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Changes in Fatty Acid Composition of Reconstituted Whole Milk Powder by Superheated Steam

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Abstract: Milk powder is an important product in the food industry that has many characteristics for improving the capacities of a food factory to attract consumers. Manufactured foods are often exposed to high temperatures during preparation and processing. Several changes occur in fatty acid profiles through oxidation. This study aimed to determine the effects of oxidation on fatty acid profiles, such as saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of reconstituted whole milk powder (WMP) by superheated steam oven in relation to thermal treatment with different temperatures and various lengths of time using GC-FID with SGE-BPX 70 column during heating. The temperature ranged from 120 to 180°C for 5 to 15 min. Results showed that in terms of time and temperature, contents of fatty acid profiles showed a decreasing trend when heating increased. SFA, MUFA and PUFA were stable at 120 to 165°C and on various lengths of time but changed at temperature at 180°C. SFA, MUFA and PUFA remained stable between 5 to 10 min but changed after 15 min at 180°C. No oxidation reaction occurred between fatty acids and oxygen molecules in superheated steam. The results also showed that milk fat stability in reconstituted WMP maintained the nutritional value of milk fat during processing.

Key words: Fatty Acids · Superheated · Steam · Lipid Oxidation · Milk Fat

INTRODUCTION

Milk and dairy products play mean roles in human nutrition which make it occupies first place in the nutrition in many countries [1]. Fat and protein are the most of important ingredient in the milk and can be effect by several factors such as feeding, cow age and processing [2]. Fatty acids quality and contain of the human foods take attention and become major nutritional even supply milk to food processing that can improve the flavor and nutritional value [3]. The nutritional value of essential fatty acids such as linoleic acid and smaller amounts of sterols and phospholipids in milk fat is necessary to provide body energy [4]. Fatty acids consist of hydrocarbon chains and high degrees of unsaturation and carbon chain length; various classifications of fatty acids include saturated fatty acids (SFAs), cis monounsaturated fatty acids (MUFAs) and cis polyunsaturated fatty acids (PUFAs) (-n-3 and –n-6 fatty acids) [5]. Heating milk is very important because of changes on carbohydrates, lipids, proteins and minerals that yield nutritional value and flavor [6]. Even the exposure of lipids to high temperatures at 150 to 180°C by cooking with other foods in the presence of oxygen and water may lead to several chemical reactions, such as hydrolysis, oxidation, polymerization, isomerization and cyclization, which can change the composition of fatty acids and produce volatile and nonvolatile compounds that can affect the sensory, functional and nutritional values [7]. Deep exposure to heating in the presence of oxygen decreases the lipid content of unsaturated fatty acids because of oxidation of unsaturated fatty acids and hydroperoxides will form because unsaturated fatty acids have double bonds that are susceptible to oxidation and may lead to

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deterioration in C18:2 caused by the decrease in iodine value and thermo-oxidation leads to desaturation of double bonds by oxidation, scission and polymerization [8]. Many people use reconstituted milk powder to prepare other food and achieve good flavor, such as in making chocolate or cakes. The amounts of milk powder are supplemented by unsaturated fatty acids to obtain high nutritional value such as linoleic and linolenic acids; these fatty acids are very sensitive to oxidation because of several double bonds that may attack oxygen and produce free radicals leading to the first step in fatty acid degradation [9, 10]. PUFAs are very important for body health because essential fatty acids cannot be produced in the human body and must be obtained from other sources, particularly EPA, 20:5 (n-3) eicosapentaenoic acid and DHA, 22:6 (n-3) docosahexaenoic acid, which are recommended for human health, specifically the DHA that has therapeutic effect on human physiology [7, 11]. These fatty acids are also primary structural components in the human brain, cerebral cortex, skin, sperm, testicles and retina [12]. PUFAs become more significant than SFAs in oxidation when treated at high temperatures with the presence of oxygen because many double bonds can attach with oxygen molecules to produce hydroperoxide, a basic compound in fatty acid degradation [13]. Penumetcha *et al*. [14]previously reported that oxidized linoleic acid can be absorbed efficiently inside the intestine and can cause many problems in the human body. Fatty acid oxidation primarily promotes atherosclerosis in terms of oxidized LDL that can lead to atherosclerotic lesion and may result in dietary problems when oxidation becomes proatherogenic [15, 16]. Many studies showed increase number of oxidized fatty acids, specifically PUFAs and these substances can undergo peroxide damage when titrated at high temperatures in the presence of oxygen and can change the fatty acid structure, which is not beneficial to the body [17, 18]. With continued heating in the presence of oxygen, hydroperoxides decomposes into many by-products including aldehydes, ketones, hydrocarbons, acids and alcohols; however, compounds have less nutritional value and undesirable flavor [19]. Processing milk to prepare safety milk by remove all the bacteria and get long shelf life it's called UHT, by this process increase the thermal treatment to milk more than 100 but to a few second because high temperature with long time can be effect on ingredient of milk [32, 33]. Many thermal methods such as air, microwave and superheated steam oven, achieved good result with the least possible effect on food components. Superheated steam has a temperature above boiling point and will form when unsaturated compared with at high temperature for superheated steam [20]. The superheated steam oven is a new method for cooking and drying food, which has many good characteristics, such as high heat transfer capability, condensed heat into contact with food, un oxidized environment and obtainable under normal pressure. The superheated steam is used to dry many products with high fat contents and exhibit lipid oxidation at high temperature where oxygen is unavailable, thereby preventing the formation of hydroperoxides [21].

