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# Effect of Simulated Gastrointestinal Digestion on Phytochemicals from Citrus-Derived Waste

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## Abstract

Citrus is one of the most consumed fruit crops in the world, its peels are often discarded, contributing to environmental pollution. This study aims at extracting the bioactive compounds in these wastes and showing the possible effect of digestion on the type and amount of the phytochemicals present. Using maceration, five solvents (Acetone, dichloromethane, methanol, n-hexane and distilled water) were used for the extraction and the phytochemical detection and quantification were performed using reagent detection methods. Simulated gastro intestinal digestions were carried out on the extracts by mimicking the digestive process that occur in the stomach and the intestine invitro and the phytochemical analysis repeated. The results before the digestion showed the presence of all the phytochemicals assayed for in the methanol and distilled water extracts. Flavonoids were also seen to be present in the extracts obtained from all the solvents used. Varying amounts of the phytochemicals were obtained after the quantitative screening; flavonoids were found to have a higher amount than other secondary metabolites with 16.68±0.1mg/100g in distilled water and 14.72±0.86mg/100g in dichloromethane extracts. During digestion, changes in pH, viscosity, aroma and color were observed in both gastric and intestinal phases. Additionally, phytochemical screening after the digestion revealed the presence of alkaloids, flavonoids and tannins in the acetone extract. Flavonoids, phenols and tannins were present in dichloromethane extract, flavonoids and tannins were present in distilled water, whereas only tannin was present in methanol. None of the phytochemical was present in extract dissolved in N-hexane while saponin was absent in all the solvents after digestion. Quantitatively, the result showed varying amounts of the phytochemical in the extract with flavonoids in acetone and dichloromethane (1.94±0.01mg/100g) and tannins in methanol (1.95±0.01mg/100g) having the highest value while phenol in dichloromethane (1.61±0.41mg/100g) has the lowest value. The results obtained showed that digestion has affected the type and quantity of phytochemicals found in citrus-derived waste.

**Keywords**:
Phytochemicals, Citrus-derived waste, Digestion, Solvents, Extraction

## INTRODUCTION

Phytochemicals are substances synthesized by plant cells, but which serve some purpose beyond the primary needs of the cell, and contribute to the survival of the whole plant as a functional organism. Some phytochemicals confer color or scent, others act as signaling molecules, either within the plant itself, or in interactions with other organisms, and many are believed to function as natural pesticides (Kumar *et al.,* 2023). Some of these substances are pharmacologically active, whilst others are either profoundly unpalatable or highly toxic (Johnson, 2013). They can be derived from various sources such as whole grains, fruits, vegetables, nuts, and herbs, and more than a thousand phytochemicals have been discovered to date (Kumar *et al.,* 2023). Some of the important phytochemicals are carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides. These phytochemicals possess strong antioxidant activities and demonstrate antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities. They also help to regulate gene transcription, enhance gap junction communication, improve immunity, and provide protection against lung and prostate cancers to ensure quality products, phytochemicals must be extracted from the source crop in a manner that retains their natural structure and properties (Andre *et al.,* 2010).

Sweet orange (*Citrus senensis*) is a small evergreen tree 7.5m high and in some cases up to 15m. Anatomically, the fruit consists of two distinct regions: the pericarp also called the peel, skin or rind, and the endocarp, or pulp and juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell. The quantity of wax is dependent on the variety, climatic conditions and growth rate. The pericarp consists of the outer flavedo, or epicarp largely made of parenchymatous cells and cuticle (Goudeau *et al*, 2008; Sharon *et al.,* 2003). The flavedo has a characteristic yellow, green or orange colour containing oliferous vesicles on the inside which can be collected by scraping on the flavedo layer. The albedo, or mesocarp lying beneath the flavedo consists of tubular-like cells joined together to constitute the tissue mass compressed into the intercellular area. The albedo is rich in flavonoids, which if transferred to the juice imparts a bitter taste (Atta *et al.*, 2012).

