Increasing Health Benefits of Milk Fat by Partial Replacement with vegetable oils Fortified with Carotenoids

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Abstract: Partial replacement of milk fat with vegetable oils meet consumer demanded for reducing the intake of saturated fatty acids and hence may contribute to reduce the risk for heart disease. Different natural sources of carotenoids as fat-soluble antioxidants (green pepper, parsley and broccoli) were mixed in emulsion form with milk fat phase to protect the polyunsaturated fatty acids of vegetable oils (olive oil, sunflower oil, sesame oil, 1:1:1, v/v/v) which used in partial replacement from the oxidization, besides acting as biological antioxidants. Carotenoids solubility efficiency through the preliminary treatments of milk such as blending, pasteurization and homogenization was monitored as well as antioxidant activity using ß-carotene/ linoleic acid assay was determined in homogenized milk with (2%) carotenoids extract emulsion. Results showed that the solubility of carotenoids increased with blinding, pasteurization and homogenization. The partial substitution of milk fat was the most suitable milk fat phase as a healthy benifits. Broccoli showed the highest carotenoid content and also recorded the highest antioxidant activity. [Journal of American Science. 2010;6(8):352-360]. (ISSN: 1545-1003).

Keywords: Milk fat, vegetable oils, carotenoids solubility, antioxidant activity.

1. Introduction:

Consumer is looking for the products can offer more health benefits. Full-fat milk is rich in saturated fatty acids, which has been shown to significantly elevate total, Low-density lipoprotein (LDL-), and high-density lipoprotein (HDL)-cholesterol and apolipoprotein B and A concentrations (Kris-Etherton *et al*. 1993; Wood, *et al.* 1993) which has long been associated with increased risk of cardiovascular disease. To meet consumer becomes demand reducing the intake of saturated fatty acids from the milk fat; partial substitution of milk fat by vegetable oils leads to reduction of saturated fatty acids. From a nutritional point of view, a different fatty acids profile of the consumer dairy product has many benefits, keeping part of milk fat has positive effect due to milk fat globule membrane (MFGM) contained signaling molecules called sphingolipids which has anticancer properties (Berra *et al.* 2002; Hertervig *et al*. 2003; Lemonnier *et al*. 2003). Cholesterol in milk fat is an essential part of sex hormones, bile acids, D vitamins and steroid hormones (Whitney and Rolfes 2002), acts also as an antioxidant (Cranton and Frackelton 1984). Conjugated linoleic acid (CLA), a component of milk fat, has also potential health benefits (MacDonald 2000; Collomb et al. 2006; Salas-Salvado et al. 2006). In same time, finding more unsaturated fatty acids from vegetable oils are desirable. Vegetable oils are rich in omega. Both linoleic and linolenic acid have a lowering effect on blood cholesterol and hence may contribute to reduce the risk for heart disease.

When vegetable oils are used, in combination with milk fat or on their own, the balance between various fatty acids can be adjusted to a higher nutritional value. On other hand; more poly unsaturated fatty acids (PUFA) are more sensitive to get rancid by lipid oxidation. Oxygen can also become activated in the presence of metals or metal complexes and initiate lipid oxidation by the formation of either free radicals or singlet oxygen ($^1O_2$). Singlet oxygen is a very efficient oxidizing agent (Frankel, 2005). The oxidation stability of vegetable oils and fats is determined by their degree of unsaturation, the presence of natural or synthetic antioxidants, the presence of pro-oxidants such as metals and the availability of oxygen.

An antioxidant can eliminate potential initiators of oxidation and thus prevent reaction. It seems, that antioxidant in milk should be applied as a systems containing compounds operating according to various mechanisms (chelators of metal ions, scavengers of free radicals or other reactive forms of oxygen, compounds deactivating enzymes), because they would be able to prove synergistic effect increasing antioxidant property of other antioxidant (Decker, 1998, Zhang and Omaye, 2000). Carotenoids, fat-soluble antioxidants can be used to protect the PUFA from the oxidized (Shany *et al.* 2006) besides acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen (Alam and Sultan

2004) and their antioxidant properties very much depend concentration (Palozza 1998).

