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Effect of Freeze and Re-freeze on Chemical Composition of Beef and Poultry Meat at Storage Period 4.5 Months (SP4.5)

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Abstract

In the present study, two fresh meat types (beef and poultry) were frozen at -20°C thawed at room temperature, refrozen at -20°C and stored for zero time (SP0), 2.0 (SP2), and 4.5 months (SP4.5) and were evaluated for moisture, protein, fat, and ash contents at the end of each Storage Period (SP). We found that poultry meat had significantly ($P \leq 0.05$) higher moisture, protein, and ash, but less fat content than beef meat. Regardless of meat type, the moisture content decreased ($P \leq 0.05$) with increased storage period. The opposite was true for the fat and ash meat compositions. When the interaction of meat type (MT) and SP was considered, beef meat had higher ($P \leq 0.05$) moisture and fat contents than poultry meat. However, poultry meat was higher ($P \leq 0.05$) in total protein content. Our study suggests that meat quality can be maintained for a prolonged time through storage at a constant low temperature.

Keywords: Frozen; Re-frozen; Beef; Poultry; Moisture; Protein; Fat; Ash

Introduction

Proteins are the building blocks of life and are important constituents for the growth and development of children, teens, and pregnant women [1,2]. Red meat consists of high biological value protein and the key micronutrients that are required for a healthy life. It also provides important fat components, including essential omega-3 polyunsaturated fats. Freezing and thawing decrease the quality of red meat versus fresh meat having high biological value proteins [3]. Protein oxidation caused by freeze-thaw cycles largely exists, especially in the manufacturing chain of commercial broiler chickens [4].

Studies conducted over the last two decades have shown that freezing leads to leaner cuts in the red meat [5]. Fresh meat progressively deteriorates due to the natural aging process and subsequent shortening of the shelf life of the meat. However, by adopting protection approaches, the shelf life of the meat could be prolonged [6]. Storage environments are crucial in reducing the rate of putrefaction, and to preserve the taste and appearance of foodstuffs [7].

Storage of meat at a continuous temperature of -20°C is reported to contribute to the safety [8] and shelf life [9] by cooling fresh meat for weeks or months, the shelf-life of meat could be substantially extended to longer periods [10-13]. Therefore, it is important to keep in mind the design and selection of the appropriate refrigeration and heat transfer mechanisms for meat preservation [14]. Dehydration, or moisture loss, causes the product to wrinkle reducing the quality [15], which should be minimized by adopting appropriate measures in food preservation [16].

Refrigeration slows down the chemical and biologic operations in the food, therefore provides protection from further quality degradation [17]. Multiple freeze-thaw cycles significantly increased the lipid and protein oxidization and reduced the color stability of broiler chicken breast [18]. The changes in yellowness and redness of the meat could result from the formation of metmyoglobin, which is a sign of protein oxidization on color modifications through multiple freeze-thaw cycles [19]. The structural modifications occurring in proteins caused by the oxidization directly influence the capability of muscles to retain water, as confirmed by the nuclear magnetic resonance relaxometry profile [20,21]. Animal tissues vary considerably in their moisture, protein, fat content, as well as in pigmentation, and the capability to bind water and fat. Therefore, studies regarding the impact of freeze-thaw cycles on protein stability and its relationship with lipid and protein oxidization require further study.

The current study was designed to assess the effects of multiple freeze-thaw cycles on the chemical composition (moisture, protein, fat, and ash) of frozen and refrozen meat (beef and poultry) at half-shelf-life designated as (SP4.5).

Materials and Methods

Materials

Samples: Fresh/frozen beef and poultry meat samples, with the observable connective tissues and fat removed, were collected from Khartoum North supermarkets and transferred aseptically into sterile food bags to the Food Research Center (FRC) laboratories. Sampled meats were without the addition of any additives and included a wide variety of the carcass (about 25 kg). Small and larger pieces were randomly selected and minced preparations were prepared under aseptic conditions according to the legal definitions. Each type of frozen meat sample was divided into two equal parts. Each part was assigned randomly to one of the two treatments: frozen, thawed, and refrozen. Each part was further subdivided into three equal parts assigned randomly to one of the three storage periods (0, 2 and 4.5 months).

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Samples were frozen at -20˚C, thawed overnight at 4˚C in their original packages and then refrozen at -20˚C.

