Microbiological profile and nutritional quality of raw foods for neutropenic patients under hospital care

Paula Cristina Galati[1]
Renata Cristina Lataro[1]
Vanessa Maciel Souza[2]
Elaine Cristina Pereira de Martinis[2]
Paula Garcia Chiarello[1]

[1]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo - USP, Ribeirão Preto, SP, Brazil
[2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo - USP, Ribeirão Preto, SP, Brazil

***Objective**: This study aimed to analyze and compare the microbiological profile and vitamin C content of raw and cooked foods destined for neutropenic inpatients.*

***Methods**: Three vegetables and nine fruits, raw and boiled, washed and sanitized were examined. Heat-tolerant coliforms and coagulase-positive staphylococci were counted and the presence of Salmonella spp was investigated. The vitamin C content was analyzed by a colorimetric reaction. The Statistical Package for Social Sciences (SPSS) software was used for statistical analysis and the nonparametric Wilcoxon test was used to compare the mean vitamin C values of the cooked and raw foods. The Spearman correlation test was applied to determine the associations between the parameters evaluated*

***Results**: Salmonella spp was absent in all samples and the populations of coagulase-positive staphylococci and heat-tolerant coliforms were below the minimum detectable limits of the methods employed (< 100 colony forming units (CFU)/g and < 3 most probable number (MPN)/g, respectively). There was a significant loss of vitamin C in the cooked foods, 38.9% on average, compared to the raw foods, a loss that was positively correlated with cooking time.*

***Conclusion**: The fresh fruits and vegetables properly sanitized in this study had a microbiological profile consistent with that required by Brazilian law. Furthermore, the nutritional value of the neutropenic diet is diminished, at least in terms of the vitamin C content.*

***Keywords**: Neutropenia; Diet; Food microbiology; Nutritive value; Immunosuppression*

Introduction

One of the major side effects of intensive chemotherapy is the increased risk of infections caused by bone marrow damage or severe marrow suppression that results in neutropenia, that is, reductions in the white blood cell count[1].

Infections in patients submitted to chemotherapy may lead to increased morbidity and mortality due to impairment of their immunological systems[2]. Thus, most oncology centers apply preventive measures to minimize exposure to infectious agents[3]. Among these measures is the use of neutropenic diets (diets containing no raw foods) with the aim of significantly reducing the number of microorganisms ingested by eating certain foods[4].

In a literature review, Wilson[5] was unable to identify a direct relationship between this type of diet and the prevention of infections despite the frequent use of neutropenic diets. In view of the lack of scientific evidence of their efficacy, institutions such as the Food and Drug Administration (FDA) and the Center for Disease Control and Prevention (CDC) in the USA do not recommend neutropenic diets for immunocompromised patients[6].

In addition to the lack of scientific evidence for the use of neutropenic diets in respect to infections, their use may interfere in the treatment and nutritional status of cancer patients as the fresh food restricted in this diet is often an alternative for individuals with nausea, altered palate, mucositis, and food aversions[7]. Furthermore, fresh foods are a source of antioxidant nutrients that may minimize the toxic effects of antineoplastic medications and contribute to a better response to cancer treatment[8]. An inadequate diet may result in a poor recovery of leukocyte and neutrophil counts, delaying the patient recovery process[9].

Hence, it is necessary to assess the microbiological safety of raw foods sanitized in accordance with technical norms, establishing the parameters and criteria for the control of food hygiene and health[10], as the use of a diet with the smallest possible number of restrictions may result in improved calorie intake and quality of life and consequently in a better nutritional status, contributing to an improved response to oncologic treatment.

The objective of the present study was to analyze and compare the microbiological quality of washed and sanitized raw foods to cooked foods and to compare the vitamin C content of foods destined to patients on the Hematology Ward in the University Hospital of the Universidade de São Paulo (USP) in Ribeirão Preto.

Methods

Thirty-four samples were evaluated in the microbiological analysis. These included

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 8/1/2012
Accepted: 10/15/2012

Corresponding author:
Paula Cristina Galati
Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo - USP
Av. Bandeirantes, 3900
14040-903 Ribeirão Preto, SP, Brazil
paulacgalatti@yahoo.com.br

www.rbhh.org or www.scielo.br/rbhh

DOI: 10.5581/1516-8484.20130028

three types of vegetables (peeled carrots, peeled beets and tomatoes) and nine types of fruits (peeled pineapple, peeled banana, persimmon, lime, apple, pear, peeled watermelon, peeled papaya, and peeled melon) in their raw, cooked, washed and sanitized forms, served in the meals of a University Hospital, between May and June 2010. The same foods, peeled, raw and cooked, were used for an analysis of vitamin C, except for the watermelon and persimmon, which were only submitted to microbiological analysis.

