

# Understanding the Amino Acid Profile of Whey Protein Products

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## Abstract

**Background:** The South African dietary supplement market will undergo a period of transition within the next few years due to the establishment of the South African Health Products Regulatory Authority (SAHPRA), which has superseded the former Medicines Control Council (MCC). While regulatory steps are yet to be fully outlined, products such as whey protein, regarded as food, will be governed by the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Foods. The guideline provides for the minimum value of essential amino acids (plus cysteine and tyrosine) per gram of protein that products claiming to contain protein will be required to comply with. Determining the compliance levels of whey protein products currently available will assist in establishing the readiness of the dietary supplement industry for regulation, and provide an indication of the overall state of the industry.

**Objectives:** To determine the amino acid profile of whey protein powder and compare analysed content to manufacturer stated content.

To compare analysed amino acid content to the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Food template amino acid profile.

**Method:** 15 of the best-selling whey protein products available in South Africa were selected for amino acid analysis. Tested amino acid content were compared to the label stated claim and the amino acid reference pattern, as stated in the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Foods.

**Results:** Sixty percent (60%) of products tested were non-compliant with the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Foods. Of the 15 products tested, 11 were manufactured in South Africa, with 8 being non-compliant to the guideline amino acid profile. Considerable variance was noted in the manufacturer stated and the tested amino acid content (ranging from 16–48% variance).

**Conclusion:** Many of the whey protein products available in South Africa are not compliant to proposed industry guidelines. The considerable variance noted highlights the need for greater oversight of the industry with clearly defined regulatory procedures.

**Keywords:** amino acid profile, amino acid spiking, athletes, content adherence, dietary supplements, sport nutrition, whey protein

## List of Abbreviations

BCAA-Branch chain amino acid; DOH-Department of Health; FAO-Food and Agriculture Organisation; HPLC-High pressure liquid chromatography; MCC-Medicines Control Council; SAHPRA-South African Health Products Regulatory Authority; SANAS-South African National Accreditation System; USA-United States of America; WHO-World Health Organisation; WPC-Whey protein concentrate; WPH-Whey protein hydrolysate; WPI-Whey protein isolate.

## 1. Background

The international dietary supplement industry has expanded exponentially over the past few years and is as lucrative in South Africa, where it is growing annually at a rate of 7.7% (Insight survey, 2016). With \$28 billion

spent on dietary supplements in the United States in 2010 (Umhau, Garg & Woodward, 2012) and 52% of Americans (Kantor et al., 2012) as well as 72% of Australians (Braun et al., 2010) using a dietary supplement within the past 12 months, dietary supplements have become staples in many households.

Despite the considerable global and local demand, this industry remains poorly regulated (Schonfeldt, Hall & Pretorius, 2015; Gabriels, Lambert & Smith, 2012; Gabriels, Lambert, Smith & Hiss, 2011). From raw ingredient contamination (Geyer et al., 2004) to label compliance discrepancies (Green, Catlin & Starcevic, 2001), the dietary supplement industry is an area of growing concern. In a largely unregulated industry, consumers supplementing their diet with supposedly safe products may in fact be doing so to their detriment. In the United States of America (USA), 23 000 emergency visits per year were attributed to health events related to the use of dietary supplements (Geller et al., 2015). Such findings are not isolated and can in part be linked to the lack of adequate safety data around many products (Raynor, Dickinson, Knapp, Long, & Nicolson, 2011) in addition to poor quality control (Risvoll, Giverhaug, Halvorsen, Waaseth, & Musial., 2017). Ninety seven cases of hepatitis have been attributed to one fat loss supplement alone (CDC, 2012), and a group of USA researchers have found that the rate of liver failure from supplement use has increased by 185% in the past 10 years (Navarro & Lucena, 2013).

The South African dietary supplement market will undergo a period of transition within the next few years, due to the establishment of the South African Health Products Regulatory Authority (SAHPRA), which has superseded the former Medicines Control Council (MCC). While the regulatory process is yet to be fully outlined, the general view (Schonfeldt et al., 2015) is that products making medicinal claims (testosterone boosters, fat loss agents etc.) are to be marketed as medicines, and will consequently fall under the Medicines and Related Substances Act 101 of 1965 (MCC, 2003). Products falling outside this bracket and not sold in tablet, capsule or soft gel dosage forms are to be marketed as foods, and will fall under the Department of Health R429 draft Regulations Relating the Labelling and Advertising of Foods (DOH, 2014). Such products include whey protein, as well as other protein powder blends, which are the focus of this study.

