

## CHEMICAL COMPOSITION OF BISCUITS SUPPLEMENTED WITH ORANGE PEEL AND PULP FLOURS

\*Nwosu A.N., Nweze B.C. and Onwuchekwa A.I.

Department of Food Science & Technology, University of Nigeria, Nsukka, Nigeria

\*Corresponding author's email: [adaora.nwosu@unn.edu.ng](mailto:adaora.nwosu@unn.edu.ng)

### ABSTRACT

*Sweet orange (Citrus sinensis) fruits were washed, peeled manually, the juice was extracted using kitchen juice extractor and the seeds were removed. The peels were separated from the pulps and sliced into thin slices of about 2 cm thick, sun dried separately till constant weight was achieved. They were milled and sieved to obtain orange peel and orange pulp flours, respectively. The orange peel and pulp flours were used to substitute 10% wheat flour. Biscuits were produced from the flour blends and 100% wheat biscuit was produced and evaluated for chemical composition (proximate analysis, mineral and phytochemical compositions). The results showed that the orange pulp biscuit was significantly higher ( $p < 0.05$ ) in moisture and fibre, while orange peel biscuit was higher in ash. The pulp and peel biscuits had lower levels in fat, protein, carbohydrate and energy content. There were no differences ( $p > 0.05$ ) in mineral content of the orange peel and pulp biscuits, though higher values were recorded for calcium and sodium when compared to 100% wheat biscuit. The orange peel biscuit had higher ( $p < 0.05$ ) levels of all the phytochemicals (saponins, alkaloids, flavonoids, anthocyanins and carotenoids) assessed. Edible biscuits can be produced from blends of wheat and sweet orange peel and pulp flours which are sources of bioactive compounds that possess nutraceutical properties.*

**Key words:** fibre, biscuit, phytochemicals, flavonoids, convenience

### INTRODUCTION

Sweet orange is an important fruit in the tropical and sub-tropical regions of the world (Waleed, 2019). The fruits are usually eaten fresh but are also used for making canned orange juice, frozen juice concentrate, jams and jellies, among others. Orange processing industries generate huge amounts of orange peel and pulp as by products from the industrial extraction of orange juices (de Castro *et al.*, 2020). The amount of peel and pulp obtained from citrus fruit processing accounts for 50 % of the original amount of the whole fruit (Schalow *et al.*, 2018). These peel and pulp contain among other things, high levels of vitamin C, dietary fibre and some bioactive substances believed to have positive health implications. These facts notwithstanding citrus peels and pulps are most often discarded. Dietary fibre has been used for the treatment of various gastrointestinal disorders and health benefits including lowering cholesterol levels, reducing risk of colon cancer and losing weight (Gill *et al.*, 2020). Dietary fibre has also been reported to have some nutraceutical potentials (Kushwaha and Maurya, 2019). Until recently, analysis of food was limited to sensory and nutritional value. However, there is growing evidence that other components of food may play an integral role in the link between food and health (Kushwaha and Maurya, 2019). Food consumers

are increasingly interested in the health benefits of food and have begun to look beyond the basic nutritional benefits to the potential disease prevention and health-enhancing compounds in food and food products (Langhans, 2018).

Biscuit is a confectionary, dried to very low moisture content (Okaka, 1997). Biscuit is a snack food which can be eaten in-between meals or at any time of the day and by any age bracket. Households spend so much on food in Nigeria and on snacks in which convenience is perceived to be the reason. Peel is the skin of some fruits and vegetables. Orange peel contains soluble sugars 16.90%, cellulose 9.21%, hemicelluloses 10.50%, and pectin 42.50% (Beatriz *et al.*, 2008). Most of the phytonutrients are found in the peel and inner white pulp of orange rather than in the juice (Brett, 2011). Hesperidin molecule has been singled out in phytonutrient research on oranges and the highest concentration can be found in the white parts of the peel and the pulps of citrus fruits (Chiba *et al.*, 2003). The peel is edible, and is consumed mostly in environments where there is scarcity of resources and where maximum nutritional value must be derived and minimal waste generated (Gargulinski, 2011). However, grating a tablespoon of orange peel each day and using it to flavour tea, salads, salad dressings, soups among others may be a practical way of harnessing the health benefits in them.

In addition to the peel, the pulp of sweet orange is a source of fibre, and other nutrients. The pulp contains total pectin 26.0 to 45.60%, neutral detergent 15.8 to 31.00% and crude fiber 9.9 to 20.60% (Porzio and Blake, 1983). The white pulpy part of sweet orange is the primary source of flavonoids, and is often discarded during processing of orange juice. This loss of flavonoids underlies eating the orange in its whole food form. Dietary fibre is the indigestible portion of plant foods, it is metabolically inert, absorbing water as it moves through the digestive system, easing defecation (Weickert and Pfeiffer, 2008). One of the actions of dietary fibre is to change the nature of the contents of the gastrointestinal tract, and to change how other nutrients and chemicals are absorbed. Soluble fibre binds to bile acids in the small intestine, making them less likely to enter the body, this in turn lower cholesterol levels in the blood (Anderson *et al.*, 2009). Soluble fibre also attenuates the absorption of sugar, reduces sugar response after eating, normalizes blood lipid levels and once fermented in the colon, produces short-chain fatty acids as by products with wide-ranging physiological activities (Weickert and Pfeiffer, 2008).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well known that plants produce these chemicals for own protection, but recent research demonstrate that they can also protect humans against diseases. There are more than a thousand known phytochemicals some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits (Weingartner *et al.*, 2008). Foods containing phytochemicals are already part of our daily diet. In fact, most foods contain phytochemicals except for some refined foods such as sugar or alcohol. Some foods such as whole grains, vegetables, fruits, herbs, etc. contain many phytochemicals. The easiest way to get them is to eat more fruits and vegetables (Weingartner *et al.*, 2008). Thus, considering the economic, nutritional health advantages of these local food sources, its promotion deserves attention from individuals and government agencies (Ojinnaka *et al.*, 2013). There is empirical evidence that phytochemicals in fruits and vegetables may reduce cancer risk, possibly due to dietary fibres, polyphenol antioxidants and anti-inflammatory effects (Brown and Arthur, 2001). An important cancer drug, Taxol, is a phytochemical initially extracted and purified from the pacific yew tree (Brown and Arthur, 2001). In the light of these, the present work was undertaken to assess the nutritional (chemical) effect of inclusion of the commonly discarded orange peel and pulp in biscuit which is a convenient food taken by all age groups to increase the intake of these phytochemicals thereby improving human health.

