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Food supplementation for workers: flour enriched with omega -3

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ABSTRACT. The objective of this study was preparing a product (omega-3 flour) to increase the nutritional value of the food for workers concerning the content of omega-3 fatty acids (n-3 FA). The omega-3 flour was prepared using waste (head sardines and leaves of carrot), flaxseed flour, manioc flour and spices. The fatty acids (FA) concentration was analyzed by gas chromatography. A total of 28 FA were identified in the omega-3 flour. The concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were 329.23mg EPA 100 g^{-1} omega-3 flour and 545.35 mg DHA 100 g^{-1} omega-3 flour. To meet the minimum requirements of omega -3, it is necessary the intake 2.5 to 3 tablespoons (soup) of omega-3 flour day^{-1}.There were analyzed two meals (A and B) generally consumed by workers without and with the addition of the omega-3 flour (1 and 2 tablespoons) to verify if there was an increase of n-3 FA. It was concluded that there was a significant increase of these FA in both meals. It was found that the omega-3 flour is constituted of a good nutritional value, especially the n-3 FA, so the product can be used as a supplement in the feeding of the workers as well as in other segments.

Keywords: polyunsaturated fatty acids, head sardines, flaxseed, carrot leaves.

Suplementação alimentar para trabalhadores: farinha enriquecida com ômega -3

RESUMO. O objetivo deste estudo foi preparar um produto (farinha de ômega-3) para aumentar o valor nutricional dos alimentos dos trabalhadores em relaçao ao teor de ácidos graxos ômega-3 (n-3 AG). A farinha de ômega-3 foi preparada utilizando resíduos (cabeças de sardinhas e folhas de cenoura), farinha de linhaça, farinha de mandioca e temperos. A concentração dos ácidos graxos (AG) foi analisada por cromatografia em fase gasosa. Um total de 28 de AG foram identificados na farinha. A concentração do ácido eicosapentaenoico (EPA) e ácido docosahexaenoico (DHA) foram 329,23 mg EPA 100 g^{-1} farinha de ômega-3 e 545,35 mg DHA 100 g^{-1} farinha de ômega-3. Para atender as exigências mínimas de ômega-3, é necessária a ingestão de 2,5 a 3 colheres (sopa) de farinha de ômega-3 dia^{-1}. Foram analisadas duas refeições (A e B) geralmente consumidos pelos trabalhadores, sem e com a adição de farinha (1 e 2 colheres de sopa) para verificar se houve um aumento de AG n-3. Concluiu-se que houve aumento significativo destes AG em ambas as refeições.Verificou-se que a farinha de ômega-3 apresenta elevado valor nutricional, especialmente em relação aos AG n-3, de modo que o produto pode ser utilizado como suplemento na alimentação dos trabalhadores bem como em outros segmentos.

Palavras-chave: ácidos graxos poli-insaturados, cabeças de sardinha, linhaça, folhas de cenoura

Introduction

Nowadays as the nutritional requirements become increasingly related to good eating habits, the insertion of functional foods in the diet, such as the series of omega-3 fatty acids (n-3 FA) among other nutrients, in the feeding of workers enables people to improve their quality of life and it contributes to the prevention of certain diseases as well.

Alternative food, which is beneficial to the human health, can be obtained with the use of animal and vegetable waste as long as it has n-3 FA in their composition. The use of waste contributes to reduce the environmental pollution and the costs of production (GALDIOLI et al., 2001).

The heads of sardines and the leaves of carrots, which are usually discarded as waste, present in their composition essential fatty acids, such as the alpha-linolenic acid (18:3 n-3 - LNA) and the linoleic acid (18:2 n-6 - LA) that are not synthesized by the human body and must be obtained through the food diet (LEONARD et al., 2004; ALMEIDA et al., 2009; STEVANATO et al., 2010). The sardine heads present in their composition the eicosapentaenoic acid (20:5 n-3 - EPA) and the docosahexaenoic acid (22:6 n3 - DHA), LNA derivatives, and the arachidonic acid (21:4 n-6 - AA) LA derivative (STEVANATO et al., 2010). Omega-3 and omega -6 play an important role on the prevention of cardiovascular diseases (MATTAR; OBEID, 2009; RUSSO, 2009), chronic inflammatory diseases (MICKLEBOROUGH, 2009), as well as on the fetal growth and on the neurodevelopment. Moreover, it acts on the prevention of cancer (CALVIELLO et al., 2007).

