Deseeded Sunflower Fractions and Their Anatomy and Cell Morphology

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Deseeded sunflower is a heterogeneous lignocellulosic biomass that has not been well utilized due to the incomplete understanding of its biological structure. In this study, deseeded sunflower was fractioned into the stalk rind, stalk pith, receptacle, bract, bractlet, leaf blade, and petiole, and their anatomy, cell morphology, and fiber dimension were studied using light microscope, environmental scanning electron microscope, and fiber quality analyzer. The results showed that the major fractions were the stalk rind and receptacle (49.4 wt% and 28.1 wt%, respectively) and each of the other fractions was less than 10 wt% of the total biomass. The pith was only composed of parenchyma tissue, and the other fractions were composed of epidermal, parenchyma, and vascular tissues. The arrangement and number of vascular tissues were different among fractions. The fiber length in the stalk rind was 0.823 mm, the width was 21.3 µm, and the aspect ratio was 38.6. The content of fiber fines in other fractions was higher than 50%, and these fractions should be developed for other uses. Fractionation was judged to be an effective way to achieve high value utilization of deseeded sunflower.

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INTRODUCTION

Fossil fuels are not sustainable and cause environmental pollution. Hence, scientists are being encouraged to explore new clean energy sources to replace them (Cai *et al.* 2023). Lignocellulosic biomass is the only known sustainable carbon resource (Sun and Cheng 2002; Sridevi *et al*. 2024). Non-wood biomass is an important material and energy resource in timber-short countries. Deseeded sunflower is an abundant and renewable non-wood biomass.

Helianthus annuus L. (sunflower) is an annual herbaceous dicotyledon in the Compositae family (Garcia *et al*. 2006). Sunflower plants are the third-largest oil crop in the world after soybean and palm (Zhai *et al.* 2014; Xu *et al.* 2020). Sunflower seed oil is used in cooking, carriers, and biodiesel (Tan *et al*. 2020). In response to the growing demand for vegetable oil, the global sunflower planting area is increasing at the rate of 10 to 20% per year. In 2019, the global sunflower planting area was 26.5 million ha (Mehdikhani *et al.* 2019). Deseeded sunflower is often abandoned in the environment. Consequently, its potential resources are being wasted.

Deseeded sunflower consists of stalk, sunflower head, and leaves. These parts vary morphologically and physicochemically, which determine the utilization method and efficiency (Marechal and Rigal 1999). The stalk rind has been used in pulping and papermaking (Khristova *et al.* 1998; Rudi *et al*. 2016), and to produce composite materials (Mati-Baouche *et al.* 2014), to generate energy products (Maroušek 2013) and to provide heavy metal adsorbents (Hashem *et al.* 2006). The stalk pith can serve as a buffer (Shi and Wang 2010) and has been used as an adsorbent (Baysal *et al*. 2018). The sunflower head is a pectin source (Sahari *et al*. 2003; Muñoz-Almagro *et al.* 2020). The leaves contain terpenoids (Macías *et al*. 2002). The tissue and cell composition of stalk rind and pith are different, showing different mechanical and moisture absorption characteristics. The Young's modulus of the stalk rind was found to be considerably higher than that of the pith. In contrast, the water diffusion coefficient of the pith was found to be considerably higher than that of the stalk rind (Sun *et al*. 2013). The parenchyma cells of sunflower pith morphologically differed from those of corn and sorghum pith. While the parenchyma cells of corn and sorghum pith are hexagonal prisms, those of sunflower pith are approximately tetrakaidehedral (Yin *et al.* 2007). The anatomy and cellular morphology of the head and leaf fractions have not yet been reported in the literature.

Although it could be regarded as an important biomass resource, research on deseeded sunflowers has mainly focused on the chemical composition and industrial application of stalks, heads, and leaves, with little research on their fraction and anatomical structure. However, the composition and anatomy of biomass resources directly affected the physical and chemical properties, which in turn could affect the technology and efficiency of the conversion and processing. Therefore, there has been a need to fractionate deseeded sunflower, systematically study the anatomy and cellular morphology of the fractions and reveal the commonalities and differences in the biological structure of each fraction. This study used deseeded sunflower as raw material and partitioned it into stalk rind, stalk pith, receptacle, bract, bractlet, leaf blade, and petiole. Light microscopy, environmental scanning electron microscopy, and fiber quality analysis were used to elucidate the cellular morphology, anatomy and fiber dimension of each fraction. The results of this study will provide a theoretical and practical basis for the efficient utilization of deseeded sunflower.

EXPERIMENTAL

Raw Material

Deseeded sunflower (variety: Sunflower 1013) was collected from Wuchuan County, Hohhot City, Inner Mongolia Autonomous Region, China. Wuchuan County is located between latitude 40°47′ to 41°23′ N and longitude 110°31′ to 111°53′ E. Wuchuan County belongs to the temperate continental monsoon climate, with an average annual temperature of 4.2 ℃ and an average annual precipitation of 360 to 366 mm.

The mature and complete sunflower 50 plants were manually collected at one time, and the sunflower seeds were removed. The stalk, head and all leaves were air-dried and separately stored in plastic bags.

