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CHEM 3170 Laboratory

Identification of major proteins in milk using MALDI-TOF Mass Spectrometry

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Experimental

Table 1. Instrument parameters for the Bruker Microflex MALDI-TOF-MS used.

Initial laser power	70%
Number of shots	300 per sample
Type of shots	10 shots at raster spot (random walk)
Detection	Detector gain: 11.4x Electronic gain: 100 mV
Spectrometer	Ion source 1: 19.99 kV Ion source 2: 17.99 kV Lens: 8.00 kV
Matrix suppression mode	Deflection

Procedure:

Each of the four milk samples (skim, 1% buttermilk, 2% and 3%) was diluted 100 fold by pipetting 100 uL into a 10.00 mL volumetric flask and diluting to the mark with TA30 solution. The TA30 solution was previously prepared as 30% of acetonitrile and 70% of 0.1% trifluoroacetic acid. Next, 50 uL of each diluted milk sample was pipetted into an Eppendorf tube along with 50 uL of sinapinic acid matrix. The matrix was previously prepared by dissolving 10 mg of sinapinic acid into 1 mL of 50/50 v/v water/acetonitrile solution. Each Eppendorf containing a sample was vortexed briefly to mix. Each sample was then spotted onto the MSP 96 ground steel target plate using a micropipette set to 3 uL. The spots were allowed to air dry on the plate and then run on the Bruker Microflex MALDI-TOF mass spectrometer.

Data and Results

Table 2. Approximate molecular weights of common proteins found in bovine milk.

Protein	**Approximate molecular weight (Da)**
α-lactalbumin	14200[3]
β-lactoglobulin	18300[3]
β bovine casein	24000[3]
αs1 bovine casein	23600[3]
αs2 bovine casein	25300[3]
κ bovine casein	19000[3]
Lactadherin	47000[6]
Lactophorin	17000 – 67000 (multiple polypeptides)[2]
Proteoso peptone	Multiple polypeptides[6]
Butyrophilin	66000[6]
Bovine serum albumin	66463[3]
Myoglobin	16940[3]

Table 3. Major protein bands of the skim milk sample determined by MALDI-TOF-MS.

Molecular weight (kDa)	Intensity (a.u.)	Corresponding Protein
13.938	2256	α-lactalbumin
16.070	769	Myoglobin
20.087	281	κ bovine casein
25.558	648	Unknown casein*
25.388	2202	Unknown casein
25.388	2169	Unknown casein

Table 4. Major protein bands of the 1% buttermilk sample determined by MALDI-TOF-MS.

Molecular weight (kDa)	Intensity (a.u.)	Corresponding Protein
13.953	315	α-lactalbumin
16.068	139	Myoglobin
25.461	165	Unknown casein*
25.411	205	Unknown casein

Table 5. Major protein bands of the 2% milk sample determined by MALDI-TOF-MS.

Molecular weight (kDa)	Intensity (a.u.)	Corresponding Protein
13.941	387	α-lactalbumin
16.062	235	Myoglobin
20.061	28	κ bovine casein
25.384	273	Unknown casein*

Table 6. Major protein bands of the 3% milk sample determined by MALDI-TOF-MS.

Molecular weight (kDa)	Intensity (a.u.)	Corresponding Protein
13.940	546	α-lactalbumin
16.079	125	Myoglobin
25.058	75	Unknown casein*
25.231	82	Unknown casein
25.254	82	Unknown casein

*Note on tables 3-6: Unknown casein represents an unidentified casein protein, either β bovine casein, αs1 bovine casein or αs2 bovine casein. Further analysis would be required to differentiate between these proteins because of their similar molecular weights.

Discussion

Casein proteins, such as as1-casein, as2-casein, and β-casein, account for about 80% of the proteins in milk, while whey proteins such as β-lactoglobulin, α-lactalbumin and serum albumin, account for the other 20%. Casein proteins are encased in micelles within milk and exist in suspension since the micelles are insoluble. Whey proteins are tightly packed globular proteins that are soluble in water and are found dissolved in milk. Casein proteins also tend to precipitate out and curdle when exposed to acid, as in the human stomach. Whey proteins stay dissolved, meaning they tend to be absorbed quicker in digestion. Casein and whey proteins also have slightly varying amino acid compositions, with caseins richer in valine, leucine and isoleucine, and wheys richer in histidine, methionine and phenylalanine.

