

# Effect of superheated steam on lipid oxidation of reconstituted whole milk powder

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**Abstract** Milk powders are ingredients that are widely used in food manufacturing. Milk powders are exposed to high temperatures during processing, which alters the physical and chemical properties of milk. In this study, the changes in acid, peroxide, thiobarbutric acid reactive substances (TBARS), and p-anisidine values were measured to reconstitute whole milk powder (WMP) in relation to thermal treatment by using a superheated steam oven with different temperatures and time periods. The temperature and time period ranged from 120 °C to 180 °C and from 5 min to 15 min. No significant differences were observed between the acid value and peroxide oxidation values of the samples and the control, which indicates that no oxidation reaction occurred between the fatty acids and oxygen in the superheated steam. TBARS and p-anisidine values did not show significant differences between samples but showed significant differences compared with the control. This study showed the ability of milk fat to reconstitute WMP and to maintain the nutritional value of milk during processing.

**Keywords:** lipid oxidation, superheated steam, milk powder, acid value, p-anisidine value

## 1 INTRODUCTION

Dairy is one of the most important products to human nutrition. Dairy is also used to prepare different types of food, such as pastries, pies, and cakes, because dairy can improve food characteristics, such as texture, color, and flavor [1]. Dietary lipids that are added in raw food materials or during food processing have an important function in the development of food flavor and nutrition [2]. However, lipid oxidation is the main reason behind the degradation of food quality. Lipid peroxidation gives rise to the development of off-flavors and wastage of essential amino acids and vitamins because of the presence of oxygen and free radicals [3]. The characteristics of milk lipids are what makes milk an outstanding constituent of food with respect to the presence of other lipid classes [4]. Milk fat may undergo physical and chemical changes, including oxidation during processing and storage [5]. Oxidation of fat milk leads to the production of low molecular weight products, such as aldehydes, ketones, and lactones; these products have undesirable odor that could decrease the quality and nutritional value of food [6]. Lipid oxidation has gained attention from researchers because of their negative effect on human health. Lipid oxidation decreases the nutritional value of food products and leads to the development of off-flavor by producing secondary oxidation product, such as alkanes, alkenes, aldehydes, and ketones [7, 8]. Oxidative stress is primarily dependent on the presence of oxygen that can be produced by hydroperoxide, which is one of the important products from fat degradation. Hydroperoxide is produced by secondary oxidation, which is responsible for the development of off-flavors [9]. The mechanism of lipid oxidation involves the deterioration of the original structure of fatty acids, particularly unsaturated fatty acids. Oxygen, temperature, and light are the catalysts of lipid peroxidation. Oxygen is important in lipid peroxidation because it is responsible for the production of free radicals that break the double bonds of unsaturated fatty acid, which leads to primary lipid oxidation, secondary lipid oxidation, and off-flavor development [10]. Milk in powder form has gained attention from manufacturers because it is not damaged by environmental factors. The advantages of dairy powders over liquid milk include lower shipping costs, increased microbial stability, unique functionality, transport convenience, good preservation, and availability for use in the manufacturing of other food [11]. Lipid oxidation in

processed dairy products is caused by accelerated oxidation, which is the reason powdered milk made from fresh milk is preferred when manufacturing other food. However, reconstituted powdered milk does not have the same quality as fresh milk [12]. For manufacturers that use confection oven or microwave, the addition of oxygen to unsaturated fatty acids and high heat transfer can cause some changes, particularly physical changes, in the composition of milk [13].

Thermal convection heating can also decrease the nutritional value of milk, which defeats the purpose of adding milk in the first place [14]. High temperature can break the double bond between carbon atoms and can produce free radicals; free radicals can attack the oxygen in the propagation step to produce hydroperoxide, which is unstable and can decompose into compounds (e.g., aldehydes, ketones, or alcohol) that are volatile and can cause off-flavors [15]. Reactive oxygen species can produce hydroxyl and peroxy radicals, which are considered essential in the oxidation process and denaturation of lipids [16]. Superheated steam is steam that has temperature above the boiling point. In temperatures above the boiling point, steam becomes unsaturated steam rather than superheated steam [17]. Superheated steam is used in the food industry because of its numerous advantages and its ability to develop desirable characteristics in food. Superheated steam is one type of unsaturated (dry) steam that is produced by the addition of sensible heat to saturated (wet) steam to obtain increased temperature of steam under normal pressure. The use of superheated steam has two advantages: lack of oxygen and availability of wet steam. Therefore, hydrolase will not be present, and lipid oxidation will not occur [18]. The objective of this study is to study the effect of superheated steam on lipid oxidation in reconstituted whole milk powder (WMP). The oxidative stability of reconstituted WMP was assessed by evaluating the total free fatty acid, peroxide values, p-anisidine value, and thiobarbutric acid (TBA).