Chemical composition analysis (moisture, protein, fat, and ash) were carried out in triplicate on samples taken at SP0 and the storage period at 2 and 4.5 months.

Therefore, the total number of samples for analysis:

- Time zero beef (0)=3
- Frozen beef=3
- Refrozen beef=3
- Time zero poultry (0)=3
- Frozen poultry=3
- Refrozen poultry=3
- Total=18
- Number of replicates × 3
- Total samples=54

Methods

Storage repeated freezing and thawing meat for nine months and we chose the 4.5 months as half-shelf life. Repeated freezing and thawing meat were common in storage, kitchens, restaurants, and retail outlets.

Proximate analysis: Moisture content, total protein, fat, and ash contents were determined according to Seweh et al. [22].

Fat content determination: The fat determination includes a partial drying of a weighed sample before Soxhlet extraction. Sand is incorporated with the sample before drying to create a greater surface area, necessary to remove moisture and prevent entrapment of fat [23]. Fat extricated is weighed and the fat content calculated.

$$\text{Fat Content } \% = \frac{(B-C)}{A} \times 100$$

Where:

A=Sample weight

B=Weight of flask after extraction

C=Weight of flask prior

Moisture content determination: Moisture content was based on weight loss of 5 g sample of meat. Meat sample was placed in an oven at 100˚C over-night, cooled in a desiccator and weighed. The moisture content was calculated as follows:

$$\text{Moisture Content } \% = \frac{W2-W1}{S} \times 100$$

Where:

W_1=Weight of the dish+dry sample

W_2=weight of dish+sample

The w=sample weight of meat

Total protein: The total protein content of the sample was determined by Gornall et al. [24]. 1 g of meat was digested with conc. H_2SO_4 and selenium copper phosphate catalyst. The digest was diluted to 100 ml with distilled water and titrated against 2% boric acid using methyl red as an indicator [25].

Ash determination: 2 g of meat sample was placed into a dried crucible of known weight. The crucible was placed inside a muffle furnace at 150˚C. The temperature was increased gradually to 600˚C and the sample was held at that temperature for 3 h. Then the crucible was taken out, cooled in a desiccator and weighed [25]. The ash percentage was calculated as follows:

$$\text{Ash Content } \% = \frac{W2-W1}{S} \times 100$$

Where:

W_1=weight of the crucible with a dry sample

W_2=weight of the empty crucible with the sample

S=weight of sample

Statistical analysis

The data collected were submitted to analysis of (ANOVA) and wherever appropriate the mean separation technique of NDRA subsisted employed [26]. The SAS program (2003-2004) was used to perform the GLM analysis [27].

Results and Discussion

Chemical Composition of beef and poultry meat

The chemical composition of beef and poultry meats namely crude fat, protein, moisture, and ash is shown in Table 1. Beef and poultry samples are considered fresh (zero time) at 4˚C. The proximate analysis of beef meat at SP0 was moisture 71.38%, crude protein 16.01%, fat 7.93%, and ash 0.79%. The poultry carcass measured at SP0, showed moisture 75.03%, crude protein 17.35%, fat 5.12%, and ash 0.86%. The proximate composition of the two species differed significantly ($P<0.05$). Poultry meat had higher ($P<0.05$) moisture, protein, and ash content than beef meat whereas beef had higher ($P \leq 0.05$) fat content than poultry meat (Table 1). The difference in the chemical composition of the two types of meat in this study is similar to results reported by several early investigations. The nutritional composition of the meat varies according to breed feeding regimen, season and meat cuts [3]. The moisture and protein contents of the beef are within the range reported [28]. The fat and ash contents in the current study are similar to and lower respectively than those reported by Spiehs et al. [29]. Regarding the chemical composition (moisture, protein, and fat) of poultry meat, our findings are comparable to those reported by de Almeida Costa et al. [30]. The effect of storage period on the chemical composition of meat is shown in the (Table 2). Large compositional changes were observed during the storage periods for the crude fat, crude protein, moisture, and ash contents of both beef and poultry meats. Crude fat, crude protein and ash content of beef and poultry meats decreased at half-shelf life. Fat content decreased from 4.83% to 3.00%, protein from 15.43% to 15.03%, and ash content from 0.87% to 0.79% in beef meat during the 2, and 4.5 months storage. Poultry meat showed a decrease in crude fat content from 7.63% to 6.90%, protein from 17.63% to 16.67%, and ash content from 0.77%

Content (%)	Beef	Poultry
Moisture	71.38^b	75.03^a
Protein	16.01^b	17.35^a
Fat	7.93^a	5.12^b
Ash	0.79^b	0.86^a

a-b means in same row bearing different superscript letters are significantly different ($P \leq 0.05$). n=9

Table 1: Chemical Composition of beef and poultry meat (time zero).

