# Presence of Tyloses in 6 Korean Oak Species for Production of Liquor Barrels and Correlation of Retention of Large Vessel Lumen

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This study was conducted to devise ways of promoting the use of deciduous trees that have increased in number with climate change. For six oak species commonly utilized to produce liquor barrels, normal wood and tension wood were classified by part. Then, both large vessel lumen area and tyloses were quantitatively assessed. The findings showed that in both normal and tension woods, large vessel lumen area was largest in sapwood, followed by heartwood and juvenile wood. Tyloses were found in both normal and tension woods of all the six species, but the number of tyloses varied according to species. It was heart wood that contained the highest amount in both normal and tension woods. It was also revealed that the number of tyloses decreased with the growing area of large vessel lumen. This study will provide fundamental data to decide oak tree species for the production of liquor barrels.

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#### INTRODUCTION

In the past, Korean alcohol was made from rice and liquor, but it began to open its eves to Western traditional liquor as it opened the door to the world. For instance, the domestic wine market has increased by more than 10% annually since 1987 (You 2013). Traditional Western spirits are mainly made from grains, potatoes, and fruits, and mostly produced by aging in oak barrels. When alcohol is matured in an oak barrel, the unique chemical ingredients of wood combine with the alcohol to form the characteristics of traditional liquor. Volatile ingredients and phenolic compounds change the flavor of wine, improving its overall quality (Cerdán et al. 2002). However, not all oak barrels have the uniform number of compounds. They all have different characteristics depending on the production area and environment (Frangipane and De Santis 2007), which play a major role in the characteristics of oak barrels. Not all timber can turn into these barrels. The name "Oak Tong," a Korean catch-all term for wooden barrels, is associated with the characteristics of oak trees. Wood is a porous material that has an intracellular pathway for transporting water and nutrients. Ordinary wood therefore tends to be leaky. Thus, only certain species have been used as liquor casks, where structures that block the fluid passage develop. The common reason why liquor containers are called "oak tong" is that they are made from oak trees.

Oak trees have 1 to 3 rows of vessels that transfer water into these cells. The wood also consists of fluid cells and wood fibers. The aforementioned structure is key to the development of tyloses in a vessel. A condition known as tylosis is produced when parenchyma cells burst open as they are in the process of entering into lumen spaces. According to the literature, cell walls of tyloses of oak trees are divided into primary and secondary walls, whose thickness ranges from 0.3 to 0.5 µm for the primary and 1.4 to 2.6 µm for the secondary (Park et al. 2006). Some species possess only primary walls (Lidija 1975). Subspecies of oak trees range from red oak to common white oak. Other species include ash (Fraxinus rhynchophylla) and chestnut (Castanea crenata), and the number of tyloses in them varies. Also, sparkling properties associated with tyloses can be observed in some species such as the black locust tree (Bruce 1990). Previous studies have identified that tyloses develop when the wood is transformed into heartwood, but for domestic mongolica oak and acacia trees, tyloses are also formed in infancy during the period when age formation is active. Sayaka et al. (2013) found that tyloses emerge in the sapwood of acacia trees but depends on the moisture content of the outermost part of the sapwood, which is active when the moisture content of the vessel is low. In other cases, tyloses may also occur in branches that have not developed into heartwood when sawtooth oak is used as materials for construction, ships and liquor barrels (Lee and Park 2009). Further research on how tyloses are generated is needed.

Tyloses in wood affect its usage. Tyloses in the vessels are produced to prevent substances from leaking. This wood could then be utilized as a vodka or wine barrel. On the other hand, tyloses impede the flow in vessel lumen, thus prolonging the time for woods to dry. It may also cause defects during such processes. Chang *et al.* (2017) argue that the absence of tyloses facilitates the drying process despite relatively poor conditions for drying compared to the conventional conditions regarding oak trees.

Deciduous tree species are spreading across South Korea with the acceleration of climate change. Oak tree species account for the largest share of the deciduous tree species, implying the importance of identifying appropriate uses of the species. In this study, therefore, six oak species most common in South Korea were chosen including Quercus mongolica, Quercus dentata, Quercus acutissima, Quercus serrata, Quercus aliena, and Quercus variabilis. All samples were collected from the six species in Yeongdong, Chungcheong province. The presence and the number of tyloses and correlations between lumen area of large vessel and tyloses in the samples were analyzed. Kim (2011) reported a difference in dynamic characteristics between normal wood and tension wood of Quercus *mongolica*, arguing that the reason for the difference lies in anatomical differences between the two types. South Korean forests also have the peculiarity that they are sloped (on average 5.7°), thus affecting the proportion of tension wood and the way that tension wood grows. Wood is classified as tension wood when the pith axis of hard wood that has grown in a slope becomes inclined to the sloped direction and the growth rings of the wood grow in an asymmetrical way around the pith. It is therefore common that tension wood can be observed in South Korea. It is against these backdrops that not only normal wood but also tension wood of the samples were examined in this study. It is expected that this study will provide fundamental data to select species when utilizing oak trees.

