

Evaluation of Antioxidant, Dietary Fibre and Microbial Properties of Spiced Tangelo Snack

Abosede O. Oduntan¹, Eniola O. Oyedeji², Bernard N. Okafor², and Balikis O. Mustapha¹

¹Product Development Programme, National Horticultural Research Institute, Jericho, Ibadan, Oyo State, Nigeria.

²Citrus Research Programme, National Horticultural Research Institute, Jericho, Ibadan, Oyo State, Nigeria.

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CORRESPONDING AUTHOR

Abosede O. Oduntan

Product Development Programme,
National Horticultural Research Institute
(NIHORT), Jericho, Ibadan, Oyo State,
Nigeria

bosetunde12@yahoo.com

+234-803-322-1716

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Abstract

*The increase in consumers' awareness of healthy snacks has called for the production of spiced snacks from citrus, such as tangelo. This study aimed to fortify tangelo with spices and evaluate the phytochemicals, dietary fibre contents, and microbial properties. In this study, fresh peeled fruits were sliced and divided into five parts, namely: tangelo fruits spiced with African cardamom (*Aframomum danielli*), Ginger (*Zingiber officinalis*), nutmeg (*Monodora myristica*), African pepper (*Xylopia aethiopica*), and an unspiced sample served as a control. The samples were dehydrated at 60 °C for 13 hours, cooled, and packed into polyethene bags before analysis. Thereafter, phytochemical, antioxidant constituents, total reducing sugar, dietary fibre, and microbial load of samples were determined for ten weeks. Results showed that dehydration generally reduced the moisture content of the spiced tangelo samples (13.78-20.67%), compared with the control (23.71%). Phenolic content (15.61-18.72 mg/g, DPPH (72.29-82.84%), tannin content (36.24-56.63 mg/g), insoluble fibre (5.68-6.27%), soluble fibre (7.56-8.32%), and total dietary fibre (13.46-14.59%) were elevated. The antimicrobial properties of the spices improved the shelf stability of the products, with *Xylopia aethiopica*-spiced tangelo showing the lowest bacterial load (11.00×10^7 CFU/mL). The inclusion of spices improved the antioxidant, shelf-life, and dietary fibre contents of the dehydrated tangelo. In conclusion, the spices, particularly *Xylopia aethiopica*, are essential antimicrobial agents in enhancing the storability and nutritional properties of tangelo snacks. Findings from this study offer a prospect for producing healthy, dried fruit snacks.*

Keywords:

Antimicrobial properties, citrus, dietary fibre, snacks, spices, tangelo

Introduction

The snacking trend has increased significantly in recent years (Almoraie et al., 2021). Nonetheless, most snacks comprise substantial amounts of starch, salt, fat, and calories (Balan et al., 2021). This has resulted in a growing demand for nutritious snack alternatives that are abundant in vitamins and fibre, and exhibit antioxidant properties similar to those found in fruits and vegetables (Draszanowska et al., 2024). The daily intake of fruits abundant in naturally occurring antioxidants as a substitute for energy-dense snacks has generated a lot of interest (Elumalai et al., 2013; DeWeese et al., 2017). Consumers are conscious of good health and are aware of the burden of living with non-communicable diseases such as diabetes, cancer, cardiovascular diseases, and high blood pressure (Choudhary et al., 2015). From the foregoing, factors affecting consumer snack selection include their nutritional values, while taste and appearance also play significant roles (Maina, 2018). This trend generates a market in the food industry for the manufacture of healthy foods, ideally from natural sources, to meet consumer requirements. Consumers are urged to consume natural products, including vegetables and fruits such as tangelo (Maoto et al., 2019).

Tangelo, a variety of sweet orange, is free of fat, sodium, and cholesterol but rich in

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vitamins and minerals (Vishvakarma, 2023). Citrus fruits and certain derivatives are linked to antioxidant, anti-inflammatory, anti-cancer, antigenic, neuroprotective, and antimicrobial properties, which are connected to their capacity to regulate hepatic lipid metabolism (Mandalari et al., 2017). Miles & Calder (2021) also noted that regular intake of oranges helps mitigate the incidence of haemorrhage, influenza, and the common cold, while contributing to healthy living and longevity.

