Detection the oxidative stability of milk fat to different types of powdered milk

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Abstract
This study was designed to show the oxidative stability of different types of powdered milk it is present in the local market and almost consumed daily. Five types of cows powder milk were selected from the markets in Salah Aldeen governorate (Almudhish, Dielac, Smartbaby, Altunsa, Commercial Milk). In this study five treatments to lipid oxidation index were carried out as follows: pH - acidity - FFA - iodine value and peroxide value. The results indicated high acidity for both Altunsa and the commercial milk with decrease in PH for both Altunsa and commercial milk. However, the statistical analysis (p<0.05) showed Altunsa milk and commercial milk recorded the highest value of the iodine and peroxide numbers, While the milk type of the smart boy and Dialac recorded the highest stability of the oxidation through the lipid oxidation index.

Key word: oxidative, commercial milk, pH - acidity - FFA – iodine.

1. Introduction
Milk has been known since ancient times as an integrated natural foodstuff because it contains most of the essential elements, which has invited many researchers to study the factors that effect on milk production and composition in order to reach high productivity for lactating animals. Main problems that milk is exposed to different changes during storage in order to reduce the quality before delivered to the consumer [1]. Spontaneous oxidation is one of the main problems that milk faces due to the undesirable oxidizing flavor resulting from the oxidation of unsaturated fatty acids, especially those involved in the synthesis of phospholipids present in the shell of the fat granule [2]. Many factors play main roles to degraded milk fat including temperature, light, naturalization of milk, peroxides, heavy metals such as iron and copper, xanthan oxidase, degree of fatty acids saturation and presence of antioxidants compounds such as vitamin A or E [3]. The most important compounds in milk fat is mucin, xanthine oxidase/dehydrogenase, butyrophilin, adipophilin and periodic acid Schiff glycopolit [4,5]. One of the criteria for measuring the stability of fat against oxidative stress is the change in the number of peroxide in the estimated amount of oxygen equivalent per kilogram of fat. The arrival of this number at 20 mmq / kg in which the fat becomes inedible according to nutritional criteria and sensory evaluation of fat [6].

Even heat processing the temperatures one of main factors play important roles to accelerated the reaction between the oxygen and lipids in food processing lead to formation various degradation product [7]. The Thermo oxidation it is an autoxidation can be proceeds by mechanism of free radical which divided to the three main steps initiation, propagation, and termination step [8]. As the mention above in lipid oxidation during heat processing the especially in initiation step the alkyl radical will form by remove one hydrogen from carbon atom in the fatty acid [8]. Many catalyzed such as temperature, light, and metal effected in initiation step as energy carrier. Lipid oxidation products can be produce off-favoure by produce volatile oxidation products, while the saturated and unsaturated aldehydes such as hexanal, 2-hexanal, 2,4-decadienal, 2,4-octadienal, 2,4-nonadienal, 2-heptenal and 2-octenal, are the major compounds responsible on the off-flavor in milk fat [9].

The objective of this study was to evaluate the lipid oxidation index to five types of powdered caws milk in market and follow the effect of storage conditions on it through the deterioration of fat and determine its for suitability to the consumers.

2. Materials and methods
2.1. Cow's powder milk
Cow's powder milk was supplied by Tikrit market in Salah Aldeen state, Iraq. The standard amounts for cow's powder milk contents were follows:

Almudhish powder milk, country of origin Sultanate of Oman, ingredient: total fat (28.0 g per 100 g), saturated fat (18.8 g per 100 g), protein (24.5 g per 100 g), carbohydrate (38.3 g per 100 g), minerals (6.0 g per 100 g), vitamin A (2100 IU), vitamin D (350 IU), sodium (280 mg per 100g) and calcium (950 mg per 100g).

