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PROTEIN QUALITY OF SOUTH AFRICAN POTATOES TO INFORM DIETARY CHOICES

Carmen Muller[1], Schönfeldt, H.C[1]

ABSTRACT: Protein content was determined using both the nitrogen to protein conversion factor of 6.25 and the summation of amino acids for four of the potato cultivars (*Solanum tuberosum* L.) most commonly consumed in South Africa. Protein content for the cultivars varied between 1.65 g/100 g and 2.14 g/100 g fresh-weight when using protein conversion and 1.64 g/100 g and 2.14 g/100 g when using the sum of amino acids. Significant differences were found between the amino acid contents of the different cultivars. Leucine had the lowest chemical score of essential amino acids. Protein quality was evaluated using various methods. Even though the protein content of the cultivars is low, it is of a high quality and can contribute to overall dietary protein consumption.

KEYWORDS: .....

INTRODUCTION

The metabolic effect of specific individual dietary amino acids is important, demanding accurate information about the amino acid profile of foods. According to the Food and Agricultural Organisation (FAO), when evaluating dietary protein: "dietary amino acids should be treated as individual nutrients and wherever possible data for digestible or bioavailable amino acids should be given in food tables on an individual amino acid basis" (FAO 2011).

Protein is considered an important macronutrient in the diet as it provides both essential amino acids and is a source of energy. There has been much discussion regarding protein and amino acid requirements for both adults and children in recent years (Ghosh et al. 2012). Internationally, the 2007 Food and Agriculture Organization/ World Health Organization (FAO/WHO) report entitled 'Protein and amino acid requirements in human nutrition', concluded that protein quality is of greater importance than actual quantity, emphasising the presence of individual amino acids in a food, instead of simply focussing on total protein (WHO, 2007). Determining the amounts of dietary essential amino acids, digestibility of protein and bioavailability of amino acids, facilitates the quantification of quality parameters of protein source foods (Gilani et al. 2005).

Protein quality has been defined as the ability of a protein to achieve metabolic actions by providing specific patterns of amino acids and it is as important as protein content (Pretorius et al. 2019; MIllward et al. 2008). The FAO report suggested that on average the recommendations for essential amino acids, particularly for lysine, should be doubled, and the recommended requirement for adults should be increased from 12 mg/ kg body mass to 30 mg/kg (WHO 2007). Data on the amino acid composition of foods is thus essential to improve global protein intake and health. The subject of the contribution of high-quality foods to the diet is questioned at length in the recently released EAT-Lancet report. In this report it is estimated that there are 820 million people suffering from low quality diets which leads

[1]University of Pretoria, Department Animal and Wildlife Sciences, Pretoria, South Africa
Corresponding author – vanniekerk.carmen@gmail.com

to a deterioration in human health. The report further promotes dietary changes to include foods that provide nutrients of high quality that can significantly decrease the number of individuals consuming low quality diets (EAT-Lancet 2019).

Alternative sources of protein can make a significant contribution to protein intake of vulnerable individuals especially in countries where high quality proteins from animal source foods is scarce. It has been noted that protein malnutrition is a causative factor of 49% of more than 10 million annual deaths of children under five (Wang et al. 2017). It is estimated that almost one third of children in developing countries are stunted, caused by long term insufficient intake of protein and energy. This emphasises the need for alternative sources of high-quality protein to meet the growing need (Global Nutrition Report, 2018). Therefore, research on various supplementary crops is encouraged to determine the true and total nutritional value of food protein (EAT-Lancet 2019).

