Original Article

Amino acid profile of rabbit meat: dietary intake and the effect of freezing on the amino acid composition

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Abstract

Rabbit meat is considered as a healthy meat due to its high protein and low fat content. This study aimed at demonstrating the amino acids profile in three rabbit breeds, namely New Zealand White, California and Balady rabbits marketed in Egypt. In addition, the estimated daily intake (EDI) of amino acids due to the consumption of the rabbit meat were calculated. Furthermore, the effect of long frozen storage for different periods on the amino acid content of the New Zealand White rabbit meat was investigated as an experimental trial. The obtained results indicate a slight variation in the amino acid profiles among the examined rabbit meat samples. Lysine was the dominant essential amino acid in all the tested rabbit breeds. Generally, California breed had the highest total amino acid content at 27.46 ± 0.30 mg/g, whereas the New Zealand White breed at 25.79 ± 0.68 mg/g, and finally the Balady breed at 25.53 ± 0.77 mg/g. The EDI values (mg/day) for the total amino acids via consumption of the rabbit meat were 52.73, 49.54 and 49.02 from California, New Zealand White and Balady rabbit meat, respectively. Freezing caused a significant reduction in the amino acid content of the rabbit meat. For instance, total amino acid content showed significant reduction trends on freezing-time-dependent manner as it was reduced to 89.01% after one month, 78.54% after two months, 57.69% after four months, and 32.12% after six months of long frozen storage. Therefore, it is not recommended to keep rabbit meat frozen at -20 °C for more than one month.

Keywords: Rabbit meat, amino acids, freezing, dietary intake

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Introduction

Rabbits are considered as a promising source of animal-derived protein. The New Zealand White and California rabbits are universal breeds that are reared for their meat and fur. These breeds are characterized by the high palatability of their meat and the high feed efficiency rates. Rabbit meat is characterized by its high protein content (about 20%) and low-fat content (about 8%) (Dalle Zotte, 2004; Hernández and Gondret, 2006).

Amino acids are small organic molecules that play essential roles in living organisms. For instance, amino acids are the building blocks of the protein, act as an energy source and serve as precursors for the biologically active molecules such as the neurotransmitters, local mediators and heme. Some amino acids act as regulators for gene expression and cellular signaling (Nelson and Cox, 2008). Amino acids are typically classified into the essential amino acids that cannot be synthesized in our body and include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Another group of amino acids is referred to as nonessential amino acids that include alanine, asparagine, aspartate, glutamate and serine. The third group of amino acids is classified as conditionally-essential, and is required to some degree during illness and at a young age. This group includes arginine, cysteine, glutamine, glycine, proline and tyrosine (Nelson and Cox, 2008). However, scarce information is available about the amino acid profiles of the different rabbit breeds or the estimated daily amino acid intake via consumption of rabbit meat in Egypt.

Freezing is a common method used for the preservation of meat. Freezing is used in the meat industry as a method for long-term preservation and improves food security, as it can significantly reduce bacterial spoilage. However, at the same time, freezing has a significant effect on the meat quality, such as loss of moisture and denaturation of protein (Muela *et al*., 2010; Leygonie *et al*., 2012). As indicated by the literature, few studies have investigated the effect of freezing for different time periods on the amino acid profile in rabbit meat.

Insight from previous facts, the first objective of the present study, was therefore to compare amino acid profiles in three rabbit breeds, namely New Zealand White, California, and Balady. Secondly, the estimated human dietary intake of amino acids via the ingestion of rabbit meat was calculated. Finally, the effect of freezing for different periods on the amino acid profile of rabbit meat was investigated.