The use of sardine heads and dried leaves of carrots in the form of flour can be inserted into workers' food through the preparation of food they customarily consume or through strategies established by their companies. Since this is a product aimed directly to the health improvement, it can also be used in other social-economic sectors, such as schools, hospitals and others, besides the domestic consumption.

This study aimed to increase the nutritional value of the food consumed by workers using a product (omega-3 flour) prepared with waste of sardine heads and carrot leaves, sources of omega-3. The flour plays an important role in the prevention of diseases and also in the use of these residues to prepare food.

Material and methods

Preparation of the Sardine Head Flour (SHF)

A total of 200 heads of sardines (*Sardinella brasiliensis*) were acquired in the fish market in the city of Maringá (Paraná State, Brazil). The sardine heads were washed with filtered water and steam-cooked (pans on the stove) for 25 minutes (STEVANATO et al., 2010). After cooking, the heads were grounded in an endless-screw grinder, placed on trays and dried in an oven for 2 - 4 hours at 180°C. Every 30 minutes the product was stirred in order to improve the cooking total. Next, the flour was sieved using a 16-mesh stainless steel sieve. The product obtained, referred to as sardine head flour (SHF), was kept in polyethylene bags in a nitrogen atmosphere, wrapped in aluminum foil and stored at -18ºC.

Preparation of Carrot Leaf Flour (CLF)

Leaves of carrot (organic, pesticide- and additive-free) were provided by a producer in the region of Maringá (Paraná State, Brazil). The leaves were collected from the same lot, washed in running water and hygienized with sodium hypochlorite (0.005%). After the removal of excess water, the leaves were dried in an oven with air circulation under the conditions of time and temperature equal to 43 hours and 70°C, respectively, according to ALMEIDA et al., 2009. The dried leaves were grounded (pulverized) in a knife mill. The product obtained, referred to as carrot leaf flour (CLF) was kept in polyethylene bags in a nitrogen atmosphere, wrapped in aluminum foil and stored at -18ºC.

Preparation of the Final Product – the Omega-3 Flour

A total of 12 kilograms of omega-3 flour was prepared. The product was prepared using different ingredients and proportions measure by weight (the amounts were expressed on mass), as described in Table 1.The mass proportions of the different constituents of the spices were: 0.25 (herbal salt), 0.125 (dehydrated garlic and onion) and 0.125 (coriander).

The herbal salt was prepared using the following ingredients: dehydrated rosemary, dehydrated basil, dehydrated oregano and salt in the ratio of 1:1:1:1. All ingredients were grounded in a blender and sieved on a kitchen strainer.The dehydrated garlic and onion was crushed and then sieved in a kitchen strainer.

The omega-3 flour was kept in polyethylene bags in a nitrogen atmosphere, wrapped in aluminum foil and stored at -18ºC

Table 1. Composition and quantity of ingredients in relational amounts.

Ingredients	Amounts
Flaxseed flour (ground flaxseed)	2
Sardine Head Flour (SHF)	1
Carrot Leaf Flour (CLF)	1
Cassava flour (obtained commercially ready)	0.5
Spices (herbal salt, dehydrated garlic and onion and coriander)	0.5

The parts are expressed in relative mass amounts by weight.

