

# Microbiological and Sensory Profile of Soymilk Based Juice Treated with Liquid Extract of *A. Danielli*

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**Abstract** The microbiological and sensory profile soymilk-based juice treated with aqueous extract of *Aframomum danielli* (1%-3%-w/v) and stored at  $27\pm 2^{\circ}\text{C}$  for twenty four weeks were examined. Synthetic additives/preservatives, which have been in use over the years, are more expensive and mostly imported with hard earned scarce foreign exchange. Adequate research work into the production and utilization of indigenous food additives on a large scale has not been undertaken in developing countries. This work was therefore planned to ascertain the usefulness of extracts of *A. danielli*, a local spice, in stabilizing the microbiological quality of soymilk-based juice. Standard methods were used for the production of juice samples from pineapple, orange, carrot and milk from soybeans. They were blended together in equal proportion and thereafter treated with *A. danielli* extract (1.0% to 3.0%) and stored at  $27\pm 2^{\circ}\text{C}$  for twenty four weeks. Infrared spectrophotometer was used to identify functional groups in *Aframomum danielli* and hence the active components responsible for the stability of the juice. Standard AOAC method was used for microbiological analysis of treated and untreated samples. Consumer evaluation of juice samples was done using analysis of variance (ANOVA). Some of the active components of the spice identified are 4-amino-acetophenone, N,N-dimethyl-2-chloroacetoacetamide, 3-beta-acetoxy-5-etienic acid, 6,10-dimethylundeca-5,9,-diene2-one, Phenyl-3-buten and 4-Phenyl butanone. There were significant differences in the microbial counts of the treated and untreated samples. Treated samples experienced little or no growth over the period of storage. The results validate previous reports that *A. danielli* extract has great preservative potentials. There were significant differences ( $P < 0.5$ ) for colour and overall acceptability of the samples. When *A. danielli* extract was added to soymilk-based fruit juice, the microbiological and sensory qualities were found to be better preserved than that of the untreated samples.

**Keywords:** *A. danielli*, Soymilk-Based Juice, Microbial load, Shelf Life

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## 1. Introduction

Fruits and vegetables are among the most important foods of mankind as they are not only nutritive but are also indispensable for the maintenance of health. They play important roles in the diet of most people in the tropics, providing essential minerals and vitamins and adding colour, flavour and variety to monotonous diets. Fruits and vegetables, in particular, are abundant sources of carotenoids in the diet and are highly perishable subject to rapid deterioration by microorganisms, enzymes, or oxidation reactions. Delgado and Sun (2000) reported the combination of freezing technology's beneficial effects of low temperature at which microorganisms cannot grow, reduced chemical reactions, and cellular metabolic reactions are delayed to retain the sensory attributes and nutritional properties of the products. They deteriorate more under tropical conditions due to the high ambient temperatures and humidity, pest and disease infestations, poor handling and storage facilities (Levi *et al*, 1983;

Aworh and Olorunda, 1988). This has led to large post harvest losses during the period of abundance, followed by season of scarcity. Fruits and vegetables are essential component of a healthy diet, able to decrease the risk of cardiovascular diseases and cancer (Allende *et al*, 2006). Apart from their nutritional and sensory properties, they are currently recognized as active and protective agents (Olivas and Barbosa-Canovas, 2005). Fruits and vegetables contain a wide variety of potential cancer inhibitory nutrients and other phytochemicals. Substantial evidence has indicated a biological link between dietary constituent and carcinogenesis (Clade, 1999; Steinmetz *et al.*, 1996).

Many fruits and vegetables are subjected to various types of processing prior to consumption. They may be canned, dried or processed into juices. Processed products are expected to be essentially uniform in composition and sensory characteristics from batch to batch. Juice processing is one of the attractive and effective means of fruit utilization and preservation. Fruit juices are becoming an important part of the modern diet in many communities (Ghengbesh *et al.*, 2005). Fruit juice with

soya, tea, milk or vegetables, with the addition of functional ingredients such as L-carnitine and vitamins, offer a wide variety of alternative in terms of product and positioning, from low-calorie to free of refined sugars, from wellness to fitness. Currently, the market demand for tropical fruit juices are increasing tremendously due to increase in consumer awareness that fruit juices are healthy and nutritious in such a way that they can beneficially affect human health (Wong *et al.*, 2003). In line with this, consumers are also becoming more sensitive and vigilant on the use of synthetic additives to preserve food safety or enhance characteristics such as colour and flavour (Corbo. 2009).

