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Original Article

DETERMINATION OF PROTEIN AND FAT OXIDATION LEVELS IN IMPORTED INFANT FORMULA AVAILABLE IN SYRIA

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ABSTRACT

Objective: The aim of this study was to evaluate fat and protein oxidation levels in imported infant formulas [infant formula (IF_{a1}) and follow up formula (IF_{a2})]; which are available in Syrian market. In addition, the aim was to determine the best conditions for preparing the feeds, and for storage of the opened cans.

Methods: Fat oxidation was evaluated by peroxide value (PV) using the iodometric method. Protein oxidation was assessed by the selective indicator protein carbonyls (PC_s), using the spectrophotometry method at 280 nm to measure the protein content. Next, the effects of storage at room temperature and refrigerator temperature on both fat and protein oxidation were studied. Furthermore, we studied the changes on fat and proteins oxidation caused by reconstituting the feeds by 40 °C and 70 °C water.

Results: PV levels of IFa_1 ranged between 0.88 and 1.30 $mEqO_2/kg$, and were higher than those of IFa_2 which ranged between 0.76 and 1.24 $mEqO_2/kg$. Similarly, PC_s levels of IFa_1 ranged between 40.5 and 87.6 m mol/kg protein, and were also higher than PC_s levels of IFa_2 which ranged between 27.78 and 82.96 m mol/kg protein. We found no differences between PC_s levels of samples stored at refrigerator and room temperature for 21 d, while PV levels of samples stored at refrigerator temperature were lower than those stored at room temperature for 21 d. For preparation conditions, no differences were observed in oxidation levels between the feed reconstituted by 40 °C and 70 °C water.

Conclusion: All IF samples available in Syrian market showed oxidation levels using PV and PC_s. Additionally, it is better to keep the opened IF cans at refrigerator temperature than keeping them at room temperature, especially for fat oxidation. Finally, no differences were observed by reconstitution IF by 40 °C and 70 °C water.

Keywords: Infant formula, Oxidation, Protein carbonyls, Peroxide value.

INTRODUCTION

Infant Formula (IF) is the breast milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants [1]. IF should mimic the breast milk composition to meet the normal growth needs for infants [2]. Milk is a complex liquid food with 87% of water; it contains proteins, carbohydrates, saturated and unsaturated fat, minerals, and vitamins [3]. Cow milk based formula is the main formula marketed in the Syrian market.

Human milk composition is different from cow milk [4] for that, many modifications should be done before the thermal treatment which is the next step of processing [5]. Heat treatment improves food safety [6, 7] and increases its shelf life [8]. However, heat treatment may enhance many modifications during both processing and storage [9] leading to decrease the nutritional value and food safety [8].

One of these modifications is the well-known Maillard reaction which occurs in milk in all thermal treatment ways [10]. In addition, Maillard reaction produces many new compounds which have negative effects on health [11]. Heat treatment also promotes both fat and protein oxidation [7]. Fat oxidation is responsible for the changes in taste and odor [12]. Lipid oxidation involves the production of free radicals which lead to Hydro peroxides; this stag is called primary oxidation. Peroxide value (PV) is considered as a selective indicator for primary oxidation [13]. Poly unsaturated fatty acids ($PUFA_S$), which are important for brain and retina development, are the highly susceptible targets to fat oxidation [12]. The second stage of fat oxidation is called secondary oxidation. Secondary oxidation produces many new compounds like Malondialdehyde MDA [14] and 4-Hydroxynonenal 4-NHE [14]. These compounds contribute to many health problems [15].

Proteins can also be oxidized during heat treatment and storage [16] especially in the IF [17] and produce a wide group of compounds like protein carbonyls PC_s [18].

For these reasons it is very important to monitor fat and protein changes caused by heat treatment to ensure all benefits and to minimize all negative effects [6, 7]. Monitoring heat treatment can be done by many selective indicators [19] like furosine, hydroxymethyl-furfural, cross-linked protein, and hexanal. Peroxide value (PV) is considered a specific indicator for primary fat oxidation [12]. There are many analytical methods to measure PV; one of these methods is the Iodometric titration [12]. MDA and 4-NHE can be measured using spectrophotometer and chromatography [13].

Protein carbonyl (PCS) is widely used as a good marker for the protein oxidation. It is also measured by many analytical methods such as the spectrophotometric method [20]. IF is not produced in Syria, however it is imported from many countries. All imported kinds are monitored by the Syrian standard (S. N. S: 197/1996)and should only be sold in pharmacies. The purpose of this study is to assess the quality, by the means of fat and protein oxidation, of the entire imported IF kinds available in the Syrian market.