Materials and Methods

Collection of samples for amino acid analysis: A total of 30 randomly selected male rabbit carcasses were collected from rabbit butchery shops in Zagazig city, Sharkia Governorate, Egypt. The collected rabbit carcasses weighed on average 1250 ± 30 g and were from three breeds, i.e. New Zealand White, California and Balady (n = 10/ each breed). From each rabbit carcass, the loin part (about 250 g) was separated for amino acid analysis. Loin parts from the New Zealand White rabbits were subdivided into five groups (each 50 g) and wrapped separately and each was analyzed fresh or kept frozen at 20 °C for 1, 2, 4, and 6 months. Amino acid analysis for all fresh and frozen samples was conducted in the Central Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Amino acid analysis: Amino acid analysis of meat samples was conducted according to the method of Ashworth (1987). In brief, one gram from each sample was weighed and dipped into diethyl ether for 24 h, at room temperature. Then, 200 µg from each sample was exposed to acid hydrolysis under vacuum in 6 M HCl at 110 °C for 24 h. The hydrolysates were concentrated to dryness under vacuum. The concentrates were dissolved in 100 µl of 0.2 M citrate buffer (pH 2.2). The amino acids were analyzed using a SYKAM Co, S433 (Germany) amino acid analyzer equipped with a polyvinyl sulfonate cationic-exchange column for physiological fluids, a post-column ninhydrin derivatization system and a two-channel detection system set at 570 and 440 nm. Analyses were repeated three times for each sample. Quantification of amino acids in samples was obtained and calculated from the area under the curves extrapolated using automatic clarity software.

Estimated daily intake of amino acid: Estimated daily intake (EDI) values of each amino acid was calculated based on the following equation:

$$EDI = C^{*} IR$$

Where EDI is the estimated daily intake for each amino acid, C is the concentration of each amino acid (mg/g) and IR is the ingestion rate which was considered as 2 g/day, based on a consumption rate of 700 g/capita/year in Egypt (Galal and Khalil, 1994).

Statistical analysis: Data is presented as means ± standard deviation (SD) (n = 10/each), statistical significance was evaluated using Tukey's Kramer HSD test (JMP program, SAS Institute, Cary, NC, USA). A $P < 0.05$ value was considered to be significant.

Results

Table 1 shows the essential amino acid profile in the loin part of New Zealand White, California and Balady rabbit breeds. Lysine was the dominant essential amino acid in all tested rabbit breeds. Balady breed had ($P < 0.05$) the highest lysine content (g/100 g protein) at 13.57 ± 1.07 g. Isoleucine was the essential amino acid with the second highest concentration in the tested samples. Similar to lysine, Balady breed had also ($P < 0.05$) the highest content of isoleucine at 8.86 ± 0.90 g, followed by New Zealand White at 8.06 ± 0.28 g, and California rabbits at 4.77 ± 0.11 g, respectively. On the other hand, California rabbits had the highest content of leucine 8.19 ± 0.27 g, followed by Balady and New Zealand White breeds at 1.78 ± 0.12 g and 1.62 ± 0.06 g, respectively. The profile of the non-essential and conditionally-essential amino acids in the examined rabbit breeds is shown in Table 1. Aspartic acid contents in the New Zealand, California and Balady breeds were 9.19 ± 0.69, 9.06 ± 0.46, and 8.35 ± 1.02 g/ 100 g protein, respectively. Alanine contents (g/ 100 g protein) were 5.66 ± 0.53 in the New Zealand White breed, 5.91± 0.17 in California breed and 6.82 ± 0.24 in the Balady breed. Serine contents (g/ 100 g protein) were 4.43 ± 0.35 g (New Zealand White), 4.19 ± 0.09 (California), 3.38 ± 0.14 (Balady). Glutamine contents were the higher conditionally-essential amino acids with mean values of 17.33 ± 0.69, 16.69 ± 0.47, and 13.53 ± 1.12 g/100 g protein in New Zealand White, California and Balady breeds, respectively.

