

Analysis of Fatty Acids in Breastmilk During a 9-month Lactation Period Using Gas Chromatography–Mass Spectroscopy (GC-MS)

Titin Aryani^{1,*}, Dwi Ernawati², Henny Parida Hutapea³

^{1,2}University 'Aisyiyah Yogyakarta, Indonesia

³Politeknik Santopaulus Surakarta

¹titinaryanipurnama@gmail.com*

* corresponding author

Submission date: 10 Juli 2018, Receipt date: 10 Oktober 2019, Publication date: 1 Juli 2020

Abstract

Breastmilk is very much needed to meet the needs of babies in every way because it contains important nutrients, carbohydrates in the form of lactose, and fatty acids in the form of polyunsaturated fatty acids. Research on fatty acid content in breastmilk with a lactation period of 9 months is needed to provide information to the public related to fatty acid content of breastmilk during 9 months lactation. The study is to analyze fatty acid content in the form of saturated fatty acids or unsaturated fat contained in breast milk. Breastmilk taken in the study was as much as 50 ml in a 250 ml erlenmeyer solution. To the sample 50 ml of concentrated, HCL concentration was added as much as 5 m and then the solution was beat for 15 minutes and was exposed ultrasonic therapy for 1 hour. The solution was then extracted using n-hexane and aquabidest solution until the milk oil solution was separated. Milk oil was extracted by adding 50 ml of Na₂SO₄ 1 m then deciphere was performed. The breastmilk milk extraction oil was weighed 0.1 gram in a Teflon-filled reaction tube. A 15% BF₃ solution in methanol was added 0.5 ml and then was heated in an air bath with a temperature of 45 ° C for 30 minutes. After cooling, solution of 0.2 ml of n-hexane was added to form two layers. The upper layer which was fatty acid methyl ester was taken by using syringe and then was injected in GC-MS. In this study, the total saturated fatty acid was 42.54% and the total unsaturated fatty acid was 57.17%. Saturated fatty acids consisted of lauric, myristic, palmitate, and stearate. Omega-6 fatty acids and monounsaturated fatty acids consisted of omega-9 fatty acids. In Mother's Milk with 9 months lactation period there are various fatty acid content of both saturated fatty acids, monounsaturated fatty acids (omega-9) and polyunsaturated fatty acids (omega-6).

Keywords: breastmilk, fatty acids, lactation period

INTRODUCTION

Dairy is the perfect food for life and growth so breastmilk is very important given to the baby. Babies need Breastmilk because breastmilk has many benefits and in addition breastmilk in the Islamic view is also a right that must be given to children. All children who are both Islamic and non-Islam have to take Breastmilk because of the importance for the child so that in certain circumstances where the mother cannot breastfeed her child, through the mother's consensus with her husband, she can choose to find nursing mother (murdli'ah) who can breastfeed her baby.



Undoubtedly, breastmilk is the best food for babies. Various studies have proven the various benefits contained in Breastmilk.