Digestive fluids are liquids originated by various organs in the digestive system to help break down food into smaller, absorbable components (Nayak, 2015). Examples include stomach acid, generated by the stomach lining, and enzymes from the pancreas and small intestine. These fluids help in the digestion and absorption of nutrients during the digestive process. Digestive fluids help digestion by reducing food to simple nutrients (Hagenlocher *et al.,* 2017). In vitro digestion models are a valid methodology to study nutrient hydrolysis by simulating standard physiological gastrointestinal conditions. However, there are pathologies in which some conditions are affected, which should be considered in the design of an in vitro digestion study (Joaquim *et al.,* 2019). Simulated digestive fluid include salivary fluid, gastric fluid and intestinal fluid. (Peng *et al.,* 2018) the elements of simulated digestive fluids include pH, digestive enzymes such as pepsin and trypsin.

Citrus-derived waste is known to contain bioactive compounds with potential health-promoting properties, Digestion sometimes may lead to changes in the type and amount of the phytochemicals ingested. This is brought about by interaction with enzymes, proteins and macromolecules. The bioavailability and bio-accessibility of such phytochemicals are reduced as a result of these interactions (Mihaylova *et al.*, 2021). This study therefore seeks to investigate the effect of simulated digestion on the serially exhaustive extracted phytochemicals in flavedo of citrus derived waste.

## METHODS

### Plant Material

*Citrus senensis*, was purchased from New Market in Gombe City, Nigeria. The authenticity of citrus-derived waste was then confirmed at the department of Botany lab Gombe, Gombe state university for identification. The fruits were washed with distilled water and the peels removed with the aid of a sharp knife. The flavedo was scrapped carefully to ensure it was not removed alongside the albedo. The flavedo was then air-dried in a shade at room temperature to prevent loss of sensitive compounds for seven days. It was then pulverized and stored properly in an airtight container.

**Serial Exhaustive Extraction**

Extraction was carried out as described by Tata *et al*., (2020) with some modification. The powdered sample was weighed (50 g/250 mL) and was prepared by consecutive extraction method using a series of solvents with increasing polarity (N-hexane, Acetone, dichloromethane, methanol, distilled water). The pulverized citrus waste was (20g) extracted in n-hexane (100 mL) for 24 h at room temperature (25±5 ºC) using a mechanical shaker. The extract was evaporated to dryness, and weighed, the total extractable components (TEC) would be calculated as TEC% = weight of extract÷ weight of sample × 100. The residue obtained after extraction in hexane was further extracted consecutively with acetone, dichloromethane, methanol, distilled water. The residue obtained in previous extraction step was extracted in the next solvent and the extracts obtained in each extraction step dried using rotary evaporator. The dried extract was stored and utilized when needed for any analysis.

**Qualitative and Quantitative Phytochemical Screening**

Qualitative and quantitative phytochemical screening was done following the procedures described by Ajuru *et al.* (2017)**.** The phytochemical classes that were tested for include alkaloids, flavonoids, saponins, Phenols, Tannins. Using different Standard reagents detection methods, the quantitative phytochemical screening was carried out to assay for the amount of each phytochemical that were detected in the qualitative assays

**Stimulated Gastrointestinal Digestion**

The invitro gastrointestinal digestion was simulated according to methodology described by Correa *et al.* (2017) with some little modification. Five different digestion tubes containing 6g of the dried extracts from five solvents: acetone, dichloromethane, distilled water, methanol and N-hexane, were each mixed with 18ml of artificial saliva (2.38g of disodium hydrogen phosphate $Na_2HPO_4$, 0.19g of potassium dihydrogen phosphate $KH_2PO_4$, 8g of sodium chloride $NaCl_2$ in 1litre of distilled water). The pH was regulated to 6.75 using NaOH and HCl, at the temperature of 37ºC and 1ml alpha-amylase was added to produce an enzyme activity of 200U. This blend was shaken at 150rpm for 10min. In sequence, the pH was adjusted to 4.2 and 18ml of artificial gastric fluid (0.32g pepsin in 100ml of 0.03M NaCl, pH 4.2) was included. The mixture was incubated on a shaker at $37^0$C for 120min, under agitation of 150rpm. Lastly, the pH was adjusted back to 6.0 following the addition of 6.5ml of NaCl, 6.5ml of KCl and 18ml of artificial intestinal fluid (0.15g of pancreatin and 0.9g of bile extract in 100ml of 0.1M $NaHCO_3$). The mixture was incubated at 37ºC for 60min, at 150rpm. Thereon the obtained digested extract was freeze-dried by first freezing to control the size and shape of ice crystals, pressure was decreased and temperature increased to sublime ice into vapour, break ionic bonds and release last water molecule. This was done on all the samples digested. Subsequently, qualitative and quantitative phytochemical analysis was carried out on the freeze- dried digested sample.