Total lipids, moisture, and fatty acid composition

Total lipids (TL) were determined according to Bligh and Dyer (1959). Fatty acid methyl esters (FAME) were prepared by methylation of total lipids by Maia and Rodriguez-Amaya (1993). The methyl esters were separated by gas chromatography using a Varian CP-3380 gas chromatographer fitted with a flame ionization detector (FID) and a fused-silica CPselect CB-Fame (MARTIN et al., 2008) capillary column (100 m x 0.25 mm id., 0.25 μm cyanopropyl). Injector and detector temperatures were at 220 and 240ºC respectively. The column temperature was maintained at 165ºC for 18 min. and programmed from 165 to 180ºC at 15ºC $min.^{-1}$ for 22 min. and from 180 to 240ºC at 15°C $min.^{-1}$ to 10 min. The ultra-pure gas flows were: 1.4 mL min^{-1} carrier gas (hydrogen), 30 mL min^{-1} make-up gas (nitrogen), 300 mL min^{-1} synthetic air and 30 mL min^{-1} hydrogen flame gas, split injection, 1:80 ratio. Samples were injected in triplicate. Retention times and peak area % values were automatically computed by a Varian 4290 integrator. For the identification of fatty acids, fatty acid retention times were compared to those of standard methyl esters (Sigma, USA), by comparison with some individual standards and by the standard addition (spiking).

Quantification of fatty acid methyl esters (FAME)

The concentration of fatty acids in $mg.g^{-1}$ of total lipids was measured against tricosanoic acid methyl ester (23:0) from Sigma (USA) as an internal standard as described by Joseph and Ackman (1992).

The concentrations of FA were obtained from the conversion of FAME for FA, calculated as Visentainer and Franco (2006). The results were expressed as mg FA per gram of TL. The analysis of fatty acids was conducted in six replicates.

Microbiological Analysis

The microbiological analyses were performed in accordance to the methods suggested by the FDA (1995). The analyses performed were: mesophilic bacteria total count, yeasts and molds counts, most probable number of fecal coliforms - *coagulase-positive staphylococci* count, investigation of *Salmonella* spp. and *Bacillus cereus* count and most probable number of *Escherichia coli*. The analyses were performed by the Department of Clinical Analyses, University of Maringá (Paraná State, Brazil).

Sensory Analysis

The omega-3 flour was sensorily evaluated by a panel of 50 tasters. The Omega-3 flour sample tasting was performed in the Pennacchi Industry of Food Products located at Avenue Gaturamo, 3333, Arapongas – Paraná State.

With a team of 50 volunteered and untrained tasters and possible potential consumers of the product, the sample was evaluated through a hedonic scale of 9 points (1 = extremely dislike to 9 = extremely like). The acceptability index (AI) of the product was calculated observing the global aspect x 100/9, where 9 is the highest score achieved on the hedonic scale. The minimum value considered for the AI is 70% as the threshold for considering that the product is well accepted by consumers (FERREIRA et al., 2006).

Statistical Analysis

The values of the means were statistically compared by Tukey's test at 5% with one-way ANOVA. The data were processed using the Statistica software 7.0 (STATSOFT, 2005).

Results and discussion

Fatty acids composition in the Omega-3 flour

The knowledge of the chemical composition, particularly with respect to the fatty acids composition in the lipid content of the foods, has aroused great interest in the scientific community because it is directly related to the human health. Moreover, it can provide an insight to evaluate the nutritional quality due to the levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), and the percentage of omega 3 and 6.

A total of 28 fatty acids (FA) were identified on the omega-3 flour (Table 2). The omega-3 fatty acids beneficial to human health, such as the alpha-linolenic acid (ALA), the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA), were found in the omega-3 flour whose values were 329.23 mg EPA 100 g^{-1} omega-3 flour and 545.35 mg DHA 100 g^{-1} omega-3 flour. The omega-3 fatty acids exert a protective effect against cardiovascular disease, the main cause of death in Brazil and in developed countries (RUSSO, 2009). The incidence of this disease is related to high levels of blood cholesterol.

Table 2. Fatty acid composition (mg 100 g^{-1}) of omega-3 flour.