## 2 MATERIALS AND METHODS

### 2.1 Whole milk powder

The WMP used in this study was Nespray, which is supplied by Nestle. The WMP was purchased from the Tesco market in Penang City, Malay-

sia. The contents of the milk powder followed that of standard milk powder, which contains fat (28.2 g per 100 g), protein (23.6 g per 100 g), and carbohydrate (39.9 g per 100 g).

## 2.2 Reconstituted whole milk powder

The WMP was reconstituted following the instructions on the package. The WMP was reconstituted by combining 33 g of powdered milk and 225 mL of water. The mixture was mixed using a magnetic stirrer for 10 min.

## 2.3 Heating reconstituted milk powder by using a superheated steam oven

The thermal coefficients of the samples in the superheated steam oven Sharp AX-1500 with a steam generation capacity of 16 cm<sup>3</sup>/min, oven capacity of 31 L, and team engine heater of 900 W were analyzed. The steam was supplied by a boiler at the beginning of the drying process at pressure of approximately 1 bar and was heated by an electric heater until the superheated state was reached. Up to 500 ml of reconstituted milk was used for each sample. The samples were placed in the processing chamber of the superheated steam processing system and exposed to different temperatures (120, 135, 150, 165, and 180 °C for 5, 10, and 15 min period time).

## 2.4 Milk fat extraction and analysis

Milk fat was extracted from the reconstituted whole milk powder using the Folch 1957 method modified with chloroform, methanol and water (2:1:1) [19]. 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 mix 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 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.

## 2.5 Peroxide oxidation value

Peroxide value was determined according to AOCS official methods described by [20]. Approximately 1 ± 0.05 g of milk fat was placed into a 250 ml conical flask with 30 ml of acetic acid and chloroform (3:2) solution. The flask was swirled until the sample dissolved in the solution. Once the fat was dissolved, 0.5 ml of saturated potassium iodide solution was added and swirled for 1 min, then 30 ml of distilled water was added. Afterward, 0.01 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> was titrated into the mixture until the color changed to light yellow. Subsequently, 0.5 ml Of 1% soluble starch indicator was titrated into the mixture placed until a blue color appears and disappears. We calculated the peroxide value as meq of peroxide/kg of oil (Eq. 1):

$$\text{Peroxide value} = \frac{(S - B) \times N \text{ thiosulfate} \times 1000}{\text{weight of sample}} \quad \dots\dots\dots 1$$

## 2.6 Acid value

After heating, 10 g of sample was placed in a conical flask. Two drops of phenolphthalein indicator and 0.1 N KOH solution was added until the advent of the color pink. Acid value was calculated (Eq. 2) [20].

$$\text{Acid value} = \frac{56.1 \times V \times C}{m} \quad \dots\dots\dots 2$$

Here, 56.1 is the equivalent weight of KOH; V is the volume in ml of standard volumetric KOH solution used; C is the exact concentration in

KOH solution used (0.1 N); and m is the mass in grams of the test portion (10 g).

## 2.7 TBARS test

The thiobarbituric acid reactive substances (TBARS) of milk fat was evaluated using the method park 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. 3.

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

## 2.8 p-Anisidine value test

P-Anisidine value was determined using the method of [22]. 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 cuvettes were used for all absorbance measurements. The p-anisidine values were calculated using Eq. 3.

$$\text{P-A.V} = \frac{25 \times (1.2A_s - A_b)}{m} \quad \dots\dots\dots 3$$

Here, A<sub>s</sub> is the absorbance of the sample; A<sub>b</sub> is the absorbance of blank; and m is the mass of fat.

## 2.9 Totox value

Totox value was collected by using an equation that used peroxide and p-anisidine values.

## 2.10 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 test was used to compare significant differences (p < 0.05).

