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ANTIOXIDANT CAPACITY OF COMMERCIAL MILK SAMPLES

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Abstract

Milk is a rich source of nutrients and has been considered by many as a valuable element of a complete diet. For instance, commercial cow's milk contains many bioactive components that boost the physiological process in the body. Among these are components exhibiting antioxidant activity. This study aims to analyze the antioxidant capacity of commercial milk samples (whole milk, reduced fat, non-fat and non-fat chocolate milk) using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay with Vitamin E (α-tocopherol) as standard. Results show that the chocolate milk has the highest antioxidant activity in comparison to the other commercially available milk samples that almost have the same antioxidant capacity. The high antioxidant capacity in non-fat chocolate milk can be due to the presence of cocoa powder, which contains phenolic compounds with antioxidant properties.

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Introduction

Dairy products such as milk are an essential part of human nutrition[1] and make up around 25-30% of the average diet.[2] The consumption of milk is estimated at billions of liters around the world, with majority of which consumed as pasteurized cow's milk[3] at an average of 10-212 kg per person annually.[4] Being the first food not only produced by humans but also in all mammals, milk provides all the necessary energy and nutrients to ensure proper growth and development.[5] Milk contains 18 of 22 essential nutrients including proteins, minerals, vitamins, and fatty acids[6] that have great impact on human metabolism and health. For instance, proximate analysis of the cow's milk yielded 88% water, 2.5% protein, 3-4% fat, 1.2% minerals and 5% lactose.[7]

A recent meta-analysis[6] of studies on the effects of consuming milk and dairy products found that consuming dairy products was associated with reduced risk of childhood obesity. In addition, preliminary evidence suggests the potential protective effects of milk against overweight, obesity, diabetes and cardiovascular disease.[8] Although milk and dairy was reported to have a positive effect on bone mineral density and was inversely related to colorectal cancer, bladder cancer, gastric cancer, and breast cancer[6] results from different meta-analysis studies were reported in one paper to show no correlation between milk intake and cancer.[8] Another study showed milk intake associated with a lower risk of cardiovascular disease, stroke, hypertension, colorectal cancer, metabolic syndrome, obesity and osteoporosis.[9] The same study also reported beneficial associations of milk intake with type 2 diabetes mellitus and Alzheimer's disease but pose higher risk on prostate cancer, Parkinson's disease, acne and Fe-deficiency anaemia.[9]

Antioxidants are molecules that scavenge of neutralize the free radicals and prevent the oxidation of other molecules or oxidative stress.[10] Free radicals are continuously produced in the body by either internal metabolic processes or by external environmental factors or lifestyle choices.[11] Uncontrolled free radicals in body can lead to oxidative stresses that have been implicated in the breakdown of vital biochemical compounds like lipids, protein, DNA resulting to diabetes, accelerated aging, carcinogenesis, and cardiovascular diseases.[12] Antioxidants can prevent this by interacting with the free radical in order to neutralize or terminate the chain reaction before there is cellular damage.[11] Since synthetic antioxidants such as BHA (butylated hydroxyl anisole) and BHT (butylated hydroxyl toluene) posed health risks, there is a demand to tap natural food sources as source of antioxidants. Dairy product such as milk can be a good source of natural antioxidants.

Among the antioxidants found in milk are the following: amino acids (cysteine and tyrosine), vitamins (A and E), carotenoids and enzymes such as superoxide dismutase, catalase and glutathione peroxidase The antioxidant capacity has a function in the shelf life of the cow's milk and also can help protect against free radicals.[13] The antioxidant capacity of milk has importance in the food industry because of the health benefits as well as taste of the milk.[14] Loss of antioxidants can promote oxidation of the milk which can result in an off-taste and a loss of the nutritional benefits.[14] The antioxidant capacity of cow's milk is directly related to the concentration of tocopherols, carotenoids, and fortification of the milk.[14] In chocolate milk, the flavonoids from the cocoa powder may add to the total antioxidant capacity.[15] The natural antioxidant capacity of cow's milk is affected by the processing, packaging, storage conditions, and duration of the storage of the milk.[14]

There are many antioxidant assays available and DPPH microplate assays using spectrophotometry is one way of determining total antioxidant capacity of milk.[16] The degree of color change of the sample in DPPH and the decrease of absorbance of the solution at a specific wavelength is correlated to an increase in concentration of antioxidants in the sample.[16] The principle of the assay is that the antioxidants in the milk scavenge the free radicals which causes the DPPH solution to change from dark violet to clear yellow. This study aims at determining the total antioxidant capacity of commercially available milk products using a microplate-based DPPH assay with Vitamin E as a standard using spectrophotometry.