## MATERIALS AND METHODS

### Procurement of Materials

Sweet orange fruits (56 kg) were purchased from Nkwo market Ibagwa in Igboeze South Local Government Area of Enugu State. Wheat flour, sugar, margarine, eggs and baking powder were purchased from Ogige Market in Nsukka town also at Enugu State of Nigeria.

### Preparation of Orange Peel and Pulp Flours

The fruits were washed thoroughly in portable water, to remove dirt and adhering extraneous materials, peeled manually with a sharp kitchen knife. Plastic kitchen juice extractor was used to extract the juice and the seeds removed. The peel and pulp were cut into tiny pieces of about 2 cm thick and sun dried to constant weight milled in attrition mill and sieved with muslin cloth with a pore size of 2 mm to obtain the flour samples. The flow diagram for the preparation of orange peel and pulp flours is shown in Figure 1.

### Flour Blending

Orange peel flour 10 g and wheat flour 90 g was weighed into a food blender. The blender was operated at full speed for 10 min. to ensure proper mixing of both flours. The same process was repeated for orange pulp flour and wheat flour.

### Production of Biscuit

The basic recipe that was used for biscuit production is shown in Table 1. The ingredients were weighed out and dry ingredients were mixed together. The fat was rubbed in and mixed with water until dough was formed. The resultant dough was kneaded and rested for about 5 min. The rested dough was rolled out into sheets and cut into shapes, using biscuit cutter. The dough was placed on greased baking trays and baked for 20 min. in an oven pre-heated to 180°C allowed to cool then packaged in high density polyethylene bags in an airtight container. The flow diagram for the production of biscuit is shown in Figure 2.

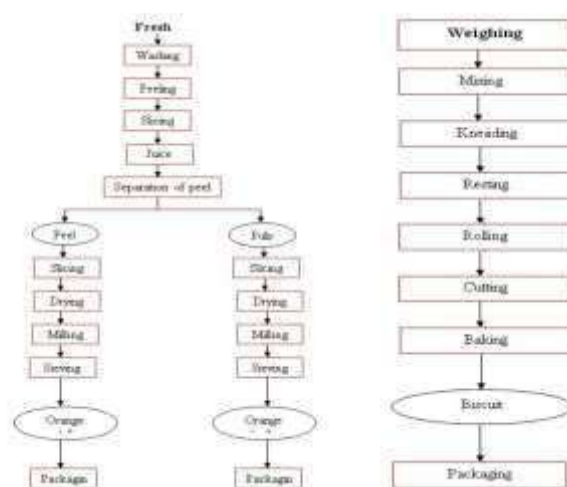


Figure 1: Preparation of orange peel and pulp flour



Figure 2: Production of biscuit

## Chemical Evaluation

### Proximate analyses

The proximate composition of biscuit samples was determined using the method of AOAC (2010).

### Determination of moisture content

Moisture content was determined by the hot air oven method. Stainless steel moisture dishes were cleaned and dried in the oven (Memmert UN30), at 100°C for 1 h to achieve constant weights. The moisture dishes were cooled in a desiccator and then weighed. Two grams of each sample was weighed into respectively labeled moisture dish and dried at 100°C, for 1 h, removed from the oven and placed in a desiccator to cool to room temperature before weighing. The moisture dishes were put back into the oven, dried and weighed intermittently until a constant weight was attained. The loss in weight from the original sample weight was calculated as the moisture content using the expression:

$$\% \text{ moisture content} = \left( \frac{W_2 - W_3}{W_2 - W_1} \right) \times \frac{100}{1};$$

where  $W_1$  is weight of empty moisture dish,  $W_2$  is weight of moisture dish + sample before drying, and  $W_3$  is weight of moisture dish + sample after drying.

### Determination of ash content

Two grams of each sample was weighed into crucibles that had been previously washed, dried and weighed. The crucibles were placed in a muffle furnace (Vecstar Lf3 and USA) and ignited at 550 ± 2°C for 4 h., cooled and weighed. The ash content was calculated with the expression

$$\% \text{ ash content} = \left( \frac{W_3 - W_1}{W_2 - W_1} \right) \times \frac{100}{1};$$

where  $W_1$  is weight of empty crucible,  $W_2$  is weight of crucible and sample before ashing,  $W_3$  is weight of crucible and sample after ashing.

### Determination of fat content

The fat content of the samples was determined using the standard AOAC (2010) method. A previously dried, cooled and weighed 250 ml round bottom flask was fixed to a Soxhlet extractor with a reflux condenser. Two grams of sample was weighed into labelled thimble and petroleum ether 60-80°C boiling point (150 ml) filled into the round bottom flask. The extraction thimble was plugged with cotton wool. The Soxhlet apparatus after assembling was allowed to reflux for 6 h. The thimble was removed with care and the petroleum ether was recovered for reuse. The round bottom flask containing the pet ether extract was removed and dried at 70°C for 1 h in an oven (Memmert UN30 Germany), cooled in desiccator and weighed. Fat content was calculated as:

$$\% \text{ fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times \frac{100}{1}.$$

### Determination of fibre content

Two grams of each sample was weighed and defatted using petroleum ether (boiling point of 40 to 60°C). The defatted sample was boiled for 30 min. in 200 ml of 1.25% H<sub>2</sub>SO<sub>4</sub> and the solution filtered through a funnel fitted with muslin cloth. It was washed with boiling water until it was free of acid. The residue was boiled for another 30 min. with 100 ml of 0.02 M NaOH. It was further washed with boiling water then with 1% hydrochloric acid and finally with boiling water to ensure that it was free of acid. The final residue was transferred into a crucible and dried in the oven for 1 h. The crucible with its content was cooled in a desiccator and weighed. The residue was transferred into crucible and dried at 100°C to a constant weight. Incineration to ash was done at 600°C for 30 min., cooled in a desiccator and weighed. The difference in weight between oven dry weight and weight after incineration was taken as the fibre content of the sample. Percent crude fibre was expressed as:

$$\frac{\text{Wt of dried sample} - \text{Wt of sample after incineration}}{\text{Initial Wt of sample}} \times \frac{100}{1}.$$

