Food Sci. Biotechnol. Vol. 17, No. 2, pp. 241 ~ 246 (2008)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Formulation and Shelf Life of a Nutritional Supplement for Undernourished Elderly People

Fabiane La Flor Ziegler, José de Assis Faria, and Valdemiro Carlos Sgarbieri*

State University of Campinas, Faculty of Food Engineering, Campinas, SP, Brazil

Abstract A nutritional supplement was developed aiming at correcting the most common nutrient and caloric deficiencies encountered in elderly people (≥60 years old). The protein source was a mixture of whey protein isolates (WPI) and bovine collagen hydrolysate (BCH) with high nutritional and functional qualities making up 12% of the formulation. The carbohydrate fraction was composed of sucrose, inulin (soluble fiber), and fructo-oligosaccharide (prebiotic). The most commonly deficient essential minerals and vitamins were also included. Acceptance of the product was good according to both an elderly panel and a laboratory panel composing of both sexes and various ages. The stability of the formulations was evaluated and the estimated shelf life at room temperature (ca. 27°C) was approximately 4 months.

Keywords: elderly, undernutrition, nutritional supplement, shelf life

Introduction

The progressive aging of the world population is a fact. In 1950 the world population of age ≥60 years was 8.2%; in 2000 this age group reached 10% and the projection for 2050 is that over 20% of the world population will reach advanced age. In Brazil this aged group is growing at about the same rate (1,2). The rapid increase of the aged population is a preoccupation of the governments of all nations, which attempt to bring down the costs of public health care, concomitant with improving the quality of life of elderly people (2). Due to several intrinsic and external factors, aged people become susceptible to chronic and disabling diseases as well as undernutrition (3).

The main intrinsic factors for this are physiological and metabolic alterations, modifications in the digestive tract and sensorial perceptions, changes in body composition, and alterations in the immune system (4-7). The most common external (environmental) factors contributing to disease and undernutrition in the elderly are poverty, social isolation, limited capacity of movement, and decline in cognitive capacity (4,8). The main nutritional deficiencies that have been reported for the elderly population include energy (5), protein (5), fiber (9), minerals, and vitamins (5,10). Accelerated shelf life tests can be conducted under abused conditions of high temperature and relative humidity and reduced storage time (11). Such option permits speed up storage time and estimate the shelf life based on mathematical models (12).

The objective of this investigation was to develop a nutritional supplement for undernourished elderly people, capable of covering the most critical deficiencies of this age group. Ideally the product should present a good acceptability by the elderly and stand storage at room temperature for at least 6 months in flexible package materials.

*Corresponding author: Tel: +55-19-35214059, Fax: +55-19-35214060
E-mail: sgarb@fea.unicamp.br
Received March 21, 2007; accepted August 29, 2007

Materials and Methods

The guidelines for the choice of ingredients (nutrients) for the formulation of the supplements were some data from recent literature (5,9) and a preliminary survey (not published) which was done at the Geriatric Sector of the Faculty of Medicine, Campinas State University, Campinas-SP, Brazil.

Materials Proteins were a whey protein isolate (WPI) – ALACEN 895, acquired from NZMP New Zealand Milk Products (São Paulo, Brazil), and a bovine collagen hydrolysate (BCH) furnished by Gelita South America, Cotia (São Paulo, Brazil); maltodextrin 1910 from Corn Products (São Paulo, Brazil); sucrose of the brand 'União®', acquired in a local supermarket, (Campinas, São Paulo, Brazil); inulin (Raftiline® GR) and fructo-oligosaccharide (Raftilose® P95) were a gift from Orafti – ActiVe Food Ingrediends (São Paulo, Brazil). The minerals magnesium oxide RA 150, zinc oxide FA, iron (Ferrochel® Amino acids chelate), sodium selenite, chromium chelavite®, magnesium chelate (20%) were supplied by M. Cassab Ltd. (São Paulo, Brazil), and calcium was a gift from Puracal PP FCC (São Paulo, Brazil). The vitamins: vitamin A acetate (325 CWS/F), dl-alpha-tocoferol, ascorbic acid, pyridoxine hydrochloride (vitamin B_6), vitamin B_{12} 0.1% WS and folic acid – BP were a gift from Roche – DSM Nutritional Products Ltd. (São Paulo, Brazil). Thiamin hydrochloride (vitamin B_1) SYNTH, was purchased from Labcenter Ltd. (Campinas, São Paulo, Brazil). Flavoring agents from various natures and procedures were also tried. The packaging material used was bioriented polypropylene (BOPP) acquired in the local market (Campinas, São Paulo, Brazil) with a thickness of 0.05 mm containing 50 g of the developed formulations which was stored at environment temperature.

