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Sato, Koji  
Umekage, Koichi  
Kumamoto, Machiko

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## (Citation)

South African Journal of Botany, 169:1-5

## (Issue Date)

2024-06

## (Resource Type)

journal article

## (Version)

Accepted Manuscript

## (Rights)

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## (URL)

<https://hdl.handle.net/20.500.14094/0100489650>



Comparison of nutritional values for *Dioscorea esculenta* grown in a cultivated sub-tropical region and a plastic greenhouse in a cold region

Koji Sato<sup>1</sup>, Koichi Umekage<sup>2</sup>, Machiko Kumamoto<sup>2</sup>

<sup>1</sup> Graduate School of Human Development and Environment, Kobe University,  
Kobe, Hyogo, Japan

<sup>2</sup> LINK's group, Kameoka, Kyoto, Japan

**Running title:** *comparison of nutritional value for dioscorea esculenta*

**Acknowledgement**

The study was supported by Kyoto grain system Co., Ltd, Kyoto, Japan, and cultivation of *dioscorea esculenta* was supported by LINK's group, Kameoka, Kyoto, Japan.

**Disclosures**

There is no conflict of interests in the present study.

**Ethical Approve**

Not Applicable

**Author contribution**

K.S: conceived and designed the study, wrote the article. K.U and M.K: conducted data gathering

**\*Corresponding author:**

Koji Sato, PhD

Graduate School of Human Development and Environment,

Kobe University, 3-11 Tsurukabuto, Kobe, Nada-Ku, Hyogo, 657-8501, Japan

TEL: 81-78-803-7812

FAX: 81-78-803-7812

E-mail: [sato712@people.kobe-u.ac.jp](mailto:sato712@people.kobe-u.ac.jp)

## Abstract

**Background:** The lesser yam (*Dioscorea esculenta*) is a phytochemical tuber that contains substantial amounts of diosgenin. *Dioscorea esculenta* is normally cultivated in tropical/sub-tropical regions such as African countries, and Okinawa in Japan. However, it remains unknown whether it can be successfully cultivated in cold regions within a plastic greenhouse. This study investigated the differences in nutritional values and diosgenin content between lesser yams cultivated in a sub-tropical climate (Okinawa) and those cultivated in a cold region (northern Kyoto) within a plastic greenhouse.

**Methods:** The tubers were planted in March 2021 and harvested in January 2022. The temperature of the plastic greenhouse was 25.6 °C throughout the cultivation period. Additionally, the soil properties were potting soil with 8% nitrogen potassium phosphate. Samples of *dioscorea esculenta* from both regions were used to produce 1kg of sliced tubers from six tubers, were dried with the peel, then pulverized. The diosgenin content was analyzed for each sample, and nutritional ingredients such as moisture, carbohydrates, protein, fat, ash, sodium, and energy were measured.

**Results:** Most of the analyzed nutritional ingredients demonstrated comparable values between plants cultivated in the sub-tropical region and within the plastic greenhouse in the cold region. The diosgenin content was 0.12g/100 g for tubers cultivated in the sub-tropical region and 0.1g/100 g for those cultivated in the cold region within a plastic greenhouse.

**Conclusion:** Nutritional ingredients and diosgenin contents were comparable between the two growing conditions. *Dioscorea esculenta* can be cultivated in sub-tropical regions and in cold regions within plastic greenhouses.

25    **Keywords:**

26    dioscorea esculenta, environment, greenhouse, climate difference, lesser yam

27

## 28    **Introduction**

29    Yams are important root crops cultivated in the humid and sub-humid tropics. They  
30    belong to the genus *Dioscorea*, which includes more than 600 species (Coursey, 1983).  
31    *Dioscorea esculenta* (*D. esculenta*) is generally cultivated in tropical/sub-tropical regions  
32    such as Latin America, east African countries, and southern Asia included Okinawa,  
33    Japan. The cultivation conditions, especially temperature, are important, as the tubers  
34    either do not survive or grow poorly at temperatures below 20 °C. The optimum  
35    temperatures for cultivation are above 30 °C (Lebot, 2019). Additionally, *D. esculenta*  
36    require adequate moisture throughout their growth period. A positive correlation was  
37    found among high and regular rainfall, vine growth, and tuber yield (Lebot, 2019).  
38    Additionally, major areas of production are centered where there is a sharply demarcated  
39    dry season of 2-5 months and a rainfall of 120-150 cm or more during the growing season  
40    (Price et al., 2017). In arid and cold regions, where temperatures are below 20 °C and  
41    rainfall is as low as 60 cm per year, cultivation and growth of *D. esculenta* are difficult  
42    and yields are poor. Therefore, cultivation of *D. esculenta* in cold regions has not been  
43    reported.