MATERIALES AND METHODS

Instrument

Electronic scales milligrams (Precisa XB 220 A), spectrophotometer (Jasco v-530 UV), and Kjeldahl device (Digestive system: Buchi, Digest system K-437. Distillation unit: B-324), centrifuges (Labofuge 200 Heraeus, REMI Labotatory centrifuge R4C), water bath (BANDELIN soronex Digitec), Micropipette (Labkit, Chemelex, S. A)

Materials

36 infant formula samples were purchased from the local pharmacies. All samples were prepared by adding distilled water before any assay. Chemicals: Urea, glacial acetic acid, tri cholor acetic acid, and ethyl acetate (Merck),(Germany). Dinitrophenylhydrazine DNPH, Chlorophorm, (Hemedia Laboratory), (India). Sodium mono and di phosphate (Labocheme), (India). All the chemicals were of analytical or HPLC grade.

Methods

Measurement of fat oxidation using peroxide value

Fat was separated by centrifugation at 3000 round/min for 30 min, and then the fat layer was collected from the surface using a spoon [21]. PV levels were determined using the Iodometric method described in the AOAC [22] and carried out in triplicate. The PV amount was expressed as mille equivalent of O_2 per kilogram of fat (mEq O_2/kg). Intraday variation was determined by analyzing two different kinds of IF for six times in one day, while inter-day variation was determine by analyzing two different kinds of IF on consecutive three days. The relative standard deviation RSD results of were 5.1% and 5.8% respectively, and both were less than 10%.

Measurement of protein oxidation using carbonyls by spectrophotometer at 370 nm

The trial was carried out according to Levin *et al.* [19]. Briefly, an aliquot of reconstituted infant formula (corresponding to 2 mg proteins) was precipitated with 10% TCA (final concentration). Then, the precipitants were incubated with 2 ml of 10 mM DNPH in 2M Hcl for 30 min at room temperature. Next, the mixture was centrifuged at 3000 round/min for 3 min, and washed three times with 1 ml of ethanol/ethyl acetate (50:50) to remove free DNPH. Finally, the precipitants were dissolved in 4 ml of 6M urea. The absorbance was measured at 370 nm on Lambda of Jasco-530 UV spectrophotometer (Jasco, Japan). The PC_S amount was expressed as mille mole of carbonyl per kilogram of protein using an absorption coefficient of 21.000M^{-1} cm^{-1} at 370 nm for protein hydrazones. Measurement was carried out in triplicate.

Determination of protein content by spectrophotometer at 280 nm

Studies mentioned that protein is lost during the procedure of Levine [23]. One way to avoid this problem was to measure the final protein amount in the final precipitates by the spectrophotometric method at 280 nm [23]. The trial was carried out at the same time of determination PC_S. However, the precipitates were incubated in 2 ml of 2M Hcl instead of DNPH. Additionally, in the final step the precipitates were dissolved in 4 ml of neutral phosphate buffer instead of 6M urea. Standard curve of bovine serum albumin was prepared in phosphate buffer in range of concentrations (0.2, 0.5, 0.7, 0.9, 1.1 mg/1 ml). The absorbance was measured at 280 nm and measurement was carried out in triplicate. The equation was $y = 0.0786x + 0.0098$ And the coefficient of determination was R^2 = 0.9997 The linearity was achieved and protein concentrations were calculated.

Effects of storage conditions on fat and protein oxidation

Two kinds of IF (A_2, F_2) were studied. The can was divided in to two portions, one kept at room temperature and the other kept at refrigerator. PV levels were measured immediately after opening the can (day 0) and after 7days, 14 d, and 21 d. PC_S levels were measured immediately after opening the can (day 0) and after 7days, 14 d, and 21 d.

Effects of IF reconstitution water temperature on fat and protein oxidation

Two kinds of IF (A_2, F_2) were studied. Infant formula powder was reconstituted using either hot water (70 C °) and wait to get cool or warm water (40 C °). Next, PV and PC_S were measured as set previously.

RESULTS AND DISCISSION

Measurement of fat oxidation

(Table 2) summarizes the PV results for all samples. IF samples showed different PV levels due to the differences in composition (table 1) and manufacturing processes. IF_{a1} showed higher PV levels than IF_{a2}. This is because IF_{a1} has higher content of $PUFA_S$ than IF_{a2} (table 1). The high content of $PUFA_S$ in IF_{a1} is necessary to achieve the nutritional requirements for normal growth of infants [24]. However, $PUFA_S$ are attractive targets for fat oxidation [12]. This means that the higher content of $PUFA_S$ leads to higher oxidation levels, and so that higher PV results. These results agree with Nadal and his colleagues results [12]. Finally, fat oxidation goes in two stages; Primary oxidation and secondary oxidation, and it is preferred to study these two stages at the same time to understand the oxidation and explain the results clearly.