This study aimed to evaluate the changes in fatty acid profiles by oxidation, particularly in unsaturated fatty acids and determine the effect of thermal heat treatment and time on reconstituted whole milk powder (WMP) using superheated steam.

MATERIALS AND METHODS

Whole Milk Powder: WMP was supplied by Nespray and purchased from the Tesco market in Penang City, Malaysia. The amounts of standard milk powder contents are as follows: fat (28.2 g per 100 g), protein (23.6 g per 100 g) and carbohydrate (39.9 g per 100 g).

Whole Milk Powder Reconstitution: The reconstituted WMP was prepared according to the instructions of the manufacturer listed on the package. The WMP was reconstituted using 33 g of milk powder and adding 225 ml of water. The mixture was shaken on a magnetic stirrer for 10 min.

Heating Reconstituted Milk Powder by Superheated Steam Oven: Thermal coefficient samples were exposed in superheated steam oven after milk reconstitution and yielded a total of 500 ml of samples. The collected samples exposed in superheated steam oven (AX1500) were infectious. The samples were exposed at various temperatures (120, 135, 150, 165 and 180°C) and period times (5, 10 and 15 min); all samples subsequently underwent analyses.

Fat Extraction: Milk fat was extracted from the reconstituted whole milk powder using the Folch 1957 method modified with chloroform, methanol and water (2:1:1) [22]. The percentage of solvent was 20, 10 and 10 respectively. The solvent mixture containing the extracted lipids was separated from the reconstituted milk by centrifugation. After that the mixture was mixed with 0.88% of KCl solution in a separating funnel with stirring

vigorously for phase separation. The upper layer was separating these contain water and methanol and non-lipid and the lower phase was separating these layer contain the chloroform and lipid. This layer was flirted by Buchner funnel using anhydrous sodium sulfate. The residue was collected out in glass vials and the solvent was removed by using rotary evaporator with temperature below 50°C.

Preparation of Fatty Acids Methyl Ester: Fatty acids were acquired by transmethylating off into fatty acid methyl ester, which was carried out by Simionato *et al*. [23]. The lipid weight was approximately 150 mg and added to 5.0 mL 0.25 mol L^{-1} sodium methoxide in methanol-diethyl ether (1:1) and it was stirred vigorously for 3 min. After that 3 mL of isooctane and 15 mL of saturated sodium chloride were added. The tube was stirred vigorously again and waiting after the phase separation. The upper layer was transfer to GC vail for analysis. Fatty acids were identified by comparison standard retention time with fatty acid retention time of samples. Fatty acids standard was purchase from Supelco (16823-0048), USA and contains 37 fatty acids.

GC-FID Analysis of Fatty Acids: GC analysis was performed using GC-FID equipped with SGE-BPX 70 column. The condition of GC during the analysis was set as follows: rates used were 1.4 mL min-1 carrier gas (H2), 30 mL min-1 make-up gas (N2) and 30 and 300 mL min-1 flame gases, H2 and flame synthetic air, respectively. The injection sample took rate 1/100 with detector temperatures 235°C. The column temperature was 65°C for 4 min, followed by a ramp of 16°C min-1 up to 185°C, kept for 12 min. A second ramp of 20° C min-1 was run up to 235°C for 14 min. The total analysis time was 40 min.

Statistical Analysis: All experiments were performed in triplicate and data were subjected in SPSS version (20) (SPSS Inc., Chicago, USA) using analysis of variance and Duncan's multiple range test for comparison of significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

Effects of Superheated Steam (Time and Temperature) on SFA Composition: Tables 1-5 show the SFA composition in reconstituted WMP at various temperatures and times in superheated steam. SFA was indicated from C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0. Changes in fatty acid composition during heating by superheated steam at 120°C did not exhibit any significant differences ($p > 0.05$) between the sample times of 5 min to 10 min compared with the control, except for C15:0 and C20:0 fatty acids with significant differences ($p<0.05$) in longer time (15 min) compared with the control. The C12:0, C14:0, C16:0 and C18:0 fatty acids did not show any significant differences compared with the control (Table 1). At 135°C, SFA did not record any significant differences ($p > 0.05$) between the sample times of 5 min to 10 min, but was significantly different at 15 min compared with the control. The C12:0 fatty acids did not show any differences compared with the control and the heating time was similar for all temperatures (Table 2). At 150°C, C10:0, C12:0, C14:0, C15:0, C16:0 and C17:0 fatty acids showed significant differences compared with the control and between the sample times. C10:0 and C16:0 fatty acids did not show any difference in values of samples at 5 min to 15 min at 150°C and all other SFA showed differences between the heating times (Table 3). These findings can be attributed to the nondegradation of SFA, in which no oxygen molecule was found inside the superheated steam oven, hence no degradation can occur in fatty acids, which require oxygen to attack free radicals and yield alkoxy radicals [9]. Alkoxy radicals can produce primary oxidation products, such as hydroperoxide and can be decomposed into many compounds that cause degradation of fatty acids, resulting in less nutritional value and undesirable flavor of the product. At 165°C, the SFA composition showed significant differences between each period and the control, except for C18:0. Each SFA did not differ at 5 min to 10 min, but the significant difference was shown after 15 min at 165°C (Table 4). At 180°C, SFA showed significant difference compared with the control. C10:0, C12:0, C14:0, C17:0 and C18:0 fatty acids have significant differences at 5 min to 10 min, whereas each SFA was different after 15 min at 180°C. C15:0 and C20:0 fatty acids did not record differences between all heating times (Table 5). Therefore, at 5 min to 10 min, SFA did not affect the fatty acid composition at 120 to 165°C; however, the fatty acid composition changed at high temperature (180°C). At high temperatures, changes in milk fat increased because of the formation of cyclic monomers, dimers and polymers, as well as changes in fatty acid structure by breaking the bonds at high heating [24].