Table 1 Amino acid content in the rabbit meat

	New Zealand	California	Balady
	Essential amino acids		
Histidine	3.64 ± 0.12[a]	3.89 ± 0.26[b]	3.72 ± 0.18[ab]
Isoleucine	8.06 ± 0.28[a]	4.77 ± 0.11[b]	8.86 ± 0.90[c]
Leucine	1.62 ± 0.06[a]	8.19 ± 0.27[b]	1.78 ± 0.12[a]
Lysine	12.24 ± 0.56[a]	9.84 ± 0.18[b]	13.57 ± 1.07[c]
Methionine	4.50 ± 0.19[a]	2.57 ± 0.12[b]	5.09 ± 0.29[c]
Phenylalanine	3.08 ± 0.11[a]	4.53 ± 0.14[b]	0.69 ± 0.06[c]
Threonine	4.08 ± 0.33[a]	4.91 ± 0.22[b]	4.33 ± 0.38[a]
Valine	5.54 ± 0.34[a]	6.03 ± 0.16[b]	5.59 ± 0.29[a]
	Nonessential amino acids		
Alanine	5.66 ± 0.53[a]	5.91± 0.17[a]	6.82 ± 0.24[b]
Aspartic acid	9.19 ± 0.69[a]	9.06 ± 0.46[a]	8.35 ± 1.02[a]
Serine	4.43 ± 0.35[a]	4.19 ± 0.09[a]	3.38 ± 0.14[b]
	Conditionally essential amino acids		
Arginine	6.43 ± 0.24[a]	5.57 ± 0.14[b]	6.75 ± 0.22[c]
Cysteine	2.08 ± 0.08[a]	2.38 ± 0.08[b]	2.28 ± 0.11[b]
Glutamine	17.33 ± 0.69[a]	16.69 ± 0.47[a]	13.53 ± 1.12[b]
Glycine	5.54 ± 0.35[a]	5.65 ± 0.18[a]	4.89 ± 0.31[b]
Proline	2.07 ± 0.06[a]	2.23 ± 0.14[a]	5.52 ± 0.24[b]
Tyrosine	4.49 ± 0.36[a]	3.58 ± 0.20[b]	4.83 ± 0.80[a]

Amino acid content (g/100 g protein) in the muscle *Longissimus dorsi* of the rabbit. Values represent mean ± SD (n = 10/each rabbit breed). Values within the same raw carrying different superscript letters (a, b, c) are statistically significant at $P < 0.05$.

The estimated daily intakes (EDI) for each amino acid via consumption of rabbit meat, particularly the loin part, from each breed were calculated. The obtained results in Table 2 showed that EDI values for the essential amino acids including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine were 1.80, 3.99, 0.80, 6.06, 2.23, 1.53, 2.02, and 2.74 mg/day respectively, in New Zealand White rabbits. These values were 2.06, 2.52, 4.32, 5.19, 1.35, 2.39, 2.59, and 3.18 mg/day, respectively, in the case of California rabbits. While in the case of Balady breeds, such values were 1.82, 4.34, 0.87, 6.66, 2.49, 0.34, 2.13, and 2.74 mg/day, respectively. EDI values for the nonessential amino acids including alanine, aspartic acid and serine via consumption of the loin part were 2.81, 4.56, and 2.19 mg/day, respectively for New Zealand rabbits; 3.12, 4.78 and 2.21 mg/day, respectively for California rabbits; 3.34, 4.10 and 1.65 mg/day for Balady rabbits (Table 2). Finally, EDI values for the conditionally-essential amino acids, including arginine, cysteine, glutamine, glycine, proline, and tyrosine, were 3.19, 1.03, 8.58, 2.75, 1.02 and 2.23 mg/day respectively, in New Zealand White rabbits. In the case of California rabbits, these values were 2.94, 1.26, 8.80, 2.98, 1.18 and 1.89 mg/day, respectively and in Balady breeds, such values were 3.31, 1.12, 6.63, 2.39, 2.70,and 2.38 mg/day, respectively.