The aim of this research was using a combination of vegetable oils with milk fat to give higher nutritional value in dairy product by partial replacement of milk fat with vegetable oils (olive oil, sunflower oil, sesame oil). Vegetable oil phase consist of olive oil which is composed mainly of the oleic acid (55-85%); sunflower oil which is high in linoleic acid (Omega 6) (67%) and sesame oil which contains 42% oleic acid, 15% saturated fat, and 43% omega- 6 linoleic acid (Alpaslan *et al.* 2001; Sankar *et al.* 2006). Many natural sources of carotenoids were mixed with different fat phases of milk and investigated for both solubility through blending, pasteurization and homogenization, and evaluate the antioxidant activity of homogenized milk with carotenoids natural sources.

2. Materials and Methods:

Materials

Preparation of carotenoids extract in emulsion forms.

Broccoli, parsley and green pepper from local market were chopped into fine pieces by a commercial blender. 10 g of fine pieces were immediately homogenized again for 30 s with the vortex blender with 2% edible oil (corn oil) and 0.5% emulsifier (tween 20) in water bath (90°C/5 min). Corn oil is exhibited as a medium stability of carotenoids and considers a very popular delivery system. Carotenoids extract emulsion was cooled in an ice bath for 5 min and filtered through a buchner funnel using Whatman filter paper No. 4.

Vegetable oils (from NRC) used in partial substitution of milk fat.

Three types of vegetable oils should have similar melting properties (melting point 58 to 60°C, Salado, et al. 2004) was chosen and used to create a balance of fatty acids by ratio (1:1:1) to get a higher nutritional value.

Methods

Preparation fat phases incorporated with carotenoids

Three milk fat phases were prepared mixed with skim milk to give 3% milk fat; 1.5% milk fat mix with 1.5% vegetable oils and 3% vegetable oils. These fat phases were mixed with carotenoids extract emulsion at different concentrate (0.5, 1.0, 1.5 and 2%), they blended, pasteurized at 65°C / 30 min and homogenized at two-stags (200, 50 Kg/cm^2) (Rannie homogenizer). Homogenization used as a mechanical emulsifying process for incorporations the emulsion of carotenoids with skim milk.

Extraction of fat phase from milk

Extraction of fat from the milk was carried out as described by (Havemose *et al.* 2004). Fat phase was extracted from milk by adding methanol and chloroform with ratio (1:1, v/v) to the milk. The mixture was shaken vigorously for 1min, and then centrifuged for 10min at 3000 × g at 4 °C. Total carotenoid content was determined in the lower phase containing the lipid fraction.

Evaluation the total carotenoid content (TC)

Carotenoids are expected to be dissolved in the dispersed fat droplets and its soluble efficiency in fat phase was evaluated by determination the total carotenoid content (TC) in fat phase after emulsion homogenization by absorption SP-2000UV UV-VIS spectrophotometer according to (Lachman *et al.* 2003). Absorbance of organic extracts was then measured in 1 cm cuvettes at λ = 444 nm and total carotenoid content in mg/kg fw of sample was expressed as lutein equivalent from the equation:

$$(K + X)L = \frac{A444.\ 25\ .\ 15}{0.259\ \mathrm{m}}\ (\mathrm{mg/kg\ fw})$$

Where:

(K + X)L is total carotenoid content (carotenes and xanthophylls)

A444 is absorbance of acetone extract at λ = 444 nm

m is sample weight (g).

Determination antioxidant activity using β-carotene bleaching assay.

Evaluation of antioxidant activity based on coupled oxidation of ß-carotene and linoleic acid was conducted as described by Taga et al. (1984). ß-carotene (6 mg) was dissolved in 20 ml of chloroform. A 3 ml of the solution was added to a conical flask with 40µl linoleic acid and 400 µl Tween 20. Chloroform was removed by using a rotary evaporator under vacuum at 35ºC. Oxygenated distilled water (100 ml) was added to the ß-carotene emulsion and mixed well. 3 ml aliquot of the ß-carotene emulsion and 0.2 ml of the diluted sample extract were placed in a test tube and mixed well. The tubes were immediately placed in a water bath and incubated at 50ºC. Oxidation of ß-carotene emulsion was monitored by measuring absorbance at 470 nm. Sample absorbance was measured at 60 min after incubation. A control consisted of 0.2 ml distilled water, instead of the extract.