## 3 RESULTS AND DISCUSSION

### 3.1.1 Effect of superheated steam treatment time on the oxidation milk fat and acid value

The effect of superheated steam time on the acid value is shown in Table 1. No significant change in acid value was observed between the samples and the control. No significant change in acid value was observed when the treatment time and temperature varied from 5 min to 15 min at 120, 135, and 150 °C. At 165 °C for 15 min, no significant differences were observed. At a high temperature of 180 °C, the samples did not show significant differences with the control when the time varied from 5 min to 15 min. However, at 10 min, the samples showed significantly different results from the control. The level of acid value explains the level of free

fatty acid because fatty acid is found in fat and oil and is associated with triglycerides. Therefore, a high acid value equates to high free fatty acid levels, which translates into decreased oil quality [20]. At 5 min to 15 min, the acid values in the superheated steam are not higher than those of the control, which means that the superheated steam maintained the milk fat from formation free fatty acids. This finding could be attributed to the fact that superheated steam uses dry steam; moreover, hydrolysis of triglycerides by heating requires wet steam to break the ester bonds between the triglyceride and fatty acids [23].

**Table 1** Effect superheated steam time on means of acid value (mg KOH/g) to reconstitute whole milk powder.

Temperature °C	Period time (min)			
	control	5	10	15
120	2.31±0.58 <sup>a</sup>	2.18±0.47 <sup>a</sup>	2.18±0.56 <sup>a</sup>	2.55±0.36 <sup>a</sup>
135	2.31±0.58 <sup>a</sup>	2.00±0.05 <sup>a</sup>	1.92±0.59 <sup>a</sup>	2.13±0.54 <sup>a</sup>
150	2.31±0.58 <sup>ab</sup>	1.84±0.27 <sup>a</sup>	2.14±0.36 <sup>ab</sup>	2.27±0.08 <sup>ab</sup>
165	2.31±0.58 <sup>a</sup>	2.07±0.11 <sup>a</sup>	2.41±0.23 <sup>abc</sup>	2.75±0.05 <sup>bc</sup>
180	2.31±0.58 <sup>a</sup>	2.99±0.05 <sup>ab</sup>	3.26±0.62 <sup>bc</sup>	3.05±0.05 <sup>ab</sup>

<sup>a-b</sup>Average with different letters in the same row indicate significant differences (p < 0.05).

### 3.1.2 Peroxide value

Lipid peroxidation is a free radical chain reaction in lipid oxidation that involves initiation, propagation, and termination. Available oxygen species are considered to be the key factors in the deterioration of polyunsaturated fatty acids through three different avenues, namely, oxygen, hydrogen abstraction, and free radical attack [24]. In this study, the peroxide value in all samples ranged from 2.20 meq O<sub>2</sub>/kg to 3.98 meq O<sub>2</sub>/kg, which is below 25 meq of active O<sub>2</sub>/kg, which is considered the acceptable level in food. The peroxide value measures lipid oxidation and is associated with the alkyl radical in hydroperoxide production. The effect of the superheated steam time on the peroxide value (see Table 2) showed no significant difference when temperature varied from 120 °C to 165 °C at 5 min to 10 min but showed significant difference at 15 min compared with the control. The POV result did not record any significant differences between 10 min to 15 min at temperatures ranging from 120 °C to 165 °C. However, between 5 min to 15 min, the results showed significant differences. The results of the peroxide value showed no significant difference compared with the control at 180 °C between 5 min to 15 min. The peroxide value can be used as an indicator of fat rancidity by measuring the primary oxidation product (hydroperoxide). The minimum and maximum peroxide values recorded in the superheated steam are 2.20 and 3.98 meq O<sub>2</sub>/kg, respectively, which did not differ significantly with the control. No differences were observed with the control after 15 min under high temperature. Given that superheated steam is did not effect on milk fat by not forming of hydroperoxide, because number of oxygen molecules in superheated steam is low [25].

### 3.1.3 TBARS value

TBARS shows the effect of superheated steam time on lipid stability (Table 3). The result shows no significant difference between samples and control at 120 °C for 5 min to 10 min. However, significant differences were recorded at 15 min. The mean temperatures were 34.45, 34.15, and 38.45

**Table 2** Effect superheated steam time on means Peroxide oxidation value (meq.peroxide/kg) to reconstitute whole milk powder.