Various preservative methods have been adopted in extending the shelf life of fruit juices including the use of spices. In recent times, spice antioxidants have raised considerable interest among food scientists, manufacturers, and consumers, because of their natural antioxidants (Lu *et al.*, 2011). Many synthetic chemicals such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), though very effective as antioxidants, have been known to have toxic and carcinogenic effects on humans (Ito *et al.*, 1985). Synthetic antioxidants may result in liver swelling and influence liver enzyme activities (Martin and Gilbert, 1968). Numerous studies have shown the antioxidant potentials of aromatic, spicy, medicinal plants (Chang *et al.*, 1977; Osawa *et al.*, 1992; Weng and Gordon, 1992). The use of these plant materials as natural antioxidants for food, cosmetics and other application becomes necessary because of food safety issues. Because of their natural nature and their scavenging properties, they are more readily accepted by consumers (Miliauskas *et al.*, 2004; Miliauskas *et al.*, 2004). Consumers are increasingly aware of the risk posed by synthetic antioxidants due to their high volatility and instability at elevated temperatures. As a result of this, focus has been shifted to the use of natural antioxidants in food preservation (Odukoya *et al.*, 2005; Oboh and Rochas, 2007; Adefegha and Oboh, 2011a).

Report has it that the main active components in spices are phenol acids, flavonoids and volatiles or essential oils (Shan *et al.*, 2005; Wojdylo *et al.*, 2007; Viuda-Martos *et al.*, 2011; Lu *et al.*, 2011). Consumption of antioxidant rich food such as spices, fruits, and vegetables could be a practical approach towards improving antioxidant status, thereby enhancing good health and preventing diseases (Chu *et al.*, 2002; Oboh and Rochas, 2007; Carlsen *et al.*, 2010). Some natural spices, herbs and flavour are known to inhibit oxidation and have been proposed for use in foods. *Aframomum danielli* is one of these numerous spices and is known to possess preservative properties (Adegoke *et al.*, 2002) and has among other characteristics, nutritive and broad antimicrobial effect (Adegoke and Skura, 1994). Antioxidant extracts are more potent than synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxianisole (BHA) (Adegoke and Copalakrishna, 1998) with potent synergistic inhibitory effects on food spoilage yeast when used in combination with hydrostatic pressure (Adegoke *et al.*, 1997).

Soymilk (also called soyamilk, or soybean juice and sometimes referred to as soydrink/beverage) is a beverage made from soybeans (*Glycine max*). It is a stable emulsion of oil, water, and protein. Soymilk is produced by soaking dry soybeans and grinding with water using Hammer mill

before cooking at boiling point for about 20 minutes. Soymilk contains similar proportion of protein as cow's milk; around 3.5% and 2% fat, 2.9% carbohydrate, and 0.5% ash. They have been blended with other food products to produce other products, most likely, with better nutritional qualities. Milk-based products all contain actual milk (and/or soymilk) or different combinations of modified proteins. Most of the popular milk-based beverages are good sources of protein, containing 10 to 40 grammes per serving.

As a result of this, new innovation/technology must be applied in the production of better quality products. The main requirement new technology must meet is to ensure product microbial safety while preserving sensory and nutritional characteristics to obtain products more similar to fresh foods (Torres and Velazquez, 2005). Producers equally face the challenge of always finding ways to preserve food in a high quality state until it reaches the consumers. The present study was carried out to investigate the effect of *A. danielli* liquid extract, a local spice, on the microbiological and sensory profile of soymilk-based juice with regard to the reported polyfunctional nature of the spice.

## 2. Materials and Methods

*A. danielli* pods were purchased from Oja-Oba and Bode, two local markets in Ibadan. The seeds were removed, cleaned of extraneous materials, winnowed and milled into powder using hammer mill. The powder was then sieved with a wire mesh to obtain fine powder. 1g, 2g and 3g respectively of the powder were each added to 100mls of distilled water and mixed thoroughly at  $-4\pm 2^{\circ}\text{C}$  for 5 days before being centrifuged at 300 revolutions per minute for 20 minutes. The supernatants were obtained as *A. danielli* extract (Adegoke and Skura, 1994).

