Qualitative Anatomical Characteristics of the Virgin Cork in *Quercus variabilis* Grown in Korea

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To provide information on the identification and quality evaluation of *Q. variabilis* virgin cork from Korea, the qualitative anatomical characteristics of the virgin cork were observed by optical and scanning electron microscopy and compared with those of *Q. suber* reproduction cork from Portugal. *Q. variabilis* showed a narrower growth ring than *Q. suber*. A dark-brown zone with sclereids was found only in *Q. variabilis* cork. The lenticular channel in *Q. variabilis* is larger than that in *Q. suber*. *Q. variabilis* virgin cork showed a distinct growth ring boundary and an abrupt transition from earlycork to latecork with a few rows of latecork cells. *Q. suber* reproduction cork showed an indistinct growth ring with a gradual transition from one to two rows of latecork cells. In the earlycork, *Q. suber* showed mild corrugation, while *Q. variabilis* displayed significant corrugation with collapsed and distorted cork cells. The lenticular channel in *Q. variabilis* virgin cork was surrounded by thick-walled cells filled with compact lenticular filling tissue. *Q. suber* reproduction cork had an opening with loose lenticular filling tissue surrounded by thick-walled cells. Prismatic crystals, thick-walled sclereid cells, and fiber-sclereids were found only in *Q. variabilis*. A few trabeculae were found in both cork samples.

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INTRODUCTION

Cork is a part of the periderm in the bark system of dicotyledonous plants with secondary growth (Pereira 2007). It is a commercially valuable material in the non-woody product industry; cork is lightweight, has low permeability to liquid and gases, is a good thermal insulator, and has high elastic compression and durability due to its closed cellular structure with high suberin and lignin content (Pereira *et al.* 1987, 2015; Aronson *et al.* 2009). Cork is commonly used in wine bottles as a stopper (Pereira 2007), flooring, walls, ceilings (Knapic *et al.* 2016), and thermal insulation and packaging (Gibson and Ashby 1997).

There are two commercial oak species used for global cork production: *Quercus suber* and *Quercus variabilis*. *Q. suber* commonly grows in the western Mediterranean basin; Portugal and Spain are the largest producers of corks from this species (Pereira and Tome 2004). *Q. variabilis* grows mostly in China, Korea, and Japan (Chen *et al.* 2012).

There are three types of cork in the production process: virgin cork, second cork, and reproduction cork. As mentioned by Pereira *et al.* (1987), virgin cork is the first cork layer produced by the original phellogen of the cork oak and is commonly harvested at an age of approximately 20 to 30 years. After removing the virgin cork, the second cork is produced by the regenerated phellogen and is removed after nine years. The successive cork layers are called reproduction corks and are harvested from the trees at nine-year intervals.

The qualitative anatomical characteristics of the cork in *Q. suber* have been reported (Pereira *et al.* 1987, 1989, 2007; Kim 1991). Pereira *et al.* (1987) reported the anatomical characteristics of the cork in *Q. suber* as follows: 1) the virgin and reproduction cork from *Q. suber* showed a honeycomb-like arrangement in the tangential surface and a brick-wall structure on the transverse and radial surfaces; 2) the earlycork cells had a larger cork cell lumina and thinner cell wall than the latecork cells; 3) the virgin cork showed a more irregular structure with narrow growth increments and higher latecork proportion than the reproduction cork; and 4) heavy corrugation and collapse in thin-walled earlycork cells were observed on the transverse and radial surfaces of the virgin cork and reproduction cork. Pereira (1989) found that trabeculae extended radially between tangential walls across a few cells in the reproduction cork of *Q. suber*. Kim (1991) explained that the reproduction cork of *Q. suber* (outer bark) on the transverse surface consisted of cork cells, lenticels, sclereids, and a dark-brown zone. Pereira (2007) reported that lenticular channels in the reproduction cork of *Q. suber* were circular to elliptical on the tangential surface and elongated to rectangular channels on the transverse and radial surfaces. Pereira (2007) also mentioned that the channels were filled with an agglomerate of cells with many intercellular spaces and often had lignified and thick-walled cells at their borders.

For *Q. variabilis* grown in China, the cork shows a honeycomb-type structure on the tangential surface and a brick wall type with an alignment in parallel rows on the transverse and radial surfaces (Lei *et al.* 2009; Miranda *et al.* 2013; Zhao *et al.* 2013; Bai *et al.* 2014; Ferreira *et al.* 2016; Yuan *et al.* 2017; Song *et al.* 2017). The lumina of *Q. variabilis* cork cells contain plenty of deposits and circular openings connecting two adjacent cells (Lei *et al.* 2009; Miranda *et al.* 2013; Bai *et al.* 2014; Song *et al.* 2017). Ferreira *et al.* (2016) explained that the virgin cork of *Q. variabilis* has strong corrugations, occasionally leading to cell collapse and distortion owing to severe growth stresses. The authors also mentioned that the lenticular channel in the virgin cork of *Q. variabilis* was filled with a loose matrix of cells, whereas the reproduction cork of *Q. variabilis* showed a fracture of tissue, which formed an empty volume at the ring boundary. Song *et al.* (2017) observed prominent corrugations and trabeculae on the transverse and radial surfaces of the reproduction cork in *Q. variabilis* from Qinling Mountain.

Q. variabilis is a common commercial wood species in Korea and has been used for a long time as timber, firewood, and raw material for charcoal (Kang and Kim 2004; Kim and Hanna 2006; Kwon *et al.* 2009). Additionally, *Q. variabilis* contains a large quantity of cork, which can be used in industry (Ferreira *et al.* 2016). However, in Korea, cork from *Q. variabilis* is still underutilized and becomes waste after harvesting wood. Furthermore, the Korean wood industry prefers to import reproduction cork from Europe and China because of the low-quality virgin cork resources in Korea.

There are few studies on the anatomical characteristics of cork in *Q. variabilis* grown in Korea. Cheong *et al.* (1988) described that the cork cells of *Q. variabilis* grown in Korea had a globular shape on the transverse and radial surfaces and a discoid shape on

the tangential surface. Kim (1993) revealed that the reproduction cork of *Q. variabilis* (outer bark) consisted of cork cells, lenticels, sclereids, and a dark-brown zone on the transverse surface. Prasetia *et al.* (2022) reported that the virgin cork of *Q. variabilis* grown in Korea had a narrow growth ring width, high proportion of latecork cells, and consisted of cork cells, lenticular channels, dark-brown zones, and sclereids. The authors also mentioned that the earlycork and latecork cells of *Q. variabilis* showed a brick wall-type structure in the transverse and radial surfaces and a honeycomb structure with various shapes, such as rectangular, pentagonal, hexagonal, heptagonal, octagonal, and nonagonal in the tangential surface. In addition, the earlycork cells had a larger cell width and lumina diameter and thinner cell wall thickness than the latecork cells.