Deseeded Sunflower Fractions

Mature, entire deseeded sunflowers, including stalks, heads, and leaves, were selected and manually fractionated. The stalks were divided into stalk rinds and stalk piths. The sunflower heads were divided into receptacles, bracts, and bractlets. The leaves were divided into leaf blades (laminae) and petioles. Namely, deseeded sunflowers were divided into 7 fractions, stalk rinds, stalk piths, receptacles, bracts, bractlets, leaf blades, and petioles. Each fraction was individually weighed, and its dry weight % was determined according to TAPPI T258 om-02 (2006). Two measurements were taken in parallel.

Light Microscopy

A light microscope (BH2, Olympus, Tokyo, Japan) was used to examine the cell morphology of deseeded sunflower fractions. The stalk rinds, receptacles, and petioles were cut into matchstick-size pieces. The piths, leaf blades, bracts, and bractlets were fragmented into small pieces. The samples were treated with acetic acid and 30% (v/v) hydrogen peroxide (1:1, v/v) at 60 °C for ≥ 24 h. When the samples turned white, the macerated fibers were removed from the reactor and thoroughly rinsed with water. The fibers were then stained with safranin O, mounted on microscope slides, and viewed.

Environmental Scanning Electron Microscopy (ESEM)

ESEM (Quanta200, FEI/Thermo Fisher Scientific, Waltham, USA) was used to view the anatomy of the deseeded sunflower fractions. The stalk rinds and piths were excised from the internodes in the middle of the stalks. The leaf blades and petioles were excised from the middle leaves on the stalks. The bracts were taken from the middle layer, and the receptacles and bractlets were removed from the edges of the heads. All fractions were cut into transverse sections (TS) and radial sections (RLS) of 2 to 3 mm in height. The stalk rinds were also cut into tangential sections (TLS). Before imaging, a gold film was applied to the sections using a sputter coater (E-1010, Hitachi, Tokyo, Japan) operating at 15 mA and 120 s sputter time. The ESEM was performed at 20 kV acceleration.

Fiber Quality Analysis (FQA)

The wet fiber widths and lengths were measured with a fiber quality analyzer (Morfi, Techpap SAS, Saint Martin d'Hères, France). The deseeded sunflower fractions were subjected to acetic acid and 30% (v/v) hydrogen peroxide (1:1, v/v) at 60 °C for ≥ 24 h. The fibers were separated in water, and their concentration was adjusted to 0.004 wt.%. In the FQA device, their widths were automatically calculated based on data obtained for 5,000 fibers. The aspect ratios were calculated from the ratios of the corresponding fiber lengths and widths.

RESULTS

Fractions

Deseeded sunflower was fractioned into stalk rind, stalk pith, receptacle, bracts, bractlets, petiole, and leaf blade (Fig. 1). The average diameter of stalk measured was ~20–30 mm, and the thickness of the stalk rind was 2 to 3 mm. The pith was well developed. The volume: mass ratios of the stalk rind and pith were 1:9 and 9:1, respectively. The stalk rind and pith densities were 328 kg/m^3 and 33.5 kg/m^3. The receptacle was the main fraction of the head. It was disk-like, 10 to 30 cm in diameter, 0.5 to 1 cm in thickness. The receptacle was composed of the epidermis and the pith. The receptacle pith was slightly yellow, filamentous, and accounted for approximately 90% of the total volume. The bracts were oval and apically acuminate, and were 2 to 3 layers, an imbricate arrangement. The bractlets were hard and membranous. They were shallow triangular openings at the top and a tight spiral arrangement throughout the receptacle. The petiole was 10 to 25 cm long. The leaf blade was broadly ovate and had three veins. The number of leaves varied greatly among varieties and locations. Generally, sunflowers have 25 to 40 leaves.

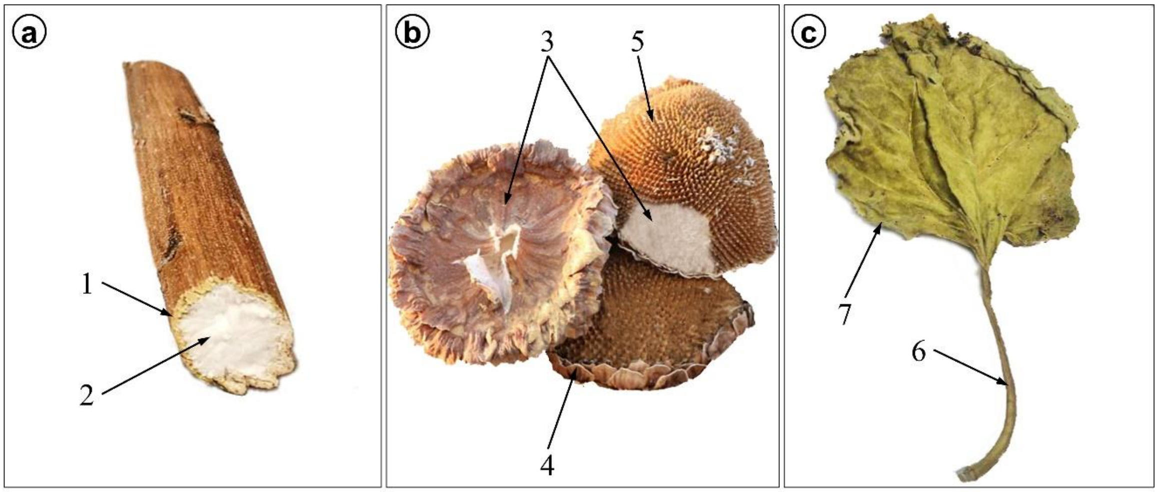

Fig. 1. Deseeded sunflower fractions. a-stalk; b-sunflower head; c-leaf; 1-stalk rind; 2-stalk pith; 3-receptacle; 4-bract; 5-bractlet; 6-leaf blade; 7-petiole