The choice of vegetables was to take into account the food preferences of patients admitted to the hospital as cooked fruit is not commonly eaten and thus is less accepted. Moreover, vegetables that have a lower risk of contamination and greater nutrition value were analyzed.

The fruits and vegetables for analysis *in natura* were washed without friction in running drinking water. Sanitation consisted of 30 minutes of immersion in a solution of 20 mL 1% sodium hypochlorite diluted in 1 liter of tap water (200-250 ppm), followed by rinsing in drinking water as recommended by Ordinance CVS-6/99[10]. After washing and sanitation, the foods were peeled and cut using a clean knife disinfected in a 1% sodium hypochlorite solution (200-250 ppm).

The fruits and vegetables to be processed were cooked in 500 mL of boiling water (100ºC) for 10 to 40 minutes depending on the consistency desired for the final product. Because of this, the food reached different temperatures at the geometric center, but all reached at least 74ºC, as recommended by the Brazilian Health Surveillance Agency (ANVISA)[10] for fruits and vegetables that do not require cleaning. The temperature of cooked food was monitored used a calibrated thermometer, whose stem was washed and disinfected in 70% alcohol before and after each time it was used. Subsequently, the samples were refrigerated (10ºC) until the time of distribution inside the hospital; the samples for the current study were collected at the time of delivery to the patients.

Samples were collected in duplicate, stored in sterilized plastic bags for sample homogenization (Interscience, France), placed in isothermal boxes and immediately sent to the Laboratory of Food Microbiology of the Faculty of Pharmaceutical Sciences of Ribeirão Preto, USP, for microbiological analysis and to the Bromatology Laboratory of the Faculdade de Medicina in Ribeirão Preto, USP for the analysis of vitamin C.

The samples were submitted to microbiological analysis as recommended by resolution RDC 12 of ANVISA of January 2, 2001[11] which establishes specifications regarding the heat-tolerant coliform and coagulase-positive staphylococci counts, and the identification of *Salmonella* spp for food consumed by immunocompromised patients.

For the microbiological analyses, two 25 g aliquots of each sample were separated. One aliquot was diluted in 225 mL of saline solution (0.85% sodium chloride, Synth), homogenized in a microbiology sample blender (Interscience) and used to prepare serial decimal dilutions. For the fecal coliform counts using the multiple tube fermentation technique (Most Probable Number, MPN), 1 mL of each solution was inoculated into three tubes containing lauryl sulfate tryptose broth (Oxoid) and incubated at 35°C for 48 h.

An aliquot from each positive tube (turbid and with gas) was transferred to brilliant green lactose bile broth (Oxoid) with a bacteriological loop and incubated at 35°C for 48 h. An aliquot from each positive tube (turbid and with gas) was transferred with a loop to Escherichia coli (EC) broth (Merck) and incubated at 45ºC for 24 h. The number of positive tubes (turbid and with gas) of EC broth was used to determine the MPN of fecal coliforms per g sample using the MPN table[12].

Coagulase-positive staphylococci (CPS) were counted by seeding serial decimal dilutions of the foods on the surface of plates containing Baird-Parker Agar (Merck). After incubation at 37°C for 24 to 48 h, typical colonies (black and with opaque and transparent halos) were seeded in slanted nutrient agar (Himedia). Smears were prepared and submitted to Gram staining. Suspect isolates (Gram-positive cocci clustered in the form of bunches of grapes) were submitted to the catalase and coagulase tests. The population of CPS/g food was calculated on the basis of the results of these tests, proportionally to the number of characteristic colonies counted on Baird-Parker Agar[12].

