Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 89 (2005) 611–615
Food Chemistry
www.elsevier.com/locate/foodchem

Oxidation of cholesterol in mayonnaise during storage

Isabel Cristina Morales-Aizpurúa, Alfredo Tenuta-Filho *

Department of Foods and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, Bloco 14, Av. Prof. Lineu Prestes, 580, 05508-900 São Paulo-SP, Brazil

Received 21 March 2003; received in revised form 11 March 2004; accepted 11 March 2004

Abstract

The oxidative stability of cholesterol in commercial mayonnaise under different storage conditions was evaluated by measuring cholesterol oxides (COs), 7-ketocholesterol (7-Keto), 25-hydroxycholesterol (25-OH), 7α-hydroxycholesterol (7α-OH) and 7β-hydroxycholesterol (7β-OH) using HPLC. Oxidation of cholesterol was indicated within about 15 days after manufacture by the presence of 7-Keto. Oxidation increased during storage at 4 and 25 °C (being greater at 25 °C) for 165 days in darkness, as indicated by the presence of 7-Keto, 25-OH, 7α-OH and 7β-OH. There was a strong correlation between COs and PV (peroxide value) [$r^2 = 0.95$ (4 °C) and $r^2 = 0.96$ (25 °C)] during the process of oxidation. The pattern of fatty acids was not affected during the period of the experiment. Temperature and time were important factors in the oxidative stability of cholesterol. Total formation of COs during 165 days was 20.3 μg/g at 4 °C and 30.2 μg/g at 25 °C.

Keywords: Cholesterol; Cholesterol oxides; Mayonnaise; Storage

1. Introduction

Cholesterol is a monounsaturated lipid with a double bond on carbon-5, and is susceptible to oxidation in the presence of oxygen, light, heat, radiation, free radicals, metal ions, and other factors. The resulting cholesterol oxides, COs, are of clinical interest, especially due to their association with cytotoxicity (Sevanian & Peterson, 1986), atherogenesis (Peng, Hu, & Morin, 1991), mutagenesis (Sevanian & Peterson, 1986) and carcinogenesis (Kendall, Koo, Sokoloff, & Kesava-Rao, 1992; Petrakis, Gruenke, & Craig, 1981; Sporer, Brill, & Schaffner, 1982).

There is abundant evidence that the COs (mentioned here by their common, systematic and trivial names), 7-ketocholesterol (3β-hydroxycholest-5-en-7-one) (7-Keto), 20-hydroxycholesterol (cholest-5-en-3β, 20-diol) (20-OH), 25-hydroxycholesterol (cholest-5-en-3β, 25-diol) (25-OH), 7α-hydroxycholesterol (cholest-5-en-3β, 7α-diol) (7α-OH), 7β-hydroxycholesterol (cholest-5-en-β, 7β-diol) (7β-OH), cholesterol-5,6α-epoxyde (5,6α-epoxi-5α-cholestan-3 β-ol) (5,6-epoxide), cholesterol-5,6β-epoxide (5,6β-epoxi-5β-cholestan-3β-ol) (5,6β-epoxide) and cholestanotriol (5α-cholestan-β, 5,6β-triol) (Triol) are cytotoxic and atherogenic to different extents (Bösinger, Luf, & Brandl, 1993). By contrast, cholesterol shows little cytotoxicity or atherogenicity (Imai et al., 1980; Imai, Werthessen, Taylor, & Lee, 1976; Peng et al., 1991).

