



## **Morphological development and starch accumulation of Konara oak (*Quercus serrata*) Acorns as a wildlife food source**

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### **ABSTRACT**

Japanese beech trees are considered to have mast seeding habit. Their acorns are consumed by wild mammals and birds. As a food for wildlife, acorns are considered a nutritional source of non-structural carbohydrates, mainly starch. The purpose of this study was to verify the morphological development of acorns of *Quercus serrata* during maturation and compare amylose and amylopectin contents as a major component of starch in the foraged seed embryo. Acorns were collected at two different sampling sites, Okutama and Ome, which are located at different altitudes. At the maturation, acorn size (major axis) and embryo weight (on a dry matter basis) at the sampling sites, Okutama and Ome, ranged from 2.13cm to 2.47cm and from 0.57g to 0.63g, respectively. Starch content in the acorn embryo was higher at Ome (40.4%) than at Okutama (30.2%) at the maturation stage. The percentage of amylopectin and amylose contents in the embryo were 52.4% and 47.6% at Okutama and 67.0% and 33.0% at Ome, respectively. Thus, the amounts of amylopectin and amylose in the acorn embryos were higher for the samples from Ome (252.2mg) than those from Okutama (174.0mg). Consequently, the present study showed that the profiles of the acorn development process were similar at both sampling sites, while starch accumulated as amylopectin and amylose in the acorn embryos of *Quercus serrata* differed between the sites. All our results are useful for understanding acorn development through its growth period and the nutritional quality of starch in acorn embryos as a food for wildlife. Furthermore, the extent of amylose and amylopectin accumulation in the embryo might be affected by the environmental factors where the mother trees grow.

**Key words:** Acorn, Amylopectin, Amylose, *Quercus serrata*, Starch

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### **INTRODUCTION**

Japanese beech trees (*Fagus crenata*), such as water oak (*Quercus mongolica*) and Konara oak (*Quercus serrata*), produce acorns in autumn. Foraged acorns are an important food for wild animals such as birds, rodents, monogastric mammals and ruminants [1 and 2]. Rodents have been observed collecting and stocking acorns in their nests, and sometimes burying them in the ground. Bears eat many acorns when accumulating body fat prior to hibernation. Earlier studies investigated removal and predation of acorns as a consumption activity by wildlife and the reproductive response of mother trees. Little research has been conducted on acorn development through seed growth and the nutritional density of acorn proteins, fats and carbohydrates.

The yield of acorns is periodical difference of two or three year intervals known as mast seeding [3-6]. For example, acorn production of *Fagus crenata* in the Tanzawa mountains, Kanagawa prefecture, Japan, was abundant in 2000, 2003 and 2006 [7]. Cyclical mast seed production is considered an important reproductive strategy for overcoming

predation by harmful insects, thus enabling mother tree species is to survive. In successful seed production years, a mother tree of *Fagus crenata* in Toshima peninsula in Hokkaido supplied 250-750kg acorns per year [8 and 9].

As human food, acorn embryos are known to be rich in carbohydrates (mainly starch) and poor in protein and fat [10 and 11]. However, there is insufficient published data about the nutritional characteristics of acorns as a food for wild animals. In addition, starch is chemically divided into amylose and amylopectin, which are the main components of carbohydrates in acorn embryos [10]. These components accumulate in the acorn embryo during its growth and maturation as a result of photo-synthesis by the mother tree. The extent to which these non-structural carbohydrates accumulate in acorns is affected by environmental conditions where mother trees grow [12]. Furthermore, it is suggested that higher deposits of amylose in acorn embryos could lead to improved tolerance for freezing conditions in winter and successful germination in the following year [13 and 14].

The goal of the present study was to compare the morphological development of acorns of *Quercus serrata* during the maturation period in the two different sampling sites, Okutama and Ome, and the accumulation pattern of amylose and amylopectin in the acorn embryo as a major component of starch.

## MATERIALS AND METHODS

### *Sampling sites and collection of acorns*

Konara (*Quercus serrata*) acorns were collected in two geographically different places at sea level. The first sampling site called Okutama, is a local mountainous area near the artificial lake Okutama. It is located at about 535m. The second site called Ome, is part of plains area near the Tokyo Metropolitan Agriculture and Forestry Research Center in Tokyo. It is located at about 162m. At each sampling site, three to five Konara (*Quercus serrata*) mother trees of similar diameter (measured at breast height) were selected for study. The development of their acorns from the beginning of flowering (the middle of June) to the end of the maturation period (the end of September) was observed at about two week intervals. Acorns were collected at least 30 samples each in both sites through the all sampling times and measured fresh weight and size (length of major and minor axis), and then stored at -20°C. In addition, as the anatomical structure of acorns in our experiment was not clearly distinguished each parts until early September, therefore, fresh weight and size were recorded as a whole seed, and then after we divided the seed into the parts of cupule, pericarp and embryo, respectively. All these parts were also stored at -20°C until analyzed.