### Determination of crude protein content

Protein was determined using the Kjeldahl method. Two grams of each sample was weighed into a Kjeldahl flask and added anhydrous sodium sulphate (5 g of Kjeldahl catalyst), concentrated H<sub>2</sub>SO<sub>4</sub> (25 ml) and few boiling chips. The samples were digested in the fume chamber to clear sample solution. Each sample digest was allowed to cool and then transferred into a 250 ml volumetric flask and made up to volume with distilled water. Five ml of 2% boric acid solution with few drops of methyl red indicator was introduced into a distillate collector (100 ml conical flask) and placed under the condenser in a distillation unit. Five ml of each sample digest was pipetted into the distillation unit, washed down with distilled water followed by addition of 5 ml of 60% NaOH solution to the digest. The sample was heated until 50 ml of the distillate was collected in the receiving flask. The distillate was titrated against 0.01 N HCl to a pink coloured end point. For the blank dilute digest from filter paper was also distilled and the distillate titrated against 0.01N HCl. Total nitrogen (%) was estimated using the expression:

$$\% \text{ Crude protein} = \frac{(T - B) - N \times 250 \times 0.0141 \times 6.25}{w \times 5} \times \frac{100}{1}.$$

Crude protein is % N × 6.25, where  $T$  is titre,  $B$  is blank,  $N$  is normality, and  $w$  is weight of acid.

**Table 1:** Recipe for biscuit production

Ingredient	Amount (g)
Flour	100.0
Margarine	22.0
Beaten egg	10.0
Baking powder	1.8
Water (ml)	45.0
Sucrose	20.0
Salt	0.3
Powdered milk	5.0

Source: Okaka (1997)

**Determination of carbohydrate content**

Carbohydrate content of each sample was calculated by difference. The difference between 100 and the sum of percentages of moisture, protein, fat, fibre and ash of each sample was calculated and the result expressed as:

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fibre} + \% \text{ ash} + \% \text{ fat}).$$

**Determination of energy value**

The values obtained for protein, fat and carbohydrate were used to calculate the energy value of the samples using the Atwater factor as described by AOAC (2010). The energy value was calculated as:

$$\text{Energy value (kCal } 100\text{-g}^{-1}) \text{ (kCal } 100\text{-g}^{-1}) \\ = P \times 4.0 + F \times 9.0 + C \times 3.75;$$

where P, F, and C are the % content of protein, fat and carbohydrate in the sample, respectively.

**Determination of Mineral Composition**

The minerals were determined using the methods described by AOAC (2010), using atomic absorption spectrophotometer (AAS). Five ml of the sample was measured using a syringe into a 250 ml Erlenmeyer flask, acidified with nitric acid and evaporated to dryness using a steam bath. Fifteen ml of HNO<sub>3</sub> was added and heated in a fume cupboard to a colourless solution after addition of 5 ml H<sub>2</sub>O<sub>2</sub>, at 400-450°C for 2 h. The ashed samples were adjusted to 50 ml in a volumetric cylinder with distilled water.

**Determination of copper content**

The samples were treated as described above. The sample was treated with 2 ml hydroxylamine-hydrochloride to reduce copper to the cuprous condition and with 0.2 ml sodium citrate to complex metallic ions. The pH adjusted to 4 and the resultant complex extracted into methanol. Standard copper solution was prepared by diluting 50 ml stock copper solution to 500 ml with redistilled water and then used to calibrate the equipment for direct copper determination. One ml of the solution contained 20 µg of Cu. The amount of copper present was determined by using appropriate copper lamp in AAS at 457 nm and the result converted to parts per million (ppm).

**Determination of iron content**

Five ml of the sample as prepared above was transferred into 50 ml volumetric flask. Then 10 ml of ammonium acetate buffer solution and 2 ml phenanthroline solution were added, and the mixture diluted to the mark with distilled water. The reagents were mixed thoroughly and allowed to stand for 10 min. for maximum colour development. Standard solution was prepared by measuring 1 g of pure iron wire into 100 ml conc. HNO<sub>3</sub>, in a water bath and was diluted to 1000 ml with distilled water. From this stock, standard solutions of 0.0, 0.5, 1.0, 2.0 and 4.0 ppm were prepared and used for equipment calibration. Total iron was determined with appropriate iron lamp.

**Determination of calcium content**

The samples were wet-ashed as described above. Five ml of each of the sample digests was measured into a conical flask using a syringe and then pipetted 1.0 ml of SrCl<sub>2</sub> solution containing 10,000 mg/ml to yield a 1,500 mg/ml of Sr<sup>2+</sup> in the final solution. Calibration curve was prepared for the element using standard solution, which was prepared by dissolving 2.497 g of oven-dried CaCO<sub>3</sub>, diluted to 100 ml with de-ionized water. From this stock solution, calcium standard solutions were prepared with the concentrations of 0.0, 3.0, 8.0 and 9.0 ml. Calcium concentration in the sample was determined using AAS with calcium filter.

**Determination of zinc content**

Five ml of each of the samples was first digested with 20 ml of acid mixture (650 ml conc. HNO<sub>3</sub>; 80 ml perchloric acid (PCA); 20 ml conc. H<sub>2</sub>SO<sub>4</sub>) and aliquot of the diluted clear digest was used for the measurement of absorbance with AAS using filters that match the element. The samples were heated until a clear digest was obtained and then diluted to the 500 ml mark with distilled water. Standard zinc solution was prepared by dissolving 1 g zinc metal in 20 ml HCl and diluted to 1,000 ml with deionized distilled water; (1 ml = 1 mg Zn).