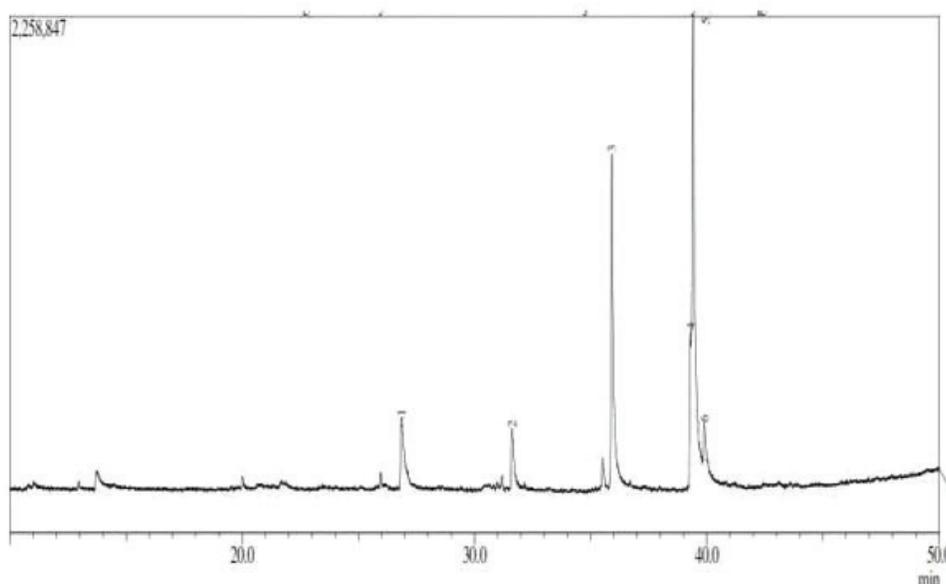
RESEARCH METHODS

In the various studies conducted, it has also been described that breastmilk consists of components of macro and micro-nutrients which includes macro-nutrients such as carbohydrates, proteins, and fats, while micronutrients includes vitamins & minerals. Breastmilk consists nearly 90% of water. Breastmilk nutrient volume and composition are different for each mother depending on the baby's needs. The above volume and composition differences are also seen during breastmilk (colostrum, transitional breastmilk, mature breastmilk and breastmilk at the time of weaning). Breastmilk nutrition in the early and latest period in each mother who breastfeeds is also different. Colostrum produced during 1-5 lactating days is rich in nutrients, especially protein (Hendarto and Pringgadini 2013). Fat is the most important milk composition, supplying energy and helps the development of the central nervous system. Moreover, milk fat is a carrier of flavor and aroma. In general, the fat content of human breastmilk ranges from 3.5% to 4.5% during breastmilk. The major lipid fraction is triglycerides which account for about 95% of total lipids. Long-chain unsaturated fatty acids, known as long-chain polyunsaturated fatty acids (LC-PUFAs) are contained in breastmilk, but are hardly found in infant formula (Horta et al., 2007). This component has an important role as a membrane structural component of the tissue system, including blood vessel tissue. Supplementation with LC-PUFA has been shown to reduce blood pressure in hypertensive people (Engler et al., 1999). Focusing on the importance of information about the content of breastmilk fatty acid during 9 months lactation, it is necessary to do research on fatty acid content contained in breastmilk.

Fatty acid content analysis was performed using gas chromatography method according to AOAC (2005). The stages generally consisted of lipid extraction, methylation, GC separation /determination, and fatty acid calculations. Total fat was extracted from 3 grams of breastmilk plus 45 ml of methanol chloroform with a 2: 1 volume ratio. After that, the liquid was emulsified with 12 ml of 0.85% NaCl and the bottom layer was collected and dried at 400C. Then the amount of fat was calculated. Then FAME (Fatty Acid Methyl Esters) was prepared with methanol-acetyl chloride with a volume ratio of 100:15 at 700C in a water bath for 3 hours and crushed with hexane. The results of FAME analysis would come out on Shimadzu GC 2010 tools plus with Quadrex 007 cyanopropyl methyl sil. Helium was used as a gas carrier with a flow rate of 2 ml / min. Thereafter, the cusp of the FAME was identified by comparing retention times according to certified standards (Supelco 37 Mixed Components of FAME). The percentage of fatty acids was calculated by the normalization method. The peak point in an area less than 0.05% in the total area was not used as a result reference.

RESULTS AND DISCUSSION

GC chromatogram data on breastmilk extension samples are shown in Figure.



