Medium chain fatty acids: extraction, isolation, purification, bioactive properties and application

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Abstract. Compared with long-chain fatty acids, medium-chain fatty acids are characterized by fast digestion and absorption, less fat accumulation and deposition, improve insulin resistance, regulate energy metabolism, inhibit microorganisms and other biological functions. They are widely used in food, medicine, genetic engineering and other nutrition-related aspects, and have great potential in the development and utilization of health food. In this paper, the definition, source, physicochemical properties, metabolic characteristics, biological functions, safety and application in nutrition-related fields of medium chain fatty acids will be reviewed and discussed.

1. Introduction

Medium chain fatty acids (MCFA) are the fatty acids consisting 8 to 12 carbon atoms, including caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0) [1]. Coconut and palm kernel oil are two major sources of MCFA [2]. Approximately 60% medium chain fatty acids naturally present in coconut and palm kernel oil; however, as the data show in Table 1, long chain saturated fatty acids and monounsaturated fatty acid also present in coconut and palm kernel oil with considerable amount [2]. Coconut oil or palm kernel oil need to be further purified to achieve pure medium chain triglycerides or oils and therefore, purified products can be sold in a medicinal form for functional and nutritional research or treatment purposes. In 1955, medium chain triglycerides first became commercially available for several clinical treatments such as fat malabsorption syndrome [2]. However, positive health effects associated with dietary intake of MCFA also been proposed by several researches recently.

The prevalence of obesity in United States has increased by 30% in past decades, and is still increasing in all gender, age and racial population groups [3]. Although scientists stated that genetic factors contribute to the development of obesity in individuals, dietary patterns such as total energy intake and fat intake significantly associated with development of obesity [3]. Therefore, consumption of saturated fatty acids (SFA) and cholesterol has being restricted as a disease risk reduction approach, which recommended by several dietary guidelines such as US Dietary Guidelines [4]. Moreover, consumption of dietary SFA may also contribute to the development of cardiovascular disease, in terms of increasing plasma LDL concentration and decreasing LDL particle size which significantly associated with the progress of atherosclerosis [5, 6].
Caprylic acid, capric acid and lauric acid are also SFA; however, became more and more attractive to nutritionists since the different metabolism mechanisms involved, including digestion, absorption and transportation [1]. For instance, MCFA can be hydrolyzed more complete and get transferred to portal blood directly, compared with LCFA [7]. Several researches have been focused on how dietary MCFAs affect human fat metabolism and metabolic syndromes risk reduction. Results indicated that MCFAs have the potential on weight management and other metabolic syndromes prevention [1]. In the following sections, the bioactive properties of MCFA in vivo and mechanisms involved will be specifically discussed.

2. Extraction, isolation, purification and identification

Extraction of palm kernel or coconut oil is slightly different than extraction from plant seeds, whereas plant seeds extraction requires several pre-extraction preparation steps such as de-hulling, conditioning and flaking processes [8]. For the extraction of palm kernel oil, separation of kernel from fruit needs to be done prior to extraction. This involved several steps, including palm oil extraction process, nuts and fiber separation, drying process and cracking followed with kernel shell separation process [8]. Winnowing columns, hydro-cloning and clay bathing are three common methods can be used to separate shell and kernel based on the density differences [8]. The miniced or grounded palm kernel can be extracted either by hard screw pressing method or solvent extraction method to achieve crude palm kernel oil. Every 1 ton crude oil usually can be achieved by using 10 tons palm kernel [8]. Similar to plant seeds or soybean oil solvent extraction method, hexane can also be used as solvent with a countercurrent flow extraction model which can increase extraction efficiency. The final step is distillation which always been required after extraction in order to remove residue of extraction solvent [8].

To achieve relative pure palm kernel and coconut oil, refining process must be applied after crude oil extraction. Refining process can remove undesirable compounds in crude oil such as protein, wax, pigments, free fatty acids and phosphatides. (Carr, 1976) There are four major steps in refining process, including degumming, neutralizing, bleaching and deodorization. In degumming step, phosphatides can be removed by adding hydrogen peroxide followed by mixing and agitating at 60 to 80°C for 30 to 60 minutes [8]. After filtration and centrifugation, up to 90% phosphatides can be removed [8]. Free fatty acids also naturally present in crude oil, eliminating free fatty acids can be accomplished by adding alkaline agents such as sodium hydroxide into crude oil [8]. Bleaching and deodorization processes can remove pigments and odors in crude oil; however, phenolic compounds such as β-carotene will also be destroyed in deodorization process where a high temperature and pressure be used [8]. After refining process, most undesirable compounds can be removed; however, as the data shown on Table 1 that LCFA and unsaturated fatty acids still existing in coconut or palm kernel oil. Therefore, fractionation MCFA from oils is next step if manufacturers would like to produce and sell the pure MCT oils as nutraceutical for research or medical treatment purposes.