Spices are dried seeds, fruit, bark, flowers, or herbs used in small amounts for food enhancement and preservation (Sharif et al., 2018). They are rich in antioxidants and inhibit microbial growth, contributing to food storability. In producing tangelo as a snack, incorporating spices would improve its taste, nutritional value, and shelf life. Thus, the phytochemical and microbial properties of tangelo fortified with spices were studied.

Horticultural products such as tangelos have a limited storage life and could be easily predisposed to spoilage caused by pathogenic microorganisms. Food spoilage caused by microbial pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* contributes to foodborne illnesses, leading to high mortality and morbidity (Almaary, 2023). To improve the shelf life, stability, and safety of processed foods as well as to manage harmful and spoilage-related microorganisms, common acids like sorbic and benzoic acids have been employed as chemical preservatives in the food manufacturing industry over the years (Sorathiya et al., 2025). Microbiological resistance has resulted from this, and chemical preservatives are unable to inhibit the growth of spoilage microorganisms or eliminate some harmful bacteria, like *Listeria monocytogenes* (*L. monocytogenes*), from food products (Zavišić et al., 2024). Consequently, considerable efforts must be dedicated to identifying natural antimicrobials capable of inhibiting fungal, viral, and bacterial infections while enhancing food quality and shelf life. Thus, this study focuses on fortifying tangelo with spices and evaluating the phytochemicals, dietary fibre contents, and microbial properties.

Materials and Methods

Sample preparation

Tangelo fruits (*Minneola tangelo*) (50 fruits) were obtained from the Citrus Program of the National Horticultural Research Institute (NIHORT), Jericho, Ibadan. The fruits were washed and air-dried, after which the peels were removed. Tangelo fruits were

sliced and spiced using ginger, nutmeg, African pepper, and African cardamom, and coded as GR, NM, AE, and AD, respectively, with an unspiced sample (Control). Five (5) grams of each spice were weighed into 50 g of honey and allowed to mix for 30 minutes. This was followed by adding each mixture to 600 g of fruit, which was allowed to diffuse into the tangelo for 30 minutes. Samples were dehydrated using dehydrator trays at 60 °C for 13 hours. Dehydrated samples were cooled and packaged in polyethene bags for analysis. These analyses were done in triplicate.

Physico-chemical analysis

Determination of moisture content

The moisture content of the sample was measured by drying at 105 °C in an oven until a constant weight was obtained (AOAC, 2015)

Determination of vitamin C

The titration method described in AOAC (2015) determined Vitamin C in fresh fruit samples.

Determination of the total phenolic content of the tangelo snack

The total phenolic content of the sample's extracts was determined using the Folin–Ciocalteu assay described by Chan et al. (2008). In this procedure, 0.5 mL of the samples was placed in a test tube, after which 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% Na₂CO₃ were added. The tubes were then allowed to stand for 30 minutes, and the absorbance was measured at 765 nm.

Determination of the carotenoid content of the tangelo snack

Ghafoor et al. (2020) developed a method to quantify carotenoids. A sample of approximately 2 grammes was combined with 25 mL of acetone. The mixture was vortexed for 10 minutes, followed by filtration with Whatman No. 1 filter paper. The filtrate was fractionated using 20 mL of petroleum ether and then washed with 100 mL of distilled water. The remaining water was eliminated using filter paper (Whatman No. 1) coated with 5 g of anhydrous sodium sulphate. Petroleum ether was used to adjust the extract volume to 25 mL, and then a spectrophotometer was used to estimate absorbance at 450 nm.

Radical scavenging properties of the snack by the DPPH method

The antioxidant activity of the sample's extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl

(DPPH) assay with slight modifications described by Rufino et al. (2011). A 0.06 mM DPPH solution was prepared in methanol, and 100 μ L of the dried spiced tangelo extract was added to 3.9 mL of this solution, after the blank had been adjusted with methanol. The absorbance at 515 nm was monitored, showing a decrease at one-minute intervals during the initial 10 minutes and stabilising at 5-minute intervals. The antioxidant capacity was expressed as the concentration of extract required to reduce the initial DPPH radical population by 50%, based on preliminary studies.

