Food Bioprocess Technol (2008) 1:223–233
DOI 10.1007/s11947-007-0049-3

REVIEW PAPER

Diacylglycerol Oil—Properties, Processes and Products: A Review

Seong-Koon Lo · Chin-Ping Tan · Kamariah Long · Mohd. Suria Affandi Yusoff · Oi-Ming Lai

Received: 8 November 2007 / Accepted: 21 December 2007 / Published online: 4 March 2008

Abstract Diacylglycerol (DAG) oil has beneficial effects on obesity and weight-related disorders. A survey of literature has shown the effects of DAG on the reduction in the accumulation of body fat in both animals and humans. The physiological effect of DAG is believed to be attributed to its metabolic pathway, which is different from triacylglycerol (TAG) metabolism. Physicochemical properties, such as melting and smoke points and polymorphic forms, of DAG are also distinct from TAG. Various patented processes for DAG oil production from several reaction routes are discussed. A review of patent literature of commercial products based on DAG oils and fats is also provided.

Keywords Diacylglycerol oil · Functional lipids · Obesity · Physicochemical properties · Production processes · Products

S.-K. Lo · O.-M. Lai (✉)
Department of Bioprocess Technology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia
e-mail: omlai@biotech.upm.edu.my

C.-P. Tan
Department of Food Technology,
Faculty of Food Science and Technology,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia

K. Long
Malaysian Agricultural Research and Development Institute,
P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

M. S. A. Yusoff
Golden Hope Research Centre Sdn. Bhd.,
P.O. Box 207, 47200 Banting, Selangor, Malaysia

Introduction

Oils and fats are important components of our daily diet. However, health experts have warned that the number of fat or obese people worldwide has risen at an alarming rate. Doctors, scientists, and nutritionists are now investigating on the role of genetics, metabolism, and drugs in contributing to this weight-related problem, as well as new methods to treat it. This, in turn, has directly or indirectly enhanced the very lucrative global antiobesity market, which currently is in excess of USD 240 billion. However, at the other end of the scale, the rapid-growing rate of obesity may bankrupt national health organizations of certain countries (Lean et al. 2006). The functional oils and fats market has certainly benefited from all this publicity. In the past decade, rising consumer awareness and mandatory governmental legislations on unhealthful fats have partly surged the sales volume of functional oil and fat products.

In early 1999, Kao introduced a novel application of diacylglycerol (DAG) oil to the Japanese consumers. Researchers at Kao have found that DAG oil has metabolic characteristics that are distinct from triacylglycerol (TAG) oils. The consumption of DAG oil is claimed to reduce postprandial serum TAG levels and thus is beneficial for the prevention and management of obesity. The physiological differences between DAG and TAG observed in animal and clinical trials are due to distinct metabolic fates of the oils after being absorbed via the gastrointestinal tract. A detailed description on the metabolic fates of DAG and TAG is provided in another section of this review. The DAG cooking oil is marketed under the brand names "Healthy Econa Cooking Oil" in Japan and "Enova Oil" in the USA, after forming a strategic partnership with Archer Daniels Midland. Unlike conventional TAG cooking oils

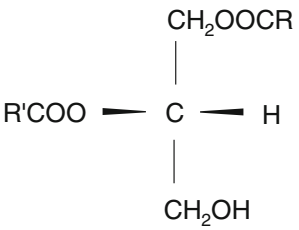
that contains only up to 10% (w/w) DAG, these rather light-tasting healthful cooking oils are claimed to contain 80% (w/w) or more of DAG as the main functional component. In this review, we look into the properties and potential health benefits and highlight patented processes and product formulations of DAG oil.

Physicochemical Properties

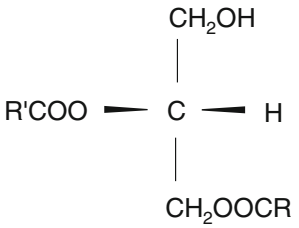
DAGs are esters of the trihydric alcohol glycerol in which two of the hydroxyl groups are esterified with fatty acids. They can exist in two structural isomers namely, 1,2-DAG and 1,3-DAG (Fig. 1). These isomers will undergo acyl migration to form equilibrium at a ratio of 3–4:7–6 between 1,2- and 1,3-DAG (Takano and Itabashi 2002) often in the presence of an acid, alkali, or heat (Sedarevich 1967). 1,3-DAG is more thermodynamically stable because of the steric effect of the molecule.

In general, the melting point of 1,3-DAG is approximately 10°C higher than TAG, and 1,2-DAG is approximately 10°C lower than 1,3-DAG, of the same fatty composition (Benson 1967; Formo 1979; Bockish 1998). The causes of these melting point differences are the strength of hydrogen bonding of the hydroxyl group and fatty acid chain arrangement of the DAG isomers. 1,3-DAG has a V-shaped fatty acid chain arrangement, while 1,2-DAG has a hairpin-shaped conformation (Fig. 2). The type of molecular arrangement of the DAG isomer relates to its polymorphic form. Unlike TAG polymorphism, DAG exhibits two types of polymorphic forms. 1,2-DAG exhibits the α- and β′-forms but has no β-form, while 1,3-DAG has no α-form but exhibits two types of β-form, β1 and the more unstable β2 (Nakajima et al. 2004).

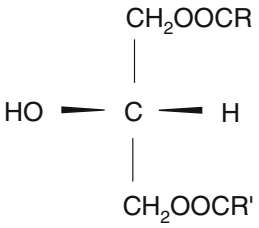
Fig. 1 Structural isomers of DAG



sn-1,2-Diacylglycerol



sn-2,3-Diacylglycerol



sn-1,3-Diacylglycerol

Digestion, Absorption, and Metabolism

DAG can be digested by the same gastrointestinal enzymes that hydrolyze TAG. However, upon digestion, DAG does not follow the resynthetic pathway of TAG, which includes the 2-monoacylglycerol (2-MAG) pathway and the glycerol-3-phosphate (GP) pathway (Friedman and Nylund 1980). In TAG digestion, the human pancreatic lipase hydrolyzes fatty acids from the terminal positions of the TAG molecule to form 1,2- and 2,3-DAG as intermediate products. These intermediate products can be further hydrolyzed by the pancreatic lipase to form 2-MAG. As such, the pancreatic lipase can also hydrolyze 1,3-DAG to form 1(3)-MAG and free fatty acids (Kondo et al. 2003). The key characteristic of DAG metabolism lies in the formation of 1(3)-MAG rather than 2-MAG as found in TAG metabolism.