Fatty acids [A]	Omega-3 Flour
12:0	285.47 ± 3.66
14:0	53.98 ± 2.15
14:1n-9	2.77 ± 0.13
15:0	5.49 ± 0.16
15:1n-9	5.65 ± 0.12
16:0	1594.74 ± 31.20
16:1n-9	21.26 ± 0.24
16:1n-7	228.41 ± 4.30
17:0	39.62 ± 1.45
17:1n-5	9.06 ± 0.07
18:0	671.55 ± 20.46
18:1n-9	2655.21 ± 20.83
18:1n-7	171.13 ± 10.23
18:2n-6 (LA)	1651.82 ±4.24
18:3n-6	10.32 ± 0.23
18:3n-3 (LNA)	5759.33 ± 33.65
20:0	30.43 ± 0.78
20:1n-9	27.50 ± 1.16
20:2n6	8.65 ± 0.50
20:3n-6	8.25 ± 0.48
20:4n-6 (AA)	60.78 ± 3.06
22:0	9.15 ± 0.56
22:1n-9	10.34 ± 0.17
20:5n-3 (EPA)	329.23 ± 4.02
24:0	37.05 ± 0.94
24:1n-9	36.87 ± 0.60
22:5n-3	40.19 ± 0.19
22:6n-3 (DHA)	545.35 ± 4.62
MUFA	3168.20 ± 45.92
SFA	2727.08 ± 33.21
PUFA	8413.92 ±32.90
n-6	1739.82 ± 0.07
n-3	6674.10 ± 32.86
PUFA/SFA	3.08 ± 0.04
n-6/n-3	0.26 ± 1,63

[A]Mean and standard deviations as an average of 6 replicates analyses. Value expressed in mg fatty acids/100 gram of omega-3 flour. Abbreviations: LA (linoleic acid), LNA (alpha linolenic acid), AA (arachidonic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), MUFA (monounsaturated fatty acid), SFA (saturated fatty acid), PUFA (polyunsaturated fatty acid), n-6 (omega-6), n-3 (omega-3).

To achieve low cholesterol levels the American Heart Association recommends a balanced diet with a lower intake of fat, cholesterol, and saturated fatty acids and a higher intake of monounsaturated and polyunsaturated fatty acids.

The adequate intake of omega-3 fatty acids vary. According to the American Heart Association the adequate intake of omega -3 fatty acids is 1.5 - 3g LNA day^{-1} and 1 – 4 g day^{-1} EPA + DHA. It is recommended 1 g day^{-1} EPA + DHA to prevent cardiovascular diseases and 2-4 g day^{-1} to decrease the triglyceride levels in patients with coronary heart disease, as high levels of triglycerides are associated with heart disease (LEE et al., 2009)

According to other authors, for patients with documented cardiovascular disease the adequate intake is 1g EPA + DHA per day (MILLER et al., 2011). According to LEE et al. (2009), patients with a known coronary artery disease should consume at least 1 g day^{-1} EPA + DHA and individuals without the disease should consume at least 250-500 mg day^{-1}. Studies by CARL et al. (2009) indicate that the consumption should be at least 500 mg day^{-1} for individuals without coronary artery disease and at least 800 to 1.000 mg day^{-1} for individuals with a known heart disease and heart failure.

According to the scientific papers mentioned, it is noted that the adequate values of EPA + DHA for the prevention of cardiovascular disease varies from 250 to 1000 mg day^{-1}. The omega-3 flour presented 874.58 mg (DHA + EPA) 100 g^{-1} omega-3 flour (Table 2). To achieve the minimum daily nutritional requirements of EPA + DHA (250 mg), the product intake should be 28.58 g or about 2 ½ tablespoons (soup) of the omega-3 flour daily.

In relation to the recommended intake of LNA, according to the American Heart Association, it is 1.5 - 3 grams per day. The omega-3 flour presented 5759.33 mg LNA 100 g^{-1} omega-3 flour. To achieve the minimum daily nutritional requirements (1.5 g day^{-1}), the product intake should be 26.04 g daily, or a little over 2 ½ tablespoons (soup) of the omega-3 flour per day. When an individual consumes food containing LNA, the body is able to synthesize EPA + DHA.

Thus, to achieve the minimum requirements of omega -3, it is necessary the intake of 2.5 to 3 tablespoons (soup) of the omega-3 flour daily.