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# EXPERIMENTAL

### Materials

Samples of tension wood, which was identified according to the width of growth rings and the pith position among all cross-section samples, were sorted out. After sorting out normal and tension woods from the six oak species that grew in Yeongdong, North Chungcheong province, the cross-sections were divided into juvenile wood, heartwood, and sapwood (Table 1). Juvenile wood samples were obtained from parts between the pith and the tenth growth ring. The criterion to obtain the juvenile wood samples was based on Hwang (2002), saying that parts between the pith and the thirteenth growth ring can be considered as juvenile wood according to his analysis of vessel lumen area.

Table 1. Specimen	and Number of Measurements
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Tree Species (English Notation, Sample ID)	Number of Samples for normal Wood	Number of Samples for Tension Wood	
Quercus aliena BI. (Galcham oak: Go)	12	12	
Quercus variabilis Blume (Oriental cork oak: Oo)	12	12	
Quercus serrata Thunb. ex Murray (Jolcham oak: Jo)	12	12	
Quercus acutissima Carruth. (Swatooth oak, So)	12	12	
Quercus dentata (Korean oak: Ko)	12	12	
Quercus mongolica Fisch. ex Ledeb. (Mongolica oak: Mo)	12	12	

#### Methods

#### Sample slice production for large vessel observation

Preparations for the observation of tyloses were made. Each *Quercus* spp. was collected from each cross-section part, cut into  $10 \times 10 \times 10 \text{ mm}^3$  dimensions. It was then softened in a solution of distilled water and glycerin at a ratio of 1:3. The slices stayed in the solution for an average of 2 to 3 days.

The softened samples were cut with a cross-section with 20- to 30  $\mu$ m thickness. Next, the slices were dyed in 1% solution of safranine (SAMCHUN/Daejung/Korea), and were dehydrated sequentially with alcohol of varying potency (30, 50, 70, and 99.9%). The last step in the preparatory stage was to apply paraffin. Two preparations of cross-sections were made according to the type of wood (normal and tension wood) and parts (juvenile wood, heartwood and sapwood) to estimate the lumen area of large vessel and observe the distribution of tyloses in a total of 50 large vessels. Statistically significant differences in the observed values of the six species were tested. The difference tests were made through F tests and T tests at a level of 0.05 in a software, Excel (Microsoft, USA).

#### Identification of tyloses in large vessels and the estimation of large vessel lumen area

The presence of tyloses in a large vessel was identified on a cross-section, and the lumen area of the large vessel was measured (Fig. 1). Thirty large vessels were examined for each region of juvenile wood, heartwood, and sapwood. The total number of vessels was determined and the number of tyloses was indicated as a percentage.

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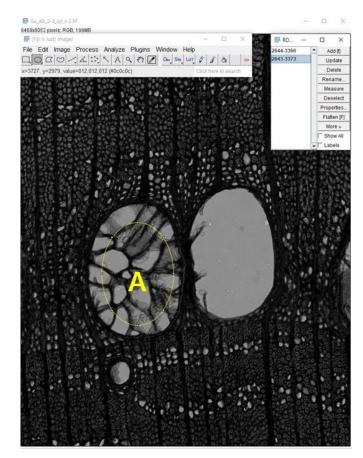


Fig. 1. Method to measure tyloses (Image J/JAVA, A: Tyloses)