Experimental Methods

Different commercial milk samples (whole milk, reduced fat milk, non-fat milk and non-fat chocolate milk) with the same expiration date were used in the analysis (Figure 1). This represented the milk marketed for direct consumption with the fat content usually standardized to the levels required by law for the three types: whole (>3.5%), semi-skimmed (1.5%–1.8%), and skimmed (<0.5%). The antioxidant capacity of these milk samples were determined by microplate-based assays using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay with Vitamin E (α-tocopherol from Sigma Aldrich) as standard and it is a modified method used by Martysiak-Zurowska and Wenta (2012).[17] The DPPH solution was prepared using 0.004g of DPPH (Sigma Aldrich) dissolved in 50.0 mL of methanol (Sigma Aldrich). In an Eppendorf tube, the milk sample (20 µL) was mixed with 1,000 µL of DPPH reagent, and 500 µL of chloroform. The resulting mixtures were incubated in the dark for 30 minutes, centrifuged for 5 minutes, and transferred into the microplate wells (200 uL). Absorbance was recorded at 515 nm using a spectrophotometer (Biotek Cytation 5). All assays were replicated at least three times. A blank made up of 1,000 µL of DPPH reagent mixed with 500 µL of chloroform was also prepared and analyzed. Different concentrations of Vitamin E solutions (0 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm) were also prepared for calibration curve. The experimental data (absorbance) were evaluated using student's t-test ($p < 0.05$) to compare the milk samples total antioxidant capacity.

Results and Discussion

Total antioxidant capacity can measure the overall antioxidant activity in foods. DPPH is an electron-transfer based assay that is used to determine the TAC and the DPPH microplate assay can assess the TAC level directly from biological fluids such as milk.[18] DPPH is a stable free radical at room temperature which produces a dark violet solution in methanol and upon exposures to antioxidant molecules in the sample, it gets lighter in response to increasing antioxidant capacity.[19] This reaction can be measured spectrophotometrically with the solution absorbance reduced and sometimes can become colorless depending on the amount of antioxidants.[19]

Based on the calibration curve prepared (Figure 2), results showed the chocolate fat free milk having the highest total antioxidant capacity (Figure 3). The main possible reason for this high total antioxidant capacity in chocolate fat-free milk is the presence of cocoa beans[15, 20] a raw material containing powerful load of antioxidants such as polyphenols. One study have shown that the higher the polyphenol content of cacao beans resulted in higher ability to scavenge free radicals or antioxidant activity.[21]

Figure 1. Commercial milk samples used in the study: Cream-O-Land whole milk fortified with vitamin D, Cream-O-Land fat free chocolate milk fortified with vitamins A and D, Cream-O-Land reduced Fat 2% milk fortified with Vitamins A and D, and Cream-O-Land fat free milk fortified with Vitamins A and D.

For the other 3 samples (whole, reduced and non-fat milk) where the level of fat content differs, results showed significant decrease in the total antioxidant capacity with decrease in fat content. The removal of milk fat significantly decreases the antioxidant content of various milk products because it also removes the fat-soluble vitamins as well. The slight but significant decrease in antioxidants shown from whole milk to 2% and from 2% to non-fat could be a result of the vitamin E lost in the skimming process.[14] However, there should be a more drastic decrease in the antioxidant levels from whole milk to 2% and from 2% to non-fat cow's milk but this was not observed in the results since the difference is not that large as compared to that of the chocolate fat free milk sample. The main reason for this can be due fortification of reduced and non-fat milk samples with Vitamin A and D. Typically, whole milk contains 5% daily value (DV) of Vitamin A and 24% DV of Vitamin D and after fortification, reduced-fat milk contains 9% DV of Vitamin A and 29% DV of Vitamin D while non-fat milk contains 10% DV value of Vitamin A and 25% DV of Vitamin D.[22]