Distilled water was acidified with 1.5 ml conc. HNO<sub>3</sub> and left for one min. The instrument was zeroed. Standard was atomized and the burner adjusted both up and down and sideways until a maximum response was obtained, and absorbance of the sample determined. The actual zinc lamp was used for its determination.

**Determination of sodium content**

Sodium was determined using AAS as described by James (1996). Five ml of each of the samples was first digested with 20 ml of acid mixture (650 ml conc. HNO<sub>3</sub>; 80 ml PCA and 20 ml conc. H<sub>2</sub>SO<sub>4</sub>) aliquot of the diluted clear digest was used for measurement of absorbance using filters that matched the element. The samples were heated until clear digest was obtained and then diluted to the 500 ml mark with distilled water. Then, 91.0%, (w/v) lithium chloride was added. A standard stock solution containing 1000 mg/ml of sodium ions was prepared by dissolving 2.542 g of oven-dried NaCl in de-ionized water and subsequently diluted to 500 ml mark. From this solution, standard solution with concentrations 0.0, 0.1, 0.2, and 0.3 ppm was prepared and then used for the calibration of the instrument. The actual sodium concentration was determined using sodium lamp at 767 nm.

**Determination of potassium content**

Potassium was determined using AAS as described by James (1996). Five ml of each of the samples was first digested with 20 ml of acid mixture (650 ml conc. HNO<sub>3</sub>; 80 ml PCA; 20 ml conc. H<sub>2</sub>SO<sub>4</sub>) and aliquots of the diluted clear digest used for atomic absorption spectrophotometry, using filters

that match the element. The samples were heated until clear digest was obtained and then diluted to the 500 ml mark with distilled water. Then, 91.0% (w/v) lithium chloride was added. A standard stock solution containing 100 mg/ml of K<sup>+</sup> ions was prepared by dissolving 1.907 g KCl in water. The solution was made to 500 ml mark with distilled water. From the stock solution, a standard solution of 0.0, 2.0, 4.0 and 6.0 ppm was prepared, to which a standard stock solution of 1% lithium-chloride was added. The potassium standard was used to calibrate the instrument while the actual potassium concentration of the samples was determined using potassium filter at 385 nm.

#### Phytochemical Analysis

Quantitative assay for flavonoids, saponins, carotenoids, tannins, alkaloids, anthocyanins, were carried out using standard methods as stated below.

#### Determination of flavonoids

This was determined according to the method of Harborne (1980). Five g of the sample was boiled in 50 ml of 2 M HCl solution for 30 min. under reflux. It was allowed to cool and subsequently filtered through a filter paper. A measured volume of the extract was recovered by filtration using weighed filter paper. The resulting difference was the weight of the flavonoid in the sample.

#### Determination of carotenoids

This was carried out in accordance with the method described by Onyeka and Nwambekwe (2007). A measured weight of the sample was homogenized in methanol using a blender (1:10, sample: methanol). The homogenate was filtered to obtain the initial crude extract using about 20 ml of distilled water in separating funnel. The other layer was recovered and evaporated to dryness at low temperature (35-50°C) in vacuum desiccator. The dry extract was then saponified with 20 ml of ethanoic potassium hydroxide and left overnight in a dark cupboard. After a day the carotenoid was taken up in 20 ml distilled water. The carotenoid extract (ether layer) was dried in a desiccator and treated with a light petroleum (Petroleum spart) and allowed to stand overnight in a freezer. The next day, the precipitated steroid was removed by centrifugation. The carotenoid extract evaporated to dryness in a desiccator, weighed and weight expressed as percentage of the sample weight.

#### Determination of saponins

The method of AOAC (2010) was used for the determination of saponin. Saponin was extracted using two different solvents. The first solvent, acetone was used to extract crude lipid from the samples while the second solvent was used for the extraction of saponin proper. Two grams of the sample was folded into a thimble and put in a soxhlet extractor and a reflux condenser fitted on top. Extraction was done with 200 ml of acetone in a 250 cm<sup>3</sup> capacity round bottom flask for 3 h after

which the apparatus was dismantled and another 250 cm<sup>3</sup> capacity round bottom flask containing 200 ml of methanol fitted to the extractor and extraction carried on for another 3 h. The weight of the flask was taken before and after the second extraction in order to obtain the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask oven dried to remove any remaining solvent in the flask. The flask was cooled in a desiccator and weighed. The percentage of saponin was calculated as follows:

$$\% \text{ Saponin} = \frac{\text{weight of dry extract}}{\text{weight of sample}}$$

#### Determination of alkaloids

The gravimetric method of Harborne (1980) was used. Five gram of sample was dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture shaken well and allowed to stand for 4 h before filtering. The filtrate obtained was evaporated to one quarter of its original volume. Concentrated NH<sub>4</sub>OH was added dropwise to precipitate the alkaloids. The precipitate was filtered with a weighed filter paper and washed with 1% NH<sub>4</sub>OH solution. The precipitate in the filter paper was dried in the oven at 60°C for 30 min. and reweighed. The alkaloid content was calculated as follows:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times 100;$$

where *W* is weight of sample, *W*<sub>1</sub> is weight of empty filter paper (without the precipitate), and *W*<sub>2</sub> is weight of filter paper plus precipitate.

#### Determination of anthocyanins

This was carried out in accordance with the method of Harborne (1980). Five grams of sample was hydrolyzed by boiling in 100 ml 2 M HCl solution for 30 min. The hydrolysate was filtered using filter paper. The filtrate was transferred into a separation funnel and equal volume of ethyl acetate was added to it, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) was recorded while the aqueous layer was discarded. The extract was separated to dryness in the crucible over steam bath. The dried extract was treated with concentrated amyl alcohol to extract the anthocyanins. After filtration, the alcohol extract and the filtrate was transferred to a weighed evaporating dish and evaporated to dryness. It was dried in the oven at 30°C for 30 min. and cooled in a desiccator. The weight of anthocyanin was determined and expressed as percentage of the original sample.