MCFA from both palm kernel oil and coconut oil can be separated and fractioned by distillation method based on boiling point differences [2]. Fatty acids can be liberated from triglycerides by splitting under high temperature and steam [9]. Hydrolyzed fatty acids can go further for distillation and separation. Usually, first two fractions contain almost all medium chain fatty acids range from C8 to C12, which have relative lower boiling points [9]. Esterification of fractionated fatty acids is the next step and should be carried out at 200°C with added glycerides. At the meantime, water need to be removed constantly in order to move forward the esterification reaction [2]. However, two times distillation usually required to further purify samples, since contamination or overlapping of fractions can occur during distillation [9]. In addition, excess fatty acids in reaction pool can be removed by

| Fatty Acid | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 |
|-----------|-----|-----|------|------|------|------|------|------|------|------|------|
| Coconut   | 0.5 | 7.1 | 6.0  | 47.1 | 18.5 | 9.1  | 2.8  | 6.8  | 1.9  | 0.1  | 0.1  |
| Palm      | 0.2 | 3.3 | 3.4  | 48.2 | 16.2 | 8.4  | 2.5  | 15.3 | 2.3  | /    | 0.1  |

Table 1. Fatty acid composition of coconut oil and palm kernel oil [2]
vacuum distillation and again, fractionated MCT oil should also go further for refining to remove possible undesirable residues and contaminants [2].

Gas chromatography (GC) widely been used as the analytical method to identify fatty acids composition in edible oil or food materials. However, fatty acid itself is not a volatile compound, whereas GC can only identify volatile compounds. Therefore, sample preparation and fatty acids methylation should always be applied prior to analyze on GC. According to Kostik’s method, dissolving 0.1-0.2 mL sample in 10 mL 0.2 M sulfuric acid prepared in methanol. Fatty acids can be methylated by refluxing for half hour at 100°C in sealed tubes. After cooling to room temperature, adding 10 mL petroleum ether and deionized water in tubes. Extracting the distinct upper layer of petroleum ether after mixing, and injecting 2μL sample into GC for analysis [10]. Abundance of fatty acids in the sample can be identified and calculated based on GC spectrum compared with standard result.

3. Efficacy testing: literature review of bioactive properties of MCFA

Debate on how saturated fats relate to metabolic syndromes is still being discussed, and no consistent conclusions have been made. Several dietary guidelines and health care organizations recommended a restriction on dietary saturated fats intake. However, the effects of consumption saturated fats may depend on the type of dietary fatty acids. For instance, MCFA may not deliver the similar adverse effects to human body as consumption of LCFA. MCFA may enhance body fat oxidation and energy expenditure, and also been demonstrated that may have anti-diabetic benefits [7]. Beyond this, researchers also indicated that MCFA also enhanced Vitamin E and calcium absorption [7].

In 2002, St-onoge and co-workers conducted a randomized-crossover controlled trial at McGill to evaluate how MCFA can affect body composition, energy expenditure and body fat oxidation in 24 general healthy overweight men [11]. Diet rich in MCFA and LCFA were two different treatments in the trial, and body energy expenditure and body composition been measured after each 4-week dietary treatment period [11]. Results indicated that body adipose tissue been significantly decreased, both energy expenditure and fat oxidation been significantly increased after diet rich in MCFA compared with diet rich in LCFA [11]. Similarly, in 2003 another trial at McGill also evaluated the effects of long-term MCFA consumption on body composition, energy expenditure and fat oxidation in 17 obese women. MCFA enriched diet and beef tallow enriched diet are two treatments with 27-day dietary intervention period [12]. Results showed that after diet rich in MCFA treatment, body fat oxidation rate and energy expenditure significantly greater than diet rich in beef tallow [12]. In addition, authors indicated that a favorable change on plasma lipids profile also been observed in diet rich in MCFA, which MCFA may also have cardiovascular disease risk reduction effects [13].

