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Development of food products using fish maw (*Pangasius hypophthalmus*) and roasted sunflower kernel (*Helianthus annuus*) for branched-chain organic acidurias patients

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ABSTRACT

Introduction: Branched-chain organic acidurias include maple syrup urine disease (MSUD), isovaleric acidemia (IVA), propionic acidemia (PA), and methylmalonic acidemia (MMA). Long term management requires diets of adequate energy and protein with restriction of the offending amino acids. Standard commercial formulas are expensive and unaffordable to patients of low socio-economic status. **Methods:** This study aimed to develop food products for branched-chain organic acidurias children aged 4-15 years using locally available raw materials in Thailand. Fish maw (*Pangasius hypophthalmus*) and roasted sunflower kernel (*Helianthus annuus*) were selected as protein sources due to their low leucine contents. Five formulations were developed, namely (i) powder (low leucine, isoleucine, and valine for MSUD) for tube feeding preparation, (ii) – (v) rice sprinkle powder, bouillon cube, instant cocoa drink, and snack bar, respectively with low leucine for IVA; low valine, isoleucine, methionine and threonine for PA and MMA. **Results:** All five formulated products provide 500-600 kcal/100 g, adequate protein in which the offending amino acids were controlled at non-harmful levels. These products were shelf stable at room temperature (Aw = 0.3-0.5). **Conclusion:** The products that were formulated from fish maw and roasted sunflower kernel provide proteins of appropriate quality and quantity for long-term management of branched-chain organic acidurias. The developed products should be further tested for efficacy among patients in accordance with an adequately powered study design.

Keywords: Branched-chain organic acidurias, *Pangasius hypophthalmus*, *Helianthus annuus*, leucine, valine

INTRODUCTION

Branched-chain organic acidurias are a group of rare inborn errors of metabolism, caused by abnormality of specific enzymes that are primarily involved in the degradation of branched-chain amino acids (leucine, isoleucine and valine) and their derivative compounds. As a result

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of degradation, an accumulation of the aforementioned amino acids and/or associated organic acids occurs in blood, cerebrospinal fluid (CSF) and urine (de Baulny, Vici & Wendel, 2012). Rapid severe accumulation of these organic acids and branched-chain amino acids, specifically leucine, can lead to cerebral oedema and early death, if left untreated. The chronically elevated blood levels of these compounds adversely affect brain microstructure and functions, resulting in psychomotor retardation (Dionisi-Vici *et al.*, 2002; Strauss *et al.*, 2010) in patients with maple syrup urine disease (MSUD), isovaleric acidemia (IVA), propionic acidemia (PA), and methylmalonic acidemia (MMA) (Knerr *et al.*, 2012; de Baulny *et al.*, 2012). Patients of these rare diseases are found worldwide. The prevalence per 100,000 has been reported to be 3.69 in British Colombia, Canada; 4.67 in Italy; 12.56 in West Midlands, United Kingdom; and 135.14 in Saudi Arabia due to the high consanguinity rate among Arab populations (Rashed *et al.*, 1994; Applegarth, Toone & Lowry, 2000; Dionisi-Vici *et al.*, 2002; Sanderson *et al.*, 2006). The prevalence of these diseases in Thailand is 50-60 cases for the whole country, which is quite low as compared to many countries.

There are two modalities for the treatment of branched-chain organic acidurias patients in relation to the phase of the diseases, namely (i) acute-phase treatment and (ii) long-term management. The acute-phase treatment aims to quickly remove the toxic metabolites by dialysis or hemofiltration in order to prevent death or severe permanent brain damage (Morton *et al.*, 2002; Saudubray *et al.*, 2002; Zand *et al.*, 2008). For the long-term management, diets of low protein and adequate/high energy in combination with limiting the offending amino acids are recommended to support normal growth and neurodevelopment (Yannicelli, 2006; Knerr *et al.*, 2012). These diets are commercially available in powder form, which are categorised as medical products. The commercial products are freed of the offending amino acids, namely (i) leucine, isoleucine and valine in the case of MSUD, (ii) leucine in IVA, and (iii) valine, isoleucine, methionine and threonine in MMA and PA. These products are also enriched with vitamins and minerals. Since the number of patients worldwide is quite low, the production and availability of commercial formulas are costly, unaffordable and unavailable to low-income patients.

