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# Sensory evaluation of a milk formulation supplemented with n3 polyunsaturated fatty acids and soluble fibres

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**Abstract**

The hypocholesterolemic effect of some polyunsaturated fatty acids and soluble fibres has been demonstrated mostly in epidemiological studies. Two types of fatty acids (eicosapentaenoic-EPA 20:5n-3 and docosahexaenoic-DHA 22:6n-3) and soluble fibres (oat flour and guar gum), were mixed in different proportions and used in a milk formulation according to a factorial design ($3^2$) with a repetition in the central point. The 10 assays were submitted to a sensory test, using a structured hedonic scale applied to heart surgery patients ($n=240$), in order to evaluate the general acceptability of each mixture. Some of these ($n=137$) answered a questionnaire, which was used to identify the target public for this product, using multivariate analysis and the correspondence technique. Response surface methodology (RSM) was used to model the polynomial quadratic regression which predicts the "sensory acceptability ($y$)" as a function of variation from selected factors: $x_1=$(n3 polyunsaturated fatty acids or n3PUFA)g/kcal and $x_2=$(soluble fibres or SF)g/kcal. The concentrations used in the mixtures were: 0.1–0.8 and 0.1–0.3 g/100 kcal, respectively. The coefficient of determination ($R^2$) was 95.76%, indicating the good fit of the model to the experimental data. The high level of n3PUFA and SF significantly decreased the sensory quality of the product, limiting the maximum expression of the functional properties of these two factors. The contour plots obtained from the quadratic model permitted determination of the acceptability of the milk formulation, which was estimated in each possible combination of n3PUFA acid and SF. These procedures were essential for developing a good product with functional properties for human consumption.


## 1. Introduction

Cardiovascular diseases are considered to be the main cause of mortality in several countries, representing a high social cost, both in terms of direct expenses for treatment and of the abrupt interruption of a productive life. Several studies have demonstrated that various risk factors are significantly associated with the incidence of cardiovascular disease; these include hypertension, plasma cholesterol, smoking habit, genetic predisposition, hormonal level, sedentary life style, age, sex, inadequate food consumption, obesity, and others. These factors may also interact, producing synergistic effects (Caggiula & Mustard, 1997; Connor, 1999; Duthie & Brown, 1999; Fuster & Pearson, 1996). Nutritional intervention through an adequate diet mainly acts on the maintenance of arterial pressure and the control of plasma triacylglycerol and cholesterol levels. These interventions have yielded positive results in terms of the reduction of cardiovascular morbidity and mortality, with recommendations about the consumption of foods rich in fibres, vitamins and polyunsaturated fatty acids, despite the great difficulty in obtaining compliance with the dietary adjustment on the part of many patients (Alexander, Lockwood, Harris, & Melby, 1999; Thinker, Parks, Behr, Schneeman, & Davis, 1999; Verges, 1998).

Many studies have revealed that some long-chain omega3 polyunsaturated fatty acids, such as C22:6 (docosahexaenoic acid—DHA), C20:5 (eicosapentaenoic acid—EPA) and C18:3 (linolenic acid) could reduce the incidence of ischemic heart disease (Clandinin, Foxwell, Yeow, Goh, & Jumpsen, 1997; Caggiula & Mustad, 1997; Harris, 1989). Omega 3 fatty acids (n3PUFA) are precursors of the eicosainoid compounds

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(3 and 5 series) which present greater antithrombotic and vasodilating effect. Proportional increases of the omega3 fatty acids in the diet are associated with reductions of total cholesterol and tryacylglycerol levels in serum, and in consequence, lower VLDL (very-low density lipoprotein) synthesis in the liver and higher removal of the cholesterol by the HDL (high density lipoprotein) in the process known as "reserve transport" (Eritsland, Arnesen, Seljeflot, & Hostmark, 1995; Yaniv, Schaffermann, Shamir, & Mader; 1999). Despite the large number of studies on supplementation with omega 3 fatty acids, both on humans and animals, the effect of these fatty acids on the plasma lipid profile is still debatable (Harris, 1996; Trautwein, 2001).

Current research also indicates the hypolipidemic effects of the ingestion of certain vegetal substances resistant to digestion by human gastrointestinal enzymes, denoted "dietary fibres", with these effects varying according to fibre solubility in water (Eastwood & Passmore, 1983; Prosky, 2000). Studies on soluble fibres such as pectin, guar resin, psyllium and oatmeal have suggested that these substances may reduce total cholesterol and LDL-cholesterol (Glore, Von Treeck, Knehans, & Guild, 1994). There is evidence suggesting that some soluble fibres may bind to bile salts or to cholesterol during the formation of intraluminal micelles, reducing the levels of cholesterol in hepatic cells, leading to a greater activation of LDL-receptors and therefore to increased LDL-cholesterol clearance. Another hypothesis centres on the inhibition of liver cholesterol synthesis by fermentation products (short-chain volatile fatty acids) or changes in intestinal motility with increased macronutrient absorption and satiety, with a reduction in total calorie ingestion (Brown, Rosner, Willet, & Sacks, 1999; Mekki et al., 1997; Trautwein, Kunath-Rau, & Erbersdobler, 1998). However, the extent of cholesterol reduction induced by soluble fibres is widely variable, especially as a function of the type of fibre consumed, the dose reached, other dietary variations, and the type of individuals evaluated in the studies (Anderson, Jones, & Riddell-Mason, 1994; Brown et al., 1999; Leontowicz, Goristein, Bartnikowska, Leontowicz, Kulasek, & Trakhtemberg, 2001; Stark & Madar, 1999). Actually, it is very difficult to isolate the fibre effect in human studies (van Horn, 1997).

The market for functional foods has reached a significant level and is expected to grow in several countries (Bland & Medcalf, 1999). However, it is important to point out that the maximum expression of the real functional properties of these foods must be reconciled with the sensory acceptance of the food that is being developed. Thus, the objective of the present study was to evaluate the sensory acceptability of a dairy formulation supplemented with different concentrations of omega-3 polyunsaturated fatty acids and soluble fibres by response surface methodology (RSM).

## 2. Material and methods

### 2.1. Ingredients

The ingredients of the dairy formulations were obtained from local sources. The n-3PUFA used was a commercial dried product (Dryn-3®) from BASF Health and Nutrition (DK), containing 35% of EPA + DHA in relation to total lipids. This is a marine fish oil concentrate with oxidative stability and sensory adequacy insured by micro encapsulation of the product, which is embedded in a matrix of gelatin and sucrose and coated with starch. The product contains sodium ascorbate, ascorbic acid and tocopherol as antioxidants and tricalcium phosphate as an anti-caking agent. This process permits its water solubility when it is premixed with other ingredients, as done in the present study.