**RESULTS AND DISCUSSION**

The digestion of extracts of citrus waste peels invitro (Tables 1 and 2) showed changes in the color of the extracts at both the gastric and intestinal phase which could be due to the breakdown of pigments or interactions with digestive enzymes and bile salts.

**TABLE 1:** Changes Observed in Gastric Phase of Digestion

| PARAMETERS | BEFORE | AFTER |
|---|---|---|
| pH | 6.75 | 4.2 |
| Color of extract | Pale yellow | Brown |
| Viscosity | High | Low |
| Aroma | Pleasant smell | Less pleasant |

**TABLE 2:** Changes Observed in Intestinal Phase of Digestion

| PARAMETERS | BEFORE | AFTER |
|---|---|---|
| pH | 4.2 | 6.2 |
| Color of extract | Brown | Dark brown/ Black |
| Viscosity | Low | Very Low |
| Aroma | Pleasant smell | Foul odor |

**Table 3:** Qualitative phytochemical analysis of citrus derived peel waste before digestion

| Solvents Phytochemical | Distilled water | Methanol | Dichloromethane | Acetone | N-hexane |
|---|---|---|---|---|---|
| Alkaloids | + | + | - | + | + |
| Phenols | + | + | + | - | - |
| Flavonoids | + | + | + | + | + |
| Saponins | + | + | + | - | - |
| Tannins | + | + | + | + | - |

All values in Tables 5 and 6 are expressed as mean ± standard deviation of triplicate measurement.

**Table 4:** Qualitative phytochemical analysis of citrus derived peel waste after digestion

| Solvents - Phytochemical | Distilled water | Methanol | Dichloromethane | Acetone | N-hexane |
|---|---|---|---|---|---|
| Alkaloids | - | - | - | + | - |
| Phenols | - | - | + | - | - |
| Flavonoids | + | - | + | + | - |
| Saponins | - | - | - | - | - |
| Tannins | + | + | + | + | - |

**KEY:** + indicates presence of phytochemicals
- indicates absence of phytochemicals

**Table 5:** Quantitative phytochemical analysis of citrus derived waste extracts before Digestion

| Solvent - Phytochemical | Distilled water | Methanol | Dichloromethane | Acetone | N-hexane |
|---|---|---|---|---|---|
| Alkaloids | 13.1±0.06 | 14.5±0.86 | - | 3.75±0.01 | 2.5±0.11 |
| Phenols | 0.32±0.03 | 3.75±0.01 | 2.6±0.94 | - | - |
| Flavonoids | 16.68±0.1 | 12.84±0.08 | 14.72±0.86 | 2.15±0.1 | 7.7±0.6 |
| Saponins | 13.4±0.01 | 16.2±0.02 | 12.8±0.02 | - | - |
| Tannins | 14.07±0.02 | 3.3±0.47 | 2.32±0.04 | 3.64±0.1 | - |

**Table 6:** Quantitative phytochemical analysis of citrus derived waste extracts after Digestion

| Solvent - Phytochemical | Distilled water | Methanol | Dichloromethane | Acetone | N-hexane |
|---|---|---|---|---|---|
| Alkaloids | - | - | - | 1.93±0.03 | - |
| Phenols | - | - | 1.61±0.41 | - | - |
| Flavonoids | 1.92±0.01 | - | 1.94±0.01 | 1.94±0.01 | - |
| Saponins | - | - | - | - | - |
| Tannins | 1.93±0.01 | 1.95±0.01 | 1.70±0.04 | 1.86±0.01 | - |

The viscosity of the extracts was also affected probably by the breakdown of the polysaccharides and protein. The emulsification of lipids, precipitation of proteins and the formation of colloidal particles might have been the factors that led to the variation in clarity (Smith *et al.,* 2020).

