

Effect of Debittering Methods on the Minerals, and Phytochemical Properties of Orange (*Citrus Sinensis*) Seeds Flour

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Abstract: The study out to determined the effect of debittering methods on the quality of flour prepared from orange seeds. Orange seeds were removed from orange fruits and then sundried at $30 \pm 2^\circ\text{C}$ for 8 h. orange seeds (20kg) was soaked in water for 12 hours, boiled at different boiling period (40, 80, 120, and 160 min) respectively. Boiled seeds were dehulled immediately to avoid spoilage, oven dried at 60°C for 14 hours, grinded to increase its surface area and defatted using soxhlet extraction method with absolute ethanol. The mineral and phytochemicals results showed that the flours had iron 0.85 mg/100g – 18.54 mg/100g, zinc 0.34 mg/100g – 0.67 mg/100g, calcium 18.23 mg/100g – 476.9 mg/100g, sodium 2.61 ppm – 8.27 ppm, potassium 6.28 ppm – 28.02 ppm, alkaloids 1.15 % – 6.95 %, saponins 0.0 – 0.38 %, flavonoids 0.55 – 29.17 % and tannins 0.94 mg/100g – 10.74 mg/100g.

Keywords: Debittering, minerals, orange seed, phytochemicals, wheat flour

I. Introduction

Citrus is one of the most abundant crops in the world with oranges, grapefruits, lemons being the most common citrus fruits. Orange belongs to the Rutaceae family which comprises grapefruits, mandarins, limes, lemons, and oranges [1]. The seeds are usually embedded at the centre of the fruit, in direct contact with the juice sacs [2]. Sweet orange seed is a by-product of juice production and constitute environmental pollution when not disposed properly. The seeds are very worrisome waste capable of causing serious environmental pollution, but these wastes are promising materials in the food industries due to their nutritional and phytochemical properties. The orange seed is a potential source of minerals and phytochemicals. Minerals and phytochemicals play vital roles in sustaining good health.

Consumption of fruits and vegetables has attracted huge attention since many epidemiological and biochemical studies have consistently demonstrated a clear and significant positive alliance between regular intake of these natural food products and reduced rates of heart diseases, common cancers, aging and other degenerative diseases [3]. The protection properties of these fruits have been attributed to the presence of several minerals and phytochemicals. Phytochemicals are non – nutritive plant chemicals that have protective or disease preventive properties [4]. Phytochemicals act as antioxidants and protect cells against free radical damage, e.g. polyphenols, alkaloids etc [5]. They also help in reducing risk of cancer by inhibiting tumor production [6].

The consumption of citrus seeds is believed to confer some protection against diseases such as cardiovascular disease and cancer [7] [8] [9]. Orange seeds were reported to contain alkaloids, steroids, flavonoids, saponins, tannins, cardiac glycosides and terpenoids [2], orange seeds act as antioxidants and protect cells against free radical damage, because of its phytochemicals contents [5]. They may also help in reducing risk of cancer by inhibiting tumor production [6]. Flavonoid (limonene) which is found in citrus seeds serve as antioxidant, inhibit tumor formation, and decreases inflammation [4]. Limonene, one of the main constituents of orange, reduces the risk of mouth, skin, lungs, breast, stomach, and colon cancer [10].

Alkaloids have pharmacological application as anasthetics and central nervous system (CNS) stimulants, muscle relaxant, antibiotics, anti-cancer, anti-arrythmic and sedative [11]. Some of the general properties of saponins include formation of foams in aqueous solution, hemolytic activity and cholesterol binding properties and bitterness [12]. Saponins natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections [12]. These compounds serve as natural antibiotics, which help the body to fight infections and microbial invasion [12]. Consumption of saponin should be encouraged, because of their hypocholesteromic activity[4].

Flavonoids are the largest group of polyphenolic compounds found in higher plants and synthesized from the shikimic acid and malonic acid pathways [13]. Flavonoids intake has been inversely linked with coronary heart disease [4]. Flavonoids are another large family of protective phytochemicals found in fruits and vegetable [4]. Tannins may decrease protein quality by decreasing digestibility and palatability [4]. Reduction of

tannin content in the dehulled flour is expected to improve its nutritional value because tannins form complexes with proteins and reduce their digestibility and palatability [14].