Table 2 Estimated daily intake (EDI) values of the amino acids via ingestion of rabbit meat of different breeds

	New Zealand	California	Balady
	Essential amino acids		
Histidine	1.80	2.06	1.82
Isoleucine	3.99	2.52	4.34
Leucine	0.80	4.32	0.87
Lysine	6.06	5.19	6.66
Methionine	2.23	1.35	2.49
Phenylalanine	1.53	2.39	0.34
Threonine	2.02	2.59	2.13
Valine	2.74	3.18	2.74
	Nonessential amino acids		
Alanine	2.81	3.12	3.34
Aspartic acid	4.56	4.78	4.10
Serine	2.19	2.21	1.65
	Conditionally-essential amino acids		
Arginine	3.19	2.94	3.31
Cysteine	1.03	1.26	1.12
Glutamine	8.58	8.80	6.63
Glycine	2.75	2.98	2.39
Proline	1.02	1.18	2.70
Tyrosine	2.23	1.89	2.38
Total amino acids	49.54	52.73	49.02

Estimated daily intake (EDI) of different amino acids (mg/day) via the consumption of rabbit meat of different breeds. The values of EDI are based on the average concentration of amino acids in the rabbit meat.

In the third part of this study, we investigated the effect of freezing on the content of amino acids in the rabbit meat. Freezing for one month slightly increased the content of amino acids, particularly histidine (105.51%), leucine (103.48%), phenylalanine (114.15%), threonine (126.58%), valine (103.38%), alanine (108.49%), aspartic acid (101.89%) and glycine (108.95%) (Fig. 2, 3, and 4). Freezing for 6 months showed a significant reduction in the content of the total amino acids (32.12%) (Fig. 5).

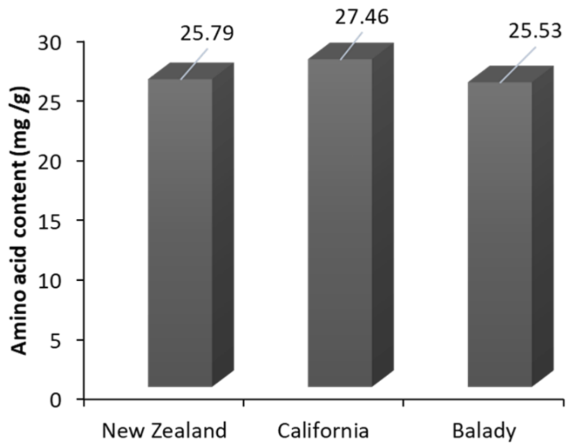

Figure 1 Total amino acids (mg/g) in the loin part of different rabbit breeds. The values are based on the average concentration of amino acids in the rabbit meat.