Capric acid (C10:0) constituted the largest portion with 14.80% at 150°C for 5 min. C12:0, C14:0, C15:0, C16:0 and C17:0 fatty acids recorded large values (10.06, 9.39, 1.56, 5.21 and 1.06%) for C12:0, C14:0, C15:0 and C16:0 at 135°C. C17:0 recorded a large value at 150°C for 5 min.

Table 1: Effect of superheated steam on percentage of fatty acids composition of reconstituted whole milk powder by temperature (120)°C and different period times

	Period time (min)			
Fatty acids (%)	Control	5	10	15
C4:0	ND	ND	ND	ND
C6:0	ND	ND	ND	ND
C8:0	ND	ND	ND	ND
C10:0	14.21±0.58^{aA}	14.62±0.54^{aA}	14.52 ± 0.58^{aA}	14.41 ± 0.55^{aAB}
C12:0	11.10 ± 0.05^{aA}	10.73±0.52^{aA}	10.27 ± 0.52^{aAB}	11.01 ± 0.02^{aA}
C14:0	9.02 ± 0.58^{aA}	8.98±0.57^{aA}	8.82 ± 0.57^{aA}	8.71±0.50^{aA}
C15:0	1.58 ± 0.05^{aA}	1.54 ± 0.01^{bAB}	1.53 ± 0.00^{bcB}	1.53 ± 0.00^{bcAB}
C16:0	5.11 ± 0.06^{aA}	5.10 ± 0.06^{aAB}	5.09 ± 0.06^{aAB}	5.07 ± 0.07^{a}
C17:0	1.07 ± 0.06^{aA}	1.07 ± 0.05^{aA}	1.06 ± 0.05^{aA}	1.05 ± 0.05^{aA}
C18:0	13.25 ± 0.56^{aA}	13.18±0.51^{aAB}	12.85 ±0.05^{abAB}	12.78 ± 0.12^{ab}
C20:0	2.55 ± 0.41^{aA}	2.06 ± 0.01^{bAB}	2.07 ± 0.00^{bAB}	2.05 ± 0.01^{bAB}
C22:0	ND	ND	ND	ND
Σ SFA*	57.89	57.28	56.21	56.61
C14:1	2.79 ± 0.19^{aA}	2.48 ± 0.01^{bB}	2.56 ± 0.19^{bAB}	2.45 ± 0.01^{bB}
C16:1n – 7	1.60 ± 0.00^{aA}	1.58 ±0.01^{aA}	1.58 ± 0.01^{aA}	1.58 ± 0.02^{aAB}
C18:1n – 9n	21.09±0.57^{aA}	20.99±0.64^{aAB}	20.81 ± 0.60^{aAB}	20.72 ± 0.62^{aAB}
Σ MUFA**	25.48	25.05	24.95	24.75
C18:2n – 6	2.40 ± 0.42^{aA}	2.15±0.11^{abA}	2.11 ± 0.05^{abA}	2.05 ± 0.01^{abA}
C18:3n – 3	1.13 ± 0.11^{aA}	1.05±0.05^{abA}	1.02 ± 0.01^{bA}	1.02 ± 0.00^{bA}
C20:4n – 6 (AA)	ND	ND	ND	ND
C20:5n – 3 (EPA)	1.54 ± 0.06^{aA}	1.45 ± 0.04^{bBC}	1.40 ± 0.01^{bcB}	1.39 ± 0.02^{bcB}
C22:2n – 6	ND	ND	ND	ND
C22:4n – 6	ND	ND	ND	ND
C22:5n – 3	ND	ND	ND	ND
C22:6n – 3 (DHA)	0.85 ±0.03^{aA}	0.80 ± 0.01^{bB}	0.82 ± 0.01^{bAB}	0.80 ± 0.01^{bA}
Σ PUFA***	5.92	5.45	5.35	5.26
PUFA\SFA	0.10	0.09	0.09	0.09
DHA\EPA	0.55	0.55	0.58	0.57
Unidentified	10.71	12.22	13.49	13.38

$^{a–c}$ Symbols bearing different letters in the same row are significantly different (P < 0.05).

