MYCOFLORA AND NUTRITIONAL CONSTITUENTS OF GREEN PEA
(Pisumsativum)

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ABSTRACT
Research on the mycoflora and nutritional constituents of P. sativum were carried out in the
Department of Plant Science and Biotechnology, Rivers State University. Proximate
investigation revealed the presence of moisture, ash, fibre, lipid, protein and carbohydrate in the
healthy and spoilt samples. However, highest values of moisture (65.5± 0.012), ash (3.2±
00.004) and carbohydrate (10.04±0. 002) were recorded for the healthy samples whereas fibre,
lipid and protein were higher for the spoilt samples. Mineral composition showed the presence of
calcium, phosphorus, potassium, iron, sodium and magnesium. All observed parameters were
higher for the healthy samples of P. sativum with an exception for iron which recorded equal
concentrations (4.0± 0.001) for both healthy and spoilt samples. Vitamin compositions found in
P. sativum were vitamins A, C, thiamine and niacin. They all had higher values for the healthy
samples. Furthermore, P. sativum also contained anti-nutrient and phytochemicals such as
phytate, oxalate, saponin, tannin, carotenoid, flavonoid, polyphenol and lignin in appreciable
concentrations. Nevertheless, three fungal organisms viz: Slerotiumrolfsii, Mucorspp and
Rhizopusstoloniferwere isolated and implicated for the spoilage of P. sativum. S. rolfsii had
highest incidence (50 ±0.003%) while Mucorspp and R. stolonifer recorded equal incidence (25±
0.023%).

Keywords: Mycoflora, Phaseolus vulgaris and nutritional constituents.

1. INTRODUCTION

Pisumsativum commonly known as green pea is an important legume that belongs to the
Fabaceae family. The plant is an annual vegetable that prefers cold season , hence is mostly
grown in the temperate region (Savage &Deo, 1989; Zohany&Hopf, 2000). P. sativum serves
several culinary purposes and it is widely consumed around the world. It can be boiled, dried or
prepared as soup and salad. Due to its high demand, they are further processed and canned for
future use (Sharma et al., 2015).

Just like every other legume, green pea is cherished because of its nutritional contents (Nguyen
et al., 2015; Ganjlooe et al., 2018). Literatures have shown that P. sativum possesses several
proximate parameters such as moisture, ash, protein, fibre and many others (Upasana&Vinay,
2018; Urbano et al., 2003). Earlier studies have also shown the plant to contain calcium, iron,
potassium, magnesium, phosphorus and several amino acids (Harmankaya et al., 2010; Igbasan et al., 1997; Amarakoon, 2012). Phytochemicals and vitamins have also been reported to be components of green pea (Xue et al., 2007; Duenas et al., 2004; Dahl et al., 2012). The research of Garg, (2015) also supported the nutritional value of *P. sativum* as he reported pea pod powder to contain proximate and mineral parameters. He also identified that the powder could be used for the preparation of Jaggery biscuits.

Vegetables have always been prone to fungal attack, leading to spoilage and economic losses. In addition, filamentous fungi are vastly distributed and available ready to cause contamination (Gourama, 2015). Kumar, (2015) implicated *Fusarium oxysporum* to be responsible for the spoilage of green pea pod sold in Panchgaon, India. Furthermore, Embaby et al., (2013) revealed several species of *Aspergillus*, *Penicillium*, *Fusarium* and *Sclerotinia* to contaminate three legume seeds including *P. sativum*. They also showed that these fungal organisms produced mycotoxins (aflatoxin and fumonisín) that were significantly high. This was supported by Ramprasad et al., (2014) as they reported the menace of filamentous fungi on green pea and other vegetables.

It was based on this available literature this research was carried out to investigate the nutritional constituents and associated spoilage fungi of *P. sativum* sold in Port Harcourt.

2. MATERIALS AND METHODS

Sample Collection

Samples of healthy fruits of *P. sativum* and partially rotted fruits were bought from the Fruit Garden Market at D. Line Diobu Port Harcourt and brought to the Department of Plant Science and Biotechnology and sent to the Plant Pathology Laboratory for further studies.