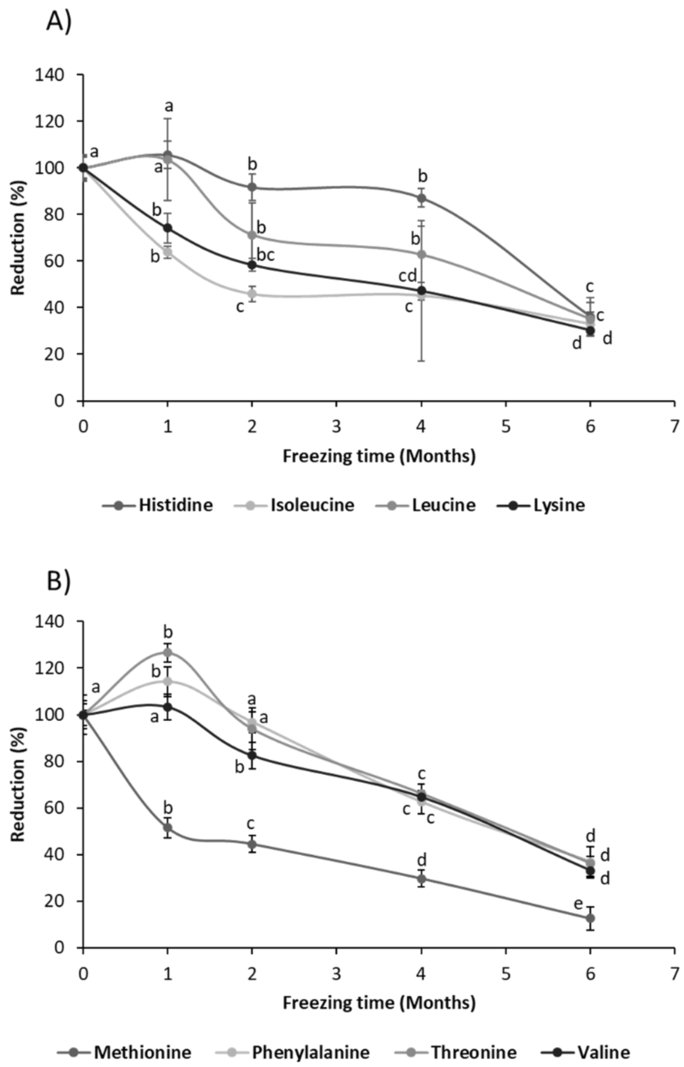

Figure 2 Effect of freezing on the essential amino acid content in the loin part of the New Zealand white rabbit. A) Histidine, isoleucine, leucine and lysine, B) Methionine, phenylalanine, threonine and valine. Values represent a reduction % (mean ± SD). Data points for each amino acid carrying different letters are significantly different at $P < 0.05$ (n = 10).

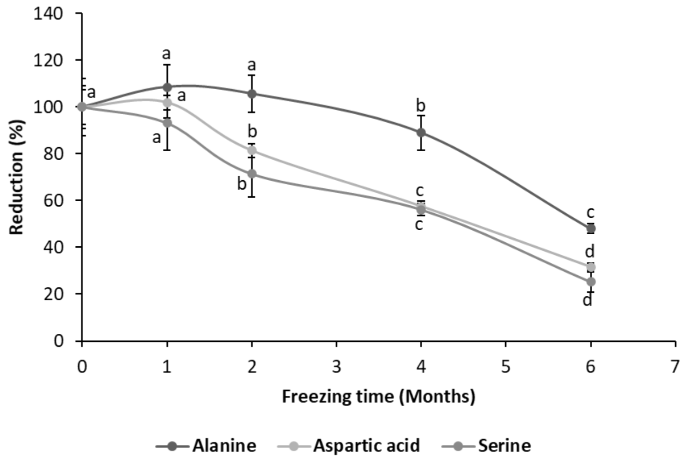

Figure 3 Effect of freezing on the non-essential amino acid content in the loin part of the New Zealand white rabbit. Values represent a reduction % (mean ± SD). Data points for each amino acid carrying different letters are significantly different at $P < 0.05$ (n = 10).

