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Assessing nutritional quality of milk-based sport supplements as determined by furosine

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Abstract

Milk proteins have a strong position in the sport nutrition markets, such as sport supplements for highly trained athletes, apart from bodybuilders. Furosine, a well-known index for the availability of lysine and subsequently of the extent of the Maillard reaction, was evaluated in different common ingredients used for formulation, as well in commercial sport supplements. Furosine content ranged from 2.8 to 1125.7 mg/100 g protein in commercial sport supplements being usually lower in samples containing mainly whey protein isolates or casein, as compared with whey protein concentrates. It is estimated that 0.1–36.7% of the lysine content is not available in this type of products. The use of high quality ingredients for the manufacture of sport supplements reveals important, since it could be the major source of protein intake of certain group of consumers in high or moderate training regime. Furosine is an appropriate indicator to estimate the nutritional quality of sport supplements. A reference value of 70 mg furosine/100 g protein content in dried sport supplements could be set up for controlling the quality of milk-based ingredients used in the formulation. Samples with higher levels are suspected of use of low quality milk-based ingredients or inappropriate storage conditions.

Keywords: Maillard reaction; Sport supplements; Protein quality; Furosine

1. Introduction

Sports supplements are usually included in the diet of highly trained athletes, as well as overall population in a moderate and high training regime, apart from bodybuilders in whom muscle mass is particularly high. The main determinants of an athlete's protein needs are their activity level and habitual protein intake; however, there is no consensus in the scientific literature, as to habitual resistance exercises increasing protein requirements (Tipton & Wolfe, 2004). Special sports foods, including some protein supplements and meal replacements, may be useful in some circumstances, but high doses of these must be avoided to prevent harmful organic effects (Maughan, King, & Lea, 2004).

The basic ingredients used in manufacturing the sport supplements are mainly caseinates and milk whey, and in a minor scale soy, wheat or egg proteins, as well as dextrinomaltose as the main carbohydrate source. But whey proteins have a strong position in the sport nutrition market based on the quality of proteins they provide. Whey proteins represent only 10% of the total solids of whey and on drying whey the resulting powders have low protein content (De Wit, 1998). Dried whey could be grouped according to the protein content and technologies applied for the production (Hoch, 1997). Then, whey protein concentrates (WPC) usually contain less than 25% of protein and whey protein isolates (WPI) usually contain more than 70% protein. Then, the addition of whey proteins from whey powders into food products has two goals: one nutritive (as dietary supplements) and the other technological (solubility, foam formation, gel formation, emulsion, water binding, viscosity, etc.). Concentrated whey powders that

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contain more than 70% proteins are used in a wide range of food applications (infant formula, health foods, and drinks) as nutritional and functional ingredients (Fox, 1989). The high nutritive value of the whey proteins is mainly due to their high content of essential amino acids as compared to the wheat, beef, soy bean or eggs (FAO, 1970).

Application of thermal treatments during the purification of different ingredients, or even in the mixture process for formulation to obtain the final product can involve the development of the Maillard reaction (MR). MR occurs between the free amino group of lysine and/or other amino acids and the carbonyl groups of reducing sugars such as glucose and maltose (Camire, Camire, & Krumbar, 1990) and is favoured in systems with intermediate moisture content, temperatures over 50 °C and pH 4–7. Lysine is commonly one of the most reactive amino acids in the early step of the MR (Ashoor & Zent, 1984). Then, loss of available lysine is the most significant consequence of the MR, and it is of great importance in those foods where lysine is the limiting amino acid (Erbersdobler, 1986). Evaluation of the early steps of the MR, as subsequently the protein quality, can be achieved by determination of furosine (ε-*N*-(furoylmethyl)-L-lysine) amino acid formed during acid hydrolysis of the Amadori compounds fructosyllysine and lactulosyllysine (Nursten, 1981).