Mycological studies

Preparation of mycological medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120°C for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask containing the mycological medium was autoclaved at 121°C and pressure of 1.1 kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40 °C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of fungi from partially rotted *P. sativum* fruits.
One gram of samples showing visible signs of spoilage by moulds was cut from the healthy portions of the fruits up to the points where rot had established and inoculated onto Sabouraud Dextrose Agar in Petri dishes onto which ampicillin was added to hinder the growth of bacteria in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25°C ± 3°C (Baudoni, 1988; Chuku, 2009; Samson et al, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure cultures of isolates were obtained after a series of isolations.

**Identification of fungal organisms from *P.sativum***

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Samson et al, 1981; Olds, 1983).

**Pathogenicity studies**

Pathogenicity studies was carried out on *P.sativum* to check if the fungi isolated from the rotted fruits were capable of causing spoilage on healthy fruits samples. The methods of (Agrios, 2005; Trigiano, 2004) was basically followed. The fungal isolates were introduced into healthy fruits and observed for seven days. The set up was monitored regularly for growth.

**Determination of nutrient components of fruits of*P.sativum***

Healthy and spoilt fruit samples of *P.sativum* were sent to the Food Science and Technology Laboratory for the determination of nutrient composition. The methods of AOAC, (2005) was used for the analysis.

**Determination of percentage incidence**

The percentage incidence of fungal occurrence was determined by the formular stated below (Nnaji&Rao, 2017):

\[ \frac{X}{Y} \times 100 = \% \text{ incidence} \]

Where:

X= total number of each organism in a variety

Y= total number of all identified organism in a variety
3. RESULTS AND DISCUSSION

Table 1: Proximate composition of healthy and spoilt samples of *P.sativum*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (%)</th>
<th>Spoilt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>65.5±0.012</td>
<td>64.2±0.011</td>
</tr>
<tr>
<td>Ash</td>
<td>3.2±0.004</td>
<td>3.1±0.022</td>
</tr>
<tr>
<td>Lipid</td>
<td>1.75±0.001</td>
<td>1.80±0.015</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>10.04±0.002</td>
<td>9.4±0.002</td>
</tr>
<tr>
<td>Fibre</td>
<td>2.5±0.003</td>
<td>3.0±0.013</td>
</tr>
<tr>
<td>Protein</td>
<td>17.0±0.002</td>
<td>18.5±0.020</td>
</tr>
</tbody>
</table>

Table 2: Mineral composition of healthy and spoilt samples of *P.sativum*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (mg/100g)</th>
<th>Spoilt (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>9.5±0.033</td>
<td>7.5±0.021</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>7.5±0.005</td>
<td>6.0±0.003</td>
</tr>
<tr>
<td>Potassium</td>
<td>98±0.021</td>
<td>96±0.005</td>
</tr>
<tr>
<td>Iron</td>
<td>4.0±0.001</td>
<td>4.0±0.001</td>
</tr>
<tr>
<td>Sodium</td>
<td>3.5±0.001</td>
<td>3.4±0.010</td>
</tr>
<tr>
<td>Magnesium</td>
<td>5.5±0.005</td>
<td>2.5±0.004</td>
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</tbody>
</table>

Table 3: Vitamin composition of healthy and spoilt samples of *P.sativum*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (mg/100g)</th>
<th>Spoilt (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>4.2±0.051</td>
<td>2.5±0.010</td>
</tr>
<tr>
<td>Thiamin</td>
<td>1.1±0.001</td>
<td>0.9±0.006</td>
</tr>
<tr>
<td>Naicin</td>
<td>0.7±0.002</td>
<td>0.4±0.023</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5.2±0.004</td>
<td>2.05±0.003</td>
</tr>
</tbody>
</table>
Table 4: Anti-nutritional and phytochemical composition of healthy samples of *P. sativum*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.11±0.012</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.05±0.023</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.02±0.001</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>0.50±0.005</td>
</tr>
<tr>
<td>Polyphenol</td>
<td>0.04±0.001</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>0.41±0.010</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.65±0.022</td>
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</tbody>
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Table 5: Fungi isolates and their percentage incidence

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Percentage incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sclerotium rolfsii</em></td>
<td>50±0.003</td>
</tr>
<tr>
<td><em>Mucor spp</em></td>
<td>25±0.023</td>
</tr>
<tr>
<td><em>Rhizopus stolonifer</em></td>
<td>25±0.023</td>
</tr>
</tbody>
</table>