Fatty acid composition of meals consumed by Industry Workers

The Tables 3 and 4 presents the concentrations in milligrams (mg) of fatty acids 100 g^{-1} of A and B meals, respectively, consumed by workers in the company researched.

The meal (A) consisted of: rice, beans, pirao hake, grilled fillet of hake, chicory, cabbage, lettuce and carrot salad.

Table 3. Fatty acid composition (mg 100 g^{-1}) of meal (A) consumed by workers.

Fatty acids[A]	Meal A		
	Without the Omega-3 flour	With 1 tablespoon (soup) of the Omega-3 flour	With 2 tablespoons (soup) of the Omega-3 flour
12:0	3.44 ± 0.04a	6.12 ± 0.04b	8.38 ± 0.33c
14:0	0.46 ± 0.02a	0.87 ± 0.03b	1.31 ± 0.05c
14:1n-9	0.13 ± 0.01a	0.14 ± 0.02a	0.17 ± 0.01b
15:0	0.12 ± 0.01a	0.13 ± 0.01a	0.17 ± 0.01b
16:0	141.77 ± 2.66a	155.46 ± 3.96b	192.22 ± 5.28c
16:1n-9	0.32 ± 0.03a	0.45 ± 0.02b	0.63 ± 0.02c
16:1n-7	5.39 ± 0.16a	10.84 ± 0.04b	9.52 ± 0.02c
17:0	1.09 ± 0.04a	1.36 ± 0.01b	2.22 ± 0.06c
17:1n-5	0.54 ± 1.45a	0.61 ± 0.03a	0.79 ± 0.02b
18:0	36.36 ± 0.63a	39.02 ± 0.19b	54.43 ± 1.71c
18:1n-9	243.35 ± 0.13a	251.03 ± 5.06b	333.91 ± 5.37c
18:1n-7	15.38 ± 1.53a	19.05 ± 0.37ab	20.13 ± 0.13b
18:2n-6 (LA	565.21 ± 24.43a	540.62 ± 4.02a	706.46 ± 21.11b
18:3n-6	3.67 ± 0.03a	3.69 ± 0.06a	4.71 ± 0.16b
18:3n-3 (LNA)	73.01 ± 2.68a	104.01 ± 1.72b	187.94 ± 5.86c
20:0	3.27 ± 0.03a	3.26 ± 0.04a	4.58 ± 0.10b
20:1n-9	2.69 ± 0.06a	3.55 ± 0.04b	3.91 ± 0.03c
20:2n6	0.36 ± 0.01	0.36± 0.01	0.36± 0.01
20:3n-6	0.59± 0.04	0.59 ± 0.03	0.58 ± 0.04
20:4n-6 (AA)	3.90 ± 0.06a	3.97 ± 0.03a	5.24 ± 0.22b
22:0	0.08 ± 0.01	0.10 ± 0.01	0.12 ± 0.02
20:5n-3 (EPA)	6.76 ± 0.10a	14.07 ± 0.88b	18.18 ± 0.20c
24:0	0.52 ± 0.14a	0.62 ± 0.01b	0.75 ± 0.03c
24:1n-9	1.88 ± 0.02a	2.11 ± 0.01b	2.26 ± 0.06c
22:5n-3	0.51 ± 0.03a	0.84 ± 0.02b	1.19 ± 0.04c
22:6n-3 (DHA)	16.99 ± 1.14a	24.26 ± 1.71b	30.04 ± 1.18c
MUFA	269.68 ± 1.13a	287.77 ± 9.40b	371.32 ± 12.11c
SFA	187.11 ± 2.02a	206.94 ± 1.08b	264.18 ± 7.88c
PUFA	671.09 ±28.36a	692.41 ± 3.12a	954.70 ± 1.75b
n-6	573.73 ± 24.40a	549.23 ± 4.02a	717.35 ± 3.92b
n-3	97.27 ± 3.96a	143.18 ± 0.90b	237.35 ± 5.67c
PUFA/SFA	3.59 ± 0.04	3.34 ± 0.06	3.61 ± 0.04
n-6 /n-3	5.90 ± 0.11a	3.84 ± 0.52b	3.02 ± 0.09b