The soluble fibre mixture was prepared by mixing oat flour, guar gum (Colloides Naturels International) and pre-gelatinized cornstarch (National Starch & Chemical) at respective proportions of 82.5/2.5/15.0, with the last hydrocolloid being added only as a thickening agent. The composition of these ingredients is shown in Table 1.

### 2.2. Experimental design

A full factorial design ($3^2$) with three levels of variation ($-1$, 0, $+1$) was employed to study the sensory response "$y$" (Barros Neto, Scarminio, & Bruns, 2001). The proportion of n3PUFA ($x_1$) and the SF ($x_2$) were independent variables studied to optimize "$y$" (Table 2). The central point (0.0) was performed twice as a true repetition. A quadratic polynomial regression model was assumed for prediction purpose of the response ($y$). The model proposed was:

$$y = \beta o + \Sigma \beta_i x_i + \Sigma \beta_{ii} x_i^2 + \Sigma\Sigma \beta_{ij} x_i x_j \quad (1)$$

where $\beta o$, $\beta_i$ $\beta_{ii}$ and $\beta_{ij}$ are intercept, linear, quadratic and interaction regression coefficients of the model, respectively, and $x_i$ and $x_j$ are coded independent variables. The Statistica 6.0 software was used for regression analysis. Response surfaces and contour plots were developed using the fitted quadratic polynomial equations.

### 2.3. Preparation of the dairy formulations

The ingredients of each formulation were mixed according to a factorial design (Table 3) and all samples were coded with three numbers and placed in plastic bottles containing 37.5 g of the powder formulation. The plastic bottles were marked with a line indicating the amount of water to be added by the taster at tasting time and were labelled with the information concerning

Table 1
Nutritional composition of ingredients selected as variables in this study: n3PUFA and soluble fibre mixture (g/100 g)

| Nutrients[a] | n-3PUFA[b] | Soluble fibre mixture[c] |
|---|---|---|
| Moisture | 5.2±0.4 | 7.9±0.1 |
| Protein (N×6.25) | 20.8±0.5 | 13.9±0.2 |
| Carbohydrate[d] | 47.6 | 56.0 |
| Lipid | 25.0±0.1 | 9.5±0.3 |
| Monounsaturated fatty acids[e] | 6.5±0.14 | 4.0±0.00 |
| Polyunsaturated fatty acids[e] | 9.1±0.35 | 3.6±0.01 |
| Saturated fatty acids[e] | 7.7±0.08 | 1.7±0.02 |
| 16:1 | 2.55±0.08 | 0.01±0.00 |
| 18:1 | 3.62±0.06 | 3.80±0.00 |
| 20:1 | 0.34±0.00 | 0.07±0.00 |
| 22:1 | – | 0.09±0.00 |
| 18:2 (n-6) | 0.37±0.01 | 3.49±0.01 |
| 18:3 (n-3) | 0.18±0.00 | 0.08±0.00 |
| 18:4 | 0.60±0.01 | – |
| 20:4 | 0.26±0.01 | – |
| 20:5 (EPA) | 4.51±0.14 | – |
| 22:5 (n-3) | 0.55±0.02 | – |
| 22:6 (DHA) | 2.65±0.08 | – |
| 14:0 | 2.10±0.06 | 0.02±0.00 |
| 15:0 | 0.14±0.00 | – |
| 16:0 | 4.56±0.14 | 1.51±0.00 |
| 18:0 | 0.93±0.01 | 0.16±0.00 |
| 20:0 | – | 0.01±0.00 |
| 22:0 | – | 0.04±0.00 |
| Not identified[e] | 1.6±0.41 | 0.2±0.00 |
| Cholesterol | 0.04±0.00 | N.D. |
| Ashes | 1.4btc0.1 | 1.7±0.1 |
| Dietetic fibre[f] | N.D. | 11.0 |
| Soluble fibre | N.D. | 3.3 |
| Insoluble fibre | N.D. | 7.7 |
| Energy (kcal) | 498.6 | 365.1 |

N.D., not determined.
[a] Values are means±SD ($n=3$).
[b] Dryn-3® (Basf Health & Nutrition S/A; Denmark) used as source of n-3PUFA.
[c] Soluble fibres mixture (%): 15.0 g of pre-gelatinized cornstarch + 82.5 g of oat flour + 2.5 g of guar gum.
[d] Values obtained by difference.
[e] Values are part of total lipids.
[f] Values are means ($n=4$).

the preparation and consumption of the product. Kits containing one sample and a questionnaire were prepared (Fig. 1).

### 2.4. Sensory analysis

For the evaluation of the overall acceptability of each dairy mixture we used a seven-point hedonic scale ranging from "dislike extremely" to "like extremely" (Morales, 1994). A total of 240 patients from the cardiology outpatient clinic of a private hospital received a sample of the mixture coded with three digits and responded to the sensory test. Each data point from sensory analysis represents an average of twenty-four panellists. Of these patients, 137 (95 female and 42 male) also responded to a questionnaire with 26 questions concerning some risk factors for cardiovascular disease. Some of these questions were selected to this study to identify the target public profile by significant correlations.

### 2.5. Statistical analysis

Data were analysed statistically by analysis of variance (ANOVA) and by the Tukey test for the identification of significant contrasts (Bower, 1998), with the level of significance set at $\alpha=0.05$ (5%) for all assays and regressions. The coefficients of the regression model, the analysis of variance and the graphic representation of the response surface were obtained using the STATISTICA 6.0 software. Pure error assessment was based on replication of the central point (0.0), as suggested in the design. The significance of the equation parameters for the sensory response variable was assessed by *F*-test. Multiple correspondence multivariate analyses were applied to the information obtained from the questionnaires using the SPAD-N 3.5 software (Crivisqui, 1997).

