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Significance of Squalene in Rice Bran Oil and Perspectives on Squalene Oxidation

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Summary As an intermediate metabolite during the biosynthesis of sterols, squalene is found ubiquitously in plants and animals. In rice, squalene is contained in rice bran, and consequently, squalene in rice bran oil has gained attention. Studies have shown that the intake of squalene from food sources demonstrate various physiological benefits such as the prevention of cancer and cardiovascular disease. Squalene is also known as an effective antioxidant in edible oils. However, due to its chemical structure, squalene is susceptible to oxidation, which may cause a decline in the nutraceutical and antioxidative effects of squalene in edible oils. Oxidative degradation of squalene also results in the formation of scission products (i.e., aldehydes and ketones) which may lead to off-flavor. Since the rate of squalene oxidation depends on the factors that induce its oxidation (i.e., light or heat), emphasis on oxidation mechanisms is necessary. It has been demonstrated in previous studies that the oxidation products formed by the singlet oxygen oxidation and free radical oxidation of squalene are different, and more recently, we demonstrated that different squalene monohydroperoxide isomers are formed by each oxidation mechanism. We herein discuss the significance of squalene in rice bran oil as well as the oxidative degradation of squalene in edible oils with focus on oxidation mechanisms.

Key Words squalene, rice bran oil, lipid oxidation, squalene monohydroperoxide isomers, LC-MS/MS

1. Nutraceutical and Antioxidative Properties of Squalene in Rice Bran Oil

Squalene is a $C_{30}H_{50}$ hydrocarbon that possesses a characteristic chemical structure consisting of six isoprene units (Fig. 1A). Squalene is known to be an intermediate metabolite during the biosynthesis of sterols and is ubiquitously found in plants and animals. Commercially, squalene is used for various purposes including vaccine adjuvants, dietary supplements, and cosmetic products, and is obtained mainly from shark liver oil. However, due to recent concerns in marine preservation, squalene from alternative sources, especially from plant origin, has attracted interest (*1*). In rice, squalene is contained in rice bran as an unsaponifiable constituent. Thus, studies have investigated the presence of squalene in rice bran oil, a product derived from rice bran. The squalene content in rice bran oil and its derivatives have been quantified with the use of different methods such as thin-layer chromatography (TLC) (*2–4*), gas chromatography with flame ionization detection (GC-FID) (*5–8*), high-performance liquid chromatography with ultraviolet detection (HPLC-UV) (*9*), and high-performance liquid chromatography with diode-array detection (HPLC-DAD) (*10*) (Table 1).

The significance of squalene in foods lies in the fact that it demonstrates various physiological effects including prevention of diseases such as cancer. For example, administration of a diet containing 1% squalene to rats was found to suppress the formation of aberrant crypt foci, an early precursor of colon cancer, suggesting the preventive effect of squalene on colon cancer (*11*). Dietary squalene was also shown to inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in rats (*12*). Other studies have also demonstrated the putative preventive effects of squalene on skin cancer (*13*) and certain types of breast cancer (*14*). Additionally, a diet supplemented with 2% squalene was found to attenuate myocardial infraction in rats (*15*) and intake of 1 g/kg squalene per day demonstrated a decrease in atherosclerotic lesion size in male *ApoE* knockout mice (*16*), which both illustrate the potential cardioprotective effect of squalene. Importantly, although squalene is an intermediate in

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the biosynthesis of cholesterol, animal studies as well as clinical trials have demonstrated that dietary intake of squalene does not affect serum cholesterol levels (*17*, *18*).

In addition to such properties as a food factor, the antioxidative property of squalene has also been thoroughly investigated. In a study that evaluated the effect of squalene on the auto and photooxidation of polyunsaturated fatty acids, the role of squalene as a peroxyl radical scavenger was suggested (*19*). Other studies have also demonstrated that squalene acts as an effective quencher of singlet oxygen (*20*) and that squalene demonstrates synergistic antioxidative properties in combination with α-tocopherol (*21*). A similar effect was observed during the photooxidation of olive oil, where it was suggested that the presence of squalene prevents the degradation of α-tocopherol via regeneration of α-tocopherol radicals (*22*). Additionally, a concentration-dependent antioxidative activity of squalene was observed during the heat-induced oxidation of rapeseed oil (*23*) and olive oil (*24*). Thus, the above studies suggesting the antioxidative effects of squalene imply, at least in part, that squalene may contribute to the prevention of oxidation in edible oils.