### 2.1. Juice Preparation

Fresh oranges, pineapple were obtained from a farm at Ajibode village in Ibadan. Carrots were purchased from Sabo in Ibadan, while soybean was sourced from International Institute for Tropical Agriculture (IITA) in Ibadan. Fresh juicy, good quality fruits and vegetable to be used for the research were sorted for processing. The fruits and vegetable were thoroughly washed under tap water to remove extraneous materials before juice was extracted from them using a modified method of Battcock *et al.*, 1998. Soymilk was equally made from the cleaned soybeans under laboratory condition. Equal quantities each of the juice from orange, pineapple, carrot and laboratory prepared soymilk were blended together. The blended juice was then treated with aqueous extract of *A. danielli* (1%-3% - w/v) and stored in plastic polyethylene containers at  $27\pm 2^{\circ}\text{C}$  for twenty four weeks with the untreated samples.

### 2.2. Microbiological Analysis

Pour plate technique was used for the microbiological examination of the various samples as described by Adegoke (2000). One gramme of each of the sample was dissolved in 1ml of 2% sterile sodium citrate solution in order to prepare a suspension. 1 ml of the suspension was

then used for serial dilution of between  $10^{-1}$  to  $10^{-6}$ . 1 ml of diluted sample was placed in sterile disposable petridishes (sterilin) in triplicates. At about  $44^{\circ}\text{C}$  –  $50^{\circ}\text{C}$ , the various media were poured on to the samples in the petridishes and allowed to set, inverted and incubated for 48hours.

### 2.3. Sensory Evaluation

Sensory evaluation of the juice was judged for color, flavour, taste and general acceptability on a nine-point hedonic scale, varying from 'dislike extremely' (score 1) to 'like extremely' (score 9) was used, according to a modified method of Stone and Sidel (1992). An informal panel of 10 untrained assessors carried out the sensory evaluation. The juice was served in a transparent white glass cups for proper assessment. Twelve (12) cups with three (3) cups each representing each treatment and coded separately in order to have a mean score of each treatment

## 3. Results and Discussion

### 3.1. Microbial Analysis

Table 1 gave the result of the microbial and mould analysis carried out on the samples. Bacterial and mould counts for untreated samples recorded high values after twenty four weeks of storage (not shown in the table). The untreated samples recorded high loads for both the mould and bacterial counts, while over half of the treated samples recorded little or no growth after the twenty four weeks of storage (table 1).

Min *et al* (2003a, 2003b) achieved a 6 log inactivation of endogenous bacteria in tomato and orange juices after thermal ( $90^{\circ}\text{C}$ , 90s) and Pulse Electric Field (PEF) treatment (40KV/CM, 5757us and  $45^{\circ}\text{C}$ ). The shelf life of the treated samples was established taking into consideration the initial microbial population of the untreated samples. The lethal effects of *A. danielli* extract on some microorganisms or the decrease in the amount of organisms in *A. danielli* treated samples could be attributed to antimicrobial effect of *A. danielli* as reported in previous research works by Zaika, 1988; Adegoke and Skura, 1994; Fasoyiro *et al.*, 2001. Likewise, Ogunwolu and Adio (2003) found *A. danielli* extract to be as effective as sodium benzoate in the reduction of microbial count and subsequent preservation of cashew juice. Similar effect of *A. danielli* on products was obtained in this study.

Some researchers have equally used synthetic and/or natural additives in preventing microbial deterioration in foods. Lavinias *et al.*, (2008) reported that the quality of juice sample was stable for 8 weeks when treated at high hydrostatic pressure of 400 MPa for 3 minutes at  $25^{\circ}\text{C}$  under refrigeration; Ashaye *et al.*, (2007) reported a reduction in microbial count and resultant preservation of cheese when treated with *A. danielli* extract. Ceylan *et al* (2004) was able to reduce the population of *E. Coli* (0157:H7) from 5.31 CFu/ml to 2.2 in 3 days in apple juice that was treated with potassium sorbate. Likewise, Crook and Boylston (2004) reported that 0.1% potassium sorbate was able to prevent deterioration in apple cider during storage, Luedtke and Powell (2002) reported increased shelf life of juice by inhibiting yeast and mould

in a low pH environment using potassium sorbate, while Hussain *et al* (2008) used 0.2% potassium sorbate to preserve apple and apricot juice blends.