Measurement of protein oxidation

(Table 2) summarized the PC_S levels for all studied samples. PCs levels ranged between 40.5 and 87.6 m mol/kg protein for IF_{a1}, and between 27.78 and 82.96 m mol/kg protein for IF_{a2}. The difference of PC_S levels between IF_{a1} and IF_{a2} depends on the composition (table 1). Whey proteins content in IF_{a1} is higher than IF_{a2} to achieve the nutritional requirements needs of infants [24], where the whey proteins/casein ratio is 60/40 in IF_{a1} while it becomes 20/80 in IF_{a2}. Whey proteins are more likely to be oxidized [25], so that the oxidation levels of proteins will increases as the whey proteins content increase and so that for PC_S levels [25]. In addition, IFa_1 is more enriched in $PUFA_S$ and thus, more susceptible to oxidation, and that may enhance protein oxidation [6]. One study showed PC_S of 8–60.9 m mol/kg protein. The difference between this study and ours may related to the different analytical techniques [6].

Table 1: Content of fat and protein in the studied samples as it is written on the cans

Infant formula IF_{a1}	Protein content%	Lipid content %	$PUFA_S$ content %	Infant formula IF_{a2}	Protein content%	Lipid content %	$PUFA_S$ content %
A_1	9.65	27.7	9.22	A_2	15	23.5	4.31
B_1	9.7	24.7	4.003	B_2	15.1	20.3	3.25
D_1	12.5	28	3.9	D_2	17	20	2.5
E_1	11	27	5.51	E_2	11.8	21.5	4.48
F_1	11.4	25.4	2.81	F_2	15	25.3	2.1

Table 2: The levels of peroxide value and protein carbonyls in the studied samples of infant formula

Infant formula IF_{a1}	The peroxide value (m molO_2/kg)	The protein carbonyls (m mol/kg protein)	Infant formula IFa_2	The peroxide value (m molO_2/kg)	The protein carbonyls (m mol/kg protein)
A_1	0.5±0.03	40.5±2.25	A_2	0.44±0.05	37.32±1.82
B_1	0.44±0.02	55.42±5.14	B_2	0.38±0.09	47.04±2.48
D_1	0.54±0.05	58.86±1.44	D_2	0.42±0.16	27.78±2.62
E_1	0.54±0.06	87.6±1.38	E_2	0.45±0.15	82.96±7.5
F_1	0.65±0.09	75.62±6.6	F_2	0.62±0.12	62.86±4.3

Effect of storage conditions on fat and protein oxidation

PV levels increased during storage time, regardless of the samples were stored at room temperature or in refrigerator (fig. 1, fig. 2). PV levels at room temperature were higher than those in refrigerator because of the difference in storage temperature. The present results are similar to those obtained by another study, where it found that PV in samples stored at 37 C ° was higher than those stored at 25 C ° [12]. For storage time, student test was made (p<95%) and showed differences between PV levels at day (0) and the day (21), for both samples stored at room and refrigerator temperature for the two studied kinds. Similarly, student test showed differences in PV levels at the day (21) between the two storage temperatures for both studied kinds.

PCs levels also increased during storage time, regardless of the samples were stored at room temperature or in refrigerator (fig. 3, fig. 4). However, student test (p<95%) showed no differences between PCs levels during storage time for samples stored at room and refrigerator temperature for both studied kinds. Additionally, there were not any differences in PCs levels between room and refrigerator temperature at the end of storage time. Results of protein oxidation were un expected because many studies said that there is a correlation between fat and protein oxidation, and assure that primary fat oxidation could enhance proteins ability to oxidation [6]. Finally, further studies should be carried out using other indicators of protein and fat oxidation to understand all results clearly.

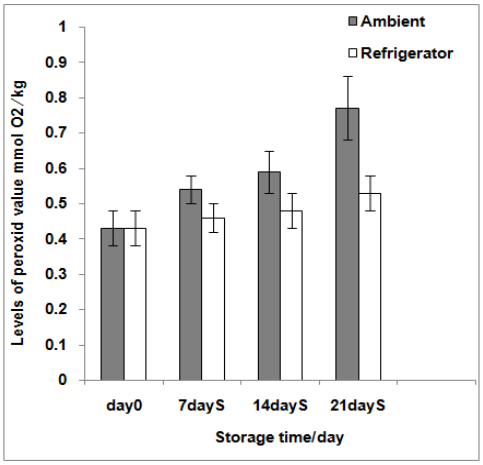

Fig. 1: The levels of peroxide value during the storage time for 21 d (A_2 infant formula)