The percentage of each fraction was determined based on the total dry weight of the sample. As shown in Table 1, the weight percentages were 49.4% (stalk rind), 5.3% (stalk pith), 28.1% (receptacle), 4.1% (bract), 3.8% (bractlet), 5.9% (petiole), and 3.4% (leaf blade). The stalk rind was the largest deseeded sunflower fraction. The second-largest was the receptacle. The weight percentages of the petioles, piths, bracts, bractlets, and leaf blades were all <10%. Hence, the stalk rind and the receptacle comprised the bulk of the deseeded sunflower mass.

Table 1. Weight Percentages of Deseed Sunflower Fractions on Dry Basis (%)

Fractions	Stalk Rind	Stalk Pith	Receptacle	Bract	Bractlet	Petiole	Leaf Blade
Weight Percentages	49.4±0.8	5.3±0.6	28.1±0.9	4.1±0.6	3.8±0.7	5.9±0.5	3.4±0.7

Fractions Anatomy

Stalk Rind

The anatomy of the sunflower stalk rind resembled that of kenaf (Abdul Khalil *et al*. 2010) but differed from that of corn stalk (Li *et al*. 2012). The stalk rind was composed of epidermal and vascular tissue and parenchyma (Fig. 2a). The epidermal tissue consisted of the epidermis and the cortex. The epidermis was a cell layer on the outside of the stalk rind that prevented excessive evaporation and bacterial invasion. The cortex was the parenchyma tissue beneath the epidermis. The vascular tissue comprised vascular bundles containing phloem, cambium, and xylem. The phloem consisted of fiber bundles that were circumferentially arranged and was relatively more developed at the corners of the stalk. The phloem fibers varied in diameter and had thick walls and obvious pits (Fig. 2b). The cambium was composed of parenchyma cells. The xylem consisted of vessels, axial parenchyma cells, xylem fibers, and xylem rays. Xylem fibers and xylem rays were radially arranged. The vessels were large in diameter and were irregularly distributed. The xylem anatomy was similar to the diffuse-porous wood (Wang *et al*. 2019) (Fig. 2c). Pith rays were located between the vascular bundles and joined the pith internally and the cortex externally. Ray cells were arranged laterally, xylem rays were generally one or more columns, and pith rays were more columns (Fig. 2d, 2e). In addition to fibers, there were more parenchyma cells in the stalk rind, which was easy to be moth-eaten or cracked. As a fiber raw material, more parenchyma cells will also affect the binding properties between the fibers (Wang *et al*. 2021).

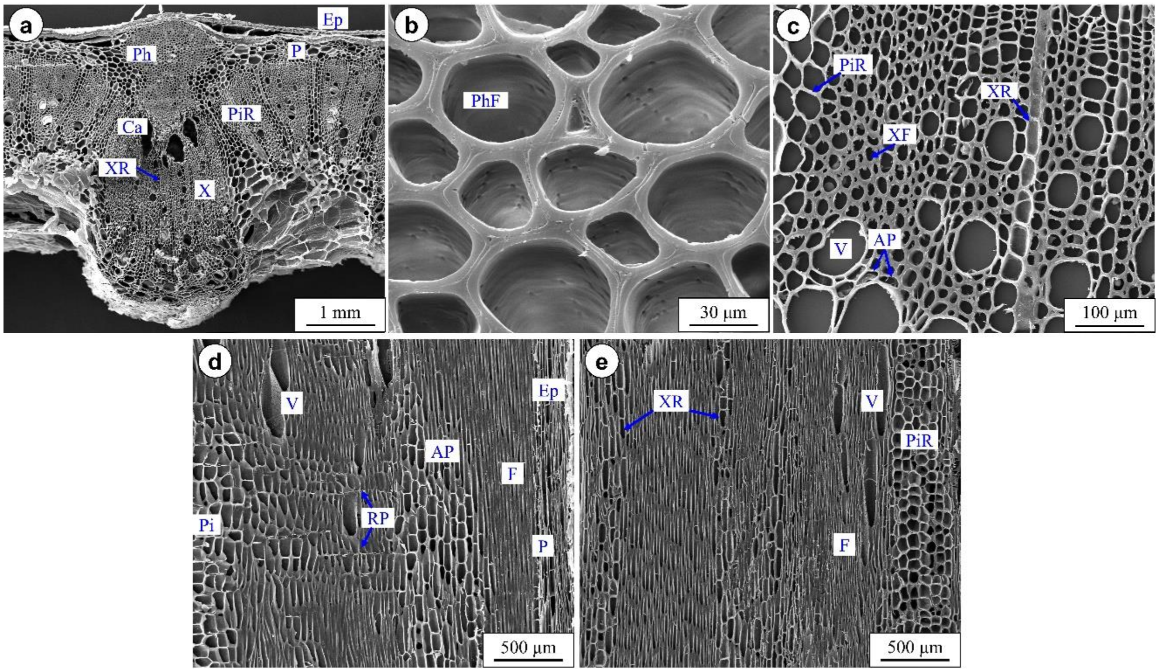

Fig. 2. Anatomy of the stalk rind. (a) transverse section of the stalk rind; (b) transverse section of the phloem fibers; (c) transverse section of the xylem; (d) radial section of the stalk rind; (e) tangential section of the stalk rind. Ep: epidermis; P: parenchyma; Ph: phloem; Ca: cambium; X: xylem; XR: xylem ray; PiR: pith ray; PhF: phloem fiber; XF: xylem fiber; AP: axial parenchyma; V: vessel; F: fiber; RP: ray parenchyma; Pi: pith