The South African Food-Based Dietary Guidelines (FBDG) encourage consumers to plan their meals around starchy foods and state that these foods should form the basis of most meals. The carbohydrates in starchy foods contribute significantly to the energy intake in the diet but can also be a source of other macronutrients and micronutrients that can contribute to human and dietary health. Potatoes are the most important non-cereal food crop with global production figure reaching 370 million tonnes (FAO 2020). These tubers are consumed worldwide and regarded as a staple food in many countries, including South Africa. The versatility of this commodity has led to an expansion in the demand for potato products which has inevitably led to an increased contribution to dietary diversity through the production and consumption of various potato products (King & Slavin 2013). In South Africa, potato consumption has increased by 26.8% associated with a 14.6% population growth showing that the total demand for potatoes increased by more than population growth, i.e., increased per capita consumption (BFAP 2019). This increase in consumption clearly shows what an important role potatoes play in the national diet. Due to some of the preparation methods, potatoes are often maligned in nutrition domains as being unhealthy and accused of contributing to the obesity epidemic. However, this is not a true reflection of the commodity, as a potato in its original form, cooked without the addition of fats and oils, can form part of a balanced diet (Bártová et al. 2015). Furthermore, it was recently reported that the biological value of potato protein is high (Camire et al. 2009).

Objective

The purpose of the study was to determine the protein content and the amino acid profile of four different potato cultivars commonly consumed in South Africa. The protein as derived from the nitrogen content of the samples and multiplied with the Jones factor was compared to the sum of total amino acids. In addition, the protein quality scores were calculated.

MATERIALS AND METHODS

Sampling

Duplicate nutrient analyses were done on composite samples of four potato cultivars obtained from various production regions across South Africa. Four cultivars that contribute to the largest market share (as shown in brackets) in South Africa were chosen for this study. Sampling was conducted from October – December 2018. Mondial (55%) and Sifra (23%) were sourced from three different production regions in South Africa, i.e., Free State, Limpopo and

Sandveld. The other cultivars, Valor (6%) and BP1 (2%), were sourced from Limpopo as this was the region that was harvesting at the time of the study. All of the potatoes were cultivated according to the common agricultural practices of each specific region under irrigation. Once harvested, 3 kg of tubers of each cultivar were packed in brown paper bags and transported to the laboratory. Upon arrival at the laboratory the tubers were stored in a cool dark room for six days to mimic market conditions. Prior to analyses, tubers were washed with water to remove all excess dirt and allowed to air-dry. Three whole tubers were randomly selected from each cultivar sample and analysed as a composite sample.

Determination of protein

Total nitrogen was determined using the Kjeldahl method. The nitrogen content was then used to calculate crude protein using the Jones conversion factor of 6.25. In 1981 a study to determine the nitrogen to protein conversion factor for potatoes was conducted using 34 different cultivars, which gave a factor of 6.24 which is similar to the empirical factor mostly used for foodstuffs. It was therefore decided that there is no reason to implement a potato specific conversion factor (Van Gelder 1981; Greenfield & Southgate 2003).

Determination of amino acids

The amino acid profile was determined by the ARC Irene Analytical Laboratory using high-performance liquid chromatography (HPLC) with florescence detection. The determination was carried out during three separate hydrolyses. The first hydrolysis analysed arginine, hydroxyproline, serine, aspartic acid, glutamic acid, threonine, glycine, alanine, tyrosine, proline, methionine, valine, phenylalanine, isoleucine, leucine, histidine and lysine. The ground freeze dried potato was weighed and hydrolysed with 6 N hydrochloric acid. An internal standard was added to the hydrolysate and filtered. A portion of the hydrolysate was dried under nitrogen-flow. The hydrolysate was derivatized with 9-fluorenylmethy chloroformate (FMOC-Cl) reagent and the amino acid content was determined by HPLC with an eluent of a tertiary gradient of pH, methanol and acetonitrile (Einarsson et al. 1983).

The second hydrolysis determined cysteine and followed an identical approach as described above with the exception that prior to hydrolysis, cysteine was oxidised to cystic acid with a peroxide formic acid solution (Gehrke et al. 1985). The third hydrolysis determined tryptophan. Ground freeze dried potato was hydrolysed enzymatically using protease. The hydrolysis was filtered through 0.45 µg filter and tryptophan was determined by means of HPLC equipped with an AMinoTAg column and florescence detection (De Vries et al. 1980).