In Thailand, these commercial products are usually available through international donations, and patients occasionally encounter shortage problems, that may result in poor control of the disorder symptoms. Therefore, there is a need to develop products for these patients by using locally available raw materials to provide a sustainable supply. Based on available database on amino acid profiles, certain kinds of locally available foods can potentially be used as raw materials for preparing food products that are low in branched-chain amino acids. The purpose of this study was to formulate food products for treating young branched-chain organic acidurias patients. Different product forms would be developed using sustainable sources of raw materials in Thailand.

MATERIALS AND METHODS

This study formulated products to be taken by patients as supplements to their usual diet of three meals a day. Patients should be able to acquire adequate energy and protein to meet the requirements of their age group. In this study, the target age was 4-15 years. Energy requirements for patients were calculated by using the Holliday-Segar equation and nutritional recommendation for severe IVA, MSUD, MMA, and PA (Wappner & Gibson, 2006; Barshop, 2006). The energy requirements of patients were expected to be met through (i) their normal diets, and (ii) the formulated food products (de Baulny *et al.*, 2012).

Total protein intake for patients aged 4-8 and 8-15 years were designed as 1.5-2.0 g/kg/day and 1.0-1.2 g/kg/day, respectively and also following individual baseline protein intake prior to entering the study (Wappner & Gibson, 2006). For MSUD and IVA, leucine was selected as the primary offending amino acid for calculation of amino acid allowance for daily intake. Total leucine intakes allowed were 500-750 mg/day for MSUD and 650-1500 mg/day for IVA. For MMA and PA patients, the intakes of valine, isoleucine, threonine and methionine were controlled at 700-1600 mg/day, 600-1300 mg/day, 500-1200 mg/day, and 250-800 mg/day, respectively (Wappner & Gibson, 2006; Barshop, 2006) and valine was chosen for calculation of the offending amino acid allowance for daily intake.

Selection of raw materials

Based on the Thai Foods database on amino acid profiles (MOPH Thailand, 2001), several food items were identified based on the ratio of leucine to protein content, and the amount of protein in the edible portion. Food items that contained reasonable protein content with minimum ratio of leucine to protein were considered ideal. Besides, they should be produced under systematic farming, which is able to maintain quality and minimise natural variations. Based on these criteria, two food items were selected, namely (i) fried fish maw and (ii) roasted sunflower kernel. Fish maw was obtained from a farmed fish called "Pla Swai" (*Pangasius hypophthalmus*), while sunflower kernel (*Helianthus annuus*) was harvested from a sunflower farm, followed by sorting and roasting. Amino acid profiles of both materials were determined, while information on their nutrient profiles was obtained from the suppliers. For food safety reasons, both materials were sent for analysis of heavy metal contamination, while information on aflatoxin contamination was obtained from the suppliers.

Additional carbohydrate and fat contents were added to improve energy density of the formulated products. Sucrose and maltodextrin were used as carbohydrate sources, while palm olein oil and non-dairy creamer provided additional fat.

Production

A total of five formulations were developed from fish maw and sunflower kernel. Formula I, low in leucine and prepared for MSUD patients, was in powder form, which could be orally consumed as a rice sprinkle. For MUSD patients, it was necessary to prepare formula I in the form of a tube-feeding diet by blending the product with selected food ingredients, such as cooked rice, soybean oil, fruits and vegetables. Formulas II - V, low in leucine and valine and prepared for IVA, PA and MMA patients, were presented in four different product forms, namely (i) rice sprinkle powder, (ii) bouillon cube, (iii) instant cocoa drink and (iv) snack bar.

Dried fish maw obtained from the farm was well mixed following which it was ground to approximately 2 mm size in an electric blender and then pasteurised in a hot air oven at 80°C for 30 min. The ground fish maw (5 kg per batch) was portioned into 1,000 g packs and vacuum-sealed in plastic laminated aluminum foil bags and stored at -20°C.

Sunflower kernel was mixed, portioned and similarly packed as for fish maw, but without the grinding step. The sunflower kernel was stored at -20°C before use.

The ground fish maw and sunflower kernel were mixed with other food ingredients selected as sources of energy, as well as salt for flavouring, glucose syrup, baking chocolate and cocoa powder were used for preparing the five different food products as mentioned above. All the Formulas, except for Formula V, were similarly prepared by weighing and blending all the ingredients in an electric blender before packing. Formula V was prepared by blending the ingredients and mixing them with melted baking chocolate bar before spreading on a tray. The solidified bar was then cut into chewable sizes (2 x 2 cm) before packing. The packing size of these food products was based on the nutrient requirements per meal of each patient (based on individual weight) and providing for 2 meals a day. All the final products were packed under vacuum in a plastic-laminated aluminium foil bag. The products were analysed for macronutrients, amino acid profile, microbiological quality and water activity.