In TAG absorption, 2-MAG will undergo re-esterification with fatty acids via the 2-MAG pathway to reform TAG, which will then be transported as a chylomicron complex into the blood stream via the lymphatic system. In the case of DAG, however, 1(3)-MAG is poorly re-esterified via the 2-MAG pathway and thus has an insignificant contribution toward TAG resynthesis (Lehner et al. 1993). Instead, substantial amounts of 1(3)-MAG are further hydrolyzed to free fatty acids and glycerol, which are precursors of the GP pathway, while some are re-esterified to 1,3-DAG (Mansbach and Nevin 1998). The efficiency of DAG digestion products to convert into TAG in the small intestines was also found to be low. Another important difference between DAG and TAG metabolism is the substrate specificity of the DAG acyltransferase (DGAT) enzymes, DGAT-1 and DGAT-2, in the small intestines (Cases et al. 1998, 2001). DGAT is involved in the final synthetic step of TAG by catalyzing the acylation of 1,2 (2,3)-DAG, which are products from the 2-MAG pathway (Bell and Coleman 1980; Lehner and Kuksis 1996). However, DGAT has low substrate specificity toward 1,3-

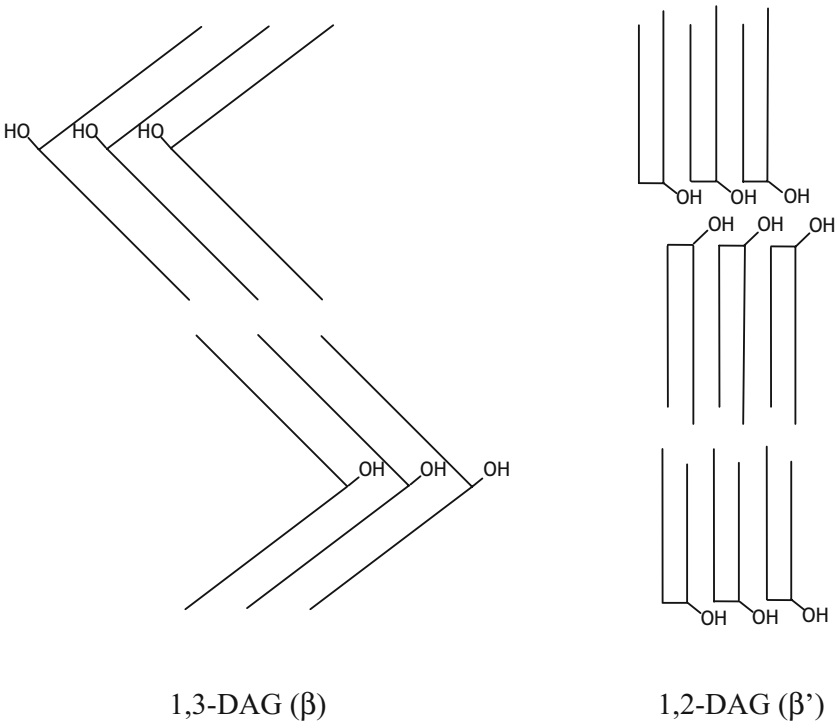


Fig. 2 Crystal arrangement of β-form 1,3-DAG and β′-form 1,2-DAG

DAG and therefore does not significantly convert 1,3-DAG to TAG (Lehner and Kuksis 1993). Conclusions from these findings on the nature of DAG digestion and absorption may explain the reduction of postprandial TAG levels in the blood.

Numerous animal (Murata et al. 1997; Watanabe et al. 1997; Murase et al. 2002a, b; Meng et al. 2004) and clinical (Kamphuis et al. 2003) studies have all shown that ingestion of DAG oil, in comparison with TAG oil, increases the rate of β-oxidation of fatty acids. Additionally, the activities of enzymes involved in fatty acid synthesis, such as glucose-6-phosphate dehydrogenase, malic enzyme, and fatty acid synthetase, were observed to significantly decrease in subjects fed with DAG oil (Murata et al. 1997). On the other hand, hepatic enzymes involved in the β-oxidation pathway, such as acyl-coenzyme A (CoA) dehydrogenase, acyl-CoA oxidase, enoyl-CoA hydratase, carnitine palmitoyltransferase, 3-hydroxyacyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, and δ3,δ2-enoyl-CoA isomerase, were found to increase in activity on a dose-dependent manner with DAG intake (Murata et al. 1997). In a clinical study (Kamphuis et al. 2003), it was interesting to note that the increase in β-oxidation occurred even without any change in daily energy expenditure resulting from resting activity or activity related to physical exertion. It is believed that the increase in β-oxidation of DAG metabolism relates to the corresponding reduction in body fat and serum TAG levels.

Potential Health Benefits

The ingestion of DAG oil has been shown to reduce body fat accumulation (Nagao et al. 2000; Murase et al. 2001, 2002a, b; Maki et al. 2002; Meng et al. 2004) and lower serum TAG levels (Hara et al. 1993; Murata et al. 1994; Taguchi et al. 2000; Tada et al. 2001; Yamamoto et al. 2001; Kondo et al. 2003; Yanagisawa et al. 2003; Yamamoto et al. 2005). Murase et al. (2001) reported a 70% reduction in body weight of mice after 5 months on a diet containing 30% DAG oil. Significant fat reductions surrounding the epididymal, mesenteric, retroperitoneal, and perirenal areas were observed. However, other animal tests (Sugimoto et al. 2003a, b) revealed that the effect of DAG oil on reducing body weight was not found at low intakes (10% or less) of DAG.

To compare the effects of DAG and TAG oil ingestion on human body fat, Nagao et al. (2000) had conducted a 16-week double-blind study on 38 healthy men with an average body mass index (BMI) of approximately 24 kg/m^2. The subjects were randomly provided with food products that contain either DAG or TAG oil at a dosage of 10 g/day during the treatment period. The results revealed that reductions in body weight, BMI, waist circumference, and total fat area were more distinct in subjects consuming the DAG oil diet (Table 1). In another related study, Maki et al. (2002) observed similar findings in a 6-month investigation on the effect of diets rich in DAG and TAG oils on obese men and women with BMI of

Table 1 Changes in anthropometric and body composition values of men consuming DAG or TAG oil as part of their daily diet in a 16-week study

		DAG oil	TAG oil
Body weight (kg)	Baseline	72.1	68.1
	Change	−2.6	−1.1
Body mass index (kg/m^2)	Baseline	24.1	23.5
	Change	−0.9	−0.4
Waist circumference (cm)	Baseline	85.0	82.0
	Change	−4.4	−2.5
Hip circumference (cm)	Baseline	97.1	96.1
	Change	−1.1	−0.7
Waist/hip ratio	Baseline	0.87	0.85
	Change	−0.04	−0.02
Body fat (g/100 g)	Baseline	21.6	20.3
	Change	−1.1	−1.5
Total fat area (cm^2)	Baseline	227	182
	Change	−38	−17
Visceral fat area (cm^2)	Baseline	79	56
	Change	−16	−5
Subcutaneous fat area (cm^2)	Baseline	148	126
	Change	−22	−8
Visceral/subcutaneous fat ratio	Baseline	0.55	0.46
	Change	−0.05	0.00
Liver/spleen ratio[a]	Baseline	1.24	1.23
	Change	0.06	0.01

Source: Nagao et al. (2000)

[a] Ratio of Hounsfield units of computed tomography.

approximately 34 kg/m^2. On the contrary, several studies did not find any significant change in body fat reduction of the subjects after the treatment period (Flatt 1988; Schutz 1995; Nagao et al. 2000).