Identification based on data from mass spectroscopy

Table 1. Data of Fatty Acid and Relative Percent of Amount of Fatty Acids In Breastmilk Extension Samples

Retention Time	Name of International Union of Pure and Applied Chemistry (IUPAC) Fatty Acid Compound	Trivial Name of Fatty Acid Compound	Percent Relatively Fatty Acid Content %	Types of Fatty Acids
28,868	metil ester dodekanoat	metil laurat	7,73	saturated
31,624	metil ester tetradekanoat	metil miristat	4,65	saturated
35,918	metil ester heksadekanoat	metil palmitat	27,91	saturated
39,308	metil ester 9,12-oktadekadienoat	metil linoleat	8,68	Omega-6
39,407	metil ester 9-oktadekenoat	metil oleat	48,49	Omega-9
39,907	metil ester oktadekanoat	metil stearate	2,25	saturated

In this study, total saturated fatty acid was 42.54% and the total unsaturated fatty acid was 57.17%. Saturated fatty acids consist of lauric, myristic, palmitate, and stearate. Meanwhile polyunsaturated fatty acids consist of omega-6 fatty acids and monounsaturated fatty acids composed of omega-9 fatty acids. Martin et al. (2012) mentioned that diet will affect the composition of fatty acid breastmilk both the direct absorption and reserves in the body. Various etiologies can affect the levels of DHA and EPA in breastmilk (Huang et al 2013; Gao et al. 2013; Saphier et al 2013; Makela et al. 2013; Urwin et al. 2013; Martin et al., 2012, Olang et al. The issue of mothers who are consuming food which are high in unsaturated fatty acids such as fish or seafood during breastfeeding is that they will have high levels of DHA and low ARA ratio with DHA. Another result found in the study is that residence may be one of the developing factors The type of dietary intake of the mother's source of fat in mothers living in coastal areas showed the higher levels of DHA and omega 3 compared to mothers living in the mainland and thus, this results in a frequency level of consumption of seafood fish containing rich unsaturated fatty acids.

In addition, marine fish (Makela et al. 2013; Huang et al. 2013; Lauritzen et al. 2002) and freshwater fish (Martin et al. 2012) can increase the milk linoleate content. Vegetable oils and processed foods could increase the level of milk linoleate (Gao et al. 2013; Martin et al. 2012; Brenna 2009; Nishimura et al., 2014; Wan et al., 2010). One type of vegetable oil which was rich in linoleic palm oil with 9660 mg / 100 g of food, rich sources of linolenic food is a group of nuts and processed (Mulyani 2014). According to Nishimura et al. (2014), in the consumption of linoleic and linolenic foods, there was a ratio of linoleic acid and linolenic acid. When the levels of linolenic acid were low, the levels of linoleic acid become high.

ARA care flow was not associated with ARA levels in breastmilk, but was associated with maternal fat factor and metabolic factors in synthesizing ARA from linoleic acid precursors (Gao et al 2013). Fatty deposits of mothers were formed from the time of pregnancy, so the period of pregnancy had an important role to fat content of breastmilk during breastmilk. The types of essential fatty acids in breastmilk were also generated by the distribution of reserves in the mother (Much et al. 2013; Lauritzen and Carlson. 2011). High carbohydrate core would affect the fat content in the body, so it would affect the fat content of milk produced (Read et al 1965).

Bertino et al. (2013) revealed that 96 hours of breastmilk in the refrigerator could maintain overall fat composition in breastmilk. Babies receiving breastmilk from the storage process still had the same full fatty acid content as breastmilk given directly. In the study, total saturated fatty acid was 42.54% and the total unsaturated fatty acid was 57.17%. Saturated fatty acids consisted of lauric, myristic, palmitate, and stearate, while polyunsaturated fatty acids consisted of omega-6 fatty acids and monounsaturated fatty acids composed of omega-9 fatty acids. The palmitate content in the study was 27.91. The study is in line with Gou's (2014) study stating that almost half of the milk fatty acids were saturated fatty acids, with 23% palmitic acid (C16: 0) in total fatty acids (Guo et al., 2014).

The results of the study were differ slightly from the results of research by Guo (2014). The results of Guo's (2014) study showed that saturated fatty acid and oleic acid (18: 1w9) were at the highest percentage content (36%) in milk. Meanwhile, in the study oleat occupied the highest percentage in percentage of fatty acid of breastmilk namely 57,17%. This was probably due to the age of breastfed babies (breastmilk) were at the age of 6 months.