#### **RESULTS AND DISCUSSION**

#### Estimation of Large Vessel Lumen Area per Species

The amount of water transported depends on the large vessel lumen area of oak trees. Ring porous woods, such as oak, are known to form large vessels in their early wood with few leaves, thus finishing the formation of cells within around 24 days (Sass et al. 2011). Therefore, late winter moisture stored in the soil is an important factor when large vessels are created (Jeong et al. 2017). Among the oak trees in this study, the large vessel area of normal wood was most abundant in sapwood, which was followed by heartwood and juvenile wood. Tension wood produced similar results. It was discovered that the large vessel area of juvenile wood of normal wood was on average 51572  $\pm 15532 \ \mu m^2$ . The average area of heartwood was 90154  $\pm$ 21021  $\mu$ m<sup>2</sup>, while that of sapwood was 101732  $\pm$  $35193 \ \mu\text{m}^2$ . Regarding tension wood, on the other hand, the area of juvenile wood was  $51259 \pm 11680 \ \mu\text{m}^2$ . That of heartwood was  $90552 \pm 26380 \ \mu\text{m}^2$  while that of sapwood was 94974  $\pm$  23852  $\mu$ m<sup>2</sup>. The followings are orders of tyloses from largest to smallest, sorted by species, type, and conditions of the wood (Table 2). For juvenile wood of normal wood, the order was Q. servata > Q. acutissima > Q. variabilis > Q. dentate > Q. aliena; for heartwood of normal wood, the order was Q. aliena > Q. acutissima > Q. variabilis > Q. serrata > Q. dentate > Q. mongolica; for sapwood of normal wood, the order was Q. variabilis > Q. acutissima > Q. variabilis > Q. dentate > Q. aliena > Q. mongolica; for juvenile wood of tension wood, the order was Q. serrata > Q. variabilis > Q. acutissima > Q. dentate > Q. mongolica > Q. aliena; for heartwood, the order was Q. variabilis > Q. serrata > Q. acutissima > Q. dentate > Q. mongolica > Q. aliena; for sapwood, the order was Q. acutissima > Q. serrata > Q. variabilis > Q. mongolica > Q. aliena; for sapwood, the order was Q. acutissima > Q. serrata > Q. variabilis > Q. mongolica > Q. aliena > Q. dentata.

Few marked characteristics were found in the type and conditions of the species. Statistically significant differences were revealed in all the three areas of both the normal wood and the tension wood. The size of the compositional cells in wood determines the material of wood (Zhang 1997). Therefore, it is determined that there is a difference in the material of normal wood and tension wood even if they are of the same species.

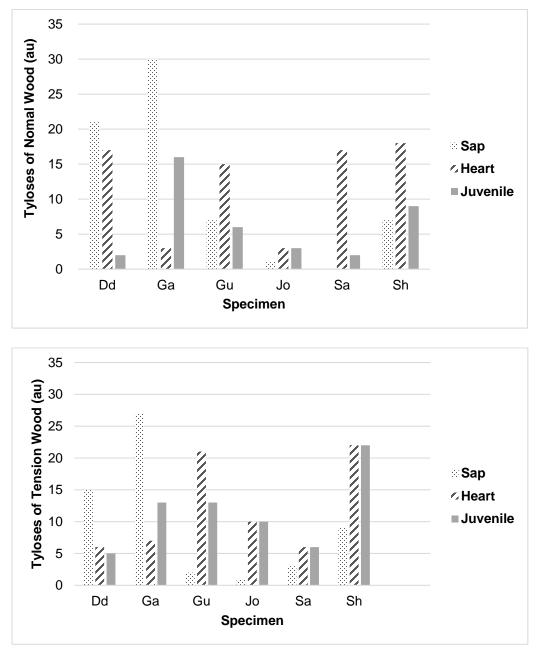


Fig. 2. The number of tyloses in normal wood and tension wood

No.	Normal Wood		Tension Wood			
	Juvenile	Heart	Sap	Juvenile	Heart	Sap
Go	33998	100961	77210	30467	67295	76772
	(± <sup>*</sup> 3303)	(± 17550)	(± 14068)	(± 7296)	(± 12141)	(± 17384)
Oo	47890	93262	102342	58676	105191	99193
	(± 9335)	(± 11654)	(± 10788)	(± 16458)	(± 15562)	(± 20281)
Jo	70356	84740	112834	60533	86710	99330
	(± 11426)	(± 18587)	(± 10736)	(± 12068)	(± 13815)	(± 19993)
So	61046	94407	104707	58100	82484	99347
	(± 9056)	(± 11720)	(± 15875)	(± 5844)	(± 7599)	(± 16100)
Ко	46816	73017	86370	55284	74246	74510
	(± 6978)	(± 6971)	(± 9323)	(± 7594)	(± 17571)	(± 21386)
Мо	33639	64552	65187	44493	72721	81690
	(± 3303)	(± 8469)	(± 6313)	(± 9874)	(± 13918)	(± 10008)

### Table 2. Lumen Area of Large Vessel (Unit: µm<sup>2</sup>)

#### Analysis and Quantitative Assessment of Tyloses by Species

Tyloses are generated when the wood becomes not just lignified but also damaged (Kwon *et al.* 2020). Tyloses also begin to emerge between May and June and the amount grows until the autumn (Gonzalo *et al.* 2018). In the present work, tyloses were observed in juvenile wood, heartwood, and sapwood. As for the normal wood, it was heartwood that had the highest number of tyloses, followed by sapwood and juvenile wood. Likewise, tyloses concerning the tension wood were largest in heartwood but bigger in juvenile wood than in sapwood.