44    In poor soils, the weak root system of these plants is unable to obtain sufficient water or  
45    nutrients to produce reasonably sized tubers. Although fertilizers are not widely used for

*D. esculenta* cultivation, the use of potassium sulphate at 125 to 370 kg/ha or a 12:12:8 nitrogen, phosphorus, and potassium (NPK) mixture at the rate of approximately 60 g per mound has been recommended in African countries (Enyi, 1972). In Eastern Caribbean countries, other types of yams are grown and yields had been substantially increased with the application of 225 kg/ha of a 9:10:23 NPK fertilizer (Enyi, 1972). The application of potash has also increased the yield and storage life of *D. esculenta* tubers, while chlorine in the fertilizer adversely influenced the starch content (Diby et al., 2011). Thus, the use of fertilizer influences the growth and yield of *D. esculenta* affect the growth of other species of yams. Moreover, in regions with lower temperatures and annual rainfall less than 300 cm, nutritional values are reduced throughout the growth period. However, the effect of different cultivation environments on their growth and nutritional values remains unclear.

Various lesser yam varieties are cultivated in Japan, including Japanese yam, water yam, and *D. alata*. In Okinawa, lesser yam especially *D. esculenta* is an important local vegetable that goes by the name “*Ku-ga imo*”. *D. esculenta* (*Ku-ga imo*) contains ten times more diosgenin than other lesser yams cultivated in Japan (Ohnogi, 2013). Diosgenin is a steroid sapogenin, and is a product of saponin hydrolysis by acids, strong bases, or enzymes; it has a steroid molecular formula similar to dehydroepiandrosterone

(DHEA) (Raju et al., 2014). *D. esculenta* produces phytochemical compounds with no side effects or toxicities reported by Raju et al (2004). The effects of diosgenin administration and intake have been reported in previous studies as improvement of hyperglycemia, glucose metabolism in skeletal muscle (Sato et al., 2017), lipid (Kusano et al., 2019) and bone metabolism (Zhang et al., 2014), and cognitive function (Tohda et al., 2017). Diosgenin is utilized as an alternative treatment involving nutrient supplements which may be beneficial in controlling glycemia, weight, and aging. Therefore, it is important to cultivate *D. esculenta* with substantial amounts of diosgenin, regardless of environment.

The aim of this study was to compare the nutritional values between *D. esculenta* cultivated in a sub-tropical region at Okinawa and that cultivated within a plastic greenhouse in a cold region in northern Kyoto. The study compared moisture, carbohydrate, protein, fat, ash, sodium, energy, and diosgenin contents in *D. esculenta* dried with the peel and pulverized.

## **Materials and Methods**

*D. esculenta* in Okinawa was made from Nakijin village, Okinawa, and soil was included red clay with organic fertilizer which was mainly cow manure (Ryukyu Eco Project, Okinawa, Japan). The local climate is sub-tropical and has a mean temperature

of 23.7 °C throughout the cultivation and growth period. In northern Kyoto, small whole tubers of *D. esculenta* were planted in March 2021 with potting soil and 8:8:8 NPK fertilizer (30\_kg10a<sup>-1</sup>) within a plastic greenhouse. There were no additional climate controls to maintain temperature and humidity for minimization of these variation factors. Whole *D. esculenta* tuber seed sets were weighed to approximately 40 g each. In March 2021, 35 individual seed sets were planted in pots of 40 cm diameter, 41 cm deep, capacity of 34 L, and filled with 38 kg of subsoil firstly. Sprouted seedlings were transplanted into the field in May 2021. The plants were established at 5.4 × 20 m spacing in a greenhouse, with 50 cm between each seedling. The mound spacing was 50 cm, the spacing between each mound were 80 cm, and soil was mounded up to 40 cm height over each furrow in Kyoto. The wide spacing and high mounds were intended to facilitate drainage and excavation with minimum disturbance to adjacent plants. Each plant was supported by a single stake of approximately 1.8 m high. The soil was well-drained, sandy loam soil with a pH level was 6.5 in the greenhouse in Kyoto, however, soil pH in Okinawa was not measured in present study. Plants were watered once a week from April to June and October to January. They were watered three times a week from July to September. The *D. esculenta* were harvested from the end of January to the beginning of February 2022. The cultivation method and plant

maintenances were conducted same way between Okinawa and Kyoto, but the height of furrow was higher in Kyoto.