Determination of tannin content of the tangelo snack

Tannin was measured using the methodology described by AOAC (2015). For 1 hour, 10 mL of distilled water was combined with half a gram of the sample. Two drops of FeCl₃ in 0.1 M HCl and about 5 ml of distilled water were added. To ensure adequate mixing, the mixture was vibrated. After adding four (4) drops of potassium ferrocyanide, a spectrophotometer was used to measure the mixture's optical density at 620 nm.

Determination of insoluble, soluble, and total dietary fibre of the tangelo snack

The dietary fibre content of the samples was determined using the enzymatic–gravimetric method per AOAC Method 991.43 (Lee et al., 1992). Briefly, 85% ethanol was used to remove sugars from the snack samples. The samples were then sequentially digested with heat-stable α -amylase to hydrolyse starch into dextrin, protease to degrade proteins into peptides and amino acids, and amyloglucosidase to further hydrolyse dextrin into glucose. The enzyme digests were subsequently clarified using glass frit crucibles covered with tar, and the precipitates were filtered after one hour. One set of soluble and insoluble fibre residues was ashed in a muffle furnace at 525 °C for five hours. The total dietary fibre content was calculated as the sum of the soluble and insoluble fibre fractions.

Determination of total reducing sugar of tangelo snack

Five millilitres (5 mL) of boiling Fehling's A and Fehling's B solutions were titrated with the sample in the burette while the methylene blue indicator was present. The sample's volume needed to reach the end point (colour change from blue to red) was established (AOAC, 2000).

Microbial load determination

Treatments used in this work include ginger (GR), nutmeg (NM), *Xylopiya aethica* (AE), *Aframomum danielli* (AD), and unspiced control. Samples of each spiced tangelo and the unspiced control were crushed and ground separately using a sterile pestle and mortar. Ten (10) grams of ground sample from each treatment were weighed into a 250-mL Erlenmeyer flask containing 100 mL of sterile distilled water, soaked for 4 hours, and then shaken vigorously using a rotary shaker. The mixture was sieved using a sterile Whatman No. 1 filter paper to obtain the stock, adopting the method described by Ayodele et al. (2009). Tenfold serial dilutions were prepared from the stock. One millilitre of dilutions (10^{-2} , 10^{-5} , and 10^{-7}) was dispensed using a sterile syringe into a 90-mm-diameter petri dish containing 10 mL of solidified nutrient agar (NA) to allow bacterial growth and potato dextrose agar (PDA) for fungal growth. Plates were incubated at 37 °C and observed daily for growth. Fungal and bacterial colonies were counted using a colony counter at 72 and 48 hours, respectively.

Statistical analysis

All the data generated in this study were subjected to statistical analysis using the SAS package, and means were separated using ANOVA and Fisher's Test for least significant differences (LSD), where the test of significance was done at 5% probability level.

Results and Discussion

The physicochemical properties of fresh and dried Tangelo samples are presented in Tables 1 and 2. The moisture contents of dried tangelo ranged from 13.78 to 23.71% (Table 2), which were significantly lower than that of fresh tangelo (80.52 %) (Table 1). Low moisture content generally reduces the susceptibility of fruits and food products to spoilage. However, the values obtained in this study were above 12%, a threshold beyond which long-term stability is considered less assured (Oduntan and Arueya, 2019). The spices added to the tangelo are in dried forms, which might have absorbed some of the moisture in the tangelo, resulting in a lower moisture content than the control, which had the highest moisture content of 23.71%. Moisture absorption in ascending order was NM < AE < GR < AD < CN. Significant differences existed between the dried spiced tangelo and the control. The total phenolic content was between 15.61 (GR) and 18.72 mg/g (NM) (Table 2). Nutmeg-spiced tangelo had the highest total phenol content, while tangelo spiced with *Aframomum danielli* had the least.