Parameter	Type of meat						C.V%	Lsd 0.05	SE ±	Level of sign. (P)
	Beef			Poultry						
	Storage period (months)									
	Zero	2.0	4.5	Zero	2.0	4.5				
Fat content (%)	$7.56^{b} \pm 0.48$	$4.83^{c} \pm 0.38$	$3.00^{d} \pm 0.17$	$9.20^{a} \pm 0.72$	$7.63^{b} \pm 0.71$	$6.90^{b} \pm 0.20$	0.59%	0.8806	0.2858	0.0067
Crude protein (%)	$17.47^{b} \pm 0.15$	$15.43^{d} \pm 0.40$	$15.03^{d} \pm 0.04$	$18.20^{a} \pm 0.70$	$17.63a^{b} \pm 0.46$	$16.67^{c} \pm 0.15$	2.33%	0.6959	0.2258	0.0211
Moisture content (%)	$70.33^{e} \pm 0.21$	$77.47^{b} \pm 0.50$	$78.15^{a} \pm 0.18$	$69.93^{e} \pm 0.15$	$71.77^{d} \pm 0.42$	$73.60^{c} \pm 0.26$	0.43%	0.5597	0.1817	0.00
Ash content (%)	$0.92^{ab} \pm 0.08$	$0.87^{ab} \pm 0.06$	$0.79^{b} \pm 0.07$	$1.00^{a} \pm 0.10$	$0.77^{bc} \pm 0.15$	$0.60^{c} \pm 0.10$	11.82%	0.1688	0.05477	0.0836
Any two mean ± SD values having same superscript are not significantly different (P ≤ 0.05)										

Table 2: Effect of storage period on proximate composition of meat.

to 0.60% during the half-shelf life. Increased moisture contents from 77.47% to 78.15% and from 71.77% to 73.60% were noted in beef and poultry respectively at 2 months storage, with further increases at 4.5 months. Initially (SP0) and throughout the storage, protein, fat, and ash contents decreased while the moisture content increased significantly (P ≤ 0.05) with increased storage period. Such a result is in line with Ranken [28] where results showed no significant (P ≤ 0.05) change with increased storage period. However such discrepancies between the two studies could be due to the nature of the storage condition (frozen and refrozen vs. refrigeration storages). The increase in moisture content and the decrease in protein content observed during the study could be connected with denaturation of meat protein that is associated with frozen meats in accordance with Arannilewa et al. [31] and Kristinsson and Rasco [32]. The changes in fat content during frozen storage up to 4.5 months could be associated with the hydrolysis of fat [33]. The effect of meat type and storage period on the chemical composition meat is shown in Table 3. Generally, the effect of the interaction of meat type and storage period on the chemical composition of meat is significantly different (P ≤ 0.05). Such a finding means that the measured dependent variables (chemical composition) react differently to the storage period tested. Hence comparison between storage periods is dependent upon the meat type. The mean of chemical composition parameters at one storage period may be the highest in beef but the lowest in poultry. Actually, the moisture content of beef *SP0 was similar (P ≥ 0.05) to that of poultry *SP0 in this study. At *SP0* the two meat species show similar (P ≥ 0.05) moisture content i.e. 70% vs. 69.9% respectively.

Content (%)	Moisture	Protein	Fat	Ash
Beef* SPO	73.30^{e}	17.50^{b}	9.20^{a}	0.92^{ab}
Beef* SP2	72.50^{b}	15.40^{d}	7.60^{b}	0.87^{ab}
Beef* SP4.5	72.10^{a}	15.00^{d}	6.90^{b}	0.79^{b}
Poultry* SPO	73.60^{e}	18.20^{a}	7.60^{b}	1.00^{a}
Poultry* SP2	71.80^{d}	$17.60a^{b}$	4.80^{c}	0.77^{bc}
Poultry* SP4.5	69.80^{e}	16.70^{c}	3.00^{d}	0.60^{c}
C.V	0.43%	2.33%	7.598%	11.82%
SE	0.18	0.23	0.29	0.06
*a-e: means in the same column bearing different superscript letters are significantly different (P ≤ 0.05) *n=3				

Table 3: the effect of meat type and store period on the chemical composition of meat (beef and poultry).