Stalk pith

Both the transverse and radial sections of the stalk pith formed a similar “honeycomb” hole structure. The pith comprised parenchyma alone without epidermal or vascular tissue. The parenchyma cells in the pith center were approximately regular hexagons but they became progressively more elongated in the radial direction and were radially distributed (Fig. 3a). The parenchyma cells were layered along the axis and interlace (Fig. 3b). The transverse and radial sections showed that the parenchyma cells in the pith were polyhedral and mostly hexagonal, radially arranged in transverse section, and layered in radial section. Yin *et al*. (2007) showed that the sunflower pith parenchyma cells were tetrakaidecahedron consisting of eight hexagons and six quadrilaterals, and the peripheral cells are long tetrakaidecahedrons.

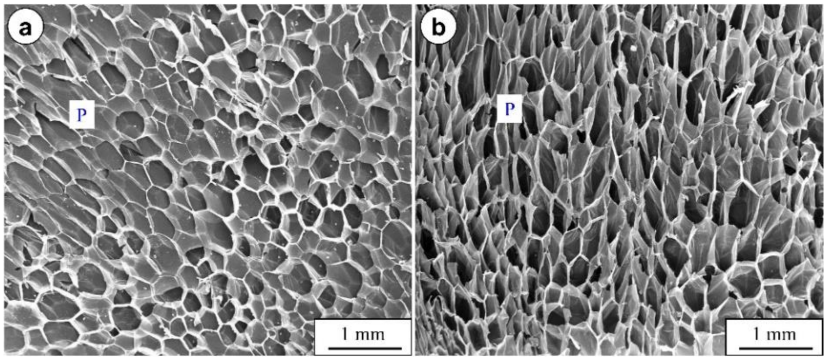

Fig. 3. Anatomy of the stalk pith. (a) transverse section of the stalk pith; (b) radial section of the stalk pith. P: parenchyma

Receptacle

The receptacle was composed of epidermis, parenchyma, and vascular tissue. Of these, the parenchyma accounted for 80% of the total volume. Vascular tissue bundles were scattered throughout the parenchyma between epidermis and the center, which varied in size and density (Fig. 4a). The parenchyma cells near the epidermis had large lumens and small intercellular spaces. Those near the center had small lumens and large intercellular spaces (Fig. 4b). The vascular bundles consisted mainly of vessels and parenchyma cells with few fibers. The vessels were mostly spiral and closely arranged (Fig. 4c and 4d).

Unlike the vessels in the stalk rind, those in the receptacle were arranged in bundles and played important roles in transport of inorganic substances during plant growth. A mechanical analysis indicated that the Young's modulus of the vessel axial compression was 105× greater than that of the parenchyma cells (Yin *et al*. 2007). Bundled vessels improved the axial mechanical strength of the receptacle and made it a strong supporting structure that ensured steady seed production.

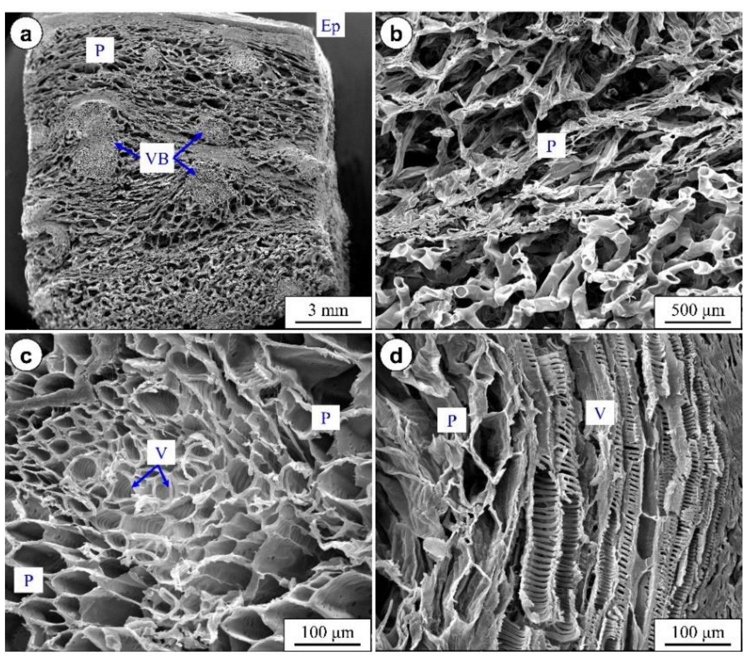

Fig. 4. Anatomy of the receptacle. (a) transverse section of the receptacle; (b) transverse section of the parenchyma; (c) transverse section of the vascular bundle; (d) radial section of the receptacle. Ep: epidermis; P: parenchyma; VB: vascular bundle; V: vessel