Methods/procedures Many ingredient combinations were tested before reaching the final formulation, which was supported from sensorial tests with 30 elderly panel

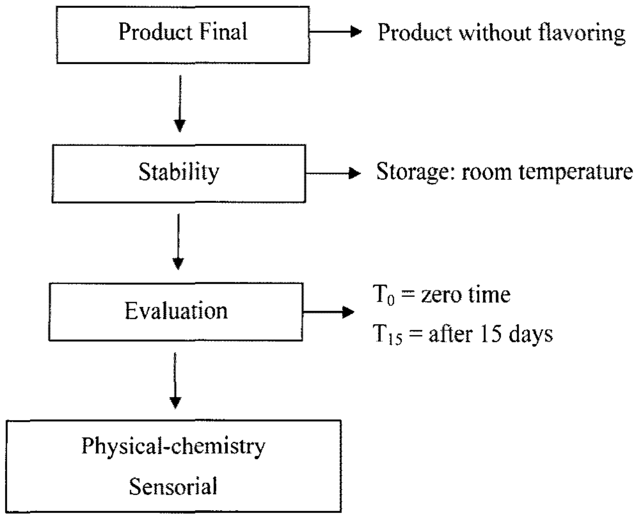

Fig. 1. Experimental design for the shelf life study (stability) for the 2 products developed.

members (≥60 years) to reach the highest acceptance among 6 different flavors of the formulations. The quantities supplemented were based on Dietary Reference Intakes (DRIs) for the age range under study and according to nutritional deficiencies encountered in this age group (13-17), respecting the maximum tolerance level for each nutrient.

Sensorial evaluation for selecting the products The following attributes were evaluated by 30 elderly consumers (70% women and 30% men) before decision of the final composition was reached.
Acceptance test were performed by using a structured hedonic scale of 9-points, in which, 9=liked very much; 1=disliked very much (18,19).
Ideal sweetness was evaluated by utilizing a 9-point scale based on the following extreme values (–4=extremely less sweet than the ideal; 4=extremely more sweet than the ideal).
Intention of buying: A 5-points scale was used in which 1=certainly not buy; 2=probably would buy; 3=have doubt about buying or not; 4=probably would buy; 5= certainly would buy.

Shelf life The experimental design for the shelf life study of the 2 chosen formulas by the elderly panel is illustrated in Fig. 1.

Chemical analysis Humidity, ash, and protein were determined by the AOAC procedures (20). Total lipids were determined by the Bligh and Dyer method (21).

Minerals and vitamins The minerals, calcium, magnesium, zinc, and selenium were analyzed by the methods of Slavin *et al.* (22) and Olson *et al.* (23). The vitamins were analyzed by the following methods: the AOAC procedures (24) for vitamin B_1 and B_6, the method of Lima *et al.* (25) for folic acid, the method of Manz and Philipp (26) for vitamin A, the method of Brubacher *et al.* (27) for vitamin E and the method of Arakawa *et al.* (28) for vitamin C.

Moisture content and water activity (Aw) determination The AOAC procedure (20) was used to determine the moisture content. Determinations were done at zero time (T_0) and after 30 days (T_{30}) of storage at room temperature (ca.27°C) and at 35±1°C, in the sample without flavoring and the one added chocolate flavor. For water activity measurement a portable pa_w kit (model 950 NE; Decagon Devices Inc., Aqualab, Pullman, WA, USA) was used. Determinations were done in duplicate at T_0 and T_{30}. For calibration of the equipment saturated $MgCl_2$ and lithium solutions (Aw 0.1-0.4) and K_2SO_4 (Aw 0.975) were used.

