# Characterization of Standard Milk Protein Samples for the Evaluation of *in-vitro* Digestion Models

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## Introduction

*In-vitro* digestion models are useful to address questions regarding physiological effects, allergic potential and food safety. The human digestive system comprises a multitude of intricate processes to break down nutrients into physiologically active compounds. Due to their complexity, it is very difficult to simulate them *in-vitro*. In recent years various digestion protocols have been developed by different laboratories. In order to harmonize these protocols, the composition of the starting material was determined.

## Methods

As reference samples dried and fat free milk protein powders were chosen due to their stability, complexity and easy handling properties. For this purpose a batch of skim milk (NZ-SMP), sodium caseinate (NZ-caseinate) and whey protein powder (NZ-WP; Fig. 1.) were kindly provided by Brent Murray.


**Fig 1.:** Sodium caseinate (A, NZ-caseinate), Skim milk powder (B, NZ-SMP) and whey protein powder (C, NZ-WP) from New Zealand were used as reference samples.

In order to define the composition, the milk protein powders were dissolved in $H_2O$ and characterized by various analytical methods. Total and non-protein nitrogen, fat, calcium, lactose, as well as protein patterns were analyzed by Kjeldahl, Roese-Gottlieb, Schmid-Bondzynski, flame-AAS, HPLC, SDS-PAGE and Protein 80 LabChip kit (Agilent), respectively.

## Results

Content in major milk proteins:
As expected NZ-WP consists to 96.20% of Whey protein, of which 59.60% is β-Lactoglobulin and 34.97% α-Lactalbumin (Fig. 3.B). NZ-Caseinat consists to 94.70% of casein, but casein is also prominent in NZ-SMP (84.23%; Fig. 2 und 3.B).

| sample | total nitrogen [g/kg] | non-protein nitrogen [g/kg] | total fat [g/kg] | calcium [mg/kg] | lactose [mmol/kg] |
|---|---|---|---|---|---|
| NZ-SMP | 64.90 | 3.33 | 7.5 | 13258.12 | 4888.67 |
| NZ-SMP | 66.37 | 3.42 | 10.39 | 13437.87 | 5116.00 |
| NZ-SMP | 64.70 | 3.32 | 8.47 | 13401.41 | 4937.73 |
| **mean NZ-SMP** | **65.33** | **3.36** | **8.79** | **13365.80** | **4980.80** |
| NZ-WP | 145.11 | 0.58 | 9.27 | 702.62 | < LOD |
| NZ-WP | 145.15 | 0.49 | 6.06 | 689.50 | < LOD |
| NZ-WP | 145.17 | 0.50 | 6.95 | 710.28 | < LOD |
| **mean NZ-WP** | **145.14** | **0.52** | **7.43** | **700.80** | **< LOD** |
| NZ-caseinate | 143.40 | 2.07 | 4.8 | 713.01 | < LOD |
| NZ-caseinate | 143.41 | - | 5.3 | 726.73 | < LOD |
| **NZ-caseinate** | **143.41** | **2.07** | **5.0** | **720.00** | **< LOD** |

**Table 1.:** Mean values [g/kg] for the content of total and non-protein nitrogen (Kjeldahl), total fat (Roese-Gottlieb), calcium (flame-AAS), lactose (enzymatic). Limit of Detection (LOD)

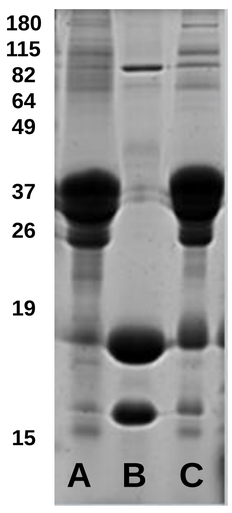


**Fig 3.:** SDS-PAGE of 2.5 µg of the three reference samples: sodium caseinate (A), whey protein powder (B) and skim milk powder (C).

Interestingly NZ-SMP comprises almost twice as much calcium as NZ-WP and NZ-caseinate (13365.80 g/kg versus 700.80 g/kg and 720.00 g/kg, Fig 3.B).
Lactose was below the detection limit in NZ-Caseinat and NZ-WP (Fig. 3.B).