Bract

The bract was composed of the epidermis, parenchyma, and vascular tissue. The parenchyma and vascular tissue were sandwiched between the internal and external epidermis. The bract parenchyma and vascular tissues resembled those of the receptacle. The parenchyma cells were irregular in shape. Those close to the outer epidermis had large lumens and small intercellular spaces, whereas those near the inner epidermis had smaller lumens and larger intercellular spaces (Fig. 5a). The vascular bundle was near the outer epidermis and consisted mainly of vessels and parenchyma cells with few fibers. The spiral vessels were arranged in bundles (Fig. 5b and 5c). The sandwich structure of bracts, which had a large surface area for photosynthesis, provided sufficient structural rigidity and strength at relatively low mass to protect the sunflower seeds better.

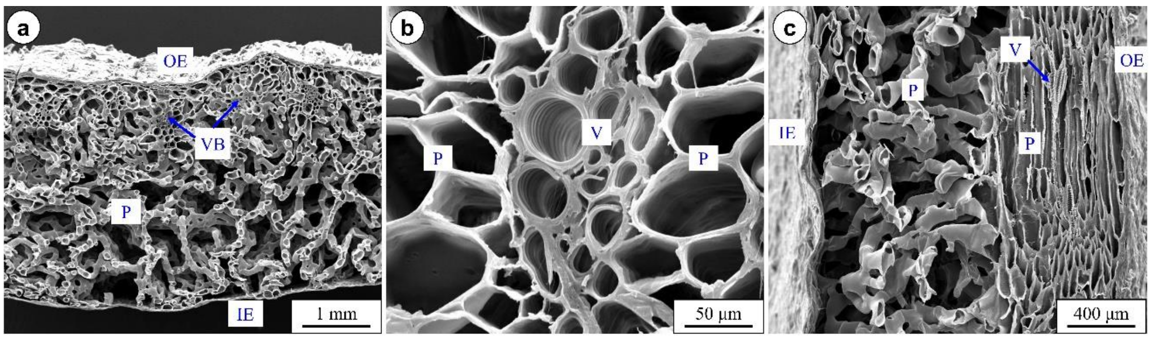

Fig. 5. Anatomy of the bract. (a) transverse section of the bract; (b) transverse section of the vascular bundle; (c) radial section of the bract. OE: outer epidermis; IE: inner epidermis; P: parenchyma; VB: vascular bundle; V: vessel

Bractlet

The bractlet comprised the epidermis, parenchyma, and vascular tissue (Fig. 6a). Like the bract, its parenchyma and vascular tissue were sandwiched between the inner and outer epidermis. The parenchyma cells were irregular in shape and had large intercellular spaces. One form of vascular tissue consisted mainly of fibers and parenchyma cells (Fig. 6b), while the other was composed mostly of vessels and parenchyma cells. The bractlet had substantially more fibers than the bract and receptacle. However, they were smaller, and their walls were thicker (Fig. 6c).

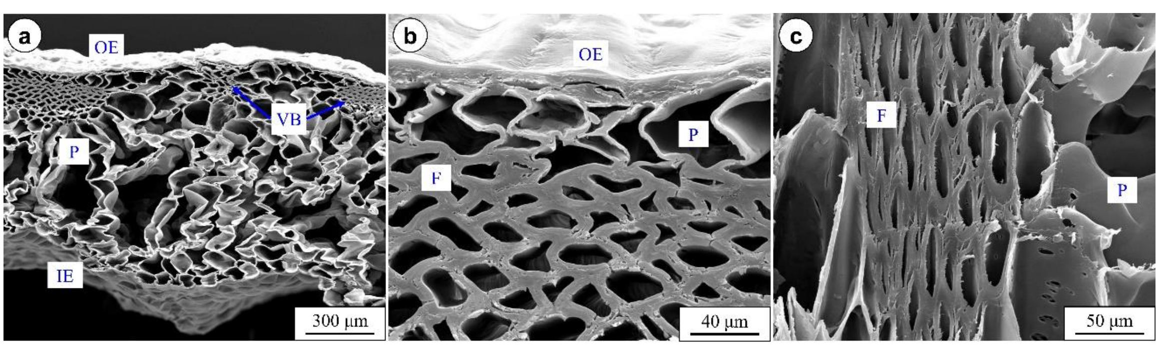

Fig. 6. Anatomy of the bractlet. (a) transverse section of the bractlet; (b) transverse section of the fibers; (c) radial section of the bractlet. OE: outer epidermis; IE: inner epidermis; P: parenchyma; VB: vascular bundle; F: fiber

The sunflower head fractions were similar in anatomy, being composed of epidermis, vascular tissue, and parenchyma. The vascular tissue consisted mainly of vessels and parenchyma cells with few fibers. The spiral vessels were arranged in bundles. The parenchyma cells were irregular in shape, and their lumens and intercellular spaces were large. The bractlet had more fibers than the bract or the receptacle, but they were short, and their walls were thick.