2. *Oxidative Degradation of Squalene*

Although squalene possesses the benefits described above, because of its characteristic chemical structure containing six double bonds, squalene is known for its susceptibility to oxidation. Since oxidation of squalene leads to the loss of its nutraceutical and antioxidative potential, it is essential to know the mechanisms by which squalene is oxidized and degraded, as well as the consequences of such oxidative degradation of squalene in foods.

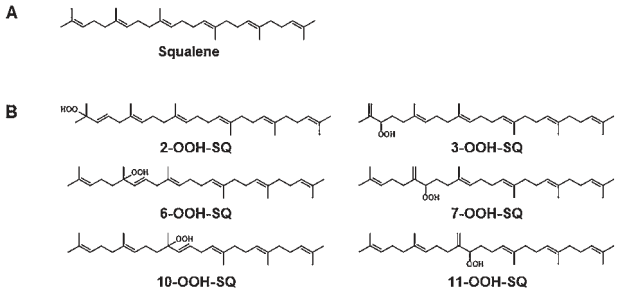

Fig. 1. Chemical structures of (A) squalene and (B) squalene monohydroperoxide isomers.

Oxidative degradation of squalene in edible oils has been investigated in several studies. In a study that evaluated the long-term storage of olive oil under room temperature and diffused lighting, squalene content was found to significantly decrease, especially under conditions where the oil was exposed to air (i.e., nearly half of the squalene was lost after 6 mo of storage) (*25*). However, a contrasting result was obtained when the long-term storage of olive oil in dark was evaluated; loss of squalene was limited to 10% even after 24 mo of storage (*26*). Likewise, when the auto and photooxidation of pumpkin seed oil was investigated, while both conditions resulted in a considerable decrease in squalene content, loss of squalene was greater during photooxidation than that during autooxidation (*27*). Such results suggest that the rate of squalene degradation depends on the cause of oxidation (i.e., light or heat) which will be discussed further in the following section.

Another potential consequence of squalene oxidation besides the loss of its positive properties is the negative effect of secondary oxidation products. Oxidation of squalene at 60°C for 24 h resulted in the formation of volatile organic hydrocarbons including methane, propene, isobutene, and isoprene (*28*). In a more recent study, heating of squalene at 120°C for a shorter time period identified abundant scission products of squalene, namely ketones and aldehydes (*29*). Although the structures of these ketones and aldehydes were not identified, we assume that these may include compounds that share a similar structure with hexanal and nonanal, which are well-known volatile components of the off-flavor of edible oils (Fig. 2). Interestingly, in the same study, such secondary oxidation products were found to demonstrate a prooxidative effect on olive oil, suggesting the further complications caused by squalene oxidation. Additional studies have shown that the photooxidation of squalene also forms formaldehyde and malonaldehyde via scission products including 6-methyl-5-hepten-2-one (*30*, *31*). Although such studies regarding the secondary oxidation products of squalene are rather limited, the above studies demonstrate the further need for investigation into ways to prevent squalene oxidation.

Table 1. Analysis of squalene content in rice bran oil and its derivatives by various methods.

