

Changes in Lipid Oxidation of Reconstituted Omega-3 Plus Milk Powder Treated by Superheated Steam

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Abstract— In this study the effect of superheated steam on the oxidative stability of reconstituted omega-3 plus milk powder was studied. The changes in fatty acids, peroxide, acids, thiobarbituric acid reactive substances (TBARS), and p-anisidine values were measured to reconstitute omega-3 plus milk powder in relation to thermal treatment by using a superheated steam (SHS) with different temperatures and time periods. The temperature and time period ranged from 120-180 °C between 5-15 min. For applying the SHS treatment did not produce changes in SFA, MUFA and PUFA at 120-150 °C to 10-15 min as compared with the control. While, the proportion of PUFA and MUFA values were recorded differences on temperature 180 °C comparison with control. The result of POV showed no changes between the temperature 120-150 °C compared with control, but indicated significant differences at the temperature 180 °C with long time 15 min. Acids and p-anisidine values did not show any differences between temperature 120-180 °C for a short period of time 5 min compare with the control, but recorded significantly on long period of time 10-15 min compared with the control. The values of TBARS showed no significant differences in temperature 120-150 for 5 min compare with control. This study showed oxidative stability of milk fat supplied long chain unsaturated fatty acids treated by SHS on temperature range 120-150 at time 5-10 min with some affected by the high temperature 180 °C at 15 min can maintain the quality of fatty acid and milk fat.

Keywords— superheated steam, omega-3 plus milk powder, fatty acids, lipid oxidation

I. INTRODUCTION

The family of omega-3 polyunsaturated fatty acid (PUFA) consists of many types of polyunsaturated fatty acids such as α -linolenic acid (C18:3), long-chain eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22: 6) [1]. The omega-3 fatty acids, especially long-chain of DHA and EPA polyunsaturated fatty acids are well known in the prevention of some types of cancer, rheumatoid arthritis and cardiovascular diseases. In Addition the DHA acts have important factor to ensuring proper development. and function of the brain and a visual functionality [2,3]. Some Researchers have shown that diets rich of omega-3-fatty acids can reduce blood triglyceride levels and platelet aggregation and show both antithrombotic and anti-inflammatory effects. [4,5]. Some health centers in the UK such as united kingdom's department of Health recommend to intake the long chain fatty acids (EPA and DHA) every week and estimated the ratio needed by 1.5 g, and that approximately equivalent 0.2 g per day, also the (EANS) European Academy of Nutritional Sciences recommends 0.2

g of EPA and DHA as the average per day [6,7]. The FDA limit daily intake of DHA and EPA and remand should be not exceed 3 g per day to adult and can take this ratio from fish oil or any food fortified source [1]. Consumption long chain unsaturated fatty acids include DHA and EPA fortified of various food that's can be increase the omega-3 average intake and that can give better results of the health protection to consumers [8-10]. Milk powder is widely used for manufacturing foods such as cakes, pastries, and chocolates, that's because milk can improve food characteristics, including texture, color, and flavor [11]. Mean compound responsible on the flavoured and nutritional value is the fat, milk fat approximately consist triglycerides, which form 98%, the 2% remaining are milk fat consists mainly of triglycerides, approximately 98%, while other milk lipids are diacylglycerol, cholesterol, phospholipids and free fatty acids [12]. The milk fat is more complex of nutrition lipids and this is due to having about 400 fatty acids [13,14]. Milk fat has two types of fatty acids, saturated fatty acids consist 70 % and unsaturated fatty acids consist 30 %, the most important of saturated fatty acids is palmitic acid its form