#### Experimental Design and Statistical Analysis

Completely randomized design was employed in the study. Using the software SPSS Version 22, data were subjected to analysis of variance. Where significant (*p* < 0.05), means were separated by Duncan's new multiple range test.

## RESULTS AND DISCUSSION

### Proximate Composition of Biscuits

The proximate composition of biscuits supplemented with orange peel and pulp flours are shown in Table 2. The biscuits which contained 10% orange pulp flour had the highest moisture content of 8.85%. The moisture contents of the wheat biscuit and the biscuit containing 10% orange peel flour were 7.08 and 8.44%, respectively. The highest moisture content of the biscuit containing orange pulp is attributed to the higher moisture content of the orange pulp flour. All the biscuit samples were below the acceptable limits of 13-15% as safe moisture content levels for storage of food and cereal products (USDA, 2017). The moisture content of a food is of significance to shelf life and packaging. Biscuits are generally low in moisture. The low moisture levels of the orange based biscuits would ensure shelf stability.

The biscuit produced from 100% wheat flour had fat content of 16.78%. Addition of orange peel and pulp flours decreased the fat content of wheat flour biscuit to 13.87 and 11.28%, respectively. This should be because of the low fat content of the orange peel and orange pulp flours. The fat contents of the biscuit containing orange peel flour was higher than that of the biscuit containing orange pulp flour because the orange peel flour contained more fat when compared to orange pulp flour. Higher fat content (16.33%) was observed by Varsha *et al.* (2020). Fats are integral part of biscuit, being the second largest component after flour in soft dough biscuits (Okaka, 1997). Fats shorten dough by weakening the dough gluten network. This results in soft biscuit which breaks easily and with a more tender mouth feel. Fat also gives a softer texture to biscuits and helps prevent the CO<sub>2</sub> bubbles from escaping from the dough too soon (Hasmadi and Sandra, 2014). Biscuits are a rich source of fat and carbohydrate, hence are energy giving foods.

The protein content of the biscuit prepared with 10% orange pulp flour was 12.84%, this value is lower than 15.19% for wheat flour biscuit and 13.06% for the biscuit containing 10% orange peel flour. Orange peel and pulp flours are not non sources of protein when compared to wheat flour. The low protein content of the orange peel and orange pulp flour may have contributed to the lower protein content of its biscuit.

The addition of orange peel and orange pulp flours increased the fibre content of the biscuits significantly ( $p < 0.05$ ). The 100% wheat flour

biscuit had fibre content of 1.33%, a value which increased to 8.17 and 11.22% in biscuits containing 10% orange peel and 10% orange pulp flours, respectively. This is because orange peel flour and orange pulp flour contained more fibre than wheat flour. Insoluble fibre aids digestion and adds bulk to stool, it hastens passage of fecal material through the gut, thus helping to prevent constipation (Bruce, 2020). Fibre also may help reduce the risk of diverticulosis, a condition in which small pouches form in the colon wall (usually from the pressure of straining during bowel movements). People who already have diverticulosis often find that increased fibre consumption can alleviate symptoms, which include constipation and or diarrhea, abdominal pain, flatulence and mucus or blood in the stool (Lemond, 2018).

The ash contents of the biscuits ranged from 1.23 to 2.13%, with the 100% wheat flour biscuit having the lowest value of 1.23%. The higher ash content of biscuit produced with orange peel (2.13%) over that containing orange pulp (1.81%) may be attributed to the higher ash content of the orange peel flour. Ash content of food is an indication of its mineral content which are good for strong bones (Agu *et al.*, 2014).

The 100% wheat flour biscuit had the highest carbohydrate content of 60.73%. This was followed by that of the biscuit prepared with 10% orange pulp flour (53.78%) and that prepared with 10% orange peel (52.19%). The energy content of the biscuit containing orange peel flour and orange pulp flour were 372.78 and 354.56 kCal, respectively. Biscuit produced with 100% wheat flour had the highest energy value of 439.52 kCal. The energy value of a food is related to its protein, fat and carbohydrate contents (Gillaspy, 2021). The higher protein, fat and carbohydrate contents of the wheat biscuits may have contributed to its higher energy value relative to the orange based biscuits. Biscuit is an energy food which is taken mostly in between meals by both young and old (Eyenga *et al.*, 2020).

### Mineral Composition of Biscuits

The mineral composition of biscuits supplemented with orange peel and orange pulp flours are shown in Table 3. Calcium content of the biscuits was in the range of 45.00-51.00 mg 100-g<sup>-1</sup>, with biscuit prepared from 100% wheat flour having the lowest value (45.00 mg 100-g<sup>-1</sup>). Youssef and Mousa (2012) reported comparable value of 50 mg 100-g<sup>-1</sup> for biscuit containing 10% orange peel flour. The RDA

**Table 2:** Proximate composition of biscuits supplemented with orange peel and orange pulp flours

Biscuit	Composition (%)						
	Moisture	Fat	Protein	Fibre	Ash	Carbohydrate	Energy (kCal/100g)
Wheat	7.08 <sup>c</sup> ± 0.08	16.78 <sup>a</sup> ± 0.07	15.19 <sup>a</sup> ± 0.04	1.33 <sup>c</sup> ± 0.19	1.23 <sup>c</sup> ± 0.03	60.73 <sup>a</sup> ± 0.21	439.52
Wheat-orange peel	8.44 <sup>b</sup> ± 0.05	13.87 <sup>b</sup> ± 0.04	13.06 <sup>b</sup> ± 0.03	8.17 <sup>b</sup> ± 0.02	2.13 <sup>a</sup> ± 0.02	52.19 <sup>c</sup> ± 0.10	372.78
Wheat-orange pulp	8.85 <sup>a</sup> ± 0.05	11.28 <sup>c</sup> ± 0.06	12.84 <sup>c</sup> ± 0.04	11.22 <sup>a</sup> ± 0.03	1.81 <sup>b</sup> ± 0.01	53.78 <sup>b</sup> ± 0.04	354.56

Values are means ± SD of triplicate determinations. Means within the same column with different superscripts were significantly different ( $p < 0.05$ ). The wheat- orange peel and wheat- orange pulp biscuits contained 10% orange peel and 10% orange pulp flour, respectively.