Quality analysis

Macronutrients

Moisture content was determined by drying in a hot air oven at 105°C for 3 h until a constant weight was obtained (AOAC International, 2012a). Protein content was determined by Kjeldahl method, in which food was digested with sulfuric acid in the presence of catalysts. Crude protein content of the food was determined by multiplying the nitrogen content with a factor of 6.25 (AOAC International, 2005; Chang, 2010). Fat content was determined using Soxhlet apparatus. Fat was extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask, and the extracted fat was dried to a constant weight and expressed as percent fat by weight (AOAC International, 2012b; Min, 2010). Ash content was determined by dry-ashing in which the sample was incinerated in a muffle furnace at $\geq$ 525°C overnight. The sample was then cooled down in a desiccator prior to weighing (AOAC International, 2012c; Marshall, 2010).

Amino acid profile

Food sample was hydrolysed by 0.1 M hydrochloric acid before being analysed for amino acid profiles using high performance liquid chromatography (HPLC). The chromatographic system was Hewlett Packard 1090 Series II/M AminoQuant™ liquid chromatograph. Separation of amino acids was performed on a narrow bore C18 HP AminoAcid Analysis (200 X 2.1 mm) column, protected by a 15 X 2.1 mm guard column to which had been injected 8 ml of the derivatives. The separated amino acids were detected in a fluorescence detector at 340/450 nm for primary amino acids and 237/340 nm for secondary amino acids (Herbert *et al.*, 2000).

Water activity

Water activity was determined by using water activity meter (Novasina sensor™, Switzerland).

Microbial quality

Aerobic plate count, most probable number (MPN) E. coli, MPN coliforms, MPN fecal coliforms, yeast and mold, and V*ibrio parahaemolyticus* were analysed based on Bacteriological Analytical Manual (USFDA, 2001; USFDA, 2002; USFDA, 2004).

RESULTS

Quality of raw materials

Based on the database on amino acid profiles and protein contents of Thai foods (MOPH Thailand, 2001), the ratios of leucine to protein content were lowest in dried fish maw and sunflower seed (Table 1). The fish maw was commercially available in fried form, while the sunflower seed was in roasted form. Both of these raw materials had the lowest amounts of leucine in their amino acid profiles as compared to those found in the other foods. Besides, they also contained reasonable contents of protein, which was required for the formulation.

In order to address the safety concern of the raw materials, the most suitable reference was the EC regulation No. 333/2007 on lead, cadmium, mercury, and inorganic tin in foods. The heavy metal contamination levels in both raw materials did not exceed the EC regulation (data not shown). No aflatoxin contamination was found in the roasted sunflower seed product (data not shown).

Formulated food products

In all formulas, fish maw and roasted sunflower kernel were used as the protein sources, but at different amounts, based on the need for leucine limitation. Formula I was developed for the MSUD patient since it needed to be a tube-feeding diet. Formulas II - V were developed for using as an instant drink (IV), snack (V) and ingredients for main dishes (II and III).

Sucrose was added to provide energy and sweet taste for most formulas except for Formula III, which did not require sweetness due to the nature of the formulation. Palm olein oil in Formula III (bouillon cube) functioned as binder and energy source. Baking chocolate was the main flavouring and structuring compound for Formula V as well as energy source. Other minor ingredients i.e. salt, monosodium glutamate (98% MSG + 1% Inosinate and 1% Guanylate), mushroom flavoured seasoning, glucose syrup, and cocoa powder were mixed into the food products to provide better flavouring (Table 2).

All the formulas provided 500-600 kcal/100 g. Energy sources were mainly from carbohydrate and fat, which were similar to the commercial products, BCAD 2® for MSUD and LMD® for IVA (Table 2). The macronutrient profiles of the developed products however were different from the commercial products, especially for the protein content. Since foods ingredients were used in the formulations, the amount of total protein was limited in order to control the blood level of undesirable branched chain amino acids at a non-harmful level (Wappner & Gibson, 2006; Barshop, 2006). Overall, Formula I had the lowest level of protein and fat but higher in carbohydrate content than Formulas II-

Table 1. Ratio of leucine to protein content based on 100 g edible portion of various protein sources