There is a lack of information on the extent of the MR in sport supplements, and special attention should be paid since it could be the major protein source in a specific group of consumers and an appropriate balance of essential amino acids should be maintained. The aim of this study is to determine the impairment of lysine due to the MR, measured as furosine, in different commercial sport supplements, as well as some of the classical ingredients used in the formulation.

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless mentioned otherwise.

2.2. Samples

2.2.1. Ingredients

Nineteen different protein ingredients, present in most formulations for sport supplements, were directly obtained from several international companies for sport supplements. The ingredients were named with letter F followed by a number (Table 1).

2.2.2. Sport supplements

Thirteen commercial sport supplements (dried) with different nutrient compositions were also supplied by international companies. Samples cover most of the types of formulations marketed, and commercial variations (i.e., flavouring) for the same product/trade mark were not considered. The samples were designed with letter S followed by a number (Table 2).

Table 1
Protein and furosine content of representative ingredients used for sport supplements formulation

Protein source[a]	Code	Protein[b]	Furosine[c]
WPC	F1	15.5	285.2 ± 2.04
	F2	10.8	319.9 ± 2.76
	F3	16.4	862.3 ± 19.59
	F4	10.9	886.3 ± 20.01
	Mean	*13.4 ± 2.97*	*588.4 ± 306.20*
WP-hydrolysed	F5	69.3*	266.6 ± 30.48
WPI	F6	78.2	16.8 ± 1.32
	F7	84.5	27.2 ± 0.97
	F8	85.5	27.3 ± 0.61
	F9	90.5	32.4 ± 1.19
	F10	79.6	68.7 ± 1.98
	F11	87.1	142.1 ± 10.62
	F12	91.1	149.7 ± 6.77
	F13	91.7	154.9 ± 21.94
	F14	93.0	261.0 ± 1.61
	Mean	*86.8 ± 5.33*	*97.8 ± 81.54*
Casein	F15	86.5	12.2 ± 0.59
	F16	82.6	20.1 ± 11.89
	F17	78.9	54.8 ± 7.01
	Mean	*82.7 ± 3.80*	*29.1 ± 21.20*
Casein-hydrolysed	F18	86.6*	23.6 ± 0.85
Soy	F19	90.0	3.3 ± 0.19

[a] Protein source: WPC, whey protein concentrate; WPI, whey protein isolate.
[b] Data are expressed as g/100 g product, and as net protein*.
[c] Data are expressed as mg/100 g of protein, respectively (means ± SE).

2.3. Protein determination

The samples (0.800–1.000 g) were heated to 1050 °C following AOAC 992.15 (AOAC, 1995) in a LECO model FP-2000 (Leco Instruments, Madrid, Spain) protein/nitrogen analyser calibrated with ethylenediaminetetraacetic acid (Dumas method). The nitrogen-to-protein conversion factor considered was 6.38, and results were expressed as grams of protein/100 g of product.

2.4. HPLC determination of furosine

Furosine determination was performed following the methods described by Resmini and Pellegrino (1991) with some modifications. Briefly, 50 mg of the sample was hydrolysed with 8 ml of 7.95 M HCl at 110 °C for 23 h in a Pyrex screw-cap vial with PTFE-faced septa. Hydrolysis tubes were sealed under nitrogen. The hydrolysates were aerated and cooled at room temperature and subsequently centrifuged at 14,000*g* for 10 min. A 0.5-ml portion of the supernatant was applied to a Sep-pak C_{18} cartridge (Millipore) pre-wetted with 5 ml of methanol and 10 ml of deion-

Table 2
Furosine and protein content in representative commercial sport supplements

