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Effect of heat treatment or frozen storage on dietary heme iron content(ผลของการใช้ความร้อนและการแช่แข็ง ต่อระดับเหล็กในรูปของฮีมในอา...

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ORIGINAL ARTICLE

Effect of heat treatment or frozen storage on dietary heme iron content

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Abstract

Dietary iron existed in two forms, heme and nonheme iron. The absorption of heme iron is higher than that of nonheme iron. Consequently, alteration of the iron forms may affect iron bioavailability. Meat, liver and heart of cow, pig and chicken were treated in different manner namely, boiling or drying or freezing. Total and heme iron contents of raw, boiled, dried and frozen samples were determined. Percent reducing of heme iron contents of samples after 15, 30 and 60 min of heat treatments were pronounced as follow; 12%, 19% and 25% for meat; 14%, 21% and 28% for liver; 10%, 14% and 20% for heart. Frozen storage of meat, liver and heart for 1, 2 and 4 weeks did not significantly affect the content of heme iron. Heme iron content in dried meat, liver and heart were decreased 51%, 30% and 23%, respectively. Changes of heme iron content due to heat treatment may decrease bioavailability of dietary iron. Therefore, to conserve nutritive value of dietary heme iron, prolong heating should be avoided. (Th. J. Pharm. Sci., Vol.12 No.3, 237-246 (1987)).

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INTRODUCTION

Low iron availability is considered to be one of the most significant factors in the etiology of iron deficiency. There are many sources of dietary iron but their bioavailability vary. The bioavailability of nonheme iron is poor compared to heme iron (1-3).

It has been established that processing and cooking methods alter heme and nonheme iron levels and also the bioavailability of iron. The absorption of intrinsically labelled boiled rabbit hemoglobin in normal and iron-deficient human subjects was decreaed relative to unheated hemoglobin (1). Igene et al. (4) showed that nonheme iron concentration in meat pigment extract was increased from 8.7% to 27.0% of total iron after cooking in boiling water to an internal temperature of 70°C. Schricker and Miller (5) also found that after heating beef and red blood cells at 100°C for 20 minutes the nonheme iron content were increased 111% and 53% respectively. They also found that a strong oxidizing agent, hydrogen peroxide, elevated nonheme iron content of beef.

Due to the difference in the availability of heme and nonheme iron, conversion of heme iron to nonheme iron by heating or chemical processing can reduce the bioavailability of meat iron (5). However, the content of heme and nonheme iron in meat after boiling, drying or freezing have not been reported. This study was design to evaluate the influence of heating, drying and freezing on the contents of heme and nonheme iron content in meat generally consumed by Thai.

MATERIAL AND METHODS

Deionized double distilled water was used throughout this study. All glasswares and sample containers used in this study were free from iron by being soaked overnight in 10% hydrochloric acid bath and thoroughly rinsed several times with deionized double distilled water.

1. Preparation of Sample

Meat samples were purchased from various markets in Bangkok. After removing all visible fat and connective tissue, the sample was cut into pieces ($4 \times 4 \times 0.5$ cm^3) and divided into 8 portions, 25-30 g each.

1.1 the first portion was untreated. About 25 g of the sample was minced for total and heme iron analysis.

1.2 The second, the third and the fourth portions were boiled in an equal or greater volume of water for 15, 30 and 60 minutes, respectively. Each sample was blended to make a slurry for total and heme iron analysis.

1.3 The fifth portion was dried at 50-60°C for 8 hours and proceeded as mentioned in 1.1.

1.4 The sixth, the seventh and the eighth portions were packed in polypropylene bags and frozen at -20°C for 1, 2 and 4 weeks, respectively. The samples were thawed at about 20°C and proceeded as mentioned in 1.1.

2. Total Iron Content Determination (6)

Minced sample or sample slurry was dried at 100°C until it was completely dry. About 300-400 mg of the sample was accurately weighed and transfered to a 50-ml Erlenmeyer flask. Four milliliters of concentrated nitric acid was added to the sample. The flask was kept at room temperature for at least 12 hours for pre-digestion. After that the flask was heated on a hot plate (100°C) for 4 hours and was allowed to cool. The digested sample was transfered to a 25-ml volumetric flask and was brought to volume with water. The digested solution was filtered through a Whatman #541 filter paper and kept in an air-tight polyethylene bottle prior to measurement of iron content. Total iron content was determined with atomic absorption spectrophotometer.