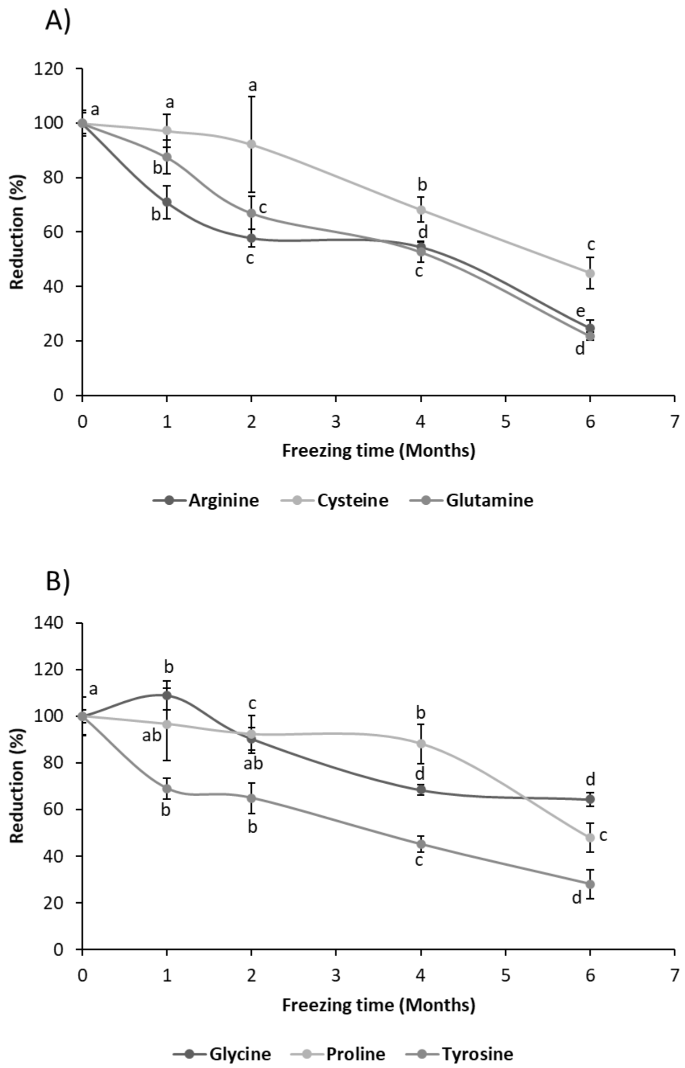

Figure 4 Effect of freezing on the conditionally-essential amino acid content in the loin part of the New Zealand white rabbit. A) Arginine, cysteine and glutamine, B) Glycine, proline and tyrosine. Values represent a reduction % (mean ± SD). Data points for each amino acid carrying different letters are significantly different at $P < 0.05$ (n = 10).

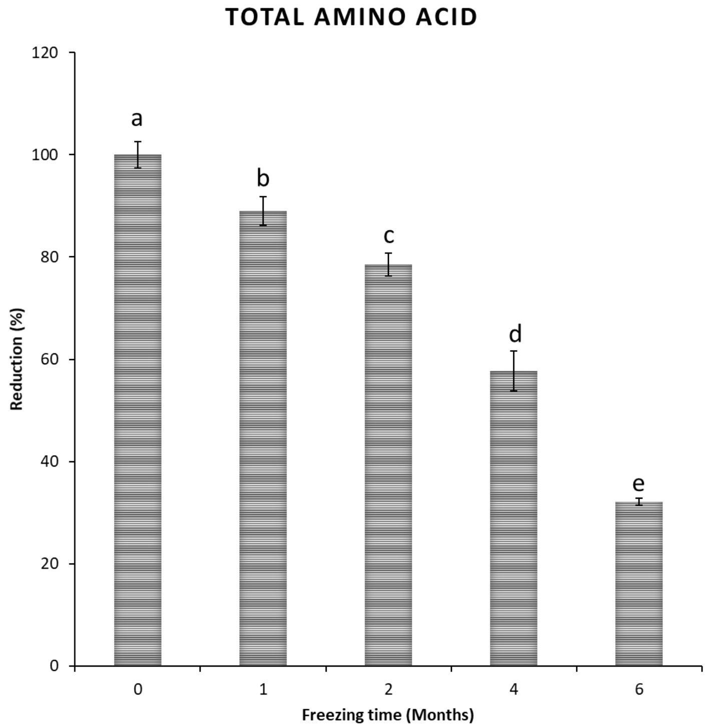

Figure 5 Effect of freezing on the total amino acid content in the loin part of the New Zealand white rabbit. Values represent a reduction % (mean ± SD). Columns carrying different letters are significantly different at $P < 0.05$ (n = 10).