Many oxidation products of cholesterol have been identified (Tai, Chen, & Chen, 1999). Those most frequently found in foods are 7-Keto, 20-OH, 25-OH, 7α-OH, 7β-OH, 5,6α-epoxide, 5,6 β-epoxide and Triol. Of these, 7-Keto occurs in relatively high concentrations in many foods (Guardiola, Codony, Rafecas, & Boatella, 1995; Nielsen, Olsen, Lyndon, Sorensen, & Skibsted, 1996; Novelli et al., 1998; Pie, Spahis, & Seillan, 1991), with the content varying, depending on the type of matrix (Lercker & Rodriguez-Estrada, 2000), and it has been proposed as an indicator of cholesterol oxidation (Gallina Toschi & Caboni, 1992; Zunin et al., 1995). By comparison, 25-OH occurs in smaller concentrations (Guardiola et al., 1995); however, 25-OH and Triol are considered to be the most atherogenic of these products (Addis, 1986; Peng et al., 1991).

* Corresponding author. Tel.: +55-11-3091-3652; fax: +55-11-3815-4410.
E-mail address: eetenuta@usp.br (A. Tenuta-Filho).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.03.016

Occurrence of total COs at up to 443 μg/g in foods of animal origin has been reported in the literature (Tai, Chen, & Chen, 2000), accounting for 1–2% to 10% of total cholesterol (Paniangvait, King, Jones, & German, 1995). COs can enter the circulation as food contaminants (Lin & Morel, 1996); however, the contribution of these exogenous oxides relative to the endogenous oxides in plasma has yet to be clarified. It is necessary to evaluate the bioavailability of each of the COs before toxicological evaluation (Linseisen & Wolfram, 1998), given that their percentages of absorption differ (Osada, Sasaki, & Sugano, 1994).

Mayonnaise is a widely consumed food product. Being made with eggs, it contains significant quantities of cholesterol. Therefore, the potential for cholesterol oxidation cannot be ignored, especially when the long shelf-life of the product (6 months) is considered. The objective of the present study was to investigate the oxidative stability of cholesterol in commercial mayonnaise by analyzing effective oxidation with formation of 7-Keto, 25-OH, 7α-OH and 7β-OH, during storage for 165 days at 4 and 25 °C in the dark.

2. Materials and methods

2.1. Materials

A brand of mayonnaise available commercially in São Paulo, Brazil was selected at random, with the samples being provided directly by the manufacturer. There was a total of 45 samples of 500 g each from five different lots with manufacturing dates spanning approximately 15 days, and one sample from each lot was analyzed. The remaining samples were stored in the laboratory at 4 ± 1 °C or at 25 ± 1 °C in the dark, and examined during 165 days. The hexane (Aldrich) and isopropanol (Sigma) used were of HPLC grade; the other chemicals were ACS reagent grade. Cholesterol (5-cholesten-3β-ol), 6-ketocholestanol (3β-hydroxy-5 α-cholestan-6-one), 7-Keto, 25-OH and 7α-OH were obtained from Sigma Chemical Co. and 7β-OH was obtained from Steraloids Inc.

2.2. Measurement of cholesterol and cholesterol oxides

2.2.1. Extraction of total lipid and saponification

Mayonnaise samples (0.25–1.0 g), reconstituted to 80% moisture by adding distilled water and homogenized, were transferred to a separating funnel with 50 ml of distilled water and extracted three times in succession with 20 ml of chloroform/methanol (v/v, 2:1) (Csallany, Kindom, Addis, & Lee, 1989). After separation and evaporation of the chloroform under vacuum, the residue was saponified by adding 20 ml of 1 M KOH in methanol and holding the mixture for 18 h at 25–30 °C, with mechanical agitation especially during the first 2 and last 2 h of the process. Ten milliliters of distilled water were added to the mixture and the unsaponified fraction was extracted three times in succession with 10 ml of hexane using a separating funnel. The organic fraction was washed with 10 ml of 1 M KOH in methanol and then three times in succession with 10 ml of distilled water. The resulting extract was filtered using Whatman No. 1 filter paper containing anhydrous sodium sulfate, and the material retained after filtration was washed with 20 ml of hexane (Chen & Chen, 1994).