approximately 30% of total fatty acids. Stearic acid and myristic acids make up 11-12 % and short-chain fatty acids C4-C10 make 10.9 % of saturated fatty acids, while the unsaturated fatty acids approximately consist 25% mono-unsaturated fatty acids oleic acid. The polyunsaturated fatty acids considered an important source of omega-3 fatty acids, which be lower in milk fat and must be fortified from another source [13,15]. Omega-3 and omega-6 considered essential fatty that's due humans and animals cannot synthesize these fatty acids and must get them from other diets. Many diets are rich in omega-3 and omega-6 such as olive oil, flax seed and fish and the researcher's use these diets as a natural source for fortified omega-3 and omega-6 to the other food [16]. Milk powder plus omega-3 and omega-6 is widely use from the consumers because has high nutrition value coming by nutritive compounds of milk and omega-3 and omega-6 fortified to it, some of reconstituted milk powder used as the addition product to the other food such as produce the biscuit, cake, pastries and chocolate all these products undergo to the high temperature during manufacturing and thus effect on the fatty acid especially omega-3 and omega-6 because it's how many unsaturated bonds that are easy breakage during exposure to high temperature with present of oxygen [17,18]. The oxygen play means the role of degraded fatty acids, especially polyunsaturated fatty acids that's because after Break down the fatty acid bonds free radicals will formation, these free radicals, unstable which can attack with the oxygen to produce proxy radicals and this can be lead to form the hydroperoxides which considered mean compounds responsible on degraded fatty acids by oxygen [19].

About 100 years ago superheated steam was discovered, but it's not taking attention and development until 20 years ago and become more used in the energy applied. Superheated steam is a clear, colorless gas obtained by heating ordinary steam at 100°C to a higher temperature under normal pressure with its high heat transfer capabilities, it is applied to a wide range of industry that's returned to attributes of the features superiority of other energy sources [20]. Superheated steam has many applied in the industry and it's developed to be more benefits in dehydration and processing foods includes herbs, lumber, paper, coal, sugar-beet, pulp, tortilla, chips, shrimp, vegetables, potato and spices [21]. In the superheated steam there is no oxygen available during the drying or process like the other thermal methods such as hot air and that's mean there are no oxidative or combustion reactions will occur during the processing [22]. The aim of this study was investigating the effect of superheated steam on oxidative stability of reconstituted omega-3 plus milk powder on the fatty acids, POV, TBRs, Acid value and P-anisidine value.

II. MATERIAL AND METHOD

A. Omega-3 plus milk powder

Omega-3 plus milk powder were supplied by Nestle and purchased from Tesco in Penang, Malaysia. The standard amounts of whole milk powder contents were follows: fat (9.4 g per 100 g), protein (20 g per 100 g), carbohydrate (57 g per 100 g).

B. Milk powder reconstitution

The reconstituted omega-3 plus milk powder was prepared according to the instructions of the manufacturer listed on the packages. The milk 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.

C. Heating reconstituted milk powder by superheated steam oven

Thermal coefficient samples were exposed in a superheated steam oven after milk reconstitution and yielded a total of 500 ml of samples. The collected samples exposed in a superheated steam oven (AX1500) were infectious. The samples were exposed at various temperatures (120,150 and 180 °C) and period times (5, 10, and 15 min) all samples subsequently underwent analyses.

D. Milk fat extraction

Milk fat was extracted from the reconstituted omega-3 plus milk powder using the Folch 1957 method with some modification chloroform, methanol and water (2:1:1) [23]. 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 separated these contain water and methanol and non-lipid and the lower phase was separated these layers contain the chloroform and lipid. This layer was filtration by Buchner funnel use anhydrous sodium sulfate. The residue was collected out in glass vials and the solvent was removed by use rotary evaporator with temperature bellow 50 °C.

E. Fatty acid analysis

Fatty acids were acquired by trans ethylating into fatty acid methyl ester, which was carried out by [24]. Approximately 150 mg of milk fat was added to 5.0 mL 0.25 mol L⁻¹ sodium methoxide in methanol-diethyl ether (1:1) and it was stirring vigorously for 3 min. After that 3 mL of isooctane and 15 mL of saturated sodium chloride were added. The tube was stirring vigorously again and waiting after the phase separation. The upper layer was transferred to GC vial for analysis. 1 ml of the upper layer was transferred to 1.5 ml GC-glass. Fatty acids were identified by comparing the retention times of FAME mixture with those of standard purchased from Supelco (16823-0048) USA and is contained 37 fatty acids. The fatty acid composition of omega-3 milk powder was directly analyzed using Gas Chromatography (GC-FID) 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 take 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.