Temperature °C	Period time (min)			
	control	5	10	15
120	2.23 ± 0.55 <sup>a</sup>	3.29 ± 0.51 <sup>ab</sup>	3.69 ± 0.88 <sup>b</sup>	2.92 ± 0.02 <sup>ab</sup>
135	2.23 ± 0.55 <sup>a</sup>	2.70 ± 0.69 <sup>ab</sup>	2.86 ± 0.90 <sup>ab</sup>	3.51 ± 0.58 <sup>ab</sup>
150	2.23 ± 0.55 <sup>a</sup>	2.29 ± 0.59 <sup>a</sup>	2.69 ± 0.25 <sup>ab</sup>	3.98 ± 1.72 <sup>b</sup>
165	2.23 ± 0.55 <sup>a</sup>	2.20 ± 0.54 <sup>a</sup>	2.50 ± 0.54 <sup>ab</sup>	2.75 ± 0.93 <sup>b</sup>
180	2.23 ± 0.55 <sup>a</sup>	2.28 ± 0.55 <sup>a</sup>	2.52 ± 0.49 <sup>a</sup>	2.81 ± 0.93 <sup>a</sup>

<sup>a-b</sup>average with different letters in the same row indicate significant differences (p < 0.05).

°C for 5, 10, and 15 min, respectively. A temperature of 135 °C did not cause a significant difference between the samples and the control. At about 150 °C, the effect of time shows no significant difference between all periods. Significant differences were recorded at 39.90, 41.60 and 44.20 °C for 5, 10, and 15 min, respectively. Significant differences were observed between samples heated for 5 and 10 min at 165 °C to 180 °C compared with those heated for 15 min at 180 °C.

No difference was observed in the samples between 5 min to 15 min at 120 °C to 150 °C. The result of TBARS showed that superheated steam time does not contribute to the reaction of TBA with malonaldehyde and to maximum color development. No oxidation reaction in milk fat occurs when superheated steam is used. Thus, milk fat stability is maintained, and degradation of unsaturated fatty acids is prevented [2, 26].

**Table 3** Effect superheated time on means TBARS (nmol/g) to reconstitute whole milk powder.

Temperature °C	Period time (min)			
	control	5	10	15
120	32.65 ± 0.37 <sup>a</sup>	34.45 ± 3.67 <sup>a</sup>	34.15 ± 0.87 <sup>a</sup>	38.45 ± 1.16 <sup>b</sup>
135	32.65 ± 0.37 <sup>a</sup>	36.05 ± 1.75 <sup>a</sup>	35.55 ± 2.16 <sup>a</sup>	37.75 ± 1.88 <sup>a</sup>
150	32.65 ± 0.37 <sup>a</sup>	39.90 ± 4.12 <sup>b</sup>	41.60 ± 2.77 <sup>b</sup>	44.20 ± 0.45 <sup>b</sup>
165	32.65 ± 0.37 <sup>a</sup>	38.35 ± 2.86 <sup>a</sup>	41.30 ± 2.52 <sup>ab</sup>	43.65 ± 1.13 <sup>c</sup>
180	32.65 ± 0.37 <sup>a</sup>	38.70 ± 0.54 <sup>b</sup>	44.95 ± 1.73 <sup>c</sup>	46.20 ± 0.39 <sup>c</sup>

<sup>a-a</sup> Average with different letters in the same row indicate significant differences (p < 0.05).

### 3.1.4 p-Anisidine Value

P-Anisidine values show the effect of superheated steam time on lipid stability (Table 4). The results showed no significant difference between samples heated at 120 °C to 135 °C for 5 min to 15 min and the control. However, significant differences were recorded between samples heated for 5 min to 10 min at 150 °C to 165 °C and the control. At 180 °C, p-anisidine showed no significant differences for all periods except for 5 min. All p-anisidine values were did not recordid significantly different (p < 0.05) at time variables. P-anisidine values measure the aldehyde compound produced from lipid oxidation, particularly 2-alkenals and 2,4-alkadienals, resulting from the decomposition of hydroperoxide in the termination step of lipid oxidation [2, 22, 27]. High levels of p-anisidine were not observed because the lack of oxygen in superheated steam promotes free radical

and hydroperoxide formation, which is considered an important compound in the oxidation of lipids and formation of undesirable flavor caused by aldehyde compounds [28-30].