2.2.2. Assay

The hexane extract obtained above (2.2.1) was evaporated under vacuum in a rotary evaporator at 40 °C. The residue was suspended in 1–2 ml of mobile phase, filtered through a 0.45-μm Durapore membrane (Millex-Millipore) and injected automatically (20–50 μl) onto an HPLC system (Shimadzu, LC-10 ADVP). A 30×0.39 cm column of μ-Porasil of pore diameter 10 μm (Waters Associates) was used in normal phase. The mobile phases used were mixtures of hexane/isopropanol at 96:4 (v/v) for cholesterol, 97:3 for 25-OH and 93:7 for 7-Keto with a flow rate of 1 ml/min, and at 91:9 (v/v) for 7α-OH and 7β-OH with a flow rate of 0.8 ml/min. Cholesterol and COs were detected at 206 nm, except for 7-Keto, which was detected at 233 nm, using a photodiode detector. Peaks for cholesterol and COs were identified by comparison of retention times with those of the corresponding reference standards. External standards were used in measuring cholesterol and 25-OH, and internal and external standards were used for 7-Keto, 7α-OH and 7 β-OH, with 6-ketocholestanol being used as the internal standard (Csallany et al., 1989; Maerker, Nunguesser, & Zulak, 1988). Oxidative stability of cholesterol during the assay was confirmed. The cholesterol was added to commercial soy oil (peroxide value of 4.34 ± 0.11 meq O_2/kg) and there was no formation of 7-Keto, 7α-OH, 7β-OH or 25-OH.

2.2.3. Validation of the method

Recovery of analytes (added to samples of mayonnaise before the lipid extraction) was 90–96% ($n = 6$); repeatability represented by the coefficients of variation, was 7.25–11.95% ($n = 8$), and the linearity of the calibration curves (0.2–1.2 μg), represented by the correlation coefficients (r^2), was 0.993–0.999. The limits of detection and quantitation were 1.09×10^{-8} and 3.62×10^{-8} g for cholesterol, 6.67×10^{-9} and 2.22×10^{-8} g for 25-OH, 2.02×10^{-9} and 6.73×10^{-9} g for 7-Keto, 3.11×10^{-8} and 1.03×10^{-7} g for 7α-OH and 6.90×10^{-8} and 2.30×10^{-7}g for 7β-OH, respectively (Long & Winefordner, 1983; Novelli et al., 1998; Razzazi-Fazeli, Kleineisen, & Luf, 2000). The structures of the COs were confirmed by gas chromatography/mass spectrometry (Sevanian et al., 1994).

2.3. Moisture content, peroxide value, pH, total lipids and fatty acids

Moisture and peroxide value (PV) were determined by the methods of the AOCS (1987) and total lipids and fatty acids by the methods in Brasil (1981) and Hartman and Lago (1973), respectively. The pH of the homogenized sample was measured directly, using a Procyon, model SA 720 potentiometer.

2.4. Statistical analyses

The data was subjected to classical regression analysis, descriptive univariate analysis, descriptive multivariate analysis (Microsoft Excel for Windows version 1997), analysis of variance (Graphpad Instat Tm 1990–93, version 2.01), mixed model analysis of variance (Minitab for Windows, version 11.12) and explanatory coefficient R^2 (SPSS for Windows, version 8.0) (Bussab & Morettin, 1986; Neter, Kutner, Nachtsheim, & Wasserman, 1996; Singer & Andrade, 1986).

3. Results and discussion

3.1. Recently manufactured mayonnaise

The results (Table 1) for moisture (16.97 ± 0.14 g/100 g) and total lipids (75.75 ± 0.77 g/100 g) obtained for the different lots of recently manufactured mayonnaise (approximately 15 days), agree with the results reported by Chirife, Vigo, Gomez, and Favetto (1989) and Stauffer (1996). The lipid content was also in accord with Brazilian legislation regarding the minimum content (65 g/100 g sample) (Brasil, 1978). The results for PV (0.75 ± 0.01 meq O_2/kg) were low; pH (3.68 ± 0.01) was adequate as regards the microbiological stability of the product (Jacobsen et al., 2001).