### 2.6. Fibre analysis

The ingredients and fibres of the formulations were analysed by an enzymatic and gravimetric method (AOAC, 1990) modified by Prosky, Asp, Schweizer, De Vries, and Furda (1992). Quadruplicate samples (1 g) were digested with thermo-stable alpha-amylase, pH 6.0, protease, pH 7.5 and amyloglucosidase, pH 4.3, to remove protein and starch. The hydrolysate was vacuum-filtered, using crucibles prewashed with an extran solution and combusted with glass wool, to separate the soluble fraction from the insoluble one. Four volumes of 98% ethanol were added to precipitate the SF. The residue was filtered, washed with 78% ethanol, 95% ethanol, and acetone, and dried and the residue was weighed. A duplicate was analysed for protein and another was incinerated at 525 °C for ash determination.

$$\%\ \text{Insoluble fibre (IF)} = [(\text{IR} - \text{P} - \text{A} - \text{Iw}/\text{W})] \times 100$$

where IR = mean insoluble residue of the sample (mg); P = mean protein content of the IR (mg); A = mean ash content of the IR (mg); W = mean sample weight (mg); Iw = insoluble white.

$$\%\ \text{Soluble fibre (SF)} = [(\text{SR} - \text{P} - \text{A} - \text{Sw}/\text{W})] \times 100$$

where SR = mean soluble residue of the samples (mg); P = mean protein content of the SR (mg); A = mean ash

Table 2
Full experimental design ($3^2$), where the independent variables n3PUFA ($x_1$) and soluble fibre mixture ($x_2$) were applied in the variation levels (−1, 0, +1)

| Tests | ($x^1$, $x^2$) | n-3PUFA ($x_1$) | | | | SF ($x_2$) | | |
|---|---|---|---|---|---|---|---|---|
| | | Dryn-3®2 | Soya oil[a] | n-3PUFA[a] | n-3PUFA[b] | SFM[a] | SF[a] | SF[b] |
| 1[c] | (−1, −1) | 0.0 | 10.0 | 0.5 | 0.1 | 0.0 | 0.4 | 0.1 |
| 2 | (0, −1) | 20.0 | 5.0 | 1.9 | 0.5 | 0.0 | 0.4 | 0.1 |
| 3 | (+1, −1) | 40.0 | 0.0 | 3.2 | 0.8 | 0.0 | 0.4 | 0.1 |
| 4 | (−1, 0) | 0.0 | 10.0 | 0.5 | 0.1 | 10.0 | 0.8 | 0.2 |
| 5 | (0, 0) | 20.0 | 5.0 | 1.9 | 0.5 | 10.0 | 0.8 | 0.2 |
| 6 | (+1, 0) | 40.0 | 0.0 | 3.2 | 0.8 | 10.0 | 0.8 | 0.2 |
| 7 | (−1, +1) | 0.0 | 10.0 | 0.5 | 0.1 | 20.0 | 1.1 | 0.3 |
| 8 | (0, +1) | 20.0 | 5.0 | 1.9 | 0.5 | 20.0 | 1.1 | 0.3 |
| 9 | (+1, +1) | 40.0 | 0.0 | 3.2 | 0.8 | 20.0 | 1.1 | 0.3 |

[a] Values expressed by g/100 g of milk formulation.
[b] Values expressed by g/100 kcal of milk formulation.
[c] This test was assumed as placebo, without any supplementation.

Table 3
Dairy milk formulations composition

| Ingredients[a] (g/100 g) | Tests |
|---|---|
| Skin milk | 31.27 |
| Artificial sweeter[b] | 0.11 |
| Cocoa powder | 5.00 |
| Natural flavour[c] | 0.60 |
| Malt extract | 3.00 |
| Vitamins A, D and E[d] | 0.02 |
| Dextrin | 0.00–50.00 |
| Soluble fibre mix[e] | 20.00–0.00 |
| Soy oil | 0.00–10.00 |
| N3PUFA[f] | 40.00–0.00 |
| Total | 100.00 |

[a] Dextrin, soluble fibre mixture, soy oil and n3PUFA values changed in function of the experimental design (Table 2).
[b] Aspartame and Acesulfame K (0.10% and 0.010% respectively).
[c] Natural Vanillin (Duas Rodas Ind.).
[d] Vitamins (Basf Health & Nutrition, DK.).
[e] Soluble fibre mixture formulated with 82.5% of oat flour, 15.0% of pre-gelatinized cornstarch and 2.5% of guar gum.
[f] Long Chain Polyunsaturated Fatty Acids – Dry-n3 (Basf Health & Nutrition, DK.) content 35% of EPA + DHA.

content of the SR (mg); W = mean sample weight (mg); Sw = soluble white.

### 2.7. Cholesterol and fatty acid analyses

Ingredients and formulation samples were submitted to lipid fraction extraction by the method of Folch, Lees, Sloanne, and Stanley (1957) and the fatty acids were esterified by the method of Hartman and Lago (1973). The solutions were injected into a gas chromatograph (GC17A Shimadzu Class CG) equipped with a 30 m×0.25 mm (inner diameter) fused silica capillary column (Supelcowax) and a flame ionization detector. Helium was used as a carrier gas and the fatty acids were separated using a gradient from 80 to 150 °C (10 °C/min) and then at 6 °C/min until 230 °C. Fatty acids were calculated from the peak areas relative to the peak area of the internal standard. The cholesterol fraction of the Dryn-3® product was determined using the same gas chromatography equipment with a 30 m×0.25 mm (inner diameter) fused silica capillary column (J & W DB-5). Helium was used as a carrier gas (1.5 ml/min) and a temperature gradient from 180 to 280 °C (20 °C/min) for 10 min was used according to the method of Naeemi, Ahmd, Al Sharrah, and Behbahani (1995).

## 3. Results and Dscussion

Table 4 presents the nutritional composition and the values of supplementation with n3PUFA and SF for the 10 formulations evaluated in the present study. Both n3PUFA and SF concentrations, in relation to total energy amount, were first established as a function of the expected real human consumption of these two nutrients and of the practical limitation of obtaining a homogeneous mixture that could be measured industrially in individual packages. Thus, an attempt was made to elaborate a study whose results could effectively be applied to the development of a new food, adequate both in terms of basic processing conditions and in terms of the normal consumption habits of the target population. The purpose was to enrich a food with functional nutrients, such as n3PUFA and SF, thus excluding any need for supplementation with capsules or other forms of medication. The range adopted for the present study was 0.1–0.8 g/100 kcal n3PUFA and 0.1–0.3 g/100 kcal SF. Based on this value and on the consumption of two glasses of dairy formula/day (≈500 ml), maximum daily ingestion represented by assay (+1.+1) would be approximately 3.2 g n3PUFA and 1.1 g SF (Table 4).