Protein source	*Per 100 g edible portion*		*Leucine:Protein ratio*
	Protein (g)	*Leucine (mg)*	
Sesame seed, black	20.3	1309	64.5
Mungbean, dried	24.4	1878	77.0
Soybean, dried	34.6	2549	73.7
Chicken, breast	25.6	1538	60.1
Pork, lean meat	20.4	1584	77.6
Pork, liver	20.4	1560	76.5
Hen egg, whole	13.1	860	65.6
Fish maw, fried	38.3	1443	37.7
Sunflower seed†, roasted	24.7	1244	50.4
Milk powder, non-fat	36.9	2927	79.3

†When sunflower seed is dehulled, the edible remainder is called the sunflower kernel
Source: The database on amino acid profiles and protein contents of Thai foods (MOPH Thailand, 2001)

Table 2. Contents of nutrients and branched-chain amino acids of the food formulations developed for patients of branched-chain organic acidurias, as compared to commercial products

Formula†	*Ingredients (g/100 g)*	*Nutrient composition per 100 g (% energy distribution)*			*Energy (kcal/100 g)*	*Branched-chain amino acid (mg/100 g)*		
		Protein	*Fat*	*Carbohydrate*		*Isoleucine*	*Leucine*	*Valine*
I	Fish maw (5), roasted sunflower kernel (30), non-dairy creamer (20), maltodextrin (30), sucrose (15), salt (1)	9.9 (7.8)	26.2 (46.1)	59.3 (46.2)	512	285	470	461
‡BCAD 2®	Corn syrup solids, amino acids, sugar, soy oil, modified corn starch (no percentages mentioned)	24.0 (23.4)	8.5 (18.7)	57.0 (55.6)	410	0	0	0
II	Fish maw (10), roasted sunflower kernel (25), non-dairy creamer (40), maltodextrin (12), sucrose (13), salt (1)	11.8 (8.6)	33.6 (55.4)	49.1 (36.0)	546	294	526	452
III	Fish maw (10), roasted sunflower kernel (25), non-dairy creamer (15), maltodextrin (30), palm oil (20), salt (1), MSG (1), mushroom flavwored seasoning (2)	12.3 (8.2)	45.0 (67.4)	36.7 (24.4)	601	268	489	516
IV	Fish maw (10), roasted sunflower kernel (25), non-dairy creamer (40), sucrose (25), salt (1), cocoa powder (1)	11.8 (8.6)	33.6 (55.4)	49.1 (36.0)	546	302	538	464
V	Fish maw (6.7), roasted sunflower kernel (16.8), non-dairy creamer (26.8), sucrose (16.8), baking chocolate (33), salt (1)	8.2 (6.2)	36.7 (58.3)	51.0 (36.0)	567	212	398	328
‡LMD®	Corn syrup solids, vegetable oil, amino acids, modified corn starch, sugar (no percentages mentioned)	16.2 (13.0)	26.0 (46.8)	51.0 (40.8)	580	580	0	630

†Formula I: Mixed powder for preparing tube-feeding diet; Formula II: Rice sprinkle powder; Formula III: Bouillon cube; Formula IV: Instant cocoa drink; Formula V: Snack bar
‡BCAD 2 and LMD: Commercial products for MSUD and IVA patients, respectively

Table 3. Water activity and microbiological quality of the food products developed for patients of branched-chain organic acidurias

Analysis	Formula†				
	I	*II*	*III*	*IV*	*V*
Water Activity (Aw)	0.3	0.4	0.4	0.4	0.5
Aerobic Plate Count, cfu/g	Not detected	Not detected	Not detected	Not detected	Not detected
MPN *E. coli* /g	< 3	< 3	< 3	< 3	< 3
MPN Coliforms /g	< 3	< 3	< 3	< 3	< 3
MPN Fecal coliforms /g	< 3	< 3	< 3	< 3	< 3
Yeast and mold, cfu/g	Not detected	Not detected	Not detected	Not detected	Not detected
Vibrio parahaemolyticus, cfu/25 g	Not detected	Not detected	Not detected	Not detected	Not detected

†Formula I: Mixed powder for preparing tube-feeding diet; Formula II: Rice sprinkle powder; Formula III: Bouillon cube; Formula IV: Instant cocoa drink; Formula V: Snack bar

V. Formula III (bouillon cube) contained slightly more energy and total fat than the others due to the added fat.

Amino acid profiles

Since branched-chain amino acids were the critical nutrients for the branched-chain organic acidurias patients, it was important to determine the amino acid profiles of the developed food products. The commercial products, LMD® and BCAD 2® did not contain the offending amino acids since they were formulated from pure amino acids. Table 2 shows that the developed formulas (Formulas I–V) contained the offending amino acids but at controlled levels that were not harmful for the IVA and MSUD patients. For example, an IVA patient of 36.5 kg weight who consumed 1,016 kcal from Formula II, III, IV or V plus 490 kcal from his/her regular low protein meal per day would obtain 1,433 mg of leucine, which was still under the maximum limit for the IVA patient of 1,500 mg/day.