Research by Guo (2014) also mentioned that breastmilk contained two essential fatty acids, linoleic acid (C18: 2w6) at 15% and alpha-linolenic acid (C18: 3w3) at 0.35% (Guo et al., 2014). But in the study, linolenic acid (omega-) was not identified. This could be caused by the factors of food consumed by mothers and the age of breastfed infants. In the study, the linoleic acid was at the levels of 8.68% which was lower than the levels that Guo found (2014). However, although omega-3 fatty acids (linolenic acid) were not identified in the study, linolenic in human metabolism could be converted into omega-6 arachidonic acid (AA, C20: 4w6) and omega-3, eicosapentaenoic acid (EPA, C20 : 5w3), and the latter can be converted to docosahexaenoic acid (DHA, 22: 6w3).

AA, EPA and DHA are important in regulating growth, inflammatory response, immune function, vision, cognitive development and motor system in newborns. Long chain polyunsaturated fatty acids are transferred from mother to fetus in the third trimester through the placenta, and the baby through breastmilk after birth (Herrera, E. 2002).

During the last trimester and neonatal period, brain tissue is quickly synthesized. Cell differentiation and development are active Synapses in the brain require the specific needs of DHA and AA. Eighty percent of brain DHA Retrieved from 26th week of pregnancy to birth. In particular, the synthesis of AA and DHA of linoleic acid (18: 2w6) and alpha-linolenic acid (18: 3w3) is limited to the fetus and neonate due to the activity of the premature enzyme. Thus, the required amount of AA and DHA should be from the mother during pregnancy, or as breastmilk after birth. One study had shown that the fat content and percentage of all polyunsaturated fatty acids in breastmilk increased significantly between the sixth week and the sixth month of lactation (Joardar et al., 2006).

The concentration of AA (omega-3) in breastmilk relies heavily on the dose with the consumption of AA rich foods in breastmilk mothers (Del Prado et al., 2001). The concentrations of Omega-3 type EPA and DHA ASI are also closely related to EPA and DHA intake during pregnancy (Weseler et al., 2008).

Breastmilk from breastfeeding women consuming vegan or vegetarian food had <0.1% DHA, compared to a mean DHA level of 0.2% -0.4% in the United States and DHA \geq 0.8% in China in which DHA comes from fish or other high sources (Makrides, 1996). It is recommended that a ~ 300 mg DHA intake per day is required to achieve a breastmilk level of 0.3% -0.35% of DHA. However, the effect of breastmilk fatty acids on neural development is complex, especially since neural development is assessed after the first six months of exclusive breastmilk.

In the results of the study, linoleic acid was at 8.68% at 9 months lactation. This possible outcome is in line with Nahrowi's (2015) study. Because according to nahrowi, the highest levels of milk linoleat was found in breastmilk with lactation period 12-23 months. According to Nahrowi (2015), the highest EPA and linoleic acid content was present in breastmilk with a 12-23 month lactation period (EPA (C20: 5n3) $0.05 \pm 0.14\text{g} / 100\text{g fat}$ and linoleic acid (C18: 2n6c) $5.50 \pm 3.40\text{g} / 100\text{g Fat}$), while the highest DHA and linolenic acid content was found in breastmilk with a lactation period of 9-11 months (DHA (C22: 6n3) $0.21 \pm 0.16\text{g} / 100\text{g fat}$ and linolenic acid $0.30 \pm 0.42\text{g} / 100\text{g fat}$). Unlike the ARA content, the highest ARA content was in breastmilk with 6-8 months lactation period (ARA (C20: 4n6) $0.13 \pm 0.14\text{g} / 100\text{g fat}$). However, statistical tests showed that all essential fatty acid levels based on lactation period were not significantly different ($P > 0.05$).

Milk production levels is decreasing as the increasing age, so at the age of 6 months and above is the age which is necessary for consumption of additional food in addition to breastmilk to meet the daily level of sufficiency of essential fatty acids (Milligan et al., 2008). The necessary ARA and DHA intake of the brain was needed maximally during the first 2 years of brain development which is rapid.