The presence or absence of *Salmonella* spp. was determined in another 25 g aliquot of the sample which was homogenized in 225 mL buffered peptone water broth consisting of 10 g/L bacteriological peptone (Oxoid), 5 g/L sodium chloride (Synth), 3 g/L disodium phosphate (Synth) and 1.5 g/L monosodium phosphate (Synth). The preparation was incubated for 24 h at 35ºC and 1 mL aliquots of this pre-enriched culture were transferred to two tubes, each containing 10 mL selective enrichment broth: Tetrathionate (Acumedia) or Rappaport Vassiliadis (Acumedia). The Tetrathionate broth tube was incubated at 35ºC and the Rappaport Vassiliadis broth tube at 42°C, both for 24 h, and the broths were used for seeding on Rambach agar (Merck) and Hektoen Enteric agar (Acumedia) exhaust plates, followed by incubation at 35ºC for 24 h. Typical colonies (blue-greenish with or without a black center on Hektoen Enteric agar or a red center on Rambach agar) were submitted to a series of biochemical tests for identification which was confirmed by a agglutination test with polyvalent sera against the somatic and flagellar antigens of *Salmonella* spp[12]

Vitamin C was determined using a previously standardized method[13]. For sample preparation, 1 g fruit or vegetable was placed in 5% trichloroacetic acid (TCA) at a proportion of 1:10 (m:v) and triturated in a Potter type homogenizer. The homogenate was centrifuged at 1000 *g* at room temperature. The supernatant (600 μL) was removed and 200 μL of a solution of the Thiourea-copper sulphate reagent (DCT) were added. The DCT solution was prepared using 2,4-dinitrophnylhydrazine (Merck), thiourea (Matherson Coleman) and copper sulfate (Merck) at a proportion of 20:1:1 (v:v:v). The samples were placed in a water bath at 37°C for 3 h and the reaction was stopped by the addition of 1 mL of 65% H_2SO_4 (Merck). The sample was left in the dark for 30 minutes and a reading was obtained with a spectrophotometer at 520 nm. The vitamin C concentration was calculated by means of an analytical curve prepared with a solution of ascorbic acid (Carlo Erba) diluted in 5% TCA (Vetec) at concentrations of 10, 20, 40 and 50 μg/mL [13].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software was used for statistical analysis and the nonparametric Wilcoxon test was used to compare the mean vitamin C values of the cooked and raw foods. The Spearman correlation test was applied to determine the associations between the parameters evaluated.

Results

Thirty-four fruit and vegetable samples were analyzed; 10 were cooked, 12 were raw and sanitized with a chlorine solution, and 12 were raw and washed under running water. Only raw sanitized and washed watermelon and persimmon samples were analyzed since these fruit are not served cooked in the hospital.

All samples were negative regarding the presence of *Salmonella* spp. Similarly, no colonies of coagulase-positive staphylococci were detected on Baird Parker plates containing the foods analyzed, with estimated populations of these bacteria of less than 100 colony forming units /g. Heat-tolerant (or fecal) coliforms were also estimated since the samples evaluated in all the MPN series of tubes were negative, showing less than 3 MPN/g (minimum detectable limits of the method).

Vitamin C content was analyzed in 20 samples; 100 g each of peeled, raw and cooked fruits and vegetables (Table 1). A mean 38.9% (18.2 ± 21.3 mg) loss of vitamin C was observed when raw foods were compared to cooked foods with this loss being significant (p-value = 0.002). All cooked foods showed a loss of vitamin C compared to foods *in natura* ranging from 5.1 to 62.3 mg of vitamin C. It should be pointed out that pineapple, beets, carrots and pears showed more than a 50% loss of vitamin C after cooking.

Table 1 - Vitamin C content in raw and cooked foods and losses resulting from cooking

Foods	Vitamin C content raw mg/100 g	Vitamin C content cooked mg/100 g	Vitamin C loss mg
Pineapple	87.96	33.57	54.39
Banana	51.31	42.90	8.41
Beet	91.40	29.10	62.30
Carrot	14.84	7.25	7.59
Lime	49.63	40.66	8.97
Apple	14.84	7.95	6.89
Papaya	71.60	66.50	5.10
Melon	28.68	19.35	9.33
Pear	15.53	6.11	9.42
Tomato	36.80	27.00	9.80

The differences were statistically significant for all comparisons of raw and cooked foods (p-value < 0.05)

When the effect of cooking time (Range: 13 - 40 minutes) and temperature (Range: 75.0 - 93.5ºC) on the percentage of vitamin C loss were evaluated (Table 2), a statistically significant association was detected between the variables "percent vitamin C loss" and "cooking time" (r = 0.7; p-value = 0.02) but no relation to "cooking temperature".