The degradation rate of the extracts was calculated by first order kinetics: Sample degradation rate = ln (a/b) ×1/t, where: ln = natural log; a = initial absorbance at time 0; b = absorbance at 60 min; t = time (min). Antioxidant activity (AA) was expressed as % inhibition relative to the control using the equation:

$$AA(\%) = \frac{\text{Degradation of control Degradation of sample X 100}}{\text{Degradation of control}}$$

Statistical Analysis

All data are presented as the mean ± SD. One-way analysis of variance (ANOVA) unpaired and paired t-tests were performed using statistical Analysis System (SAS Institute, 1988) ($P \leq 0.05$) was the level of significance.

3. Results:

Total soluble carotenoids content (TC) in fat phase (3%) supplemented with green pepper extract.

Total carotenoids content (mg/kg fw) resulted in full fat phase (3%) supplemented with different ratio of green pepper extract (0.5, 1.0, 1.5 and 2.0%) at different treatments showed at Tab. 1. The average of soluble carotenoids percentage of the green pepper in fat phases was different as showen in Fig 1. The average soluble carotenoids percentage to the absolute emulsion at different concentrate in blending extract/ full fat milk was 46.0% increased in extract/partial substitution milk fat to 60.5% and extent in increasing in extract/vegetable fat milk to 71.4%. At pasteurization stage, the average soluble carotenoids percentage from the absolute emulsion in extract/ full fat milk was also 49.25% increased also to 63.5% in extract/partial substitution milk fat and to 72.0% in the extract/vegetable fat milk. At the following stage, the average soluble carotenoids percentage in homogenized extract/ full fat milk with different concentrate was 57.7% increased to 73.0 and 82.6% in homogenized extract/partial substitution milk fat and extract/vegetable fat milk respectively.

Total soluble carotenoids content (TC) in fat phase (3%) supplemented with parsley extract

Total carotenoids content (mg/kg fw) resulted in full fat milk (3%) supplemented with different ratio of parsley extract through different treatments showed at Tab. 1. The average soluble carotenoids percentage from the absolute emulsion in blending extract/ full fat milk was 59.5% increased in extract/partial substitution milk fat to 69.5% and extent in increasing in extract/vegetable fat milk to 78.7%. At pasteurization stage, the average soluble carotenoids percentage of absolute emulsion in extract/ full fat milk was 62.82% increased also to 69.65% in extract/partial substitution milk fat and to 78.77% in the extract/vegetable fat milk. At the following stage, the average soluble carotenoids percentage in homogenized extract/ full fat milk was 67.87% increased to 76.64 and 85.73% in homogenized extract/partial substitution milk fat and extract/vegetable fat milk respectively. These gradually increasing of soluble carotenoids in different fat phase through different treatments showed in Fig 2.

Total soluble carotenoids content (TC) in fat phase (3%) supplemented with broccoli extract

It is obviously that the broccoli has the highest source of carotenoids compared to the other sources. Resulted showed that soluble carotenoids percentage from the absolute broccoli extract through different treatments showed at Tab. 1. With blending the average soluble carotenoids percentage calculated was 64.47% in extract/ full fat milk increased to 74.29% and 84.28% in extract/partial substitution milk fat and in the extract/vegetable fat milk respectively. At pasteurization the milk, the average soluble carotenoids percentage was 70.72, 79.02 and 87.19% in extract/ full fat milk, in extract/partial substitution milk fat and in the extract/vegetable fat milk respectively. The solubility of carotenoids was increased after homogenization that was 73.43, 82.28 and 95.25% in extract / full fat milk, extract/partial substitution milk fat and in the extract/vegetable fat milk respectively Fig 3.