2.1 Heme Iron Content (7, 8)

a) Preparation of Acid Acetone Solution

Diluted hydrochloric acid (2N) 100 ml was transfered to a 1000-ml volumetric flask and was brought to volume with acetone. This acid acetone solution was kept in a tight, light-resistant container to prevent color developing.

b) Determination of Heme Iron Content

About 2 g of minced sample or sample slurry was accurately weighed and transfered to a polypropylene tube wrapped with aluminium foil. The sample was extracted with 9 ml of acid acetone solution. The solution was filtered through a Whatman # 42 filter paper. Optical density of the filtrate was read with UV-Visible spectrophotometer at wavelength 640 nm.

2.2 Nonheme Iron Content

The content of nonheme iron in the sample was calculated as the difference between total iron and heme iron contents.

3. Statistical Analysis

Results were analyzed statistically by one-way and two-way analysis of variance. Individual means and regression lines were compared when "F" was statistically significant at the 5 percent level of probability (9).

RESULTS AND DISCUSSION

Heme and nonheme iron content of raw and boiled meat are presented in tables 1 and 2. Heat treatment resulted in substantial decrease in heme iron content and consequently increase in nonheme iron content. This study was agreed with the results observed in meat pigment extracted by Igene et al. (4) and Chen et al. (10) Jansuittivechakul et al. (11) reported that boiling for 30 minutes caused 13% reduction of heme iron content of lyophilized meat, which was similar to the present study. In addition, Schricker (5) reported that other household cooking methods such as braising, roasting and microwave cooking increased nonheme iron content of beef round generally less than 10%. Therefore, it appeared that various cooking methods altered heme and nonheme iron contents.

Upon heating, protein moieties of heme iron were oxidized and the grayish-brown denatured globin hemichrome was resulted. Schricker and Miller (5) reported that much of the additional nonheme iron was appearently derived from heme iron of hemoglobin and myoglobin. They also proposed that heating caused oxidative cleavage of prophyrin ring, thus releasing nonheme iron. This was supported by the results of Chen et al. (10) which indicated that the increase of nonheme iron was due to the cleavage of iron from prophyrin ring rather than the cleavage of heme, porphyrin with iron, from the globin portion of hemoglobin and myoglobin. However, the exact mechanism of nonheme iron liberation has been questioned.

A gradual decrease of 12%, 19% and 25% in heme iron content of meat was observed after boiling meat for 15, 30 and 60 minutes, respectively. The decreasing in the early period may occured from the alteration of pigment color but in the late period it may also be the result of the cleavage of porphyrin ring.

A negative relationship between boiling time and the heme iron content of boiled meat and a positive relationship between boiling time and the nonheme iron content of boiled meat were observed in this study.

A regression equation was $Y = 96.54 - 0.40X$, where X was the length of boiling time and Y was the heme iron content of boiled meat expressed as a percent of that of raw sample and the corresponding

Table 1 Heme Iron Contents of Raw and Boiled Meat

	Heme Iron Content[1] (μg/g dry weight)			
	Raw	Boiled 15 min	Boiled 30 min	Boiled 60 min
Beef				
round	107.48 ± 1.95 (100.00)	99.48 ± 0.95 (92.56)	93.31 ± 0.37 (86.82)	88.52 ± 0.78 (82.36)
tenderloin	99.59 ± 21.07 (100.00)	90.22 ± 20.86 (90.59)	86.81 ± 21.00 (87.17)	81.05 ± 22.07 (81.38)
liver	222.58 ± 19.32 (100.00)	200.44 ± 14.42 (90.05)	189.39 ± 10.63 (85.09)	177.98 ± 2.32 (79.96)
heart	121.86 ± 4.33 (100.00)	112.60 ± 3.40 (92.40)	105.30 ± 3.28 (86.41)	96.38 ± 1.27 (79.10)
Pork				
round	23.58 ± 1.79 (100.00)	19.53 ± 0.71 (82.81)	17.37 ± 1.41 (73.67)	15.50 ± 0.58 (65.72)
tenderloin	12.56 ± 1.11 (100.00)	9.53 ± 1.56 (75.85)	7.27 ± 0.18 (57.86)	6.37 ± 0.17 (50.74)
liver	128.07 ± 15.84 (100.00)	104.56 ± 2.69 (81.64)	92.03 ± 1.42 (71.86)	83.19 ± 5.67 (64.95)
heart	95.25 ± 21.77 (100.00)	81.44 ± 10.08 (85.46)	77.91 ± 8.93 (81.76)	72.38 ± 7.20 (75.96)
Chicken				
leg	12.95 ± 0.14 (100.00)	10.20 ± 0.28 (77.41)	7.82 ± 1.48 (60.38)	6.21 ± 2.22 (47.29)
breast	4.78 ± 0.95 (100.00)	3.16 ± 0.43 (75.65)	2.95 ± 0.22 (61.72)	2.62 ± 0.25 (54.77)
liver	70.71 ± 13.80 (100.00)	61.93 ± 9.56 (87.57)	56.19 ± 7.18 (79.45)	50.34 ± 3.30 (71.19)
heart	70.92 ± 2.15 (100.00)	65.64 ± 3.25 (92.55)	63.37 ± 2.72 (89.36)	59.24 ± 1.31 (83.53)

