Pomegranate (Punica granatum L.) is rich in bioactive compounds and presents potential beneficial effects on human health, including the prevention of cancer. Pomegranate seed oil (PSO) is rich in conjugated isomers of linolenic acid (CLnA) and it is believed that CLnA effects are associated with inhibition of growth and apoptosis induction in different types of cancer. The aim of this study was to investigate the effects of PSO on breast cancer cells bioenergetics. MDA-MB-231, a metastatic human breast carcinoma cell line, was treated with 10 mM PSO for 24 hours and we evaluated (a) cell viability by neutral red assay; (b) oxygen consumption by high-resolution respirometry; and (c) $^{13}$C lactate production via nuclear magnetic resonance. Additionally, PSO composition was determined by gas chromatography. The results of PSO composition showed that punicic acid (36%), $\alpha$-eleostearic acid (18%), catalpic acid (15%) and $\beta$-eleostearic acid (3%) were the most abundant CLnA. Bioactivity assays revealed that PSO did not affect MDA-MB-231 viability. However, respiration rate and the fraction of oxygen consumption associated with ATP synthesis were significantly lower in PSO-treated compared to control cells. Additionally, we observed a 10% decrease in lactate production in PSO-treated cells. Interestingly, by adding antimycin A – an inhibitor of complex III from the electron transport chain - lactate production was 30% lower in PSO-treated cells when compared to controls. The attenuated effect of antimycin A on lactate production suggests that PSO interferes with ATP synthesis in MDA-MB-231 cells, causing mitochondrial dysfunction. These results indicate that changes in energy metabolism of MDA-MB-231 cells are early signs of cellular dysfunction. Thus, PSO has potential beneficial effects on MDA-MB-231 cells proliferation by adversely affecting mitochondrial bioenergetics and glucose utilization. Mechanistic studies are being performed to identify the role of CLnA in these changes and its possible chemotherapeutic effects.