$^{A–D}$ Symbols bearing different letters in the same column are significantly different (P < 0.05)

*SAF saturated fatty acids.

** MUFA monounsaturated fatty acids.

*** PUFA polyunsaturated fatty acids.

ND Not detected.

Total percentages of SFA were between 57.28 to 56.61 at 120°C, 57.28 to 56.61% at 135°C, 56.79 to 55.25% at 150°C, 55.42 to 55.41% at 165°C and 54.87 to 54.43% at 180°C (Tables 1-5). These ranges slightly declined when the time and temperature were advanced. This finding is ascribed to the decrease of SFA at high temperature during heating, in which glycerol and free fatty acids partially evaporate; this reaction shifts the equilibrium to other hydrolysis products [25].

The SFA content at all temperatures and times was between 57.28 to 54.43% compared with that of the control (57.89%). This finding indicates no significant difference between the samples and low difference compared with the control. SFA/PUFA ratio demonstrated a dominant percentage of saturation relative to PUFA, but the ratio of (MUFA + PUFA)/SFA showed a dominant percentage of MUFA and PUFA relative to SFA [26].

Tables 1-5 show the effect of various temperatures at a fixed time. The results showed no significant differences between 120 to 180°C at 5 min to 10 min on the fatty acid samples compared with the control. C10:0, C12:0, C14:0, C15, C16, C17:0 and C18:0 fatty acids recorded no significant differences between each sample treatment at 120 to 165°C for 5 min to 15 min. C12:0 showed a

Table 2: Effect of superheated steam on percentage of fatty acids composition of reconstituted whole milk powder by temperature (135)°C and different period times

	Period time(min)			
Fatty acids (%)	Control	5	10	15
C4:0	ND	ND	ND	ND
C6:0	ND	ND	ND	ND
C8:0	ND	ND	ND	ND
C10:0	14.21±0.58^{aA}	14.62±0.54^{aA}	14.52 ± 0.58^{aA}	14.41 ± 0.55^{aAB}
C12:0	11.10 ± 0.05^{aA}	10.73±0.52^{aA}	10.27 ± 0.52^{aABC}	11.01 ± 0.02^{aB}
C14:0	9.02 ± 0.58^{aA}	8.98±0.57^{aA}	8.82 ± 0.57^{aA}	8.71±0.50^{aA}
C15:0	1.58 ± 0.05^{aA}	1.54 ± 0.01^{bB}	1.53 ± 0.00^{bcB}	1.53 ± 0.00^{bcB}
C16:0	5.11 ± 0.06^{aA}	5.10 ± 0.06^{aB}	5.09 ± 0.06^{aAB}	5.07 ± 0.07^{aB}
C17:0	1.07 ± 0.06^{aA}	1.07 ± 0.05^{aA}	1.06 ± 0.05^{aA}	1.05 ± 0.05^{aA}
C18:0	13.25 ± 0.56^{aA}	13.18±0.51^{aBC}	12.85 ±0.05^{abABC}	12.78±0.12^{abABC}
C20:0	2.55 ± 0.41^{aA}	2.06 ± 0.00^{bAB}	2.07 ± 0.00^{bAB}	2.05 ± 0.01^{bAB}
C22:0	ND	ND	ND	ND
Σ SFA*	57.89	57.28	56.21	56.61
C14:1	2.79 ± 0.19^{aA}	2.48 ± 0.01^{bBC}	2.56 ± 0.19^{bBC}	2.45 ± 0.01^{bBC}
C16:1n – 7	1.60 ± 0.00^{aA}	1.58 ±0.01^{aA}	1.58 ± 0.01^{aA}	1.58 ± 0.02^{aC}
C18:1n – 9n	20.09±0.57^{aA}	20.99±0.64^{aAB}	20.00 ± 0.60^{aA}	20.03 ± 0.62^{aAB}
Σ MUFA**	24.48	25.05	24.14	24.06
C18:2n – 6	2.40 ± 0.42^{aA}	2.15±0.11^{abA}	2.11 ± 0.05^{abA}	2.05 ± 0.01^{abA}
C18:3n – 3	1.13 ± 0.11^{aA}	1.05±0.05^{abA}	1.02 ± 0.00^{bA}	1.02 ± 0.00^{bA}
C20:4n – 6 (AA)	ND	ND	ND	ND
C20:5n – 3 (EPA)	1.54 ± 0.06^{aA}	1.45 ± 0.04^{bBC}	1.40 ± 0.01^{bcBC}	1.39 ± 0.02^{bcBC}
C22:2n – 6	ND	ND	ND	ND
C22:4n – 6	ND	ND	ND	ND
C22:5n – 3	ND	ND	ND	ND
C22:6n – 3 (DHA)	0.85 ±0.03^{aA}	0.80 ± 0.01^{bB}	0.82 ± 0.01^{bB}	0.80 ± 0.01^{bA}
Σ PUFA***	5.92	5.45	5.35	5.26
PUFA\SFA	0.10	0.09	0.09	0.09
DHA\EPA	0.55	0.55	0.58	0.57
Unidentified	11.71	12.22	14.30	14.07

$^{a–c}$ Symbols bearing different letters in the same row are significantly different (P < 0.05).