Omemu *et al* (2006) reported high microbial load in locally prepared roselle juice hawked daily in our local markets, Al-Jedah and Robinson (2002) reported high microbial load ( $1.4-3.2 \times 10^8$ ) for juices sold in Qatar, while Gbenghesh *et al* (2005) reported  $1.8-8.4 \times 10^6$  for various single strength juices sold in Tripoli, Libya. On yeast and mould counts, Omemu *et al.*, (2006) reported  $1.2 \times 10^4$  in fresh fruit juices, while  $1.0-3.3 \times 10^4$  was reported in fresh fruit juices sold in Qatar (Al-Jedah and Robinsin, 2002).

There were reductions in the microbial populations of the treated samples when compared with those of the untreated samples. The reduction experienced could be attributed to the potency of *A. danielli* extract which could be compared to that reported for other modes of treatments such as the use of PEF reported by Jia *et al.*, 1999; Yeom *et al.*, 2000; Elez *et al.*, 2006; Rivas *et al.*, 2006 in extending the shelf life of the products and even better, as local spice in this case *A. danielli*, has no reported or recorded side effect on consumers or quality of the products, most especially now that most of the synthetic preservatives in use come with their attendant problems. It was observed that the quality of the juice was stable for over 10 weeks and that bacteria and mould load increases afterwards, which might be due to the germination of spores as the effect of the preservatives decreases gradually or could be as a result of the presence of natural microorganisms in *A. danielli* extract that are present in the unpurified spice.

**Table 1. Microbiological Quality of the Preserved Blend of Juice from Carrot, pineapple, orange with Soymilk**

No. of Wks.	Bacteria (CFU/ml x $10^{-3}$ )	Moulds (CFU/ml x $10^{-3}$ )
2	Nil	N
4	Nil	il Ni
6	Nil	l Ni
8	Nil	l Ni
10	Nil	l Ni
12	Nil	l Ni
14	Nil	l Ni
16	<1	l Ni
18	<1	l Nil
20	<1	<1
22	<1	<5
24	<5	<5

**Sensory analysis:** There were significant differences in the colour and general acceptability of the samples. The result from the sensory evaluation demonstrated that the use of *A. danielli* in soymilk-based juice did not have any negative effect on sensory quality attributes. After about three and half months of storage, the taste and colour of the treated samples began to depreciate and did not have much effect on other quality attributes of the juice. There were no significant differences in the taste and aroma of both the untreated and treated samples as shown in table 2. The overall acceptability assessment for the juice was put at liked moderately.

Table 2. Mean Scores of Sensory Acceptance of CPOS Juice

Sample	Colour/App	Taste	Aroma/Flavour	Acceptability Overall
Untreated	6.07 <sup>a</sup>	6.17 <sup>a</sup>	5.83 <sup>a</sup>	5.97 <sup>b</sup>
A1	5.83 <sup>c</sup>	6.20 <sup>a</sup>	6.20 <sup>a</sup>	6.10 <sup>a</sup>
A2	5.97 <sup>b</sup>	5.77 <sup>a</sup>	5.87 <sup>a</sup>	5.93 <sup>b</sup>
A3	6.07 <sup>a</sup>	5.80 <sup>a</sup>	5.87 <sup>a</sup>	5.57 <sup>c</sup>

\*CPOS- Blend of carrot, pineapple, orange and soymilk.

\*A1-Sample treated with 1% *A. danielli* extract.

\*A2-Sample treated with 2% *A. danielli* extract.

\*A3-Sample treated with 3% *A. danielli* extract.

\*Mean + SD down a column with superscript are significantly different with a>b>c. Mean separation done with Duncan Multiple Test Range.

\*Means for each attributes followed by the same letter are not significantly different at 5% level by Turkey.

## 4. Conclusion

This study has been able to highlight once again the preservative potentials of *A. danielli* extract on soymilk-based juice for up to three and half months. *Aframomum danielli* was efficient in decreasing or preventing the growth of micro organisms over the period of storage. The juice was also accepted in terms of sensory attributes. The indigenous spice is cheap and easily accessible thus leading to a reduction in the cost of production on a large scale. The utilization of this spice should be encouraged and recommended to people as healthy drink.

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