Table 1: Antioxidant, moisture, dietary fibre, and reducing properties of a fresh sample of Tangelo fruit

| Properties | Result |
|--------------------------|--------|
| Carotenoid (mg/100g) | 0.38 |
| Vitamin C (mg/g) | 0.06 |
| DPPH (%) | 82.28 |
| Tannin (mg/g) | 30.52 |
| Moisture (%) | 80.52 |
| Total dietary fibre (%) | 7.90 |
| Total reducing sugar (%) | 9.26 |

The benefits of phenols to humans include protection from heart disease and cancer (Rahman *et al.*, 2021). Furthermore, it was reported that phenolic compounds are linked with antioxidant properties and perform a crucial role in regulating lipid peroxidation (Socrier *et al.*, 2019). Also, phenols exhibit health-promoting effects by modifying the metabolic activities of humans (Kumar *et al.*, 2021). This could be of high potential health value when spiced tangelo is consumed.

The carotenoid content of the dried spiced tangelo ranged from 0.27 to 0.41 mg/100g. A significant decrease was evinced in contrast to the control. The snack's carotenoid content was affected by the addition of the spices. Values differ, with the control having the highest, which might result from the carotene, which had not reacted with any spice during dehydration. According to Tufail *et al.* (2024), eating foods high in carotenoids lowers the likelihood of developing conditions like cancer, cardiovascular disease, age-related macular degeneration, cataracts, immune system-related illnesses, and other degenerative diseases.

The vitamin C content of spiced tangelo ranged from 0.02 to 0.05 mg/g (Table 2). The highest vitamin C content was observed in ginger-spiced tangelo, while the sample spiced with nutmeg had the least. No

significant difference was observed among control, AD, GR, and AE; variations were observed between NM and the others, except AE ($P \leq 0.05$). The variations found in the vitamin C contents might be attributed to the processing method (i.e., the exposure of the tangelo snack to heat during drying) because vitamin C is heat-labile; hence, resulting in the low vitamin C contents of dried spiced tangelo samples as compared to fresh tangelo (Table 1). Vitamin C is a cofactor in manufacture of collagen, which supports cardiovascular function, maintains cartilage, bones, and teeth, and is necessary for wound healing (Gallie, 2013).

The free radical scavenging ability of the dried spiced tangelo samples by the DPPH method ranged from 72.49 to 82.84% (Table 2). AD-spiced tangelo had the highest free radical scavenging ability, while NM-spiced had the least. The scavenging ability of NM was not substantially different from that of the control, which was significantly different ($P \leq 0.05$) from AD, AE, and GR. The increase in the scavenging ability of spiced tangelo compared to the control may be attributed to the addition of different spices. The values recorded were AD > GR > AE > CN > NM. It can be implied that the addition of *Aframomum daniellii* to the tangelo snack had the most significant antioxidant activity in comparison to the addition of the other spices, and could exhibit the potential to reduce diseases related to the oxidation of lipids, such as cardiovascular diseases (Hussain *et al.*, 2021)

The tannin content of spiced tangelo ranged from 36.24 to 56.63 mg/g. NM contributed the most to the tannin content, while AE contributed the least. The control and GR tannin contents were not significantly different, and those for NM and AD were also not significantly different. The observed high content of tannin in the tangelo snacks spiced with nutmeg correlates with the high antioxidant activity because tannin is a natural source of antioxidants (Antasionasti *et al.*, 2021).

Table 2: Phytochemical and antioxidant properties of dried spiced Tangelo

| Sample | Total Phenolic (mg/g) | Carotenoid (mg/100g) | Vitamin C (mg/g) | DPPH (%) | Tannin (mg/g) | Moisture (%) |
|----------|-----------------------|----------------------|--------------------|--------------------|--------------------|--------------------|
| CN | 17.30 ^b | 0.41 ^a | 0.04 ^a | 72.49 ^d | 44.72 ^b | 23.71 ^a |
| NM | 18.72 ^a | 0.39 ^b | 0.02 ^b | 72.29 ^d | 56.63 ^a | 13.78 ^c |
| AD | 15.04 ^d | 0.31 ^d | 0.04 ^a | 82.84 ^a | 54.56 ^a | 20.67 ^b |
| AE | 17.08 ^b | 0.27 ^c | 0.03 ^{ab} | 74.99 ^c | 36.24 ^c | 15.43 ^d |
| GR | 15.61 ^c | 0.37 ^c | 0.05 ^a | 76.85 ^b | 45.19 ^b | 18.45 ^c |
| F- value | 0.0001 | 0.0001 | 0.0845 | 0.0001 | 0.0001 | 0.0001 |
| LSD | 0.42 | 0.01 | 0.02 | 0.83 | 4.03 | 0.34 |