The term 'whey protein' includes whey protein concentrate (WPC), whey protein isolate (WPI) and whey protein hydrolysate (WPH). WPC is the least processed and is 35-80% protein by weight, whereas WPI is further processed to remove fat and carbohydrates to produce a product that is 90% protein by weight (Whetstone, Croissant & Drake, 2005). WPH is enzymatically and acid pre-treated to reduce particle size and is the fastest digesting protein of the three variants. Whey protein products are commonly sold in blends of WPC and WPI, which may include WPH. Whey protein typically comprises of an abundance of amino acids (building blocks of protein), which can be divided into essential, non-essential and conditionally essential amino acids.

An essential amino acid is one that the body cannot synthesize on its own and must therefore be obtained through diet. The nine essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Helmenstine, 2016). Conditional essential amino acids are amino acids that are essential only at certain stages of growth or for individuals unable to synthesize them, thus requiring their addition through diet (WHO, 2007). These amino acids are: arginine, cysteine, glutamine, tyrosine, glycine, ornithine, proline and serine. Non-essential amino acids are those that the human body is able to synthesize and is therefore not required through diet, and include: alanine, asparagine, aspartic acid and glutamic acid (WHO, 2007).

The Department of Health R429 draft Regulations Relating the Labelling and Advertising of Food guidelines provide a template for protein quality in any product making a protein claim. The indicator of protein quality is derived from the Food and Agriculture Organization / World Health Organization guidelines (FAO, 2007). The guidelines establish the minimum content of essential amino acids (plus cysteine and tyrosine) required for a product to make a protein claim (per gram of protein). Products containing below the specified amino acid profile (in any single amino acid value) are deemed non-compliant.

Previous research on whey protein products have indicated products on the market containing up to 80% less protein than the manufacturer stated content (Schonfeldt et al., 2015). Traditionally, detecting the protein content of food products has relied on identifying the nitrogen content of a sample. Such detection methods have been exploited by manufacturers in the food industry, with the widespread adulteration of food products with non-protein nitrogen containing substances (Moore, DeVries, Lipp, Griffiths & Abernethy, 2010; Ingelfinger, 2008). Due to such exploitation, a more reliable means of ascertaining protein or amino acid content adherence is needed, such as by the amino acid analysis of products (Levinson & Gilbride., 2011)

Due to the lack of oversight in the dietary supplement industry, consumers face significant risk associated with poor quality control and lack of product adherence to label claims (Schonfeldt et al., 2015; Gabriels et al., 2011; Geyer et al., 2004), with calls for greater regulation of the dietary supplement industry having been made for a number of years (Gabriels et al., 2012). With the advent of the South African Health Products Regulatory

Authority and the Department of Health R429 draft guidelines Relating to the Labelling and Advertising of Food, the adherence of the products to the guideline requirements can be used to determine the readiness of the dietary supplement industry for regulation and from there assist in paving the way towards industry governance. Product non-adherence to label specified guidelines and adulteration/contamination of dietary supplements have been noted in prior studies (Geyer et al., 2004 (Geyer et al., 2004; Ingelfinger, 2008) and have been attributed to numerous adverse health events (Navarro et al., 2013; Levinson et al., 2011). Once the extent of adherence to label claims and industry requirements is determined, further action can be taken to improve quality control and outline clearly defined regulatory steps.

## 2. Method

In this study, 15 of the best selling whey protein products available in South Africa were selected for testing, having been identified by online resources. The top 3 retailers of dietary supplements in South Africa were determined by online search engines and the top 5 whey protein products from each retailer (as indicated by each specified retailer) were combined to create a list of 15 products. An analysis of the amino acid profile of the products was conducted and compared to the label stated claim as well as to the nine amino acid reference patterns indicated in the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Foods (Table 1).

Products making a protein containing claim are required to comply with the specified minimum values per gram of protein made. Any individual amino acid levels falling below the reference pattern deem the product non-compliant.

Table 1. The Department of Health draft Regulations Relating the Labelling and Advertising of Foods R429 minimum amino acid values per gram of protein

<b>Reference Amino Acid (per gram of protein)</b>	<b>Quantity (mg)</b>
Histidine	17.0
Isoleucine	30.5
Leucine	62.0
Lysine	50.0
Methionine plus Cysteine	24.0
Phenylalanine plus Tyrosine	43.5
Threonine	26.0
Tryptophan	6.8
Valine	40.5

(DOH, 2014).