**Table 3:** Mineral composition of biscuits supplemented with orange peel and pulp flours

Biscuit	Composition (mg/100g)					
	Calcium	Copper	Iron	Zinc	Sodium	Potassium
Wheat	45.00 <sup>b</sup> ± 0.03	0.90 <sup>a</sup> ± 0.02	4.90 <sup>a</sup> ± 0.06	5.07 <sup>a</sup> ± 0.11	238.00 <sup>b</sup> ± 2.00	210.67 <sup>a</sup> ± 3.06
Wheat-orange peel	50.39 <sup>a</sup> ± 1.55	0.26 <sup>b</sup> ± 0.21	3.04 <sup>b</sup> ± 0.51	1.11 <sup>b</sup> ± 0.06	262.67 <sup>a</sup> ± 6.43	174.67 <sup>b</sup> ± 5.03
Wheat-orange pulp	51.00 <sup>a</sup> ± 2.00	0.26 <sup>b</sup> ± 0.03	3.03 <sup>b</sup> ± 0.06	1.12 <sup>b</sup> ± 0.03	260.00 <sup>a</sup> ± 2.00	176.67 <sup>b</sup> ± 5.03

Values are means ± SD of 3 replications. Means within the same column with different superscripts were significantly different ( $p < 0.05$ )

for calcium for an adult is 200 mg (NRC, 1989). Calcium is an important mineral in the body. About 99% of the body's calcium is contained in the bone, and the rest in teeth, other tissues and in circulation (Pu and Xue, 2016). Calcium is needed for the growth and maintenance of bones, teeth and muscles.

Biscuits containing 10% orange peel and 10% orange pulp flours had the same copper content of 0.26 mg 100-g<sup>-1</sup>. This value was lower than 0.90 mg 100-g<sup>-1</sup> for the wheat flour biscuit. The copper content of the orange based biscuits compared well with 0.24 mg 100-g<sup>-1</sup> reported by Youssef and Mousa (2012). For orange peel and pulp based biscuits, the copper contents of the biscuit samples were well above the United States recommended daily allowance of 0.9 mg per day for adults (Broadley and White, 2007). The iron contents of biscuits ranged from 3.03-4.90 mg 100-g<sup>-1</sup>. There was no significant difference ( $p > 0.05$ ) between the biscuit containing 10% orange peel flour and 10% orange pulp flour in their iron contents.

However, the 100% wheat flour biscuit contained higher iron than the orange based biscuits. The iron content obtained in this study was higher than those reported for biscuits containing orange peels by Youssef and Mousa (2012). Iron is an important element in the diet of pregnant women, nursing mothers, infants, convulsing patients and elderly where it prevents anemia and other related diseases (Coad and Pedley, 2014). The recommended daily allowance (RDA) for iron per day for men (19 years and older) and women (over 50 years) is 8 mg/day, while that for girls and women (11-50 years old) is 18 mg/day (FNB, 2001). However, according to Fairweather-Tait *et al.* (2020), neither the total iron content nor the nutrient density of the individual food constitutes an accurate guide for choosing dietary sources of iron. Rather, bioavailability of iron depends on its presence in the gastrointestinal tract which is as a result of the physiochemical form of iron in the lumen. Also, form and the presence or absence of factors that influence absorption and the body's need for iron determine how much iron that is delivered to the body.

The zinc content of the 100% wheat flour biscuit (5.07 mg 100-g<sup>-1</sup>) was higher than those of the biscuits containing orange peel (1.11 mg 100-g<sup>-1</sup>) and pulp (1.12 mg 100-g<sup>-1</sup>). Zinc is an essential trace element for protein and nucleic acid synthesis and normal body development during periods of rapid growth such as infancy and it is also essential in maintaining a healthy immune system especially during recovery from illness (Gammoh and Rink,

2017). The RDA for male adult is 12.15 mg (NRC, 1989). The sodium content of the biscuit increased with the addition of 10% orange peel and 10% orange pulp flours. The biscuit containing 10% orange peel flour and 10 % orange pulp flour had 262.67 mg 100-g<sup>-1</sup> and 260.00 mg 100-g<sup>-1</sup> sodium, respectively, values were significantly ( $p < 0.05$ ) higher than 238.00 mg 100-g<sup>-1</sup> for 100% wheat flour biscuit. The RDA value for sodium for adults is 500 mg (NRC, 1989). Reduced intake of sodium is encouraged to reduce the risk of elevated blood pressure. The higher an individual's salt intake, the higher the blood pressure and the risk of coronary heart diseases (NRC, 1989). The range of 238 to 260 mg 100-g<sup>-1</sup> sodium content obtained for the orange based biscuits agreed well with those reported by Youssef and Mousa (2012).

Potassium content of the 100% wheat biscuit was 210.67 mg 100-g<sup>-1</sup>, while 174.67 and 176.67 mg 100-g<sup>-1</sup> were obtained for the biscuits containing 10% orange peel flour and 10% orange pulp flour, respectively. The values for the orange based biscuits were higher than the values obtained by Youssef and Mousa (2012). The RDA for potassium is 200 mg for adults (NRC, 1989). Potassium is very important in maintaining the body fluid volume and osmotic equilibrium (Brinkman *et al.*, 2021).

#### Phytochemical Composition of Biscuits

Phytochemicals composition of biscuits supplemented with orange peel and pulp flours are shown in Table 4. Biscuits prepared with 10% orange peel flour had the highest contents of the phytochemicals assessed. The levels of these phytochemicals were significantly different ( $p < 0.05$ ) from those in the other biscuit samples. Phytochemicals have biological significance but are not established as essential nutrients (FDA, 2010). They are biologically very active. They include anti-oxidants, and compounds that modify potential toxins and carcinogens.

Biscuit containing orange peel had significantly ( $p < 0.05$ ) higher saponin content than the other samples. Saponins are a class of phytochemicals (bio-organic compounds) abundant in the plant kingdom. They are naturally occurring glucosides described by the soap-like foaming property. Consequently, they produce foams when shaken in aqueous solutions. Literature shows that saponins exhibit a biological role and medicinal properties such as cholesterol lowering action in animals and humans (Eskandar and Somayeh, 2015), antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory, among others (Armelle *et al.*, 2018).