F. Lipid Oxidation analysis

Peroxide value and acid value were determined according to AOCS official methods described by [25]. The thiobarbitric acid reactive substances (TBARS) of milk fat were evaluated using park method 1997 with some modifications. Approximately 2 g of milk was mixed with 2 ml of 20% trichloroacetic acid solution with 4 ml of 0.01 M TBA solution. Subsequently, the sample was heated in a water bath at 90 °C for 15 min. The TBA solution with milk fat was cooled in an ice bath for 15 min. The solution of pyridine and isoamylalcohol (2:1) was mixed with the TBA solution and centrifuged at 2400 rpm for 15 min. The supernatant was separated, and absorbance in the spectrophotometer (UV mini- 240, Japan) at 550 nm was converted to the TBARS by using Eq. 1.

$$\text{TBARS} = \text{absorbance} \times 100 \times (3/2) \quad (1)$$

P-Anisidine value was determined using the method of [26]. The p-anisidine was dissolved in glacial acetic acid to make a 0.25 g/100 ml solution. The solvent used was isooctane. The test was conducted in triplicate for all samples. Up to 0.3 g of the milk fat samples were accurately weighed and placed into 25 ml volumetric flasks and diluted with isooctane. Up to 5 ml of the solution was pipetted into a test tube; 5 ml of isooctane was added, followed by 1 ml of the p-anisidine solution. The mixture was mixed thoroughly in the test tube. After 10 min, the absorbance of the sample was measured using a spectrophotometer (UV mini-1240, Japan) with 350 nm. Isooctane was used as the reagent blank. Glass corvettes were used for all absorbance measurements. The p-anisidine values were calculated using Eq. 2.

$$\text{P-A. } V = 25 \times (1.2A_s - A_b) / m \quad (2)$$

As is the absorbance of the sample; A_b is the absorbance of blank; and m is the mass of fat.

G. Statistical analysis

The experiments were performed in triplicate, and the data were analyzed using SPSS version 20–2013 (SPSS Inc., Chicago, USA). Analysis of variance was performed, and Duncan's multiple range tests was used to compare significant differences ($p < 0.05$).

III. RESULT AND DISCUSSION

A. Changes in fatty acid composition during superheated steam heat treatment

Fatty acid composition of reconstituted omega-3 plus milk powder that was heated in superheated steam oven on different temperature and periods of time shown in Table 1. The SFA is present in higher concentration of fatty acids in milk fat and the palmitic acid (C16:0) recorded the highest value in saturated fatty acids. The PUFA was present the second values after SFA and that return to fortify the Omega-3 and Omega-6 fatty acids to this type of milk sample and α - linoleic acid (C18: 3n-3) is the dominated on the PUFA. Linoleic acid (C18: 2n-6) is second fatty acids

dominated in the PUFA and its present omega -6 fatty acids in milk fat. At various heat treatment of superheated steam the fatty acids present no significant differences on temperature 120-150 °C in time 15-10 min, respectively to the SFA, MUFA and PUFA with slight difference on the high temperature 180 °C for 5 min. Table 1 present the amount of SFA, MUFA and PUFA to reconstituted omega 3-6 plus milk powder treated by superheated steam oven. Whilst, the Valuable of SFA, MUFA and PUFA were indicate decrease, but significantly did not show any differences compare with the control (sample without heating).

TABLE I
FATTY ACID COMPOSITION (MG. G-1) OF RECONSTITUTED OMEGA-3 PLUS MILK POWDER TREATED WITH SUPERHEATED STEAM.

Fatty acids (mg. g ⁻¹)	Control	Superheated steam		
		120 °C, 15 min	150 °C, 10 min	180 °C, 5 min
C8:0	10.18±0.31 ^a	10.06±0.13 ^a	10.02±0.13 ^a	9.80±0.10 ^a
C10:0	5.58±1.00 ^a	4.41±0.87 ^a	4.97±0.86 ^a	5.19±0.92 ^a
C12:0	27.81±1.15 ^a	27.76±0.05 ^a	27.43±0.48 ^a	27.02±0.06 ^a
C14:1	8.19±0.42 ^a	7.83±0.30 ^a	7.78±0.04 ^a	7.18±0.03 ^b
C16:0	116.37±1.22 ^a	116.05±0.59 ^a	115.94±2.61 ^a	108.43±1.02 ^b
C18:0	10.25±0.64 ^a	10.31±0.35 ^a	9.47±0.39 ^a	9.41±0.28 ^a
C18:1n9	31.39±2.09 ^a	31.08±1.01 ^a	30.08±0.68 ^a	30.28±0.53 ^a
C18:2n6	33.83±5.14 ^a	33.44±5.34 ^a	32.25±13.02 ^a	32.64±9.29 ^b
C18:3n3	38.13±0.82 ^a	37.99±0.63 ^a	37.46±0.40 ^a	36.84±0.40 ^a
C20:0	9.54±0.67 ^a	9.56±0.99 ^a	9.16±0.75 ^a	9.28±0.67 ^a
C20: 5	2.75±0.24 ^a	2.47±0.24 ^a	2.69±0.06 ^a	2.64±0.30 ^a
(n3) EPA				
C22: 6	4.18±0.43 ^a	4.16±0.41 ^a	3.76±0.99 ^a	3.70±1.03 ^a
(n3) DHA				
Σ SFA*	179.73	178.15	176.99	169.13
Σ MUFA**	39.58	38.09	37.86	37.46
Σ PUFA***	77.89	77.06	71.16	68.82