[1] values is parentheses represent heme iron content of boiled sample expressed as percent of that of raw sample.

Table 2 Nonheme Iron Contents of Raw and Boiled Meat

	Nonheme Iron Content[1] (μg/g dry weight)			
	Raw	**Boiled 15 min**	**Boiled 30 min**	**Boiled 60 min**
Beef				
round	63.10 ± 9.07	72.62 ± 6.44	77.78 ± 6.26	83.18 ± 8.85
	(100.00)	(115.08)	(123.25)	(131.81)
tenderloin	63.84 ± 4.93	73.05 ± 2.02	76.09 ± 5.03	83.26 ± 3.45
	(100.00)	(114.42)	(119.18)	(130.42)
liver	225.17 ± 16.34	246.93 ± 24.08	255.12 ± 27.07	267.51 ± 39.18
	(100.00)	(109.66)	(113.30)	(118.80)
heart	113.08 ± 7.24	123.89 ± 7.26	130.26 ± 9.45	138.96 ± 13.47
	(100.00)	(109.55)	(115.19)	(122.88)
Pork				
round	25.77 ± 0.46	29.01 ± 1.54	29.37 ± 0.51	33.83 ± 4.73
	(100.00)	(112.58)	(113.98)	(131.29)
tenderloin	20.47 ± 4.73	24.16 ± 5.48	25.19 ± 3.80	27.35 ± 1.33
	(100.00)	(118.02)	(123.04)	(133.61)
liver	733.81 ± 12.23	758.93 ± 10.09	767.25 ± 21.45	779.28 ± 6.21
	(100.00)	(103.42)	(104.56)	(106.20)
heart	146.05 ± 47.05	167.15 ± 68.93	163.74 ± 60.22	172.83 ± 65.10
	(100.00)	(114.45)	(111.43)	(118.34)
Chicken				
leg	34.24 ± 14.29	35.96 ± 16.09	39.42 ± 17.77	40.46 ± 19.72
	(100.00)	(105.01)	(115.12)	(118.16)
breast	41.87 ± 7.39	42.02 ± 8.04	42.64 ± 6.84	44.43 ± 5.82
	(100.00)	(100.37)	(101.84)	(106.12)
liver	288.58 ± 49.86	301.73 ± 50.91	304.31 ± 54.56	308.02 ± 60.19
	(100.00)	(104.56)	(105.45)	(106.73)
heart	138.58 ± 80.73	147.78 ± 84.20	145.24 ± 84.28	150.33 ± 82.67
	(110.00)	(106.64)	(104.81)	(108.48)

[1] values in parentheses represent nonheme iron content of boiled sample expressed as percent of that of raw sample.

correlation coefficient(r) was -0.9505. (Figure 1). This observation was similar to that reported by Schricker and Miller (5) who demonstrated a linear relationship between nonheme iron content of meat and cooking time in a 176°C oven. Chen et al. (10) who worked with meat pigment extract suggested that heating time or rate of heating instead of temperature was the major factor responsible for the alterations of heme and nonheme iron levels.