Studies investigating the effect of DAG oil on serum TAG levels in animals and humans also showed inconsistent results. Reductions in serum TAG levels were observed in some studies (Hara et al. 1993; Murata et al. 1994; Taguchi et al. 2000; Tada et al. 2001; Yamamoto et al. 2001, 2005; Kondo et al. 2003; Yanagisawa et al. 2003), while in others, there was no change in serum TAG levels (Nagao et al. 2000; Meguro et al. 2001; Soni et al. 2001; Maki et al. 2002; Kamphuis et al. 2003; Sugimoto et al. 2003a, b). Hara et al. (1993) investigated on the effect of feeding diets containing 10% DAG oil to rats and concluded that DAG has an ability to reduce serum TAG levels. Murata et al. (1994) suggested that the ingestion of DAG oil decreased serum TAG levels by retarding the chylomicron assembly that is essential for TAG transportation. In another study, Kondo et al. (2003) reported that TAG synthesis in DAG-infused rats was less pronounced than in TAG-infused rats. On the other hand, Taguchi et al. (2002) did not find any decrease in serum TAG levels of rats fed with diets containing 30% DAG oil. It is interesting to note that it was found that the DAG oil suppressed the hepatic activity of microsomal TAG transfer protein (MTP) and MTP messenger ribonucleic acid. MTP is an important component for the transportation of TAG from the site of synthesis to the site of assembly with apolipoprotein B at the endoplasmic reticulum. In a study using obesity-prone mice, the effect of DAG diet on reducing serum TAG level was shown after 8 months of treatment (Murase et al. 2001). Murase et al. (2001) suggesting that the upregulation of lipid metabolism and β-oxidation may be partially responsible for the lower serum TAG levels. Murase et al. (2001) also observed that significant reductions in serum cholesterol levels were observed in these obese-prone rats. Other studies (Murase et al. 2002a, b) suggest that DAG stimulates intestinal lipid metabolism by changing the mucosal lipid profile, which, in turn, affects the regulation of gene expression in the small intestine.

Similar discrepancies were also observed in various clinical studies, where some studies confirmed that DAG intake was able to decrease serum TAG levels (Taguchi et al. 2000; Tada et al. 2001; Yamamoto et al. 2001, 2005; Yanagisawa et al. 2003), while others reported otherwise (Nagao et al. 2000; Meguro et al. 2001; Maki et al. 2002; Kamphuis et al. 2003). However, DAG intake was shown to be beneficial in reducing serum TAG levels in young women with hyperlipidemia-prone variants of fatty acid-binding protein 2 and MTP (Yanagisawa et al. 2003) and in type 2 diabetic patients (Yamamoto et al. 2001). Additionally, the consumption of DAG oil also reduces glycosylated hemoglobin (HbAlc) concentrations in these diabetic patients (Yamamoto et al. 2001). The level of HbAlc is an accurate indication of the recent overall blood glucose concentration in diabetic patients. However, no significant reductions in serum TAG levels were noted in studies involving overweight and obese subjects (Nagao et al. 2000; Maki et al. 2002). These inconsistent results may be attributed to different quantities of DAG in the study diets, as well as different physiological condition of the subjects, e.g., healthy, hyperlipidemic, diabetic, overweight, and obese. DAG oil is also beneficial in reducing remnant-like lipoprotein particles (RLPs; Tada et al. 2001). RLPs are atherogenic metabolites of TAG-rich lipoproteins such as chylomicrons and very-low-density lipoproteins. However, in another study, high-density lipoprotein (HDL) cholesterol was found to increase with DAG ingestion (Teramoto et al. 2004). In spite of these inconsistencies, DAG oil has proved to be beneficial for reducing body fat accumulation in both animal and human subjects.

Production Process Patents

Various routes for the production of DAG oil have been reported in patent literature. In general, DAG oil can be

produced via glycerolysis between TAG and glycerol, esterification of fatty acids or its derivatives to glycerol, hydrolysis of TAG, or a combination of methods thereof. These processes often involve either a chemical or an enzyme catalyst. Apart from these methods for the production of DAG, other related technologies such as DAG separation and purification methods that complement well with DAG production have also been patented. In this section, patent literature on processes related to DAG oil is reviewed.

There are a number of patents for the production of DAG oil via esterification of fatty acids or its derivatives such as fatty acid anhydrides and fatty acid methyl esters. Mazur and Hiler's (1992) process involves the esterification of 3–40% of fatty acid anhydride with 3–40% of glycerol in the presence of a water-immiscible hydrocarbon or chlorinated hydrocarbon solvent such as methylene chloride. The reaction is catalyzed by a 1,3-position-specific immobilized lipase. DAG yield of 41% (w/w) is reported. This method involves the use of chlorinated solvents, which may pose a problem in waste management. Additionally, the use of such solvents may not be an attractive marketing option for DAG oil. Lo and Baharin (2001) reported on a solvent-free method for the production of DAG from fatty acid deodorizer distillates obtained from edible oil refineries. In this method, DAG oil is produced by esterification of free fatty acids present in the deodorizer distillates with glycerol, which is added into the reaction. Similarly, the process is catalyzed by a 1,3-position-specific immobilized lipase. A yield of 60% (w/w) of DAG oil can be obtained. The advantage of this process is in its use of a lower-cost raw material compared with refined fatty acids and thus may positively reflect on the production cost of the DAG oil. However, because the process requires the use of an immobilized enzyme as catalyst, the process cost may not be low. To provide a solution to the high cost of enzymes, Lai et al. (2007) reported on the use of a strongly acidic cation exchange resin as a chemical catalyst for the synthesis of DAG oil from free fatty acids. The application of the heterogenous catalyst will allow for easy separation of the catalyst from the reaction products. The cost of the ion-exchange resins is also significantly cheaper than that of commercial immobilized lipases. Another advantage of this process involves the use of relatively lower temperatures as compared to other chemically catalyzed synthetic processes. However, one major drawback of this process is the use of more expensive free fatty acids compared with TAG as raw materials. Yoon et al. (2004) disclosed a process for the production of DAG oil containing conjugated linoleic acids, comprising of esterifying MAGs with free fatty acids in the presence of a lipase. A similar process for DAG production involving transesterification between MAG and TAG has been invented by Toshinori et al. (2000). These processes utilizing MAG as raw materials for DAG production may not be industrially attractive as the cost of MAG is relatively high.

DAG oil can also be produced from partial hydrolysis of TAG. Lai et al. (2006) discloses an enzymatic process for partial hydrolysis of TAG to produce DAG. A commercial immobilized lipase was used to catalyze the hydrolysis of TAG under controlled conditions to produce DAG oil. The advantage of this process lies in the single-step hydrolytic reaction of TAG without further addition of other substrates such as glycerol. However, precise control of water content in the reaction system is required for optimal DAG yield.

Sugiura et al. (2002a) disclosed a glycerolysis process to produce DAG from TAG and glycerol, in the presence of small quantities of water and lipase to assist catalysis. The glycerolysis reaction is conducted at relatively lower temperatures (0–25°C). As such, the DAG product is removed by crystallization during the course of reaction. This method of DAG separation may not be cost effective in large-scale production as longer reaction times are required (20–100 h). Jacobs et al. (2003) reported on a glycerolysis process for producing DAG oil from TAG and glycerol using potassium acetate as a catalyst. The process is claimed to provide a crude DAG product with good color. However, the process requires a relatively high reaction temperature of 190–240°C and therefore may translate to a significant energy cost. Nevertheless, the economics of this process is compensated by the use of low-cost raw materials and catalyst.