The concentrations of cholesterol (71.4 ± 5.49 mg/100 g) were close to those reported by Feeley, Criner, and Watt (1972) (50–70 mg/100 g), while those of 7-Keto (1.99 ± 0.59 μg/g), the only oxide detected, accounted for no more than 0.5% of cholesterol (Table 1). According to Paniangvait et al. (1995), total COs in foods can account for 1–2% to 10% of cholesterol.

3.2. Stored mayonnaise

With the exception of 7-Keto, none of the oxides studied was detected in the control sample or during storage for up to 75 days at 25 °C or up to 135 days at 4 °C (Table 2). In most cases, formation of 7-Keto was more elevated during the experimental period. At the end of the experiment, 7-Keto was 3.7 and 3.6 times higher than the control at 4 and 25 °C, respectively.

25-OH, 7α-OH and 7β-OH were only detected after 135 days at 25 °C (Table 2). The occurrence of 25-OH toward the end of the experimental period is in agreement with the report of Maerker (1987), who considered that COs as products of oxidation of carbon-7 such as 7-Keto are formed more readily than products of side-chain oxidation such as 25-OH, which occur by transfer of free radicals. The predominance of 7-Keto, compared with 7-OH (α and β) during storage suggests that dehydration is more important than reduction in the oxidation of cholesterol. Toward the end of storage at 25 °C, 25-OH exceeded 7-Keto, with a decrease in the rate of formation of 7-Keto.

The quantities of 7-Keto measured after 165 days of the experiments at 4 and 25 °C amounted to about 1% of those for cholesterol (Table 1), and the sum of 25-OH, 7α-OH and 7β-OH amounted to 2.9% (4 °C) and 4.2% (25 °C), respectively. These values can be considered significant, given that total COs content measured in different foods has been reported to be 1–2% to 10% of cholesterol content (Paniangvait et al., 1995). The results in Table 2 very probably underestimate the degree of oxidation of cholesterol in mayonnaise, since they present only values for 7-Keto, 25-OH, 7α-OH and 7β-OH. At least four other oxides – 20-OH, 5,6α-epoxide, 5,6β-epoxide and Triol – could also be present as mentioned previously.

The pH remained stable and suitable for maintaining the microbiological stability of the product and the PV within acceptable limits. According to Jacobsen et al. (2001), the pH should not be above 4.2. Brazilian legislation (São Paulo, 1992) stipulates a maximum tolerated value for PV of 10 meq O_2/kg. Fatty acids content was not affected by storage conditions, irrespective of the small quantities of peroxide.

Table 1
Moisture, total lipids, peroxide value (PV), pH, cholesterol and 7-Keto in recently manufactured mayonnaise (approximately 15 days)

Lots	Moisture (g/100 g)	Total lipids (g/100 g)	VP (meq O_2/kg)	pH	Cholesterol (mg/100 g)	7-Keto (μg/g)
1	17.04	75.64	0.75	3.68	66.6	2.87(0.43)
2	16.96	75.27	0.74	3.67	69.3	1.28(0.18)
3	16.91	74.88	0.75	3.68	66.8	2.18(0.33)
4	16.79	76.86	0.75	3.68	74.9	1.94(0.26)
5	17.15	76.09	0.74	3.67	79.2	1.69(0.21)
Mean ± SD	16.97 ± 0.14	75.75 ± 0.77	0.75 ± 0.01	3.68 ± 0.01	71.4 ± 5.49	1.99 ± 0.59

(% relative to cholesterol); SD – standard deviation ($n = 5$).