Table 2 - Cooking time and temperature and percentage of vitamin C loss in cooked compared to raw fruits and vegetables

Foods	Cooking time (min.)	Cooking temperature (ºC)	Loss (%)
Pineapple	22	80.3	61.8
Banana	18	84.0	16.4
Beet	40	93.5	68.2
Carrot	20	80.3	51.1
Lime	18	80.7	18.1
Apple	16	77.4	46.4
Papaya	13	92.8	7.10
Melon	15	90.3	32.5
Pear	20	88.2	60.7
Tomato	13	75.0	26.6

Discussion

Neutropenic diets are recommended in order to reduce the risk of infections, however evidence supporting this conduct is lacking, because there are no studies that show any reduction in the incidence of infections with the use of such diets[14].

In Brazil, neutropenic diets are still used, but the recommended diet varies very much between hospitals. Vicenski et al. found that of the 17 hematopoietic stem cell transplantation centers studied in Brazil, 100% forbid thin-skinned fruits and 88% forbid thick-skinned fruit[15]. In international centers, thin-skinned fruits are allowed as long as they have been washed well[5].

Fruits and vegetables contain natural microbiota from the environment which are influenced by the structure of the plant, cultivation techniques, transportation, and storage[16,17]. Consequently, the microbiota detected in minimally processed foods is similar to that occurring in nature, typically consisting of microorganisms that are not pathogenic to humans[18].

Chlorine products should be used to reduce the microbial load of fresh vegetables[19]. However, there are few studies in the literature evaluating the microbiological contamination of fruits and vegetables *in natura* and sanitized with a chlorine solution.

Some studies have shown that free chlorine concentrations of 60 to 200 ppm can inactivate the vegetative cells of bacteria and fungi and that contamination with *Salmonella* spp. on the surface and in nucleus tissues of tomatoes can be substantially reduced by immersing the fruits in a chlorine solution for two minutes[20,21].

In the present study, the fruits and vegetables were sanitized as recommended by ANVISA[10] i.e., by immersion in a chlorine solution (200-250 ppm) and then peeled with clean and sanitized utensils. According to the results obtained in the microbiological evaluation, all vegetable samples studied were free of pathogens and thus within the microbiological standards required by law.

In a study by Oie et al.[22], microbial contamination was significantly reduced after the fruits and vegetables were disinfected with sodium hypochlorite compared to washing with water only. Nevertheless, microorganisms on the peel of the fruits were not completely eradicated. Thus, grapes and lemons that were not peeled were inadequate for consumption by immunocompromised patients. In this same study, the pulp

of the peeled fruits was not contaminated or presented very low contamination after washing with water and sanitation.

The differences detected between studies evaluating the efficiency of the cleaning and sanitation process may be due to factors such as concentration of the sanitizing product, time of contact with the food and the cleanliness of the utensils used to cut and peel the samples[22]. On this basis, there is an obvious need for the elaboration of protocols and for the training of the staff responsible for the sanitation of fruits and vegetables *in natura* destined for immunocompromised patients.

Some antioxidant nutrients such as vitamins A, C and E minimize the toxic effects of antineoplastic medications and contribute to a better response to the treatment employed[8]. The interactions between antineoplastic agents and antioxidant agents enhance the mechanism of action of these drugs, resulting in reduced side effects, improved quality of life and longer survival[23,24].

More than 90% of the vitamin C in the human diet comes from fruit and vegetables[25]. This vitamin is one of the most sensitive components of foods and therefore is frequently used as an indicator of food processing: if it is well retained in the food, the percentage of retention of all other vitamins should be similar or higher[22].

Data regarding the conventional process for the cooking of fruits are scarce in the literature, possibly because these foods are not normally consumed in the cooked form with the process, in Brazil, being used only in specific situations such as in the diet of neutropenic patients.

Some studies have demonstrated a reduced vitamin C content in cooked vegetables[26,27] due to its high solubility and heat instability. In addition, this process causes changes to the physical characteristics and chemical composition of vegetables[28].

The effect of blanching on the vitamin C content of various vegetables, including carrots, spinach and potatoes, was studied by Puupponen-Pimiä et al.[29], who observed that about one third of the vitamin C content was lost during cooking in water.

The form of cooking influences the percent change in antioxidant content. A study on cooking losses showed a 34% reduction of vitamin C in broccoli after cooking in water for 15 minutes, a 22.4% reduction after cooking in steam for 23 minutes, an 8% reduction after pressure cooking for 2 minutes, and a 9% reduction after cooking in a microwave oven for 11 minutes[29].