CN- Control, Ginger - GR, nutmeg - NM, *Xylopia aethiopica* - AE, *Aframomum danielli* - AD, Results are mean±SD, and the same superscript in a column are not significantly different ($n=3$)

Tannin contributes to the bitter taste of food samples, which is responsible for the sensory perception of some food products (Soares *et al.*, 2020). A similar report was evinced by Antasionasti *et al.* (2021). Insoluble dietary fibre (IDF) values were between 5.68 and 6.27% (Table 3). The highest IDF was reported for AD-spiced tangelo, while NM-spiced tangelo had the least. Significant variations occurred among the insoluble dietary fibre contents of the dried spiced tangelo snacks. Adding different spices to the tangelo increased its insoluble fibre content, except for the one with nutmeg. Consuming foods rich in insoluble dietary fibre helps prevent gastrointestinal tract diseases by adding bulk to the stool, excreting bile acids, and decreasing intestinal transit time (Mudgil, 2017).

Values for soluble dietary fibre (SDF) increased from 7.56% in the control to 8.32% in *Aframomum danielli* spiced tangelo. The amount of SDF significantly varies among the samples and the control. Soluble dietary fibre is known to help reduce cholesterol in the blood, thus preventing diseases such as diabetes and heart disease. Soluble dietary fibre was more prevalent in all the spiced tangelos than insoluble dietary fibre. This addition provided some nutritional benefits to the snack, as SDF is associated with a reduced risk of numerous chronic diseases compared to insoluble dietary fibre, as Bader-UI-Ain *et al.* (2019) reported.

Table 3: Dietary fibre and total reducing sugar of spiced dried tangelo

| Sample | Insoluble Dietary fibre (%) | Soluble dietary fibre (%) | Total Dietary fibre (%) | Total Reducing sugar (%) |
|--------|-----------------------------|---------------------------|-------------------------|--------------------------|
| CN | 5.91 ^d | 7.56 ^e | 13.46 ^e | 16.82 ^a |
| NM | 5.68 ^e | 8.14 ^b | 13.82 ^d | 17.22 ^b |
| AD | 6.27 ^a | 8.32 ^a | 14.59 ^a | 17.71 ^a |
| AE | 6.12 ^b | 7.81 ^d | 13.93 ^c | 16.24 ^c |
| GR | 6.06 ^c | 8.05 ^c | 14.10 ^b | 17.10 ^c |
| F | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| LSD | 0.04 | 0.05 | 0.09 | 0.03 |

CN- Control, Ginger - GR, nutmeg - NM, *Xylopiya aethiopic* - AE, *Aframomum danielli* - AD. Results are Mean±SD, and the same superscript in a column are not significantly different (n=3)

Microbial load

The fungal load at 2 weeks of storage was generally low. However, Tangelo snacks spiced with African pepper had significantly (P≤ 0.05) lower fungal loads in comparison with other treatments (Table 4). There was no significant difference (P≤ 0.05) in fungal load between tangelo snacks spiced with *Aframomum*

danielli and *Monodora myristica*. In contrast, the highest load was observed in ginger-spiced snacks and unspiced controls (Table 4). A similar trend was observed at the 6-week storage period, where the microbial load recorded in snacks spiced with *Aframomum danielli*, *Monodora myristica*, and *Xylopiya aethiopic* was significantly lower (P≤ 0.05) than in ginger-spiced and unspiced snacks (Table 4). As the storage period progressed to 8 weeks, there was a drastic reduction in the fungal load, most notably in the tangelo snack spiced with *Aframomum danielli* and *Monodora myristica*. The trend at 10 weeks was similar to that observed at 8 weeks, where the fungal load in all snacks was low, except in the snack spiced with ginger (Table 4). Among all the treatments, the bacterial load in the tangelo snack spiced with African pepper was consistently lower throughout the storage period (Table 5). General observation revealed that snacks spiced with African pepper had the least fungal and bacterial growth.