^{a-d}Average with different letters in the same row indicate significant differences ($p < 0.05$).

* SFA: saturated fatty acids.

** MUFA: monounsaturated fatty acids.

*** PUFA: poly unsaturated fatty acids

Generally the fatty acids take decrease when treated with heat treatment [27]. Decrease the unsaturated fatty acid in milk treated with heat treatment due to the lipid oxidation initiated by the oxygen therefor, the superheated steam has free oxygen that due dry steam and can limit the oxidation and degradation fatty acids [28,29]. From the other hand, milk and milk product undergo to the convention heat treatment during preparation (microwave, boiling and cooking) and that's lead to undesirable changes in lipids and fatty acids by the oxidation steps that's leads to produce low molecule weight of products (aldehydes, ketones and lactones) which responsible on undesirable flavored in milk and milk products [11]. Microwave heat treatment recorded effect on the fat oxidation and fatty acids and isomer changes [30]. Interpretation of probability in the most non-degradation of fatty acids in milk treated by superheated steam that's the SHS work without present of oxygen with this advantage can prevent or reduce the oxidation process resulting maintains the fatty acids from degradation to increase the nutrition values of the products [31].

B. Effect superheated steam on Lipid Oxidation of reconstituted omega-3-6 plus milk

The effect of superheated steam on the acid value, Peroxide oxidation value (POV), p-Anisidine value and TBRS value of reconstituted omega3-6 plus milk were determined and the results show in fig 1, 2 and 3.

Fig 1 show affects SHS temperatures and periods of time on the acid value. The results indicate no significant differences at the 120-180 °C for 5 min compare with the control. Among SHS heat treatment results recorded no significant differences between 120-180 °C but there are significant differences compared with the control at periods of time 10-15 min. All the results of acid value have not much changes from the control (sample without heating) even after long time, SHS has given good results of acid value from these treated by pasteurization or UHT process [32]. Increase the acid value in the samples indication to increase the free fatty acids in milk sample and that Interpretation of degradation lipids specially unsaturated fatty acids and reduce quality of products [33].

Effect SHS on the POV shown in fig 2. POV is a free radical chain produce from reaction between free radicals and the oxygen described in the initiation and propagation steps of oxidation. The Oxygen species play the mean rolls to produce the hydroperoxides by the free radicals and the hydrogen abstract [34]. SHS heat treatment indicates no significant differences between an SHS heat treat and the control (sample without heating). In this results the peroxide value ranged between (6.66- 8.00 meq/kg) were below 25 meq of active O₂/kg which considered as limit of acceptability in the food [22]. Luck of POV in the SHS may explain that oxygen inside SHS which prevent or minimize and that limited the lipid oxidation in the samples [29].

Effect SHS on the TBARS shown in fig 3. The results show no significant differences between SHS heat treatment, but indicate significant differences with the control. Heat treatment temperature 120,150 and 180 C show stability of TBRS on parodies of time 5-10 min with slight changes on the long time 15 min. TBARS is measure secondary lipid oxidation by reacting TBA thiobarbituric acid with alkenals and alkadienals resulting from secondary oxidation specially from unsaturated fatty acids more than 3 double bounds to give term of also react thiobarbituric acid reactive substances (TBARS) [35].