Table 2
COs (μg/g), PV (meq O_2/kg), pH [a] and fatty acids (g/100 g) [b] in mayonnaise stored at 4 and 25 °C for up to165 days in the dark

	Control	45 days		75 days		135 days		165 days	
		4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
7-Keto	1.99 ± 0.59	3.16 ± 0.27	5.76 ± 1.22	4.41 ± 0.81	5.72 ± 0.73	5.78 ± 0.61	7.61 ± 1.14	7.38 ± 1.14	7.15 ± 1.61
25-OH	nd	nd	nd	nd	nd	nd	2.02 ± 0.73	6.72 ± 0.66	9.13 ± 1.44
7α-OH	nd	nd	nd	nd	nd	nd	5.43 ± 1.29	2.32 ± 0.42	6.84 ± 0.39
7β-OH	nd	nd	nd	nd	nd	nd	5.23 ± 1.13	3.93 ± 0.75	7.02 ± 0.17
Total COs	1.99 ± 0.59	3.16 ± 0.27	5.76 ± 1.22	4.41 ± 0.81	5.72 ± 0.73	5.78 ± 0.61	20.20 ± 2.58	20.34 ± 1.34	30.16 ± 1.13
PV	0.74 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.80 ± 0.00	0.86 ± 0.00	0.83 ± 0.00	2.52 ± 0.12	0.92 ± 0.00	5.03 ± 0.49
pH	3.68 ± 0.01	3.66 ± 0.01	3.65 ± 0.01	3.67 ± 0.01	3.67 ± 0.01	3.67 ± 0.01	3.69 ± 0.01	3.66 ± 0.02	3.77 ± 0.04
SFA	14.6	–	–	14.5	14.5	–	–	14.1	14.2
MUFA	25.2	–	–	25.3	25.3	–	–	24.9	25.3
PUFA	58.5	–	–	59.0	59.2	–	–	58.7	59.0

Control – recently manufactured mayonnaise (approximately 15 days); nd – not detected; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.
[a] Mean of five different samples ($n = 5$) ± SD.
[b] Sample 5 (Table 1) analyzed in duplicate.

COs content was strongly correlated with PV, both at 4 °C ($r^2 = 0.95$) and at 25 °C ($r^2 = 0.96$). Adjusted regression models showed that COs content tended to increase during the period of the experiment after 75 days of storage with the rate being faster at 25 °C, showing the same trend as PV. Thus, decreasing storage temperature and time is an important factor in suppressing the oxidation of cholesterol in mayonnaise. There was no concrete justification of the increase in COs after 165 days/4 °C without significant increase in PV.

We have not found any information in the literature relating to the stability of cholesterol in mayonnaise to oxidation during storage. Sarantinos, O'Dea, and Sinclair (1993) appear to be the only workers to have measured COs in mayonnaise. In a relatively small number of samples ($n = 3$) these authors found 7-Keto at 13 ± 13 μg/g, 25-OH at 9 ± 4 μg/g, 7α-OH at 5 ± 5 μg/g, 4β-OH (4β-hydroxycholesterol) at 32 ± 5 μg/g, 20α-OH (20α-hydroxycholesterol) at 7 ± 7 μg/g and Triol at 2 ± 2 μg/g. The quantities of oxides found by Sarantinos et al. (1993) are significant, especially those for 4β-OH, but at the same time they show substantial variation.

Further studies are necessary in order to reach a better understanding of the oxidative stability of cholesterol in mayonnaise. These studies should focus on the quality of the egg used as starting material, the efficiency of the antioxidant systems used and the form, temperature and time of storage of products produced on an industrial scale.

Acknowledgements

The authors wish to thank CNPq and FAPESP, Brazilian Research Agencies, for support of this work.