$^{A–D}$ Symbols bearing different letters in the same column are significantly different (P < 0.05)

*SAF saturated fatty acids.

** MUFA monounsaturated fatty acids.

*** PUFA polyunsaturated fatty acids.

ND Not detected.

significant decrease at 120 to 180°C for 15 min. At 180°C for all time periods, the SFA result was slightly different from the control. Subsequently, C10:0 differed from the control at 15 min. The stability of SFA at high heating is attributed to the absence of oxygen in superheated steam oven, which is needed for fatty acid degradation that produces primary and secondary oxidation products; therefore, the superheated steam could maintain the SFA and nutritional value of milk fat [27].

Effects of Superheated Steam (Time and Temperature) on MUFA Composition: Tables 1-5 show the effects of various time periods on MUFA composition of three Westphalian MUFAs, namely, myristoleic acid (C14:1), palmitoleic acid (C16:1) and oleic acid (C18:1). Oleic acid is the major MUFA in milk with percentages of 20.99 to 20.72%, 20.99 to 20.03%, 20.27 to 20.14, 20.21 to 20.73% and 20.10 to 20.09% at 120°C to 180°C for 5 min to 15 min. C14:1 and C16:1 fatty acids recorded 1.62 to 2.48% and 1.46 to 1.95%. The percentage of oleic acid did not show any significant difference between various times of heat treatment compared with the control for 5 min to 15 min at 120°C to 135°C. At longer time (15 min) with high temperature (180°C), the result of oleic acid did not show significant difference between the heat treatment samples and the control at temperature 120 and 135°C but showed significant difference between heat treatment samples at temperature 150-180°C especially at long time 15 min. The C14:1 and C16:1 fatty acids did not show any significant differences between all time periods at 120°C

Table 3: Effect of superheated steam on percentage of fatty acids composition of reconstituted whole milk powder by temperature (150)°C and different period times

	Period time(min)			
Fatty acids (%)	Control	5	10	15
C4:0	ND	ND	ND	ND
C6:0	ND	ND	ND	ND
C8:0	ND	ND	ND	ND
C10:0	14.21 ± 0.58^{a}	14.80 ±0.09^{abA}	14.26 ± 0.11^{abA}	14.03 ± 0.60^{bAB}
C12:0	11.10 ± 0.05^{a}	10.06 ± 0.52^{abA}	10.72 ± 0.51^{abAB}	10.21 ± 0.25^{bB}
C14:0	9.02 ± 0.58^{ab}	9.20 ± 0.08^{aA}	9.16 ± 0.13^{aA}	9.09 ± 0.08^{abA}
C15:0	1.58 ± 0.05^{a}	1.41 ± 0.02^{bC}	1.40 ± 0.01^{bcC}	1.37 ± 0.02^{bcC}
C16:0	5.11 ± 0.06^{a}	5.05 ± 0.02^{abB}	5.03 ± 0.02^{abB}	5.01 ± 0.03^{abB}
C17:0	1.07 ± 0.06^{a}	1.06 ± 0.06^{aA}	1.05 ± 0.06^{aA}	1.04 ± 0.06^{aA}
C18:0	13.25 ± 0.56^{a}	12.72 ± 0.13^{bBC}	12.38 ± 0.05^{bcBC}	12.13 ± 0.11^{cdBC}
C20:0	2.55 ± 0.41^{a}	2.49 ± 0.37^{aB}	2.43 ± 0.32^{aAB}	2.37 ± 0.27^{aB}
C22:0	ND	ND	ND	ND
Σ SFA	57.89	56.79	56.43	55.25
C14:1	2.79 ± 0.19^{a}	2.42 ± 0.03^{bBCD}	2.43 ± 0.02^{bCD}	2.42 ± 0.02^{bB}
C16:1n – 7	1.60 ± 0.00^{a}	1.52 ± 0.01^{bB}	1.50 ± 0.01^{bcB}	1.49 ± 0.01^{bcC}
C18:1n – 9n	20.09 ± 0.57^{a}	20.27 ±0.06^{bcAB}	20.35 ± 0.04^{cB}	20.14 ± 0.05^{abAB}
Σ MUFA	24.48	24.21	24.28	24.05
C18:2n – 6	2.40 ± 0.42^{a}	2.35 ± 0.40^{aA}	2.32 ± 0.37^{aA}	2.30 ± 0.35^{aA}
C18:3n – 3	1.12 ± 0.11^{a}	1.05 ± 0.06^{abA}	1.09 ± 0.13^{abA}	1.08 ± 0.11^{abA}
C20:4n – 6 (AA)	ND	ND	ND	ND
C20:5n – 3 (EPA)	1.54 ± 0.06^{a}	1.40 ± 0.05^{aBC}	1.30 ± 0.04^{aC}	1.28 ± 0.05^{aC}
C22:2n – 6	ND	ND	ND	ND
C22:4n – 6	ND	ND	ND	ND
C22:5n – 3	ND	ND	ND	ND
C22:6n – 3 (DHA)	0.85 ± 0.03^{a}	0.81 ± 0.01^{bB}	0.79 ± 0.01^{bAB}	0.79 ± 0.01^{bA}
Σ PUFA	5.91	5.61	5.50	5.45
PUFA\SFA	0.10	0.09	0.09	0.09
DHA\EPA	0.55	0.57	0.60	0.60
Unidentified	8.72	10.93	10.79	12.25

$^{a–c}$ Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

$^{A–D}$ Symbols bearing different letters in the same column are significantly different ($P < 0.05$)

*SAF saturated fatty acids.