**Table 4** Effect superheated steam time on means of P-Anisidine value to reconstitute whole milk powder.

Temperature °C	Period time (min)			
	control	5	10	15
120	5.22 ± 0.15 <sup>a</sup>	5.70 ± 0.53 <sup>a</sup>	6.91 ± 0.91 <sup>a</sup>	5.83 ± 1.59 <sup>a</sup>
135	5.22 ± 0.15 <sup>a</sup>	6.17 ± 0.82 <sup>a</sup>	6.30 ± 0.40 <sup>a</sup>	5.44 ± 1.37 <sup>a</sup>
150	5.22 ± 0.15 <sup>a</sup>	6.87 ± 0.76 <sup>b</sup>	6.88 ± 0.49 <sup>b</sup>	5.56 ± 0.51 <sup>a</sup>
165	5.22 ± 0.15 <sup>a</sup>	6.77 ± 0.65 <sup>a</sup>	6.67 ± 0.62 <sup>a</sup>	6.46 ± 1.24 <sup>a</sup>
180	5.22 ± 0.15 <sup>a</sup>	6.92 ± 0.79 <sup>b</sup>	6.59 ± 1.00 <sup>ab</sup>	6.64 ± 0.70 <sup>ab</sup>

<sup>a,b</sup>Average with different letters in the same row indicate significant differences (p < 0.05).

**3.1.5 Totox value**

The value of total oxidation is represented by the totox value (see Table 5). The totox values were collected using an equation that required the peroxide and p-anisidine values. The totox value also measures lipid oxidation and explains the formation of primary and secondary lipid oxidation [31]. In this study, the totox value recorded ranged from 9.87 to 13.47. The totox value showed stability to time in the superheated system. This value led to the stability of the peroxide value and p-anisidine value from lipid oxidation in reconstituted WMP, which led to reduced oxidation and deterioration of milk fat.

**Table 5** Effect superheated steam time on means of Totox value to reconstitute whole milk powder.

Superheated steam temperature °C	Period time (min)			
	control	5	10	15
120	9.87 ± 1.08 <sup>a</sup>	10.09 ± 1.53 <sup>a</sup>	12.54 ± 0.18 <sup>ab</sup>	13.43 ± 2.40 <sup>b</sup>
135	9.87 ± 1.08 <sup>a</sup>	12.98 ± 0.85 <sup>a</sup>	12.65 ± 2.08 <sup>a</sup>	13.07 ± 3.30 <sup>a</sup>
150	9.87 ± 1.08 <sup>a</sup>	11.27 ± 1.44 <sup>a</sup>	12.27 ± 0.26 <sup>a</sup>	13.53 ± 3.96 <sup>a</sup>
165	9.87 ± 1.08 <sup>a</sup>	11.19 ± 1.68 <sup>ab</sup>	11.67 ± 1.46 <sup>ab</sup>	13.29 ± 1.70 <sup>b</sup>
180	9.87 ± 1.08 <sup>a</sup>	12.82 ± 0.75 <sup>b</sup>	11.64 ± 0.04 <sup>b</sup>	12.27 ± 1.15 <sup>b</sup>

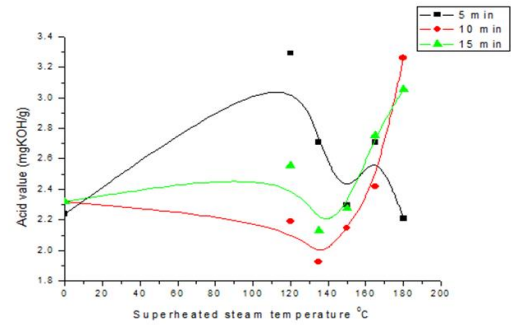
<sup>a,b</sup>Average with different letters in the same row indicate significant differences (p < 0.05).

**3.2 Effect of superheated steam temperature on reconstituted whole milk powder**

**3.2.1 Acid value**

Fig. 1 shows the effect of superheated steam temperature on acid formation. The total free fatty acid in WMP was observed at 120, 135, 150, 165, and 180 °C. The result showed no significant difference at temperatures ranging from 120 °C to 165 °C. At 180 °C for 10 min to 15 min, results showed significant difference compared with the control (Fig. 1). The chemical changes occurring during lipid heating are shown in two types of reactions, namely, oxidative and thermolytic reactions [32]. The thermolytic reactions include hydrolysis, isomerization, polymerization, and cyclisation. Lipid hydrolysis can cause undesirable flavors in food [33]. Increased acid value in food is an indication of the deterioration of fat because separation of fatty acid from glyceride composition causes increased undesirable flavor in food [34]. In the superheated steam, the acid value did not change, and the superheated steam maintained the structure of triglycerides because of the use of dry steam, which does not break the bonds

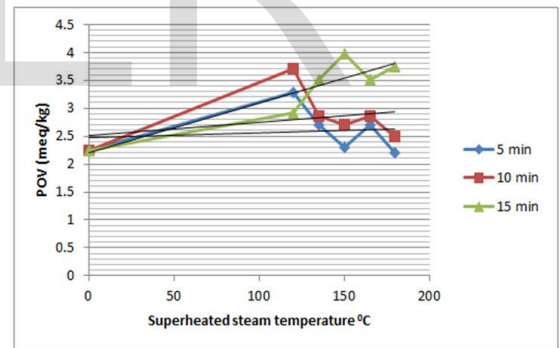
between fatty acids and glycerol.