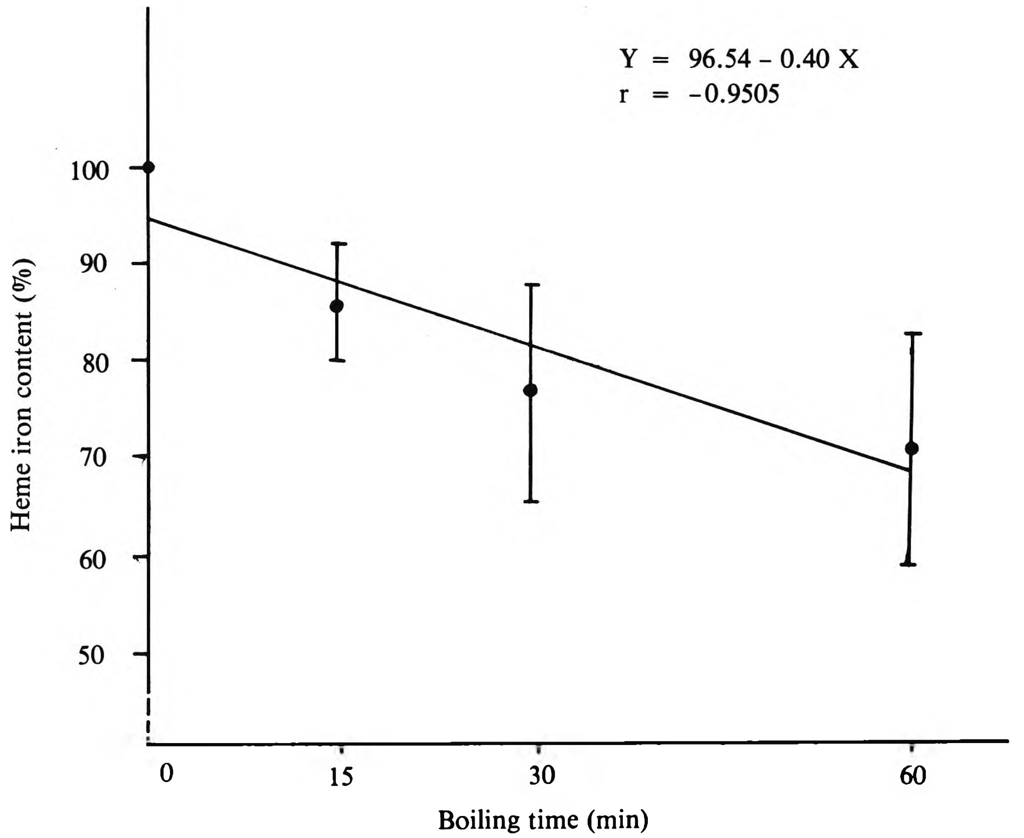

Figure 1 Relationship between heme iron content of boiled meat and the length of boiling time.

Heme and nonheme iron content of fresh, frozen and dried meat are presented in tables 3 and 4. Heme and nonheme iron content of frozen meat were not significantly different from that of fresh meat. After freezing for 1, 2 and 4 weeks, the average heme iron content in meat were 98.80%, 98.10% and 97.94% of that of fresh sample, respectively. Field et al. also found that freezing at -29°C for 60 days had no effect on the concentration of total pigment of muscle and marrow (12). They suggested that there was no breakdown of pigment during freezing.

Drying is a dehydration method used to preserve meat. Exposure of meat to sunlight is the general drying procedure. In this study, drying at 50-60°C for 8 hours was used to simulate heat exposure in sun drying since the method could provide a constant condition for various kinds of meat studied.

Drying fresh meat at 50-60°C for 8 hours resulted in 38.84% reduction of heme iron content. Average heme iron content in dried meat was 61.1% of that of fresh meat. It was shown that drying caused alterations of iron contents in the same manner as of boiling. However, the decrease of heme iron and the increases of nonheme iron resulted from drying were significantly ($P<0.05$) greater than those of boiling. This was mainly due to the fact that boiling and drying were different cooking methods. Boiling is a moist-heat method and drying is a dry-heat one. Even though, water was a good heat conductor and its presence aided in the penetration of heat into deeper part of sample, it was found that boiling had less effect on heme and nonheme in meat than did drying. This suggested that other factors such as temperature and the length of heat exposure time may be concerned.