Code	Protein[a] source	Carbohydrate source	Protein[b]	Furosine[c]
S1	WPI	Not available	83.1	2.8 ± 0.13
S2	Caseinate WPI-Lactoalbumin	Not available	79.7	6.9 ± 0.42
S3	WPI hydrolysed egg albumin	Not available	83.2	7.5 ± 0.83
S4	WPI + Caseinate hydrolysed egg albumin	Not available	86.9	16.3 ± 1.48
S5	WPC/WPI hydrolysed-WPC	Dextrinomaltose	84.8	27.7 ± 0.54
S6	WPC + Caseinate	Dextrinomaltose fructo-oligosaccharides	61.5	31.1 ± 3.28
S7	WPI + Caseinate	Not available	89.7	41.0 ± 3.13
S8	Not available	Not available	81.3	58.0 ± 1.42
S9	WPC/WPI hydrolysed egg albumin	Dextrinomaltose	71.1	94.2 ± 3.27
S10	WPI	Fructo-oligosaccharides	74.8	107.3 ± 6.15
S11	WPC + Caseinate	Not available	88.2	203.5 ± 23.33
S12	WPC + Caseinate	Dextrinomaltose Glucose	56.9	221.7 ± 34.08
S13	WPC	Dextrinomaltose	19.7	1125.7 ± 31.21

[a] Protein source: WPC, whey protein concentrate; WPI, whey protein isolate.
[b] Data are expressed as g/100 g product.
[c] Data are expressed as mg/100 g of protein, respectively (means ± SE).

ized water and was then eluted with 3 ml of 3 M HCl. The sample was analysed according to Delgado, Corzo, Santa-María, Jimeno, and Olano (1992). The dried sample was dissolved in 1 ml of a mixture of water, acetonitrile and formic acid (95:5:0.2). A degassed mobile phase was prepared with 5 mM sodium heptane sulphonate, including 20% of acetonitrile and 0.2% of formic acid. An Excell-ODS analytical column (25 × 0.46 cm, 5-μm particle size, Tecknokroma, Barcelona, Spain) was used at 32 °C. The elution was isocratic and flow rate was 1.0 ml/min. The injection volume was 20 μl and detection was done at 280 nm. Furosine was quantified by the external standard method. Calibration curve was built from a stock solution (1.2 mg/ml of furosine) in the range 1.2–42.8 mg/l.

2.5. HPLC equipment

The HPLC system consisted of a MD-420 pump, a MD-465 autosampler, a MD-432 ultraviolet–visible detector and a DT-450/MT v.3.90 computing integrator connected to a PC, all from Kronton Instruments (Milan, Italy).

2.6. Statistical analysis

All of the analyses were performed at least in duplicate. The Statgraphics v. 5.1 software package (Statistical Graphics Corp., Rockville, MD, USA) was used for statistical analysis. Data were statistically tested by one-way analysis of variance (ANOVA), followed by Duncan's test to compare means that showed significant variations ($P < 0.05$).

3. Results and discussion

Typical chromatograms of a standard solution of furosine, WPC ingredient, and of a commercial sport supplement are shown in Fig. 1. Furosine eluted at 6.85 min

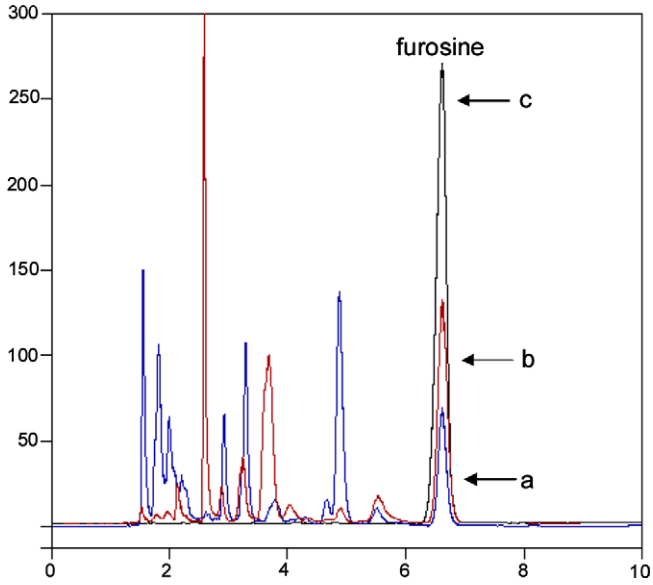

Fig. 1. Ion-pairing HPLC profile from sample F2 (a), sample S10 (b), and 8.13 mg/l furosine standard solution (c).