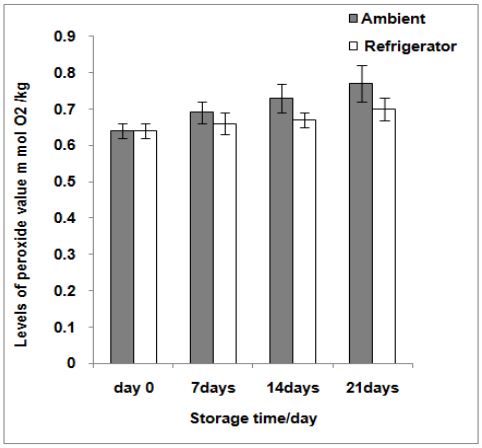

Fig. 2: The levels of peroxide value during the storage time for 21 d (F_2 infant formula)

Effects of IF reconstitution water temperature on fat and protein oxidation

PCs and PV levels at 70 C ° were similar to those at 40 C ° (fig. 5, fig. 6). For A_2 formula PV levels at 40 °C and 70C ° were (0.86, 0.87 mEq O2/kg), and for F_2 formula were (1.28, 1.30 mEq O2/kg). PCs levels at 40 °C and 70 C ° were (36.18, 36.75 m mol/kg protein) for A_2 formula, and for F_2 formula were (61.1, 61.55 m mol/kg protein). Student test showed (p<95%) no differences between the results at two temperatures for both kinds of infant formula

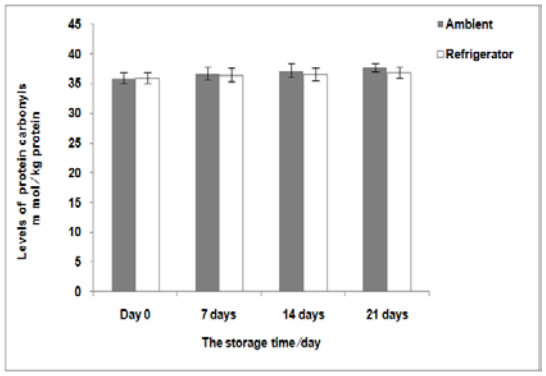

Fig. 3: The levels of protein carbonyls during the storage time for 21 d (F_2 infant formula)

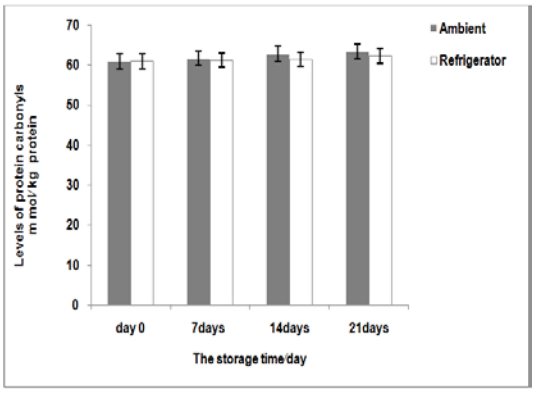

Fig. 4: The levels of protein carbonyls during the storage time for 21 d (F_2 infant formula)

Temperature is known to be as an important influent factor for oxidation in food emulsions [26], it could be that higher temperature than 70 C ° is needed to show this effect on fat and protein oxidation in IF [27]. The samples were analyzed immediately after the cans have been opened, so the oxidation could increase by passing the time.

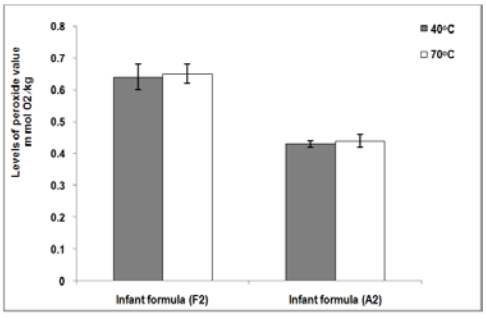

Fig. 5: The levels of peroxide value after reconstituting infant formula using heated water (40, 70 °C) for A_2 and F_2 samples.

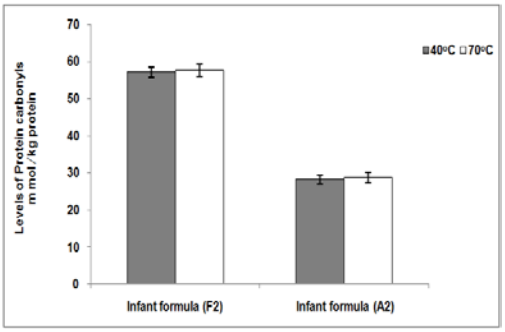

Fig. 6: The levels of protein carbonyls after reconstituting infant formula using heated water (40, 70 °C) for A_2 and F_2 samples

CONCLUSION

All kinds of Infant Formula available in the Syrian market were studied and showed different levels of peroxide value and protein carbonyls as an indicator for fat and protein oxidation, respectively. Further studies are required to understand completely the oxidation process in infant formula, by studying many other specific indicators, in addition to studying the well-known Maillard reaction.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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