The current manipulations and inaccuracies of nitrogen content analysis as a means to determine protein content of food products (Moore et al., 2010; Ingelfinger, 2008; Smith & Bonwick, 2007) has resulted in amino acid analysis being regarded as a more accurate means of determining product authenticity (FAO, 2003). While High Performance Liquid Chromatography (HPLC) as a means of ingredient verification has drawbacks, such as the equipment costs, specialized skills required and the long analysis times (Moore et al., 2010), it is regarded as an invaluable technique to detect individual amino acid (Levinson et al., 2011; Bartolomeo & Maisano, 2006). The amino acid detection procedure for this study was outsourced to a South African National Accreditation System affiliated (SANAS) facility in Pretoria and was conducted using HPLC. They were provided with the 15 samples which were previously dispensed into individually marked containers blinded to the testing facility. Products were within specified expiry dates and stored at specified storage conditions.

The procedure involved the acid hydrolysis of individual amino acids in order to produce free form amino acids of the 15 specified samples, pre column derivatization and separation by HPLC, and detected by use of a fluorescence detector. The individual amino acid levels for each sample were tabulated and compared to both the manufacturers stated content as well as the Department of Health R429 draft Regulations Relating to the Labelling and

Advertising of Foods template amino acid pattern. The reference amino acid pattern provided the amino acid level per gram of protein, with the levels detected being divided by the manufacturer stated protein content of each sample, providing an amino acid value per gram of protein.

### 3. Results

Of the 15 products tested, four were manufactured internationally (USA and Europe) and 11 were produced in South Africa. Whey protein is derived from cow's milk and is presented in a flavoured or unflavoured powder. At present, whey protein is regarded as a food product in South Africa and is thus required to comply with the Department of Health R146 Regulations Relating to the Labelling and Advertising of Foods which is due to be superseded by the R429 draft regulations.

Considerable variance was noted in the amino acid profile of many products with the amino acid glycine testing higher in 8 products than indicated on the label claim. The glycine variance ranged from 0.11g more per 100g to up to 10.25g more than indicated per 100g. Leucine, Isoleucine and valine collectively are referred to as branch chain amino acids (BCAA). BCAA content was typically less than manufacturer stated claim with the exception of 2 products that contained higher than stated valine content.

From the 15 products tested, only six met compliance to the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Foods R429. Of the 4 products manufactured internationally, three were compliant to the Department of Health (DOH) draft guidelines with one product not meeting regulatory requirements. Eight products manufactured locally were not compliant to the draft R429 guidelines; hence 72.73% of locally manufactured products do not adhere to the R429 draft guidelines.