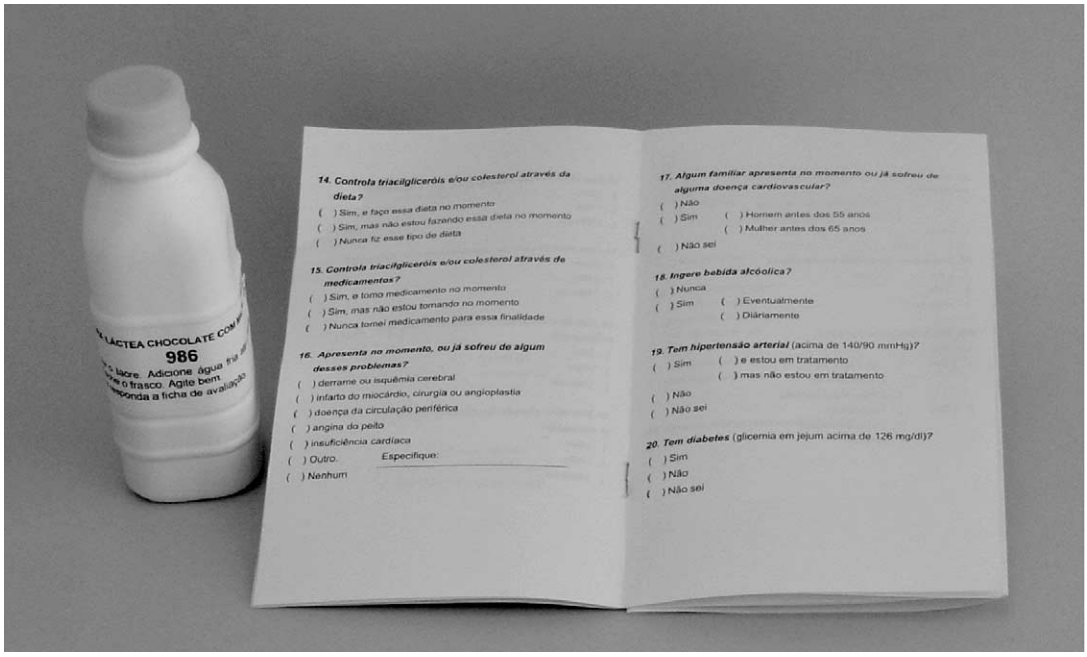


Fig. 1. Individual "kit" for sensory analysis content of one sample of the dairy formulation "easy-to-drink" and one questionnaire.

Table 4
Nutritional values of the dairy formulations

| Nutritional value[a] (g/100 g) | (−1,−1) | (0,−1) | (+1,−1) | (−1,0) | (0,0) | (0,0)[b] | (+1,0) | (−1,+1) | (0,+1) | (+1,+1) | (−0.2,−0.3) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Moisture | 7.6±0.6 | 7.2±0.5 | 7.6±0.4 | 9.4±0.4 | 7.9±0.6 | 9.0±0.5 | 7.2±0.3 | 8.9±0.1 | 8.0±0.8 | 8.4±0.7 | 5.7±0.3 |
| Protein[c] | 12.8±0.3 | 16.9±0.6 | 24.4±0.2 | 14.4±0.1 | 18.3±0.7 | 19.1±3.5 | 25.0±0.6 | 15.5±0.4 | 19.7±0.5 | 25.9±0.9 | 17.06±0.9 |
| Fat | 13.3±0.4 | 11.4±0.2 | 11.4±0.2 | 12.5±0.4 | 12.2±0.1 | 12.6±0.4 | 10.8±0.1 | 12.9±0.2 | 13.0±0.5 | 13.0±0.1 | 10.0±0.4 |
| n3PUFA[d] | 0.5 | 1.9 | 3.2 | 0.5 | 1.9 | 1.9 | 3.2 | 0.5 | 1.9 | 3.2 | 1.6 |
| Carbohydrates[e] | 60.9±0.5 | 59.0±0.7 | 51.3±0.6 | 58.1±0.6 | 56.0±1.2 | 53.8±2.8 | 51.4±0.6 | 56.9±0.6 | 53.7±1.7 | 47.1±1.1 | 62.2±0.8 |
| Ashes | 5.4±0.1 | 5.5±0.1 | 5.3±0.1 | 5.6±0.2 | 5.6±0.1 | 5.5±0.1 | 5.6±0.1 | 5.8±0.1 | 5.6±0.1 | 5. 7±0.1 | 5.6±0.1 |
| Soluble fibres[f] | 0.4 | 0.4 | 0.4 | 0.8 | 0.8 | 0.8 | 0.8 | 1.1 | 1.1 | 1.1 | 0.6 |
| Energy (kcal)[g] | 414.5 | 406.2 | 405.4 | 402.6 | 409.8 | 405.2 | 402.7 | 405.8 | 410.3 | 408.5 | 407.6 |
| n3PUFA g/100 kcal | 0.0 | 0.5 | 0.8 | 0.0 | 0.5 | 0.5 | 0.8 | 0.0 | 0.5 | 0.8 | 0.41 |
| SF g/100 kcal | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.15 |

[a] Mean±SD ($n=3$).
[b] Central point replication.
[c] This difference was due the gelatine present in Dryn-3®, without nutritional value (Castro, Tirapegui, & Silva, 2000).
[d] These values are means of each group content different proportion of fatty acids.
[e] Values are obtained by difference.
[f] These values are means of each group contain different proportion of soluble fibres.
[g] Energy values were calculated using the Atwater factors of 4,4, and 9 kcal/g for protein, carbohydrate and fat, respectively.

Harris (1996) reviewed some studies of dietary supplementation with n3PUFA (EPA + DHA) in humans with a mean daily ingestion always lower than 7 g and observed a substantial, persistent and dose-dependent hypotriglycemic effect. Lovegrove, Brooks, Murphy, Gould, and Williams (1997) supplemented nine healthy normotriacylglycerolemic male volunteers with 1.4 g EPA + DHA a day for two periods of 22 days each. The authors did not observe a significant hypotriglycemic effect and attributed this negative result to the increased fat ingestion necessary for supplementation with n3PUFA itself and a consequent weight gain by the individuals. However, as pointed out by Gibney (1997), this was one of the few studies in which supplementation with n3PUFA consisted exclusively of food enrichment. In that study the authors also evaluated the sensory acceptability of the products and observed no significant difference between the products enriched with n3PUFA and their controls, suggesting that the development of functional foods could be an excellent alternative to increase the ingestion of nutrients with nutraceutic components, such as n3PUFA and fibres.