Six tubers of *D. esculenta* were harvested (~450 to 600 g per plant). Flours with and without peels were prepared as analytical samples from the harvested tubers. To prepare flour without peels, tubers were washed, peeled, and sliced with a peeling machine to produce 1 kg of sliced tubers. In contrast, samples containing peels were sliced without peeling. Sliced tubers were dried at approximately 70–75 °C for 20 h, pulverized to a powder, and heat-treated at temperatures of > 200 °C for 60 s (Kyoto Grain System Co., Ltd, Kyoto, Japan). Each pulverized *D. esculenta* powder sample was analyzed for moisture content and nutritional ingredients such as carbohydrate, protein, fat, ash, sodium, energy and diosgenin content. Moisture content was analyzed by a loss-on-drying method according to Ileleji et al (2010). Protein content was analyzed by the combustion method (King-Brink & Sebranek, 1993). Fat content was analyzed by the acid hydrolysis method (Xiao et al., 2012). Ash content was determined by the dry ashing method (Sahrawat et al., 2002) and sodium was measured by atomic absorption spectrophotometry (Julshamn et al., 2005). The amount of carbohydrate was calculated by the formula: 100 - (moisture + protein + fat + ash contents) according to a study by Schakel et al (1997). Energy content was also calculated by converting it to calories at

the rates of 4 calories per gram of carbohydrate or protein and 9 calories per gram of fat (Fenton & Elmrayed. 2021). Finally, the diosgenin content was analyzed by high performance liquid chromatography (HPLC) on a Shimadzu 20AT instrument equipped with SPD-M20A detector. *D. esculenta* contains a substantial amount of dioscin combined with glycoside with diosgenin. Therefore, removal of glycoside was conducted by hydrolysis with using hydrochloric acid according to Yu et al (2019). Briefly, 10 g of *D. esculenta* powder was hydrolyzed in 60 mL of water containing 5 mL of H<sub>2</sub>SO<sub>4</sub> for 3 h. The hydrolyzed sample was filtered and the residue was washed using 100 mL of hot water, dried at 80 °C, and extracted in a Soxhlet extractor with 80 mL of petroleum ether at 120 °C for 4 h. After cooling to ambient temperature, the solution was transferred to a 10 mL volumetric flask and diluted to the final volume with 60% ethanol. The final solution was passed through a 0.22-μm membrane filter, then the sample was quantified by C18 reverse-phase column of analytical HPLC with elution using an isocratic binary system of acetonitrile/water (90:10) at flow rate of 1 mL min<sup>-1</sup> (Jasim et al., 2015). Quantification of the diosgenin produced was calculated from the area of the peak using the equation according to previous study (Jasim et al., 2017).

### **Statistical Analysis**

Six tubers of *D. esculenta* was harvested and 1kg each was dried and heated powder was stored separately for 4°C, then analyzed each separated powder. All values are expressed as the mean  $\pm$  standard error (SE). The differences between groups were compared using the nonpaired Student's *t* test. Differences were considered significant when  $P < 0.05$ . Diosgenin contents with and without peel analysis was conducted with repeated-measures ANOVA. A post hoc comparison test was used to correct for multiple comparisons (Bonferroni test), when significant differences were observed. For ANOVA,  $p < 0.05$  indicated significance.

## Results

### *Nutritional Ingredients*

The moisture content tended to be higher tubers grown in the cold region within the plastic greenhouse. However, there was no significant difference between those cultivated in the sub-tropical region and those grown in the cold region within the plastic greenhouse (Fig. 1A). Ash and sodium contents were not significantly different between groups (Fig. 1B and C). The amounts of protein, fat, carbohydrate and energy were comparable and not significantly different between groups (Fig. 2A-D). The nutritional status of *D. esculenta* was analyzed, and with the exception of diosgenin content, no significant differences were seen in nutrient contents with peel and without peel.