Another process for the production of DAG oil involves a combination of hydrolysis and esterification reactions (Yamada et al. 1999). The process comprises of hydrolyzing TAG oil to obtain free fatty acids, followed by esterification of these free fatty acids, without further purification, with glycerol to produce DAG. The hydrolysis step may be performed using steam or in the presence of a lipase. For the esterification step, an immobilized lipase is required for catalysis. In comparison with other enzymatic processes for DAG oil production, this process has the potential for industrial feasibility. As a follow-up to this process, Sugiura et al. (2002b) up-scaled this process into a production plant setting and described that high-purity DAG oil can be produced at high yields and in a short time by carrying out esterification reaction of fatty acids with glycerol in an immobilized enzyme-packed tower. Sugiura et al. described that the residence time for the reaction substrates in the tower should not be more than 120 s to prevent increased concentrations of TAG. From an engineering viewpoint, the superficial velocity of the substrate is preferably not lower than 1 mm/s so as to minimize mass transfer resistance between solid and liquid and to reduce the reaction rate. Based on Kozeny-Carman's equation, Sugiura et al. determined that the ratio between the packing

thickness of the immobilized lipase, L, and the squared average particle diameter of the immobilized lipase, d^2; defined as L/d^2, should be controlled between a value of 3–25 to maintain a pressure drop of 20 kg/cm^2 or less. A lower pressure drop is desirable in minimizing plant cost. In addition, water formed during the reaction is simultaneously removed under reduced pressure. The effects of various process parameters on the yields of DAG and TAG are shown in Table 2. Based on this method, a DAG yield of 65% (w/w) is reported.

Another interesting method of producing DAG oil was disclosed by Choo et al. (2007). According to the invention, an edible oil with high DAG content of at least 8% (w/w) can be produced from TAG oil of vegetable origin by subjecting the vegetable oil to short-path distillation under vacuum of not more than 0.01 Torr and at temperature of 300°C and below, wherein the DAG oil is obtained as the distillate. As mentioned earlier, vegetable oils generally do not contain more than 10% (w/w) of DAG. Because of the relatively low DAG content in vegetable oils, the DAG oil yield obtained from this process will be at most 10% (w/w). The low DAG yield will translate to a high production cost of DAG oil, thus making this process industrially unattractive.

Product Application Patents

The versatility of DAG oil is evident as numerous applications, for example, as a cooking oil, frying oil, salad oil, salad dressing and mayonnaise, shortenings and margarines, chocolates, ice cream fats, confectioner's fats, specialty oils with enriched essential fatty acids, fried and baked food products, beverages, formulations with phytonutrients, and products with specific physiological benefits, are reported in patent literature. The following is a summary of product applications comprising DAG oil as the functional component.

DAG Oil Composition for Specific Physiological Benefits

The main physiological benefit of DAG oil is its ability to reduce body fat and therefore prevent or treat obesity. Koike et al. (2003) disclosed an oil composition for such a purpose, which comprises 5–100% (w/w) of a MAG and/or a DAG with a fatty acid composition of 15–90% (w/w) of ω-3 unsaturated fatty acids based on MAG and/or DAG content. Additionally, the oil composition contains an antioxidant to prevent oxidation. Another DAG oil composition claimed to exhibit excellent inhibitory effect on body

Table 2 Effects of various process parameters on DAG and TAG yields

	Run number						
	1	2	3	4	5	6	7
Batch size (kg)	100	100	100	100	100	100	1
Oleic acid (kg)	86	86	86	86	86	86	0.86
Glycerol (kg)	14	14	14	14	14	14	0.14
Immobilized lipase	Lipozyme IM	Lipozyme IM	Lipozyme IM	Lipozyme IM	Lipozyme IM	Lipozyme IM	Lipozyme IM
Average particle diameter, d (mm)	0.43	0.43	0.43	0.43	0.43	0.08	0.43
Amount (kg)	5	5	5	5	20	5	0.1
Packing thickness, L (m)	0.18	0.18	0.18	0.18	0.7	0.18	0.33
Superficial velocity, U (mm/s)	4.4	2.2	4.4	1.1	3.7	0.5	2.0
Residence time (s)	40	79	40	158	190	351	164
L/d^2	0.95	0.95	0.95	0.95	3.80	27.50	1.80
Pressure loss, P (kg/cm^2)	2.6	1.5	2.6	0.7	9.5	8.5	2.5
Reaction time (h)	3.5	3.5	3.5	3.5	3.5	3.5	7.0
Oleic acid residue (wt%)	14.1	15.4	16.4	43.1	11.6	47.8	12.4
Glycerol residue (wt%)	0.3	0.7	0.5	2.1	0.4	2.7	0.1
Monoacylglycerol (MAG) product (wt%)	14.1	18.3	16.8	15.3	15.0	16.4	9.3
Diacylglycerol (DAG) product (wt%)	65.6	58.1	59.5	32.8	55.7	26.8	63.0
Triacylglycerol (TAG) product (wt%)	5.9	7.5	6.8	6.7	17.3	6.3	15.2
Yield (DAG + TAG)	71.5	65.6	66.3	39.5	73.0	33.1	78.2

Source: Sugiura et al. (2002b).

fat accumulation and having good flavor, color, and hydrolytic and oxidative stabilities was invented by Takase et al. (2003). The oil is comprised of 15–70% (w/w) of DAG in which less than 15% (w/w) of the fatty acids are ω-3 unsaturated fatty acids and 30–85% (w/w) of TAG in which at least 15% (w/w) of the fatty acids are ω-3 unsaturated fatty acids. This composition also contains a small amount of an antioxidant.

Masui et al. (2001) described a DAG oil composition capable of reducing arteriosclerotic factors in the blood and thereby lowering the risks of arteriosclerosis and other degenerative diseases. The authors have observed that when an oil composition comprising of at least 35% (w/w) of DAG, wherein the constituents of the fatty acids of the DAG oil satisfy the following equation: Amount of cis-form unsaturated fatty acid/amount of saturated fatty acid + amount of trans-form unsaturated fatty acid≥6, wherein the amount of trans-form fatty acid is not exceeding 5% (w/w) based on fatty acids of DAG oil, the intake of DAG oil with such composition will increase HDL cholesterol level and reduce the activity of plasminogen activator inhibitor type 1 (PAI-1), which controls the production of plasmin in the blood. A lower activity of PAI-1 is essential for the prevention of arteriosclerosis. Another oil composition for the prevention of arteriosclerosis was invented by Koike et al. (2001). It is claimed by the inventors that an oil composition containing 10–40% (w/w) of DAG, in which at least 55% (w/w) of the fatty acids are unsaturated and 15–100% (w/w) of these fatty acids are ω-3 unsaturated fatty acids with at least 20 carbon atoms, and 40.1–89.8% (w/w) of TAG, in which at least 70% (w/w) of the fatty acids are unsaturated and 5–80% (w/w) of these fatty acids comprise of linoleic acid, could prevent arteriosclerosis. In addition to being antiarteriosclerotic, it is also claimed that such an oil composition has excellent oxidation stability and good flavor. Another closely similar oil composition was again invented by Koike et al. (2002c). The authors describe an oil or fat with excellent visceral fat-burning property, body fat-burning property, and stability against autoxidation. According to this invention, the oil comprises of 60–100% (w/w) of DAG, wherein the fatty acid composition of the oil consists of 15–90% (w/w) of an ω-3 unsaturated fatty acid having less than 20 carbon atoms and the weight ratio of *cis*-ω-3 unsaturated fatty acid to the sum of *cis*-ω-6 unsaturated fatty acid, saturated fatty acid, and *trans*-unsaturated fatty acid is from 1 to 6.