Also according to Harris et al. (1990), 10–15% reduction in total cholesterol in hypercholesterolaemic individuals (>300 mg/dl) would be reached only after the ingestion of relatively large amounts of n3PUFA (3–6 g/day) during a period of at least 5–8 weeks. In a study conducted on hypertriglycemic patients ingesting 3.4 g/day of n3PUFA (EPA + DHA) for 2 weeks, the authors observed that n3PUFA reduced the serum concentrations of triacylglycerols in addition to also improving various mechanisms related to lipidic risk factors.

If there is no consensus about the doses used in studies of dietary supplementation with n3PUFA in humans, the picture is even less clear with respect to soluble fibres supplementation. Most studies favoured doses ranging from 34 g total dietary fibre (2.5 g soluble fibre) to 123 g (10.3g soluble fibre). In a meta-analysis, Brown et al. (1999) concluded that, within a possible range of soluble fibre consumption (2–10 g/day), a small but significant reduction in total cholesterol could be observed. As an example, the ingestion of 3 g soluble fibre derived from oats could reduce LDL-cholesterol by about 0.13 mmol/l. On average, blood cholesterol levels can be reduced by an estimated 5–15% through the diet approach, with some hyperlipidemic patients experiencing even great reductions (van Horn, 1997).

Table 5 presents the mean sensory score defined by the 240 patients (panellists) for the 10 formulations evaluated. None of the formulations was scored less than "regular" in terms of mean evaluation. This result may have been due to the addition of flavouring substances, such as soluble powdered cocoa, malt extract and vanilla, to an appropriate combination of sweeteners, or to the addition of antioxidants, such as α-tocopherol, which prevent the oxidation of the fat present and consequently conserve taste. These factors may have contributed to the reduction of the negative sensory effect, especially of powdered marine fat, the source of n3PUFA used in the assays. Another factor that justifies the good acceptability of these formulations may be the place where the sensory evaluation was done (hospital). The tasters may have compared the formulations with medicine and not with food.

Table 5
Sensory evaluation of the dairy formulations in the full factorial design ($3^2$)

| Treatment ($x_1$,$x_2$) | Formula code | Acceptability[a] |
|---|---|---|
| (−1,−1) | 967 | 6.17 ± 1.01ab |
| (0,−1) | 541 | 5.71 ± 1.20ab |
| (+1,−1) | 195 | 4.50 ± 0.51cd |
| (−1,0) | 219 | 6.38 ± 0.82a |
| (0,0) | 352 | 6.04 ± 0.91ab |
| (+1,0) | 674 | 5.17 ± 1.20bc |
| (−1,+1) | 436 | 4.75 ± 1.11cd |
| (0,+1) | 823 | 4.63 ± 1.66cd |
| (+1,+1) | 986 | 4.08 ± 1.10d |
| (0,0)[b] | 709 | 5.46 ± 1.28abc |

[a] Mean ± SD ($n = 240$). Means with same letters are not significantly different ($P > 0.05$) using ANOVA and the Tukey test.
[b] True Central Point Replication.

The data in Table 5 were processed in order to find a polynomial model that could estimate the sensory response ($y$) as a function of variations in $x_1$ and $x_2$ (n3PUFA and SF) in the composition of the formulations. The RSM developed by Box and Draper (1987) has been applied with success in some sensories studies (Castro, Tirapegui, & Silva, 1998). The regression models (linear and quadratic) were significant ($P < 0.05$), suggesting the rejection of the null hypothesis for the fitted coefficients involved. Since the lack of fit of the linear model was significant, we opted for a more complete model, such as quadratic regression, that would include the effects of the interactions in the response. Data were analysed by non-linear regression to fit the following second order equation:

$$y(x) = 5.84 - 0.59x_1 - 0.49x_2 - 0.16\ x_{11} - 0.76x_{22} + 0.25x_1x_2$$

where $y(x)$ = estimate of the sensory score and $x_i$ = proportion of each independent variable as g/100 kcal formulation. The model showed a non-significant lack of fit ($P > 0.05$) and a coefficient of determination ($R^2$) of 95.76%, considered adequate as percentage of the variation explained (Cornell, 1990). In short, the regression coefficients showed that n3PUFA and SF had linear effects on overall acceptability. The SF had a more important quadratic effect, and it is not necessary to pay attention to the interaction effect.

Using the Response Surface obtained by quadratic regression (Fig. 2), we observed a negative effect of the addition of n3PUFA to the formulation, as well as a positive sensory effect of the intermediate values of soluble fibres. Despite the high level of refinement of the raw material used as a source of n3PUFA (EPA + DHA), this is a product of marine origin (refined fish oil) with greater susceptibility to oxidative degradation. Because of potential contamination, rancidity and the occurrence of unwanted tastes and flavours, alternatives to fish oils are currently being explored; these include n3PUFA extractions from fungi and algae (Trautwein, 2001). In the case of soluble fibres, their ability to thicken without heating confers a bulkier aspect, a fact of great sensory importance when skim milk is used. However, an excess of these hydrocolloids may leave the product too thick, impairing its full dilution.

The optimization of the sensory response ($y$) by derivation techniques led to a value outside the range evaluated for $x_1$. On this basis, we opted for the graphic identification (Fig. 2) of a "compromise solution" that would satisfy a critical sensory value of 6.0 (very good

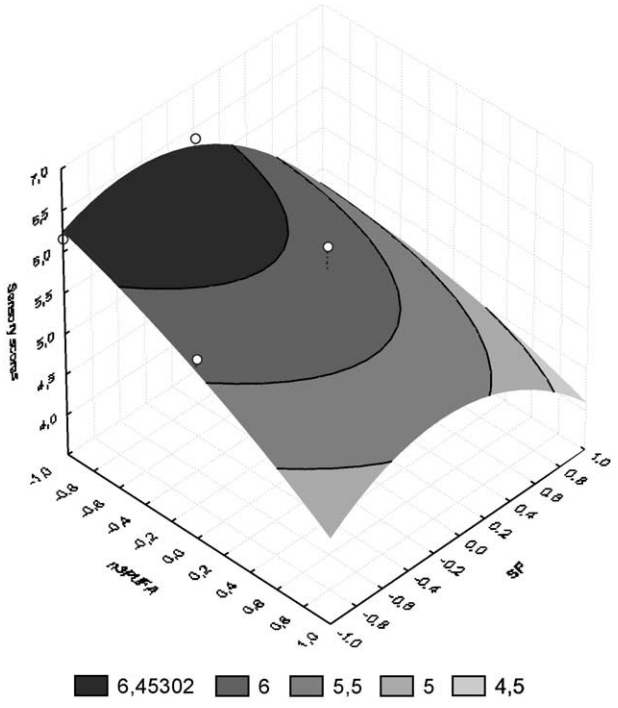


Fig. 2. Response surface plot of acceptability (overall liking) as a function of n3PUFA and SF concentrations.