### *Chemical analysis and measurement of gross energy content*

Stored samples were dried in a forced-air oven at 70°C for 2 to 3 days, ground through a 1mm screen in a Wiley Mill, and stored in sealed bottles until analyzed. The moisture content of each sample was calculated by determining the difference of weight loss after drying. Crude protein and fat contents were determined in duplicate by the Kjeldahl method and ether-extracts procedure according to the AOAC [15], respectively. Amylose and amylopectin contents were analyzed using an amylose-amylopectin assay kit purchased from Megazyme International Ireland Ltd, Wicklow, Ireland. The gross energy content of each sample was measured by the bomb-calorie meter (IKA C-5000, IKA Japan, Co. Ltd.).

### *Statistical analyses*

All data were analyzed statistically by the analysis of variance and statistical (significant) differences between two sampling sites were determined by the Student's t-test. The difference between mean values and among variants was calculated using the least significant difference (LSD) method with a 5% level of significance [16].

## RESULTS AND DISCUSSION

### *Morphological development of acorn*

At the first sampling period in this experiment the acorn size measurements for both the major and minor axis were similar. This similarity continued to for nearly one month (Figure 1).

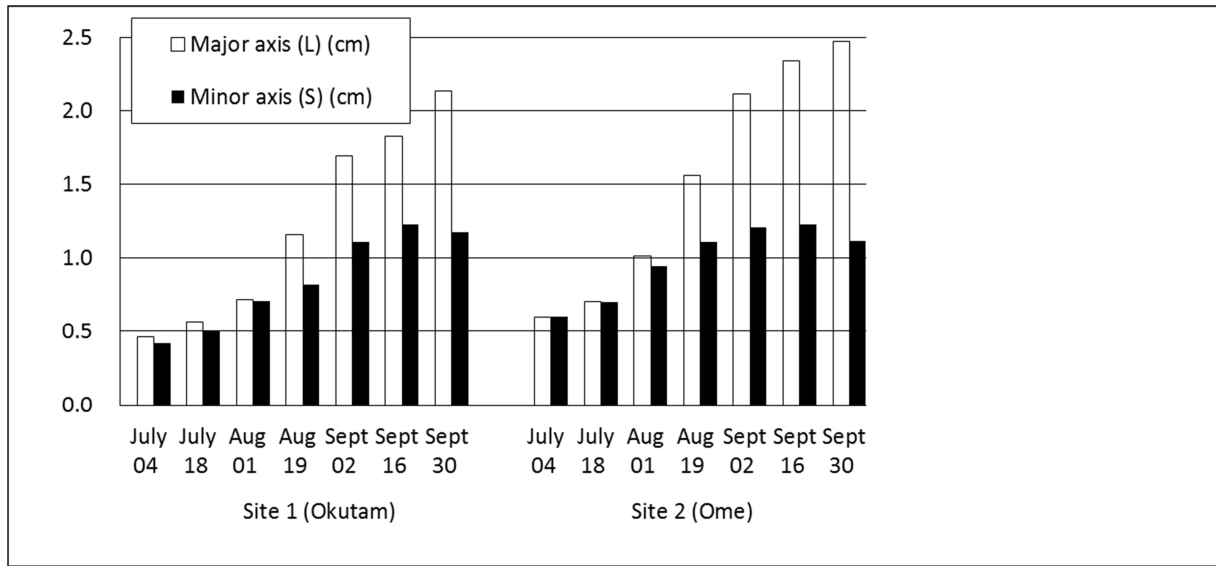


Figure 1. Changes in major and minor axis of acorns of *Quercus serrata* during maturation at Okutama and Ome

After that, the size of the major axis increased linearly and reached a maximum of 2.1cm at the Okutama site and 2.5cm at the Ome site at the maturation period. Seed weight also increased rapidly after the middle of maturation period and the individual morphological structures of the acorns were anatomically distinguishable as cupule, pericarp and embryo (Figure 2).