Evaluation of the protein quality of foods for human consumption

In addition to the concentration of amino acids in foods, it is important to consider the digestibility of essential and non-essential amino acids in foods. Protein quality is typically measured using biological assays or chemical analysis as discussed below.

Analyses

Table 1 shows the summary of adult essential amino acid requirements which will be used for further comparison, discussion and calculation in this article. The values presented in Table 1 are the best currently available estimates for essential amino acid requirements (FAO/WHO/UNU 2007).

Table 1 Summary of the adult essential amino acid requirements (FAO/WHO/UNU 2007)

Amino Acid[a]	mg/kg body weight/day	mg/g maintenance protein
Histidine	10	15
Isoleucine	20	30
Leucine	39	59
Lysine	30	45
Sulphur Amino Acids	15	22
Methionine	10	16
Cysteine	4	6
Aromatic Amino Acids (Phenylalanine and Tyrosine)	25	38
Threonine	15	23
Tryptophan	4	6
Valine	26	39
Total essential amino acids	184	277

[a]Mean protein requirement of 0.66 g protein/kg per day

Chemical score of essential amino acids

Once the quantity of amino acids in the different cultivars was determined, the chemical score (CS) of the essential amino acids (CSEAA) was calculated in relation to the amino acid pattern of the reference requirements (Table 1) proposed by the FAO (FAO/WHO/UNU 2007; FAO 2013), using the following equation (Eq 1) as first described by Mitchell and Block (1946).

$$\text{CSEAA} = \left[\frac{(\text{gEAA in test protein})}{(\text{gEAA in reference protein})}\right] \quad \text{(Eq 1)}$$

Essential amino acid index

The essential amino acid index (EAAI) measures the presence of amino acids that the human body cannot synthesise and gives a stronger indication of potential nutritive value. The essential amino acids index (IEAA) was calculated using the following equation (Eq 2) described by various researchers (Oser 1959; Huang et al. 2018; Rolinec et al. 2018; Abdualrahman et al. 2019).

$$\mathbf{EAAI} = \mathbf{100} \times \sqrt[n]{\frac{a}{ap} \times \frac{b}{bp} \times \frac{c}{cp} \times \ldots\ldots\ldots \frac{i}{ip}} \quad \text{(Eq 2)}$$

where a, b, c, . . ., i = content of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine and valine in each sample; ap, bp, cp, . . ., ip = content of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine and valine in the reference protein (FAO, 2013); n = number of amino acids used (counting pairs such as methionine and cysteine, and phenylalanine and tryptophan, as one).

Protein digestibility corrected amino acid score (PDCAAS)

Protein digestibility-corrected amino acid score (PDCAAS) is a method of evaluating the quality of a protein based on both the amino acid requirements of humans and their ability to digest the protein. The protein digestibility can be determined analytically, or can be approximated by using existing tables on protein digestibility of foods. PDCAAS was calculated using the equation proposed by the FAO technical working group on protein quality and human requirements (Eq 4) (FAO/WHO 1991; FAO/WHO/UNU 2007). PDCAAS values > 1 were truncated to 1 (FAO 2013).

$$\text{PDCAAS} = \left[\frac{(\text{g of limiting amino acid in test protein})}{(\text{g of same amino acid in reference protein})}\right] \times \text{digestibility} \quad \text{(Eq 4)}$$

Digestible indispensable amino acid score (DIAAS)

The FAO developed the digestible indispensable amino acid score (DIAAS) in 2013 as the new recommended method to determine protein value (FAO 2013; FAO 2014). This method takes into account both the individual amino acid concentration and its digestibility at the end of the small intestine (Ileal), determined best with human models, and if not, with pig or rat models,

respectively (Rutherfurd et al. 2015). It is calculated by dividing the digestible dietary indispensable amino acid (mg) in one gram of the dietary protein by the same dietary indispensable amino acid (mg) in one gram of the reference protein (Eq 5) (FAO, 2013). For the calculation of DIAAS, the 2013 FAO Report recommends using human true ileal amino acid digestibility coefficients, or pig true ileal amino acid digestibility coefficients, or rat true ileal amino acid digestibility coefficients, in that order of preference (FAO, 2013). An ileal digestibility value of 90 was used for the calculation (Branco-Pardal, et al., 1995). DIAAS values were not truncated.

