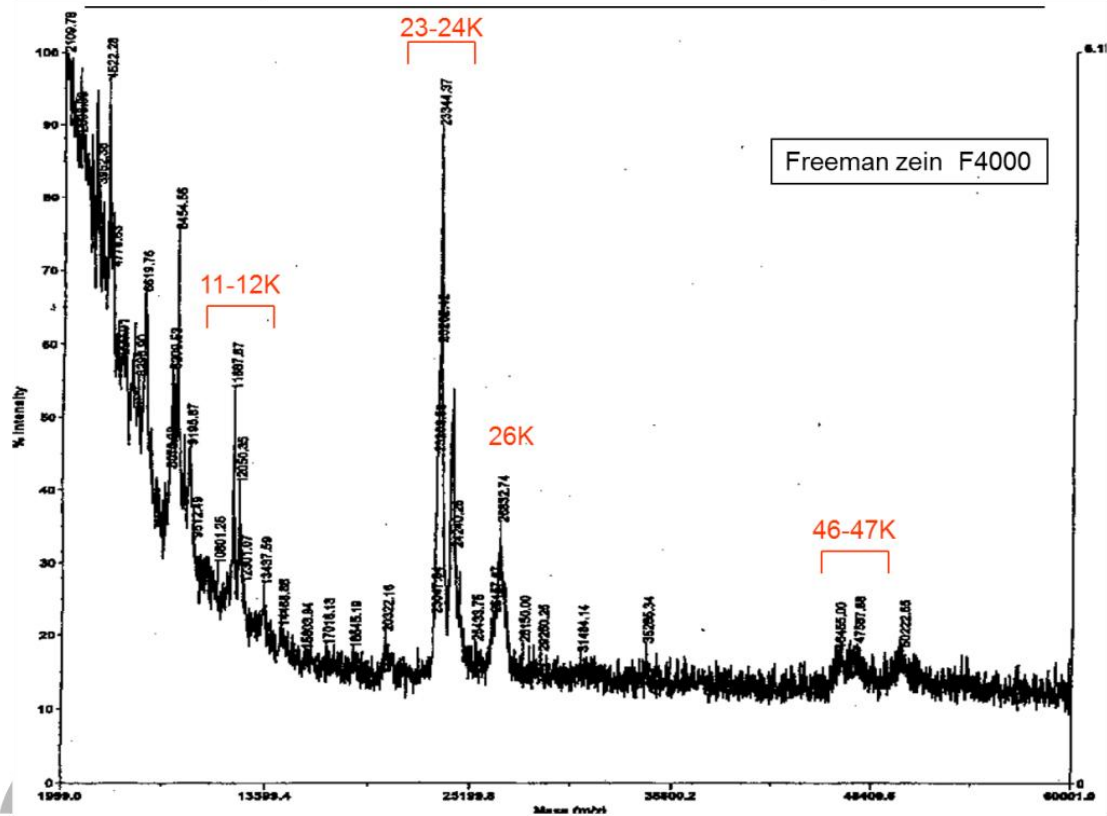


Figure 7. MALDI-MS of Freeman zein F4000



Certain processing methods, e.g., addition of reducing agents, could break disulfide bonds and eliminate or reduce the proportion of some of the zein fractions. Different solvents can change the relative proportions of the zeins being co-extracted. In addition, individual zeins vary in composition and differ in their amino acid sequences and surface charges based on corn genotypes, location of protein bodies in the kernel and age of the kernel.

The composition of the zein products, in terms of the relative proportions of zein fractions, could have an effect on the functional properties of zein and its utilization. Amazein™, the only zein product extracted from raw corn, will have a protein profile that reflects the corn that was used as the raw material and in all likelihood will contain many of the zein fractions present in corn in varying proportions. This should be taken into account when using Amazein™ in certain applications.

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