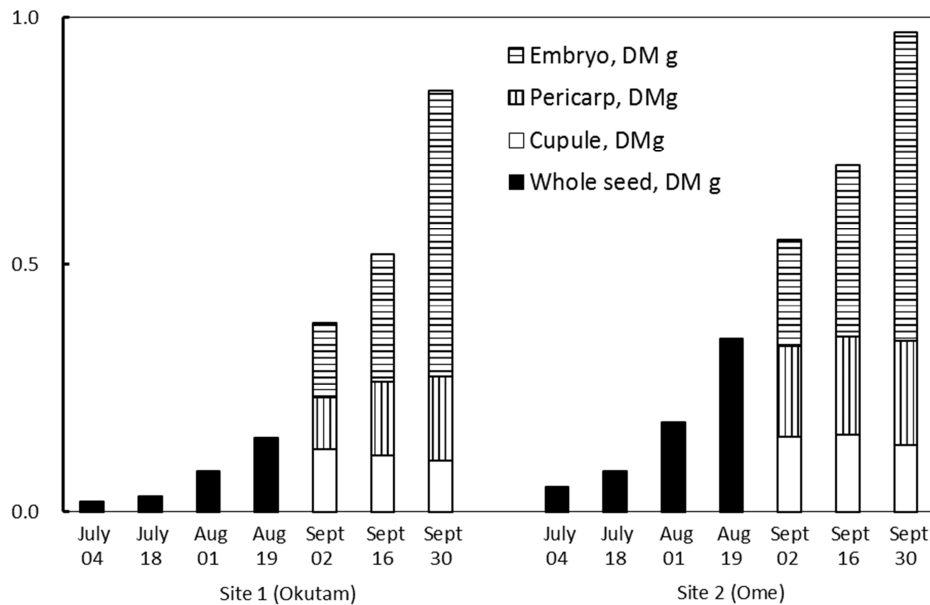


Figure 2. Changes in weights of acorns and major organs of *Quercus serrata* during maturation at Okutama and Ome

Although the weights of the cupule and pericarp remained constant through the seed growth period at both sampling sites, the weight of the acorn embryo was 0.58g (dry matter) at the Okutama site and 0.62g at Ome site at maturation. There were no statistical differences between each measurement at both sampling sites because the variance of each replicant value was very small.

Size and weight of matured acorns, in general, differs among *Quercus* tree species in eastern North America [17] and in Japan [18]. The acorn of *Quercus liaotungensis* in China is ovoid, 1.2 – 1.5cm wide and 1.7 – 1.9cm long, and 2-3g (raw weight basis) [19]. Iwabuchi *et al.* [20] also showed the morphological variation of acorns of *Quercus*

*serrata* collected at a different altitudes (60-1100m above sea level) in central Japan and suggested that acorn traits were correlated negatively with altitude and precipitation during the acorn growing season, and positively with the Warm Index [21] and annual mean temperature. This means, the higher the precipitation and the lower the Warm Index, the smaller the size and volume of acorns. In our experiment we had no environmental data for the sampling sites, but acorn size (major axis) and weight were slightly smaller in Okutama compared with Ome. Although in our experiment the locations of the two sampling sites had different altitudes, determining the environmental factors affecting morphological development of acorns at the two sampling sites would require further study. Suda *et al.* [22] defined the morphological traits of acorn as the ratio of the major axis measurement to diameter and showed the value of 1.27 to 2.17 for *Quercus serrata* tree species. Our data followed this result and it will be possible to consume acorns not only for large mammals (deer, wild boar and bear) but also for small mammals (rodents) and birds for their food.

#### **Chemical composition and energy content of acorn**

When the size of sampled acorns was small and not divided anatomically into each organ (cupule, pericarp and embryo) during immature stages, chemical components and energy content were analyzed as a whole seed (Table 1.). The crude protein content of whole seeds ranged from 3.4% to 7.1% on a dry matter basis at the Okutama site and from 1.8% to 6.2% at the Ome site, respectively. Crude fat content of whole seeds was below 1% at both sites. The starch content of whole seeds was also a constant 5% in immature stages. The gross energy content of whole seeds ranged from 17.6kJ/DM g to 18.9kJ/DM g. These values were similar at immature stages at both sampling sites.