Dielac powder milk, country of origin Vietnam, ingredient: total fat (25.0 g per 100 g), protein (16.2 g per 100 g), carbohydrate (51.6 g per 100 g), minerals (0.502 g per 100 g), vitamin A (1600 IU), vitamin D (336 IU), sodium (200 mg per 100g) and calcium (475 mg per 100g).
Smart baby powder milk, country of origin Australia, ingredient: total fat (24.8 g per 100 g), protein (16.4 g per 100 g), carbohydrate (50.6 g per 100 g), minerals (0.473 g per 100 g), vitamin A (1653 IU), vitamin D (342 IU), sodium (210 mg per 100 g), and calcium (481 mg per 100 g). Altunsa powder milk, country of origin Turkey, ingredient: total fat (28.0 g per 100 g), saturated fat (18.3 g per 100 g), protein (25.0 g per 100 g), carbohydrate (38.0 g per 100 g), minerals (5.2 g per 100 g), vitamin A (1800 IU), vitamin C (80 IU), sodium (335 mg per 100 g), and calcium (860 mg per 100 g). Commercial powder milk, country of origin India, ingredient: total fat (29.0 g per 100 g), saturated fat (18.2 g per 100 g), protein (25.0 g per 100 g), carbohydrate (38.0 g per 100 g), minerals (7.2 g per 100 g), vitamin A (1800 IU), vitamin D (355 IU), sodium (291 mg per 100 g), and calcium (855 mg per 100 g).

2.2. Milk reconstituted
powdered milk for five types (Almudhish, Dielac, Smartbaby, Altunsa, Commercial Milk), were reconstituted by weighed 33 grams of from each powdered milk and mix with 225 ml distilled water according to the manufacturer's instructions listed on the packages.

2.2. Determination of milk composition
The proportions of the milk ingredients were estimated using a Lactoscan milk analyzer German-made No 131301-3530 milk component estimator at the College of Agriculture, Tikrit University.

2.3. Determination of milk pH
The pH was measured by pH meter (Mettler Toledo S20-K Germany). The sample was taken at triplicate. pH meter was calibrate before start to use by different buffer solution.

2.4. Determination of acid value
Acid value was estimate according to [10]. After milk reconstituted 10 gram of sample was placed in a conical flask. Two drops of phenolphthalein indicator and 0.1 N KOH solution were added until the advent of the color pink. Acid value was calculated Eq. 1:

\[
\text{Acid value} = \frac{56.1 \times V \times C}{m}
\]

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)

2.5. Milk fat extraction
Milk fat was extracted from the reconstituted powder milk according to [8], method with some modification. The chloroform, methanol and water were used to ratio of (2:1:1) [22]. The solvent mixture containing the extracted lipids was separated from the reconstituted milk by centrifugation. The mixture was mix with 0.88% of KCl solution in a separating funnel with stirring vigorously for phase separation. The upper layer was separate, which contain water, methanol and non-lipid. Lower phase was separate, contain chloroform and lipid. This layer was filtrate 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.

2.6. Determination of Free fatty acid
Free fatty acids were estimated according to the method mentioned by [11]. 10 ml of reconstituted milk was taking and placing it in a mixture of ethanol and diethyl ether 50/50 with phenonphthalene as indicator and titration with NaOH.

\[
\text{FFA} = \frac{\text{NaOH} \times 2.28}{10 \text{ml}}
\]

2.7. Determination of the iodine number
The iodine number is defined as the number of grams iodine absorbed by 100 g oil or fat. The iodine number is used to find the degree of unsaturation of free fatty acids, as well as to control the hydrogenation process and in detecting oil adulteration as a constant of fat or oil. [11]. Weigh 0.25 g was taken from milk fat from each of sample and mix with 10 ml chloroform and it was added 30 ml of Hans solution, cover the beaker with shaking well and placed in the dark place for 30 minutes, stirring it from time to time. After that 10 ml of 15% solution of potassium iodide was adding and mixed well. The mixture was added to 100 ml distilled water previously boiled and cooled by washing. The mixture was mixed with 0.1N sodium thiosulfate until the color is close to yellow, then added the starch index (2 ml of starch 1%) (when adding the starch guide a blue color is formed) and complete the titration until the blue color disappears.