$$\text{DIAAS} = \left[\frac{\text{(mg of digestable dietary indispensable amino acid in 1 g test protein)}}{\text{(mg of the same dietary indispensable amino acid in 1 g reference protein)}} \right] \times 100 \qquad \text{(Eq 5)}$$

Statistics

Data received from the laboratory was arranged in tabular format and statistically analysed using the GenStat for Windows (2008) statistical computer programme (Payne et al. 2012). A one-way analysis of variance (ANOVA) test was applied with Fisher's protected *t*-test least significant difference at the 5% level of significance among cultivar means.

RESULTS AND DISCUSSION

This study measured and compared the amino acid profile of four different potato cultivars. These four cultivars form 86% of the South African potato market and are subsequently the most commonly consumed tubers. In Table 2 the protein and amino acid content of these four cultivars are reported.

As shown in Table 2, protein values using Nitrogen conversion did not differ significantly (P=0.055) between the various cultivars, ranging from the lowest value for Valor (1.65 g/100 g) to the highest value for BP1 (2.14 g/100 g). These values are similar to those found in the United States Department of Agriculture Food Composition Tables for the protein content of potatoes (2.03 g/100 g) (USDA 2019), as well as for traditional potatoes cultivated in the Canary Islands (1.94 g/100 g) (Galdon et al. 2010).

The protein content of foodstuffs is determined by using a nitrogen to protein conversion factor. In the case of potatoes, the standard Jones factor of 6.25 is used. Critical reviews of the accuracy of the Jones factor for protein determination have delivered varying results depending on the food analysed. Some researchers state that the Jones factor is merely nitrogen expressed using a different unit and does not provide accurate results for true protein content (Mariotti et al. 2008). A study of red meat found an underestimation of protein content when the Jones factor of 6.25 was used in the determination (Hall & Schönfeldt, 2013). In this study it was found that the Jones factor of 6.25 delivered accurate results when determining the protein content of potatoes. Variance between the calculated protein content and the sum of amino acids differed between 0% and 1% allowing the researchers to accept that the nitrogen to protein conversion factor of 6.25 can be used.

It is known that the nutritional, and more specifically the amino acid, contents of potatoes do vary and that the three greatest influencers, in order of effect are cultivar (34.3%), nitrogen fertilisation (17.9%) and the conditions that occur during the growth phase (2.1%), i.e., year and site interactions (Bártová et al.,2015; Grubben,et al. 2019). These findings were borne out when comparing the results from a previous study on the nutritional content of South African potatoes conducted in 2013 where tubers were planted under dry land conditions and had an overall lower protein content than tubers planted under irrigation as seen in the current study. Tubers from the 2013 study

