

Dietary Antioxidant Profile and Control of Lipid Oxidation Characteristics of Daniellin™

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Abstract The aim of this present work is to study the possibility of using Daniellin™ as a dietary antioxidant and for controlling lipid oxidation in dairy milk. Daniellin™ found earlier to be able to control the production of ochratoxin A in a non-alcoholic beverage was produced in tablet form and it had (mg/g): vitamin A (6.3×10^{-4}) and vitamin C (2.64). Daniellin™ dietary antioxidant with a moisture content of 2.1 %, pH 7.0 played an important role in lipid peroxidation because when it was added to fresh dairy milk at 50, 150, and 200 ppm it had antioxidant effectiveness* of 69.2%, 76.9% and 84.6% respectively.

* Antioxidant effectiveness (AE) = $\frac{\text{Peroxide value (PV) of control} - \text{PV of test}}{\text{PV of Control}}$

Keywords Daniellin™, Antioxidant, Lipid Oxidation, Dairy Milk

1. Introduction

Dietary antioxidants are molecules that prevent cell damage against free radicals and are critical for maintaining optimum health in both animals and humans and apart from their beneficial effects on immune cell function dietary antioxidants are also useful for protection against infectious microorganisms(1). In order to help the prevention of or at least, delay the onset of several degenerative disorders, it is essential to have an adequate intake of antioxidant nutrients from an early age(2).

Diets rich in antioxidant-containing components such as polyphenolic compounds, vitamins E and C, and carotenoids are believed to be useful nutrients in the prevention of oxidative stress-related diseases(3). Furthermore, diets rich in antioxidant-containing compounds have been reported to relate with lower risks of some cancers and coronary heart disease(4) and antioxidants have been found to have the ability to protect the body from damage caused by free radical oxidative stress(5). At 750 µg / ml, a fraction from the leaf of *Aframomum daniellii* was reported to be potent in scavenging 1,1-diphenyl 1, 2-picrylhydrazyl (DPPH) free radicals(6). The antioxidants obtained from *A. daniellii*(7), the source of Daniellin™, are more potent than synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

When Daniellin™ was incorporated into a non-alcoholic beverage with storage at 26± °C for 5 days, the protein content and calorific value of treated samples increased and there was a 100 % reduction in the level of ochratoxin A in the samples(8). However, with a paucity of data on the application of Daniellin™ in food matrices, and with milk being a source of organic compounds and minerals(9), we became interested in the possibility of utilizing Daniellin™ as a dietary antioxidant and in order to control lipid oxidation in milk as *A. daniellii* from where Daniellin™ was produced, has previously been used to control lipid oxidation in different food systems(10,11).

2. Materials and Methods

2.1. Milk

Fresh cow's milk obtained in Ibadan, Nigeria and was kept at 4-10°C under aseptic conditions until used for analyses.

2.2. Preparation of Dietary Antioxidant

Arising from experimental trial runs in order to determine the concentrations of Daniellin™ ingredient to excipient for use in tablet formulation, the following (w/w) were chosen: Daniellin™, 4.8 %; ascorbic acid, 32% and lactose, 20 %, thereafter the materials were mixed by gentle turning using pestle in a mortar. The method described by Adebayo and Itiola(12) for tablet production was modified as follows: 500 mg of mixture (Daniellin™-ascorbic acid- lactose) was compressed on a Carver hydraulic hand press ma-

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chine(Model C, Carver Inc, USA) using a 10.50 mm diameter die and flat-faced punches lubricated with 0.3 % (w/v) of magnesium trisilicate dispersed in acetone with dwell-time of 1 min and compression pressure of 1.0 Torr. After ejection, Daniellin™ dietary antioxidant tablets were stored over silica gel in a desiccator for 24-36 h to facilitate hardening and elastic recovery.

2.3. Moisture Content and pH

For moisture content and pH determinations, the methods described by AOAC (13) were employed.

2.4. Minerals

Into a macro-Kjeldal digestion flask was placed 5g of Daniellin™ dietary antioxidant, 20 ml of HNO_3 and 20 ml of distilled water. The mixture was boiled to half of its original volume after which it was cooled to $26 \pm 1^\circ\text{C}$ and 10ml of H_2SO_4 was added followed by heating until white fumes appeared. The mixture was cooled, boiled till white fumes appeared, filtered and used for detection of minerals with Atomic Absorption Spectrophotometer (13). The procedure was done in duplicate.

2.4. Vitamin A

The method described by Kirk and Sawyer (14) was modified as follows: to 2 g of Daniellin™ dietary antioxidant were added: hydroquinone, 10g; ethanol, 40 ml and potassium hydroxide solution, 10 ml followed by boiling under reflux action for 30 min. The mixture after cooling was transferred along with 20 ml of distilled water into a 500 ml separatory funnel. Extraction was then carried out twice using 25 ml of diethyl ether. Washing of the extract was done using 50 ml of distilled water and ether was thereafter distilled off. Absolute alcohol, 5 ml was added to the extract, evaporated to 5 ml in order to remove excess water.