** MUFA monounsaturated fatty acids.

*** PUFA polyunsaturated fatty acids.

ND Not detected.

to 135°C compared with the control. At 150°C for 5 min to 15 min, the C14:1 and C16:1, fatty acids did not show any significant difference between the sample treatments, but showed a significant difference between the sample and the control. The C18:1 differed from the control for 5 min to 10 min at 150°C, which recorded 20.27 and 20.35%. The C14:1 fatty acid did not show any significant differences for 5 min to 15 min at 165°C between the sample treatments, but C18:1 disagreed with the control its recorded similar data to the treatment sample, which also disagreed at 15 min. For 5 min to 10 min, the result for MUFA recorded no significant differences between the sample treatments at 180°C, but the result showed significant differences at 15 min for the C14:1, C16:1 and C18:1 fatty acids with heat treatment samples and the control.

Tables 1-5 show the effects of various temperatures on MUFAs. The effects of temperatures were shown in five systems at 120 to 180°C in the superheated steam oven. At 120°C, no significant difference was recorded for all time periods for the C16:1 and C18:1 compared with the control. However, for the C14:1 at 120°C, significant difference was shown with the control at 5 and 15 min. At 120°C to 165°C for 5 min, the MUFA did not show any significant differences between the heat treatment samples but showed difference at 180°C compared with

Table 4: Effect of superheated steam on percentage of fatty acids composition of reconstituted whole milk powder by temperature (165)°C and different period times

	Period time(min)			
Fatty acids (%)	Control	5	10	15
C4:0	ND	ND	ND	ND
C6:0	ND	ND	ND	ND
C8:0	ND	ND	ND	ND
C10:0	14.21 ± 0.58^{Aa}	14.13 ± 0.39^{abA}	13.96 ± 0.50^{abcA}	13.87 ± 0.46^{abcAB}
C12:0	11.10 ± 0.05^{aA}	10.39 ± 0.55^{ba}	10.05 ± 0.06^{bBC}	10.04 ± 0.06^{bB}
C14:0	9.02 ± 0.58^{aA}	9.20 ± 0.08^{aA}	9.28 ± 0.08^{aA}	9.09 ± 0.08^{aA}
C15:0	1.58 ± 0.05^{aA}	1.42 ± 0.05^{bC}	1.38 ± 0.01^{bCD}	1.32 ± 0.06^{bcC}
C16:0	5.11 ± 0.06^{aA}	5.04 ± 0.01^{aC}	5.02 ± 0.03^{abB}	5.93 ± 0.07^{bC}
C17:0	1.07 ± 0.06^{aA}	1.06 ± 0.06^{aA}	1.05 ± 0.06^{aA}	1.04 ± 0.06^{aA}
C18:0	13.25 ± 0.56^{aA}	12.10 ± 0.46^{bBC}	11.98 ± 0.62^{bC}	12.05 ± 0.47^{bBC}
C20:0	2.55 ± 0.41^{aA}	2.08 ± 0.03^{abB}	2.07 ± 0.04^{abAB}	2.07 ± 0.04^{abB}
C22:0	ND	ND	ND	ND
Σ SFA	57.89	55.42	54.79	55.41
C14:1	2.79 ± 0.19^{aA}	2.21 ± 0.01^{bCD}	2.18 ± 0.03^{bCD}	2.18 ± 0.02^{bC}
C16:1n – 7	1.60 ± 0.00^{aA}	1.51 ± 0.01^{Bb}	1.50 ± 0.01^{bcB}	1.48 ± 0.01^{bcC}
C18:1n – 9n	20.09 ± 0.57^{abAB}	20.21 ± 0.03^{aAB}	20.02 ± 0.02^{bA}	20.73 ± 0.09^{cAB}
Σ MUFA	24.48	23.93	23.7	24.39
C18:2n – 6	2.40 ± 0.42^{aA}	2.06 ± 0.08^{bA}	2.05 ± 0.08^{bA}	2.04 ± 0.05^{bA}
C18:3n – 3	1.13 ± 0.11^{aA}	1.08 ± 0.06^{Ba}	1.04 ± 0.05^{bA}	1.04 ± 0.05^{bA}
C20:4n – 6 (AA)	ND	ND	ND	ND
C20:5n – 3 (EPA)	1.54 ± 0.06^{aA}	1.37 ± 0.02^{bC}	1.28 ± 0.06^{bcC}	1.25 ± 0.07^{bcCD}
C22:2n – 6	ND	ND	ND	ND
C22:4n – 6	ND	ND	ND	ND
C22:5n – 3	ND	ND	ND	ND
C22:6n – 3 (DHA)	0.85 ± 0.03^{aA}	0.81 ± 0.01^{bB}	0.80 ± 0.00^{bAB}	0.79 ± 0.01^{bA}
Σ PUFA	5.91	5.61	5.50	5.45
PUFA\SFA	0.10	0.10	0.10	0.09
DHA\EPA	0.55	0.59	0.62	0.63
Unidentified	11.72	15.04	16.01	14.75