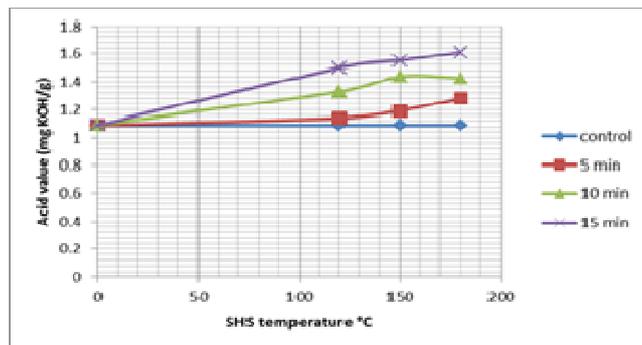


Figure 1: Effect superheated steam (temperature and time) on the acid value to reconstituted omega3-6 plus milk powder.

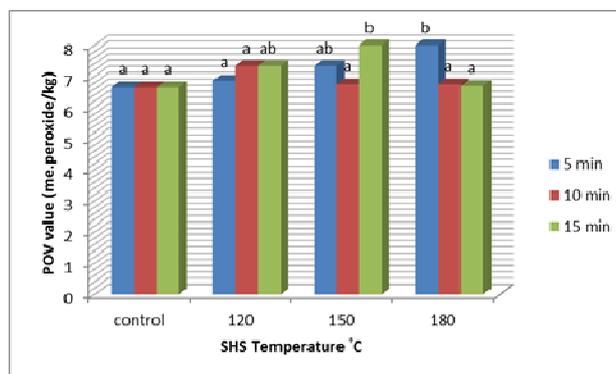
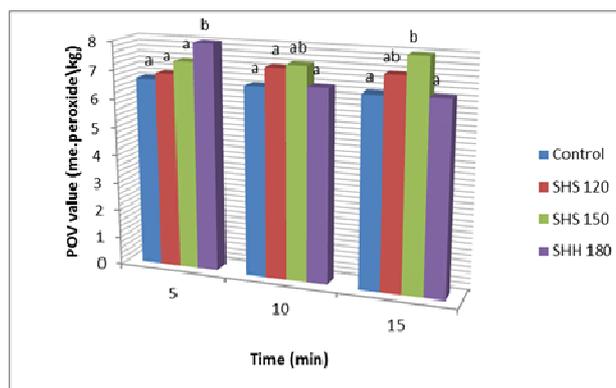
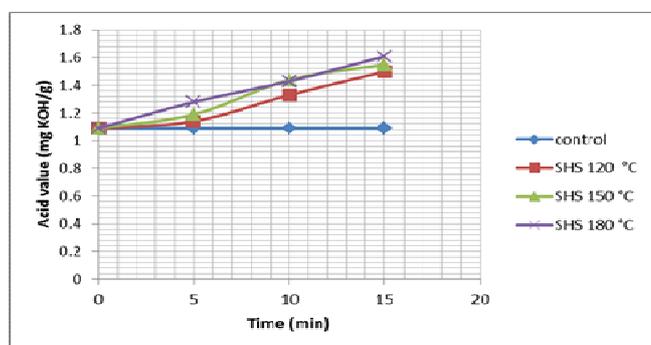


Fig 2: Effect superheated steam (temperature and time) on the POV value to reconstituted omega3-6 plus milk powder.



Effect SHS on the TBARS shown in fig 3. The results show no significant differences between SHS heat treatment but indicate significant differences with the control. Heat treatment temperature 120,150 and 180 C show stability of TBRS on parodies of time 5-10 min with slight changes on the long time 15 min.