Table 2 Amino acid content (mg) of four different potato cultivars

Analysis	P-value	Mondial	Sifra	Valor	BP1
Protein (N × 6.25) (g/100 g)	0.055	1.770	1.940	1.650	2.140
Sum of amino acids		1.750	1.930	1.640	2.140
Essential amino acids (EAA)		0.675	0.740	0.675	0.860
Arginine	≤0.05	0.135^{a}	0.135^{a}	0.105^{b}	0.12^{ab}
Histidine	0.603	0.055	0.050	0.040	0.040
Isoleucine	≤0.05	0.05^{c}	0.06^{b}	0.065^{b}	0.08^{a}
Leucine	≤0.05	0.075^{c}	0.095^{b}	0.09^{bc}	0.115^{a}
Lysine	0.052	0.115	0.120	0.110	0.145
Methionine	*	0.030	0.030	0.030	0.040
Phenylalanine	≤0.05	0.06^{b}	0.065^{b}	0.07^{b}	0.095^{a}
Threonine	≤0.05	0.055^{c}	0.07^{b}	0.06^{c}	0.08^{a}
Tryptophan	*	0.02	0.02	0.02	0.03
Valine	≤0.05	0.080^{b}	0.095^{b}	0.085^{b}	0.115^{a}
Non-essential amino acids (NEAA)		1.075	1.185	1.965	1.275
Alanine	≤0.05	0.055^{c}	0.08^{a}	0.06^{c}	0.07^{b}
Aspartic acid	≤0.05	0.345^{b}	0.315^{b}	0.355^{b}	0.475^{a}
Cysteine	0.258	0.025	0.025	0.020	0.040
Glutamic acid	≤0.05	0.42^{b}	0.495^{a}	0.27^{c}	0.375^{b}
Glycine	*	0.05	0.05	0.05	0.06
Proline	0.4	0.055	0.075	0.08	0.08
Serine	≤0.05	0.055^{c}	0.07^{b}	0.06^{c}	0.08^{a}
Tyrosine	≤0.05	0.06^{b}	0.065^{b}	0.06^{b}	0.085^{a}
Sulphur Amino Acids Methionine + Cysteine	0.08	0.055	0.055	0.050	0.080
Aromatic Amino Acids Phenylalanine +Tyrosine	≤0.05	0.12^{b}	0.13^{b}	0.13^{b}	0.18^{a}
EAA/NEEA Ratio		0.628	0.624	0.699	0.675
PDCAAS		1	1	1	1
DIAAS		101	99	108	114
Essential amino acid index (EAAI)		1.37	1.37	1.49	1.52
Essential amino acid index %		137	137	149	152

*Values for these amino acids did not show variance and therefore no statistical analysis was possible
#Means with different superscripts in a row differ significantly

found overall lower protein values: Mondial 1.45 g/100 g, Sifra 1.00 g/100 g, Valor 1.35 g/100 g and BP1 1.96 g/100 g (van Niekerk et al. 2016).

In the current study, of the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), five amino acid values differed significantly between the four cultivars (Table 2). The values for methionine and tryptophan were too similar for statistical analysis. Amino acid values for histidine and lysine did not differ significantly between the cultivars.

There are numerous methods to assess the nutritional value of proteins. Oser's

method (one of the earlier methods) was used to rate the quality of protein based on the contribution of all the essential amino acids (Rao et al. 1959). The essential amino acid index (EAAI) ranged from 1.367 for Mondial to 1.518 for BP1. All four cultivars had an EAAI value above 0.90 and can therefore be classified as a good quality protein according to Oser's method.

In Table 3 the chemical score of essential amino acids (CSEAA) with the lowest value indicates the first limiting amino acid. For potatoes leucine was found to be the first limiting amino acid as it had a score of less than 1 for all four cultivars. These results are in agreement with the results of a study conducted in Denmark (Jorgens & Lauridsen, 2004). Leucine is one of the three branched chain amino acids (BCAA) which play an essential role in protein synthesis and is critical in metabolic processes (Layman, 2003). Consuming plant foods, such as beans, soya, lentils or split peas, which are high in leucine, together with potatoes, can contribute to the intake of complete protein (Pretorius et al. 2019). Lysine is an essential amino acid and the contribution of lysine from plant sources broadens the intake of essential amino acids within a changing modern diet. From this study it became evident that potatoes are a plant based source of lysine (Table 3).