## **Diosgenin Contents**

The diosgenin content for *D. esculenta* with the peel, analyzed after hydrolysis, was comparable for plants harvested in Okinawa (0.12 g/100 g) and for those harvested in the greenhouse in Kyoto (0.10 g/100 g; Fig. 3). Alternatively, diosgenin contents for tubers without the peel were significantly lower (0.03 g/100 g for plants grown in Okinawa and 0.028 g/100 g for those grown in Kyoto; Fig. 3) compared to those with the peel.

## **Discussion**

*D. esculenta* is normally cultivated in tropical/sub-tropical regions and is not suitable for cultivation in cold regions. However, it can be cultivated in cold regions where the minimum temperature continues under 20 °C, as it is in northern Kyoto, Japan, if grown in a plastic greenhouse. Moreover, comparison of the nutritional ingredients for *D. esculenta* did not differ between those cultivated in a sub-tropical region and those grown within a plastic greenhouse in a cold region. To the best of our knowledge, this is the first report that *D. esculenta* can be cultivated in a cold region within a plastic greenhouse and yield the same quality and similar nutritional ingredients as *D. esculenta* cultivated in a sub-tropical region.

In the present study, the moisture content in powdered *D. esculenta* tended to be higher in those grown in the cold region within a plastic greenhouse, and the sodium content was

slightly higher in those grown in the sub-tropical region. One of the reasons that tubers cultivated in Kyoto had higher moisture content was the difference of climate compared to Okinawa. Kyoto is surrounded by mountains in a natural amphitheater and the temperature variation between day and night is larger compared to the sub-tropical region. Furthermore, the months from May to July experience higher temperature and humidity, with temperatures elevated to approximately 35 °C. In contrast, the climate is dry and cold with substantial snowfall throughout December through February, especially in northern Kyoto where *D. esculenta* was cultivated in the present study. The climate variability and changes pose a threat to crop growth, yield, moisture content, and nutrients (Dwamena et al., 2021). Though a plastic greenhouse was used for *D. esculenta* cultivation in Kyoto, the minimum air temperatures during October through December were lower compared to Okinawa. Thus, the moisture content of powdered *D. esculenta* cultivated in the cold region within the plastic greenhouse was higher than for those cultivated in the sub-tropical region. Additionally, sodium content was lower for tubers cultivated in the cold region and owing to the cultivation location. As previously described, Kyoto is a montane basin, distant from the sea. Conversely, Okinawa is in southern island in Japan, surrounded by the sea. It may be that a higher soil sodium

187 content and local rainfall, affected the amount of sodium content in *D. esculenta* grown  
188 there, however, it needs to measure soil sodium content in both regions for future study.

189 *D. esculenta* also contains other nutritional ingredients such as vitamins and minerals.  
190 Previously, the effect of cooking on nutrients in *D. esculenta* have been demonstrated.  
191 Fresh *D. esculenta* tubers contained 16.7 mg/100 g and vitamin C which was reduced by  
192 heating, whether by boiling, steaming, or baking. Additionally, the contents of minerals  
193 such as magnesium, potassium, zinc, iron, and copper, also found in *D. esculenta*,  
194 remained constant when exposed to heat (Wanasundera & Ravindran, 1992). In our study,  
195 *D. esculenta* were dried with the peel at approximately 70-75 °C for 20 hours, then  
196 pulverized and heat-treated at temperatures over 200 °C. Therefore, the heat treatment  
197 would have reduced vitamin contents for both cultivated Okinawa and Kyoto plants. For  
198 this reason, this study included no measurement of vitamin and mineral content.  
199 Therefore, the vitamin and mineral contents of pulverized *D. esculenta* cultivated both in  
200 Okinawa and Kyoto should be compared.

201 Additionally, the diosgenin content was analyzed for pulverized powder with the peel  
202 and without the peel in the present study. The value of diosgenin was  $0.03 \pm 0.005$  g/100  
203 g in samples without the peel: about one-third less than the diosgenin content in samples

with the peel. Therefore, diosgenin was highly concentrated in *D. esculenta* peel (or between *epicarp* and *mesocarp*).

Moreover, *D. esculenta* included a higher level of steroidal saponins compared to other *Dioscorea* species; total steroidal saponins was 17.65 mg/g in *D. esculenta* compared with 42.15 and 17.44 mg/g in *D. cayenesis* and *D. rotundata*, respectively (Lebot et al., 2019). These *Dioscorea* species include large variation of steroidal saponins such as allantoin, dioscin, gracillin, protodioscin and protogracillin (Lebot et al., 2019). To analyze the diosgenin content, we extracted these saponins and removed glycoside by hydrolysis with hydrochloric acid. In future studies, other hydrolysis methods, as well as advanced measurements using high performance thin-layer chromatography (HPTLC) should be conducted.