Koike et al. (2002b) revealed another DAG oil composition with a specific physiological effect. As described in this invention, the consumption of an oil or fat with a composition comprising of 5–99.9% (w/w) MAG having a fatty acid composition of 15–90% (w/w) of an ω-3 unsaturated fatty acid of less than 20 carbon atoms, 1–80% (w/w) of an ω-9 unsaturated fatty acid, and 2–50% (w/w) of an ω-6 unsaturated fatty acid and 0.1–49.9% (w/w) DAG, wherein the weight ratio of DAG to MAG is below 1 and the content of polyunsaturated fatty acid with four double bonds is 20% (w/w) or less based on total fatty acid composition, has a beneficial effect of lowering glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in the blood. High levels of GOT and GPT is often released into the blood when there is a liver or heart malfunction. This formulation is claimed to be useful in pharmaceutical products and foods for the prevention of hepatic function disturbances and obesity.

Another DAG oil formulation for inhibition of platelet aggregation and reduction in body fat, while simultaneously having excellent oxidative stability, flavor, and flowability, was again invented by Koike et al. (2002a). According to the invention, the oil formulation is made up of 0.1–59.8% (w/w) TAG, 40–99.7% (w/w) DAG, 0.1–10% (w/w) MAG, and 0–5% (w/w) free fatty acids, wherein the DAG consists of 15–89.5% (w/w) of ω-3 unsaturated fatty acids with at least 20 carbon atoms and 10–84.5% (w/w) of monounsaturated fatty acids.

DAG oil is also formulated to lower blood sugar level, improve insulin resistance, and reduce the effect of leptin, in addition to resisting the accumulation of body and visceral fat (Koike et al. 2002d). Serum leptin levels are reported to be closely linked to the amount of fat in the body (William et al. 2001). In this invention, an oil or fat composition comprising of 10.1–94.9% (w/w) TAG, 0.1–30% (w/w) MAG, and 5–59.9% (w/w) DAG, which consists of 15–90% (w/w) of an ω-3 unsaturated fatty acid with less than 20 carbon atoms, is described to possess such properties.

Oil-in-Water Type Emulsion Foods

Oil-in-water type emulsion (O/W) food products are commonly represented by mayonnaise and salad dressings. In general, mayonnaise and salad dressings contain oil, egg yolk, vinegar, and seasonings (salt, sugar, spices, flavors, etc.). The major difference between mayonnaise and salad dressings is in the oil content. Mayonnaise has an oil content of 65–85% (w/w), while salad dressing has less than 60% (w/w) oil. The application of DAG oil in O/W products was first patented by Nomura et al. (1992). According to the invention, the O/W product has an oil phase comprising of 30–100% (w/w) DAG with a melting point of 20°C or less. The O/W composition is claimed to exhibit a rich fatty savor even at a low fat content. Several years later, Kawai and Konishi (2000) describes an O/W composition that has excellent storage stability, good appearance, taste, and physical properties. The composition has an oil phase that is comprised of 30% (w/w) or greater

of DAG oil and a yolk wherein the ratio of lysophospholipids to the whole phospholipids is at least 15% (w/w) based on phosphorous content. Shiiba et al. (2002) provided further improvements by disclosing an O/W composition that has excellent shelf stability at low temperatures comprising at least 20 (w/w) and 0.5–5% (w/w) of a crystallization inhibitor. The crystallization inhibitor is a polyglycerol fatty acid ester, sucrose fatty acid ester, or sorbitan fatty acid ester.

Water-in-Oil Type Emulsion Foods

The other form of emulsified food product has a water-in-oil type emulsion (W/O). Examples of such products are margarine, spreads, butter cream fillings, and icings used in baking and the confectionery industry. Mori et al. (1999a) invented a W/O-emulsified fat composition that has good stability and spreadability and is suitable for use as a margarine. The W/O composition is made up of 40 to less than 95% (w/w) of DAG and 5 to less than 60% (w/w) of TAG, wherein the DAG comprises of 0.5 to less than 20% (w/w) DAG containing two saturated C14–C22 fatty acid groups, 20 to less than 55% (w/w) DAG containing one saturated C14–C22 fatty acid group and one unsaturated C14–C22 fatty acid group, and 25 to less than 70% (w/w) DAG containing two unsaturated C14–C22 fatty acid groups, and the weight ratio of total C14 and C16 saturated fatty acid groups in DAG to total C18, C20, and C22 saturated fatty acid groups in DAG is in the range from 1 to 8. Another W/O composition claimed to have excellent flavor release during the time of ingestion was invented by Masui and Konishi (2001). According to this invention, it is claimed that 30% (w/w) of the W/O oil or fat composition is being able to reverse in phase within 1 min after coming into contact with water at 36°C, thereby releasing the flavor component. The W/O composition comprises of water as the aqueous phase, 15% (w/w) or more of DAG as the oil phase, and a demulsifier, which comprises at least a polyglycerol fatty acid ester having a hydrophilic–lipophilic balance (HLB) value of 8 or more, a water-soluble decomposed protein, lysolecithin having a HLB value of 8 or more, a sucrose fatty acid ester having a HLB of 5 or more, a MAG organic acid ester having a HLB of 8 or more, and a sorbitan fatty acid ester having a HLB of 8 or more. A W/O composition by Masui and Yasunaga (2001) describes a W/O product that is stable in spite of containing a high water content and has good storage and mouth feel. The product composition comprises of water as the aqueous phase, 35–95% (w/w) of DAG having a melting point of below 20°C, and the remainder as TAG, which is composed of 13–60% (w/w) palmitic acid and 5% (w/w) or less of fatty acid having 12 carbons or lower, as the oil phase. Additionally, the TAG has to possess a stable polymorphic form of β′.

DAG Oil Composition Containing Phytosterols

P1hytosterols are lipid compounds that have been shown to lower serum cholesterol levels in humans (Ling and Jones 1995; Jones et al. 1997). Because of its limited solubility in oil (approx. 1% w/w) and insolubility in water, normal intake of phytosterols are not efficiently absorbed by the intestines and therefore ineffective in lowering serum cholesterol levels. Efforts were made to increase solubility of phytosterols in oil by converting it into phytosterol fatty acid esters (Hendriks et al. 1999). However, Meguro et al. (2001) reported that phytosterols can achieve higher solubility in DAG oil without the need of esterification. Several patent literatures were found on DAG oil composition containing dissolved phytosterols.