without interferences. Limits of quantification and detection were 0.3 mg/l (corresponding to 2.4 mg/100 g product) and 0.1 mg/l, respectively. The precision of the procedure (including acid hydrolysis) was 3.12% (relative standard deviation) for repeatability (same day) and 7.28% at reproducibility conditions (three independent preparations for three days). Recovery was performed in acid hydrolysates of a WPI ingredient in the range of 48.2–841.5 mg of furosine/100 g of protein, with an average value of 95.1%.

3.1. Furosine in ingredients used for sport supplements formulation

Two main protein sources were identified, caseinates and whey proteins (both WPC and WPI), whereas only few supplements contained other protein sources than milk

proteins such as hydrolysed egg albumin and hydrolysed wheat proteins. On the other hand, 19 different protein-based ingredients from different producers were assayed in order to assess their nutritional quality by means of their blocked lysine content, measured as furosine. As shown in Table 1, ingredients were classified into six groups: WPC, WPI, calcium caseinate, hydrolysed whey proteins, hydrolysed casein and soy proteins.

Furosine is expressed as a fraction of protein (mg of furosine/100 g protein), since protein content in the formulas is the limiting factor for its formation and assesses the effect of both formulation and processing on the nutritional quality of the product. Furosine ranged from 3.3 to 886.3 mg/100 g of protein. Lowest values were obtained in soy protein mainly due to the lower content of lysine while milk proteins, mainly whey proteins, are especially rich in lysine residues. Focussing on milk-derived proteins as ingredients, is noteworthy the low level obtained in caseinate (29.1 mg/100 g protein) and WPI (97.8 mg/100 g protein) as compared with WPC (588.4 mg/100 g protein). These differences were statistically significant when one-way ANOVA followed by Duncan's test was applied. Fig. 2 depicted the distribution of samples in a box and whistler plot where outliners were not observed. Furosine content among different types of ingredients can be explained by a combination of several factors; the technological process applied (a), net content of protein (b), type of protein and number of lysine residues per molecule (c), presence of reducing sugars in the formulation and/or ingredient (d), and previous thermal-treatment applied to milk used for protein isolation (e). Usually, caseinates are obtained by precipitation from milk at pH 4.6 and they suffer a very low heat treatment (Guo, Flynn, & Fox, 1999) and significant high amount of lactose is not expected to react during drying. On the contrary, whey proteins are obtained from the resulting milk whey obtained after casein precipitation and are directly dried by means of spray-drying or roller-drying (Hoch, 1997). In the case of WPI, a great part of sugars are removed and the proteins are concentrated previously to the drying step by applying ultrafiltration, microfiltration, dialysis or some combined strategies (De Wit, 1998). Then, MR is limited due to the lower reducing sugar content and the high ratio of protein enrichment. In addition, whey proteins have higher lysine content as compared with caseins, then giving rise to higher furosine values for similar technological process and reducing sugar content. WPC contains higher lactose proportions (6–8%) compared with WPI (<1%) (Holt, McPhail, Nevison, Nylander, & Otte, 1999). Caseinates and WPI have a mean protein content of 82.7% and 86.8% whereas WPC have only 13.4%. In the cases of caseinates and WPI, the differences could be attributed to the different heat treatment and lysine content. On the other hand, the differences obtained between WPI and WPC could come from the higher sugar content of WPC, which give rise to a higher development of MR. Finally, extent of the thermal-treatment applied to milk is an important source of Amadori compound formation. It is well described that furosine values are higher in classical sterilisation as compared to conventional pasteurisation.