**Figure 1** Effect superheated steam temperature on total free fatty acids to reconstitute whole milk powder.

**3.2.2 Peroxide value**

Fig. 2 shows the changes in peroxide value in a superheated system using five different temperatures (120, 135, 150, 165, and 180 °C) and three different periods of time. The results recorded a stable peroxide value with slight rise at 180 °C for 15 min (Fig. 1), which caused the superheated steam to form hydroperoxide. Hydroperoxide is the compound responsible for the degradation of fats. Hydroperoxide formation resulted from the addition of oxygen to the alkyl radical output, i.e., the addition of one atom of hydrogen and carbon atom in fatty acids [35]. Superheated steam measures the stability of peroxide value. Hydroperoxide formation is limited in superheated steam because of the lack of oxygen; thus, lipid quality



**Figure 2** Effect superheated steam temperature on peroxide value to reconstitute whole milk powder.

is maintained [36].

**3.2.3 TBARS value**

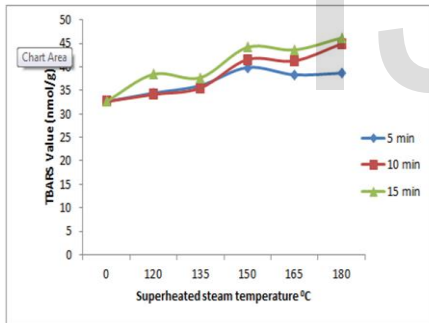
Fig. 3 show changes in TBARS in superheated steam. The result shows TBARS ranging from 32.65 nmol/g to 44.20 nmol/g. The results showed no significant differences under 120 °C to 150 °C for all time periods. At 165 °C to 180 °C, the results recorded slight changes compared with the control (Fig. 3). The stability of TBARS in reconstituted WMP with superheated steam can be explained on the basis of the mechanism of lipid oxidation. During lipid oxidation of malonaldehyde (MA), three or more double bonds from the degradation of polyunsaturated fatty acids can be detected spectrophotometrically on a wavelength of 530 nm to 535 nm by binding with TBA [37]. The result of superheated steam is stable. Therefore, superheated steam maintains fat milk from MA oxidation and formation.

**3.2.4 p-Anisidine value**

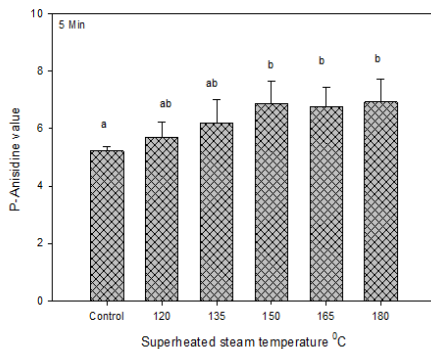
Figs. 4, 5, and 6 show the effect of superheated steam on p-anisidine under five different temperatures (120, 135, 150, 165, and 180 °C) at three different periods of time (5, 10, and 15 min). The results show no significant difference at 120 °C to 165 °C between samples under 5 min to 10 min but showed significant difference ( $p < 0.05$ ) when compared with the control (Figs. 2 and 5). However, the p-anisidine value showed no significant changes ( $p < 0.05$ ) at 120 °C to 180 °C for 15 min.