[A]Mean and standard deviations as an average of 6 replicates analyses. Value expressed in mg fatty acids 100 g^{-1} of food. Averages followed by different letters in the same line are significantly different ($p < 0.05$) by Tukey's test. Meal A: rice. Beans, pirao hake, grilled fillet of hake, chicory, cabbage, lettuce and carrot salad. Abbreviations: LA (linoleic acid), LNA (alpha linolenic acid), AA (arachidonic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), MUFA (monounsaturated fatty acid), SFA (saturated fatty acid), PUFA (polyunsaturated fatty acid), n-6 (omega-6), n-3 (omega-3).

The meal (B) consisted of: rice, beans, beef cubes with cabotiá pumpkin, farofa of vegetables, chard, grated beet. A total of 26 fatty acids were identified on meal A and B (Tables 3 and 4 respectively).

To the meals A and B, it was added 1 and 2 tablespoons (soup) of the omega-3 flour. The fatty acid concentrations (mg fatty acids 100 g^{-1} meal) with the addition of the omega-3 flour are presented in Tables 3 and 4.

A total of 26 fatty acids were identified in both meals, with the addition of 1 and 2 tablespoons (soup) of the omega-3 flour (Tables 3 and 4).

Regarding the meal (A), the content of the omega-3 fatty acids was higher than on the meal (B), since the fish (hake) in meal (A) is the source of the omega-3 fatty acids, especially EPA and DHA, whose values were: EPA of 6.76 mg 100 g^{-1} in meal (A) and 1.24 mg 100 g^{-1} in meal (B) and DHA of 16.99 mg 100 g^{-1} in meal (A) and 0.51 mg 100 g^{-1} in meal (B), according to Tables 3 and 4.

Table 4. Fatty acid composition (mg 100 g^{-1}) of meal (B) consumed by workers.

Fatty acids[A]	Meal B		
	Without the Omega-3 flour	With 1 tablespoon (soup) of the Omega-3 flour	With 2 tablespoons (soup) of the Omega-3 flour
12:0	11.20 ± 0.50	15.16 ± 0.04b	13.81 ± 0.08c
14:0	2.16 ± 0.06a	2.77 ± 0.03b	2.70 ± 0.01b
14:1n-9	0.29 ± 0.01a	0.44 ± 0.02ab	0.37 ± 0.03b
15:0	2.72 ± 0.05	2.43 ± 0.03b	2.16 ± 0.05b
16:0	258.15 ± 10.49	267.11 ± 4.19	265.80 ± 2.37
16:1n-9	1.62 ± 0.08a	2.05 ± 0.04b	1.70 ± 0.05a
16:1n-7	16.14 ± 0.47a	24.70 ± 0.34b	16.59 ± 1.24a
17:0	5.28 ± 0.30	5.93 ± 0.16	5.34 ± 0.15
17:1n-5	6.88 ± 0.47	7.95 ± 0.05	6.93 ± 0.50
18:0	108.50 ± 6.95	104.56 ± 1.88	97.97 ± 2.93
18:1n-9	503.69 ± 11.99a	511.54 ± 4.46a	457.73 ± 8.50b
18:1n-7	22.41 ± 1.18	25.58 ± 1.98	22.49 ± 1.93
18:2n-6 (LA	700.43 ± 2.72a	610.05 ± 9.24b	625.22 ± 1.96b
18:3n-6	3.91 ± 0.03	4.24 ± 0.03	3.89 ± 0.25
18:3n-3 (LNA)	95.92 ± 2.72a	146.24 ± 3.06b	150.50 ± 8.21c
20:0	4.45 ± 0.05	4.43 ± 0.03	4.29 ± 0.09
20:1n-9	3.45 ± 0.14	3.42 ± 0.12	3.47 ± 0.29
20:2n6	0.12 ± 0.01a	0.37± 0.03b	0.35± 0.01b
20:3n-6	0.28± 0.04a	0.62 ± 0.02b	0.58 ± 0.03b
20:4n-6 (AA)	4.77 ± 0.17	5.07 ± 0.05	5.02 ± 0.04
22:0	0.08 ± 0.01	0.13 ± 0.01	0.22 ± 0.02
20:5n-3 (EPA)	1.24 ± 0.11a	4.12 ± 0.24b	4.00 ± 0.25b
24:0	0.32 ± 0.03	0.39 ± 0.01	0.36 ± 0.02
24:1n-9	2.27 ± 0.02	2.54 ± 0.16	2.66 ± 0.23
22:5n-3	2.41 ± 0.04a	2.65 ± 0.07b	2.66 ± 0.05b
22:6n-3 (DHA)	0.51 ± 0.01a	3.84 ± 0.25b	4.38 ± 0.06b
MUFA	556.75± 9.68a	578.22 ± 6.44a	511.94 ± 1.73b
SFA	392.86 ± 2.93	402.91 ± 2.16	392.65 ± 5.20
PUFA	809.59 ± 2.33	777.19 ± 12.98	796.60 ± 10.54
n-6	709.51 ± 2.57a	620.34 ± 9.35b	635.06 ± 2.21b
n-3	100.08± 2.79a	156.85 ± 3.63b	161.54 ± 8.32b
PUFA/SFA	2.06 ± 0.09	1.93 ± 0.04	2.03 ± 0.05
n-6/n-3	7.09 ± 0.22a	3.95 ± 0.03b	3.93 ± 0.20b