Whey proteins serve a variety of functions in bovine milk, including biomolecule transportation and synthesis, or even cancer prevention. α-lactalbumin is an especially important protein found in the whey fraction, as it is partially responsible for lactose production. The protein is produced in the mammary glands where it combines with another polypeptide to form lactose synthase. Lactose synthase catalyzes the formation of the disaccharide lactose from glucose and galactose. Lactose production is important for milk production since it helps create osmotic pressure.

Casein proteins are also a significant component of milk. They mostly exist in suspension as micelles encasing the insoluble polypeptides, which allows them to act as the primary emulsifiers for other hydrophobic components of bovine milk. Calcium phosphate also readily binds to the micelles, stabilizing them and making milk an excellent source of calcium.

For all four of the milk samples analyzed, the most dominant protein was identified as α-lactalbumin, which was identified in all four samples with a molecular weight around 13950 Da. It was the only whey protein identified in any of the four samples. Myoglobin, as well as varying casein proteins were also identified in all four samples (see tables 3-6). κ casein was identified in only skim and 2% samples at approximately 20 kDa, and at a low intensity relative to the dominant proteins. All samples had between 1-3 other peaks indicating casein proteins, either as1-casein, as2-casein, and β-casein. These peaks could not be definitively matched to any one of these proteins since their literature molecular weights are so similar (see Table 2). For skim, 1% and 2% milk samples, the unidentified casein proteins were the second most dominant analytes by peak intensity. Myoglobin was identified at about 16 kDa in all four samples at a moderate intensity.

Conclusion

Four bovine milk samples were analyzed for major proteins using MALDI-TOF-MS and α-lactalbumin was determined to be the dominant protein in all samples. Myoglobin, κ-casein and other undifferentiated casein proteins were also identified in the samples.