to excellent acceptability) for the final formulation using the maximum possible concentration of n3PUFA and SF. We then defined the values of $x_1 = -0.20$ and $x_2 = -0.30$, with their sensory estimate reaching 6.05 and fully satisfying the preestablished criterion for acceptability of the dairy formulation. Thus, the "compromise solution" would correspond to about 0.41 g n3PUFA and 0.15 g SF in 100 kcal of dairy formulation, presenting a sensory concept equivalent to "very good". Considering a consumption of two daily glasses (≈500 ml), the daily supplementation with n3PUFA would be of the order of 1.6 g and the daily supplementation with SF would be 0.6 g (Table 4). Allied to an effective dietary reduction of consumption of other fats, especially saturated fats, trans fatty acids and cholesterol, and to a healthier life style, including the practice of physical exercise, this supplementation could significantly contribute to a reduction of risk factors for cardiovascular disease.

Multivariate analysis, using the correspondence technique, was applied, in order to identify the characteristics of the target population for the "functional dairy formulation" developed in the present study. This procedure allows plotting individuals and characteristics in the same *p*-dimensional space (Castro, Tirapegui, &

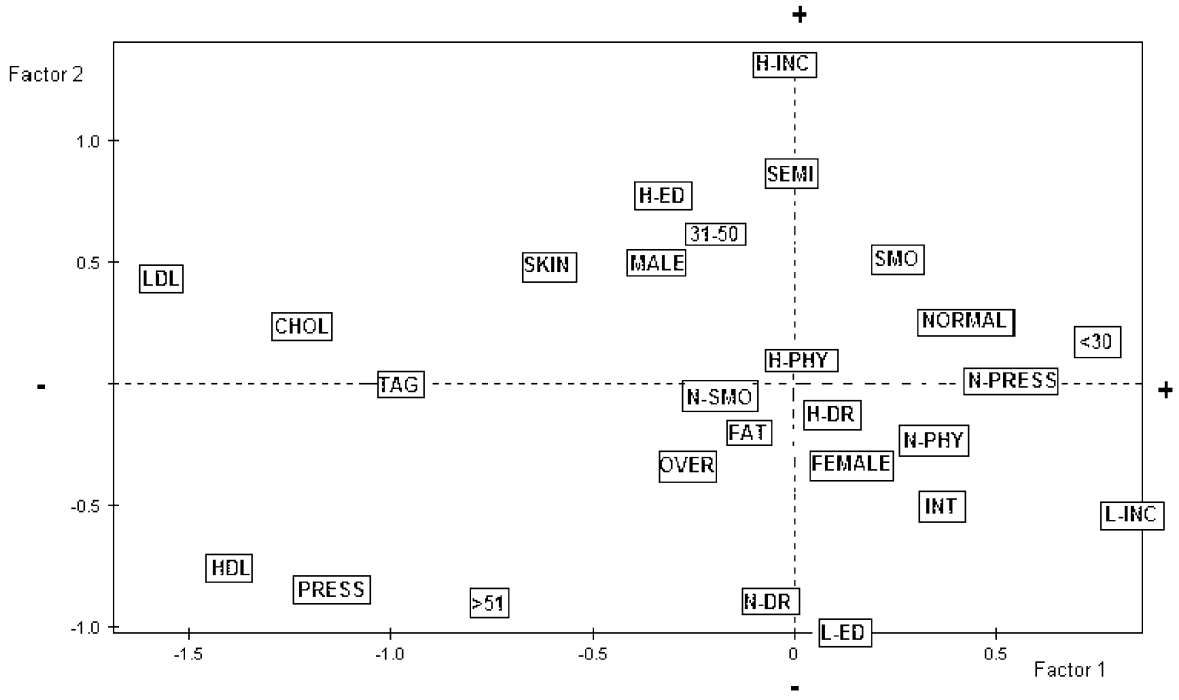


Fig. 3. Graphic presentation of the multivariate analysis using correspondence technique. Key abbreviations—(>30): age <30 years old, (31–50): age: 31–50 years old, (>50): age >50 years old; (CHOL): serum cholesterol level >200 mg/dL; (TAG): serum triacylglycerol level >200 mg/dL; (LDL): serum LDL-cholesterol level >130 mg/dL; (HDL): serum HDL-cholesterol level <35mg/dL; (SMO): smoke more than 20 cigarettes/day; (N-SMO): no smoke; (H-INC): high income (>27.000 US/year); (L-INC): low income (<4.500 US/year); (H-DR): alcohol consumption daily; (N-DR): no alcohol consumption; (FAT): BMI >27; (OVER): for women and 25 for men BMI >27; (NORMAL): BMI normal; (SKIN): skin milk consumption; (SEMI): half-skin consumption; (INT): integral milk consumption; (H-ED): high educational level; (L-ED): low educational level; (PRESS): control of blood pressure with drugs; (N-PRESS): normal blood pressure <140/90 mmHg; (PHY): intense practice of physical activities; (N-PHY): no practice of physical activities.

Silva, 2002). A total of 137 patients were interviewed with respect to different risk factors for cardiovascular disease. Fig. 3 presents some interesting correlations detected in the profile of the patients evaluated. The variables that contributed most to the factor 01 were the lipoprotein serum (LDL, HDL), total cholesterol, triacylglycerol and the arterial pressure. On the other hand, the variables income, education and sex contributed much more to the factor 02 (Table 6). Male patients were found to have higher educational levels and incomes, factors that might explain the greater access to the information and consumption of skim or semi-skim milk. Female patients showed a higher association with lower educational levels and incomes, absence of practice of physical exercise, lower alcohol and cigarette consumptions, and greater consumption of whole milk. As expected, there was a strong correlation between high total cholesterol, LDL-cholesterol and triacylglycerol levels. An association between HDL-cholesterol concentration and high blood pressure was also observed among patients older than 50 years. For women, after their 40s, the progressive decline in estrogen production is probably the most important factor responsible for the higher total cholesterol levels, secondary to an increase in low density lipoprotein (LDL) cholesterol and a decrease in high density lipoprotein (HDL) cholesterol levels (Guimarães et al., 1998). Lower income, normal BMI and regular arterial pressure were more associated with younger people (>30) while lower alcohol ingestion was associated with lower education and income level of the patients.