Determination of adsorption isotherms The adsorption isotherms were determined by measuring the Aw with a portable Decagon pa_w kit (model 950 NE) and the moisture gain of the products, according to Teixeira Neto and Jardim (29).

Package permeability The water vapor permeability ratio (g water/m^2 · day) was determined by a methodology similar to that of the American Society for Testing and Materials (ASTM) described in terms of 2 parameters: a diffusion constant (D) and solubility coefficient (S) according to Fujita (30) and Crank (31).

Instrumental color determination Changes in color were determined in the system CIElab with the colorimeter Color Eye 2020 with the software COMCOR 1500 Plus (Macbeth, New Windsor, NY, USA). Readings were done directly from capsules containing the sample, under the conditions: observation at 10°, illuminant D65 and configuration DREOL, obtaining the values L* (luminosity), +a* (red), –a* (green), and +b* (yellow), according to Ferreira (32).

Sensory evaluation The samples without flavoring and chocolate flavor were the formulations with highest acceptance by sensorial evaluation done by elderly. Acceptance tests with these formulations were performed at different times (days) of the shelf life study (T_0, T_{15}, and T_{30}) with 30 adult panel members. The samples (powder) stored at room temperature (ca.27°C) and at 35°C were dispersed in milk, served in plastic cups of 50 mL in a temperature controlled room and analyzed in relation to appearance, aroma, taste, and global impression; by using a structured hedonic scale of 9-points (9=liked very much; 1=disliked very much). Buying intention was also evaluated (19) by the scale already described.

Statistical analysis All the results were analyzed by analysis of variance and differences among means by the Tukey's test (33). The software used was 'Statistica-Basic Statistics and Tables' (StatSoft, Tulsa, OK, USA) and the SAS statistical program.

Results and Discussion

Centesimal composition The centesimal composition of the 2 formulas preferred by the panel in this study is shown in Table 1. The main component of the formula is the carbohydrate, composed of sucrose, maltodextrin, and inulin reaching 79.8% of the composition. The second major component is protein (ca.13%), a mixture of 60% WPI and 40% bovine collagen hydrolysate.

Nutritional composition The basic nutritional composition of the 2 products is shown in Table 2, which also shows the formula contribution of the various nutrients, as a percentage of the Daily Recommended Intakes. It is recommended the dose to be dispersed in 200 mL of liquid which could be water, fruit juice, or milk. The panel preference was for milk as the diluting fluid and chocolate or instant coffee as flavoring.

Moisture content and water activity The contents of water and water activities of the formulations with and without chocolate at the beginning (T_0) and after 30 days of storage at room temperature (ca.27°C) and at 35°C, are shown in Table 3.

The data of Table 3 indicate a significant increase in humidity by comparing the values at T_0 with T_{30} (room temperature) whereas when the comparisons are made between the storage at room temperature and at 35°C, both samples lost humidity at the end of 30 days of storage. The moisture content fluctuations in the samples reflected the corresponding variations in the Aw values.

Table 1. Centesimal composition of the product without flavoring and with chocolate flavor

Component (%)	Products	
	No flavor added	Chocolate flavor
Protein %N×F[1)]	12.7 ± 0.3^{a}	12.8 ± 0.02^{a}
Total lipids	0.00	0.00
Ashes	2.4 ± 0.01^{b}	2.5 ± 0.02^{a}
Moisture	5.1 ± 0.03^{a}	4.9 ± 0.03^{b}
Carbohydrate[2)]	79.8	79.8

[1)]F=6.05 (mixture of 60% WPI: 40% BCH).
[2)]Estimated by difference [100−(protein+total lipids+ash+moisture)]. Results are means of 3 determinations±SEM. Different superscript letters (lines) indicate statistical differences (Tukey's test, $p<0.05$).

Table 2. Nutritional composition of the products (g/dose of 200 mL and % of the DRIs)[1)]

Nutrients quantity	g/dose (200 mL)	% DRIs/dose
Protein (g)	7.5	14
Carbohydrate (g)	40.7	~30
Prebiotic		
Inulin (g)	4	-
FOS (g)	2	-
Vitamins		
B_1 (mg)	0.2	17
B_6 (mg)	0.7	43
B_{12} (μg)	1.2	50
C (mg)	60.0	73
A (μg)	450.0	56
E (mg)	15.0	100
Folic acid (μg)	133.3	33
Minerals		
Ca (mg)	400.0	33
Mg (mg)	140.0	38
Zn (mg)	5.5	58
Se (μg)	18.3	33

[1)]Source: DRI (1997); DRI (1998); DRI (2000); DRI (2002).