[a–d] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

[A–D] Symbols bearing different letters in the same column are significantly different ($P < 0.05$)

*SAF saturated fatty acids.

** MUFA monounsaturated fatty acids.

*** PUFA polyunsaturated fatty acids.

ND Not detected.

the control which ranged from 120 to 180°C, resulting in 2.48 to 2.26% for C14:1, 1.58 to 1.51% for C16:1 and 20.99 to 20.10% for C18:1. At 120 to 180°C for 10 min, the C14:1 showed no significant difference between the heat treatment samples at 2.56 and 2.26%. The C16:1 did not show any significant differences between 150 to 180°C, but differed from 120° to 135°C, at 120 to 180°C by 1.58 to 1.48%. At 120°C to 180°C for 10 min, C18:1 showed no significant difference between the heat treatment samples, but exhibited difference with the control.

At 15 min and temperature of 135 to 150°C, the C16:1 and C18:1 did not show any significant differences between the heat treatment samples, but recorded significant differences at 120°C and with the control. The C14:1 showed significant differences at 165 and 150°C. At 180°C for 10 min, the C14:1 and C16:1 showed significant differences compared with other temperatures and the control and the C18:1 did not show significant differences at 120 to 135°C, but showed difference with 150 to 180°C compared with the control. After 15 min, the C14:1 and C18:1 recorded significant differences at 180°C between the temperature and the control. The C16:1 did not record any significant differences between 120 to 135°C compared with the control, as well as between 150 to 180°C, but showed significant differences at 180 with 120 to 135°C and the control.

The MUFA results showed stability of the C14:1 and C18:1 to the heating in superheated steam with slight change in the C18:1. These findings led to lime degradation of fatty acids [28]. Hui, 1992 proved the

Table 5: Effect of superheated steam on percentage of fatty acids composition of reconstituted whole milk powder by temperature (180)°C and different period times

	Period time(min)			
Fatty acids (%)	Control	5	10	15
C4:0	ND	ND	ND	ND
C6:0	ND	ND	ND	ND
C8:0	ND	ND	ND	ND
C10:0	14.21 ± 0.58^{aA}	13.89 ± 0.26^{aA}	13.78 ± 0.39^{aA}	13.52 ± 0.50^{abB}
C12:0	11.10 ± 0.02^{aA}	10.38 ± 0.54^{abA}	9.92 ± 0.16^{bcC}	10.30 ± 0.58^{abB}
C14:0	9.02 ± 0.58^{aA}	9.18 ± 0.06^{aA}	9.15 ± 0.06^{aA}	8.50 ± 0.44^{abA}
C15:0	1.58 ± 0.05^{aA}	1.35 ± 0.04^{bC}	1.35 ± 0.01^{bD}	1.29 ± 0.06^{bC}
C16:0	5.11 ± 0.06^{aA}	5.03 ± 0.04^{abC}	5.35 ± 0.53^{cC}	5.88 ± 0.02^{abC}
C17:0	1.07 ± 0.06^{aA}	1.05 ± 0.^{06aA}	1.05 ± 0.06^{abA}	1.04 ±0.06^{abA}
C18:0	13.25 ±0.56^{bB}	11.98± 0.54^{bC}	11.85± 0.67^{bC}	11.90 ± 0.62^{bC}
C20:0	2.55 ± 0.41^{aA}	2.01 ± 0.02^{bB}	2.01 ± 0.02^{bB}	2.00 ± 0.01^{bB}
C22:0	ND	ND	ND	ND
Σ SFA	57.89	54.87	54.46	54.43
C14:1	2.79 ± 0.19^{aA}	2.26 ± 0.05^{bD}	2.26 ± 0.10^{bD}	1.90 ± 0.09^{cD}
C16:1n – 7	1.60 ± 0.00^{aA}	1.51 ± 0.01^{bB}	1.48 ± 0.01^{bcB}	1.47 ± 0.01^{cC}
C18:1n – 9n	20.09 ± 0.57^{aA}	20.10 ± 0.10^{aB}	20.08 ± 0.12^{aAB}	20.09 ± 0.29^{bB}
Σ MUFA	24.48	23.87	23.82	23.47
C18:2n – 6	2.40 ± 0.42^{aA}	2.06 ± 0.08^{bA}	2.05 ± 0.08^{bA}	1.97 ± 0.02^{bA}
C18:3n – 3	1.13 ± 0.11^{aA}	1.04 ± 0.06^{bA}	1.04 ± 0.05^{bA}	1.03 ± 0.06^{bA}
C20:4n – 6 (AA)	ND	ND	ND	ND
C20:5n – 3 (EPA)	1.54 ± 0.06^{aA}	1.25 ± 0.03^{bD}	1.19 ± 0.01^{cD}	1.18 ± 0.01^{cdD}
C22:2n – 6	ND	ND	ND	ND
C22:4n – 6	ND	ND	ND	ND
C22:5n – 3	ND	ND	ND	ND
C22:6n – 3 (DHA)	0.85 ± 0.03^{aA}	0.78 ± 0.01^{bB}	0.76 ± 0.01^{bB}	0.75 ± 0.01^{bcA}
Σ PUFA	5.92	5.13	5.04	4.93
PUFA\SFA	0.10	0.09	0.09	0.09
DHA\EPA	0.55	0.62	0.63	0.63
Unidentified	11.71	16.13	16.68	17.17