Petiole

The petiole was composed of the epidermis, collenchyma, parenchyma, and vascular tissue. The parenchyma occupied the largest area. There were more vascular bundles, not uniform sizes, arranged along the circumference of the petiole (Fig. 7a). The phloem consisted mainly of parenchyma cells. The xylem comprised mainly reticulated and spiral vessels arranged radially. The reticulated vessels were wide with thick inner walls and were complete in form. The spiral vessels were fragile and narrow with thin inner walls (Fig. 7b and 7c).

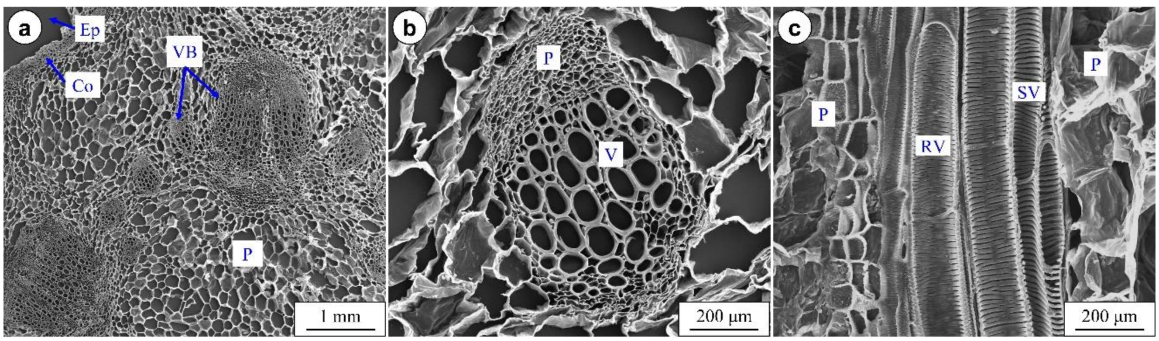

Fig. 7. Anatomy of the petiole. (a) transverse section of the petiole; (b) transverse section of the vascular bundle; (c) radial section of the petiole. Ep: epidermis; Co: collenchyma; P: parenchyma; VB: vascular bundle; V: vessel; RV: reticulated vessel; SV: spiral vessel

Leaf Blade

The leaf blade was composed of inner and outer epidermis, mesophyll, and vascular tissue (Fig. 8a). The mesophyll consisted of palisade and spongy tissue and was composed of parenchyma cells (Fig. 8b). The vascular bundles were distributed in the veins and consisted of vessels and parenchyma cells with few fibers (Fig. 8c).

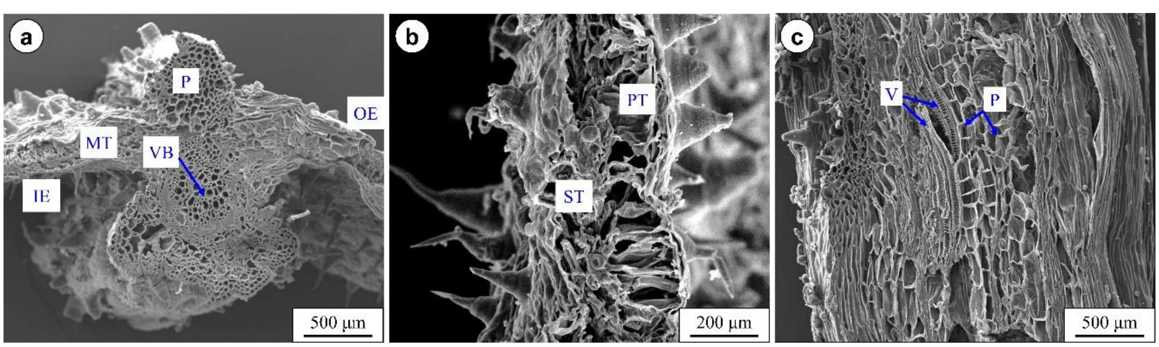

Fig. 8. Anatomy of the leaf blade. (a) transverse section of the leaf blade; (b) radial section of the mesophyll; (c) radial section of the vein. OE: outer epidermis; IE: inner epidermis; MT: mesophyll tissue; VB: vascular bundle; PT: palisade tissue; ST: spongy tissue; P: parenchyma; V: vessel

Fractions Cell Morphology

Stalk rind and stalk pith

The stalk rind had fibers, parenchyma cells, vessels, and epidermal cells (Fig. 9a). The fibers in the phloem were long and thick-walled, while those in the xylem were short and thin-walled. The pith ray parenchyma cells were round, while the xylem ray parenchyma cells were claviform or oval. The reticulated vessels were numerous and short, whereas the spiral vessels were sparse and long. The epidermal cells were rectangular. Some were dissociated, while others were "flaky," which maintained the arrangement of the plant materials.

The stalk pith consisted entirely of parenchyma cells and had neither fibers nor vessels (Fig. 9b). Compared with parenchyma cells of the stalk rind, parenchyma cells in the pith were similar in shape, larger in size, and thinner in the cell wall.