In future experiments the 20 most abundant proteins will be quantified by selected reaction monitoring mass spectrometry [1].

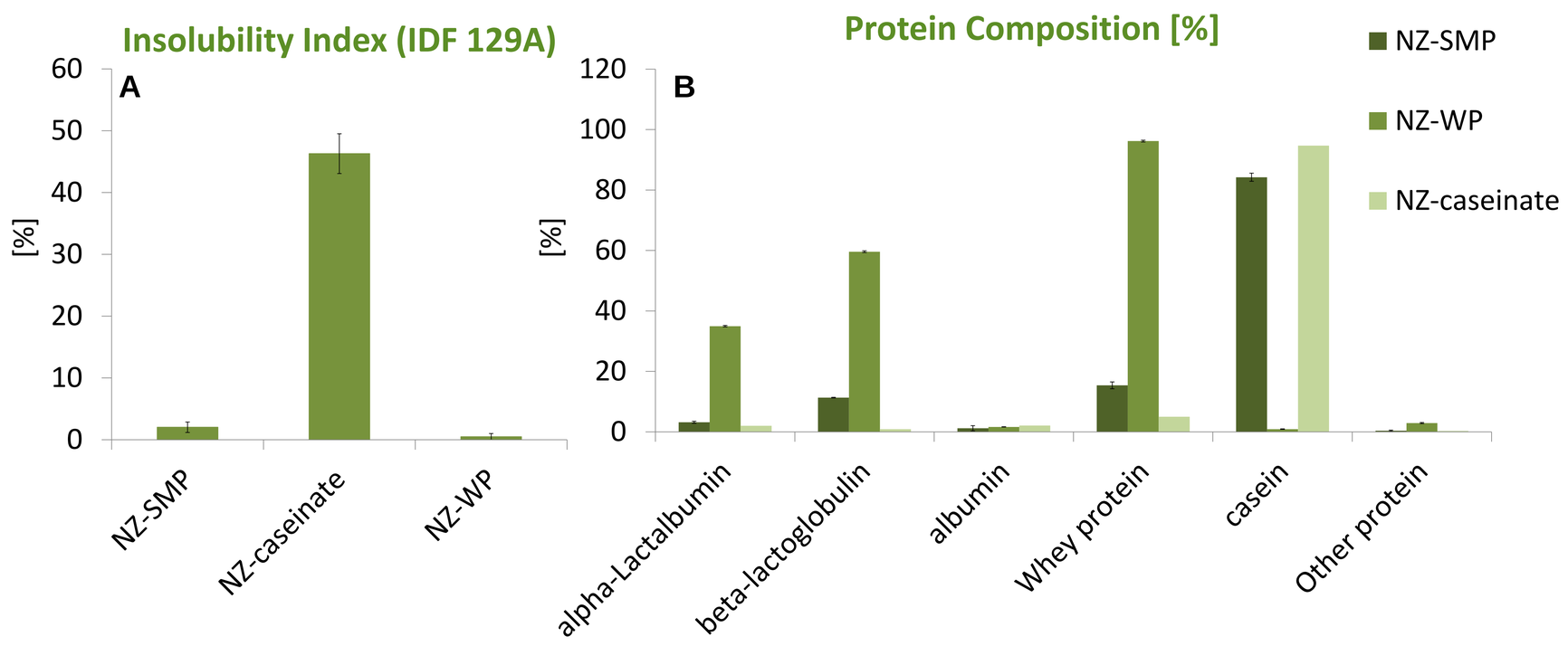


**Fig 3. A:** Insolubility Index (IDF 129A) [%] for NZ-SMP, NZ-caseinate and NZ-WP. **B:** Protein composition [%] of NZ-SMP, NZ-caseinate and NZ-WP, identified by Protein 50 LabChip kit (Agilent).

## Application

By using the here presented data on the provided reference samples the fate of the principle ingredients can be followed during *in-vitro* digestion. These reference samples can also be used to control repeatability in future experiments.

[1] Mathis D., Schwander F., Kopf-Bolanz K., Egger C., Portmann R., *Absolute Quantification of 20 Major Proteins in Dairy Products by LC-MS/MS*, 2012

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