Adsorption isotherms The equilibrium moisture content (on dry basis) was determined for each value of water activity for both formulations.

The experimental values of equilibrium moisture (% d.b.) were plotted against water activity. The adsorption isotherms for the formulations without flavoring and with chocolate flavor are represented in Fig. 2.

According to Brunauer *et al.* (34) the curves are similar to isotherms of type II. A sigmoid configuration is typical of isotherms type II which is commonly found for the majority of foods (35). By the use of desiccators and saturated saline solutions, the critical points of moisture and Aw limits were determined for both products at 26.8±1°C. Agglomeration/stoning, change in color, and microorganisms development were visually inspected.

Beginning of agglomeration occurred at Aw of 0.65 for both formulations, which did not occurred at Aw of 0.43. Slight color alteration was observed for the chocolate formula at 0.43 Aw and this change was accentuated for both products above 0.65 Aw.

Color instrumental analysis The luminosity (L*) and the chromaticity coordinates (a* and b*) remained practically constant during the whole period of storage, for the formulation with no flavoring. Similarly, the formula with chocolate flavor did not change significantly the value L*,

Table 3. Humidity and water activity at T_0 and after 30 days (T_{30}) under room temperature and 35°C

Storage conditions and time	Formulations	
	No flavor added	Chocolate flavored
Humidity[1)]		
T_0 (initial)	5.09 ± 0.03^{Ba}	4.86 ± 0.03^{Bb}
T_{30} (room temp.)	5.78 ± 0.11^{Aa}	5.71 ± 0.40^{Aa}
T_{30} (35°C)	4.73 ± 0.05^{Cb}	4.87 ± 0.03^{Ba}
Water activity[2)]		
T_0 (initial)	0.28	0.29
T_{30} (room temp.)	0.42	0.39
T_{30} (35°C)	0.28	0.30

[1)]Means of 3 determinations±SEM. Different capital letters (columns) or small letters (lines) indicate statistical differences (Tukey's test, $p<0.05$).
[2)]Means of only 2 determinations.

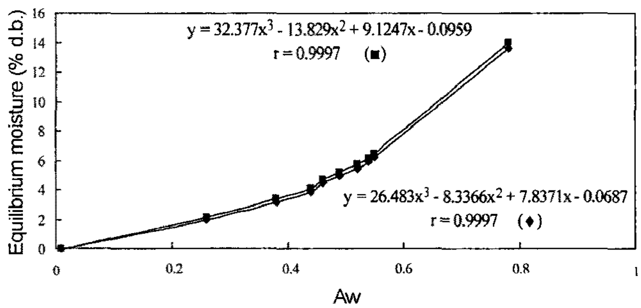

Fig. 2. Adsorption isotherms for the formulations without flavor (◆) and with chocolate flavor (■) at room temperature (ca.27°C).

Table 4. Values of L* (luminosity), a*, and b* (chromaticity coordinates), and ΔE (difference with a standard measured at T_0) for the formulations without flavor (A) and with chocolate flavor (B) at room temperature (ca.27°C)[1)]

Aw	L*		a*		b*		ΔE	
	A	B	A	B	A	B	A	B
0.22	85.5	65.1	-0.5	4.5	5.7	8.7	14.10	14.54
0.33	85.5	64.2	-0.5	5.1	11.1	9.5	14.42	15.35
0.43	87.3	64.3	-0.7	4.4	7.0	8.7	11.86	15.39
0.65	84.2	59.1	0.2	5.4	10.1	10.1	15.80	20.67
0.75	79.4	51.9	0.4	5.7	13.0	10.5	21.78	27.95
0.90	50.8	21.9	6.1	5.3	20.6	5.7	52.87	54.41
1.00	49.0	20.8	7.1	4.1	20.4	3.3	54.64	55.41

[1)]Means of 2 determinations.