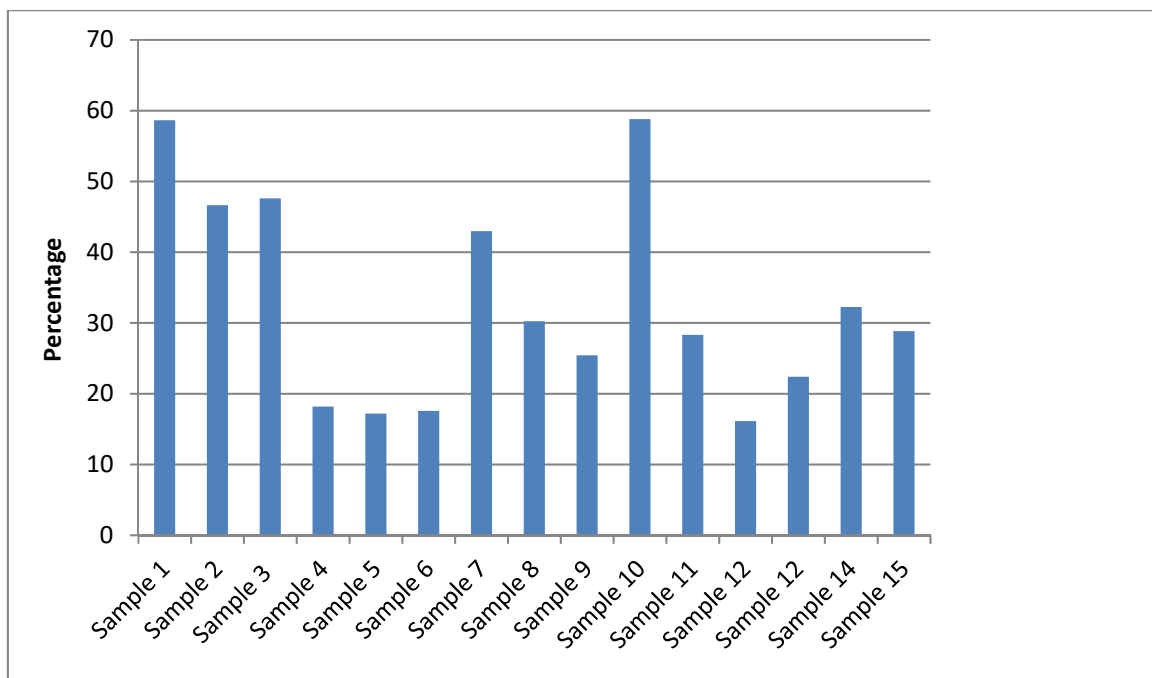


Figure 1. Percent variance from manufacturer stated amino acid values

Figure 1 indicates the average percentage variance between the manufacturer stated value and that reported from the 17 amino acids analysis performed. In all instances, the amounts identified from the amino acid analysis test were lower than that stated on the packaging. Cysteine and tryptophan values have been omitted due to their instability under acid hydrolysis.

Table 2. Comparison of manufacturer stated amino acid content versus actual (tested) amino acid content

AA	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested
Alanine	4.90	1.86	5.80	2.92	5.40	2.54	3.35	2.67	4.00	3.46	3.84	2.98	3.65	1.86	4.20	3.02
Arginine	2.10	1.36	2.90	1.97	2.80	1.71	1.83	1.76	2.00	2.00	1.86	2.15	1.53	1.36	1.76	1.86
Aspartic Acid	10.80	4.02	11.00	6.012	11.30	5.16	7.63	5.61	7.50	6.71	6.62	5.67	8.03	4.02	9.24	5.97
Glutamic Acid	16.70	6.72	19.00	10.52	18.24	9.07	12.58	9.58	13.57	10.94	11.13	10.82	13.21	6.72	15.2	10.37
Glycine	1.80	0.80	23.00	5.13	2.10	1.05	1.28	1.00	1.60	1.82	1.51	1.27	1.02	0.80	1.17	8.37
Histidine	2.20	0.97	2.00	1.35	2.07	1.55	1.53	1.45	1.38	5.37	1.40	1.59	1.24	0.97	1.42	2.20
Isoleucine	5.80	2.14	6.20	3.79	7.00	3.30	4.15	3.65	4.67	4.13	4.31	3.85	4.67	2.14	5.37	3.52
Leucine	10.80	3.56	11.80	5.98	11.40	5.62	7.55	5.90	9.05	6.71	7.38	6.45	7.38	3.56	8.90	5.85
Lysine	9.60	3.21	10.10	5.75	9.04	6.37	6.49	5.67	7.08	6.61	6.74	6.07	7.01	3.21	8.06	5.57
Methionine	1.90	0.82	2.10	1.2	2.60	1.04	1.53	1.08	1.47	1.59	1.62	1.33	1.61	0.82	1.85	1.43
Phenylalanine	3.30	1.15	3.90	1.84	3.50	1.78	2.27	1.84	2.69	2.11	2.50	2.14	2.19	1.15	2.52	1.80
Proline	5.80	2.24	6.50	3.71	6.50	3.11	4.74	3.31	4.50	3.90	5.25	3.88	4.01	2.24	4.62	3.67
Serine	4.70	1.96	N/A	3.03	5.40	2.58	3.65	2.59	3.67	4.38	4.39	3.22	3.36	1.96	3.86	3.29
Threonine	7.20	2.65	7.50	4.43	7.40	3.48	4.63	3.72	5.60	4.53	5.27	4.09	4.89	2.65	5.62	4.21
Tyrosine	1.80	1.08	-	1.92	3.50	2.16	1.95	2.01	2.34	2.41	2.44	1.63	1.90	1.08	2.18	1.80
Valine	5.80	2.01	6.4	3.52	6.40	3.06	3.86	3.37	4.91	3.90	2.65	3.6	4.31	2.01	4.96	3.33