The reproduction cork of *Q. suber* from Europe is the main raw material for various products in Korean cork industry, such as wine stoppers, insulation boards, pavements, and sidewalks. However, as the demand for cork increases for the products, the Korean cork industry is trying to find alternative cork resource from domestic wood species. The virgin cork of *Q. variabilis* is the only resource currently available in Korea. So far, there is still a lack of information regarding the quality of cork resource from this species. This study investigated the qualitative anatomical characteristics of the virgin cork in *Q. variabilis* that grows in Korea and compared its characteristics with the reproduction cork of *Q. suber*. This study will provide valuable information for the effective utilization of *Q. variabilis* cork growing in Korea.

EXPERIMENTAL

Materials

The virgin cork of *Q. variabilis* was collected from the breast heights of three trees in the research forest of Kangwon National University (Chuncheon, Korea) (37°77 N, 127°81 E). Two planks of reproduction cork from Castelo Branco cork forest of Amorim Group (Mozelos, Portugal) were provided by FC Korea Land Co., Ltd. (Seoul, Korea). Photographs and basic information of the virgin cork of *Q. variabilis* and the reproduction cork of *Q. suber* are presented in Fig. 1 and Table 1, respectively.
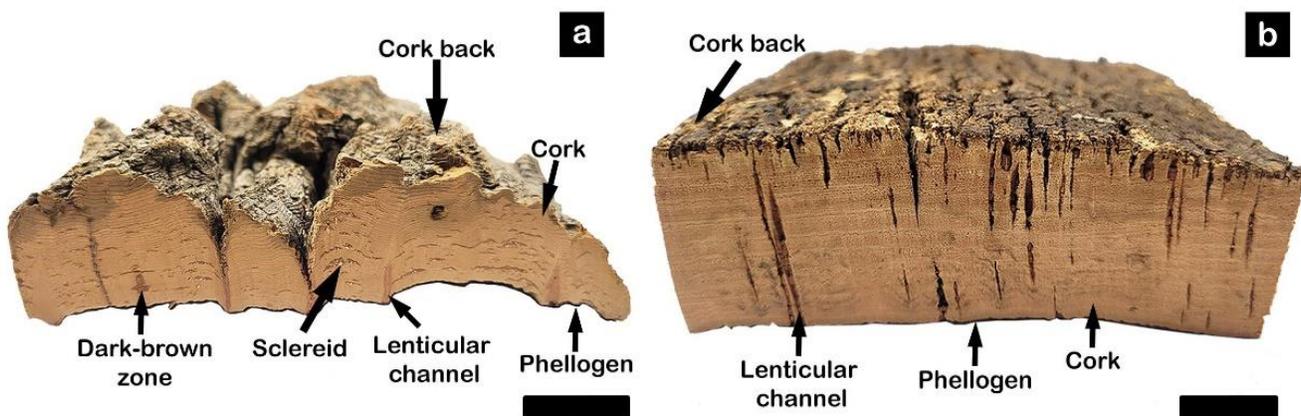
Fig. 1. Virgin cork of *Q. variabilis* (a) and reproduction cork of *Q. suber* (b). Scale bars: 20 mm

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Table 1. Basic Information of the Sample Cork

Species	Cork type	Location	Cork thickness (mm)
Q. variabilis	Virgin cork	Research forest of Kangwon National University, Chuncheon, Korea (37°77 N, 127°81 E)	10-30
Q. suber	Reproduction cork	Castelo Branco cork forest of Amorim Group, Mozelos, Portugal	30-40

Observation and Optical Microscopy

To observe the macroscopic characteristics of the transverse, radial, and tangential surfaces in both species, 40 cork samples with the dimensions of 20 (Radial) × 20 (Tangential) × 20 (Longitudinal) mm^3 from each species were trimmed using a sliding microtome (MSL-H model; Nippon Optical Works, Nagano, Japan) and captured using a mobile phone (Samsung Note 20, 12MP with F1.8, Suwon-si, Korea). The most representative sample from each species, consisting of each element in a cork tissue, such as lenticular channel, dark-brown zone, and sclereid, was compared and presented.

For optical microscopy, cork cells and the dark-brown zone with sclereid were separated using knife blades (Snap-off knife blades, Whashin, Seoul, Korea). The cork samples were converted into the dimensions of 5 (R) × 5 (T) × 10 (L) mm^3, while the dark-brown zone with sclereid were cut into the dimensions of 1-5 (R) × 5-10 (T) × 5-10 (L) mm^3. The cork cells and the dark-brown zone included sclereid were soaked separately in Schultze reagent for three days and heated at 60 °C for 1 h until the samples disintegrated (Franklin 1945). The delignified samples were neutralized with sodium hydroxide solution, stained with 1% safranin solution, dehydrated using a graded series of alcohol (50%, 70%, 90%, 95%, and 99%), and observed under an optical microscope (Zeiss Axioskop 2 Plus, Göttingen, Germany) connected to an images analysis system (Infinity Capture: Infinity 5, Teledyne Lumenera, Ottawa, Canada).

Scanning Electron Microscopy

To perform scanning electron microscopy of the three surfaces, the air-dried cork samples with dimensions of 10 (R) × 10 (T) × 10 (L) mm^3 were prepared and trimmed using a sliding microtome (MSL-H model; Nippon Optical Works, Nagano, Japan). To isolate the cork cells and lenticular filling tissue, the delignified samples were sonicated with an ultrasonic disintegrator (Sonics and Materials, VCX130PB, 130W, Newtown, CT US).