[A]Mean and standard deviations as an average of 6 replicates analyses. Value expressed in mg fatty acids 100 g^{-1} of meal. Averages followed by different letters in the same line are significantly different ($p < 0.05$) by Tukey's test. Meal B: rice, beans, beef cubes with cabotiá pumpkin, farofa of vegetables, chard, grated beet. Abbreviations: LA (linoleic acid), LNA (alpha linolenic acid), AA (arachidonic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), MUFA (monounsaturated fatty acid), SFA (saturated fatty acid), PUFA (polyunsaturated fatty acid), n-6 (omega-6), n-3 (omega-3).

A very long chain of fatty acids EPA and DHA is very important for the human health.

DHA has an important role in the retinal development (CHEATHAM et al., 2006) and in the brain (WURTMAN, 2008) and EPA and AA originates eicosanoids, which are inflammatory mediators, being AA the main substrate for the synthesis of eicosanoids in the human body (CALDER, 2006; GAROFOLO; PETRILLI, 2006).

By adding 1 tablespoon of the prepared flour (soup) to the meal (A), there was a significant increase ($p < 0.05$) in the amount of EPA and DHA, that is, 108.14 and 42.79% respectively (Table 3), in relation to the meal without the addition of the omega-3 flour. By adding 2 tablespoons (soup) of the omega-3 flour to the meal (A) there was a significant increase ($p < 0.05$) in the amount of EPA and DHA, that is, 168.93 and 76.81% respectively (Table 3), in relation to the meal with the addition of 1 tablespoon (soup) of the omega-3 flour only.

By adding 1 tablespoon (soup) of the omega-3 flour to the meal (B), there was a significant increase ($p < 0.05$) in the amount of EPA and DHA, that is, 323.26 and 652.94% respectively (Table 4), in relation to the meal without the addition of the omega-3 flour.

However, when 2 tablespoons (soup) of omega-3 flour were added to the meal (B), there was no significant difference ($p > 0.05$) in the amount of EPA and DHA (Table 4) in relation to the meal with the addition of 1 tablespoon (soup) of the omega-3 flour only.

Microbiological Analysis of the omega-3 flour

Table 5 presents the results of the microbiological analysis on the omega-3 flour. According to Franco and Landgraf (2005), indicator microorganisms are groups that, when present in the food, can provide information about the occurrence of fecal contamination, the presence of pathogens or the potential deterioration of the food, and they could indicate inadequate sanitary conditions during the food processing, manufacturing or storage. Because it is a product made out of various ingredients, the Brazilian law does not establish acceptance values for the microbiological analyzes, so that it can not be compared to a standard pattern.