Within the WPI group of ingredients, two subgroups can be distinguished according to the furosine content; those with values lower than about 100 mg/100 g protein and those with a higher content. The appearance of these two subgroups could be attributed to differences in their manufacture such as milk used for the obtention of whey, drying conditions, reducing sugar content or inadequate storage conditions, since their protein contents did not differ (Fig. 3). Similar results were obtained for WPC. Those with lower values are WPC spray-dried, whereas those with values close to 900 mg/100 g protein (samples F3 and F4) were dried by means of rolling, a more damaging process.

Since the final protein content is an indirect indication of the technological processes applied being higher protein

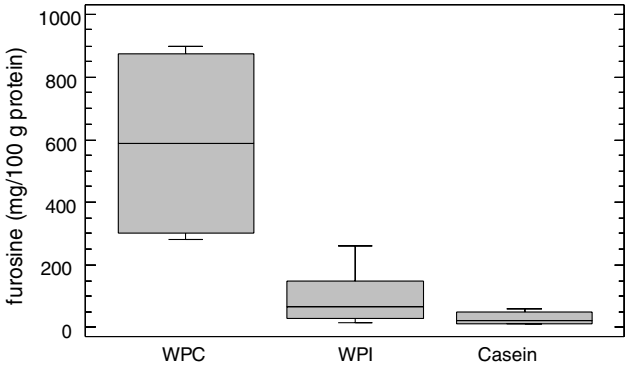

Fig. 2. Box and whistler plot of furosine content in ingredients used for sport supplement formulation. Median, first/third quartiles and standard deviation.

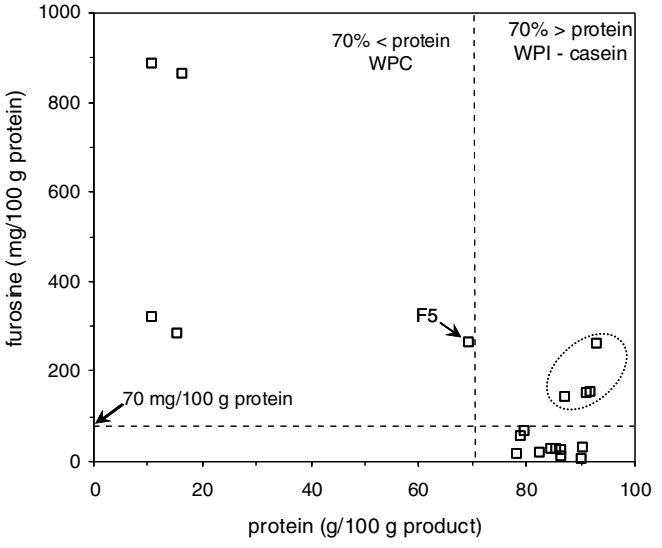

Fig. 3. Furosine content in classical ingredients for sport supplements formulas as function of protein content. Dotted lines denote estimated limit for furosine in WPI and caseinates (70 mg furosine/100 g protein) and protein content.

content for WPI and lower protein content for WPC, the relationship between furosine vs. protein content (Fig. 3) was studied. WPC and WPI ingredients are clearly classified according to the protein content and a maximum furosine level for WPI and caseinates (about 70 mg furosine/100 g protein) was set up from the median. WPI samples lying out of this area are suspected of inadequate storage conditions or a more drastic drying process was applied, as grouped in a dotted circle shown in Fig. 3.

The results obtained showed that caseinates have the lowest average furosine values (except soy proteins) and that the procedure did not significantly increase its level (sample F8). In this sense it could be thought that caseinates would be mostly selected to elaborate sport supplements, since they present lower levels of blocked lysine. However, because whey proteins are better digested and have higher lysine contents, they are preferred for nutritional purposes (Holt et al., 1999).