AA	Sample 9		Sample 10		Sample 11		Sample 12		Sample 13		Sample 14		Sample 15	
	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested	Stated	Tested
Alanine	4.00	2.5	3.68	2.45	3.70	2.66	3.83	3.28	3.58	3.25	3.60	2.80	-	3.06
Arginine	2.00	2.00	1.85	1.80	1.55	1.80	1.88	2.04	1.69	1.96	1.51	1.72	-	2.04
Aspartic Acid	8.75	4.99	7.95	4.90	8.14	4.99	8.75	6.75	7.52	6.44	7.92	5.28	-	6.22
Glutamic Acid	14.00	10.6	12.68	9.34	13.39	9.34	13.77	11.28	11.97	10.77	13.03	9.31	-	11.15
Glycine	1.50	10.63	1.37	9.20	1.04	8.70	1.37	1.52	1.26	1.37	1.08	11.08	-	1.45
Histidine	1.50	1.04	1.39	1.47	1.26	1.29	1.42	1.52	1.28	3.19	1.22	5.51	1.37	1.80
Isoleucine	3.50	3.35	3.19	2.70	4.74	3.13	4.70	4.14	4.63	3.61	4.61	2.87	4.55	3.19
Leucine	9.75	5.60	8.63	4.67	7.84	5.22	8.10	6.53	7.21	6.15	7.63	4.86	7.10	5.92
Lysine	7.75	5.01	6.91	3.94	7.10	5.48	7.86	6.92	6.52	6.10	6.91	4.99	5.86	5.75
Methionine	1.50	1.22	1.38	1.02	1.63	1.08	1.81	1.21	1.53	1.35	1.58	1.30	1.74	1.32
Phenylalanine	2.75	2.11	2.44	1.57	2.22	1.68	2.47	2.03	2.16	1.84	2.16	1.63	2.79	1.91
Proline	4.00	4.33	3.60	3.31	4.07	3.24	4.69	3.69	4.40	3.50	3.96	3.28	-	3.9
Serine	3.75	2.90	3.43	2.71	3.40	2.71	3.59	3.17	3.21	3.55	3.31	4.07	-	3.54
Threonine	4.25	3.51	3.84	3.52	4.96	3.57	5.21	4.43	5.24	4.34	4.82	3.59	5.18	4.19
Tyrosine	2.75	2.55	2.50	1.09	1.92	1.95	2.36	2.81	1.98	2.22	1.87	2.18	-	2.20
Valine	3.25	3.45	2.89	2.81	4.37	2.92	4.63	3.69	4.29	3.41	4.25	2.90	4.21	3.12

\*Amino acid values not indicated (-) not stated by the manufacturer.

Table Indicating the Compliance of protein powder samples to the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Food (presented as amino acids per gram of protein)

AA	DOH	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Histidine	17.00	12.44	16.88	18.02	20.14	71.6	21.49	13.29	26.19	13.0	20.42	17.43	19.0	40.9	76.53	27.27
Isoleucine	30.50	27.44	47.38	38.37	50.69	55.07	52.03	29.32	41.9	41.88	37.5	42.3	51.75	46.28	39.86	48.33
Leucine	62.00	45.64	74.75	65.35	81.94	89.47	87.16	48.77	69.64	70.0	64.86	70.54	81.63	78.85	67.5	89.7
Lysine	50.00	41.15	71.88	74.07	78.78	88.13	82.03	43.97	66.3	62.63	54.72	74.05	86.5	78.21	69.31	87.12
Meth + Cyst																
Pheny + Tyro	43.50	28.59	47.0	45.81	53.47	60.27	50.95	30.55	42.86	58.25	36.94	49.05	60.5	52.05	52.92	62.27
Threonine	26.00	33.97	55.38	40.47	26.0	60.4	55.27	36.3	50.12	43.88	48.89	48.24	55.38	55.64	49.86	63.48
Tryptophan																
Valine	40.80	25.77	44.0	35.58	46.81	52.0	48.65	27.53	39.28	43.13	39.03	39.46	46.13	43.72	40.28	47.27

\*Cysteine and tryptophan values not tested due to instability in testing protocol.

#### 4. Discussion

The dietary supplement industry is now a multi-billion dollar enterprise (Umhau et al., 2012), growing considerably over the past few years (Insight survey, 2012). Numerous studies have highlighted the need for more stringent and clearly defined dietary supplement regulations within South Africa. However, few have tested the content adherence of products such as through amino acid analysis. This study sheds light on whey protein products available in South Africa (and internationally) as well as the dietary supplement industry as a whole. Indicating the current state of the dietary supplement industry constitutes the first step toward more accountability by manufacturers and greater consumer confidence.