The fortification of milk with Vitamin A and Vitamin A began

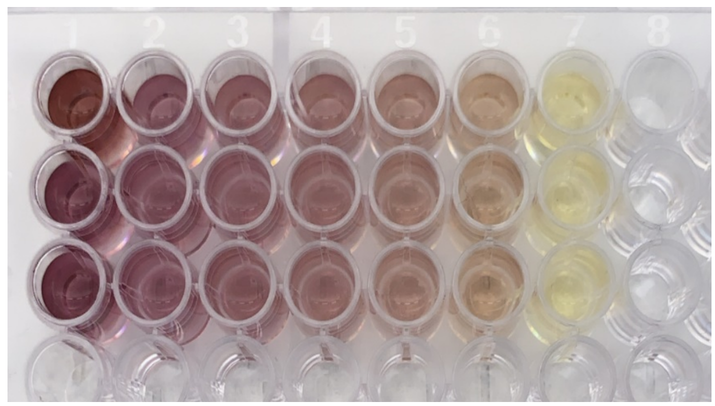

Figure 2. Microplate showing the color change in the DPPH mixture with increasing antioxidant levels (Vitamin E concentration) 0 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm, 250 ppm, and 500 ppm.

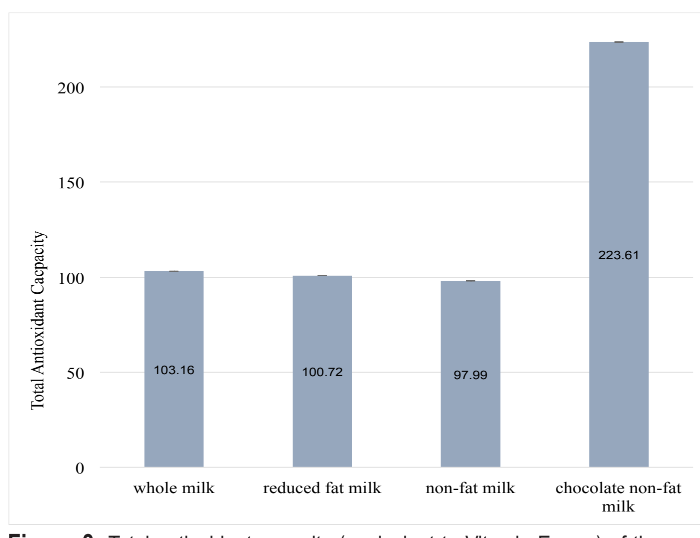

Figure 3. Total antioxidant capacity (equivalent to Vitamin E ppm) of the commercially available cow's milk samples that were tested using the DPPH microplate assay using Vitamin E standard.

in the 1930s in the United States in order to prevent rickets in children.[23] Vitamin A is important for cell division, organ and skeletal growth and maturation, immune system strength, and development and maintenance of eye health and night vision[24] while Vitamin D plays an important role in calcium absorption, bone growth and phosphate homeostasis.[25] Although fortification is optional for whole milk, reduced-fat and skim milks in the United States must be fortified with Vitamin A and Vitamin D with at least 2,000 IU/quart and 400 IU/quart, respectively.[23]

Conclusions

The total antioxidant capacity of the commercial milk samples (whole milk, reduced fat, non-fat and chocolate milk) were analyzed using a microplate assay using DPPH with Vitamin E as standard. Results show that the chocolate milk has the highest antioxidant activity in comparison to the other milk samples (whole, reduced and non-fat milk) that have close but significantly different antioxidant capacity due to the fortification process in the milk. The high antioxidant activity in chocolate milk can be due to the presence of cocoa added. The cocoa powder added to the chocolate milk contains polyphenols that are loaded with antioxidants.

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