Antioxidant activity (%)

Results showed that a positive correlation between dissolved natural source percent and its soluble carotenoids, so that we choose (2% emulsion) after homogenized stage to determination the antioxidant activity of the carotenoids soluble in fat phase

Results showed that antioxidant activity was increased significantly with increasing the carotenoids content (Table 1). Broccoli extracts dissolved in modified fat showed promotion of bleaching of the β-carotene emulsion; it was 11.46% in milk broccoli mixture (as antioxidant activity) followed by 11.05% in parsley and 10.28% in green pepper mixture as antioxidant activity as show in Table 1. A correlation coefficient (r^2) between total carotenoids (TC) and antioxidant activity was 0.95. The results revealed that the correlation between total carotenoids (TC) and antioxidant activity was strongly positive.

Table 1: Total carotenoid content (mg/Kg fw) in 3% fat phase and antioxidant activity of homogenized milk with 2% carotenoids extract emulsion.

Treatment	Conc.	Green pepper				Parsley				Broccoli			
		Milk fat	Mix*	Oil**	Absolute green pepper.	Milk fat	Mix*	Oil**	Absolute Parsley	Milk fat	Mix*	Oil**	Absolute Broccoli
Blending	0.5	173^{d}	225^{c}	265^{b}	401^{a}	238^{d}	284^{c}	341^{b}	423^{a}	252^{d}	314^{c}	370^{b}	430^{a}
	1	193^{d}	264^{c}	311^{b}	438^{a}	284^{d}	327^{c}	362^{b}	454^{a}	310^{d}	338^{c}	385^{b}	456^{a}
	1.5	216^{d}	287^{c}	334^{b}	454^{a}	297^{d}	341^{c}	379^{b}	475^{a}	322^{d}	363^{c}	407^{b}	489^{a}
	2	237^{d}	300^{c}	358^{b}	475^{a}	322^{d}	364^{c}	398^{b}	508^{a}	335^{d}	388^{c}	427^{b}	512^{a}
Pasteurization	0.5	191^{d}	238^{c}	288^{b}	418^{a}	251^{d}	305^{c}	357^{b}	431^{a}	282^{d}	335^{c}	376^{b}	432^{a}
	1	214^{d}	279^{c}	324^{b}	445^{a}	311^{d}	338^{c}	384^{b}	488^{a}	333^{d}	364^{c}	406^{b}	458^{a}
	1.5	237^{d}	304^{c}	351^{b}	475^{a}	332^{d}	363^{c}	399^{b}	513^{a}	356^{d}	391^{c}	428^{b}	491^{a}
	2	269^{d}	335^{c}	366^{b}	494^{a}	341^{d}	375^{c}	419^{b}	518^{a}	378^{d}	417^{c}	451^{b}	525^{a}
Homogenization	0.5	224^{d}	290^{c}	325^{b}	425^{a}	290^{d}	329^{c}	391^{b}	441^{a}	318^{d}	351^{c}	428^{b}	436^{a}
	1	261^{d}	338^{c}	375^{b}	454^{a}	327^{d}	362^{c}	413^{b}	492^{a}	341^{d}	388^{c}	464^{b}	486^{a}
	1.5	304^{d}	376^{c}	426^{b}	509^{a}	346^{d}	399^{c}	436^{b}	515^{a}	390^{d}	431^{c}	488^{b}	510^{a}
	2	335^{d}	418^{c}	486^{b}	522^{a}	400^{d}	449^{c}	481^{b}	530^{a}	410^{d}	466^{c}	507^{b}	553^{a}
Antioxidant activity (%)	2	8.24^{d}	10.28^{c}	11.96^{b}	13.05^{a}	9.84^{d}	11.05^{c}	11.83^{b}	13.25^{a}	10.09^{d}	11.46^{c}	12.47^{b}	13.83^{a}