Table 4: Fungal load (×10⁷ CFU/mL) in spiced tangelo snack at different storage intervals

| Treatments | Storage interval (weeks) | | | | |
|------------|--------------------------|-------|-------|-------|-------|
| | 2 | 4 | 6 | 8 | 10 |
| GR | 5.67 | 70.67 | 55.00 | 33.67 | 13.33 |
| AD | 4.33 | 45.00 | 38.67 | 33.33 | 5.00 |
| NM | 3.00 | 45.00 | 36.33 | 7.67 | 7.33 |
| AE | 2.33 | 35.33 | 41.00 | 8.00 | 5.00 |
| CN | 1.33 | 68.00 | 65.33 | 24.00 | 6.00 |
| LSD (0.05) | 4.70 | 18.16 | 20.11 | 8.65 | 3.01 |

CN- Control, Ginger - GR, nutmeg - NM, *Xylopiya aethiopic* - AE, *Aframomum danielli* - AD

Table 5: Bacterial load (×10⁷ CFU/mL) in spiced tangelo snack at different storage intervals

| Treatments | Storage interval (weeks) | | | | |
|------------|--------------------------|--------|-------|-------|-------|
| | 2 | 4 | 6 | 8 | 10 |
| GR | 117.00 | 75.67 | 50.33 | 38.33 | 27.00 |
| AD | 61.00 | 100.00 | 54.67 | 37.00 | 15.00 |
| NM | 36.00 | 120.33 | 29.00 | 16.33 | 95.33 |
| AE | 48.00 | 76.67 | 31.00 | 11.67 | 11.00 |
| CN | 74.57 | 96.67 | 77.00 | 18.00 | 19.67 |
| LSD (0.05) | 31.84 | 27.96 | 24.82 | 10.95 | 17.97 |

CN- Control, Ginger - GR, nutmeg - NM, *Xylopiya aethiopic* - AE, *Aframomum danielli* - AD

Recently, consumers have increasingly opted for minimally processed foods over their processed counterparts, leading to a high demand for natural products that can serve as alternatives to food preservatives (Maddaloni *et al.*, 2025). Natural products are increasingly accepted as substitutes for synthetic chemical preservatives because they are

more naturally tolerated in the human body and offer inherent benefits to the food industry (Silva and Domingues, 2017). Spices have been used as food and flavouring agents since ancient times, and as medicine and food preservatives in recent times (Khan et al., 2024). Studies have shown that spices such as clove, oregano, thyme, and cinnamon possess antimicrobial activities against pathogenic and spoilage bacteria and fungi (Liu et al., 2017). Furthermore, the secondary metabolites of these spices are referred to as antimicrobial agents; most of these substances are widely accepted as safe food ingredients with negligible adverse effects (Nabavi et al., 2015). Therefore, spices are good candidates for developing new antimicrobial agents against food spoilage and pathogenic organisms.

CONCLUSION

The fortification of tangelo spices significantly influenced its phytochemical content, dietary fibre composition, and microbial stability. Snacks prepared from spiced tangelo had higher insoluble and soluble dietary fibre than the control, making them promising promoters of gastrointestinal health and mitigators of chronic disease risk. Phenolic and tannin contents were greater, particularly in nutmeg- and African cardamom-fortified samples, contributing to stronger antioxidant activities and better health benefits. Though vitamin C and carotenoid contents declined due to heat sensitivity during processing, antioxidant activity was not diminished. It remained high, with Aframomom danielli-spiced tangelo exhibiting maximum radical scavenging capacity. Including spices, especially *Xylopiya aethiopica* and *Aframomum danielli*, also significantly suppressed microbial growth, thereby increasing shelf life. These results indicate that spice-fortified tangelo snacks are nutritionally better, functionally beneficial, and microbiologically safer than regular snacks, and they have high commercial potential as natural, health-promoting foods.

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