## Conclusions

In conclusion, although *D. esculenta* is generally cultivated in tropical/sub-tropical climate regions, it can be cultivated in cold regions if a plastic greenhouse is used to protect the plants. Additionally, the nutritional values were similar between plants cultivated in the sub-tropical region, Okinawa and within a plastic greenhouse in the cold region, Kyoto. Further studies should compare other nutrients, as well as the difference in nutrients in samples with and without peel.

222    **Acknowledgement**

223    The study was supported by Kyoto Grain System Co., Ltd, Kyoto, Japan, and cultivation  
224    of *D. esculenta* was supported by LINK's group, Kameoka, Kyoto, Japan.

225    **Duality of Interests**

226    There is no conflict of interests in the present study.

227    **Author contribution**

228    K.S: conceived and designed the study, wrote the article. K.U and M.K: conducted data  
229    gathering

230

231    **References**

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300

301 Figure Legends

302 **Figure 1:** Moisture (A), ash (B) and sodium (C) contents in *D. esculenta* (expressed as  
303 mean  $\pm$  SE).

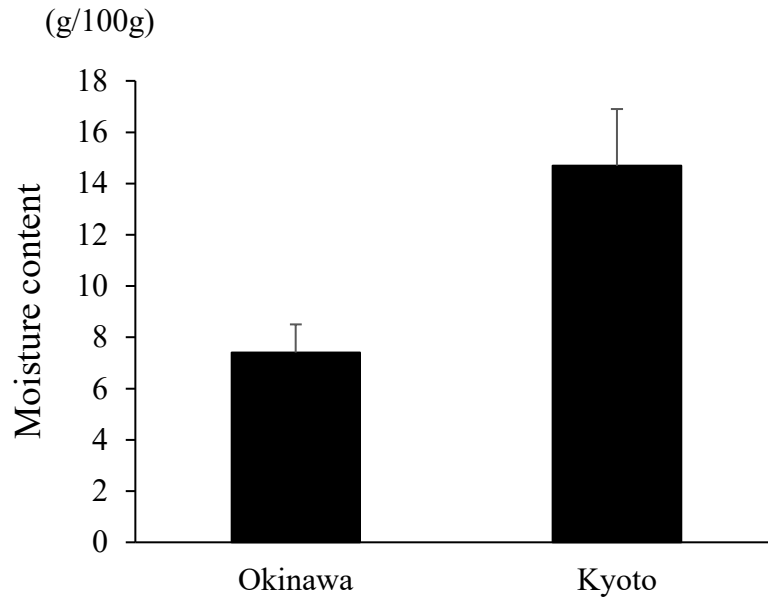
304 **Figure 2:** Protein (A), fat (B), carbohydrate (C) contents and energy (D) in *D. esculenta*  
305 (expressed as mean  $\pm$  SE).

306 **Figure 3:** Diosgenin content in *D. esculenta* with and without peel (expressed as mean  
307  $\pm$  SE). \*  $p < 0.05$  compared to without peel