Iron deficiency is the most common nutritional disorder in the world [15]. According to WHO, around 0.8 million deaths can be attributed to iron deficiency each year [15]. In terms of the loss of healthy life, expressed in disability-adjusted life years (DALYs), iron-deficiency anemia results in 25 million DALYs lost [15]. Iron deficiency causes growth retardation, low birth weight, increased prenatal mortality, increased maternal morbidity and mortality [16]. Iron-deficiency anemia is also a serious public health problem in all countries comprising the Eastern Mediterranean Region, Middle East and North Africa Region [15]. Iron functions as hemoglobin in the transport of oxygen and function as essential components of enzymes involved in biological oxidation such as cytochromes [17].

The bitter fraction of orange fruit arises from a tetracyclic triterpenoid called limonin. The limonin is produced from limonic acid or limonin monolactone, which is found in the seeds and membranes of most *Citrus* fruits [18]. Limonin or its precursor is mainly in the seeds, including peels, albedo and segment wall of the orange fruit [19]. Bitterness due to limonin in a variety of *Citrus* products is a major problem of the worldwide citrus industry and causes significant negative impact on orange product processing. Excess bitterness affects the quality of many processed orange products. This results in significant loss of products due to rejection by consumers, thus, there is need to reduce the level of bitterness of orange seed.

Wheat flour is low in phytochemicals and mineral constituents. Sweet orange seed flour, however, has high levels of calcium, potassium and essential phytochemicals [20]. Sweet orange seed flour could be used to supplement wheat flour. The production of orange seed flour would not only increase the source of flour for food processing but would also act as a waste management strategy. The purpose of this investigation was to determine the effect of the debittering methods such as soaking, boiling and defatting on the mineral and phytochemical composition of orange seed flour.

II. Materials And Methods

Sweet orange (*Citrus sinensis*) fruits were purchased from a local market in Enugu State, Nigeria.

2.1 Preparation of debittered orange seed flour

The fruits were cut into halves with a clean sharp knife and the seeds were removed and sundried at 34 ± 2 °C, for 8 h. The orange seed were cleaned with tap water. The orange seeds (20 kg) were soaked in tap water for 12 h. Thereafter, the hydrated seeds were boiled for 40, 80, 120 and 160 min, respectively. All samples were dehulled manually, winnowed, oven dried at 60 °C for 12 h, ground with corolla hand grinding machine. The oils were extracted with ethanol in a soxhlet apparatus and milled. Flours were analyzed for bitterness by sensory evaluation, the 100 % wheat flour served as control. The flow chart for the preparation of debittered orange seed flour is shown in figure 1.

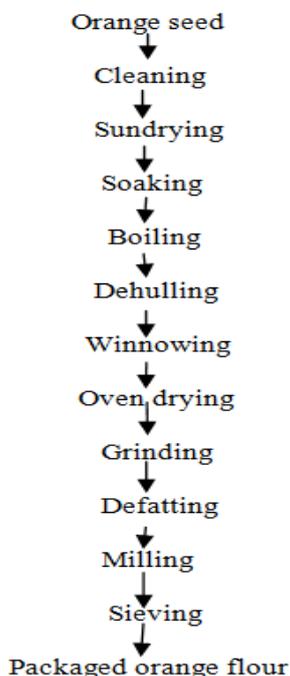


Fig 1. Processing of debittered orange seed flour

2.2 Determination of mineral composition

Zinc was determined by wet ashing [21]. Potassium and sodium were determined using Atomic Absorption Spectrophotometry method as described by [22]. Calcium was determined using the method of [21].

2.3 Determination of phytochemical and anti-nutrient composition

Flavonoid was determined using the method of [23]. Alkaloid was determined using the method of [23]. Saponin was determined using the method of [21]. Tannin was determined using the method described by [24].