**Table 4:** Phytochemical composition of biscuits supplemented with orange peel and pulp flours

Biscuit	Composition (%)				
	Saponins	Alkaloids	Flavonoids	Anthocyanins	Carotenoids
Wheat	0.02 <sup>b</sup> ± 0.00	0.04 <sup>ab</sup> ± 0.01	0.52 <sup>b</sup> ± 0.03	0.85 <sup>c</sup> ± 0.03	0.80 <sup>b</sup> ± 0.03
Wheat-orange peel	0.03 <sup>a</sup> ± 0.00	0.05 <sup>a</sup> ± 0.00	0.71 <sup>a</sup> ± 0.03	1.12 <sup>a</sup> ± 0.03	0.86 <sup>a</sup> ± 0.02
Wheat-orange pulp	0.02 <sup>ab</sup> ± 0.00	0.03 <sup>b</sup> ± 0.00	0.44 <sup>c</sup> ± 0.04	1.05 <sup>b</sup> ± 0.02	0.75 <sup>b</sup> ± 0.04

Values are means ± SD of 3 replications. Means within the same column with different superscripts were significantly different ( $p < 0.05$ )

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atoms in their structure, which makes them alkaline. Based on structure alkaloids can generally be divided into indoles, quinolines, isoquinolines, pyridines, steroids among others (Kurek, 2019). They often have bitter taste and are probably responsible for the bitter taste of orange peels. They play important biological function in plants.

Flavonoids have been reported to function as pigments and antioxidants (Agati and Tattini, 2012). The most important flavonone in oranges is hesperidin which has been reported to lower high blood pressure as well as cholesterol in animal studies and have strong anti-inflammatory properties (CSIRO, 2004). In plants, they are not involved in photosynthesis, respiration or protein synthesis. When foods that contain phytochemicals are eaten, the phytochemicals will activate a group of enzymes that go round cleaning up the free radicals before they cause any harm to the body (Onimawo and Akubor, 2012). Some phytochemicals work as antioxidants (Onimawo and Akubor, 2012).

Studies have shown that the total antioxidant capacity of some market fruits and vegetables highly correlated with anthocyanin content (Dai and Mumper, 2010). Fruits and vegetables rich in anthocyanins had the total antioxidant activity, followed by those rich in flavonones and flavonols (Dai and Mumper, 2010). Carotenoid is a class of micronutrient that cannot be replaced in the diet of humans. There are well over 800 natural carotenoids that have been discovered in different colours such as red, yellow, orange among others (Lu *et al.*, 2021). They are lipophilic natural pigments stored in chloroplast; they exist in green tissues as photosynthetic pigments. Almost all fruits contain carotenoids. Carotenoids are effective oxygen scavengers that have pro-vitamin A activity and thus can reduce oxidative stress (Roohbakhsh *et al.*, 2017).

## CONCLUSION

The study reveals that edible and acceptable biscuits can be produced from blends of wheat and sweet orange peel and pulp flours, which are sources of bioactive compounds with various nutraceutical properties. The consumption of the biscuits will help in improving health of consumers.

## REFERENCES

Agati G. and Tattini M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Sci. J.*, **196**, 67-76

- Agu H.O., Eze G.C. and Jideani A.I.O. (2014). Quality assessment of acha-based biscuit improved with bambara nut and unripe plantain. *Afr. J. Food Sci.*, **8** (5), 278-285
- Anderson J.W., Baird P. and Davis R.H. (2009). Health benefits of dietary fiber. *Nutr. Rev.*, **67** (4), 188-205
- AOAC (2010). *Official Methods of Analysis* 18<sup>th</sup> ed., Association of Official Analytical Chemists, Washington DC, USA
- Armelle T.M., Ndontsa B.L. and Kuate V. (2018). A naturally occurring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi-factorial drug resistant cancer cells via ferroptotic and apoptotic cell death. *Phytomedicine*, **43** (1), 78-85
- Beatriz R., Ana T., Paolo T., Atilio C. and Jose M.D. (2008). Submerged citric acid fermentation on orange peel auto-hydrolysate. *J. Agric. Food Chem.*, **56** (7), 2380-2387
- Brett B. (2011). Enjoy the health benefits of oranges. Retrieved 06/03/2021 from: <https://www.ktradio.network.com/tag/british>
- Brinkman J.E., Dorius B. and Sharma S. (2021). *Physiology, Body Fluids* (2<sup>nd</sup> edition), StatPearls Publishing LLC, Treasure Island FL, USA p. 45
- Broadley M.R. and White P.J. (2007). Improving the micronutrient content of crops using agronomic genetic approaches: Selenium (Se) as a case study. EPSO Workshop: The Feed Value Chain, June 26-27, Faculty of Life Sciences, University of Copenhagen
- Brown K.M. and Arthur R.R. (2001). Selenium, selenoproteins and human health: A review. *Pub. Health Nutr.*, **4** (2B), 593-599. Retrieved 05/03/2021 from: <http://en.wikipedia.org/wiki/phytochemical>
- Bruce D.F. (2020). Dietary fiber for constipation. Retrieved 23/09/2021 from: <https://www.webmd.com>
- Chiba H., Uehara M. and Wu J. (2003). Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. *J. Nutr.*, **133** (6), 1892-1897
- Coad J. and Pedley K. (2014). Iron deficiency and iron deficiency anemia in women. *Scand. J. Clin. Lab. Invest. Suppl.*, **74** (244), 82-89
- CSIRO (2004). The Commonwealth Scientific and Industrial Research Organization Report, February 26, 2004
- Dai J. and Mumper R.J. (2010). Plant phenolics: Analysis and their antioxidant and anti cancer properties. *Molecules*, **15**, 7313-7352
- de Castro L.A., Lizi J.M., das Chagas E.G.L., de Carvalho R.A. and Vanin F.M. (2020). From orange juice by-product in the food industry to a functional ingredient: Application in the circular economy. *Foods*, **9** (5), 1-17
- Eskandar M. and Somayeh H. (2015). Saponin: Properties, methods of evaluation and applications. *Ann. Res. Rev. Biol.*, **5** (3), 207-220
- Eyenga E., Tang E.N., Achu M.B., Boulanger R., Mbacham W.F. and Ndingeng S.A. (2020). Physical, nutritional and sensory quality of rice-based biscuits fortified with safou (*Dacryodes edulis*) fruit powder. *Food Sci. Nutr.*, **8** (7), 3413-3424