Discussion

Profile of amino acids in New Zealand White, California and Balady rabbit breeds: There was a clear difference in meat amino acid profile among the examined breeds. Essential amino acid content in the meat samples from New Zealand White breed had the following order: lysine > isoleucine > valine > methionine > threonine > histidine > phenylalanine > leucine. Meat samples from Balady breed had almost the same essential amino acid profile to that of New Zealand White breed with the only difference being that phenylalanine levels were higher than that of leucine, which is possibly attributed to their genetic buildup. However, the amino acid profile in meat samples from the California breed was slightly different and it was as follows: lysine > leucine > valine > threonine > isoleucine > phenylalanine > histidine > methionine. Meat samples from all breeds had the same nonessential amino acid profile as follows: aspartic acid > alanine > serine. The third group of amino acids is classified as conditionally-essential, which is required at a young age and during illness (Nelson and Cox, 2008). Glutamine was the dominant conditionally-essential amino acid in all breeds. The profile of the conditionally-essential amino acid in meat samples from the New Zealand White had the following order: glutamine > arginine > glycine > tyrosine > cysteine > proline. This profile in the case of the California breed was as follows: glutamine > glycine > arginine > tyrosine > cysteine > proline; while in case of Balady breed, it was as follows: glutamine arginine > proline > glycine > tyrosine > cysteine. The recorded concentrations of the amino acids in the present study were in accordance with previously published reports. For instance, Bivolarski *et al*. (2011), estimated the amino acid content in the muscle *Longissimus Lumborum* of New Zealand White rabbits as arginine (6.23 ± 0.16), histidine (4.37 ± 0.42), isoleucine (5.30 ± 0.15), leucine (9.15 ± 0.22), lysine (9.97 ± 0.18), methionine (2.02 ± 0.16), tyrosine (2.75 ± 0.21), phenylalanine (4.35 ± 0.19), threonine (3.33 ± 0.15), tryptophan (6.23 ± 0.16), valine (6.26 ± 0.31), Alanine (6.05 ± 0.26), aspartate (9.68 ± 0.28), glutamate (17.98 ± 1.18), serine (1.75 ± 0.18), cysteine (1.13 ± 0.14), glycine (4.96 ± 0.23) and proline (4.73 ± 0.45). In addition, Nasr *et al*. (2017), measured the amino acid content in the muscle *Longissimus Lumborum* of the pure breed of New Zealand White rabbits in Egypt. Essential amino acid contents (%) were as follows: arginine (3.17%), histidine (4.52%), isoleucine (5.97%), leucine (9.9%), lysine (9%), methionine (2.57%), tyrosine (3.22%), phenylalanine (4.37%), threonine (4.65%) and valine (6.77%). The concentration of nonessential amino acids was: Alanine (6.35%), aspartic acid (9.2%), glutamine (17.6%), alanine (6.35%), serine (4.06%), glycine (5.97%), cysteine (1.29%), and proline (1%). It is noteworthy to mention that the recorded concentration of phenylalanine in the current study is much lower than that reported in other studies, which could be attributed to differences in the genetic buildup of the individual rabbits or differences in the chemical composition of the used animal feed. In general, the California breed had the highest total meat amino acid content at 27.46 ± 0.30 mg/g, followed by the New Zealand White breed at 25.79 ± 0.68 mg/g and finally the Balady breed at 25.53 ± 0.77 mg/g (Fig. 1). The recorded concentrations are comparable to that reported by Pla *et al*. (2004), who recorded that amino acid content as 20.15 ± 0.72 in the forelegs, 18.69 ± 1.19 in the thoracic cage, 22.10 ± 0.59 in the *Longissimus dorsi* muscle, 20.91 ± 0.75 in the abdominal walls, 20.68 ± 0.69 in the spine portion and 21.24 ± 0.29 in the hind legs, respectively. The differences in the amino acid profile among the different breeds can be attributed to genotype, weaning time, age and the effect of feed and nutritional supplements during the growth cycle of the rabbits. In agreement with this assumption, Bianospino *et al*. (2006), recorded clear effects for the breed on rabbit growth, weight and carcass quality. Besides, Nasr *et al*. (2017), stated that breed has significant effects on the amino acid profile of rabbit meat. As the current investigation was limited to samples collected from butchery shops, a future study is still needed to precisely investigate the effect of the ration, sex and age on the amino acid profile in rabbit meat in an experimental approach.