III. Results And Discussion

3.1 Effect of debittering methods on mineral composition of flours

The mineral contents of orange seed flour, debittered orange seed flour and wheat flour are shown in Table 1.

Table 1: Mineral composition of orange seed flours and wheat flour

Flour	Iron (Mg/100g)	Zinc (Mg/100g)	Calcium (Mg/100g)	Sodium (Ppm)	Potassium (Ppm)
Wheat flour	1.14 ^c ±0.01	0.34 ^d ±0.01	18.23 ^c ±0.09	2.61 ^c ±0.03	6.28 ^c ±0.01
OSF	18.54 ^a ±0.70	0.67 ^a ±0.02	476.90 ^a ±0.97	8.28 ^a ±0.06	28.02 ^a ±0.02
OSFB40	8.11 ^b ±0.15	0.61 ^{ab} ±0.01	171.92 ^b ±0.87	7.92 ^{ab} ±0.07	25.23 ^b ±0.03
OSFB80	6.18 ^c ±0.15	0.59 ^b ±0.01	170.56 ^b ±0.16	7.56 ^{bc} ±0.21	25.10 ^b ±0.03
OSFB120	1.94 ^d ±0.14	0.58 ^b ±0.07	158.06 ^b ±0.48	7.21 ^{cd} ±0.01	25.00 ^b ±0.02
OSFB160	0.85 ^e ±0.06	0.45 ^c ±0.04	152.01 ^b ±0.18	7.15 ^d ±0.00	24.46 ^b ±0.02

Means within a column with the same superscript were not significantly different ($P>0.05$). OSF=orange seed flour, OSFB40 = orange seed flour boiled for 40 min, OSFB80 = orange seed flour boiled for 80 min, OSFB120 = orange seed flour boiled for 120 min, and OSFB160 = orange seed flour boiled for 160 min. All the treated flours were defatted.

The orange seed flour had the highest iron content of 18.54 mg/100g. The iron content of wheat flour was 1.14 mg/100g. The debittering methods reduced the iron content of the orange seed flour. The iron contents of the debittered orange seed flours ranged from 0.85 mg/100g – 8.11 mg/100g. Flour produced from 160 min boiled seed had the least Iron content (0.85 mg/100g). Probably due to leaching boiling had negative effect on the iron content. Increased boiling time resulted in decreased iron content. Boiling reduced the content of iron in seeds [25].

There were significant ($p<0.05$) differences in the zinc contents which ranged from 0.34 – 6.73 mg/100g with the highest value in the orange seed flour. Wheat flour had the lowest zinc content (0.34 mg/100g). However, flours produced from 80 and 120 min boiled seeds had high value of zinc. Low zinc intake in young children increases the rate of diarrhea and acute lower respiratory infection and reduces linear growth and physical development [26]. Deficiencies of zinc in man include hypogonadism, growth failure, and impaired wound healing [27]. Zinc is essential for the function of many enzymes [28]. Zinc is required for maintenance of normal mobilization of vitamin A from the liver [28]. Low zinc intake results in hyponadism, dwarfism, low serum and red blood cell in humans and retarded growth [28]. In children, suffering from marginal zinc deficiency, impaired taste acuity, poor appetite and suboptimal growth were reversed with zinc supplementation [28]. As an antioxidant, Zinc is important to the immune, reproductive and central nervous systems and is present in many areas of the brain, particularly in the hippocampus and amygdala [29], influencing brain structure and function [30] [31]. Zinc deficiency ranging from slower wound healing to delayed physical and cognitive development [32]. Inadequate Zn in nutrition has been identified in both developing and developed countries, particularly in infants, pregnant and lactating women and older adults [33] [34] [30]. Zinc supplementation studies in infants and pregnant women have demonstrated benefits in terms of the physical health, growth rate and cognitive development of the infants and babies born to the mothers taking supplements [35] [36] [37].