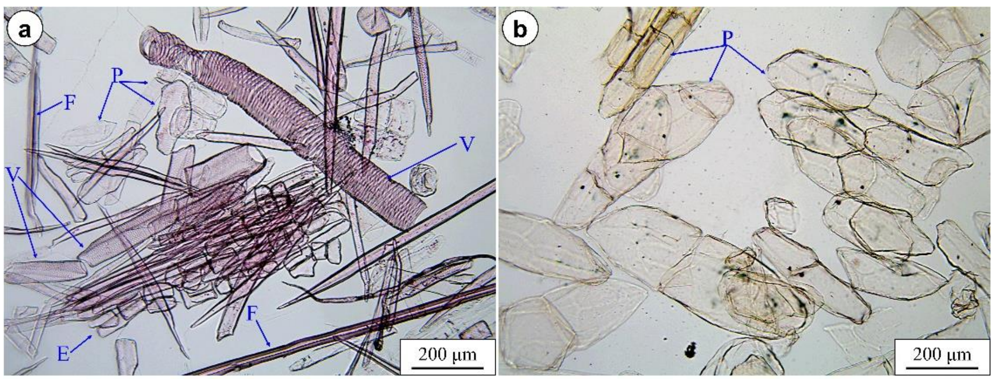

Fig. 9. Stalk rind and stalk pith cell morphology. (a) stalk rind; (b) stalk pith. F: fiber; E: epidermal cell; V: vessel; P: parenchyma cell

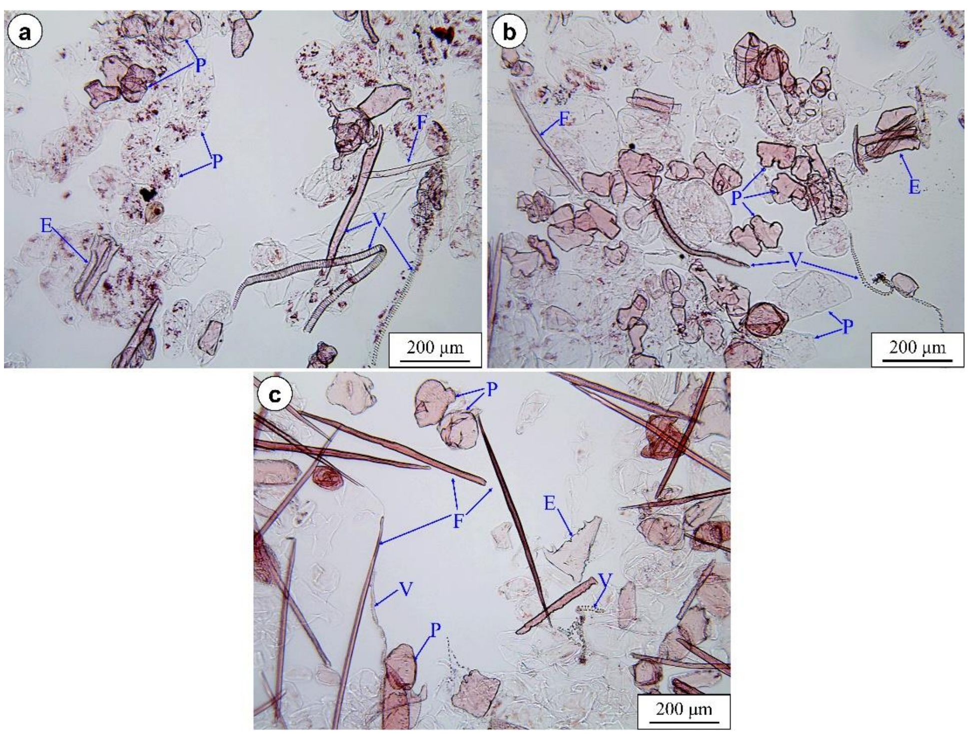

Fig. 10. Receptacle, bract, and bractlet cell morphology. (a) receptacle; (b) bract; (c) bractlet. F: fiber; E: epidermal cell; V: vessel; P: parenchyma cell

Receptacle, bract, and bractlet

The receptacles, bracts, and bractlets were composed of epidermal cells, vessels, parenchyma cells, and fibers (Fig. 10). The epidermis was readily isolated into single cells, which were serrated. The vessel ends were tubular or lingual, and the spiral vessels were slender. The parenchyma cells were abundant in sunflower head fractions but had irregular morphology and differed significantly in volume. The bractlet fibers were more numerous than those in the receptacles and bracts.

Leaf blade and petiole

The leaf blade and petiole consisted of epidermal cells, parenchyma cells, vessels, and fibers. The parenchyma cells were the most abundant, followed by the vessels and epidermal cells. There were few fibers (Fig. 11). The parenchyma cells were irregular in shape, and the vessels were mostly spiral. The leaf blade epidermal cells were round and had distinct stomatal apparatus. The petiole epidermal cells were square like those of the stalk rind.

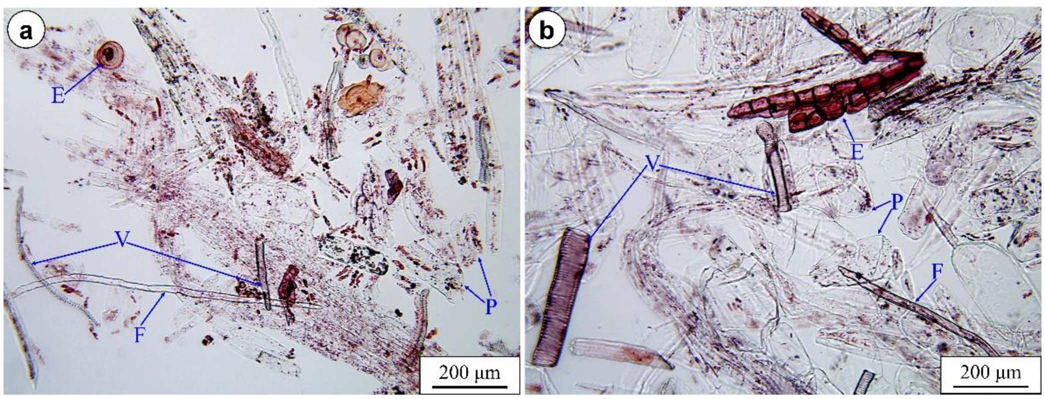

Fig. 11. Leaf blade and petiole cell morphology. (a) leaf blade; (b) petiole. F: fiber; E: epidermal cell; V: vessel; P: parenchyma cell.