As it has been mentioned before, Milk production levels is decreasing as the increasing age, so at the age of 6 months and above is the age which is necessary for consumption of additional food in addition to breastmilk to meet the daily level of sufficiency of essential fatty acids. According to Marangoni et al. (2000) and Milligan et al. (2008), ARA and DHA intake of the brain was needed optimally during the first 2 years of a period of rapid brain development.

A study had evaluated the longitudinal effects of fish oil on pregnancy on the composition of breastmilk fatty acids and baby outcomes. In a randomized controlled trial, 98 women received 2.2 g docosahexaenoic acid (DHA) and 1.1 g eicosapentaenoic acid (EPA) or olive oil from 20 weeks of pregnancy until delivery. The composition of

fatty acids in breastmilk (at 3 days, 6 weeks, and 6 months) and the baby's erythrocyte membrane (at 1 year) were determined by gas liquid chromatography. Breastmilk fatty acids were examined in association with growth and development. Compared to the control group, breastmilk from women consuming fish oil had DHA and EPA levels was proportionately higher at 3 days and 6 weeks after delivery, but the difference was not seen by 6 months.

The status of DHA (omega-3) infants at 1 year of age is directly related to DHA levels at 3 d, 6 weeks, and 6 months postmenatal (but not for antenatal supplementation). Both EPA and DHA in breastmilk are positively correlated with Griffith's developmental value including hand and eye coordination. Thus, supplementation in pregnancy is associated with increased long-chain n-3 polyunsaturated fatty acids (LCPUFAs) in breastmilk, particularly in early lactation, and is positively related to the infant DHA status at 1 year of age (Janet et al., 2007). In the study, researchers analyzed the results of fatty acid content test of saturated breastfeeding extension. The data in the study showed that there was a presence of omega-6 content and also increase in omega-9 during lactation over 6 months. Previous research had shown that fatty acid levels in breastmilk under 6 months were highest in omega-3 levels (Aryani and Utami 2017). The average of breastmilk over 6 months almost contained no omega-3. Based on the finding, babies should be given complementary foods milk after 6 month old baby. Feeding supplements in infants aims to provide omega-3 intake in infants because omega-3 fatty acids are essential fatty acids that cannot be produced by the body. Omega-3 fatty acids are very influential in the growth and development of infant intelligence (Diana, 2013).

The study is in line with the Nahrowi study (2015). Infants with a lactation period of 12-23 months had the highest total fatty acid content (77.59 ± 5.98 g / 100g fat), while the lowest levels were in infants with 3-5 months lactation period (70.61 ± 7.63 g / 100g fat). Compared with the Saphier (2013) study, the total fatty acid content of Indonesian samples is higher than that of Israeli women (72%). It is different if it is examined in 100 ml of breastmilk, the total fatty acid content in breastmilk ranges from 1.87-4.11 g and the lowest content was found in breastmilk with lactation period of 3-5 months. The result of statistical test showed that based on the lactation period, the total fatty acid content of ASI was significantly different ($p = 0.019$), while the milk fat content was not significantly different ($p = 0.076$). The more the lactation period increases, the total fatty acid content of the ASI increases ($p = 0.002$; $r = 0.36$), whereas the fat content does not. This is according to Finley et al (1985) study that the lactation rate is positively correlated with total fatty acid levels.

CONCLUSION

The study concludes that in breastmilk with a lactation period of 9 months there are various fatty acids content of saturated fatty acids, monounsaturated fatty acids (omega-9) and polyunsaturated fatty acids (omega-6). 2 In the study, the total saturated fatty acid was 42.54% and the total unsaturated fatty acid was 57.17%. Saturated fatty acids consist of lauric, myristic, palmitate, and stearate. Meanwhile polyunsaturated fatty acids consist of omega-6 fatty acids and monounsaturated fatty acids composed of omega-9 fatty acids.

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