The result for the proximate composition of healthy and spoilt *P. sativum* presented in Table 1 showed the presence of moisture, ash, lipid, carbohydrate, fibre and protein. The healthy samples of *P. sativum* recorded higher values for moisture (65.5±0.012), ash (3.2±0.004) and carbohydrate (10.04±0.002) while lipid (1.80±0.015), fibre (3.0±0.013) and protein (18.5±0.020) were higher for the spoilt samples. The proximate parameters assessed in this study confirmed the report of early findings (Savage & De, 1989). Kumar, (2015) also evaluated the proximate composition of healthy and spoilt green pea. The carbohydrate and protein values as reported are lower than those found in this study for both healthy and spoilt *P. sativum*. However, the fibre concentrations of the present study are lower than those reported. The proximate parameters of the current study also agree with those investigated by Upasana&Vinay, (2018) for the peel of green pea; even though higher values for moisture (83.41%), protein (19.79%) and ash (5.65%) were reported. Meanwhile, the lipid value recorded in this study is in line with the 1.2 to 2.4 lipid concentration reported by Dahl *et al.*, (2012) for *P. sativum*. 
Mineral composition of *P. sativum* presented in Table 2. revealed that calcium, phosphorus, potassium, iron, sodium and magnesium were present. The healthy samples recorded higher values for all the parameters assessed with an exception for iron which had equal values for both healthy and spoilt samples. The calcium and phosphorus values recorded in this study are higher than those reported by Igbasan *et al.*, (1997) for twelve cultivars of green pea. Harmankaya *et al.*, (2010) reported higher values for all the assessed parameters in this study. Nevertheless, the iron concentration (4.0±0.001) of this present study conforms to the 2.19 to 5.84 mg/100g range that was reported for several genotypes of green pea.

The result of vitamins composition presented in Table 3. indicated the presence of vitamins A, C, niacin and thiamin in both healthy and spoilt *P. sativum*. Higher values of 4.2± 0.051, 1.1± 0.001, 0.7± 0.002 and 5.2± 0.004 were recorded for vitamin A, thiamin, niacin and vitamin C respectively for the healthy samples. The presences of these vitamins have been reported earlier in *P. sativum* (Savage & Deo, 1989). Higher values of these vitamins have also been reported by early researchers in green pea (Robertson & Sissons, 1986; Wills *et al.*, 1984).

The result of anti-nutrients and phytochemicals of green pea presented in Table 4. showed the presence of phytate, oxalate, saponin, tannin, carotenoid, polyphenol, flavonoid and lignan. The result of the current study is in agreement with earlier works anti-nutrients and phytochemicals have been associated with several legumes including green pea (Elkowicz & Sosulski, 1982). The research of Martens *et al.*, (2017) also showed the presence of phytochemicals in *P. sativum* and the flavonoid concentration in this study is in line with the 0.45 and 0.43 they reported.

The nutritional quality of *P. sativum* cannot be overlooked as these parameters are necessary and vital for healthy living. Inasmuch as it serves as a source of amino acids (protein), energy (carbohydrate) and fibre; it can also support the body immune system as phytochemicals possess antimicrobial and anticarcinogenic activities (Campos-Vega *et al.*, 2010; Mathers, 2002; Martens *et al.*, 2017).

Three fungal organisms viz: *Sclerotium rolfsii*, *Mucorspp* and *Rhizopus stolonifer* as presented in Table 5. were isolated and implicated through pathogenicity test to cause spoilage of *P. sativum*. Highest percentage incidence (50±0.003) was recorded for *S. rolfsii* while *Mucor* and *Rhizopus* recorded equal incidence (25±0.23). The isolates of this present study disagrees with Kumar, (2015) as he implicated only *F. oxysporum* to be responsible for the spoilage of green pea. Furthermore, the isolation of *S. rolfsii* is in line with the *Sclerotinasclerotiorum* reported by Embaby *et al.*, (2013). The occurrence and equal incidence of *Mucor* and *Rhizopus* could be attributed to their taxonomic relationship as they both belong to the Mucorales order and are always associated with food spoilage (Salako & Anjorin, 2012). The deteriorative activities of these organisms stand as a treat to the consumption of *P. sativum*.

4. CONCLUSION

Legume crops have always been referred to be rich in nutrient and the current study has shown that *Pisumsativum* is neither an exemption. However, the presence of spoilage organisms makes it unsafe for consumption and reduces its market values. Hence proper hygienic measures should be adopted to guide against contamination.
REFERENCES