The samples were filtered using a 0.2 μm pore size membrane filter (PTFE Membrane, ADVANTEC, Tokyo, Japan), stored in tert-butanol solution for 1 h, and then place refrigerator (WSM-1243RF, 220 V/60 Hz, Seoul, Korea) for 2 h. The samples were freeze-dried using a bench top freeze dryer (OPR-FDB-5503, OPERON, Gimpo-si, Korea) for 12 hrs. Air-dried and freeze-dried samples were coated with gold using a sputter coater (Cressington sputter coater 108; Watford, UK) and observed under a scanning electron microscope (JSM-5510, 15 kV, Tokyo, Japan).

RESULTS AND DISCUSSION

Macroscopic Structure

Macrographs of the transverse, radial, and tangential surfaces of cork in both species are shown in Fig. 2. Growth rings were observed on the transverse and radial surfaces of both species. The growth ring width in the virgin cork of *Q. variabilis* was narrow as 0.4 to 0.6 mm, whereas the width in the reproduction cork of *Q. suber* was wide as 2.0 to 3.0 mm. Cork cells, lenticular channels, sclereids, and dark-brown zones were observed in the virgin cork of *Q. variabilis*. In contrast, the reproduction cork of *Q. suber* consisted of cork cells and lenticular channels. The lenticular channel in the virgin cork of *Q. variabilis* was larger than that in the reproduction cork of *Q. suber*. The lenticular channel of virgin cork in *Q. variabilis* showed a light brown color, while that of the reproduction cork showed a dark-brown color on the three surfaces. The lenticular channels on the radial surface of both species were wider than those on the transverse surface. On the tangential surface, the lenticular channel of the virgin cork in *Q. variabilis* was observed less frequently than that of the reproduction cork in *Q. suber*, which was abundant and randomly distributed. Sclereids had a light brown color with various shapes and sizes, and were commonly included in the dark-brown zone. The dark-brown zone was randomly scattered in the virgin cork of *Q. variabilis* and showed a blade-like shape on the transverse surface and vertically elongated shape on the radial and tangential surfaces but was wider on the tangential surface.
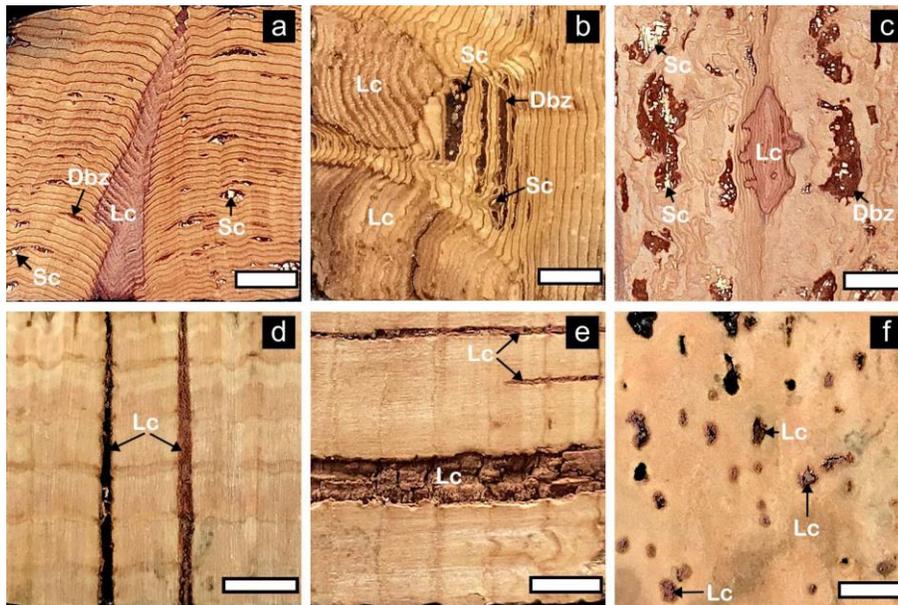
Fig. 2. Transverse (a, d), radial (b, e), and tangential (c, f) surfaces in *Q. variabilis* virgin cork (a-c) and *Q. suber* reproduction cork (d-f). Lenticular channel (Lc), sclereid (Sc), and dark-brown zone (DBZ). Scale bars: 4 mm

There are several studies on the macroscopic characteristics in the cork of *Q. variabilis* and *Q. suber* that support the present study. Kim (1993; 1991) reported that the reproduction cork (outer bark) of *Q. variabilis* and *Q. suber* contained cork cells, lenticels, sclereids, and a dark-brown zone on the transverse surface. In contrast, the dark-brown zone and sclereid was hardly observed in the reproduction cork of *Q. suber* in the present

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study. Zhao *et al.* (2013) and Bai *et al.* (2014) reported that the cork of *Q. variabilis* contained impurities in the tissue of cork, such as lenticels tissue, dark-brown zone, and sclereids. Li *et al.* (2019) reported that the virgin cork and reproduction cork of *Q. variabilis* showed lenticular channels, sclereids surrounded by dark and hard layers, and a smaller lenticular channel in the reproduction cork than in the virgin cork. Additionally, lenticular channels and sclereids in the dark and hard layers of the reproduction cork were less frequent compared to the virgin cork. In the authors' previous study (Prasetia *et al.* 2022), the virgin cork of *Q. variabilis* showed a narrower growth ring width than the reproduction cork of *Q. suber* grown in Portugal. Cork cells, lenticular channel, and dark-brown zone included sclereid were observed in the virgin cork of *Q. variabilis*, while dark-brown zone and sclereid were absent in the reproduction cork of *Q. suber*.