The residue was dissolved in isopropyl alcohol, centrifuged and optical density taken at 520 nm was used to calculate concentration of vitamin A:

$$\frac{\text{Concentration of standard} \times \text{absorbance of standard}}{\text{Absorbance of standard W}} \times V$$

Where V = volume of solution containing sample weight
W = weight of sample taken

2.5. Vitamin C

For the determination of vitamin C (ascorbic acid), the method described elsewhere (13) was employed.

2.7. Microbial counts

Bacterial and fungal counts of Daniellin™ dietary antioxidant were carried out as described by Adegoke (15).

2.6. Antioxidant Effectiveness

The methods for adding Daniellin™ to milk, determination of peroxide value (PV) and antioxidant effectiveness (AE) were similar to methods described earlier (7).

3. Results and Discussion

3.1. Chemical analysis of Daniellin™ Dietary Antioxidant

Daniellin™ dietary antioxidant with a moisture content of 2.1% had some minerals and vitamins (Table 1). The plant material from where Daniellin™ was produced had minerals like magnesium and calcium (10).

Daniellin™ dietary antioxidant had vitamins E and C and other nutrients. In the prevention of oxidative stress-related diseases (3), vitamins A and C and other nutrients present in fruits and vegetables are known to be useful particularly as dietary antioxidants have been found to modulate the host susceptibility or resistance to infectious pathogens (1).

With evidence-based science linking diet to reduction in non-transmissible chronic disease risks (15), the relevance of foods that provide attributes beyond nourishing properties (16) are relevant. Furthermore, in order to help prevent the development of, or at least delay the onset of several degenerative disorder, it might be essential to have adequate intake of antioxidant nutrients from early age as adequate amounts of antioxidants that neutralize oxidant-mediated tissue injury can help prevent damage to the immune cells (2). When it is realized that intake of dietary antioxidant supplement can help to reduce the adverse effects of oxidative stress in man (17), Daniellin™ with its nutrients and an antioxidant effectiveness of 84.6% at after 9 hours of its addition to dairy milk (table 2), can be further explored for use in stabilizing fat-containing foods and also providing additional benefits to consumers.

With the use of Daniellin™ in the stabilization of the milk in this present study, it is possible that Daniellin™ can also be useful in other dairy products packed using transparent materials where light oxygen and light-induced changes have been reported (18).

Microbiological characters of Daniellin™ dietary antioxidant

Using standard microbiological methods, Daniellin™ dietary antioxidant before being added to test milk was found to have an acceptable microbiological quality (data not shown) had a count of 7.3×10^1 and this count is within the acceptable limit for orally administered solid in pharmaceutical preparations (19). Daniellin™ has earlier been found to have potent inhibitory activities against ochratoxin A and aflatoxins and extending the shelf life of a perishable beverage (10).

Table 1. Moisture content, pH, Vitamins and Minerals in Daniellin™ Dietary Antioxidant

M.C.* (%)	pH	Vitamin (mg/kg)	
		A	C
2.1	7.0	6.3×10^{-4}	2.64

*: Moisture content (%)

While good manufacturing practices at all levels of the production of milk are essential for product safety and to avoid deterioration, however, chemical and microbiological deteriorative changes still do occur(20), thus DaniellinTM with its effect on the stability of dairy milk in this study, we have more interested interests in further studies of DaniellinTM for use in controlling deteriorative changes in dairy products.

Table 2. Peroxide Values and Antioxidant Effectiveness of DaniellinTM in Dairy Milk

Daniellin TM (ppm)	Time (h)			
	0	3	6	9
50	2.4	1.4(30)*	1.0(58.3)	0.8 (75)
150	2.0	1.2(40)	0.7(70.8)	0.6 (83.3)
200	1.8	1.1(45)	0.5(79.2)	0.4 (91.7)
Control	2.0	2.0	2.4	2.6

*: % antioxidant effectiveness: $\frac{\text{Peroxide value (PV) of control} - \text{PV of test}}{\text{PV of control}}$

4. Conclusions

DaniellinTM stabilized dairy milk and the dietary antioxidant has vitamins A and C and minerals like iron, calcium and magnesium.

As some antioxidants of plant origin have been recommended for use as potential chemotherapeutic agents in place of the ones currently being used for treatment of some degenerative processes like cancer(21), and with the report of the protective effects of *Aframomum danielli* (source of DaniellinTM) on liver cells and lowering of levels of some enzymes associated with liver dysfunction(22) DaniellinTM is being examined for its potential applications in agriculture and medicine. Furthermore, as DaniellinTM was able to control lipid peroxidation in fresh milk, our future research works will highlight the synergistic effects of DaniellinTM with antioxidant nutrients in dairy milk.

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