\[
100 \times 127 \times N \times \text{iodine} = A - B \times \text{Sample weight}
\]

A = The number of ml of sodium thiosulfate required to calibrate the plank.
B = the number of ml of sodium thiosulfate required to sample the sample.
N = standard sodium thiosulfate

2.8. Determination of Peroxide value (PV)

Peroxide value was determined according to [12] official methods described by [4]. 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. After fat was dissolved, 0.5 mL of saturated potassium iodide solution was added and swirled for 1 min. Subsequently, 30 ml of distilled water was added. Exactly 0.01 N Na₂S₂O₃ 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 until a blue color disappears. Peroxide value was calculated as eq of peroxide/kg of fat (Eq. 3):

\[
Peroxide\ value = \frac{(S - B) \times N\ thiosulfate \times 1000}{(weight\ of\ sample)} \quad (3)
\]

3. Results and Discussion

The study indicated that are a few differences in the tests conducted on the powdered milk, as the first thing we did was to conduct pH meter tests, where the reading showed that the Smart baby milk had a percentage of (6.6), while it was Almudhish milk with limits (6.5) and Altunsa milk with limits (6.7). The Dielac milk had limits (6.7) and finally the ratio of commercial milk was (6.5). The calculations or outputs of the acidity number were as follows: Altunsa was up to (4.8), Dialect milk was limited (4), Almudhish milk was limited (4) Smart baby milk was (4) and finally the sample of commercial milk was (4.8) and the analyzes were used on milk to estimate the percentage of fat on the Milko Scan where the results gave these ratios of the quantities of fat in milk types as shown in Table 1 and 2.

| Milk type  | Fat %       | Solid Fat % | Lactose % | Protein % | Density % | Freezing point |
|------------|-------------|-------------|-----------|-----------|-----------|----------------|
| Almudhish  | 2.23±0.74a  | 7.55±0.10a  | 4.11±0.40a| 2.67±0.25a| 1.011±0.53a| 0.23±0.11a     |
| Dielac     | 2.41±0.14b  | 7.55±0.23a  | 2.24±0.28b| 2.70±0.39a| 1.022±0.17b| 0.24±0.11b     |
| Smart baby | 2.41±0.13c  | 7.55±0.11b  | 2.18±0.50c| 2.70±0.12a| 1.022±0.75b| 0.24±0.10b     |
| Altunsa    | 2.66±0.10d  | 7.60±0.31b  | 4.24±0.21d| 2.91±0.11b| 1.022±0.51b| 0.24±0.10b     |
| Commercial Milk | 2.66±0.11d | 7.60±0.31b  | 4.23±0.51d| 2.86±0.10b| 1.022±0.31b| 0.24±0.10b     |

Means in same column bring different superscripts are significantly different (P ≤ 0.05).

| Milk type  | pH       | Acid value |
|------------|----------|------------|
| Almudhish  | 6.5±0.31b| 4.8±0.11c  |
| Dielac     | 6.4±0.44c| 4.1±0.31b  |
| Smart baby | 6.6±0.72ab| 4.0±0.22a |
| Altunsa    | 6.7±0.69a| 4.8±0.31c  |
| Commercial Milk | 6.5±0.33b | 4.8±0.24c |
Means in same column bring different superscripts are significantly different (P ≤ 0.05).

After completing the results, Free fatty acid was estimated and the ratios were as follows:
Where the proportions of Al-Mudhisheh milk were in the range of (0.57), while the ratio of the Smart baby milk was in the range of (0.57), the proportion of Altunsa milk was in the range of (0.68), and the ratio of the Dielac milk was in the range of (0.57). Table (3) shows the ratios of Free fatty acid for different types of powdered milk.