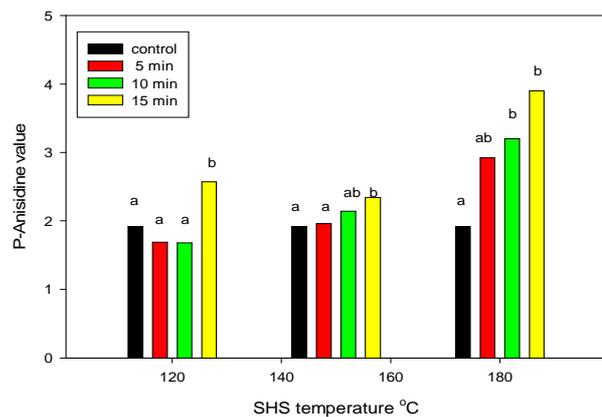
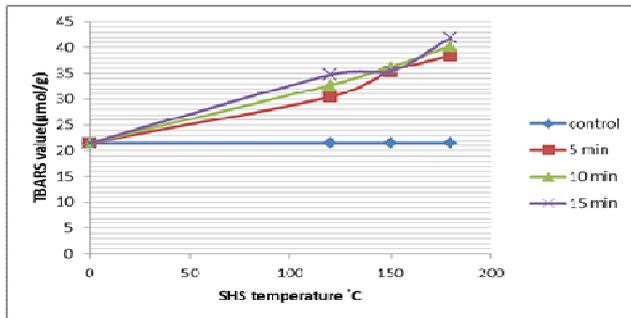
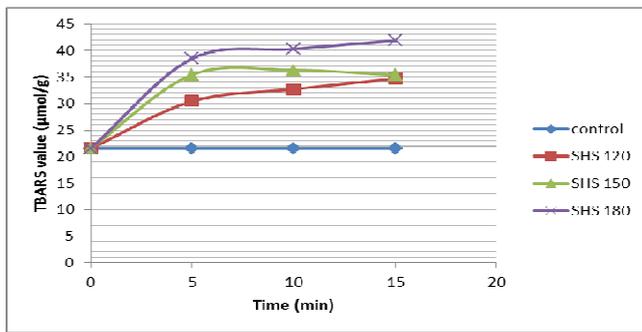


Fig 3: Effect superheated steam (temperature and time) on the TBARS values to reconstituted omega3-6 plus milk powder.

TBARS is measure secondary lipid oxidation by reacting TBA thiobarbituric acid with alkenals and alkadienals resulting from secondary oxidation specially from unsaturated fatty acids more than 3 double bounds to give term of also react thiobarbituric acid reactive substances (TBARS) [35].

The p-anisidine value (p-AnV) explain the secondary lipid oxidation, especially aldehydes principally 2-alkenals and 2,4-alkadienals produce by hydroperoxides in termination steps of oxidative mechanism [35]. Stability of P-anisidine value in reconstituted omega3-6 plus milk powder treated by superheated steam attributed to lack oxygen in SHS even prevent hydroperoxides formation resulting prevent aldehydes format [28].

Fig 4 show affects SHS temperatures and periods of time on the P-anisidine value the result shows no significant differences at the 120-150 °C for 5-10 min compare with the control with some changes on the long time 15 min of 150

°C. At the high temperature 180 °C superheated steam present significant different compare with the SHS heat treatment and the control. All the results of P-anisidine have not much changed from the control (sample without heating) even after long time.

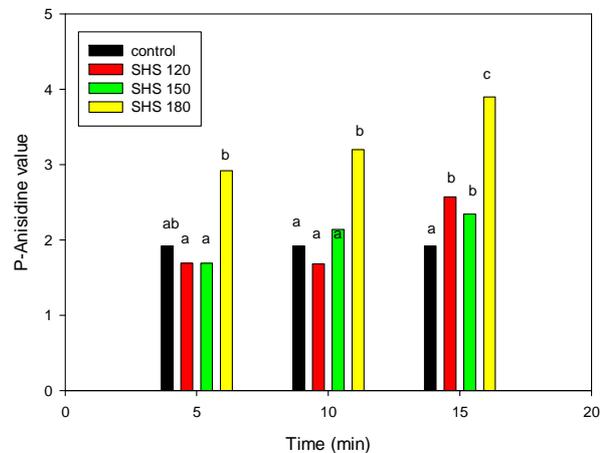


Fig4: Effect superheated steam (temperature and time) on the P-Anisidine value to reconstituted omega3-6 plus milk powder.

IV. CONCLUSIONS

During heated reconstituted omega3-6 plus milk powder by superheated steam and indicated on changes in fatty acids and lipid oxidation by different methods of analysis. SHS temperature and time range used to indicate changes in milk fat. However, the superheated steam leads to decrease SFA content 179.73-169.13 mg. g⁻¹ to maintain PUFA and MUFA from changes that explain no oxidation or degradation occur to the fatty acids during heat processing. Superheated steam show stability of lipid oxidation at different time and temperature with some changes of acid value, POV value, TBARS value and P-anisidine value of the high temperature 180 °C and long-time 15 min. By the results the temperature between 120-150 °C with periods of time 5-10 min present no significant differences on the changes in fatty acids or lipid oxidation that can be recommended to use this rang for treating milk or any product included milk by superheated steam.

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