3.2. Furosine analysis in commercial sport supplements

The results for furosine analysis in commercial dried sport supplements are shown in Table 2. Values ranged from 2.8 to 1125.7 mg/100 g protein for samples S1 and S13, respectively, with a mean value of 149.5 and a median of 41.0. Five samples (S9, S10, S11, S12, and S13) showed a furosine content higher than 70 mg/100 g protein, indicating the use of highly damaged proteins for their manufacture, or inadequate storage conditions. Samples S11, S12, and S13 contain WPC as ingredient. As shown previously for the analysis of furosine in ingredients (Table 2), WPC reached furosine values higher than 300 mg/100 g protein. In this sense, although there are no information supplied by the manufacturers about the relative proportion of the different protein sources used for the sport supplements studied, it can be hypothesised that those supplements with a high concentration of WPC are responsible for the higher levels of furosine (such as samples S11, S12, and S13). In the case of sample S10, the high levels of furosine could indicate that the WPI used for its manufacture has similar heat damage to those WPI ingredients with high furosine levels (samples F11, F12, F13, and F14, Table 2). The rest of the samples showed low levels of furosine, which indicates the use of proteins with low heat damage. A non-significant relationship between protein content in the commercial sport supplements and furosine was found. Usually, commercial products with higher protein content imply lower furosine levels, meaning higher lysine availability and overall protein quality, except for sample S11.

MR may compromise the nutritional value of foods through the impairment and destruction of essential amino acids and by limiting the bioavailability of lysine and other essential nutrients (Finot, 1990). An average rate of lysine, as described by the manufacturers in the product label, of 8.5 g lysine/100 g of protein was obtained. The impairment of lysine residues might be estimated by the furosine content. Amadori compounds (lactulosyllysine, fructosyllysine), referred to as unavailable or blocked lysine, under acid hydrolysis conditions described above, convert to lysine (40%), pyridosine (24%), and furosine (36%). Then, blocked lysine might vary between 0.1% and 36.7% from sample S1 and S13, respectively. Assuming a daily intake of 50 g of the studied sport supplements and taking into account that only a 36% of furosine is generated from the acid hydrolysis of the corresponding Amadori product (Erbersdobler, 1986), the Amadori compounds intake from these supplements ranges widely from 3 to 310 mg/day. Amadori products have a limited absorption of only 1–3% (Erbersdobler & Faist, 2001) because the main part of them are metabolised by the gut flora. In this sense, furosine analysis results especially interesting to know the protein nutritional value related to blocked lysine. Therefore, it is a good indication for the manufacturing companies in order to select high quality ingredients as well as checking the real nutritional quality of sport supplements once the production has finished.

4. Conclusions

One of the most efficient parameters for evaluating the loss of lysine availability in foodstuffs is the measurement of furosine (Resmini & Pellegrino, 1991). The manufacture and consumption of supplements should be carefully evaluated, as the ingredient formulation and thermal treatment may impair the availability of amino acids. This would be of great importance since Maillard glycosylation will influence physiological processes in the human body by a reduced digestibility of proteins and excretion of peptide fragments in the faeces, excretion of non-hydrolysed peptides in the urine, and retarded release of amino acids from glycated proteins by the MR giving a more time spread supply of amino acids for anabolism. This finally results in lower values and reduced activity of several enzymes due to interference with non-digested peptides. Then, a reduction of the lysine availability also indicates a reduction in the efficiency of protein digestion by gastric enzymes due to the formation of isopeptides or protein crosslink.

This study illustrates the relationship among levels of furosine, as marker of lysine impairment in sport supplements, and compositional parameters such as the type of protein used in their manufacture. Since the specific processing conditions of each of the samples tested were not known, any conclusion about the influence of processing on the lysine impairment, measured as furosine, can be drawn. Secondly, the type of protein used in the formulation is critical for the furosine levels, being WPC the most damaged proteins followed by WPI and caseinates. Finally, this study evidenced that furosine could be applied for routine assessment of the protein quality by the administration or manufacturers in order to define regulatory basis or an internal quality control in sport supplements production. Special attention should be paid on samples with high protein rate and furosine content higher than 70 mg/100 g protein, which could be suspected by overprocessing,

inadequate storage practices, and subsequently a reduced nutritional quality of the protein.

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