Material	Method	Squalene concentration	Reference
Rice bran oil	GC-FID	0.53 mg/g	(*8*)
Rice bran oil	HPLC-DAD	3.189 mg/g	(*10*)
Crude rice bran oil	HPTLC-UV (214 nm)	0.36 mg/g	(*4*)
Crude and refined rice bran oil	HPTLC-UV (214 nm)	0.40–0.48 mg/g	(*2*)
Rice bran oil fatty acid distillate	GC-FID	0.69–2.09 mg/g	(*7*)
Rice bran oil fatty acid distillate	HPLC-UV (210 nm)	2.2 wt%	(*9*)
Rice bran oil deodorization distillate	TLC-FID	8.5 wt%	(*3*)
Rice bran oil unsaponifiable concentrate	GC-FID	1.06 wt%	(*6*)
Milled rice	GC-FID	4.0–14.3 μg/g	(*5*)

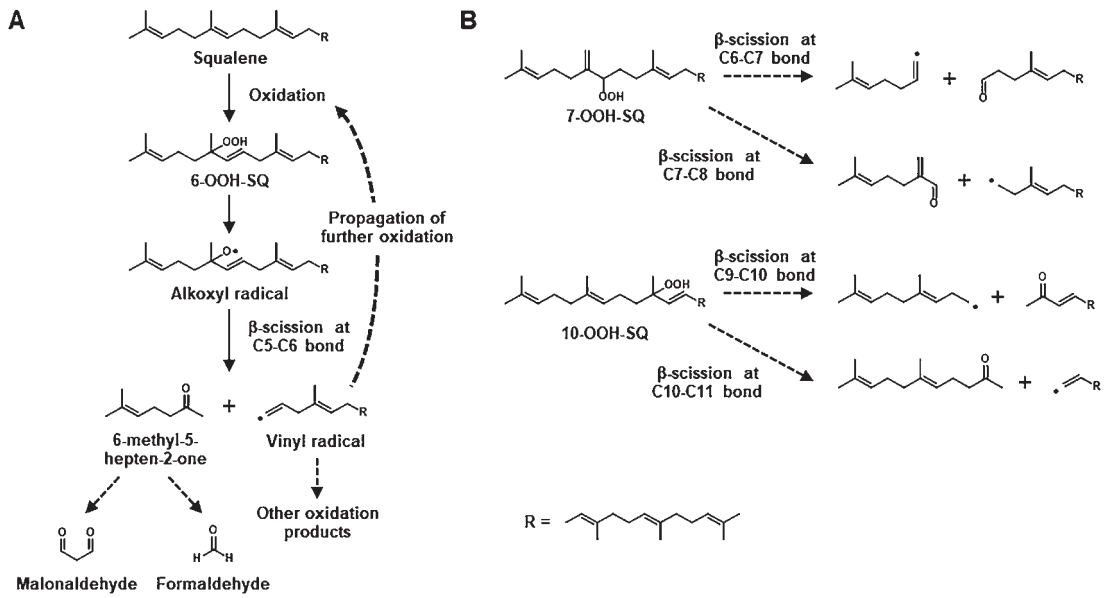

Fig. 2. Proposed pathways by which scission products (i.e., aldehydes and ketones) are formed. (A) Yeo et al. reported the formation of malonaldehyde and formaldehyde from 6-methyl-5-hepten-2-one found in photooxidized squalene samples (*31*). The formation of 6-methyl-5-hepten-2-one is presumably derived from the β-scission of a particular squalene monohydroperoxide isomer (6-OOH-SQ). (B) Since various monohydroperoxide isomers are produced by squalene oxidation, hypothetically, other scission products may also be produced (β-scission of 7-OOH-SQ and 10-OOH-SQ are shown as examples).

3. Evaluation of Squalene Oxidation Mechanisms: LC-MS/MS Analysis of Squalene Monohydroperoxide (SQOOH) Isomers

As described above, evaluation of squalene oxidation with focus on the factors that induce its oxidation (i.e., oxidation mechanisms) is crucial. Oxidation mechanisms, in a more general sense, can be considered as the type of reactive oxygen species that induce lipid oxidation. For example, light-induced oxidation (i.e., photooxidation) is typically caused by singlet oxygen, which directly reacts with double bonds via the ene reaction to form lipid hydroperoxides as the primary oxidation product (*32*, *33*). Such reaction of singlet oxygen with squalene is known to be rather reactive (*20*), and previous studies have demonstrated that squalene monohydroperoxide (SQOOH) was mainly formed (*34*, *35*). On the other hand, heat-induced oxidation (i.e., autooxidation) of lipids is generally caused by free radicals such as the hydroxyl radical and alkoxyl radical. Free radical oxidation is known to initiate from the abstraction of a hydrogen atom to form a lipid radical, and the subsequent addition of molecular oxygen results in the formation of lipid oxidation products (*32*, *36*). With regard to squalene, studies on the free radical oxidation of squalene has led to contrasting views. Whereas early studies identified cyclic dihydroperoxides as the main oxidation product (*37*), more recent studies have reported that alcohols and epoxides are mainly formed (*29*, *38*).