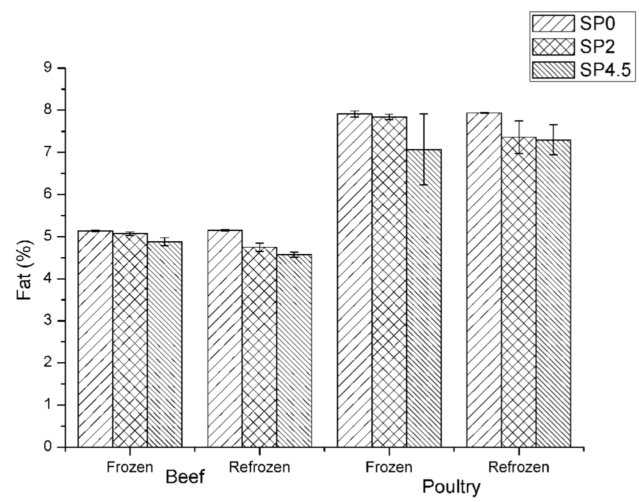

Figure 1: The effect of storage period on crude fat. The effects in fats were slight change at SP2 in frozen and refrozen beef and significantly (P ≤ 0.05) increased at SP4. Moreover, the effects of fat were higher decreased in both of frozen and refrozen at SP4.5. About poultry there were slight changes in frozen and high significant effects at SP2. Moreover, higher effects were appeared at SP4.5 in frozen and refrozen compared with SP0. Frozen and refrozen poultry had significantly (P ≤ 0.05) higher changes than beef.

Crude fat

The chemical composition of the frozen and refrozen meat samples is shown in Table 2. The crude fat of both beef and poultry significantly decreased to 3.00%, and 6.90% respectively. Respective of the storage period, the fat content of the two species differ significantly (P<0.05). The % fat decreased during storage to 3% after SP4.5 for both frozen and refrozen meat. The changes in fat resulting from frozen beef significantly (P ≤ 0.05) decreased from 7.56% at SP0 to 4.83%, and 3.00% at SP2, SP4.5 respectively. Also, a small change at SP4.5 and higher decreases in fat during the storage of poultry were determined as 9.20%, 7.63%, and 6.90% at SP0, SP2, and SP4.5 respectively (Table 3). A comparison of fat at SP0 and during the freeze-thaw cycles at SP4.5 is illustrated in (Figure 1). There are decreases of the fat from initial (SP0) and after SP2 with a further large decrease after SP4.5. The changes in fat content during frozen storage up to SP4.5 could be associated with the oxidation or hydrolysis of fat. The results corroborate findings from Soyer et al. [34]. However, there are no changes in SP0 of both meat type and frozen type. Frozen and refrozen poultry meats showed a significantly (P ≤ 0.05) high decrease in fat compared with beef samples at SP4.5 (Figure 1) The result of Van Zyl and Ferreira [35] were not in agreement with our finding which might be attributed to the difference in the temperature, period, type of the meat, and fodder type.

Crude protein

Protein contents are 16.01% and 17.35% in beef and poultry respectively as shown in Table 1. The protein content of the meats differed significantly (P<0.05). Poultry meat has higher (P<0.05) protein than beef. The difference in the chemical composition and nutritional composition of poultry and beef meats in this study are not in agreement with the literature [36], where no significant change was reported with an increase in storage period. However, such discrepancies between the two studies could be due to the meat type and the nature of the storage conditions. The decrease in protein content observed in the study could be connected with the denaturation of meat protein that is associated with frozen meats. The protein contents of the beef were similar to results obtained by Humaeda [36]. The effect of storage on crude protein in beef meat resulted in composition 17.50%, 15.40%, and 15.00% at SP0, SP2, and SP4.5 respectively. The effect of storage on crude protein in poultry meat resulted in composition of 18.20%, 17.60%, and 16.70% at SP0, SP2, and SP4.5 respectively. The crude protein of beef meat showed a significantly ($P \leq 0.05$) higher decrease than poultry meat from SP0 up to SP4.5 (Table 3). In this study, protein decreased significantly ($P \leq 0.05$) with an increase in storage period. Our results did not correspond to reported literature [37]. For protein in beef during frozen storage up to SP4.5, there were no significant changes in both meat type and frozen type. The effect of frozen type and storage period of protein in both meat types resulted in a slight decrease at and significantly ($P \leq 0.05$) higher decrease at SP4.5. The effect of frozen and SP2refreeze of beef meat at SP2 on protein resulted in protein values significantly lower than frozen poultry meats. Also, poultry protein showed a significantly ($P \leq 0.05$) higher decrease than beef at SP4.5 (Figure 2). Poultry protein deteriorated during storage up to SP2; afterward values are similar to the protein in beef at SP0. The results are similar to reported results of Anon and Calvelo [38], Mietsch et al. [39], Ngapo et al. [40].