Microscopic Structure
Growth ring characteristics

The transverse and radial surfaces of the virgin cork of *Q. variabilis* and the reproduction cork of *Q. suber* are displayed in Fig. 3. The virgin cork of *Q. variabilis* had two to eight rows of latecork cells in each growth ring, whereas the reproduction cork of *Q. suber* showed only one to two rows of latecork cells. Additionally, there was an abrupt transition from earlycork to latecork in the virgin cork of *Q. variabilis*, whereas the reproduction cork of *Q. suber* showed a gradual transition from earlycork to latecork. The growth ring boundary was distinct in the virgin cork of *Q. variabilis* and indistinct in the reproduction cork of *Q. suber*.
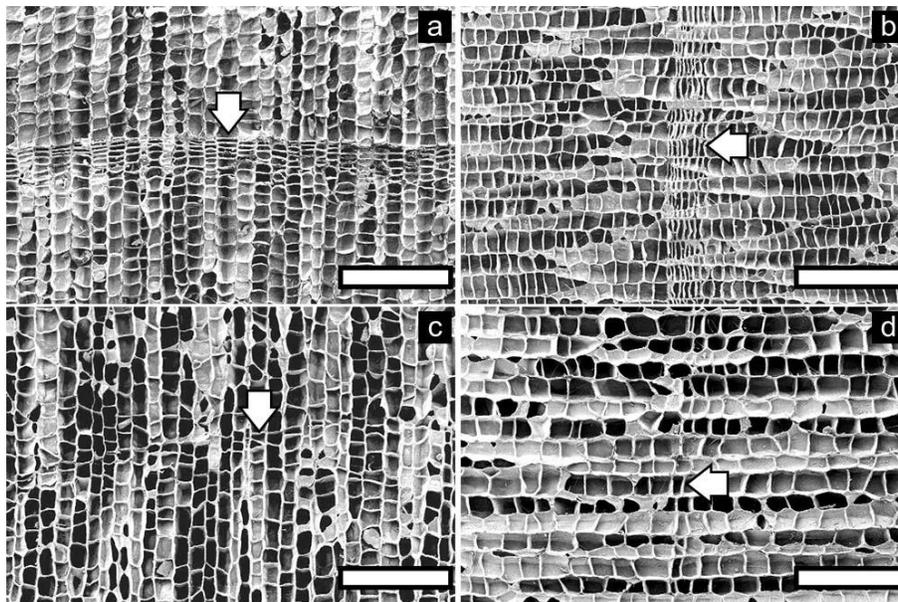
Fig. 3. Transverse (a, c) and radial (b, d) surfaces in *Q. variabilis* virgin cork (a, b) and *Q. suber* reproduction cork (c, d). White arrows indicate growth ring boundary. Scale bars: 150 µm

Miranda *et al.* (2013) reported that the reproduction cork of *Q. variabilis* showed two to four latecork cells in each growth ring. As mentioned by Ferreira *et al.* (2016), the virgin cork and reproduction cork of *Q. variabilis* had two to six rows of latecork cells in each growth ring. The results from Miranda *et al.* (2013) and Ferreira *et al.* (2016) are consistent with the growth ring characteristics of the virgin cork in *Q. variabilis* of the

present study. Prasetia *et al.* (2022) revealed that the virgin cork of *Q. variabilis* had a higher proportion of latecork cells than the reproduction cork of *Q. suber*. Pereira *et al.* (1987) also reported that within one growth ring, the virgin cork of *Q. suber* showed a higher proportion of latecork cells than the reproduction cork of *Q. suber*.

The morphology of corrugation in the virgin cork of *Q. variabilis* and the reproduction cork of *Q. suber* are presented in Fig. 4. On the transverse and radial surfaces, the virgin cork of *Q. variabilis* showed severe corrugation in earlycork, while the reproduction cork of *Q. suber* showed a mild corrugation. Significant corrugation in the virgin cork of *Q. variabilis* was accompanied by the collapse and distortion of cork cells in the earlycork. On the tangential surface, *Q. suber* showed heavily deformed cork cell walls compared to *Q. variabilis*.

The heavy corrugation in the cork of *Q. variabilis* was also observed in reproduction cork and virgin cork of *Q. variabilis* in China (Miranda *et al.* 2013; Bai *et al.* 2014; Ferreira *et al.* 2016; Yuan *et al.* 2017). Miranda *et al.* (2013) reported that the reproduction cork of *Q. variabilis* showed a heavy corrugation in earlycork cells. Bai *et al.* (2014) reported that the earlycork cells of the *Q. variabilis* virgin cork showed severe corrugation and cell collapse. Ferreira *et al.* (2016) reported that the virgin cork of *Q. variabilis* exhibited strong corrugations, occasionally leading to cork cell collapse and distortion. Yuan *et al.* (2017) also mentioned that cork cells from the virgin and reproduction cork in *Q. variabilis* showed obvious corrugation without collapse. As mentioned by Pereira *et al.* (1987), the virgin cork and reproduction cork of *Q. suber* also showed a heavy corrugation in thin-walled earlycork cells.
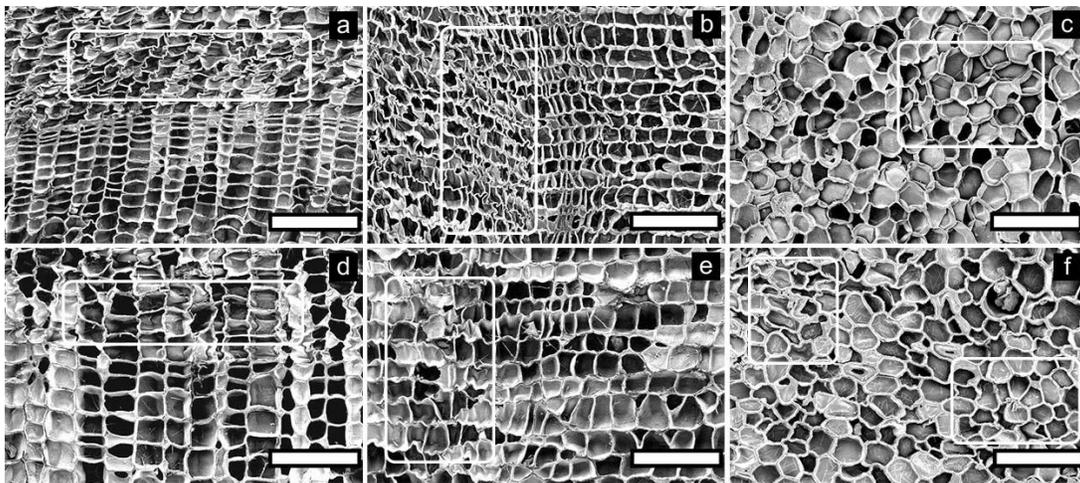
Fig. 4. Transverse (a, d), radial (b, e), and tangential (c, f) surfaces of corrugated earlycork cells in *Q. variabilis* virgin cork (a-c) and *Q. suber* reproduction cork (d-f). White rectangles indicate a severe corrugation in the virgin cork, a mild corrugation in the reproduction cork, and deformed cork cells. Scale bars: 100 µm