In the hospital where the fruit and vegetable samples used in the present study were collected, these foods destined for immunocompromised patients are all cooked in water, a fact explaining the significant loss of vitamin C observed.

The significant positive association between vitamin C loss and cooking time obtained in this study confirms the results of Zhang & Hamauzu[30] who, on investigating the influence of cooking time (30, 60, 90, 120 and 300 seconds in a microwave oven) on the content of antioxidant compounds in broccoli, observed an increasing loss with longer cooking times. Processing for 30 seconds led to a 19% loss of ascorbic acid with increasing the cooking time to 300 seconds causing a 66% loss. The choice of cooking time was related to the final consistency desired for each food.

Although vitamin C oxidation mainly occurs at high temperatures[31], there was no correlation between cooking temperature and percent loss of vitamin C in the foods analyzed. Bessi[32], on evaluating the loss of vitamin C in kale, cauliflower and peppers, observed that the loss during cooking in water was due to solubilization and not to thermal degradation

Conclusive studies are lacking to maintain the use of neutropenic diet and the use of such diets may interfere with the treatment and nutrition status of cancer patients, as they limit food choice and restrict food with high nutritional value. The data from this study showed that fresh fruits and vegetables, sanitized correctly, have a microbiological profile consistent with that required by Brazilian law on food intended for immunocompromised patients. Furthermore, since the loss of vitamin C in cooked food predicts the loss of other nutrients, we may conclude that neutropenic diets lose their nutritional value, a fact that suggests that this diet is not an effective alternative in the maintenance of an adequate nutritional status of these patients.

These results may contribute to the discussion about the dietary restrictions imposed by neutropenic diet, contributing to the upgrading of nutritional management for immunocompromised patients.

Finally, these results cannot be generalized to all institutions since the microbiological quality depends on the hygienic conditions of the raw material, of the place where the foods are processed and on the cleanliness of the utensils used. We recommend the elaboration of protocols and training programs for staff on the effective sanitation of raw vegetables with chlorine solution, in order to reduce microbiological danger. Another important topic of nutritional care in this ward is to counsel patients and caregivers on hygiene procedures at home, and to provide information allowing a safe and effective nutritional recovery.

References

1. DeMille D, Deming P, Lupinacci P, Jacobs LA. The effect of the neutropenic diet in the outpatient setting: A pilot study. Oncol Nurs Forum. 2006;33(2):337-43.
2. Zitella LJ, Friese CR, Hauser J, Gobel BH, Woolery M, O'Leary C, et al. Putting evidence into practice: prevention of infection. Clin J Oncol Nurs. 2006;10(6):739-50. Comment in: Clin J Oncol Nurs. 2007;11(2):185-6.
3. Tarr S, Allen DH. Evidence does not support the use of a neutropenic diet. Clin J Oncol Nurs. 2009;13(6):617-8.
4. van Tiel F, Harbers MM, Terporten PH, van Boxtel RT, Kessels AG, Voss GB, et al. Normal hospital and low-bacterial diet in patients with cytopenia after intensive chemotherapy for hematological malignancy: A study of safety. Ann Oncol. 2007;18(6):1080-4.
5. Wilson BJ. Dietary recommendations for neutropenic patients. Semin Oncol Nurs. 2002;18(1):44-9.
6. CDC. Infectious Disease Society of America and the American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoetic stem cell transplant recipients. Recommendations of CDC, the Infectious Disease Society of America, and the American Society of Blood and Marrow Transplantation [Internet]. Atlanta: CDC; 2000 [cited 2011 September 14]. Available from: http://www.cdc.gov/MMWR/Preview/mmwrhtml/rr4910a1.htm
7. Todd J, Schmidt M, Christain J, Williams R. The low-bacterial diet for immunocompromised patients: reasonable prudence or clinical superstition? Cancer Pract. 1999;7(4):205–7.
8. Santos HS, Cruz WM. A terapia nutricional com vitaminas antioxidantes e o tratamento quimioterápico oncológico. Rev Bras Cancerol. 2001;47(3):303-8.
9. Carter LW. Influences of nutrition and stress on people at risk for neutropenia:

nursing implications. Oncol Nurs Forum. 1993;20(8):1241-50.