There were significant differences in the calcium contents of the flour samples, which ranged from 18.23 – 476.90 mg/100g with the highest value in the orange seed flour (476.90 mg/100g). Wheat flour had the lowest calcium content of 18.23 mg/100g. However, the debittering methods reduced the calcium content of orange seed flour. There were no significant differences in the calcium contents of the debittered orange seed flours, values varied from 152.01 – 171.92 mg/100g. Calcium is an essential nutrient needed to help muscles to contract, blood to clot and nerves to carry message [28]. Calcium also helps build bones and teeth and keep them strong. Poor calcium intake is a risk factor for osteoporosis, a crippling disease. Calcium is the major component of bone and assists in teeth development [38].

The sodium contents of the flours ranged from 2.61 – 8.28 ppm. There were significant ($p < 0.05$) differences in the sodium content of the flour samples. The orange seed flour recorded the highest sodium content of 8.28 ppm, wheat flour had the least sodium content of 2.61 ppm. Sodium ion acts in concert with other electrolytes, to regulate the osmotic pressure and to maintain the appropriate water balance of the body and acid-base balance (pH) [28].

The potassium content of the flours varies from 6.28 – 28.02 ppm. Orange seed flour had the highest value of 28.02 ppm while wheat flour had calcium content of 6.28 ppm. The debittering methods reduced the potassium content of the dehulled orange seed flour. However, there was no significant difference in the potassium content of the various debittered orange seed flours, values ranged from 24.46 – 25.23 ppm. The decrease in potassium content may be due to leaching in the cooking water during boiling. Potassium plays important roles in the maintenance of normal glucose tolerance and in the release of insulin from cells of islets of langerhans [39]. The high level of potassium is of nutritional benefit especially for patient with high blood pressure [40]. Potassium plays an important role in normal cellular function and metabolism, such as regulating the activity of nerves and muscles, maintaining proper electrolyte balance and acid-base balance in the body, preventing hyper-acidity, controlling high blood pressure and also reducing risk of stroke [28]. Potassium ion acts as a catalyst in the intracellular fluid, in energy metabolism and is required for carbohydrate and protein metabolism [28].

Boiling drastically reduced the mineral and antinutritional composition of the flour [41]. The decrease in the minerals appeared to be more pronounced for iron and calcium in the debittered orange seed flours when compared to the orange seed flour.

3.2 Phytochemicals composition of flour

Table 2: Phytochemical composition of orange seed flours and wheat flour

Flour	Alkaloids (%)	Saponins (%)	Flavonoids (%)	Tannins (mg/100g)
Wheat	1.15 ^c ±0.06	Nd	0.55 ^d ±0.07	0.94 ^a ±0.12
Orange seed	6.95 ^a ±0.68	0.38 ^a ±0.01	29.17 ^a ±0.66	10.74 ^a ±1.12
OSFB40	6.37 ^{ab} ±0.14	0.31 ^b ±0.01	12.11 ^b ±0.48	8.25 ^b ±0.26
OSFB80	6.28 ^{ab} ±0.04	0.28 ^c ±0.01	11.91 ^b ±0.21	8.25 ^b ±0.49
OSFB120	5.81 ^b ±0.53	0.15 ^d ±0.02	11.69 ^b ±0.80	7.34 ^b ±0.92
OSFB160	5.70 ^b ±0.60	0.14 ^d ±0.01	9.72 ^c ±0.04	7.34 ^b ±0.60

Means within a column with the same superscript were not significantly different ($P > 0.05$). OSF=orange seed flour, OSFB40 = orange seed flour boiled for 40 min, OSFB80 = orange seed flour boiled for 80 min, OSFB120 = orange seed flour boiled for 120 min, and OSFB160 = orange seed flour boiled for 160 min