Lenticular channel

SEM images of the lenticular channel in the virgin cork of *Q. variabilis* and the reproduction cork of *Q. suber* are presented in Figs. 5 and 6. On the transverse and radial surfaces, the virgin cork of *Q. variabilis* showed a larger lenticular channel than that of *Q. suber*. The lenticular channel in the virgin cork of *Q. variabilis* was surrounded by lignified thick-walled cells and was filled with compact lenticular filling tissue (Figs. 5 and 6A). In contrast, the lenticular channel had an opening with loose lenticular filling tissue

surrounded by thick lignified cells in the reproduction cork of *Q. suber* (Figs. 5 and 6B). The thick-walled cell layer of the lenticular channel in the virgin cork of *Q. variabilis* was wider than that in the reproduction cork of *Q. suber*. As shown in Fig. 7, the delignified cells of lenticular filling tissue in both species showed a round shape and varied in size within a diameter of 10 to 40 μm. There were a few pits on the surface of the lenticular filling tissue cells in both species. The cells of lenticular channel filling tissue in the virgin cork of *Q. variabilis* were smaller than those in the reproduction cork of *Q. suber*.
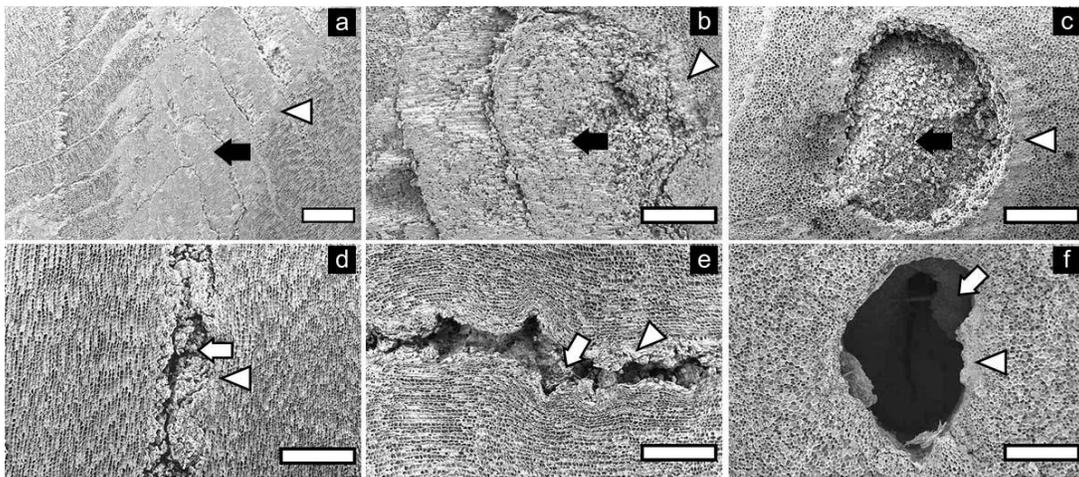
Fig. 5. Transverse (a, d), radial (b, e), and tangential (c, f) surfaces of lenticular channel in *Q. variabilis* virgin cork (a-c) and *Q. suber* reproduction cork (d-f). Compact lenticular filling tissue in the virgin cork in *Q. variabilis* (black arrows) and loose lenticular filling tissue or opening in reproduction cork of *Q. suber* (white arrows). Lenticular channels surrounded by thick-walled cells (arrowheads). Scale bars: 500 μm
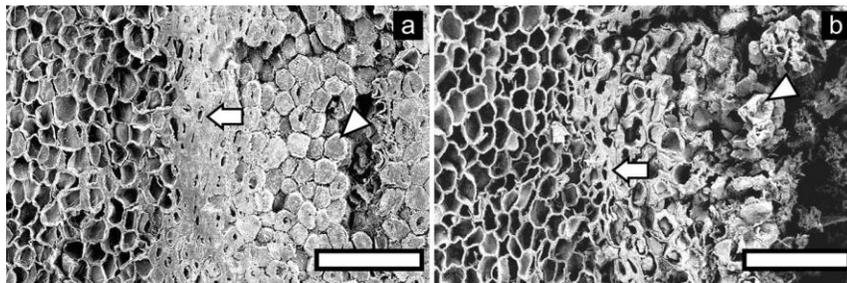
Fig. 6. Thick-walled cells (white arrows) and lenticular filling tissue (arrowheads) in the lenticular channel of *Q. variabilis* virgin cork (a) and *Q. suber* reproduction cork (b). Scale bars: 100 μm

The characteristics of lenticular channel in this work were in line with the results of Pereira (2007) and Ferreira *et al.* (2016). The corks of *Q. suber* and *Q. variabilis* contain a lenticular channel that radially crosses the cork layers from the phellogen to the cork back (Pereira 2007 and Ferreira *et al.* 2016). Pereira (2007) reported that the lenticular channel in *Q. suber* was filled with lenticular filling tissue with many intercellular spaces and lacked a regular structural arrangement. Additionally, the lenticular filling tissue in *Q. suber* is rounded, almost spherical, with small dimensions of diameter approximately 10 to 20 μm and often surrounded by thick-walled lignified cells. Ferreira *et al.* (2016) also reported that the lenticular channel in the reproduction cork of *Q. variabilis* showed a circular or elliptical outline on the tangential surface and was completely filled with a loose matrix of cells surrounded by thick-walled lignified cells.
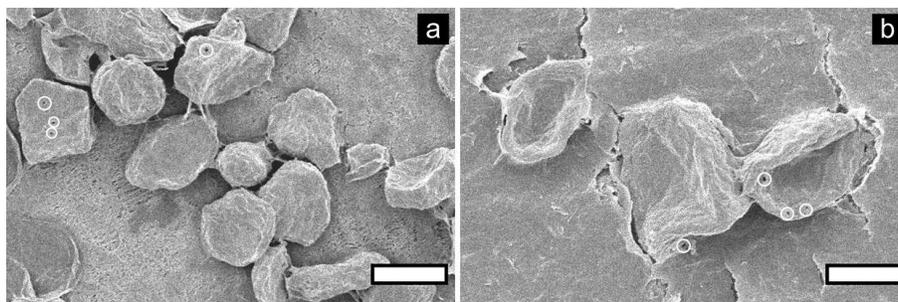
Fig. 7. Delignified cells of lenticular filling tissue in *Q. variabilis* virgin cork (a) and *Q. suber* reproduction cork (b). A few pit-like openings in the cell wall of the circular or elliptical lenticular filling cells in both species (white circles). Scale bars: 20 μm

