Sulfur plays an important role in the rubber industry as a vulcanizing agent, providing an enhancement in mechanical properties relative to unvulcanized rubber. The sulfur used in industry originates from natural and petroleum sources. Petroleum-based sulfur (PS), usually a by-product from a refinery process, was used as the vulcanizing sulfur in the present work. The chemical structure of PS was characterized and compared to that of a commercial rhombic sulfur (RS) using sulfur K-edge X-ray absorption near-edge structure (XANES) spectroscopy. The results reveal that sulfur K-edge XANES spectroscopy has good sensitivity to the chemical structure of sulfur, and the chemical structures of sulfur in rubbers vulcanized with PS and RS are very similar. Furthermore, different types of rubbers vulcanized with PS or RS exhibit comparable mechanical properties. This work shows that PS can be used to replace RS as a vulcanizing agent for rubber.