10. Secretaria de Estado de Saúde. Centro de Vigilância Sanitária do Estado de São Paulo. Portaria CVS-6 de 10.03;1999: dispõe sobre os parâmetros e critérios de controle higiênico-sanitário em estabelecimentos de alimentos [Internet]. São Paulo; 1999. [cited 2011 September 14]. Available from: http//www.cvs.saude.sp.gov.br/zip/E_PT-CVS-06_100399.pdf
11. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC nº 12, de 2 de Janeiro de 2011. Regulamento técnico sobre padrões microbiológicos para alimentos [Internet]. Brasília; ANVISA; 2001 [cited 2011 Sep 14]. Available from: http://www.sfdk.com.br/imagens/lei/MS-RDC12.htm
12. Food and Drug Administration. Bacteriological analytical manual online. [Internet]. Silver Spring, MD: FDA; 2001 [cited 2011 Sept 14]. Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm
13. Bessey AO. Ascorbic acid microchemical methods. In: Gyiuirgy P, editor. Vitamin methods. New York: Academic Press, 1960. p.303.
14. French MR, Levy-Milne R, Zibrik D. A survey of the use of low microbial diets in pediatric bone marrow transplant programs. J Am Diet Assoc. 2001;101(10):1194-8.
15. Vicenski PP, Alberti PA, C DJ. Dietary recommendations for immunosuppressed patients of 17 hematopoietic stem cell transplantation centers in Brazil. Rev Bras Hematol Hemoter. 2012;34(2):86-93.
16. Pacheco MA, Fonseca YS, Dias HG, Cândido VL, Gomes AH, Armelin IM, et al. Condições higiênico-sanitárias de verduras e legumes comercializados no Ceagesp de Sorocaba- SP. Hig Aliment. 2002;16(101):50-5.
17. Rosa OO, Carvalho EP. Características microbiológicas de frutos e hortaliças minimamente processados. Bol SBCTA. 2000;34(2):84-92.
18. Zagory D. Effects of post-processing handling and packaging on microbial populations. Postharvest Biol Technol.1999;15(3):313-21.
19. Moretti, C. Manual de processamento mínimo de frutas e hortaliças [Internet]. Brasília: SEBRAE; 2007. [cited 2010 Jun 21]. Available from: http://ag20.cnptia.embrapa.br/Repositorio/MPMFH_Cap02_Alteracoes_metabolicas_000ga5h444402wx5ok0821iy5wax60zk.pdf
20. Simons LK, Sanguansri R. Advances in the washing of minimally processed vegetables. Food Australia. 1997;49:75-80.
21. Zhuang RY, Beuchat LR, Angulo FJ. Fate of Salmonella montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl Environ Microbiol. 1995;61(6):2127-31.
22. Oie S, Kiyonaga H, Matsuzaka Y, Maeda K, Masuda Y, Tasaka K, et al. Microbial contamination of fruit and vegetables and their disinfection. Biol Pharm Bull. 2008;31(10):1902-5.
23. Weijl NI, Hopman GD, Wipkink-Bakker A, Lentjes EG, Berger HM, Cleton FJ, et al. Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. Ann Oncol. 1998;9(12):1331-7.
24. Lamson DW, Brignall MS. Antioxidants in cancer therapy: their actions and interactions with oncologic therapies. Altern Med Rev.1999;4(5):304-29. Comment in: Altern Med Rev. 1999;4(5):303.
25. Moody K, Charlson ME, Finlay J. The neutropenic diet: what's the evidence? J Pediatr Hematol Oncol. 2002;24(9):717-21.
26. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem. 2002;50(10):3010-4.
27. Özkan M, Kirca A, Cemeroglu B. Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. Food Chem. 2004;88(4):591-7.
28. Lisiewska Z, Kmiecik W. Effects of level of nitrogen fertilizer processing conditions and period of storage of frozen broccoli and cauliflower on vitamin C retention. Food Chem. 1996;57(2):261-70.
29. Puupponen- Pimiä R, Häkkinen ST , Aarni M, Suortti T, Lampi AM, Eurola M, et al. Blanching and long term freezing affect various bioactive compounds of vegetables in different ways. J Sci Food Agric. 2003;8:1389-402.
30. Zhang D, Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry. 2004; 88:503-9.
31. Howard LA, Wong AD, Perry AK, Klein BP. β-carotene and ascorbic acid retention in fresh and processed vegetables. J Food Sci. 1999;64(5):929-36.
32. Bessi MT. Análise dos efeitos de diferentes parâmetros na estabilidade de Vitamina C em vegetais processados [Thesis]. Campinas: Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas; 1990.

xxx