References

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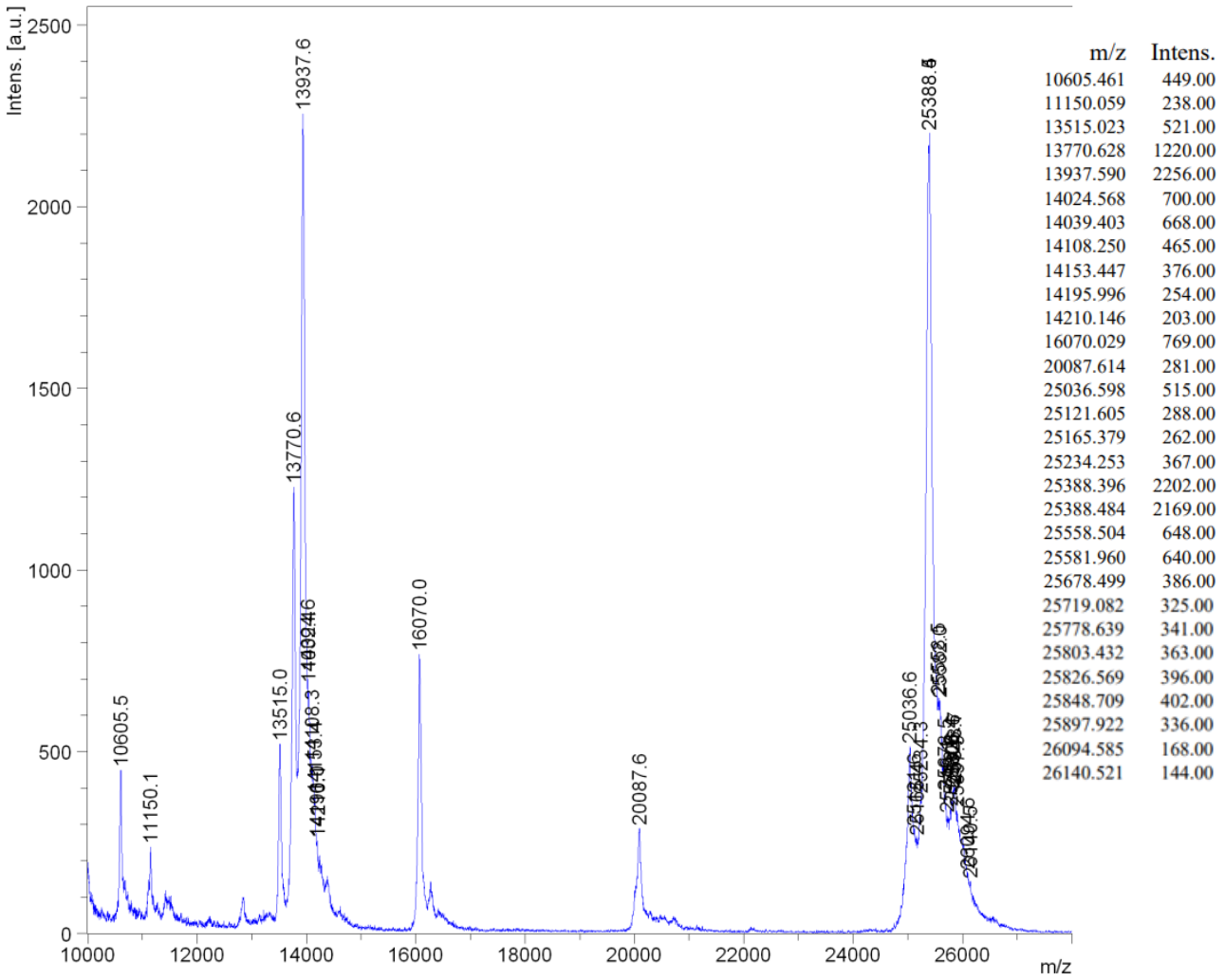
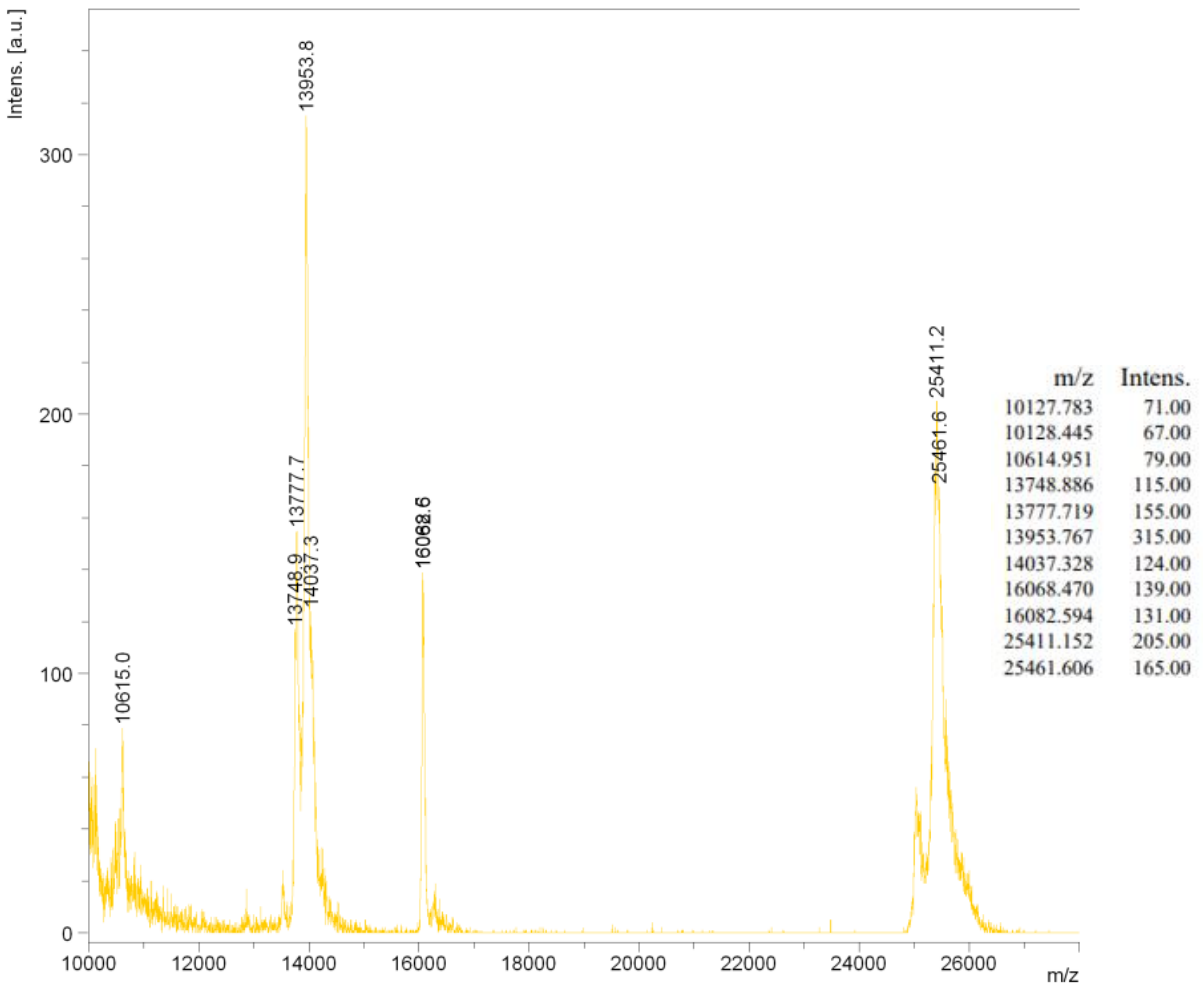
Appendix