Data obtained by the contingence table, used for the multiple correspondence multivariate analysis, containing 137 cases and 26 variables of the questionnaire, also showed that, although 53% of the patients had a BMI above normal (>24 for women and >25 for men), 67% did not follow any type of dietary control. A positive result, however, was that 88% did not smoke or had stopped smoking for more than 2 years. This agrees with the studies of Guimarães et al. (1998), in which 75.9% of the evaluated population ($n = 8.045$) did not smoke and, among the smokers, the predominance was of the men of higher social class. These features reflect the importance of public awareness campaigns that effectively contributed to the reduction of the proportion of smokers, especially in populations with a tendency to develop cardiovascular disease. It is also clear that these campaigns should be continued, also directed at better nutritional orientation, reduction in alcohol consumption and encouragement of the practice of physical activity. The lack of information mainly involves lower socioeconomic classes. Among the patients who habitually consumed milk on a daily basis, 66% consumed whole milk even though they had access to skim or semi-skim milk. This is a high level in a population at risk for cardiovascular disease.

## 4. Conclusions

A food with functional properties should be developed with sensory acceptability and a level of daily ingestion, compatible with the habits of the consumer. The "functional" nutrient used to enrich this food should be ingested in adequate amounts for maximal expression of its potentially beneficial effects on human health.

The statistical methodology of surface response (RSM) used in the present study was very efficient for optimization of a milk formulation for human consumption enriched with two different types of functional

Table 6
Contribution of the variables to the factors (1) and (2)

| Variables[a] | Factor 1 | | Factor 2 | |
|---|---|---|---|---|
| Distribution (side) | Left (−) | Right (+) | Top (+) | Bottom(−) |
| Higher % of contribution | ↑[CHOL, TAG, LDL,HDL] | ↓[CHOL, TAG, LDL,HDL] | H-INC<br>H-ED | L-INC<br>L-ED |
| | PRESS | N-PRESS | MALE | FEMALE |
| Age | >50 | >30 | 31–50 | >50 |
| Alcohol consumption | – | H-DR | – | N-DR |
| BMI | FAT/OVER | NORMAL | – | – |
| Physical activities | – | – | H-PHY | N-PHY |
| Milk consumption | SKIN | INT | SEMI/SKIN | INT |
| Smoke | – | – | H-SMO | N-SMO |

[a] (>30): age <30 years old, (31–50): age: 31–50 years old, (>50): age >50 years old; (CHOL): serum cholesterol level >200 mg/dL; (TAG): serum triacylglycerol level >200 mg/dL; (LDL): serum LDL-cholesterol level >130 mg/dL; (HDL): serum HDL-cholesterol level <35 mg/dL; (SMO): smoke more than 20 cigarettes/day; (N-SMO): no smoke; (H-INC): high income (>27.000 US/year); (L-INC): low income (<4.500 US/year); (H-DR): alcohol consumption daily; (N-DR): no alcohol consumption; (FAT): BMI >27; (OVER): for women and 25 for men BMI >27 ; (NORMAL): BMI normal; (SKIN): skin milk consumption; (SEMI): half-skin consumption; (INT): integral milk consumption; (H-ED): high educational level; (L-ED): low educational level; (PRESS): control of blood pressure with drugs; (N-PRESS): normal blood pressure <140/90 mmHg; (PHY): intense practice of physical activities; (N-PHY): no practice of physical activities.

nutrients, i.e. n3PUFA and SF. Doses defined according to a sensory criterion could contribute to a supplementation of approximately 1.6 g n3PUFA and 0.6 g SF, considering a daily consumption of only two glasses (500 ml) of the diluted product. These values may seem to be low compared to the mega doses commonly reported in other studies, but they imply a real possible consumption of these nutrients through food enrichment.

Multiple correspondence factorial analysis, applied to the characterization of the profile of functional food consumers, clearly demonstrated the importance of public campaigns for the control of risk factors for cardiovascular disease, providing guidelines for better nutrition, reduction of alcohol consumption and the practice of moderate physical activity.

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## References

Alexander, H., Lockwood, L., Harris, M. A., & Melby, C. L. (1999). Risk factors for cardiovascular disease and diabetes in two groups of Hispanic Americans with differing dietary habits. *Journal of American College of Nutrition*, *18*(2), 127–136.

Anderson, J. W., Jones, A. E., & Riddell-Mason, S. (1994). Ten different dietary fibres have significantly different effects on serum and liver lipids of cholesterol-fed rats. *Journal of Nutrition*, *124*, 78–83.

Association of Official Analytical Chemists AOAC. (1990). *Official methods of analysis of the AOAC*. Washington, DC: Association of Official Analytical Chemists.

Barros Neto, B., Scarminio, I. S., & Bruns, R. E. (2001). *Como fazer experimentos: pesquisa e desenvolvimento na ciência e na indústria*. Campinas, SP: Editora da Unicamp.

Bland, J., & Medcalf, D. G. (1999). Future prospects for functional foods. In I. Goldberg (Ed.), *Functional foods. Designer foods, pharmafoods, nutraceuticals* (pp. 537–553). Maryland: Aspen Punblishers.

Bower, J. A. (1998). Statistics for food science V: ANOVA and multiple comparisons (Part B). *Nutrition and Food Science*, *1*, 41–48.

Box, G. E. P., & Draper, N. R. (1987). *Empirical model-building and response surfaces*. New York: John Wiley & Sons.

Brown, L., Rosner, B., Willet, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fibre: a meta-analysis. *American Journal of Clinical Nutrition*, *69*(1), 30–42.

Caggiula, A. W., & Mustad, V. A. (1997). Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiologic studies. *American Journal of Clinical Nutrition*, *65*(5suppl), 1597S–1610S.

Castro, I. A., Tirapegui, J., & Silva, R. S. S. F. (1998). Development of protein mixtures and evaluation of their sensory properties using the statistical response surface methodology. *International Journal of Food Science and Nutrition*, *49*, 453–461.

Castro, I. A., Tirapegui, J., & Silva, R. S. S. F. (2000). Protein mixtures and their properties optimized by Statistical Response Surface Methodology. *Nutrition Research*, *20*, 1341–1353.

Castro, I. A., Tirapegui, J., & Silva, R. S. S. F. (2002). Application of multivariate statistical methods to the analysis of cost, nutritional and sensorial quality for some proteins used in food formulation. *Journal of Food Quality*, *25*, 83–90.