Table 3 Heme Iron Contents of Fresh, Frozen and Dried Meat

	Heme Iron Content[1] (μg/g dry weight)				
	Fresh	**Frozen[2] 1 week**	**Frozen 2 week**	**Frozen 4 weeks**	**Dried[3]**
Beef					
round	107.48 ± 1.95 (100.00)	106.28 ± 1.05 (98.89)	106.46 ± 1.61 (99.05)	105.96 ± 0.59 (98.59)	66.18 ± 21.67 (61.58)
tenderloin	99.59 ± 21.17 (100.00)	99.16 ± 21.90 (99.56)	98.50 ± 22.25 (98.90)	97.69 ± 23.14 (98.09)	49.42 ± 11.89 (49.62)
liver	222.58 ± 19.32 (100.00)	216.62 ± 23.99 (97.32)	215.16 ± 25.04 (96.67)	215.84 ± 24.44 (96.97)	161.56 ± 2.80 (72.59)
heart	121.86 ± 4.33 (100.00)	122.31 ± 3.19 (100.37)	122.47 ± 2.13 (100.51)	121.18 ± 2.09 (99.45)	73.30 ± 23.90 (60.15)
Pork					
round	23.58 ± 1.79 (100.00)	22.97 ± 0.99 (97.43)	23.41 ± 2.36 (99.27)	22.64 ± 0.89 (96.03)	12.01 ± 4.61 (50.49)
tenderloin	12.45 ± 1.11 (100.00)	12.26 ± 0.95 (97.66)	11.47 ± 1.76 (91.30)	11.49 ± 1.87 (91.51)	4.80 ± 1.46 (38.23)
liver	128.07 ± 15.84 (100.00)	125.40 ± 15.26 (97.91)	126.02 ± 17.32 (98.40)	126.07 ± 15.91 (98.44)	79.11 ± 21.81 (61.77)
heart	95.29 ± 21.77 (100.00)	94.58 ± 22.61 (99.26)	93.69 ± 23.03 (98.33)	93.65 ± 21.47 (98.28)	80.71 ± 14.76 (84.70)
Chicken					
leg	13.35 ± 0.97 (100.00)	13.44 ± 0.81 (100.64)	12.99 ± 0.24 (97.27)	12.47 ± 0.45 (93.38)	5.74 ± 2.70 (43.01)
breast	4.43 ± 0.23 (100.00)	4.37 ± 0.51 (98.67)	4.16 ± 0.34 (93.89)	4.06 ± 0.38 (91.67)	2.10 ± 1.23 (47.36)
liver	78.32 ± 5.02 (100.00)	76.38 ± 4.31 (97.52)	74.88 ± 4.95 (95.61)	78.58 ± 4.31 (100.33)	61.08 ± 1.81 (77.99)
heart	74.90 ± 2.61 (100.00)	75.45 ± 2.74 (100.73)	75.28 ± 3.43 (100.50)	74.82 ± 3.56 (99.89)	64.46 ± 2.49 (86.06)

[1] values is parentheses represent heme iron content of frozen or dried sample expressed as percent of that of fresh sample.
[2] frozen at -20° C and thawed at 20° C prior to analysis.
[3] baked at 50-60° C for 8 hours.

Table 4 Nonheme Iron Contents of Fresh, Frozen and Dried Meat

	Nonheme Iron Content[1] ($\mu g/g$ dry weight)				
	Fresh	Frozen[2] 1 week	Frozen 2 weeks	Frozen 4 weeks	Dried[3]
Beef					
round	63.10 ± 9.07 (100.00)	65.14 ± 9.25 (103.23)	64.32 ± 9.60 (101.92)	65.66 ± 9.91 (104.06)	106.29 ± 34.05 (168.44)
tenderloin	63.84 ± 4.93 (100.00)	63.47 ± 4.52 (99.42)	64.03 ± 5.69 (100.29)	65.86 ± 9.37 (103.16)	114.90 ± 8.54 (179.97)
liver	225.17 ± 16.34 (100.00)	229.61 ± 14.17 (101.97)	230.90 ± 11.18 (102.55)	230.61 ± 13.85 (102.24)	284.67 ± 47.49 (126.43)
heart	113.08 ± 7.24 (100.00)	112.82 ± 10.94 (99.76)	110.94 ± 10.73 (98.10)	113.18 ± 10.36 (100.08)	162.80 ± 43.44 (143.96)
Pork					
round	25.77 ± 0.46 (100.00)	24.97 ± 1.64 (96.90)	26.21 ± 0.36 (101.74)	25.61 ± 2.68 (99.39)	35.74 ± 4.73 (138.70)
tenderloin	20.47 ± 4.73 (100.00)	21.91 ± 3.51 (107.02)	23.07 ± 4.36 (112.67)	20.96 ± 5.85 (102.37)	29.19 ± 1.33 (142.60)
liver	733.81 ± 2.23 (100.00)	734.88 ± 1.54 (100.15)	740.17 ± 6.24 (100.87)	737.84 ± 2.57 (100.55)	783.92 ± 6.21 (106.83)
heart	146.05 ± 47.05 (100.00)	149.59 ± 52.29 (102.43)	148.84 ± 49.41 (101.91)	149.65 ± 48.33 (102.47)	166.23 ± 65.10 (113.82)
Chicken					
leg	23.87 ± 0.77 (100.00)	22.79 ± 1.96 (95.90)	22.47 ± 1.76 (94.16)	23.91 ± 1.84 (100.17)	29.28 ± 13.58 (122.67)
breast	41.11 ± 8.88 (100.00)	41.30 ± 8.21 (100.47)	41.93 ± 7.37 (102.00)	41.14 ± 6.93 (100.06)	44.70 ± 7.35 (108.74)
liver	323.10 ± 1.05 (100.00)	324.08 ± 6.03 (100.30)	326.59 ± 3.48 (101.08)	319.54 ± 8.13 (98.90)	339.35 ± 75.41 (105.03)
heart	83.78 ± 3.24 (100.00)	81.36 ± 3.82 (97.11)	83.04 ± 1.02 (99.11)	81.65 ± 0.59 (97.45)	88.13 ± 46.45 (105.19)