Moisture content

The moisture of the two meat species differed significantly ($P<0.05$) as illustrated in Table 1. The moisture contents at SP0 are 71.38% and 75.03% for beef and poultry respectively. Poultry meat has higher ($P<0.05$) moisture content than beef meat. This finding was not in agreement with reported literature [41]. The higher moisture for poultry meat might be attributed to water absorption from the atmosphere or decomposition of some meat components to release bound water. The moisture content of the beef is within the range reported by Peter Williams [3,5] but lower than that reported by Huff-Lonergan and Lonergan [42].

The moisture contents of poultry meat were significantly ($P \leq 0.05$) higher than beef meat from SP0 to SP4.5 (Table 3), are observed for moisture during the whole storage period under both frozen and refrozen conditions in beef and poultry meat. Moisture contents in beef meat were 71.80%, 72.50%, and 72.10% at SP0, SP2, and SP4.5 respectively, while moisture contents in poultry meat were 75.30%, 71.80%, and 69.80% at SP0, SP2, and SP4.5 respectively. These results indicate that freeze-thaw cycles and the storage period considerably affect the moisture quality of meat. The results were agreed with Ali, et al. [18]. The effect of freeze and refreeze of meat at SP2 on moisture resulted in a significantly ($P \leq 0.05$) higher increase in frozen and refrozen in both meat types. As indicated there were no significant changes in moisture contents during storage of refrozen poultry meat. In contrast, moisture showed varying results (i.e lower counts in one type but higher counts in another type of meat) depending upon the meat type and freeze-thaw cycles. However, there have been variable results due to moisture loss during the freeze-thaw cycle (Figure 3).

Ash content

The ash contents are 0.79% and 0.86% in beef and poultry respectively. As shown in Table 1, ash content is significantly different ($P<0.05$). The ash content for beef is similar to the storage period SP0, and SP2. Storage resulted in beef meat ash values of 0.92%, 0.87%, and 0.79% at SP0, SP2, and SP4.5 respectively. For poultry meat, ash composition was 1.00%, 0.77%, and 0.60% at SP0, SP2, SP4.5 and respectively. The effect of ash for both meat types was significantly ($P \leq 0.05$) higher during storage (Table 3). Our findings show that the ash deteriorated during the storage period. Deterioration might be attributed to fat, protein, and water hydrolysis. Ash results indicate that there are no changes at SP0 for both meat types and frozen type. Ash content showed a significant decrease in both frozen and refrozen beef at SP2. Moreover, ash content showed a significant ($P \leq 0.05$) decrease at SP4.5 in refrozen poultry. However, the ash content showed only a

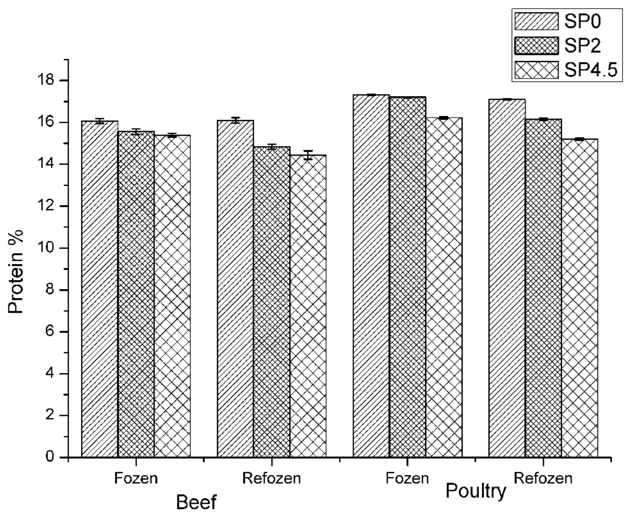

Figure 2: The effect of storage period on crude protein. The effects on protein were no significant changes in frozen and refrozen beef at SP2 and significantly ($P \leq 0.05$) high effects to the end of SP4.5 compared with SP0. The effect of frozen type and storage period of protein in poultry shown slight decrease at SP2 of frozen and significantly ($P \leq 0.05$) decreased in refrozen at SP2. Moreover, the effects of protein were increased in frozen and high significantly ($P \leq 0.05$) increased at SP4.5 in poultry.