Table 5. Average acceptance for aroma, taste, appearance, and global impression of the sensory evaluation at T_0, T_{15}, and T_{30} for the formulations without flavor and with chocolate flavor[1)]

Attributes	Average acceptance without flavor			Average acceptance chocolate flavor		
	T_0	T_{15}	T_{30}	T_0	T_{15}	T_{30}
Aroma	5.76^{Bb}	5.71^{Cb}	6.24^{Ab}	6.88^{Aa}	6.71^{Ca}	6.79^{Ba}
Taste	5.79^{Aa}	5.41^{Cb}	5.71^{Bb}	6.27^{Ca}	6.50^{Ba}	6.76^{Aa}
Appearance	6.79^{Ba}	6.59^{Ca}	6.94^{Aa}	6.70^{Ba}	6.82^{Aa}	6.82^{Aa}
Global Impression	6.03^{Ba}	5.97^{Cb}	6.12^{Ab}	6.45^{Ca}	6.79^{Aa}	6.76^{Ba}

[1)]Different capital letters (lines) compare the sample without flavor or chocolate flavor as a function of the time (Tukey's test, $p<0.05$). Different small letters (lines) compare each attribute between the 2 samples (without flavor or chocolate flavor) at the same time (Tukey's test, $p<0.05$).

a*, and b* during the 30 days of storage under both room temperature and 35°C. A small decrease in the red color (a*) and in the yellow (b*) was observed during the storage period. However the values ΔE (difference with a standard measured at T_0) exhibited a greater change in the chocolate formula, compared with the one with no flavoring added.

A very significant change in color (ΔE) occurred in both samples at Aw above 0.75, as shown in Table 4.

Sensory evaluation The results of the sensory evaluation of the non flavored and chocolate flavored samples for aroma, taste, appearance, and global impression at T_0, T_{15}, and T_{30} did not show significant change. The averages acceptance values in the 9-points scale are shown in Table 5 for the product without flavoring and chocolate flavored. The acceptance tests of the 2 products selected by the elderly panel showed a preference for the chocolate flavor (score 6.60) followed by the product without added flavor. The preferred material to use as diluent was the non-fat milk as compared to water or fruit juice. The acceptance of the products remained unchanged for the whole period of storage.

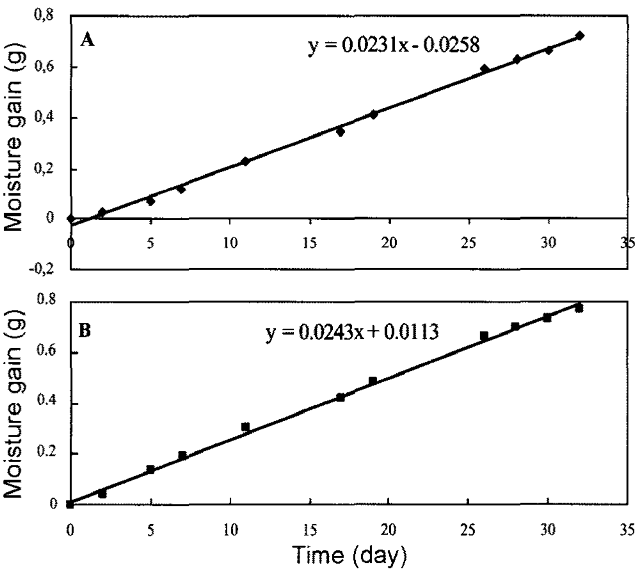

Fig. 3. Values of the weight gain (g) vs. time (day) for the formulations without flavor (A) and with chocolate flavor (B) evaluated during 30 days for the shelf life estimate.

Package permeability The permeability of the BOPP film was estimated by the moisture change obtained through the average angular coefficient of the straight line (Fig. 3 A, B), dividing by the exposed area of the package. The calculated value was 1.0128 g $H_2O/m^2 \cdot day \cdot 58\% RU \cdot 27°C$.

For flexible plastic packaging material the moisture change will depend on the type of material used, regarding to type of resin, thickness, temperature, and relative humidity of the storage environment (36).

Shelf life estimation The shelf life can be estimated by the use of mathematical model, as described by Azanha and Faria (12). However, in the present work the weight gain of the packed product in a period of 30 days was determined. The plot of moisture gain (g) vs. time (day) are shown in Fig. 3 (A, B) representing the linear regression equation for the product without flavoring and with chocolate flavor added, respectively.