Microbial quality and water activity

For safety reasons, the developed products were analysed for the microbiological quality based on the nature of the products. Table 3 indicates that no growth of the tested bacteria was detected, including mesophilic aerobic bacteria (aerobic plate count), pathogenic/sanitation indicator bacteria (MPN *E. coli*, MPN coliforms, MPN fecal coliforms), yeast and mold, and *Vibrio parahaemolyticus*. Water activities of the developed products were 0.3-0.5 which were not conducive for microbial growth.

DISCUSSION

After reviewing the locally available food items in Thailand, it was found that fish maw and sunflower kernel contained reasonable amounts of protein with limited content of branched chain amino acid. The selected fish maw was derived from farmed pangasius fish (*Pangasius hypophthalmus*), in which the variety and feeding environment were controlled. Sunflower kernel was also obtained from systematic cultivation of *Helianthus annus* farm. Under such conditions, both raw materials were more stable in their nutrient profiles. Obtaining the raw materials from farmed sources ensures continued availability of the study materials.

Both raw materials were preserved in dried/roasted and dried/fried forms, which made them available all year round. These raw materials were used as the main sources of amino acids and at the same time they also provided energy from fat that is naturally found in sunflower kernel and from the cooking oil used in frying of fish maw. For safety reasons, fish maw was tested for contaminant heavy metals while sunflower kernels were tested for pesticide and aflatoxin. In the product development process, the products were treated as low water activity (Aw<0.85), which did not allow any growth of pathogens. However, the microbiological qualities of all products were also tested since the products were used with no further heating. By using the developed processes, the products passed the relevant microbial standards for the products (FAO/WHO, 1993).

For long term use, all forms of products should be made available as choices to the patients. As compared to the commercial products, our products still contained certain amounts of branch-chained amino acids, however the amount of leucine per day was 1,000–1,471 mg/day, which was at a level that is below the harmful level for patients (more than 1,500 mg/day).

Total cost of the locally available raw materials that were used for preparing these products ranged from US$ 0.42-0.51 per 100 g, while the costs of the commercial products i.e. BACD2 and LMD were up to US$ 15.6 and US$ 12.3 per 100 g, respectively.

CONCLUSION

It is feasible to formulate and produce food products that used locally available raw materials, namely fried fish maw and roasted sunflower kernel as alternatives to commercial products for branched-chain organic acidurias patients. Leucine in the developed products could be controlled to be less than 1,500 mg/day. The products were shelf-stable and could be easily controlled for safety. Several forms of products could be produced as choices for patients and parents. The products should be beneficial for patients in developing countries where the commercial disease-specific amino acid formulas are unaffordable and often unavailable.

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Authors' contributions

CS, conducted the study and wrote the manuscript; CV, designed the study, advised on food science and nutrition issues and wrote manuscript; WD, designed the study and advised on the medical issues; SU, designed the study and advised on medial issues; CN, advised on medical issues.

Conflict of interest

The authors declared no conflict of interest.

References

AOAC International (2005). Crude protein in cereal grains and oilseeds content. *Official Methods of Analysis of AOAC International* 992.23 (18th ed.). Gaithersburg, MD, USA.

AOAC International (2012a). Loss on drying (moisture) in sugars. *Official Methods of Analysis of AOAC International* 925.45 (19th ed.). Gaithersburg, MD, USA.

AOAC International (2012b). Fat in milk. *Official Methods of Analysis of AOAC International* 989.05 (19th ed.). Gaithersburg, MD, USA.

AOAC International (2012c). Ash of sugar and syrups. *Official Methods of Analysis of AOAC International* 938.08 (19th ed.). Gaithersburg, MD, USA.

Applegarth DA, Toone JR & Lowry RB (2000). Incidence of inborn errors of metabolism in British Columbia, 1969-1996. *Pediatrics* 105(1):e10.

Barshop BA (2006). Disorders of valine-isoleucine metabolism. In N Blau, GF Hoffmann, J Leonard & J Clarke (eds). *Physician's guide to the treatment and follow-up of metabolic diseases* (pp. 81-92). Springer, Germany.