Dark-brown zone and sclereid

SEM images of the dark-brown zone and sclereid in the virgin cork of *Q. variabilis* are shown in Fig. 8. The dark-brown zone and sclereid occasionally caused distortion in the growth rings and significant corrugation in the earlycork. The dark-brown zone, the so-called non-conducting secondary phloem commonly consist of parenchyma cells, phloem fibers, sclereid, and crystals. The parenchyma cells were circular to angular in shape and varied in size. In addition, the parenchyma cells had thicker cell walls than cork cells. The phloem fibers in the dark-brown zone typically showed tapered tips (Fig. 9A) but sometimes bifurcated tips (Fig. 9B), and crystals were occasionally observed in parenchyma cells near the phloem fibers (Fig. 9C). As shown in Fig. 10, the sclereid cells had no lumen and had irregular shapes with a number of pits on the surface. The crystals in the sclereid commonly exhibited a rhomboidal shape with a rough surface. Additionally, the fiber-sclereid had an elongated form with a thick wall and was much shorter than the phloem fiber.

There are some published studies comparable to the present data on the anatomical characteristics of the dark-brown zone. Bai *et al.* (2014) reported that the dark-brown zone of the virgin cork of *Q. variabilis* consisted of sclereids and crystals. Sen *et al.* (2011a, 2011b) reported that the dark-brown zone of the bark of *Quercus cerris* consisted of fibers, cluster sclereids, fiber-sclereids, prismatic crystals, and parenchyma cells. The tapered fibers with a narrow lumen had crystals in the parenchyma cells of a circular to rectangular shape. Sclereid cells had no lumen and very thickened and polylamellated cell walls with a few pit channels, and large prismatic crystals. The authors also mentioned that the druses and prismatic crystals are probably calcium oxalate in the phloem. Additionally, the fiber-sclereids were similar to the fibers but shorter. Quilho *et al.* (2013) and Sousa *et al.* (2021) also reported fibers, parenchyma cells, crystals, and clustered sclereids in the non-conducting secondary phloem of the bark of *Quercus faginea* and *Quercus rotundifolia*. The fibers in the secondary phloem were bordered by crystals in the parenchyma cells. Furthermore, in *Q. faginea*, the fibers occasionally showed bifurcated tips (Quilho *et al.* 2013). Thick-walled sclereid cells with various shapes and large prismatic crystals have also been observed in the non-conducting secondary phloem of *Q. faginea* and *Q. rotundifolia* (Quilho *et al.* 2013; Sousa *et al.* 2021). In addition, fiber-sclereids have been found in *Q. rotundifolia* (Sousa *et al.* 2021).
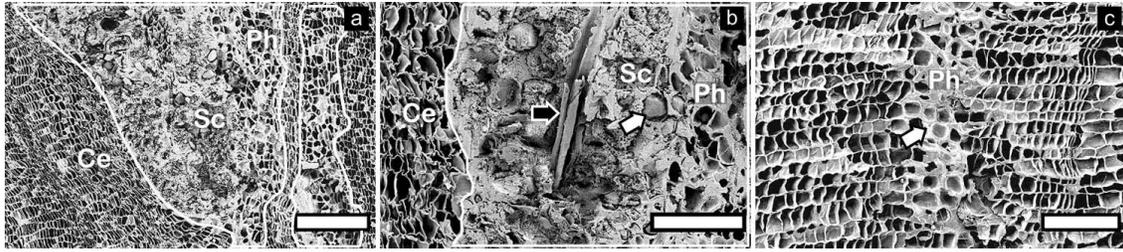
Fig. 8. Radial surface of *Q. variabilis* virgin cork. a: Phloem tissue (Ph) and sclereid (Sc) in the dark-brown zone (white solid line) and corrugated earlycork cells (Ce). b: Prismatic crystals (white arrows) and phloem fibers (black arrow) in sclereid. c: Thick-walled parenchyma cells (white arrow) in phloem tissue. Scale bars: 200 μm (a); 300 μm (b); 100 μm (c)

Fig. 9. Tapered (white arrows) and bifurcated phloem fiber (black arrow), and prismatic crystals in parenchyma cells (arrowheads) in the dark-brown zone of *Q. variabilis* virgin cork. Scale bars: 200 μm (a); 40 μm (b, c)
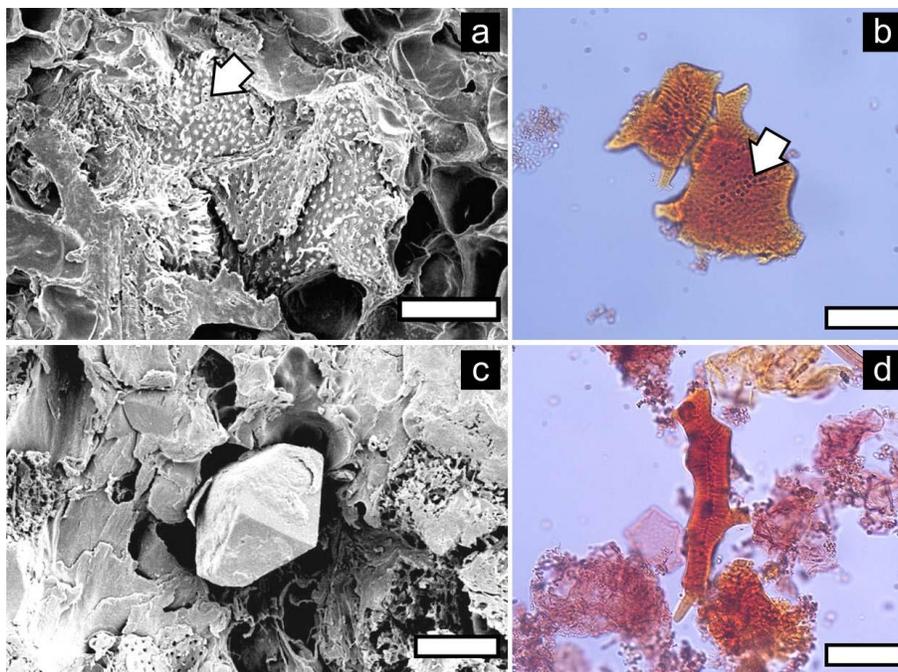
Fig. 10. Sclereid cells (a, b), a prismatic crystal (c), and a fiber-sclereid (d) in the dark-brown zone of *Q. variabilis* virgin cork. Many pits are observed in the sclereid cells surface (white arrows). Scale bars: 30 μm (a); 20 μm (c); 40 μm (b, d)