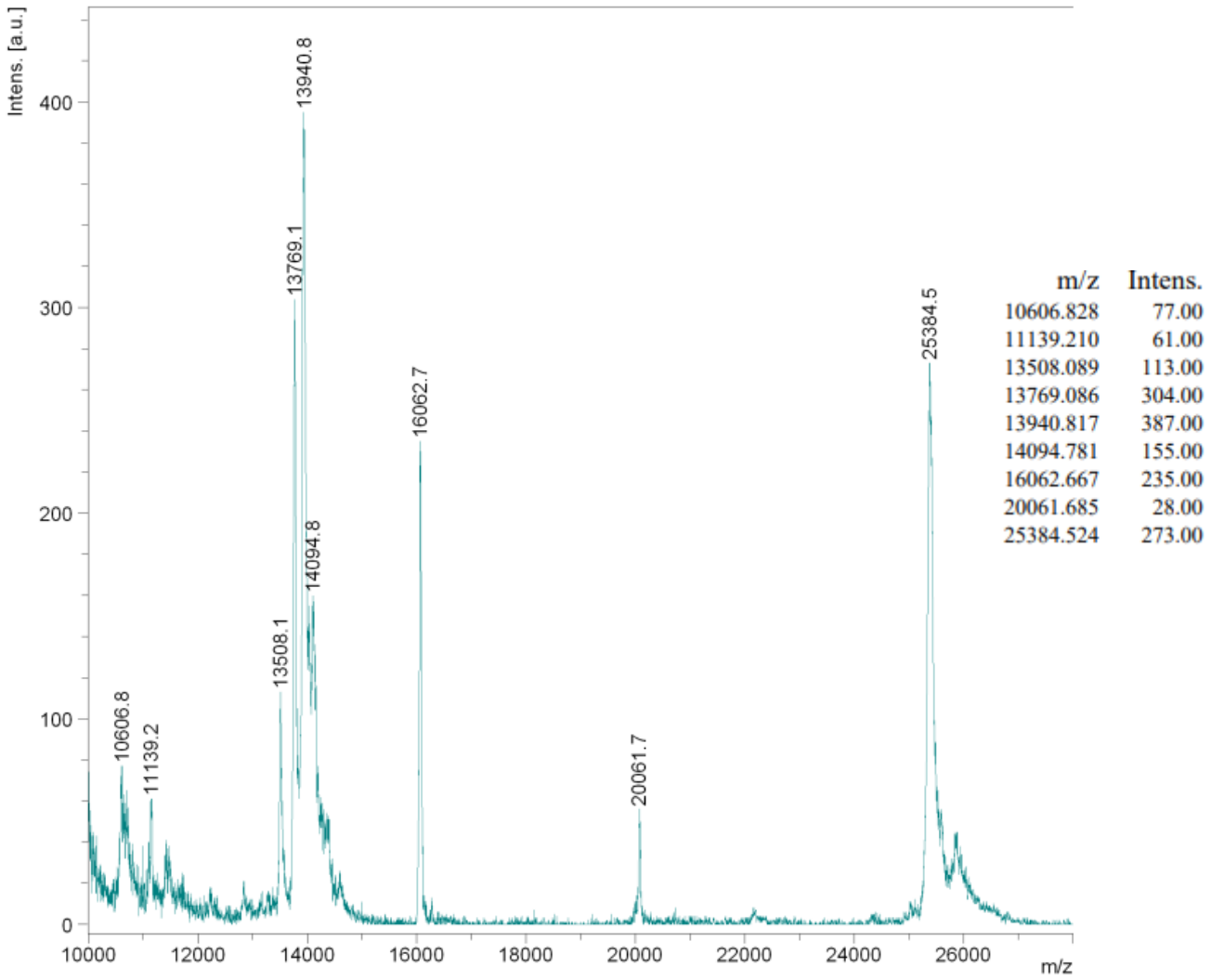
m/z	Intens.
10605.461	449.00
11150.059	238.00
13515.023	521.00
13770.628	1220.00
13937.590	2256.00
14024.568	700.00
14039.403	668.00
14108.250	465.00
14153.447	376.00
14195.996	254.00
14210.146	203.00
16070.029	769.00
20087.614	281.00
25036.598	515.00
25121.605	288.00
25165.379	262.00
25234.253	367.00
25388.396	2202.00
25388.484	2169.00
25558.504	648.00
25581.960	640.00
25678.499	386.00
25719.082	325.00
25778.639	341.00
25803.432	363.00
25826.569	396.00
25848.709	402.00
25897.922	336.00
26094.585	168.00
26140.521	144.00