Chang SK (2010). Protein analysis. In SS Nielsen (ed). *Food analysis. Fourth edition* (pp. 133-146). Springer, New York.

de Baulny HO , Vici CD & Wendel U (2012). Branched-chain organic acidurias/acidaemias. In JM Saudubray, GVD Berghe & JH Walter (eds). *Inborn metabolic disease* (pp. 278-296). Springer-Verlag, Berlin.

Dionisi-Vici C, Rizzo C, Burlina AB, Caruso U, Sabetta G, Uziel G & Abeni D (2002). Inborn errors of metabolism in the Italian pediatric population: a national retrospective survey. *J Pediatr* 140(3):321-327.

FAO/WHO (1993). *Codex Alimentarius vol.4. Foods for special dietary uses (including foods for infants and children)* (pp. 53-64). Joint FAO/WHO Food Standards Programme of the Codex Alimentarius Commission. Food and Agriculture Organization of the United Nations and World Hhealth Organization, Rome.

Herbert P, Barros P, Ratola N & Alves A (2000). HPLC determination of amino acids in musts and port wine using OPA/FMOC derivatives. *J Food Sci* 65(7):1130-1133.

Knerr I, Weinhold N, Vockley J & Gibson KM (2012). Advances and challenges in the treatment of branched-chain amino/keto acid metabolic defects. *J Inherit Metab Dis* 35:29–40.

Marshall MR (2010). Ash analysis. In SS Nielsen (ed). *Food analysis. Fourth edition* (pp. 105-115). Springer, New York.

Min DB (2010). Fat analysis. In SS Nielsen (ed). *Food analysis. Fourth edition* (pp. 117-132). Springer, New York.

MOPH Thailand (2001). Amino acid content of Thai foods. Ministry of Public Health of Thailand. From http://nutrition.anamai.moph.go.th/temp/files/amino acid of Thai foods.pdf. [Retrieved August 10 2013].

Morton DH, Strauss KA, Robinson DL, Puffenberger EG & Kelley RI (2002). Diagnosis and treatment of maple syrup disease: a study of 36 patients. *Pediatrics* 6:999-1008.

Rashed M, Ozand PT, Aqeel A & Gascon GG (1994). Experience of King Faisal Specialist Hospital and Research Center with Saudi organic acids disorders. *Brain* Dev 16(16 Suppl):1-6.

Sanderson S, Green A, Preece MA & Burton H (2006). The incidence of inherited metabolic disorders in the West Midlands, UK. *Arch Dis Child* 91(11):896-899.

Saudubray JM, Nassogne MC, Lonlay PD & Touati G (2002). Clinical approach to inherited metabolic disorders in neonates: an overview. *Semin Neonatol* 7:3–15.

Strauss KA, Wardley B, Robinson D, Hendrickson C, Rider NL, Puffenberger EG, Shelmer D, Moser AB & Morton DH (2010). Classical maple syrup urine disease and brain development: principles of management and formula design. *Molecular Genetics and Metabolism* 99: 333–345.

USFDA (2001). *In: Bacteriological Analytical Manual (BAM): Aerobic Plate Count. U.S. Food and Drug Administration.* From https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm063346.htm. [Retrieve December 15 2013].

USFDA (2002). *In: Bacteriological Analytical Manual (BAM): Enumeration of Escherichia coli and the Coliform Bacteria.* U.S. Food and Drug Administration. From https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm064948.htm. [Retrieved December 15 2013].

USFDA (2004). *In: Bacteriological Analytical Manual (BAM): Vibrio. U.S. Food and Drug Administration.* From https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm070830.htm. [Retrieved December 15 2013].

Wappner RS & Gibson KM (2006). Disorders of leucine metabolism. In N Blau, GF Hoffmann, J Leonard, J Clarke (eds). *Physician's guide to the treatment and follow-up of metabolic diseases* (pp. 59-79). Springer, Germany.

Yannicelli S (2006). Nutrition therapy of organic acidaemias with amino acid-based formulas: emphasis on methylmalonic and propionic acidaemia. *J Inherit Metab Dis* 29:281-287.

Zand DJ, Brown KM, Lichter-Konecki U, Campbell JK, Salehi V & Chamberlain JM (2008). Effectiveness of a clinical pathway for the emergency treatment of patients with inborn errors of metabolism. *Pediatrics* 122:1191-1195.