Goto et al. (2000a, b) claimed that the solubility of phytosterol can be increased by 1.2–20% (w/w) when 15% (w/w) or more of DAG oil is used as solvent. Additionally, the authors claimed that 80% (w/w) or more of DAG oil can dissolve 0.05–20% (w/w) of phytosterols. However, 55% (w/w) or more of unsaturated fatty acids have to be present in the DAG oil for effective solubilization of the phytosterols. In another patent (Goto et al. 2001), an oil composition containing 15% (w/w) or more of DAG and up to 2,000 ppm of tocopherol was reported to effectively dissolve 1.2–20% (w/w) of phytosterols. The DAG component is comprised of at least 70% (w/w) unsaturated fatty acids. Nakajima et al. (2002) made further improvements of phytosterol solubility in DAG oil in a disclosure whereby an oil composition containing 15–95% (w/w) is used to dissolve 2–10% (w/w) of phytosterol and the resultant oil composition remains a transparent liquid at temperatures of 0–30°C.

Shortenings

DAG also finds an application in the formulation of shortenings. Doucet and Olathe (1999) invented a shortening composition comprising a nonhydrogenated vegetable oil and a stearine fraction containing 50–60 mol% of DAG. It is claimed that the shortening formulation has a synergistic amount of solids and crystal matrices that imparts superior organoleptic properties to the food product, without the incorporation of trans-fatty acids commonly found in partially hydrogenated fats. Concomitantly, Doucet et al. (1999) claimed that the above effects can also be made possible with the addition of MAG composed predominantly of saturated fatty acids.

Frying Applications

Because DAG has a lower molecular weight than TAG, DAG oil has a significantly lower smoke point (30–40°C)

than TAG oil with similar fatty acid compositions. Therefore, frying applications with DAG oil will be problematic. Several DAG compositions suitable for use as frying oil have been reported. Sakai et al. (2002a) reported a fat composition containing at least 15% (w/w) of DAG, a fatty acid L-ascorbic ester, and a component such as catechin or a natural plant extract such as rosemary, sage, and turmeric extracts. The authors claimed that the DAG composition has excellent stability toward oxidation, while providing good flavor and appearance. Another DAG composition reported by Sakai et al. (2002b) contains 15% (w/w) or more of DAG and 70 ppm or more of one or more types of organic carboxylic acids such as two- to eight-carbon hydroxycarboxylic or dicarboxylic acids and their derivatives thereof. The composition is claimed to resist thermal oxidation or hydrolysis after prolonged heating or storage, as well as to reduce smoking when the oil composition is used for frying purposes.

Foods Containing DAG Oil

Foods that are prepared with or contain DAG oil are summarized in this section. Mori et al. (1999b) disclosed a fried food with a fat composition containing 55 to less than 95% (w/w) of DAG, which composed of 55 to less than 93% (w/w) of unsaturated fatty acids. The authors claimed that when the food is fried with DAG oil of such composition, the resultant fried food will have a low water content and will not likely get moist and reduce in crispiness over a prolonged period of time. The fried foods that are covered in this patent are fried cakes, French fried potatoes, fried chicken, and doughnuts. A disclosure from Mori and Watanabe (2000) described a food that is comprised of 0.5–85% (w/w) DAG with C2–C10 fatty acids. When ingested, the food composition is claimed to possess good organoleptic properties, as well as to reduce body fat accumulation and provide energy at times of exhaustion and fatigue.

Another DAG-containing food product as reported by Kudo et al. (2002) is fried or baked potatoes, which comprise of 3–50% (w/w) of oil or fat, wherein the DAG content is 15% to less than 50% (w/w). The fatty acids of the DAG oil are composed of 15–100% (w/w) of ω-3 unsaturated fatty acids of less than 20 carbon atoms. Similar to the findings of Mori et al. (1999b), potatoes fried or baked in such a DAG oil composition was reported to provide a product that has a low water content, favorable texture and taste, and good storage stability.

Ice Cream Coating Fats

Ice cream coating fats are generally TAG of medium-chain fatty acids, such as lauric acid-rich coconut oil. The first use of DAG as an ice cream coating fat was reported by Cain et al. (1999). The fat composition is comprised of 50–90% (w/w) DAG and 10–50% (w/w) TAG of vegetable origin. The DAG oil is composed of 75–90% (w/w) diunsaturated DAG, less than 5% (w/w) disaturated DAG, and 10–25% (w/w) DAG with one unsaturated and one saturated fatty acid. The TAG composition in this fat composition is such that the sum of triunsaturated and diunsaturated TAG is at least 50% (w/w). According to this invention, the ice cream coating fat had resulted in a product that is softer and less brittle but had quicker and smoother meltdown, than cocoa butter-based coating fats.

Conclusions and Outlook

Numerous scientific reports have shown the effectiveness of DAG in preventing body fat accumulation and obesity-related disorders. The commercial potential of DAG has prompted various patent publications on production technologies and product applications of DAG oil. However, greater emphasis is required to further reduce the overall cost of DAG oil to meet consumer expectations. At the current growth rate of obese population throughout the world, it can be expected that the global market demand for DAG oil will increase in the future.

Acknowledgment The authors thank Golden Hope Research Centre Sdn. Bhd. for financial support.

References

Bell, R. M., & Coleman, R. A. (1980). Enzymes of glycerolipid synthesis in eukaryotes. *Annual Review of Biochemistry*, *49*, 459–487.

Benson, F. R. (1967). Polyol surfactants. In M. J. Shick (Ed.), *Nonionic surfactants* (pp. 247–299). New York, NY: Marcel Dekker.

Bockish, M. (1998). Composition, structure, physical data, and chemical reactions of fats and oils, their derivatives, and their associates. In M. Bockish (Ed.), *Fats and oils handbook* (pp. 53–120). Illinois, USA: AOCS.

Cain, F. W., Manson, H. G. A., Quinlan, P. T., & Moore, S. R. (1999). *Ice-cream coating fats*. US Patent no .US5891495 (in English).

Cases, S., Smith, S. J., Zheng, Y. W., Myers, H. M., Lear, S. R., Sande, E., et al. (1998). Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 13017–13023.

Cases, S., Stone, S. J., Zhou, P., Yen, E., Tow, B., Lardizabal, K. D., et al. (2001). Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. *Journal of Biological Chemistry*, *276*, 38870–38876.

Choo, Y. M., Puah, C. W., Ma, A. N., & Basiron, Y. (2007). *Production of edible oil*. US Patent Application no. US20070021625 (in English).

Doucet, J., & Olathe, K. (1999). *Shortening system, products therewith, and methods for making and using the same*. US Patent no. US5908655 (in English).

Doucet, J., Olathe, K., Rethwill, C. E., McHugh, K., & Willhelm, C. L. (1999). *Shortening system*. European Patent Application no. EP1057887 (in English).

Flatt, J. P. (1988). Importance of nutrient balance in body weight regulation. *Diabetes/Metabolism Reviews*, *4*, 571–581.

Formo, M. W. (1979). Physical properties of fats and fatty acids. In D. Swern (Ed.), *Bailey's industrial oil and fat products* (vol. 1, 4th ed., pp. 53–120). Illinois, USA: AOCS.

Friedman, H. I., & Nylund, B. (1980). Intestinal fat digestion, absorption and transport. *American Journal of Clinical Nutrition*, *33*, 1108–1139.

Goto, N., Nishide, T., Tanaka, Y., & Yasukawa, T. (2000a). *Oil and fat composition containing phytosterol*. US Patent no. US6025348 (in English).

Goto, N., Nishide, T., Tanaka, Y., Yasukawa, T., & Masui, K. (2000b). *Phytosterol-containing fat composition*. European Patent Application no. EP0990391 (in English).