Table 3. The changes in peroxide value, Iodine value and free fatty acid of powdered cow's milk during storage.

| Milk type | Peroxide value | Iodine Value | FFA  |
|-----------|----------------|--------------|------|
| Almudhish | 6.5±0.22a      | 4.8±0.71b    | 0.57±0.13a |
| Dielac    | 6.4±0.34a      | 4.1±0.44a    | 0.75±0.12d |
| Smart baby| 6.6±0.56b      | 4.0±0.42a    | 0.55±0.10a |
| Altunsa   | 6.7±0.50c      | 4.8±0.29b    | 0.68±0.11c |
| Commercial Milk | 9.5±0.43d | 4.8±0.24b | 0.92±0.15e |

Means in same column bring different superscripts are significantly different (P ≤ 0.05).

Table 3 shows the ratios of the iodine value and the peroxide value. The percentages of acidity in the milk of Altunsa and commercial milk were high level, reaching 4.8%, and the proportions were normal for the Almudhish milk types. Dielac and smart baby milk, as it reached 4%, and this is consistent with [13]. The differences in the ratio between casein and carbon dioxide, as well as salts of stearate and phosphates, and this corresponds to also with what mentioned by [14]. The percentage of free fatty acids was high in the samples of Altunsa and commercial milk, where the ratios reached to 0.68%, while it was 0.58% for Al-Mudhahesh, Dielac and the Smart Baby, due to the decomposition of triglycerides. In the types of Altunsa and commercial milk. The percentage of the iodine number was high in the Altunsa samples, where it reached 35.4%, while it recorded a decrease in the Almudhish sample, which amount of 32.9%, and the increase was due to the fact that the Altunsa milk fat is rich in unsaturated fatty acids, as the iodine number is a measure of the extent of the unsaturation in the fat or oil, so the higher the value The iodine number is high, indicating that the fat or oil is rich in unsaturated fatty acids [15]. The percentage of peroxide was high in the Almudhish sample, as it reached 0.43%, while it decreased in Almudhish milk and the smart baby. The percentage was 0.37%, and this rise in the number Iodine due to oxygen and a number of minerals, including the copper and iron. The oxygen range is one of the main factors that play an important role to accelerate the reaction between oxygen and lipids leading to the formation of various degradation product. The oxygen-oxidation process is an autooxidation can namely proceed by mechanism of free radical which divided to the three main steps initiation, propagation, and termination step. During heat processing to the any foods include high concentration of fat there are some changes will occur in the stricture of fatty acids make it undergo to the external changes and produce different compounds. As mention earlier, during heat processing when milk convert to powder face in the initiation step, alkyl radical will form by the remove one of hydrogen from carbon atom in the fatty acid. Many catalyzed such as temperature, light, and metal effected in initiation step as energy carrier [16]. The free radicals formation required amount of energy to remove hydrogen atom which its take different energy depend it position on the carbon chain. For example, remove one hydrogen from C11 of the linoleic fatty acid required amount of energy estimate as 50 (Kcal/mol) from its position of fatty acids while remove same hydrogen atom but from C8 or C14 to the seam fatty acid require 75 Kcal/. However, the energy required to remove one hydrogen atom from saturated carbon bonds such as C17 and C18 in linoleic acid about 100 (Kcal/mol) [8]. On propagation step, the free radicals formation in initiation steps can be react with oxygen to form the peroxy radical which it is unstable also rapidly abstract hydrogen atom from another free radicals to produce the hydroperoxides [7]. The lipid hydroperoxides are considered primary products to the oxidation reaction rapidly decompose by heat processing to the other product such as aldehydes, alcohols, ketones, lactones, esters, acids, hydrocarbons and acids which considered volatile compounds responsible on the off-flavour in oil and lipids.
Conclusion

Powdered milk samples were carried out on selected milk (Almudhish, Dielac, Smartbaby, Altunsà, Commercial Milk). Lipid oxidation treatment significantly influenced on physical properties such as the pH and acid value with slight changes on the density values. However the chemical treatment indicated changes in milk fat oxidation in terms of PV, Free Fatty acid and Iodine values. This is because of the presence of free oxygen environment during the powdered packaging and storage levels. The Smart baby milk which provides lower lipid oxidation index more than other powdered milk on the Iodine value and FFA. However, the Dielac milk recorded lower PV form the other milk powdered which recommended to use from consumers in this study.

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