The different letters means significant

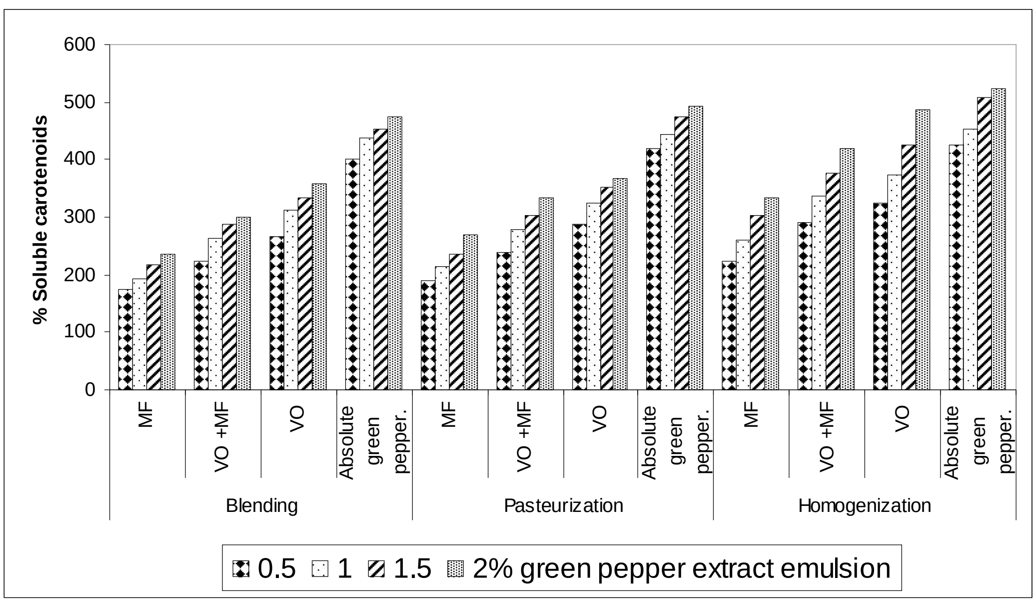

Figure 1: The average of soluble carotenoids percentage of the green pepper in different fat phases, at each technological treatments.

MF: Milk fat

VO+MF: Milk fat +vegetable oil (1:1)

VO: Vegetable oil

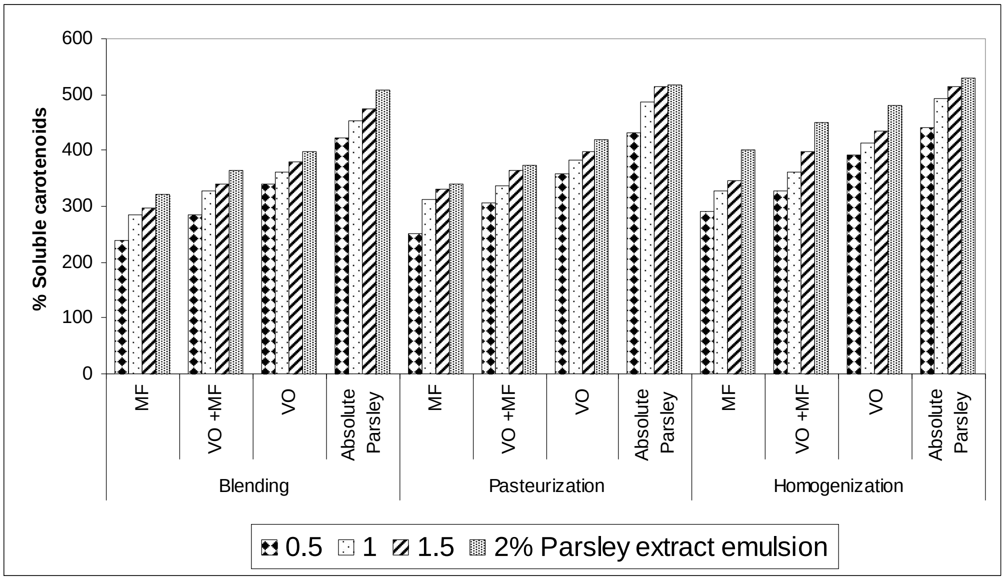

Figure 2: The average of soluble carotenoids percentage of the parsley in different fat phases, at each technological treatments.