The results by species showed that in the normal wood, *Quercus aliena* had the largest tyloses, followed by *Quercus dentata*, *Quercus mongolica*, *Quercus variabilis*, *Quercus acutissima*, and *Quercus serrata* (Fig. 2). In contrast, for the tension wood tyloses were highest in *Quercus mongolica*, followed by *Quercus aliena*, *Quercus variabilis*, *Quercus dentata*, *Quercus serrata*, and *Quercus acutissima* in order (Fig. 3). It can be argued that tyloses exist in all the species, with no clear differences between species, conditions, and parts. Tyloses are generated even in drought or frost. More embolized stems arise from drought and frost (Hervé and Melvin 1990). Thus, it can be assumed that the number of tyloses is likely to be affected by its surroundings.

#### Tyloses Existence Analysis by Large Vessel Lumen Area

The large vessel lumen area depends on the rainfall a year before it is formed, and the flow amount of nutriment through the area is determined by the area size (Jeong *et al.*, 2017). A test of significant differences between species (Table 2) revealed no distinct trends, despite the differences between some species. Analysis of a relationship between the large vessel lumen area and the existence of tyloses showed that a larger vessel lumen area resulted in the smaller number of tyloses. In contrast, an inverse trend was found in heartwood of the tension wood. According to previous research, no correlations are identified between the diameter of large vessels and the number of tyloses (Gonzalo *et al.* 2018). Far more samples are needed in further research to establish the correlations.

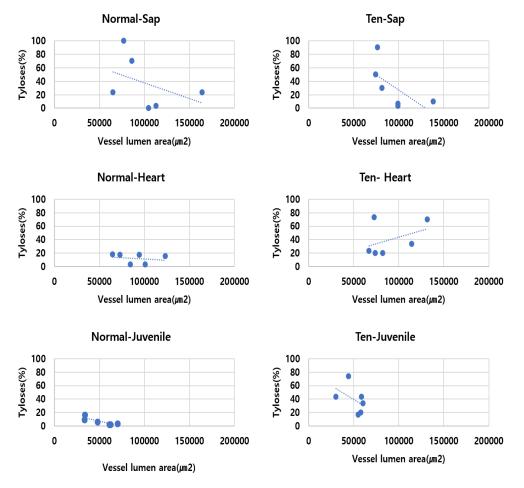


Fig. 3. Relationships between vessel lumen area and tyloses

# CONCLUSIONS

This study was conducted to devise ways of expanding the use of deciduous tree species that are gradually dominating South Korean forests. To this end, six oak species that are commonly used to produce liquor barrels in the country were selected. Then, normal wood and tension wood were sorted out from the samples. Next, they were also divided according to three parts including juvenile wood, heartwood and sapwood. The presence and the number of tyloses, as wells as the lumen area of large vessels were estimated.

- 1. It was revealed that all the six oak species that are most common in South Korea are white oak and they show the normal type of tyloses.
- 2. In both normal and tension woods, the large vessel area was biggest in sapwood, followed by heartwood, and then juvenile wood. Significant differences were found between all the given subjects. It can thus be assumed that quality differences would exist between the normal and tension woods even in an identical species.

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- 3. The difference in the number of tyloses between species was discovered despite their presence in both the normal and tension woods of the six oak species. As for normal wood, it was heartwood that formed the largest number, followed by sapwood and juvenile wood. Regarding the tension wood, it was also heartwood that had the biggest number of tyloses, but the number was larger in juvenile wood that in sapwood. Then, a comparison of the number of tyloses between species revealed that, in the normal wood, *Quercus aliena* BI. had the largest tyloses, followed by *Q. dentata*, *Q. mongolica* Fisch. ex Ledeb., *Q. variabilis* Blume, *Q. acutissima* Carruth., and *Q. serrata* Thunb. ex Murray. In contrast, for the tension wood tyloses were highest in *Q. mongolica* Fisch. ex Ledeb., followed by *Q. aliena* BI., *Q. variabilis* Blume, *Q. dentata*, *Q. serrata* Thunb. ex Murray, and *Q. acutissima* Carruth.
- 4. The analysis of the relationship between the large vessel lumen area and tyloses showed that tyloses decreased with the growing area. Yet, the inverse tendency was observed in heartwood of the tension wood, thus meaning that species are not attributable to the difference. Therefore, for the relationship to be determined, far much larger samples are needed in further research.

It is expected that the results this study proposes will be used as fundamental data to determine species when utilizing oak species that are spreading in South Korea with climate change.

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