Characterizing the commercially available whey proteins through mass spectrometry methods

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Introduction

Whey is the liquid remaining after milk has been curdled and strained. It is a byproduct of the manufacture of cheese or casein and has several commercial uses. The major protein components of the whey protein fraction occurring in cow milk are β-lactoglobulin (~65%), α-lactalbumin (~25%), bovine serum albumin (~8%) and immunoglobulins [1]. Whey protein is commonly marketed as a dietary supplement, and various health claims have been attributed to it in the alternative medicine community [2]. Although whey proteins are responsible for some milk allergies, the major allergens in milk are the caseins not abundant in whey [3]. Whey supplements are less tightly regulated by the Food & Drug Administration compared to pharmaceuticals, and numerous claims of adulterated and tainted supplements have been reported. Here, we integrated different bioanalytical techniques to carry out an independent analysis of the commercially available whey proteins to assess the quality of these products.

SDS-PAGE analysis

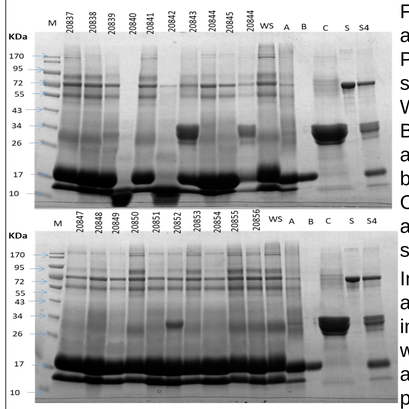


Figure 1: SDS-PAGE analysis of Whey Proteins along with the standards from Sigma. WS: Whey standard from Bovine milk (Sigma), A-alpha-lactalbumin, B-beta-lactaoglobulin, C-Caesin, S- Bovine serum albumin, S4- all four standard mixed together

In sample 20843, 20846, and 20852, no labelling information for casein was found, yet, gel analysis suggests the presence of significant amounts of casein.

Elemental analysis by ICP-MS (mg/serving)

#	NCNPR #	Na	Mg	K	Cr	Mn	Fe	Cu	Zn	Se
1	20837	86.5 (60)	21.7	177.8 (190)	0.0003	0.004	0.3	0.3	0.3	0.02
2	20838	83.3 (130)	21.3	195.3	0.0004	0.01	0.3	0.1	0.2	0.02
3	20839	95.8 (100)	23.4	193.6 (100)	0.0002	0.005	0.2	0.1	0.1	0.02
4	20840	219.8 (230)	51.3	536.7 (380)	0.6 (0.5)	0.2	2.0 (0.6)	0.3	0.4	0.04
5	20841	93.6 (120)	20.2	203.2	0.000	0.007	0.3 (0.36)	0.1	0.2	0.02
6	20842	228.5 (150)	27.5	199.0 (110)	0.001	0.01	0.3 (0)	0.2	0.2	0.02
7	20843	150.0 (150)	22.1	136.1 (95)	0.001	0.02	0.2	0.3	1.3	0.02
8	20844	123.7 (240)	127.2 (100)	567.3 (370)	0.031 (0.03)	0.6 (0.5)	0.3	0.8 (0.6)	4.0 (4.2)	0.04 (0.017)
9	20845	226.3 (230)	8.7	80.0	0.001	0.003	0.3	0.4	0.1	0.02
10	20846	370.9 (330)	292.7 (220)	1733.9 (1210)	0.5 (0.2)	3.2 (2.5)	11.7 (9)	3.7 (1.5)	20.4 (15)	0.25 (0.07)
11	20847	68.9 (65)	26.1 (20)	268.7 (150)	0.01	0.10	1.1 (0.3)	0.2	0.2	0.01
12	20848	70.1 (50)	26.1 (26)	289.6 (190)	0.038 (0.036)	0.08	0.2	0.8 (0.602)	4.2 (4.5)	0.04 (0.021)
13	20849	113.0 (96)	20.4 (22)	201.4 (150)	0.001	0.03	0.2	0.2	0.1	0.02
14	20850	137.3 (140)	40.4	441.4	0.02	0.1	1.5	0.3	0.3	0.02
15	20851	117.8 (110)	23.2	201.9 (150)	0.002	0.005	0.3	0.2	0.1	0.02
16	20852	49.4 (41)	23.7	151.3 (83)	0.0002	0.004	0.1	0.1	0.1	0.02
17	20853	52.6 (60)	30.6	274.2 (175)	0.02	0.1	1.6 (0.8)	0.2	0.3	0.02
18	20854	116.7 (95)	16.6 (15)	120.5 (75)	0.002	0.003	0.4	0.4	0.1	0.02
19	20855	138.5 (85)	18.9	199.0 (190)	0.002	0.004	0.4 (0)	0.3	0.1	0.02
20	20856	131.7 (110)	26.9	270.6	0.002	0.01	0.5	0.6	0.2	0.03