Figure 1. Spectrum and table of values obtained from the skim milk sample.



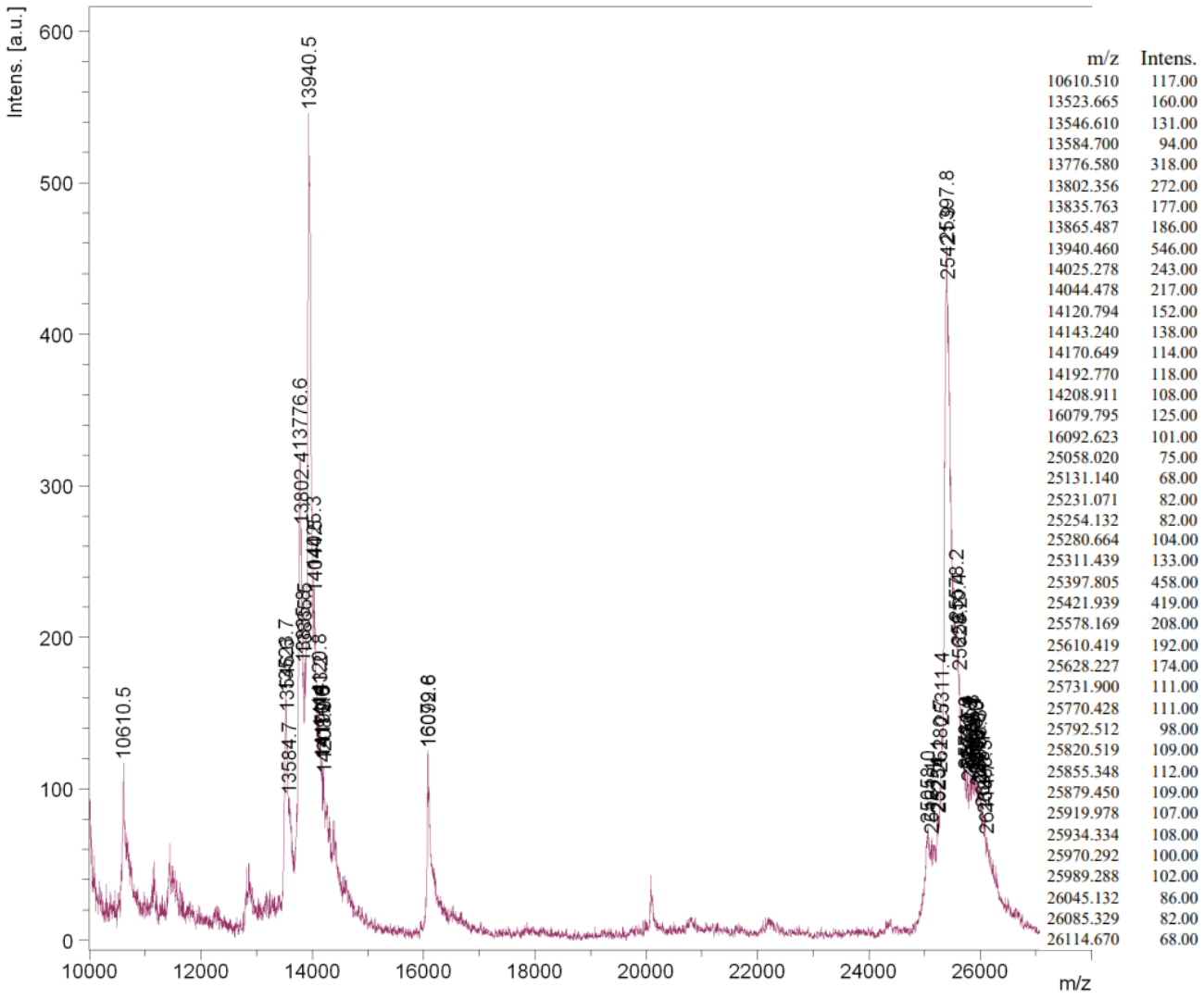
m/z	Intens.
10127.783	71.00
10128.445	67.00
10614.951	79.00
13748.886	115.00
13777.719	155.00
13953.767	315.00
14037.328	124.00
16068.470	139.00
16082.594	131.00
25411.152	205.00
25461.606	165.00

Figure 2. Spectrum and table of values obtained from the 1% buttermilk sample.



m/z	Intens.
10606.828	77.00
11139.210	61.00
13508.089	113.00
13769.086	304.00
13940.817	387.00
14094.781	155.00
16062.667	235.00
20061.685	28.00
25384.524	273.00

Figure 3. Spectrum and table of values obtained from the 2% milk sample.



m/z	Intens.
10610.510	117.00
13523.665	160.00
13546.610	131.00
13584.700	94.00
13776.580	318.00
13802.356	272.00
13835.763	177.00
13865.487	186.00
13940.460	546.00
14025.278	243.00
14044.478	217.00
14120.794	152.00
14143.240	138.00
14170.649	114.00
14192.770	118.00
14208.911	108.00
16079.795	125.00
16092.623	101.00
25058.020	75.00
25131.140	68.00
25231.071	82.00
25254.132	82.00
25280.664	104.00
25311.439	133.00
25397.805	458.00
25421.939	419.00
25578.169	208.00
25610.419	192.00
25628.227	174.00
25731.900	111.00
25770.428	111.00
25792.512	98.00
25820.519	109.00
25855.348	112.00
25879.450	109.00
25919.978	107.00
25934.334	108.00
25970.292	100.00
25989.288	102.00
26045.132	86.00
26085.329	82.00
26114.670	68.00

Figure 4. Spectrum and table of values obtained from the 3% milk sample.