Based on the study of the isotherms of the products, the values of initial and critical moisture were determined for the 2 products, which were 2.1 and 2.2%(w/w) for the non-flavored and chocolate flavored product, respectively. It was observed that the critical moisture (5.1%, w/w) was the same for both products. The moisture variation (U) was estimated by subtracting the initial moisture (Ui) from the critical moisture (Uc). The U value estimated for the product without flavoring was 3 g/100 g sample and 2.9 g/100 g for the chocolate flavored sample. Using these values and the linear regression equations for A ($y=0.02331x-0.0258$) and B ($y=0.0243x+0.0113$), where y= variation of U, and x=time (day), the shelf life for both products was estimated. Shelf life at room temperature was 131 days (ca.4.4 month) for the product without flavoring and 119 days (ca.4 month) for the chocolate flavored product.

It is concluded that a more protective packaging material should be used in order to increase shelf life of the products.

Vitamin analysis Determination of vitamins B_1, C, A, and E was performed in both products at T_0, T_{15}, and T_{30}. A substantial loss of these vitamins was registered in both products, under the 2 conditions of storage. Considering the total period (T_0-T_{30}) the vitamin losses in the non flavored product ranged from 0 to 24.7% at 35°C and from 26 to 56% at room temperature (ca.27°C) for the chocolate flavored product, from 8 to 63% at room temperature and from 7 to 45% at 35±1°C.

Curiously, in both products the vitamin losses were higher at room temperature (ca.27°C) than at 35°C, probably due to the environment temperature fluctuations (21-29°C).

Considering the substantial losses of vitamins in a relatively short period of time, it is recommended a packaging material more appropriate in terms of barrier to light, oxygen, and water vapor (37).