Table 1: Elemental analysis of 20 whey protein products by collision/reaction cell ICP-MS. Samples were digested with concentrated nitric and hydrochloric acid (8:2) using a closed vessel microwave system

Methods

20 different whey protein powder samples were collected from online markets. From each sample, 10mg of whey powder was weighted and dissolved in 1ml of 50mM sodium bicarbonate buffer pH 8 with vortexing. 100µl of solution was taken from each sample and filtered using a 3kDa molecular weight cutoff spin filter (GE Healthcare, UK). Proteins were denatured by heating the samples at 95ºC for 15min in the presence of 10mM DTT. Cysteine reduction was carried out by incubating the samples with 20mM idoacetamide for 45min in the dark. Sequencing grade trypsin was added to a 1:40 weight ratio of trypsin/protein to the protein sample and incubated it at 37 °C overnight. Peptides were analyzed by LC-MS/MS using a Thermo Orbitrap Fusion Tribrid.

Proteomics analysis of Whey Proteins

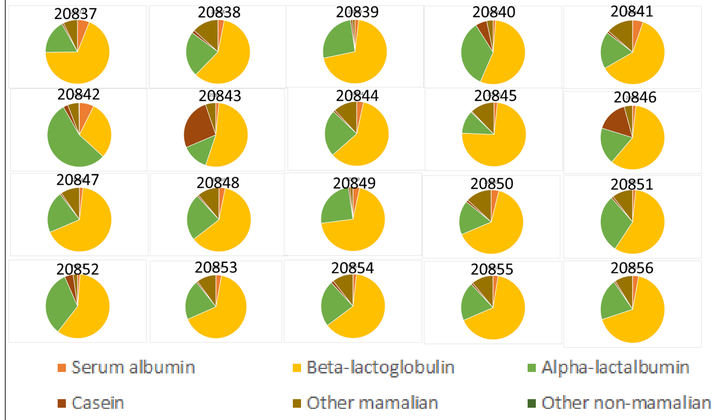
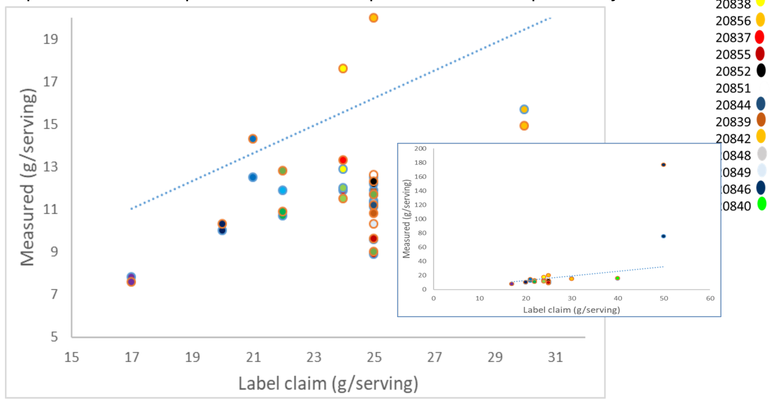


Figure 2: The major whey protein components identified using a Byonic search after LC-MS/MS analysis using the Swiss-Prot non-redundant database. The decoy database was created by reversing the sequence of the target database. Search parameters: precursor mass tolerance 100ppm, fragment mass tolerance 0.4Da, enzymatic cleavage with two possible missed cleavages, modification with idoacetamide (57.021464Da). The proteins were identified with 1% FDR and only those identifications which showed minimum three peptides in protein search were considered for analysis. No significant amount of adulterant protein was detected.

Total protein quantification

Figure 3: Total protein quantification by Bradford reagent. Standard curve was prepared using BSA. 5µl of protein samples were added to 96 well plates and immediately 250µl of Bradford reagent was added to each well. After 30 minutes and later absorbance was measured at 595nm using a plate reader. The experiments were repeated two months apart to measure repeatability.



Conclusions:

- 20 commercial whey protein products were analyzed to assess the quality of these products
- Major whey proteins components were identified with SDS-PAGE analysis with no adulterating protein from non-dairy sources
- Bradford assay data shows, total protein amount deviates from the label claim significantly for most samples
- Elemental analysis of whey proteins revealed the presence of unlabeled element for some samples, all at safe levels

Acknowledgements

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