**3.2.5 Totox value**

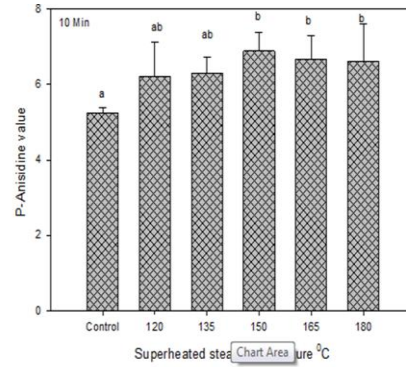
The effect of superheated steam temperature on totox value is shown in Figs. 7, 8 and 9. The result recorded no significant difference between temperatures 120, 150, and 165 °C compared with the control for 5 min. The results also showed no significant difference at 135 °C to 180 °C. At 180 °C, the results of the samples were significantly different from that of the control. In Fig. 7, no significant difference was observed among the samples under 120,135, and 150 °C compared with the control. At 165 and 180 °C, no significant difference was observed compared with the control. In Fig. 8, the effect of superheated steam temperature on the totox value showed no significant difference between all superheated steam temperatures compared with the control. Oxidation was more stable because of less oxygen in superheated steam, which prevented free radical generation. Lack of oxygen maintained lipid integrity, high nutritional value, and good flavor [31].



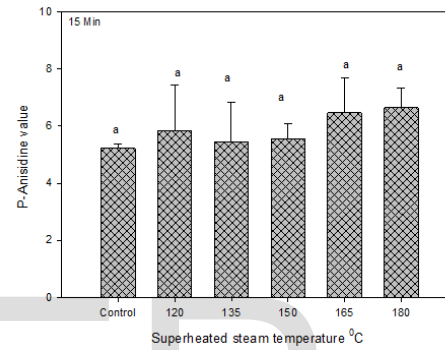
**Figure 3** Effect superheated steam temperature on TBARS value to reconstitute whole milk powder.



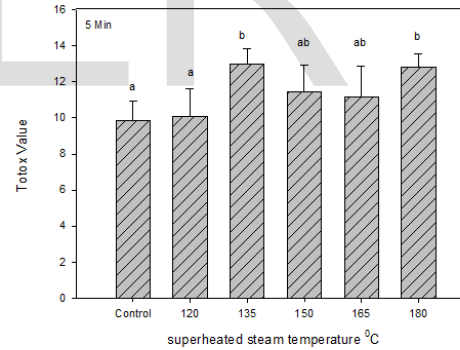
**Figure 4** Effect superheated steam temperature on P-Anisidine value to reconstitute whole milk powder on time 5 min.



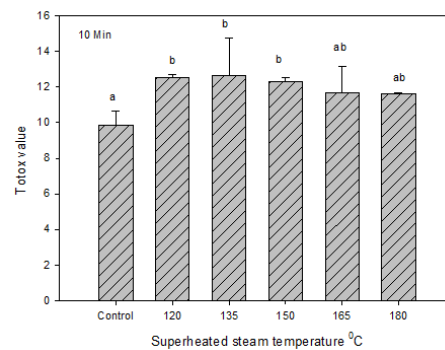
**Figure 5** Effect superheated steam temperature on P-Anisidine value to reconstitute whole milk powder on time 10 min.



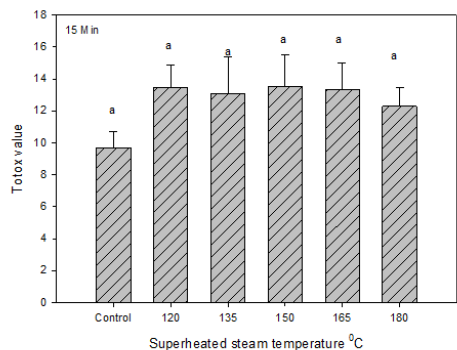
**Figure 6** Effect superheated steam temperature on P-Anisidine value to reconstitute whole milk powder on time 15 min.



**Figure 7** Effect superheated steam temperature on Totox value to reconstitute whole milk powder on time 5 min.



**Figure 8** Effect superheated steam temperature on Totox value to reconstitute whole milk powder on time 10 min.



**Figure 9** Effect superheated steam temperature on Totox value to reconstitute whole milk powder on time 15 min.

### CONCLUSION

We heated reconstituted WMP by using a superheated steam technique at different temperatures and time periods. We also used different tests to analyze lipid oxidation. We tested the acid, peroxide, TBARS, p-anisidine, and totox values of the samples. The results showed that at 120 °C to 165 °C for 5 min to 10 min, no significant difference in acid, peroxide, TBARS, and p-anisidine values were observed between the samples and the control. p-Anisidine values showed no significant difference at 120 °C to 180 °C for 15 min. The totox value reflected the stability of oxidation at different temperatures. The superheated steam did not have an effect on lipid oxidation in reconstituted WMP. Therefore, superheated steam improves the flavor of products and maintains nutritional value by inhibiting oxidation.

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