MF: Milk fat
VO+MF: Milk fat +vegetable oil (1:1)
VO: Vegetable oil

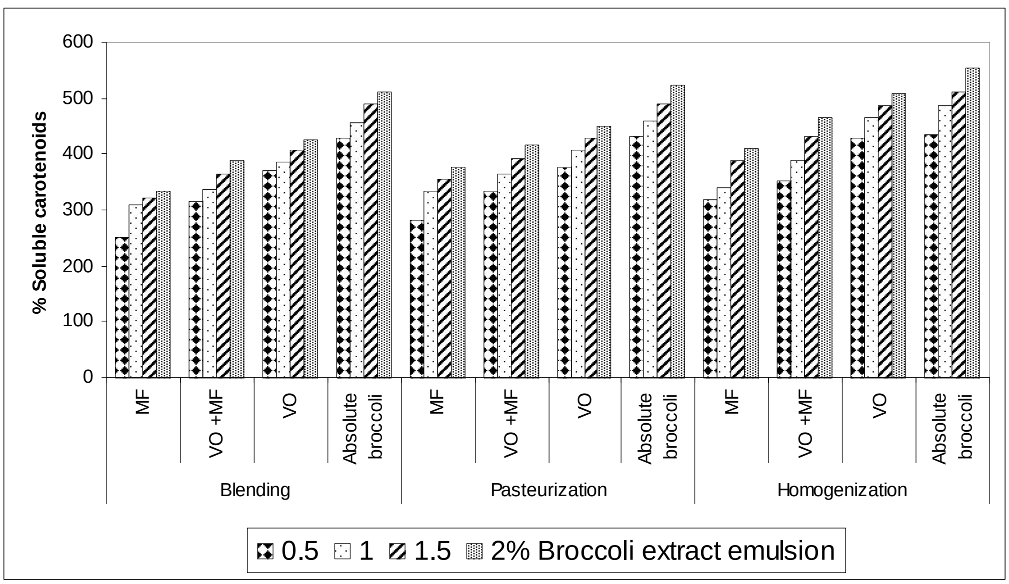

Figure 3: The average of soluble carotenoids percentage of the broccoli in different fat phases, at each technological treatments.

MF: Milk fat
VO+MF: Milk fat +vegetable oil (1:1)
VO: Vegetable oil

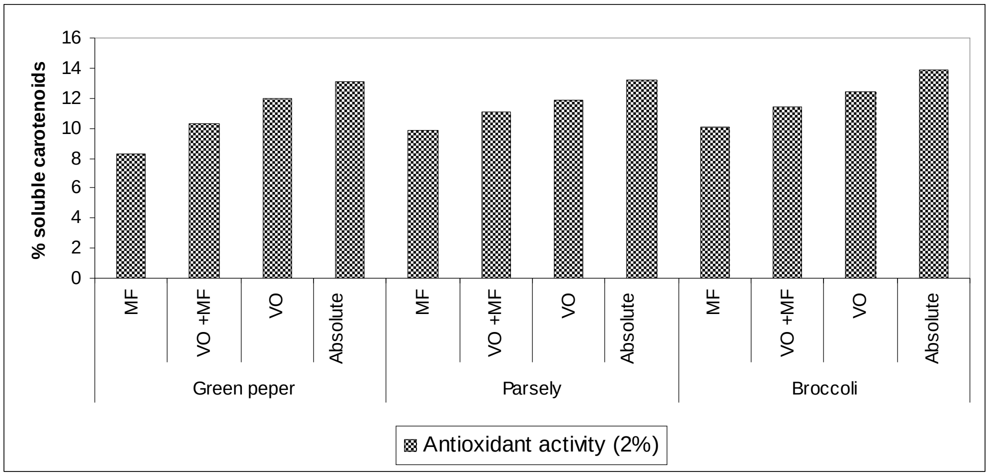

Figure 4: Antioxidant activity (%) of homogenized milk with 2% carotenoids extract emulsion from green pepper, parsley and broccoli.
MF: Milk fat
VO+MF: Milk fat +vegetable oil (1:1)
VO: Vegetable oil

4. Discussion:

It is obviously that total carotenoids in absolute emulsion were decreased after technological treatments. After blending carotenoids in the vegetable particles were transferred from the vegetable matrices into the two phases of milk; fat phase and aqueous phase, therefore total carotenoids in fat phase was decreased than in absolutely of the three different vegetable matrices extract emulsion. The carotenoids in the aqueous phase were bound to bovine serum albumin (BSA) forming the carotenoid–protein complex (Wackerbarth *et al.* 2009).