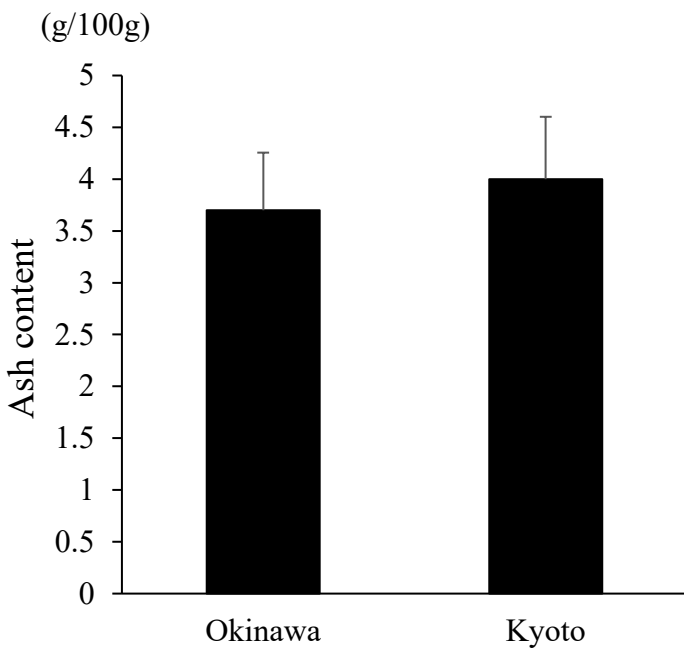
308

Figure 1  
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A



B



C

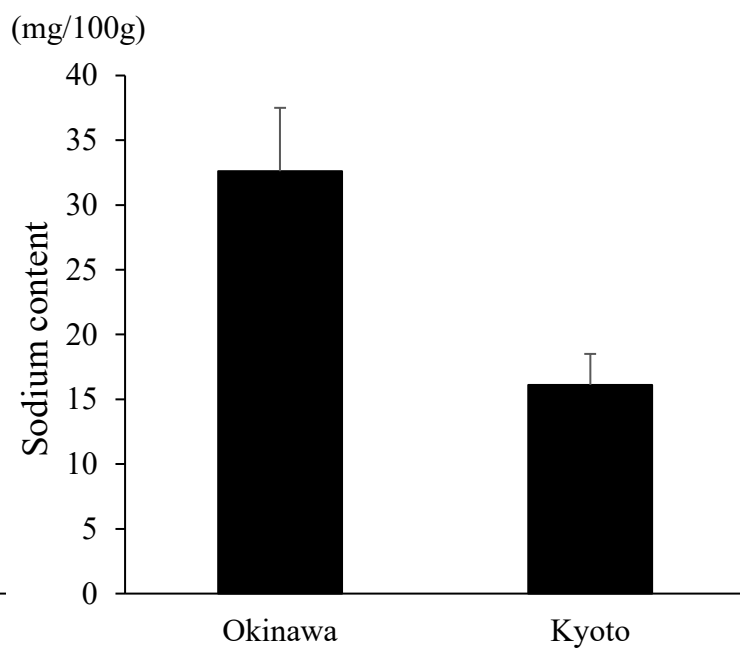


Figure 2  
Sato et al.