**Table 1. Chemical composition and energy content of acorns of *Quercus serrata* at the two experimental sites, Okutama and Ome**

Sampling date	Part	Fat (DM %)	Protein (DM %)	Starch (DM %)	Energy (kJ/DM g)
Site 1 (Okutama)					
July 04	Whole	1.0	5.1	3.6	18.1
July 18	Whole	0.8	7.1	4.3	18.6
Aug 01	Whole	0.4	6.5	4.1	18.7
Aug 19	Whole	0.6	3.7	5.0	18.9
Sept 02	Whole	0.5	3.4	9.6	-
	Cupule	0.4	3.8	1.3	19.3
	Pericarp	0.9	3.2	3.5	18.7
	Embryo	0.8	3.8	34.8	16.4
Sept 16	Whole	0.8	3.6	15.0	-
	Cupule	0.3	3.7	1.3	19.2
	Pericarp	0.5	4.8	3.2	18.7
	Embryo	0.7	2.1	21.1	16.5
Sept 30	Whole	1.4	4.1	21.3	-
	Cupule	0.5	3.3	1.2	19.3
	Pericarp	0.6	4.4	3.3	19.2
	Embryo	1.8	4.1	30.2	16.8
Site 2 (Ome)					
July 04	Whole	0.8	1.8	4.4	18.2
July 18	Whole	0.6	6.2	3.6	18.7
Aug 01	Whole	0.5	5.8	4.5	18.4
Aug 19	Whole	0.6	4.9	4.8	17.6
Sept 02	Whole	0.8	4.5	6.9	-
	Cupule	0.6	1.2	1.1	19.6
	Pericarp	0.8	7.1	3.0	18.7
	Embryo	0.9	4.8	14.3	16.6
Sept 16	Whole	0.9	3.9	18.8	-
	Cupule	0.5	2.1	1.3	19.6
	Pericarp	1.0	4.7	4.3	19.1
	Embryo	1.1	4.2	35.0	16.6
Sept 30	Whole	1.2	2.4	27.4	-
	Cupule	0.5	2.7	1.5	19.6
	Pericarp	0.8	4.6	5.4	19.2
	Embryo	1.5	1.6	40.4	16.5

At the maturation period, the end of September in this experiment, crude protein and fat contents were slightly greater, but no significant difference among those components was found. Starch contents in acorn embryos increased with seed growth and maturation, and the values were significantly higher than that in the cupule and

pericarp ( $p < 0.05$ ). Starch content of the embryo was also significantly different between the sampling sites. It was higher at Ome (40.4%) than at Okutama (30.2%) ( $p < 0.05$ ).

The general composition of sweet acorns and chestnuts as a human food are summarized in the Standard Tables of Food Composition in Japan [23]. The protein, fat and carbohydrate contents are in ranges of 2.8-3.2%, 0.5-0.8% and 36.9-57.6, respectively. The ingredients of acorn embryos of *Quercus serrata* in Kagawa prefecture [24] was 4.3% protein, 3.1% fat and 9.4 kJ/DM g, respectively. Protein and fat contents of acorn embryos collected from the two experimental sites were higher than protein and fat contents found by previous researchers. Therefore, the energy contents of acorn embryos from our two sample sites were about 1.8 times greater. Our results show that acorn embryos of *Quercus serrata* are nutritious, especially rich in carbohydrates (starch) and energy, and are good food sources for wild animals.

#### **Amylose and amylopectin content in the acorn embryos**

At the maturation period, the starch contents of acorn embryos were 174.0mg at Okutama than 252.5mg at Ome, and they were significantly different ( $p < 0.05$ ) (Figure 3). The percentages of amylose and amylopectin to the starch contents were 47.6% and 52.4% at Okutama and 33.0% and 67.0% at Ome, respectively. Consequently, the amylopectin content of the acorn embryos was higher than that of amylose at both sampling sites, while the weight ratio of amylose to amylopectin contents was calculated as 0.91 at Okutama and 0.49 at Ome ( $p < 0.05$ ).

