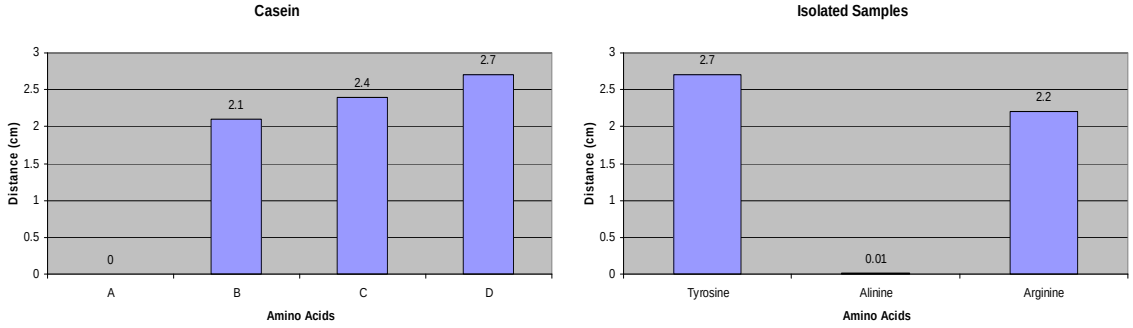
Analysis of Proteins

Summary: My group and I attempted to break down the milk protein casein into its smaller subunits and then determine whether it contained the amino acids Tyrosine, Alanine and Arginine. This was done with thin-layer chromatography: in this case by taking samples of casein and the isolated amino acids and applying them to silica gel-coated plastic strip at equal measured distances from the bottom. A special solvent was then slowly absorbed from the bottom, traveling up the strip over a period of time. This caused individual amino acids to complete their reactions and leave visible marks that were both measureable and comparable to other marks. We found that casein has at least four different amino acids, and that Tyrosine, Alanine and Arginine are present. The existence of more amino acids in casein is not surprising as there are 20 common amino acids in proteins *(Raven et al., 2008)*. Lysine is also known to be one of the amino acids that makeup casein *(Eldred & Rodney, 1946)*.

Methods & Results: My group and I began the experiment by obtaining a silica gel-coated plastic strip, which was handled carefully so that the surface would not be contaminated. Using a pencil, we made four small dots on the powder side of the strip approximately one inch from the bottom—these marks were also horizontally equidistant. Using a capillary tube, a small amount of casein was taken from a provided sample and then applied to the first of the dots on the strip. We repeated this procedure for Tyrosine, Alanine, and Arginine with fresh capillary tubes and applied them to the second, third and fourth dots, respectively. Our strip was then placed in a "developing jar" for amino acids, with the bottom ¾" submerged in a special solvent containing 65.5% butanol, 17.25% acetic acid, and 17.25% H_20. The entire strip was contained and then left to slowly absorb the solvent for one hour.

Upon completion, we placed the strip on a hot plate to expedite drying, removed it, sprayed it with Ninhydrin and then left it to dry again for about five minutes. We noticed that purple colored spots appeared, which indicated the locations of the completed reactions for each amino acid. Pencil markings were made in these places and at the end of the solvent front (the total distance traveled by the solvent upward along the strip).

From the casein sample, we found that four markings appeared. Expectedly, only one purple spot appeared for each of the other amino acid samples. Measuring these marks from their starting locations with a ruler produced the following distances (in centimeters):



To determine the relative distance of migration (R_f) value for the markings, we divided each individual marking's distance by the total distance traveled by the solvent front, which was a measured 4.4cm from starting location of the samples. This R_f value was then used to make comparisons between both data sets. Amino acid A (R_f=0.00) matched with Alanine (R_f=0.00), amino acid B (R_f=0.48) was a very close match for Arginine (R_f=0.50), and amino acid D (R_f=0.61) matched Tyrosine (R_f=0.61). Amino acid C (R_f=0.55) was unaccounted for and therefore represents an unknown amino acid.

References:

Eldred, N.R. & Rodney, G., 1946. The Effect of Proteolytic Enzymes on Raw and Heated Casein, *The Journal of Biological Chemistry*, 162, pp. 261-265.

Raven, P. & Johnson, G. & Losos, J. & Mason, K. & Singer, S., 2008. *Biology*, 8th ed., McGraw-Hill.