Cork cellular features

The transverse, radial, and tangential surfaces of the virgin cork in *Q. variabilis* and the reproduction cork of *Q. suber* are shown in Fig. 11. On the transverse and radial surfaces, the latecork cells of both species showed a brick-wall-type structure with a narrow cell lumen and thick cell wall, while the earlycork cells showed rectangular, pentagonal, and hexagonal shapes with a wide cell lumen and thin cell wall. In both species, undulations were commonly found in the radial cell wall of earlycork cells, but they were rarely observed in the tangential wall. Undulations were rarely found in the radial walls of the latecork cells and occasionally in the tangential wall. On the tangential surface, the cork cells of both species showed a honeycomb structure of rectangular, pentagonal, hexagonal, heptagonal, and octagonal shapes. Undulated cork cells were observed on the tangential surface. Needle-like deposits were observed on the transverse, radial, and tangential surfaces of both corks, but no intercellular voids were found on any surfaces.
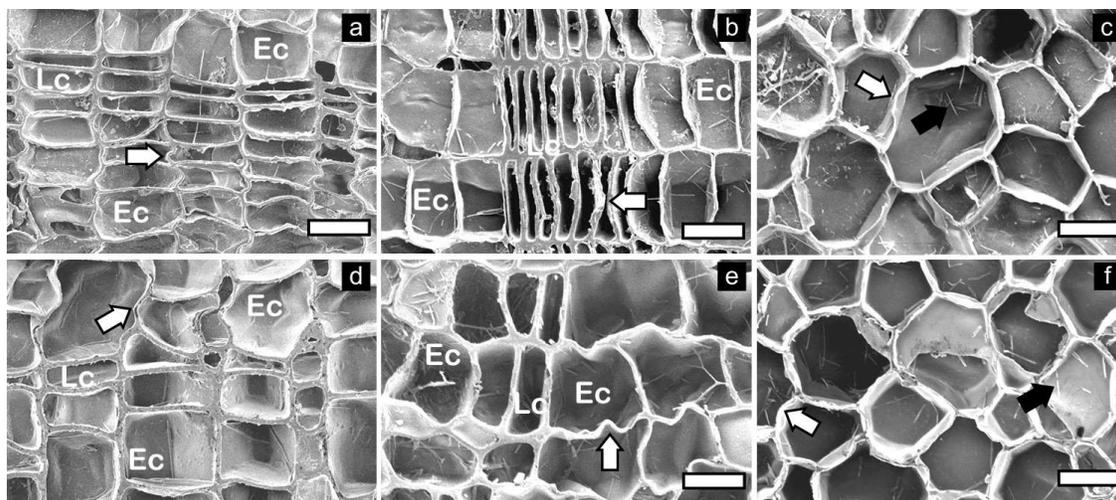
Fig. 11. Transverse (a, d), radial (b, e), and tangential (c, f) surfaces in *Q. variabilis* virgin cork (a-c) and *Q. suber* reproduction cork (d-f). Earlycork (Ec), latecork (Lc), needle-like deposits in the lumen of cork cells (black arrows), and undulated cork cells (white arrows). Scale bars: 20 μm

So far, there have been many studies on the cellular features of the virgin and reproduction cork of *Q. variabilis* from China and *Q. suber* from Portugal. The cork of *Q. variabilis* and *Q. suber* showed a brick-wall type appearance on the transverse and radial surfaces and a honeycomb structure on the tangential surface, and no intercellular voids were found in all surfaces (Pereira *et al.* 1987; Lei *et al.* 2009; Miranda *et al.* 2013; Zhao *et al.* 2013; Bai *et al.* 2014; Ferreira *et al.* 2016; Yuan *et al.* 2017; Xiaozhou *et al.* 2017). Earlycork cells had a larger cork cell lumen with a thinner cell wall than the latecork cells (Bai *et al.* 2014; Ferreira *et al.* 2016; Yuan *et al.* 2017; Additionally, the cork cell walls of both species showed undulations on the transverse and radial surfaces and buckling on the tangential surface (Pereira 2007; Lei *et al.* 2009; Miranda *et al.* 2013; Bai *et al.* 2014; Ferreira *et al.* 2016; Yuan *et al.* 2017). The lumen surface of the cork in *Q. variabilis* was rough and contained several deposits, whereas that of *Q. suber* was smooth with occasional deposits (Miranda *et al.* 2013; Ferreira *et al.* 2016; Xiaozhou *et al.* 2017).

In the authors' previous study, the earlycork and latecork cells of *Q. variabilis* virgin cork from Korea and *Q. suber* reproduction cork from Portugal showed a brick wall-type structure in the transverse and radial surfaces, and a honeycomb structure in the

tangential surface. The earlycork cells of both species had larger cell width and lumina diameter and thinner cell wall thickness than the latecork cells (Prasetia *et al.* 2022).