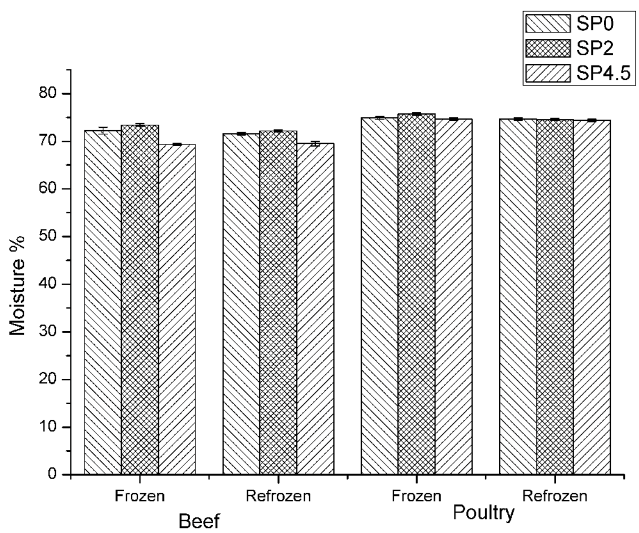

Figure 3: The effect of storage period on moisture contents. The moisture contents were slight increase in frozen and significantly decreased in refrozen poultry at SP2. The moisture was slight increase in frozen and slight decreased in refrozen poultry at SP2. The effects on moisture contents were no significantly ($P \leq 0.05$) change in frozen and refrozen poultry at SP04.5 In contrast to this, moisture showed extraordinary results in beef in both of SP and in poultry at SP2.

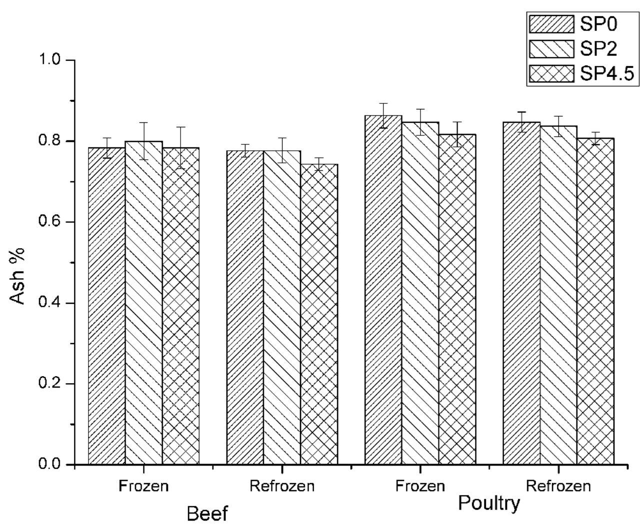

Figure 4: The effect of storage period on ash contents. The ash contents results were indicated that there no significant changes at SP2 of refrozen and refrozen at 4.5 in beef meat. The change in ash contents as results were significant decreased of frozen and refrozen in both of MT exception of at SP2 in frozen beef. However, the ash contents were decreased in both of freezing type's in poultry meat in all of SP.

slight decrease in frozen and refrozen poultry meat at SP2 and up to SP4.5 refrozen (Figure 4).

Conclusion

This study was done to determine the chemical composition (fat, protein, and ash) quality of frozen and refrozen meats (beef and poultry) up to SP4.5 under abused freezing conditions. Our findings show that the chemical composition decreased while moisture increased during storage. Poultry meat had higher % moisture, protein and ash contents but less % fat than beef meat. Our recommendations are to store meat (beef and poultry) at constant freeze temperature to avoid temperature fluctuation and product composition instability. Also, never refreeze a completely thawed poultry and beef meat, and frozen meats should be properly packaged to reduce the airspace and its insulating effect and the resulting high freezing costs.

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