Goto, N., Nishide, T., Tanaka, Y., Yasukawa, T., & Masui, K. (2001). *Oil or fat composition containing phytosterol*. US Patent no. US6326050 (in English).

Hara, K., Onizawa, K., Honda, H., Otsuji, K., Ide, T., & Murata, M. (1993). Dietary diacylglycerol-dependent reduction in serum triacylglycerol concentration in rats. *Annals of Nutrition and Metabolism*, *37*, 185–191.

Hendriks, H. F. J., Weststrate, J. A., van Vliet, A. T., & Meijer, G. W. (1999). Spread enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. *European Journal of Clinical Nutrition*, *53*, 319–327.

Jacobs, L., Lee, I., & Poppe, G. (2003). *Chemical process for the production of 1,3-diglyceride oils*. PCT International Patent no. WO03029392 (in English).

Jones, P. J. H., MacDougall, D. E., Ntanious, F., & Vanstone, C. A. (1997). Dietary phytosterols as cholesterol-lowering agents in humans. *Canadian Journal of Physiology and Pharmacology*, *75*, 217–227.

Kamphuis, M. M., Mela, D. J., & Westerterp-Plantenga, M. S. (2003). Diacylglycerol affects substrate oxidation and appetite in humans. *American Journal of Clinical Nutrition*, *77*, 1133–1139.

Kawai, S., & Konishi, Y. (2000). *Acid oil-in-water emulsified composition*. PCT International Patent Application no. WO0078162 (in English).

Koike, S., Hosaya, N., & Yasumasu, T. (2001). *Oil composition and use thereof*. European Patent Application no. EP1135991 (in English).

Koike, S., Hosoya, N., & Yasumasu, T. (2002a). *Fat compositions*. European Patent Application no. EP1211305 (in English).

Koike, S., Yasumasu, T., Hase, T., Murase, T., & Yasukawa, T. (2002b). *Oil/fat composition*. PCT International Application no. WO0211551 (in English).

Koike, S., Yasumasu, T., Hase, T., Murase, T., Yasukawa, T., Katsuragi, Y., et al. (2002c). *Oil/fat composition*. PCT International Application no. WO0211552 (in English).

Koike, S., Yasumasu, T., Hase, T., Murase, T., Yasukawa, T., Katsuragi, Y., & Takei, A. (2002d). *Oil/fat composition*. PCT International Patent Application no. WO0211550 (in English).

Koike, S., Murase, T., & Hase, T. (2003). *Oil or fat composition*. US Patent Application no. 2003/0054082 (in English).

Kondo, H., Hase, T., Murase, T., & Tokimitsu, I. (2003). Digestion and assimilation features of dietary DAG in the rat small intestine. *Lipids*, *38*, 25–30.

Kudo, N., Kameo, Y., & Mizuno, W. (2002). *Oil-cooked or baked potatoes*. European Patent Application no. EP1203534 (in English).

Lai, O. M., Yusoff, M. S. A., Lo, S. K., Long, K., Tan, C. P., Lim, J. W., et al. (2006). *Process for the production of diacylglycerol*. PCT International Application no. PCT/MY2006/000034 (in English).

Lai, O. M., Yusoff, M. S. A., Lo, S. K., Long, K., Tan, C. P., Tahiruddin, S., et al. (2007). *Production of acylglycerol esters*. PCT International Application no. PCT/MY2007/000025 (in English).

Lean, M., Gruer, L., Alberti, G., & Sattar, N. (2006). Obesity—Can we turn the tide? *British Medical Journal*, *333*, 1261–1264.

Lehner, R., & Kuksis, A. (1993). Triacylglycerol synthesis by sn-1,2 (2,3)-diacylglycerol transacylase from rat intestinal microsomes. *Journal of Biological Chemistry*, *268*, 8781–8786.

Lehner, R., & Kuksis, A. (1996). Biosynthesis of triacylglycerols. *Progress in Lipid Research*, *35*, 169–201.

Lehner, R., Kuksis, A., & Itabashi, Y. (1993). Stereospecificity of monoacylglycerol and diacylglycerol acyltransferases from rat intestineas determined by chiral phase high-performance liquid chromatography. *Lipids*, *28*, 19–34.

Ling, W. H., & Jones, P. J. H. (1995). Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Science*, *57*, 195–206.

Lo, S. K., & Baharin, B. S. (2001). *Process for producing phytonutrient-enriched diacylglycerols*. Malaysian Patent Application no. PI20014817 (in English).

Maki, K. C., Davidson, M. H., Tsushima, R., Matsuo, N., Tokimitsu, I., Umporowicz, D., et al. (2002). Consumption of diacylglycerol oil as part of a reduced-energy diet enhances loss of body weight and fat in comparison with consumption of a triacylglycerol control oil. *American Journal of Clinical Nutrition*, *76*, 1230–1236.

Mansbach II, C. M., & Nevin, P. (1998). Intracelllular movement of triacylglycerols in the intestine. *Journal of Lipid Research*, *39*, 963–968.

Masui, K., Katsuragi, Y., Toi, T., & Yasukawa, T. (2001). *Fat or oil composition*. PCT International Application no. WO0113733 (in English).

Masui, K., & Konishi, Y. (2001). *Water-in-oil type emulsified fat and/or oil composition*. PCT International Patent Application no. WO0101787 (in English).

Masui, K., & Yasunaga, K. (2001). *Water-in-oil type emulsified fat and/or oil composition*. PCT International Patent Application no. WO0115542 (in English).

Mazur, A. W., & Hiler, G. D. (1992). *Regioselective synthesis of 1,3-disubstituted glycerides*. Canadian Patent no. CA2106316 (in English).

Meguro, S., Higashi, K., Hase, T., Honda, Y., Otsuka, A., Tokimitsu, Y., et al. (2001). Solubilization of phytosterols in diacylglycerol versus triacylglycerol improves the serum cholesterol lowering effect. *European Journal of Clinical Nutrition*, *55*, 513–517.

Meng, X., Zou, D., Shi, Z., Duan, Z., & Mao, Z. (2004). Dietary diacylglycerol prevents high-fat diet-induced lipid accumulation in rat liver and abdominal adipose tissue. *Lipids*, *39*, 37–41.

Mori, H., Masui, K., Tanaka, Y., & Yasukawa, T. (1999a). *Water-in-oil emulsified fat composition*. PCT International Patent Application no. WO9959422 (in English).

Mori, H., Sakai, H., Tanaka, Y., & Yasukawa, T. (1999b). *Fried food and shortening*. PCT International Patent Application no. WO9959424 (in English).

Mori, H., & Watanabe, T. (2000). *Food containing fat or oil*. European Patent no. EP0970615 (in English).

Murase, T., Aoki, M., Wakisaka, T., Hase, T., & Tokimitsu, I. (2002a). Anti-obesity effect of dietary diacylglycerol in C57BL/6J mice: dietary diacylglycerol stimulates intestinal lipid metabolism. *Journal of Lipid Research*, *43*, 1312–1319.

Murase, T., Mizuno, T., Omachi, T., Onizawa, K., Komine, Y., Kondo, H., et al. (2001). Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice. *Journal of Lipid Research*, *42*, 372–378.