Table 5. Microbiological Analysis of the omega-3 flour.

Microorganisms	Omega-3 Flour
Mesophilic bacteria (CFU g^{-1})	5.6 x 10^4
Yeast count (CFU g^{-1})	< 1 x 10^2
Mold count (CFU g^{-1})	2,1 x 10^3
Fecal coliforms (MPN g^{-1})	< 3
Coagulase-positive staphylococc (CFU g^{-1})	< 1 x 10^2
Samonella spp em 25 g	Absent
Escherichia coli (MPN g^{-1})	< 3
Bacillus cereus (CFU g^{-1})	< 1 x 10^2

Values expressed in CFU g^{-1} (colony-forming units per gram) and MPN g^{-1} (most probable number per gram). Results are obtained from analysis of the technical report from the microbiology laboratory and the microscopic food from the Department of Clinical Analyses, State University of Maringá.

The mesophilic bacteria are associated to the food hygienic–sanitary quality and values lower than 10^6 CFU g^{-1} are acceptable according to Lira et al. (2001).

The presence of coliforms in the food is important for the indication of contamination during the manufacturing process or post-processing. The presence of the total coliforms and Escherichia coli in processed foods, according to JUNQUEIRA et al. (1997), is considered a useful indication of post-sanitization or post-processing contamination, evidencing that the hygiene practices are out of the the standards required for the food processing. Fecal coliforms are often used as indicators of the sanitary quality of water and they do not represent on their own a health hazard, but they do serve as indicators of the presence of other organisms that cause health problems. As the coliforms are easily destroyed by the heat, analyzing them may be useful for the food post-processing contamination test (FORSTHE, 2002).

The values found for fecal coliforms (< 3) are within the recommended ones when compared to the products ready for consumption that are seafood based (2 x 10 MPN g^{-1}) in accordance to the Resolution No. 12, January 2, 2001 approving the regulation for technical standards on microbiological food (BRASIL, 2001). The microorganisms that cause foodborne illnesses can work by releasing toxins, such as *Staphylococcus* aureus and *Bacillus cereus,* and by causing infections, such as *Salmonella* and *Escherichia coli.*

No *Salmonella* was found in the omega-3 flour, the *Coagulase-positive staphylococci* and Bacillus cereus values obtained are respectively acceptable (< 1 x 10^2 and 10^2 CFU g^{-1}) according to BRASIL (2001), compared to food ready for consumption that are based on fish, whose values recommended are: 10^3 CFU g^{-1} for *coagulase -positive staphylococci*, absence of *salmonella* and 10^3 CFU g^{-1} for *Bacillus cereus.*

Sensory Analysis of the Omega-3 flour

The sensory analyses of food determine the acceptability of the product in the consumer market. The degree of acceptability depends on factors such as age and sensitivity, varying from person to person. To get an overall assessment of a omega-3 flour sample, depending on its sensory characteristics perceived by tasting, the acceptability was calculated by the acceptability index (AI). The AI for the omega-3 flour was 78%, which indicates a value above the recommended one for a product to be accepted, which is 70% (FERREIRA et al., 2006).

Conclusion

According to the results obtained, it was found that the omega-3 flour is a food with a high nutritional value compared to other food, particularly with regard to omega-3 fatty acids. So that the product can be used as supplementation for the feeding of workers, as well as in other segments (kindergartens, hospitals, nursing homes, schools) in order to enrich the food consumed. Thus, in order to meet the minimum requirements of omega -3 for the human body, it is necessary to intake 2.5 to 3 tablespoons (soup) of the omega-3 flour daily. By adding the product to meals eaten by selected workers, there has been a significant increase in the level of the Omega-3 fatty acids, especially the eicosapentaenoic acid (EPA) and the docosahexaenoic acid. Moreover, to the improvement on the content of these fatty acids are beneficial to the human health when food is regarded.

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