$^{a–d}$ Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

$^{A–D}$ Symbols bearing different letters in the same column are significantly different ($P < 0.05$)

*SAF saturated fatty acids

** MUFA monounsaturated fatty acids

*** PUFA polyunsaturated fatty acids

ND Not detected

relationship between the ratio of oxygen and the deterioration of unsaturated fatty acids. The relative auto-oxidation rates of oleic, linoleic and linolenic acids at 1:40 to 50:100 based on the oxygen were the rate of interactions between C18:0, C18:1, C18:2 and C18:3 with the oxygen at 1.2 × 104, 5.3 × 104, 7.3 × 104 and 10.0 × 104 $M^{-1}s^{-1}$, respectively [27].

Effects of superheated steam (time and temperature) on PUFA composition: Tables 1–5 show the effects of superheated steam at 5 min to 15 min on the PUFA as shown in four fatty acids (C18:2, C18:3, EPA and DHA). Table 1 shows that the C18:2, C18:3, EPA and DHA did not show any significant differences between the treatment samples for 5 o 15 min at 120°C. Therefore, the period time of 5 to 15 min did not affect the PUFA at 120°C. Table 2 shows that the period time of 5 to 15 min at 135°C did not show any significant difference between C18:2, C18:3 and EPA compared with the control. DHA showed significant difference at 15 min but did not record any significant differences compared with the rest of the samples with the control. Table 3 shows that the C18:2 and EPA did not show any significant difference between the treatment samples and the control for 5 min to 15 min at 150°C, whereas DHA showed a significant difference compared with control, but did not show any difference with each treatment sample at 5 min to 15 min. C18:3 did not show any significant difference at period time of 5 to 15 min compared with the control but showed significant difference with the control at 180°C. Table 4 displays that C18:2, C18:3 and DHA showed significant differences

between the period time compared with the control, but did not record any significant differences between the treatment samples for 5 min to 15 min at 165°C. Table 5 shows significant changes in EPA and DHA between the treatment samples compared with the control for 5 min to 15 min at 180°C, but did not show any significant difference between 5 min to 10 min at 180°C.

Tables 1-5 show the effects of superheated steam temperatures at 120°C to 180°C on the PUFA. C18:2 and C18:3 did not record any significant differences between 120 to 180°C for 5 to 10 min. DHA did not show significant difference between the temperatures 120 to 180°C, but was different from the control, whereas EPA showed significant difference between the control and each heat treatment sample at 180°C with 120 to 180°C. EPA and DHA also recorded significant differences between 180°C with 120 to 165°C and the control. Table 1-5 shows no significant differences for 15 min to C18:2, C18:3 and DHA at all temperatures from 120 to 180°C compared with the control. EPA showed significant difference between each temperature compared with the control and between 180 and 120 to 135°C. For all times and temperatures from 120 to165°C, the result for PUFA recorded stability to the heating with minor change to a high degree of thermal 180°C and this stability is attributed to the non-oxidation reaction between the fatty acid and the oxygen because no oxygen is available in superheated seam oven. [29] interpreted this decline when the temperature increased above 180°C and the rate of decline in the PUFA will be increased from 30 to 80%. This finding can decrease back to the double bonds in the PUFA which can be associated with other compounds, particularly proteins. The lipid–protein interactions occur naturally in tissue and may be induced in food products during processing and storage [30].

CONCLUSIONS

By comparison of the aforementioned results, the superheated steam period time 5 min to 10 min did not affect the SFA for all temperatures from 120 to 165°C with slight changes in the SFA after 15 min. At 180°C, the SFA changed after 15 min. MUFA recorded changes on the period time of 15 min at 180°C, but for 5 to 10 min at 120 to 165°C, no changes were shown in the heat treatment samples. Superheated steam time of 5 to 10 min at 120 to 180°C did not affect C18:2 and C18:3, but showed changes after 15 min at 180°C. The EPA and DHA superheated steam did not change after 5 to 10 min at 120 to 180°C and showed slight changes after 15 min at 180°C. In summary, the superheated steam maintained the fatty acid profile and prevented lipid oxidation of milk fat because the process occurred in an atmosphere with less oxygen, which is the main factor in oxidation.

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