The study found only 40% of products tested, comply with proposed industry guidelines. A further analysis of the results reveals that 75% of international products meet proposed industry guidelines while only 27% of locally manufactured products are compliant. The discrepancy between locally manufactured and international products can be attributed to many potential factors. Such include the smaller sample size of international products selected and the variance in manufacturing facilities. The lack of adherence to industry guidelines can be attributed to large discrepancies between manufacturers stated content and actual product content as products adhering to manufacturer stated content would naturally comply with the reference amino acid pattern of the Department of Health draft R429 Regulations Relating to the Labelling and Advertising of Foods.

Higher than stated glycine content was noted in 8 of the products tested, reaching up to 10 times more than manufacturer stated content. Such is indicative of the adulteration of products (Moore et al., 2010). This is further highlighted by the large variance in the amino acid content of products. The BCAA content was lower (ranging from minimal to significant) in 13 of the 15 products tested. Of the 2 products containing higher than stated BCAA content, such was in valine with lower than stated values for both leucine and isoleucine.

Previous research into the dietary supplement industry of South Africa found a majority of products to meet adhered content. With only five (5) of 70 products found to under represent protein content according to industry requirements (Schonfeldt et al., 2015). Products containing less than specified protein content varied from 40% to 80% less protein than indicated by the manufacturer. The study looked at protein products as a whole, including protein derived from a combination of sources and carbohydrate containing products as opposed to whey protein products exclusively. The means of analysis also does vary significantly. Previously, researchers detected the protein content of protein products through use of the Dumas method, detecting protein values through the measurement of the nitrogen content. While the test provided an indication of the current state of whey protein supplements, the addition of non-protein nitrogen containing substances through adulteration was not taken into account. Adulteration of food is the fraudulent substitution of an authentic food ingredient with a cheaper, non-authentic component (Moore et al., 2010; Smith et al., 2007). In 2007, concerns regarding food safety due to adulteration of products were highlighted with the widespread melamine poisoning experienced in China and parts of Asia. 294 000 children were believed to have been affected by tainted baby formula, resulting in the hospitalisation of 50 000 and at least 6 deaths (Ingelfinger et al., 2008). Melamine, containing 66% nitrogen is believed to have been added to manipulate perceived protein and such instances have been detected worldwide

(Ingelfinger et al., 2008). In this study, elevated glycine levels of up to 10 times more than the manufacturer stated levels were noted. Such is indicative of adulteration and is likely to be widespread in whey protein products given the large variance between manufacturer stated amino acid content and actual amino acid content.

A possible limitation that must be mentioned is that one sample from each manufacturer was selected for testing. Further research should look at obtaining samples from different batches with possibly multiple tests per batch performed. The amino acid profile did not include tryptophan, cysteine and asparagine. Further research can obtain the individual values of these amino acids. Whey protein, while hugely popular only constitutes a fraction of the dietary supplement market, the content of pre-workout supplements, amino acid powders, fat loss agents and testosterone boosters to name a few require content verification to ensure that the manufacturer stated content is adherent to the actual content as well as to ensure the absence of pharmaceuticals or other banned substances.

Calls for greater oversight within the dietary supplement industry are warranted and regulations need to be developed and implemented for the safety of consumers. Regulatory steps need to involve clearly defined procedures that improve quality control and provide penalties for manufacturers engaging in adulteration of products and other unethical practices.

## 7. Conclusion

The vast majority of bestselling whey protein products available in South Africa do not adhere to manufacturer stated claims and 60% do not meet the amino acid levels required by the Department of Health R429 draft Regulations Relating to the Labelling and Advertising of Foods. The study indicates that the dietary supplement industry in South Africa needs greater industry oversight in order to ensure consumer safety. Poor manufacturer quality control and adulteration of products are concerns highlighted and without addressing such, the dietary supplement industry presents great consumer risk.

## Declarations

### Ethics Approval

The research paper has been granted full approval by the University of KwaZulu Natal Humanities and Social Sciences Research Ethics Committee (HSS/0408/017M).

### Availability of Data and Material

Individual amino acid chromatogrammes available on request.

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### Authors' Contributions

KN conceptualized and wrote the article. VB and RN provided feedback and editing assistance.

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None.

### Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

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