Meanwhile, we have been conducting studies on SQOOH, the primary oxidation product of squalene oxidation, with special focus on its isomers. Squalene, when oxidized, is known to form six SQOOH isomers (Fig. 1B), but the analysis of SQOOH at the isomeric level had not been achieved previously. We were first able to achieve SQOOH isomer analysis by using LC-MS/MS with atmospheric pressure chemical ionization (APCI), but due to the nature of APCI fragmentation, fragment ions with similar patterns were produced, making it difficult to selectively analyze SQOOH isomers (*35*). Hence, we recently developed an improved method that enabled for the highly selective detection of six SQOOH isomers (*39*), based on evidence that the use of sodium ions during LC-MS/MS analysis enables the selective analysis of other lipid hydroperoxide isomers (*40–43*). With the use of this method, it was identified that squalene oxidation by singlet oxygen oxidation and free radical oxidation yield different SQOOH isomers; singlet oxygen oxidation formed six SQOOH isomers in nearly equal amounts whereas free radical oxidation mainly formed 2-OOH-SQ and 3-OOH-SQ (*44*). Moreover, when two antioxidants, α-tocopherol and β-carotene, were evaluated

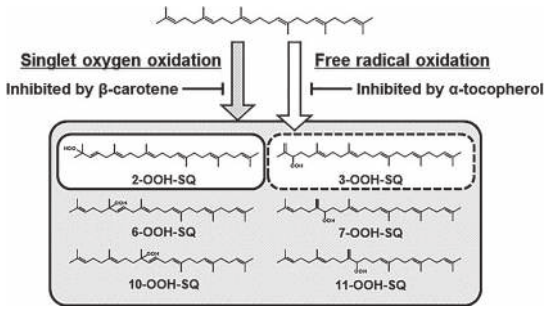

Fig. 3. Singlet oxygen oxidation and free radical oxidation of squalene form different SQOOH isomers and are prevented by different antioxidants.

for their antioxidative effect to prevent squalene oxidation, each antioxidant was shown to be effective mainly towards only one of either singlet oxygen oxidation or free radical oxidation. Such recent results suggest that identifying oxidation mechanisms by analyzing SQOOH isomers, and then applying an antioxidant that is effective towards the identified oxidation mechanism is an effective way to prevent squalene oxidation (Fig. 3).

4. Conclusions and Future Perspectives

Squalene contained in foods including rice bran oil, possesses positive characteristics including its nutraceutical benefits and antioxidative properties. However, squalene is known to be susceptible to oxidation, and oxidative degradation of squalene may cause a decline in such properties. Studies have suggested that the rate of squalene degradation depends on the cause of oxidation, and additionally, secondary oxidation products of squalene have been suggested to negatively affect the properties of various edible oils. As such, it is necessary to evaluate the oxidation of squalene in food products with focus on oxidation mechanisms. With regard to oxidation mechanisms, previous studies have demonstrated that the photooxidation (i.e., singlet oxygen oxidation) of squalene results in the formation of mainly SQOOH, whereas heat-induced oxidation (i.e., free radical oxidation) of squalene has led to contrasting views. Such information regarding the oxidation products derived from the free radical oxidation of squalene is yet to be clarified. Moreover, studies on the detection of squalene oxidation products from actual edible oil samples including rice bran oil is highly anticipated. Finally, our recent results concerning the analysis of SQOOH isomers for determination of squalene oxidation mechanisms may be valuable for prevention of squalene oxidation, and its application to edible oils are targets for future studies.

Disclosure of State of COI

The authors declare no conflicts of interest.

Acknowledgments

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