Murase, T., Nagasawa, A., Suzuki, J., Wakisaka, T., Hase, T., & Tokimitsu, I. (2002b). Dietary alpha-linolenic acid-rich diacylglycerols reduce body weight gain accompanying the stimulation of intestinal beta-oxidation and related gene expressions in C57BL//KsJ-db/db mice. *Journal of Nutrition*, *132*, 3018–3022.

Murata, M., Hara, K., & Ide, T. (1994). Alteration by diacylglycerol of the transport and fatty acid composition of lymph chylomicrons in rats. *Bioscience, Biotechnology and Biochemistry*, *58*, 1416–1419.

Murata, M., Ide, T., & Hara, K. (1997). Reciprocal responses to dietary diacylglycerol of hepatic enzymes of fatty acid synthesis and oxidation in the rat. *British Journal of Nutrition*, *77*, 107–121.

Nagao, T., Watanabe, H., Goto, N., Onizawa, K., Taguchi, H., Matsuo, N., et al. (2000). Dietary diacylglycerol suppresses accumulation of body fat compared to triacylglycerol in men in a double-blind controlled trial. *Journal of Nutrition*, *130*, 792–797.

Nakajima, Y., Fukasawa, J., & Shimada, A. (2004). Physicochemical properties of diacylglycerol. In Y. Katsuragi, T. Yasukawa, N. Matsuo, B. D. Flickinger, I. Tokimitsu, & M. G. Matlock (Eds.), *Diacylglycerol oil* (pp. 182–196). Illinois, USA: AOCS.

Nakajima, Y., Nishide, T., & Sakuma, T. (2002). *Edible oil and production process thereof*. United States Patent Application no. US20020045000 (in English).

Nomura, M., Koike, S., Yamashita, K., Okisaka, K., Sano, Y., Omura, H., et al. (1992). *Edible oil-in-water emulsion*. US Patent no. US5160759 (in English).

Sakai, H., Ishibashi, M., & Kohori, J. (2002a). *Fat compositions*. European Patent Application no. EP1186648 (in English).

Sakai, H., Katada, M., & Ishibashi, M. (2002b). *Oil or fat composition*. European Patent Application no. EP1249173 (in English).

Schutz, Y. (1995). Macronutrients and energy balance in obesity. *Metabolism*, *44*(3), 7–11.

Sedarevich, B. (1967). Glyceride isomerisation in lipid chemistry. *Journal of the American Oil Chemists' Society*, *44*, 381–393.

Shiiba, D., Asabu, Y., Kawai, S., & Nakajima, Y. (2002). *Acidic oil-in-water type emulsion composition*. United States Patent Application no. US20020119239 (in English).

Soni, M. G., Kimura, H., & Burdock, G. A. (2001). Chronic study of diacylglycerol oil in rats. *Food Chemistry and Toxicology*, *39*, 317–329.

Sugimoto, T., Fukuda, H., Kimura, T., & Iritani, N. (2003a). Dietary diacylglycerol-rich oil stimulation of glucose intolerance in genetically obese rats. *Journal of Nutritional Science and Vitaminology*, *49*, 139–144.

Sugimoto, T., Kimura, T., Fukuda, H., & Iritani, N. (2003b). Comparisons of glucose and lipid metabolism in rats fed diacylglycerol and triacylglycerol oils. *Journal of Nutritional Science and Vitaminology*, *49*, 47–55.

Sugiura, M., Shimizu, M., Yamada, Y., Mine, K., Maruyama, E., & Yamada, N. (2002a). *Process for producing partial glyceride*. US Patent no. US 6337414 (in English).

Sugiura, M., Yamaguchi, H., & Yamada, N. (2002b). *Preparation process of diglyceride*. US Patent no. US6361980 (in English).

Tada, N., Watanabe, H., Matsuo, N., Tokimitsu, I., & Okazaki, M. (2001). Dynamics of postprandial remnant-like lipoproteins particles in serum after loading of diacylglycewrols. *Clinica Chimica Acta*, *311*, 109–117.

Taguchi, H., Omachi, T., Nagao, T., Matsuo, N., Tokimitsu, I., & Itakura, H. (2002). Dietary diacylglycerol suppresses high fat diet-induced hepatic fat accumulation and microsomal triacylglycerol transfer protein activity in rats. *Journal of Nutritional Biochemistry*, *13*, 678–683.

Taguchi, H., Watanabe, H., Onizawa, K., Nagao, T., Gotoh, N., Yasukawa, T., et al. (2000). Double-blind controlled study on the effects of dietary diacylglycerol on postprandial serum and chylomicron triacylglycerol responses in healthy humans. *Journal of the American College of Nutrition*, *19*, 789–796.

Takano, H., & Itabashi, Y. (2002). Molecular species analysis of 1,3-diacylglycerols in edible oils by HPLC/ESI-MS. *Bunseki Kagaku*, *51*, 437–442.

Takase, H., Koike, S., Sakai, H., Nishide, T., Hase, T., & Murase, T. (2003). *Oil composition*. PCT International Patent Application no. WO03024237 (in English).

Teramoto, T., Watanabe, H., Ito, K., Omata, Y., Furukawa, T., Shimoda, K., et al. (2004). Significant effects of diacylglycerol on body fat and lipid metabolism in patients on hemodialysis. *Clinical Nutrition*, *23*, 1122–1126.

Toshinori, I., Yasuharu, N., Shinichi, H., & Shoichi, K. (2000). *Production of diglyceride-containing fat and oil composition and fat and oil composition using the same*. Japanese Patent no. JP2000345189 (in Japanese).

Watanabe, H., Onizawa, K., Taguchi, H., Kobori, M., Chiba, H., & Naito, S. (1997). Nutritional characterization of diacylglycerols in rats. *Journal of the Japanese Oil Chemists' Society*, *46*, 301–307.

William, C. L., Gulli, M. T., & Deckelbaum, R. J. (2001). Prevention and treatment of childhood obesity. *Current Atherosclerosis Reports*, *3*, 486–497.

Yamada, Y., Shimizu, M., Sugiura, M., & Yamada, N. (1999). *Process for producing diglycerides*. PCT International Application no. WO9909119 (in English).

Yamamoto, K., Asakawa, H., Tokunaga, K., Meguro, S., Watanabe, H., Tokimitsu, I., et al. (2005). Effects of diacylglycerol administration on serum triacylglycerol in a patient homozygous for complete lipoprotein lipase detection. *Metabolism*, *54*, 67–71.

Yamamoto, K., Asakawa, H., Tokunaga, K., Watanabe, H., Matsuo, N., Tokimitsu, I., et al. (2001). Long-term ingestion of dietary diacylglycerol lowers serum triacylglycerol in type II diabetic patients with hypertriglyceridemia. *Journal of Nutrition*, *131*, 3204–3207.

Yanagisawa, Y., Kawabata, T., & Hasegawa, K. (2003). Improvement in blood lipid levels by dietary sn-1,3-diacylglycerol in young women with variants of lipid transporters 54T-FABP2 and -493g-MTP. *Biochemical and Biophysical Research Communications*, *302*, 743–750.

Yoon, D.-H., Han, G.-W., Hong, S.-G., & Lee, Y.-H. (2004). *Preparation method of conjugated linoleic acid diglycerides*. PCT International Application no. WO2004096748 (in English).