Table 3 Chemical score of essential amino acids (CSEAA)

Analysis	Mondial	Sifra	Valor	BP1
Histidine	2.10	1.73	1.63	1.25
Isoleucine	0.95	1.04	1.32	1.25
Leucine	0.73	0.84	0.93	0.91
Lysine	1.46	1.39	1.49	1.51
Sulphur Amino Acids Methionine and Cysteine	1.43	1.30	1.39	1.70
Aromatic Amino Acids Phenylalanine and Tyrosine	1.80	1.78	2.09	2.22
Threonine	1.37	1.58	1.59	1.63
Tryptophan	1.90	1.73	2.03	2.34
Valine	1.17	1.27	1.33	1.38

The protein digestibility– corrected amino acid score (PDCAAS), was adopted in 1993 as the preferred method for measurement of the protein quality in human nutrition. However, the truncation of PDCAAS to 1.0 means that important information on highly nutritious proteins is discarded. This truncation is done to 1 if the protein value exceeds 1 using the PDCAAS calculation (Millward, 2012; FAO, 2013; Joye, 2019). This is the case in the current study where all the PDCAAS values were truncated to 1 and therefore no conclusions could be drawn. A more recent protein quality score, as proposed by the FAO in 2013, is the digestible indispensable amino acid score (DIAAS), that compares the content of all digestible essential amino acids in a protein to the level of these digestible amino acids in a reference protein. The adoption of this new method to measure the quality and digestibility (bioavailability) of dietary proteins reflects the advances made in analytical methods in order to provide more accurate data (British Nutrition Foundation, 2013). The DIAAS values for the cultivars ranged from 99 for Sifra to 114 for BP1. In comparison, soya, which is commonly regarded as a high-quality protein plant-based food, has a DIAAS value of 90 (Pretorius et al. 2019).

In Table 4, the protein, sum of amino acids, PDCAAS, DIAAS, EAAI and ratio of essential to non-essential amino acids of potatoes is compared to high protein plant-based foods. The values for potatoes are the averaged values of the four cultivars analysed in this study. In this comparison potatoes are the food with the lowest protein content on a fresh-weight basis. The PDCAAS values for potatoes, beans, lentils and soy were truncated to one. Chickpeas and split peas had PDCAAS values of 0.96 and 0.92,

Table 4 Protein quality measures for potatoes and high protein plant-based foods

	Potatoes	Dry Haricot Beans*	Tinned Chick-peas*	Dry Lentils*	Dry Split peas*	Dry Soy*
Protein (N × 6.25) (g/100 g)	1.88	20.87	19.87	24.83	21.77	36.45
Sum of Amino Acids	1.87	19.93	18.69	22.56	21.06	35.78
PDCAAS	1	1	0.96	1	0.92	0.6
DIAAS	106	81	94	132	106	90
Essential amino acids index EAAI	1.44	1.98	1.66	1.65	1.66	1.53
EAA/NEAA ratio	0.66	0.84	0.61	0.62	0.66	0.64

*(Pretorius et al. 2019)

respectively. DIAAS values ranged between 81 for beans and 132 for lentils. Potatoes had a DIAAS value of 106 which is an average score compared to the other foods in Table 4. Potatoes had the lowest score for EAAI at 1.44 with beans scoring highest at 1.98. All the foods had an EEAI score above 0.90, which indicates that they are all classified as a good quality protein. Potatoes, chickpeas, lentils, split peas and soy had similar EAA/NEEA ratios, and beans had the highest score of 0.84.

Conclusions and recommendations

On average raw potatoes contained 1.6 – 2.1 g/100 g protein. In general crude protein content as calculated using the Jones factor of 6.25 corresponded well with the sum of amino acids. When using the essential amino acid index and DIAAS all four potato cultivars that were analysed can be classified as containing protein of good quality. Leucine was found to be the first limiting amino acid in potatoes, which is an amino acid that is commonly limited in plant-based products. If PDCAAS was used to describe the protein quality, all values were truncated to 1, which may mean that valuable information was lost due to truncation. DIAAS values ranged from 99 for Sifra to 114 for BP1. This is numerically higher than the DIAAS values for soya of 90.

Even though potatoes are not typically considered a source of protein, due to their low protein content, the unique amino acid composition allows potatoes to be regarded as a plant-based food that contains high quality protein. Investigations to identify cultivars that contain more protein are recommended. The development and cultivation of such cultivars should be studied.

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