Fraction Fiber Dimensions

The dimensions of the fiber in deseeded sunflower fractions are listed in Table 2. The order of the length of each fraction fiber was as follows: stalk rind > bractlet > petiole > leaf blade > bract > receptacle > stalk pith. The order of the width of each fraction fiber was as follows: stalk pith > receptacle > bract > leaf blade > petiole > bractlet > stalk rind. The order of the aspect ratio of each fraction fiber was as follows: stalk rind > bractlet > petiole > leaf blade > bract > receptacle > stalk pith. The fiber length in the stalk rind was 0.823mm, the width was 21.3 μm, the aspect ratio was 38.6, and the content of fine fibers was only 31.6%. Based on these numbers, the stalk rind has the potential to be used as a lignocellulosic feedstock for biorefining. The fiber coarseness of other fractions was high, which was characterized by coarse and short fibers, poor fiber quality, and the fine fiber content was greater than 50%. These fractions were appropriate to consider developing and utilizing other uses.

Table 2. Fiber Dimension in Deseeded Sunflower Fractions

Fractions	Stalk rind	Stalk pith	Receptacle	Bractlet	Bract	Leaf Blade	Petiole
Fiber Length (mm)	0.823	0.343	0.363	0.51	0.374	0.448	0.464
Fiber Width (μm)	21.3	68.7	33.2	22.5	36.5	31.5	29.4
Fiber Aspect Ratio	38.6	4.99	10.9	22.7	10.2	14.2	15.8
Fiber Coarseness （mg/100m）	9.2	31.7	24.5	11.6	33.0	18.3	10.9
Fiber Kink（%）	7.4	25.6	11.8	15.7	16.6	16.7	19.1
Fiber Curl（%）	5.4	20.2	12.9	8.2	14.4	10.1	10.6
Fiber Fines Content (%)	31.6	84.9	80.4	50.1	86.7	82.7	67

DISCUSSION

Based on the difference of macroscopic biological structure, the deseeded sunflower was systematically fractionated. The tissue structure and cell morphology of each fraction were studied respectively, and the fiber quality of each fraction was measured. The results indicated that deseeded sunflower exhibited a high degree of heterogeneity in organs, tissues, and cells, which directly affected its processing and utilization characteristics.

Compared with corn, wheat, and other crop straws (Li *et al*. 2012), sunflower stalk rind had more parenchyma cells due to its more developed pith rays. Although it was not as extensive as corn stalk in fiber utilization, the sunflower salk rind could still be used as lignocellulosic fiber for processing and utilization. The parenchyma cells in the pith were polyhedral in shape and exhibited a large cell cavity, which can be regarded as a natural excellent foam material (Gibson 2005). The proportion of parenchyma tissues in receptacles, bracts, bractlets, petioles and leaves were between that of stalk and pith. The parenchyma cells of these fractions were irregular in shape and loose in structure, which could be used as adsorption materials. In addition, unlike the petioles of coconut trees and palms (Satyanarayana *et al*. 1982; Sugiyama *et al*. 2014), sunflower petioles were not suitable as fiber raw materials.

Deseeded sunflowers were found to be complex biomass materials composed of multiple fractions, each with distinct differences in appearance and anatomical structure. The tissue composition, tissue arrangement, cell type and cell morphology of each fraction were also significantly different. These differences directly affected the technology and efficiency of the processing and utilization. In the future, the chemical composition of fractions of deseeded sunflowers will be studied, to comprehensively understand the biological, physical, and chemical characteristics of each fraction.

CONCLUSIONS

1. The deseeded sunflower was divided into seven fractions of stalk rind, stalk pith, receptacle, bract, bractlet, leaf blade, and petiole. The weight percentages of stalk rind and receptacle were 49.4% and 28.1%, respectively, while the weight percentages of the other fractions were less than 10%. The stalk rind and receptacle were the main fractions of deseeded sunflower.

2. The pith was only composed of parenchyma tissue, and the other fractions were composed of epidermal, parenchyma and vascular tissue. The arrangement and quantity of vascular tissue varied among different fractions.
3. The length of stalk rind fibers was 0.823 mm, the width was 21.3 µm, the aspect ratio was 38.6, and the content of fine fibers was 31.6%. The stalk rind can be used as a lignocellulosic feedstock. The content of fine fibers in other fractions was higher than 50%, and they were suitable for developing other uses.
4. The biological structure of deseeded sunflower was highly inhomogeneous, which directly affected the physical and chemical properties of each fraction. Fraction was an effective way for high-value utilization of deseed sunflowers.

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