- Fairweather-Tait S., Speich C., Mitchikpe C.E.S. and Dainty J.R. (2020). Dietary iron bioavailability: A simple model that can be used to derive country-specific values for adult men and women. *Food Nutr. Bull.*, **41** (1), 121-130
- FDA (2010). Guidance for Industry: Evidence-Based Review System for the Scientific Evaluation of Health claims. Food and Drug Administration (FDA)
- FNB (2001). Dietary reference intakes for vitamin A and K, arsenic, boron, molybdenum, zinc, silicon and nickel. Washington D.C. Food and Nutrition Board (FNB), Standing Committee on Scientific Evaluation of Dietary reference intake. National Academy Press, pp. 28-30
- Gammoh N.Z. and Rink L. (2017). Zinc in infection and inflammation. *Nutrients*, **9**, 1-25
- Gargulinski R. (2011). Is it healthy to eat orange peels? Retrieved 17/11/2020 from: <https://www.livestrong.com>
- Gill S.A., Rossi M. and Whelan K. (2020). Dietary fibre in gastrointestinal health and disease. *Nat. Rev. Gastroenterol. Hepatol.*, **18**, 101-116
- Gillaspy R. (2021). Energy yielding nutrients: Carbohydrate, fat and protein. Retrieved 18/01/2022 from: <http://www.study.com>
- Harborne J.B. (1980). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Chapman and Hall publisher, New York, pp. 32-35
- Hasmadi M. and Sandra E. (2014). Effect of fat types on the structural and textural properties of dough and semi-sweet biscuit. *J. Food Sci. Technol.*, **51** (9), 1998-2005
- James C.S. (1996). *Analytical Chemistry of Foods* (pp. 267-496). Blackie Academic & Professional, New York
- Kurek J. (2019). Alkaloids- their importance in nature and for human life. *IntechOpen*. <https://doi.org/10.5772/intechopen.85400>
- Kushwaha R. and Maurya N.K. (2019). Nutraceutical potential of dietary fibers. In: Hussain M. (ed.), *Research Trends in Medicinal Plant Sciences* (pp 1-16). New Delhi: Aki-Nik Publications
- Langhans W. (2018). Food components in health promotion and disease Prevention. *J. Agric. Food Chem.*, **66** (10), 2287-2294
- Lemond A. (2018). The best and worst food for managing or preventing diverticulitis. Retrieved 23/08/2021 from: <https://www.everydayhealth.com>
- Lu W., Shi Y., Wang R., Su D., Tang M., Liu Y. and Li Z. (2021). Antioxidant activity and healthy benefits of natural pigments in fruits. A review. *Int. J. Mol. Sci.*, **22**, 4945
- NRC (1989). Recommended Dietary Allowances. National Research Council (NRC). National Academy Press, Washington DC
- Ojinnaka M.C., Ofoelo M.U. and Ezenwa L.I. (2013). Nutritional evaluation of wheat cakes enriched with edible African termites (*Macrotermes nigeriensis*). *Agro-Science*, **12** (3), 35-42
- Okaka J.C. (1997). *Cereals and Legumes Storage and Processing Technology*. Obio Printing and Publishing Company, Enugu, pp. 117-130
- Onimawo I.A. and Akubor P.I. (2012). *Food Chemistry: Integrated Approach with Biochemical Background*. Joytal Printing Press, Ibadan, Nigeria, pp. 297-300
- Onyeka E.U. and Nwambekwe I.O. (2007). Phytochemical profile of some leafy vegetables in south East Nigeria. *Nig. Food J.*, **25** (1), 67-79
- Porzio M.A and Blake J.R. (1983). Unconventional sources of dietary fiber. *ACS Symp. Series*, **214** (14), 191-204
- Pu F. and Xue S. (2016). Calcium intake, calcium homeostasis and health. *Food Sci. Human Wellness*, **5** (1), 8-16
- Roohbakhsh A., Karimi G., Iranshahi M. (2017). Carotenoids in the treatment of diabetes mellitus and its complications: A mechanistic review. *Biomed. Pharmacother.*, **91**, 31-42
- Schalow S., Baloufaud M., Cottancin T. and Fischer J. (2018). Orange pulp and peel fibres: Pectin-rich-by-products from citrus processing for water binding and gelling in foods. *Europ. Food Res. Technol.*, **2**, 235-244
- USDA (2017). US-Nigeria commodity storage workshop VII-I stored grain management in on-farm storage and warehouse structures. International Institute of Tropical Agriculture (IITA) Ibadan
- Varsha R., Veeru S., Varsha R. and Partibha M. (2020). Orange peel powder: A potent source of fiber and antioxidants for functional biscuits. *Int. J. Curr. Microbiol. Appl. Sci.*, **9** (9), 1319-1325
- Waleed F.A. (2019). Nutritional benefits of citrus fruits. *Am. J. Biomed. Sci. Res.*, **3** (4), 303-306
- Weickert M.O. and Pfeiffer A.F. (2008). Metabolic effects of dietary fiber consumption and prevention of diabetes. *J. Nutr.*, **138** (3), 439-442
- Weingartner O., Bohm M. and Laufs U. (2008). Controversial role of plant sterol esters in the management of hypercholesterolaemia. *Europ. Heart J.*, **30** (4), 404-409
- Youssef H.M.K.E. and Mousa R.M.A. (2012). Nutritional assessment of wheat biscuits and fortified wheat biscuits with citrus peel powders. *J. Food Pub. Health*, **2** (1), 55-60