[1] values in parentheses represent nonheme iron content of frozen or dried sample expressed as percent of that of fresh sample.
[2] frozen at -20° C and thawed at 20° C prior to analysis
[3] baked at 50-60° C for 8 hours.

Schricker and Miller (5) stated that a 10% reduction of heme iron had only a minimal effect on total absorbable iron in a meal. The severe heat treatments used in this study such as boiling more than 30 minutes and drying at 50-60°C for 8 hours resulted in an approximately 20-30% reduction of heme iron content. Change in heme iron of this magnitude may cause a significant decrease in absorbable iron. Furthermore, decrease in total absorbable iron resulting from heme destruction is greater than that from the increase of nonheme iron (5) because heme iron is highly available and its absorption is unaffected by the composition of diet. The availability of nonheme iron is greatly influenced by a variety of enhancing and inhibiting substances present in foodstuffs. Therefore, heat treatment not only decreased heme iron content of meat but also reduced the bioavailability of meat iron.

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ผลของการใช้ความร้อนและการแช่แข็งต่อระดับเหล็กในรูปของฮีมในอาหาร

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*นัยนา วัฒนเพ็ญไพบูลย์ ภ.ม.***

บทคัดย่อ

เหล็กในอาหารมี 2 รูปแบบคือ เหล็กในรูปของฮีม และเหล็กที่ไม่ได้อยู่ในรูปของฮีม การดูดซึมของเหล็กในรูปของฮีมจะดีกว่าเหล็กที่ไม่ได้อยู่ในรูปของฮีม ดังนั้นถ้ารูปแบบของเหล็กเปลี่ยนไปก็จะมีผลต่อปริมาณเหล็กที่ร่างกายจะดูดซึมและนำไปใช้ได้ การศึกษานี้ได้นำเนื้อ ตับ และหัวใจ ของโค หมู และไก่มาต้ม, ทำให้แห้ง และเก็บในตู้เย็น เพื่อดูผลต่อปริมาณเหล็กที่อยู่ในรูปของฮีมในตัวอย่างอาหาร การต้ม 15, 30 และ 60 นาที จะทำให้เหล็กในรูปของฮีมในเนื้อลดลง 12%, 19% และ 25% ในตับลดลง 14%, 21% และ 28% และในหัวใจลดลง 10%, 14% และ 20% ตามลำดับ การแช่แข็งนาน 1, 2 และ 4 สัปดาห์ไม่มีผลเปลี่ยนแปลงปริมาณเหล็กในรูปของฮีม ส่วนการทำให้แห้งทำให้ปริมาณเหล็กในรูปของฮีมในเนื้อ ตับ และหัวใจลดลง 51%, 30% และ 23% ตามลำดับ การเปลี่ยนแปลงรูปแบบของเหล็กเนื่องจากความร้อนนี้อาจมีผลต่อการดูดซึมและการที่ร่างกายจะนำเหล็กไปใช้ได้ ดังนั้นเพื่อถนอมคุณค่าอาหารจึงไม่ควรให้ความร้อนแก่อาหารนานเกินไป (ไทยเภสัชสาร ปีที่ 12(3) : หน้า 237-246 (2530)).

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