In the stomach, the pH drops significantly due to the secretion of hydrochloric acid by parietal cells. This acidic environment is essential for the activation of some proteolytic enzymes. The pH rises due to the secretion of bicarbonate ions from the pancreas, which neutralize the acidity of the chyme (Smith *et al.,* 2020). This change in pH from acidic to alkaline (Table 1 and 2) indicates the transition from gastric to intestinal digestion. Enzymatic reactions during digestion release aroma compounds, potentially altering their flavor profile. Digestive processes also lead to chemical reactions that modified the flavor compounds present, thus, influencing their overall taste perception (Smith *et al.,* 2020). As the phytochemicals move from the gastric phase to the intestinal phase, the viscosity is lowered, this may be attributed to the nature of the phytochemical and the enzymes involved in the process. Low viscosity leads to fast release, can expose phytochemicals to enzymatic degradation, reduced solubility and bio-accessibilty (Tanaka *et al.,* 2019)

Phytoconstituent screening of the peels of the orange before and after digestion shows the presence of different phytochemicals in different solvents (Table 3 and 4). Methanol and Distilled water extracts showed the presence of all the tested phytochemicals before digestion but flavonoids and tannins were the two phytochemicals present upon digestion. Tannins have been reported to exert physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease serum lipid level, produce liver necrosis, and modulate immuno-responses. They play protective role from predators, and might help in regulating plant growth (Ferrell, 2006). Saponins were absent in all the solvents after digestion but present in distilled water, methanol and dichloromethane extracts before digestion. Their absence after digestion could be as a result of a series of structural changes that occur by a stepwise desugaring process (He *et al*., 2019). The absence of other phytoconstituents after digestion could be attributed to the presence of other compounds in the digestive tract and individual differences in metabolism (Johnson, 2013). Similarly, many phytochemicals chemically degrade when subjected to certain conditions like changes in pH, heat, prooxidants etc. (Hu *et al*., 2022). The polarity of the various solvents used also played a key role in the presence or otherwise of the phytochemicals in table 5. Alkaloids for instance was detected in all the solvents used. This could be due to the fact that most alkaloids are weak bases and some can be amphoteric (Spiller, 2019).

The quantitative phytochemical analysis showed varying amounts of bioactive component, this agrees with the studies conducted by Johnson, (2013) which also found out that not all phytochemicals are present in all plants part in large amounts and those present differ according to the type of extraction method used. Flavonoids were present in varying amounts in all the solvents used for extraction (Table 5) but absent in the methanol and N-hexane extracts, after digestion (Table 6). Flavonoids are a collection of phenolic compounds with free hydroxyl groups which results in very rapid conjugation by glucuronidation, sulfation. Therefore, methylation is used to caps all free hydroxyl groups in order to eliminate conjugation as the primary metabolic pathway, which leads to improvements in metabolic stability and it has been observed that methylated flavones are metabolically much more stable than their unmethylated analogues (Naeem *et al*., 2022). This could be the reason why it is detected in varying amount after digestion. There was also a general decrease in the amounts of the phytochemicals present after digestion when compared with the amounts obtained before digestion. Alkaloids were present in all extracts except dichloromethane before digestion with the highest amount found in the methanol extract (14.5±0.86 mg/100g). Upon Digestion, no amount of alkaloid was detected in all the extracts, with the exception of acetone extract that has 1.93±0.03 mg/100g. Lack of stability upon digestion could have led to converting these phytochemicals into their metabolites as such the amounts decreased or became absent (Hayes *et al*., 2021). The interaction with other compounds in the simulated digestive fluid might result in changing the structure and function of the phytochemicals present, thus lowering their concentration or even led to an entirely different compound. Polyphenols have been found to be very responsive to slightly alkaline environments, like those found in the intestines. In this environment, some of the compounds undergo transformation into various structural forms with distinct chemical characteristics. Furthermore, during the process of digestion, phenolic compounds can easily interact with other food components that are released, including iron, minerals, dietary fiber or protein, resulting in the loss of phytochemicals (Mihaylova *et al*., 2021).

## CONCLUSION

This study clearly showed that citrus-derived waste can be put to use, serving as a source of phytochemicals that possess biological activity. Process like encapsulation can be employed to ensure that majority of the phytoconstituent are not lost during digestion as the results obtained showed that digestion has affected the phytochemicals found in *citrus sinensis* derived wastes. Conducting in vivo studies to provide more insight on the behavior of the phytochemicals in the digestive system and in-silico studies is necessary to elaborate more on the nature of the changes that occurred following digestion.

**Data Availability Statement:** Data sharing not applicable.

**Declaration of competing Interest:** The authors declare no conflict of interest.

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