References

1. Heredia OC. Demographic characteristics of third age in Latin América and Brazil. Interdisc. Stud. Aging 2: 7-21 (1999)
2. Pessini L. Aging and health: Impacts of the II World Assembly on the Aging. World Health 26: 457-463 (2002)
3. Aranha FQ, Barros ZF, Moura LSA, Gonçalves MCR, Barros JC, Metri JC, Souza MS. The role of vitamin C on the organic alteration in the elderly. Braz. J. Nutr. 13: 89-97 (2000)
4. Campos MTFS, Monteiro JBR, Ornelas APRC. Factors affecting the food consumption and the nutrition of the elderly. Braz. J. Nutr. 13:157-165 (2000)
5. Pallàs MC. Importance of nutrition in the elderly people. Novartis Consumer Health S.A., 2002. Available: http://www.novartismedicalnutrition.com/es. Accessed June 28, 2004.
6. Nicolas AS, Andrieu S, Nourhashemi F, Rolland Y, Vellas B. Successful aging and nutrition. Nutr. Rev. 59: S88-S92 (2001)
7. Chandra RK. Nutrition and the immune system: An introduction. Am. J. Clin. Nutr. 66: 460S-463S (1997)
8. Nogués R. Factors affecting the nutrients intake in the elderly which condition its correct nutrition. Clin. Nutr. 15: 39-44 (1995)
9. Robinson GE, Leif BJ. Nutrition Management & Restorative Dining for Older Adults – Practical Interventions for Caregivers. 1st ed. Vol. 14. American Dietetic Assoc., Chicago, IL, USA (2001)
10. Elsborg L, Nielsen JA, Bertram U. The intake of vitamins and minerals by the elderly at home. Int. J. Vitam. Nutr. Res. 53: 321-329 (1983)
11. Speigel A, Brown WE. Shelf life testing. p. 96. In: Flexible Packaging Technology. The Center Professional Advancement, Brunswick, NJ, USA (1990)
12. Azanha AB, Faria JAF. Use of mathematical models for estimating the shelf life of corn-flakes in flexible packaging. Packag. Technol. Sci. 18: 171-178 (2005)
13. Dietary Reference Intakes; DRI for calcium, phosphorous, magnesium, vitamin D, and fluoride. The National Academies Press, 1997. Available: http://www.nap.edu/. Accessed May 20, 2005.
14. Dietary Reference Intakes; DRI for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline. The National Academies Press, 1998. Available:_http://www.nap.edu/. Accessed May 20, 2005.
15. Dietary Reference Intakes; DRI for vitamin C, vitamin E, selenium, and carotenoids. The National Academies Press, 2000. Available: http://www.nap.edu/. Accessed May 20, 2005.
16. Dietary Reference Intakes; DRI for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. The National Academies Press, 2001. Available: http://www.nap.edu/. Accessed May 20, 2005.
17. Dietary Reference Intakes; DRI for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. The National Academies Press, 2002. Available: http://www.nap.edu/. Accessed May 20, 2005.
18. Vickers Z, Holton E, Wang J. Effect of ideal-relative sweetness on yogurt consumption. Food Qual. Prefer. 12: 521-526 (2001)
19. Meilgaard M, Civille GV. Sensory Evaluation Techniques. Florida CRC Press, Inc., Boca Raton, FL, USA. p. 281 (1999)
20. AOAC. Official Methods of Analysis. 15th ed. Horwitz W (ed). Association of Official Analytical Chemists, Washington DC, USA. p. 1141 (1990)
21. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phys. 37: 911-917 (1959)
22. Slavin S, Petersen GE, Lindhal PC. Determination of heavy metals in meats by atomic absorption spectroscopy. Atom. Abs. Newslett. 14: 57-59 (1975)
23. Olson OE, Palmer IS, Cary E. Modification of the official fluorimetric method for selenium in plants. J. Assoc. Off. Anal. Chem. 58: 117-121 (1975)
24. AOAC. Official Methods of Analysis. 17th ed. Horwitz W (ed). Vol. 2, Method 961-15. Association of Official Analytical Chemists, Washington DC, USA (2000)
25. Lima JA, Catharino RR, Godoy HT. HPLC methodology for folic acid determination in enriched wheat flour and bread. Tech. Molitoria Intern. 55: 151-158 (2004)
26. Manz U, Philipp K. Determination of vitamin A in complete feeds and premixes and vitamin concentrates with HPLC. pp. 5-7 In: Analytical Methods for Vitamins and Carotenoids in Food. Roche, Roche, Switzerland (1988)
27. Brubacher G, Müller-Mulot W, Southgate DAT. Methods for the Determination of Vitamins in Food – Recommended by COST 91. Elsevier, New York, NY, USA. pp. 97-106 (1985)
28. Arakawa N, Otsuka M, Kurata T, Inagki C. Separative determination of ascorbic acid and erythorbic acid by high-performance liquid chromatography. J. Nutr. Sci. Vitaminol. 27: 1-7 (1981)
29. Teixeira Neto RO, Jardim DCP. Transformation reactions in foods. pp. 1-18. In: Transformation Reactions and Shelf Life in Processed Foods – Technical Manual. Campinas, São Paulo, Brazil (1996)
30. Fujita J. Diffusion in polymer-diluent's systems. Fortsch. Hochpolym. Forsch. 3: 1-47 (1961)
31. Crank J. The Mathematics of Diffusion. 2nd ed. Clarendon Press, Oxford, UK. pp. 1-10 (1975)
32. Ferreira VLP. Food Colorimetry. Food Technology Institute, Campinas, São Paulo, Brazil. p. 43 (1991)
33. Gomes FP. Experimental Statistical Course. 10th ed. Nobel, São Paulo, Brazil. p. 430 (1982)
34. Brunauer S, Emmett PH, Teller E. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60: 309-312 (1938)
35. Bell LN, Labuza TP. Moisture sorption isotherms. p. 20. In: Moisture Sorption Practical Aspects of Isotherm Measurement and Use. 2nd ed. AACC Eagan Press, St. Paul, MN, USA (1996)
36. Padula M. Packaging influence on shelf life of foods. pp. 4-17. In:

Transformation Reactions and Shelf Life in Processed Foods – Tecnical Manual. Campinas, São Paulo, Brazil (1996)
37. Alves RMV. Package specification by the use of sorption isotherm using mathematical model. pp. 7-20. In: Course on Water Activity in Foods. Food Technology Institute, Campinas, São Paulo, Brazil (1997)