All the phytochemical in the orange seed flours were higher than in wheat flour in Table 2. Significant differences existed in the phytochemical contents. Alkaloids level in the flours ranged from 1.15 - 6.95 %. Orange seed flour had the highest alkaloid content of 6.95 %. Wheat flour had the lowest value of alkaloids (1.15 %). The debittering methods reduced the alkaloids level significantly ($p < 0.05$). However, there were no significant differences in the alkaloids contents of debittered orange seed flours. The presence of alkaloids in the orange seeds indicates that the orange seed should not be eaten raw. Most alkaloids are known for their pharmacological effects rather than their toxicity, however, when alkaloids occur in high levels in foods, they cause gastro-intestinal upset and neurological disorders [42]. Alkaloids are best known as drugs, however, several occurs in foods, potential foods or food contaminants. Appropriate screening of certain common food commodities and foods under development from new sources is important in quality assurance so that risks may be evaluated or eliminated [28]. Phytochemicals may display their health protective effects in diverse ways. Saponin was absent in wheat flour. Saponin contents of the flour ranged from 0 – 0.38 %. The highest value was showed by the orange seed flour (0.38 %). Debittering methods reduced the saponin concentration. The saponin concentration reduced in the orange seed flour with increased in boiling time of the seed. The saponins contents of the debittered orange seed flours varied from 0.14 – 0.31 %. There was generally a gradual decrease in the saponin contents of the debittered orange seed flours with boiling time. Saponin is an anti-nutritional factor whose toxicological effects should be balanced with its benefits. Some of the general properties of saponins include formation of foams in aqueous solution, hemolytic activity and cholesterol binding properties and bitterness [12]. Consumption of saponin should be encouraged, because of their hypocholesteromic activity [4]. The flavonoid concentrations were higher in the orange seed flour and debittered orange seed flours than in the wheat flour. The flavonoid contents of the flours ranged from 0.55 % – 29.17 %. The orange seed flour had the highest value (29.17 %) while the wheat flour had the lowest value (0.55%). However, the debittering methods reduced the flavonoid contents. The flavonoid contents of the debittered orange seed flours ranged from 9.72 –

12.11 %. Flavonoid concentrations were significantly ($p < 0.05$) affected by boiling. Flavonoids are destroyed by heat processing methods like drying, roasting and boiling [43].

There were significant ($p < 0.05$) differences in the tannin concentration of the flour samples. The tannins values varied from 0.94 – 10.74 mg/100g. The orange seed flour had the highest value (10.74 mg/100g) while the wheat flour had the least value (0.94 %). The debittering methods reduced the tannins concentration of the orange seed flour. The tannins contents of the debittered orange seed flour ranged from 7.343 – 8.25 mg/100g. There were no significant differences in the tannins contents of the debittered orange seed flours. The reduction of tannin contents of the boiled orange seeds was in agreement with the earlier report that processing methods such as soaking, boiling and fermentation lowered the tannin contents of foods [44]. The decrease in the levels of tannin during cooking may be due to the thermal degradation and denaturation of the tannin as well as the formation of insoluble complexes [45]. Tannin affects the nutritive value of food products by forming complex with protein (both substrate and enzyme) thereby inhibiting digestion and absorption [46].

Thermal processing can affect the phytochemicals by thermal breakdown that affect the integrity of the cell structure which then results in the migration of components leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen [47]. Boiling, soaking, sun drying, defatting, and oven drying resulted in reduction of all the phytochemicals analyzed in this study. Most anti-nutritional factors in food can be reduced by proper application of heat [48] [49]. The reduction of phytochemical/anti-nutrients increased as boiling period increased [49]. The determination of the anti-nutritional substances was of interest because of their toxicity in orange seeds, and negative effects on mineral bioavailability.

Soaking prior to boiling reduced the concentrations of the flavonoids, alkaloids, saponins and tannins. Soaking in water or periodic changing of the water during soaking, would give a more appreciable reduction in the phytochemicals concentrations. These processes are used for detoxification and debittering of the seeds [50].

IV. Conclusion

The debittered orange seed flours were higher in calcium, iron, zinc and potassium content than wheat flour. However, the minerals decreased with increased in the boiling time of the seeds. Flours produced from debittered orange seed flour were richer in phytochemical constituents such as flavonoids, tannins, saponins and alkaloids than in wheat flour. However, the debittering methods reduced the phytochemical content of orange seed flour and its bitter taste.

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