Human estimated daily intake of different amino acids via consumption of rabbit meat in Egypt: Rabbit meat is popular in many European countries such as Spain (EscribaPérez *et al*., 2017) and Bulgaria (Bivolarski *et al*., 2011). For instance, EscribaPérez *et al*. (2017), found that around 20% of the Spanish population consumes rabbit meat at least once a week. The consumers' age was mainly over 55 years old, probably due to the meat's high nutritive value, palatability and digestibility. However, the rabbit industry in Egypt has not reached a high level of organization. In addition, rabbit meat consumption in Egypt is still quite low, estimated to be around 0.70 kg per capita/ year (Galal et al., 1994). This low consumption rate is probably due to both cultural and market reasons. However, rabbit meat is considered to be healthier than other meat types due to its high protein and low-fat content (Nasr *et al*., 2017). The EDI values for the total amino acids via consumption of the rabbit meat indicate that California rabbit meat can provide 52.73 mg/day, whereas New Zealand White and Balady rabbit meat can provide 49.54 and 49.02 mg/day, respectively. Generally, there is a clear lack of information about the calculation of EDI values for different amino acids via the consumption of meat. The National Research Council in the United States (NRC, 1989) has estimated the required daily intake for amino acids in the adult to be 8-12, 10, 14, 12, 13, 14, 7, 3.5, and 10 mg/Kg body weight for histidine, isoleucine, leucine, lysine, methionine plus cysteine, phenylalanine plus tyrosine, threonine, tryptophan and valine, respectively. Symptoms of protein deficiency are most commonly observed in deprived children in poor countries. Where protein intake is exceptionally low, there are physical signs such as stunted growth, poor body mass, edema, fragile hair and skin lesions. Other biochemical changes include low serum albumin and hormonal imbalances while the major signs in adults are edema and loss of muscle mass and hair (NRC, 1989). Therefore, it is highly recommended to increase the consumption of rabbit meat as a good source for amino acids required for proper growth and for the maintenance of high health standards. Similarly, Petracci *et al*. (2018), recommends the development of new strategies to encourage consumers to rediscover rabbit meat as an attractive alternative to the commonly consumed pork, beef and poultry meat.

Effect of freezing on the amino acid content of rabbit meat: Freezing is one of the most common preservation methods used to increase the shelf life of the meat and to improve food security for both retailers and consumers (Pietrasik and Janz, 2009; Muela *et al*., 2010). In the present study, we further investigated the effect of freezing after different storage periods on the amino acid content in rabbit meat. The obtained results (Fig. 2-5) indicate that freezing had a biphasic impact on the content of the amino acids. For instance, freezing for one month slightly increased the content of amino acids as in histidine, leucine, phenylalanine, threonine, valine, alanine, aspartic acid and glycine. However, freezing for two months or more caused a significant reduction in the content of the amino acids, in a time-dependent manner. Freezing for six months reduced the content of the histidine to 36.06%, isoleucine to 33.07%, leucine to 35.03%, lysine to 30.31%, methionine to 12.51%, phenylalanine to 36.94%, threonine to 36.16%, valine to 33.11%, alanine to 47.99%, aspartic acid to 31.62%, serine to 25.25%, arginine to 24.68%, cysteine to 44.92%, glutamine to 21.72%, glycine to 64.41%, proline to 47.93% and tyrosine to 28.02%. In general, total amino acid content showed a significant reduction trend in a freezing-time-dependent manner as it was reduced to 89.01% after one month, 78.54% after two months, 57.69% after four months and 32.12% after six months freezing time. Reduction of the amino acid content after a long time of freezing can be attributed to protein denaturation (Leygonie *et al*., 2012) and the formation of the large ice crystals, particularly at the slow freezing (-20 °C), which can destroy the myofibrillar protein (Sun *et al*., 2016). In agreement with the results of the present study, Seong *et al*. (2017), reported that the freezing of fresh meat entails a significant variation in its quality traits. Indeed, depending on their size and location, the formation of ice crystals profoundly alters the ultrastructure of the meat. Furthermore, Soglia *et al*. (2019), observed a significant reduction in the histidine-containing dipeptides in the frozen broiler breast compared to the fresh.

In conclusion, the assessment of 17 amino acids in three breeds of rabbit, including New Zealand White, California and Balady, revealed slight inter-breed variation in their amino acid profile. Rabbits are considered as an outstanding source of animal-derived protein that can partially cover human needs for amino acids. Freezing had significant reducing effects on the amino acid content in the rabbit meat. Therefore, it is not recommended to keep rabbit meat frozen at -20 °C for more than one month.

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