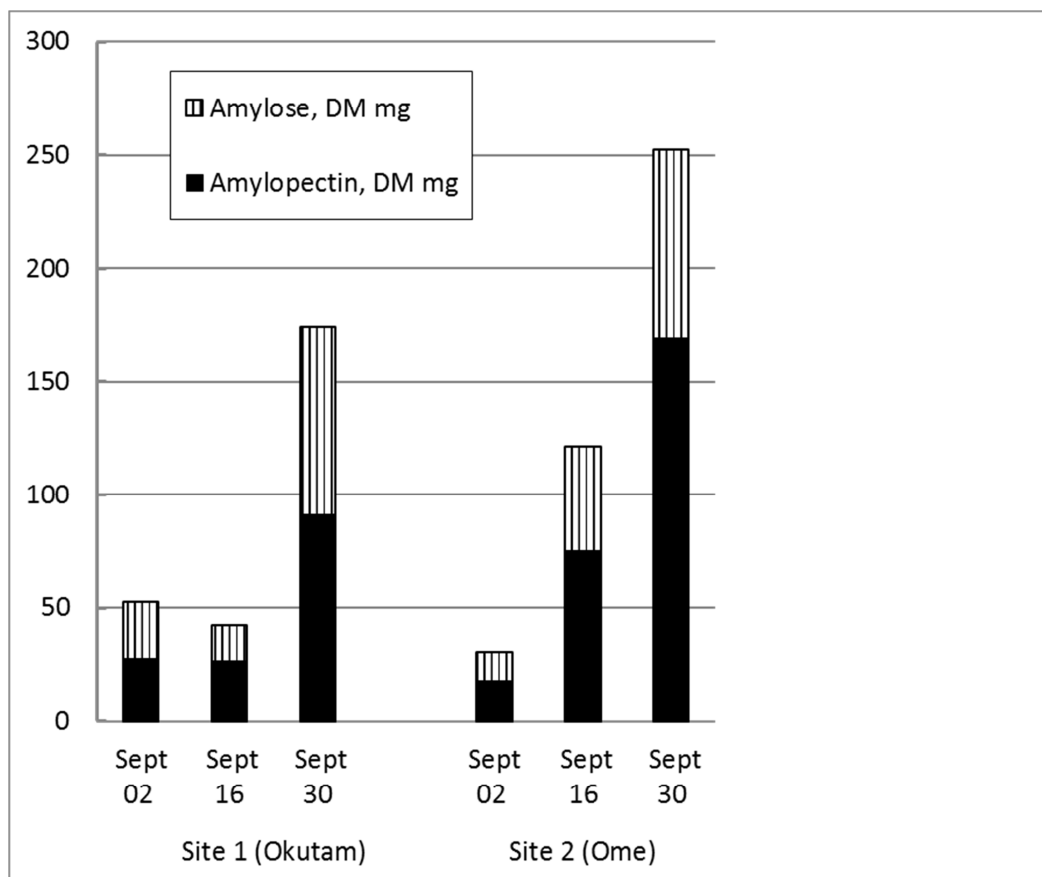


Figure 3. Changes in amylose and amylopectin contents in the acorn embryo of *Quercus serrata* at Okutama and Ome

Starch is a major preserve polysaccharide of green plants. Acorn embryos, the seed of the *Quercus* tree species, is one such source of starch that is important food of energy for wildlife. In our experiment, at the Ome sampling site, starch accumulation in acorn embryos was rather stable until the middle of acorn development and increased rapidly at the end of the maturation period. At Okutama, starch accumulation increased at the middle phase of seed growth

and reached maximum quantities at the end of the maturation period. The amount of starch in acorn embryos was also different between the two sampling sites.

Furthermore, the ratio of amylose and amylopectin contents in the acorn embryos between the two sampling sites was not similar in this experiment.

Stevenson *et al.* [10] analyzed the acorn starch of pin oak (*Quercus palustris* Muenchh.) collected in October and reported that the acorn starch content was 17% (dry weight basis). Although this result was considerably lower than the 55% [25] and 59% [26] reported for other *Quercus* species, the amylose content of acorn starch was 31.4%. Yoo *et al.* [27] (2012) compared the proximate composition of starches from chestnuts (*Castanea crenata*) and acorns (*Quercus acutissima*), and showed that amylose content was 29.5% and 30.6%, respectively. These results were similar to our data for Ome, but were considerably different for Okutama.

Although amylose and amylopectin are glucose homopolymers and composed starch molecules, the chemical structure of amylose is linear  $\alpha$ -1,4 glucan and that of amylopectin is  $\alpha$ -1,4 glucan with  $\alpha$ -1,6 glucan. These compositional differences were also interested in freezing resistance of woody plant. Sakai [13] pointed out that the content of carbohydrates such as starches increased with the decreasing temperature of late autumn and that this response might prevent the freezing of plant organs. In other words, a higher accumulation of amylose in the acorn may be related to an increased tolerance for freezing temperatures and may maintain the germinability of the embryo. However, the properties of acorn starches may also be affected by the botanical conditions and environmental factors where the mother tree grows.

## CONCLUSION

We have verified the morphological development of acorns of *Quercus serrata* during the maturation period in the different sampling sites, and the accumulation pattern of amylose and amylopectin in the acorn embryos. Highlights of the study are following; (1) Morphological development in size and weight of acorns had increased rapidly at the end of maturation period and this tendency was similar at the both sampling sites. (2) Starch composition of acorns mainly consisted of amylose and amylopectin, but the content of amylopectin was slightly greater than that of amylose. (3) Our results show that acorn embryos of *Quercus serrata* are nutritious, especially rich in carbohydrates (starch) and energy, and are good food sources for wild animals.

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