An important step is to measure and predict the solubility of target components (carotenoids) in the fat phase of milk at pasteurization and homogenization conditions, the preliminary steps towered dairy manufacture to optimize the extraction process. At pasteurization stage to inactivation of microorganisms, resulted showed increasing in the total carotenoids content although the carotenoids are sensitive to heat, and may varying percentages of desirable constituents such as nutrients, colour, aroma and texture are destroyed (Alwazeer *et al*. 2002; Blasco *et al*. 2004). This increased may relate to lower the density of fat phase that had influence increasing the solubility of carotenoids. A second hypothesis is that the different vegetable matrices (green pepper, parsley and broccoli) have different abilities to release carotenoids (due to different fiber composition or different intracellular locations of carotenoids) during heat treatment. After homogenization, the total carotenoids content was extent increased as showed in Table.1. A first hypothesis is that homogenization pressures size-reduced the fat droplets and further the carotenoids were associated with lipid portions thus enhancing the total soluble carotenoids. The solubility of natural sources of targeted carotenoids (green pepper, parsley and broccoli) is related to its physical and chemical properties such as polarity, molecular structure, and nature of the material particles and it is also related to the operating conditions such as temperature, pressure, density of fat (Shi *et al.* 2007). The degree of saturation of fat phase was also significantly affect ($P \leq 0.05$) on the carotenoids solubility. Oils rich in unsaturated fatty acids promote carotenoid solubility. Results showed that vegetable oils which are usually rich in polyunsaturated fatty acids were enhanced the release of carotenoids from vegetable matrices; whereas in partial substitution of milk fat (saturated fat milk) by vegetable oils the release of total corotenoids was more than in milk fat (saturated fat milk). Solubility of carotenoids depends on the presence of polar groups in the carotenoid and fatty acid pattern of the vegetable oil (Borel *et al.* 1996).

In dairy products, in addition to carotenoids function as the natural pigments and provitamin A precursor role, carotenoids has another function that is the role the proteins with respect to other molecules (Britton 1995). Partial substitution of milk fat is important not only because of their nutritional significance, but also due to their role in providing the required fatty acids to the body that may render health benefits to the consumer. In this condition, carotenoids present in these vegetables have a strong ability to neutralize active oxygen species (Howard et al. 2000) and therefore can protect unsaturated fatty acids against oxidation free radical damage (Surita *et al.* 2008) and off-flavor development.

In the β-carotene/linoleic acid assay, antioxidant activity of the carotenoids extracts from fat phase of homogenized milk (2%) varied and broccoli was higher in antioxidant activity than parsley and green pepper respectively ($P<0.05$). There are positive correlation between carotenoids content and its antioxidant activity. Antioxidants play an important role in the protection of human body against damage by reactive oxygen species (Govindarajan, 2005). Furthermore previous epidemiological studies have shown that the intake of natural antioxidants has been associated with reduced risks of cancer and other diseases associated with oxidative damages (Rietveld and Wiseman, 2003).

The stability and nutritional properties can be optimized for different applications, depending on the demands on the final product.

5. Conclusion:

The reduction of saturated fatty acids and cholesterol content in the human diet has thus become an increasingly important goal in modern medicine, especially in view of the preponderance of evidence connecting these components of the diet are increased risk for heart disease, etc. Partial replacement of milk fat (saturated fatty acids) with vegetable oils and enrichment milk with natural source for carotenoids (as antioxidant) is an excellent way to develop optimum health benefits of dairy products. When vegetable oils are used, in combination with milk fat or on their own, the balance between various fatty acids can be adjusted to a higher nutritional value. Supplementation milk with natural source of carotenoids play an important role as antioxidant for protect the PUFA from the oxidation and as a fortified product. Choosing the type of carotenoids source depend on the sensory evaluation of milk supplemented and the product need to manufacture. Therefore, the consumption of the described modified dairy product may meet the demand of subjects who wish to lower their risk for atherosclerosis and cardiovascular disease.

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