References

Addis, P. B. (1986). Occurrence of lipid oxidation products in foods. *Food and Chemical Toxicology, 24*(10/11), 1021–1030.
American Oil Chemists' Society. (1987). *Official methods and recommended practices*. Champaign: AOCS.
Bösinger, S., Luf, W., & Brandl, E. (1993). Oxysterols: Their occurrence and biological effects. *International Dairy Journal, 3*, 1–33.
Brasil. (1978). *Leis, decretos, etc. Portaria 12l78 da CNPA*. Brasília: Diário Oficial da União.
Brasil. (1981). *Métodos analíticos oficiais para controle de produtos de origem animal e seus ingredientes: métodos físicos e químicos*. Brasília: LANARA.
Bussab, W. O., & Morettin, P. A. (1986). *Estatística básica*. São Paulo: Atual.
Chen, B. H., & Chen, Y. C. (1994). Evaluation of the analysis of cholesterol oxides by liquid chromatography. *Journal of Chromatography A, 661*, 127–136.
Chirife, J., Vigo, M. S., Gomez, R. G., & Favetto, G. J. (1989). Water activity and chemical composition of mayonnaises. *Journal of Food Science, 54*(6), 1658–1659.
Csallany, A. S., Kindom, S. E., Addis, P. B., & Lee, J. H. (1989). HPLC method for quantitation of cholesterol and four of its major oxidation products in muscle and liver tissues. *Lipids, 24*(7), 645–651.
Feeley, R. M., Criner, P. E., & Watt, B. K. (1972). Cholesterol content of foods. *Journal of the American Dietetic Association, 61*, 131–149.
Gallina Toschi, T., & Caboni, M. F. (1992). Cholesterol oxides: Biological behaviour and analytical determination. *Italian Journal of Food Science, 4*, 223–228.
Guardiola, F., Codony, R., Rafecas, M., & Boatella, J. (1995). Formación de derivados oxidados del colesterol en alimentos. *Grasas y Aceites, 46*(3), 202–212.
Hartman, L., & Lago, R. C. A. (1973). Rapid preparation of fatty acid methyl ester from lipids. *Laboratory Practice, 22*, 475–476.
Imai, H., Werthessen, N. T., Subramanyam, V., LeQuesne, P. W., Soloway, A. H., & Kanisawa, M. (1980). Angiotoxicity of oxigenated sterols and possible precursors. *Science, 207*, 651–653.
Imai, H., Werthessen, N. T., Taylor, C. B., & Lee, K. T. (1976). Angiotoxicity and arteriosclerosis due to contaminants of USP – grade cholesterol. *Archives of Pathology and Laboratory Medicine, 100*, 565–575.

Jacobsen, C., Hartvigsen, K., Thomsen, M., Hansen, L. F., Lund, P., Skibsted, L. H., Holmer, G., Adler-Missen, J., & Meyer, A. S. (2001). Lipid oxidation in fish oil enriched mayonnaise: Calcium disodium ethylenediaminetetraacetate, but not gallic acid, strongly inhibited oxidative deterioration. *Journal of the Agriculture and Food Chemistry, 49*, 1009–1019.

Kendall, C. W., Koo, M., Sokoloff, E., & Kesava-Rao, G. (1992). Effects of dietary oxidized cholesterol on azoxymethane induced colonic preneoplasia in mice. *Cancer Letters, 66*, 241–248.

Lercker, G., & Rodriguez-Estrada, M. T. (2000). Cholesterol oxidation: Presence of 7-Ketocholesterol in different food products. *Journal of Food Composition and Analysis, 13*, 625–631.

Lin, C.-Y., & Morel, D. W. (1996). Esterification of oxysterols in human serum: Effects on distribution and cellular uptake. *Journal of Lipid Research, 37*, 168–178.

Linseisen, J., & Wolfram, G. (1998). Absorption of cholesterol oxidation products from ordinary foodstuff in humans. *Annals of Nutrition and Metabolism, 42*, 221–230.

Long, G. L., & Winefordner, J. D. (1983). Limit of detection. A closer look at the IUPAC definition. *Analytical Chemistry, 55*(7), 712A–724A.

Maerker, G. (1987). Cholesterol autoxidation-current status. *Journal of the American Oil Chemists Society, 64*(3), 388–392.

Maerker, G., Nunguesser, E. H., & Zulak, I. M. (1988). HPLC separation and quantitation of cholesterol oxidation products with flame ionization detection. *Journal of the Agricultural and Food Chemistry, 36*, 61–63.