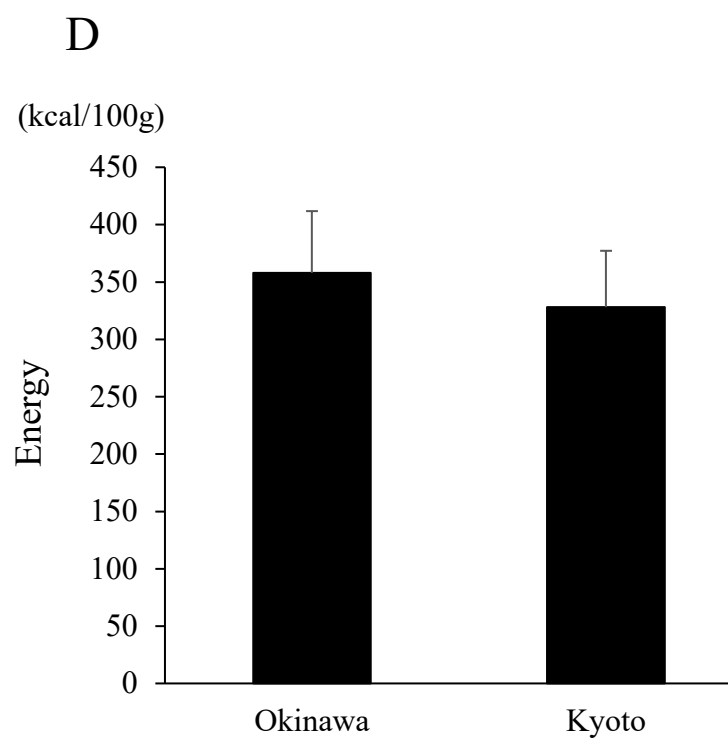
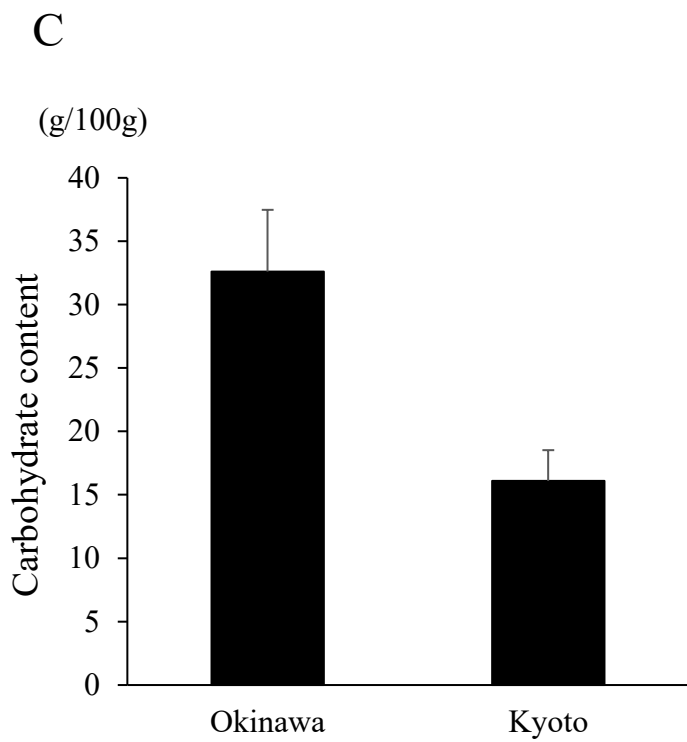
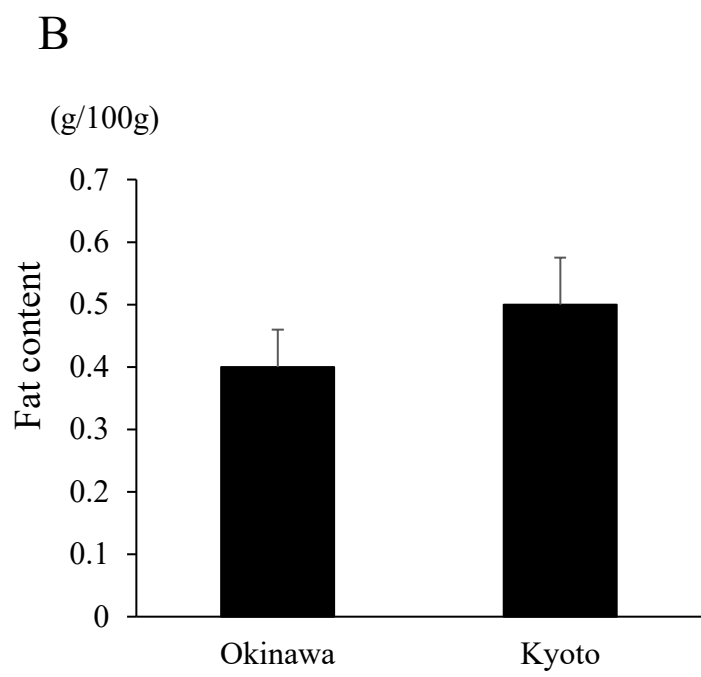
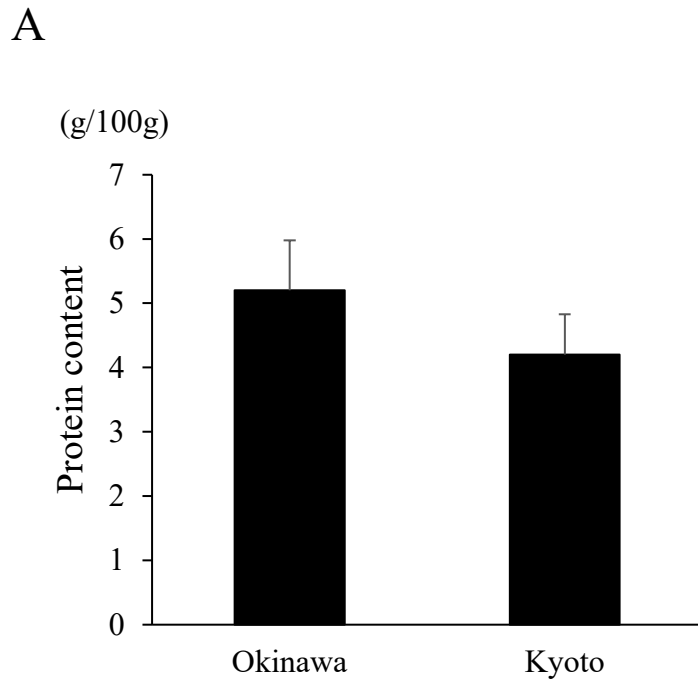


Figure 3  
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