Delignified cork cells from the virgin cork of *Q. variabilis* and the reproduction cork of *Q. suber* are presented in Fig. 12. Needle-like deposits were still observed after delignification (Fig. 12A and 12B). The cork cells had a polygonal shape with a smooth surface and a few circular pits on the surface of the cell wall. The results on the isolated cork cells are consistent with previous studies. Lei *et al.* (2009) reported that the isolated cork cells from the reproduction cork in *Q. variabilis* showed a three-dimensional structure of pentagonal and hexagonal prisms. The cork of *Q. variabilis* showed circular openings connecting two cells in the cell wall surface (Lei *et al.* 2009; Miranda *et al.* 2013; Bai *et al.* 2014; Xiaozhou *et al.* 2017).
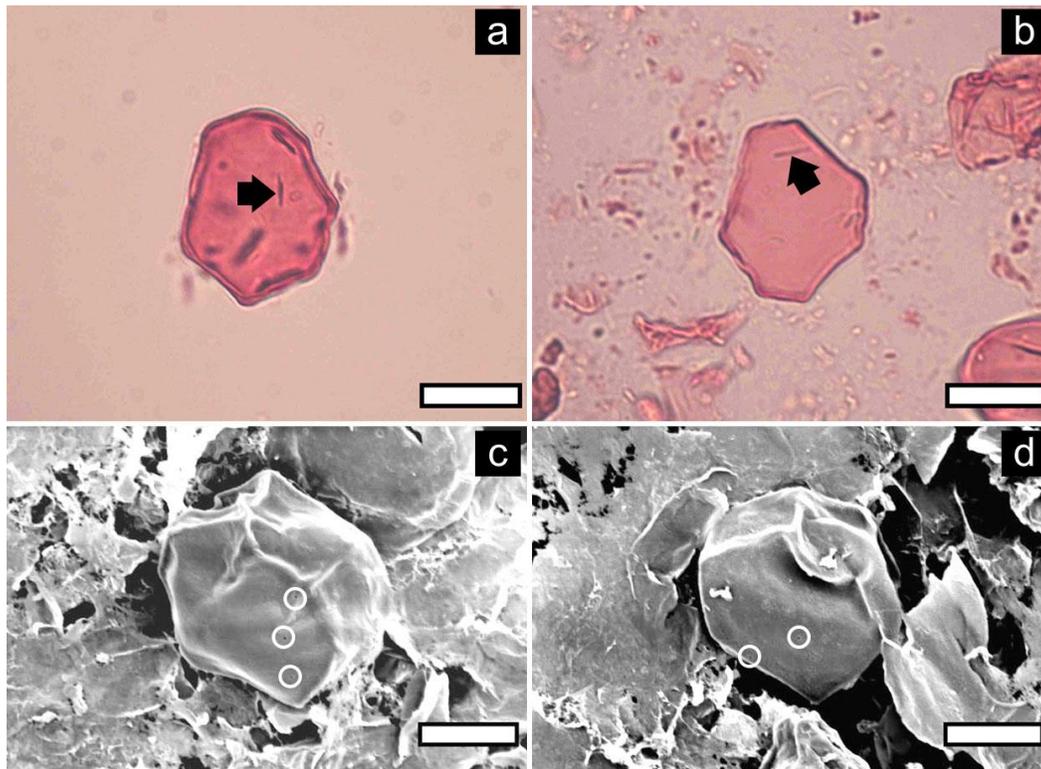
Fig. 12. Earlycork cells in *Q. variabilis* (a, c) and *Q. suber* (b, d). Needle-like deposits in the lumen of earlycork cells (black arrows). Pit-like openings on the cell wall surface of both corks (white circles). Scale bars: 20 μm (a, b); 15 μm (c, d)

Figure 13 shows trabeculae in the earlycork and latecork of *Q. variabilis* virgin cork and reproduction cork of *Q. suber*. Trabeculae showed a rod-like appearance with a diameter of 1 to 2.5 μm on the transverse surface and elongated between tangential walls. A few trabeculae in the cork cells of both species showed a transversal split. A few previous studies on the trabeculae in cork cells support the present results. Trabeculae crossing the cell lumen radially between tangential walls with a rod-like appearance were observed in the reproduction cork of *Q. variabilis* (Xiaozhou *et al.* 2017) and reproduction cork of *Q. suber* (Pereira 1989). Pereira (1989) also reported that trabeculae extended, in general, from three to six cells with an average diameter of 1 to 2 μm.
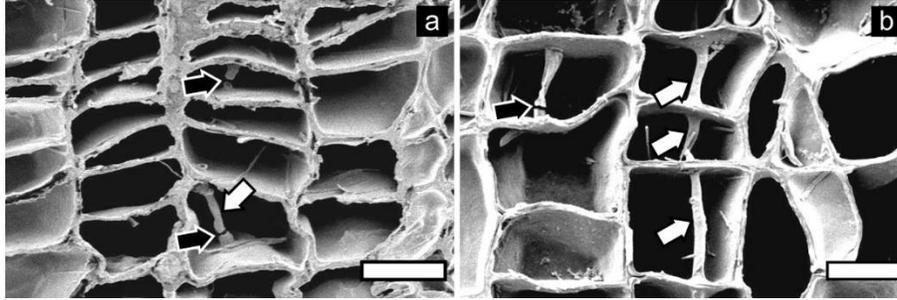
Fig. 13. Trabeculae (white arrows) in the transverse surface of *Q. variabilis* virgin cork (a) and *Q. suber* reproduction cork (b). Transversally splitted trabeculae (black arrows). Scale bars: 15 μm

CONCLUSIONS

1. Macroscopically, the virgin cork of *Quercus variabilis* has a narrower growth ring than the reproduction cork of *Q. suber*. Cork cell and lenticular channel were found in both species but dark-brown zone and sclereid were observed only in the virgin cork of *Q. variabilis*. In addition, the virgin cork of *Q. variabilis* clearly showed larger lenticular channel than the reproduction cork of *Q. suber* on the three surfaces.

2. Microscopically, the cork of *Q. variabilis* showed a distinct growth ring boundary with an abrupt transition from earlycork to latecork, whereas the cork of *Q. suber* showed an indistinct growth ring with a gradual transition. The earlycork of *Q. variabilis* displayed significant corrugation with collapse and distortion, whereas that of *Q. suber* showed only mild corrugation.

3. The lenticular channel in the cork of *Q. variabilis* was widely surrounded by lignified thick-walled cells and filled with compact lenticular filling tissue, whereas that of *Q. suber* was narrowly surrounded by lignified thick-walled cells and had an opening.

4. The dark-brown zone consisted of phloem fibers, parenchyma cells, crystals, and sclereids in the virgin cork of *Q. variabilis* and was occasionally accompanied by a distorted growth ring and significant corrugation of earlycork cells. Thick-walled sclereid cells, prismatic crystals, and fiber-sclereid were found in the sclereid of *Q. variabilis*.

5. Both species showed a comparable earlycork and latecork cell features, and also trabeculae, needle-like deposits in the lumen, pit-like openings on the cell wall were observed in both species.

There were distinct differences in the macroscopic and microscopic characteristics between *Q. variabilis* virgin cork and *Q. suber* reproduction cork. Even though there are different qualitative characteristics in the cork between both species, the virgin cork of *Q. variabilis* could be potential for alternative cork resource for *Q. suber*. The results of the present study can be used for identification and quality evaluation for further effectively utilization of the cork in *Q. variabilis* grown in Korea. Additionally, due to the structural characteristics of the virgin cork in *Q. variabilis*, it is suggested that the applications of the cork require trituration to cork granules and agglomeration to produce cork composite products, while its cellular features support its use for insulation, surfacing, and sealant products.

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