Clandinin, M. T., Foxwell, A., Yeow, K., Goh, K. L., & Jumpsen, J. A. (1997). Omega-3 fatty acid intake results in a relationship between the fatty acid composition of LDL cholesterol ester and LDL cholesterol content in humans. *Biochemical and Biophysical Acta—Lipids and Lipid Metabolism*, *1.346*(3), 247–252.

Connor, S. L. (1999). The healthy heart: challenges and opportunities for dietetics professionals in the 21st century. *Journal of American Diet Association*, *99*(2), 164–165.

Cornell, J. A. (1990). *Experiments with mixtures. designs, models and the analysis of mixture data*. Nova York: John Wiley & Sons.

Crivisqui, E. (1997). *Programme de reserche et dénsignement en statistique appliquée (PRESTA)*. Belgique: Presta.

Duthie, G. G., & Brown, K. M. (1999). Reducing the risk of cardiovascular disease. In I. Goldberg (Ed.), *Functional foods. Designer foods, pharmafoods, nutraceuticals* (pp. 19–39). Maryland: Aspen Publishers.

Eastwood, M. A., & Passmore, R. (1983). Dietary fibre. *Lancet*, *2*, 202–206.

Eritsland, J., Arnesen, H., Seljeflot, I., & Hostmark, A. T. (1995). Long-term metabolic effects of n-3 polyunsaturated fatty acids in patients with coronary artery disease. *American Journal of Clinical Nutrition*, *61*(4), 831–836.

Folch, J., Lees, M., Sloanne, S., & Stanley, G. H. (1957). A simple method for the isolation and purification of total lipid from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Fuster, V., & Pearson, T. A. (1996). 27th Bethesda Conference: matching the intensity of risk factor management with the hazard for coronary disease events. *Journal of the American College of Cardiology*, *27*, 957–1047.

Gibney, J. M. (1997). Incorporation of n-3 polyunsaturated fatty acids into processed foods. *Guest Editorial. British Journal of Nutrition*, *78*, 193–195.

Glore, S. R., Von Treeck, D., Knehans, A. W., & Guild, M. (1994). Soluble fibre and serum lipids: a literature review. *Journal of American Diet Association*, *94*(4), 425–436.

Guimarães, A. C., Lima, M., Mota, E., Lima, J. C., Martinez, T., Filho, A. C., Paes, J. N., Bertolami, M., Lion, M. F., Maranhão, M., Silva, O. F., Bodaneze, L. C., Dias, G. C., Macedo, V., & Neto, A. A. (1998). The cholesterol level of a selected Brazilian salaried population: biological and socioeconomic influences. *CVC Prevention*, *1*, 306–317.

Harris, W. S. (1989). Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *Journal of Lipid Research*, *30*, 785–807.

Harris, W. S. (1996). N-3 fatty acids and lipoproteins: comparison of results from human and animal studies. *Lipids*, *31*, 243–252.

Harris, W. S., Rothrock, D., Fanning, A., Inkeles, S., Goodnight, S. H., Illingworth, D. R., & Connor, W. E. (1990). Fish oils in hypertriglyceridemia: a dose–response study. *American Journal of Clinical Nutrition*, *51*(3), 399–406.

Leontowicz, M., Goristein, S., Bartnikowska, E., Leontowicz, H., Kulasek, G., & Trakhtemberg, S. (2001). Sugar beet pulp and apple pomace dietary fibres improve lipid metabolism in rats fed cholesterol. *Food Chemistry*, *72*, 73–78.

Lovegrove, J. A., Brooks, C. N., Murphy, M. C., Gould, B. J., & Williams, C. M. (1997). Use of manufactured foods enriched with fish oils as a means of increasing long-chain n-3 polynsatured fatty acids intake. *British Journal of Nutrition*, *78*, 223 – 236.

Mekki, N., Dubois, C., Charbonnier, M., Cara, L., Senft, M., Pauli, A. M., Portugal, H., Gassin, A. L., Lafont, H., & Lairon, D. (1997). Effects of lowering fat and increasing dietary fibre on fasting and postprandial plasma lipids in hypercholesterolaemic subjects consuming a mixed Mediterranean-western diet. *American Journal of Clinical Nutrition*, *66*(6), 1443–1451.

Morales, A. A. (1994). *La evalucación sensorial de los alimentos en la teoria y la práctica*. Zaragoza: Editorial Acribia.

Naeemi, E. D., Ahmd, N., Al Sharrah, T. K., & Behbahani, M. (1995). Rapid and simple method for determination of cholesterol in processed food. *Journal of AOAC International*, *78*, 1522–1525.

Prosky, L.(2000). What is dietary fibre? *Journal of AOAC International*, *83*, 985–987.

Prosky, L., Asp, N. G., Schweizer, T. F., De Vries, J. W., & Furda, I. (1992). Determination of insoluble and soluble dietary fibre in food and food products: collaborative study. *Journal of AOAC International*, *75*, 360–367.

Stark, A., & Madar, Z. (1999). Dietary fibre. In I. Goldberg (Ed.), *Functional foods. Designer foods, pharmafoods, nutraceuticals* (pp. 355–393). Maryland: Aspen Punblishers.

STATISTICA. (2002). *v.6.0. for window Vol. IV*. Tulsa-OK, USA: Statsoft Inc (software).

Thinker, L. F., Parks, E. J., Behr, S. R., Schneeman, B. O., & Davis, P. A. (1999). n-3Fatty acid supplementation in moderately hypertriglyceridemic adults changes post-prandial lipid and apolipoprotein B responses to a standardized test meal. *Journal of Nutrition*, *129*, 1126–1130.

Trautwein, E. A. (2001). n-3 Fatty acids—physiological and technical aspects for their use in food. *European Journal of Lipid Science and Technology*, *103*, 45–55.

Trautwein, E. A., Kunath-Rao, A., & Erbersdobler, F. (1998). Effect of different varieties of pectin and guar gum on plasma, hepatic and biliary lipids and cholesterol gallstone formation in hamsters fed on high-cholesterol diets. *British Journal of Nutrition*, *79*, 463–471.

van Horn, L. (1997). Fibre, lipids and coronary heart disease. A statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation*, *95*, 2701–2704.

Verges, B. (1998). Cardivascular risk and dyslipidemias. *Annales d'Endocrinologie*, *59*(4), 335–343.

Yaniv, Z., Schaffermann, D., Shamir, I., & Mader, Z. (1999). Cholesterol and triglyceride reduction in rats fed *Matthiola incana* seed oil rich in (n-3) fatty acids. *Journal of Agricultural Food Chemistry*, *47*, 637–642.