Using stable isotopes to study lipid metabolism has been introduced and widely used over past few decades. Delany and his colleagues used 13C labeled fatty acids to evaluate the oxidation rate in four general healthy men. Participants consumed 13C labeled fatty acid with hot food after 1-week body weight maintaining diet. 13C labeled lauric acid, palmitic acid, stearic acid and long chain unsaturated fatty acids were administrated to each participant in the study. Results indicated that lauric acid showed a 40% cumulative oxidation rate which is much higher than all other fatty acids tested [14]. Scientists believed that oxidation rate should depend on the chain length and degree of unsaturation of the fatty acid, which unsaturated fats should give higher oxidation rate than saturated fats. However, in Delany et al research, 13C labeled lauric acid showed a highest oxidation rate. Papamandjaris et al. in 2000 conducted a trial to compare fat oxidation differences between diet rich in MCFA and LCFA in 12 healthy young women. MCT diet rich in butter and coconut oil, whereas LCT diet rich in beef tallow. Each dietary treatment is 6-day in total and followed by 8-day post-treatment measurement period. A mixture of 13C labeled myristic acid, palmitic acid and stearic acid been administrated to participants during post-treatment period at daily basis. Results indicated that endogenous LCFA oxidation rate was significant higher in MCT treatment, compared with LCT treatment [15].

Based on current evidences from clinical trials as well as animal studies, consumption of MCFA may enhance body fat oxidation and postprandial energy expenditure and therefore, may significantly contribute to body fat loss. However, further studies are still needed to be more accurately and
specifically identify the mechanisms involved. Beyond this, anti-diabetic effects also been demonstrated after consumption of diet rich in MCFA. Both in human and animal studies, a relative lower body fat accumulation and better glucose tolerance been observed after consuming diet in MCT [7]. Meanwhile, increased insulin sensitivity and reduced adipose tissue also been observed by researchers [7]. The possible mechanism of anti-diabetic effect might be increased concentration of circulating adiponectin, which been demonstrated that can improve insulin action in vivo and vitro [7]. Researchers also indicated that MCT may also improve human brain function and cognitive ability due to the rapid oxidation and formation of ketone bodies [2]. However, evidences are not well documented and further studies should be done to clarify and identify the mechanisms.

4. Mechanism of action of MCFA
MCFA have relative smaller molecule size and behave more polar than LCFA. Therefore, metabolic pathways of dietary MCFA and LCFA might be different, including digestion, absorption, transportation and oxidation [1]. Figure 1 gives the example of the metabolic differences between MCFA and LCFA.

Unlike LCT, digestion of MCT is more complete due to the preferential of pancreatic lipase [2]. After digestion, LCFA need to be converted to chylomicrons by incorporating apolipoproteins, cholesterol esters and triglycerides, thus can be transferred through lymphatic system to liver for further processing. Since chylomicron contains apolipoproteins and phospholipids, thus it can circulate in lyphatic and blood systems due to the partial hydrophilic properties. In contrast, MCFA do not need this transformation, MCFA can bound with albumin, bypassing lymphatic system and get directly transferred into liver for oxidation via portal blood [1]. Therefore, MCFA have a relative shorter travel time and can reach liver faster than LCFA. Beyond this and at cellular level, when fatty acid acyl-CoA ready for β-oxidation, acyl-CoA molecule needs to be converted to acyl-carnitine form. Carnitine palmityl transferase I and II are responsible for this conversion so that fatty acid can move into mitochondria inner membrane for β-oxidation, whereas MCFA can penetrate mitochondria inner membrane without this media [1, 7]. Therefore, MCFA can be oxidized as rapid as glucose, and be oxidized much faster than LCFA upon the differences between absorption, digestion and transportation. Meanwhile, since MCFA can be transferred to liver directly without transformation into chylomicron, thus only few amount of MCFA present in circulating lipids. Almost only LCFA can be absorbed into peripheral tissues. Therefore, lower recovery of MCFA in adipose tissue after long term consumption of MCT diet been observed in some studies. Based on current well documented evidences, MCFA been believed that significantly contribute to body fat loss and energy expenditure. However, more researches are still needed to further clarify and validate the effects and mechanisms.

Figure 1. Metabolism pathways of MCFA and LCFA [1]. A, B, C, D stand for chylomicron, lymphatic system, portal blood
5. Conclusion

Though weight management effect is the major health benefit been proposed by researchers, anti-diabetic and anti-Alzheimer’s disease benefits are also been demonstrated in some other studies. Similarly, proposed mechanisms associated with these two benefits are rapid fatty acid oxidation, enhanced ketone formation and possible less fat accumulation in body. However, there are insufficient studies and well documented evidences to illustrate mechanism at physiological level.

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