Neter, J., Kutner, M. H., Nachtsheim, C. J., & Wasserman, W. (1996). *Applied linear statistical models*. Boston: McGraw Hill.

Nielsen, J. H., Olsen, C. E., Lyndon, J., Sorensen, J., & Skibsted, L. H. (1996). Cholesterol oxidation in feta cheese produced from high-temperature bleached and from non-bleached butteroil from bovine milk. *Journal Dairy Research, 63*, 615–621.

Novelli, E., Zanardi, E., Ghiretti, G. P., Campanini, G., Dazzi, G., Madarena, G., & Chizzolini, R. (1998). Lipid and cholesterol oxidation in frozen stored pork, salame milano and mortadella. *Meat Science, 48*(1/2), 29–40.

Osada, K., Sasaki, E., & Sugano, M. (1994). Lymphatic absorption of oxidized cholesterol in rats. *Lipids, 29*(6), 555–559.

Paniangvait, P., King, A. J., Jones, A. D., & German, B. G. (1995). Cholesterol oxides in foods of animal origin. *Journal of Food Science, 60*(6), 1159–1174.

Peng, S., Hu, B., & Morin, R. J. (1991). Angiotoxicity and atherogenicity of cholesterol oxides. *Journal of Clinical Laboratory Analysis, 5*, 144–152.

Petrakis, N. L., Gruenke, L. D., & Craig, J. C. (1981). Cholesterol and cholesterol epoxides in nipple aspirates of human breast fluid. *Cancer Research, 41*, 2563–2565.

Pie, J. E., Spahis, K., & Seillan, C. (1991). Cholesterol oxidation in meat products during cooking and frozen storage. *Journal of the Agricultural and Food Chemistry, 39*, 250–254.

Razzazi-Fazeli, E., Kleineisen, S., & Luf, W. (2000). Determination of cholesterol oxides in processed food using high-performance liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization. *Journal of Chromatography A, 896*, 321–334.

São Paulo. (1992). *Código Sanitário: Decreto Nº 12.342 de 27 de setembro de 1978*. São Paulo: IMESP.

Sarantinos, J., O'Dea, K. O., & Sinclair, A. J. (1993). Cholesterol oxides in Australian foods. Identification and quantification. *Food Australia, 45*(10), 485–490.

Sevanian, A., Seraglia, R., Traldi, P., Rossato, P., Ursini, F., & Hodis, H. (1994). Analysis of plasma cholesterol oxidation products using gas- and high-performance liquid chromatography/mass spectrometry. *Free Radical Biology and Medicine, 17*(5), 397–409.

Sevanian, A., & Peterson, A. R. (1986). The cytotoxic and mutagenic properties of cholesterol oxidation products. *Food and Chemical Toxicology, 24*(10/11), 1103–1110.

Singer, J. M., & Andrade, D. F (1986). *Análise de dados longitudinais*. Campinas: IMECC.

Sporer, A., Brill, D. R., & Schaffner, C. P. (1982). Epoxycholesterols in secretions and tissue of normal, benign, and cancerous human prostate glands. *Urology, 20*, 244–250.

Stauffer, C. E. (1996). *Fats and oils*. Saint Paul: Eagan Press.

Tai, C.-Y., Chen, Y. C., & Chen, B. H. (1999). Analysis, formation and inhibition of cholesterol oxidation products in food: An overview (Part I). *Journal of Food and Drug Analysis, 7*(4), 243–257.

Tai, C.-Y., Chen, Y. C., & Chen, B. H. (2000). Analysis, formation and inhibition of cholesterol oxidation products in food: An overview (Part II). *Journal of Food and Drug Analysis, 8*(1), 1–15.

Zunin, P., Evangelisti, F., Caboni, M. F., Penazzi, G., Lercker, G., & Tioscornia, E. (1995). Cholesterol oxidation in baked foods containing fresh and powdered eggs. *Journal of Food Science, 60*(5), 913–916.