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Protein and amino acid profiles of frozen and fresh broiler meat*

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It is commonly believed that the quality and nutritional value of different brands of both frozen and fresh broiler meat in the retail markets is similar. Hence, broiler meat from two types of carcasses (three per type; frozen: A, B and C, and fresh: D, E and F) was evaluated in terms of the crude protein, amino acids (AAs), protein quality, flavours and antioxidant AAs and compared according to the recommended daily allowance (RDA) of protein/AAs for adults. The total essential amino acids (TEAAs) were significantly greater in sources A and C of frozen meat and source F of fresh meat than in sources B and E of frozen and fresh meat, respectively. Levels of glycine, as a nonessential amino acid (NEAA), and flavour-related amino acids (FRAAs) were higher in frozen meat compared to fresh meat. The values recorded for the total amino acids (TAAs), predicted-protein efficiency ratio (P-PER) and total flavour amino acids (TFAAs) of meat were significantly greater in source A of frozen meat and source F of fresh meat compared to sources B and E of frozen and fresh meat, respectively. Levels of total aromatic amino acids (TAAAs), as the antioxidant index, were significantly higher in source F of fresh meat than sources B and E of frozen and fresh meat, respectively. In conclusion, broiler meat can meet 71.4-98.9% of the RDA for essential amino acids (EAAs) for adults, depending on the type of meat source, suggesting an opportunity for enhancing the nutritional and qualitative value of broiler meat through husbandry and dietary manipulation.

KEYWORDS: amino acids / broiler meat / daily allowance / protein / retail market

Poultry is one of the most popular and affordable meats consumed globally, and its nutritional composition is generally affected by different factors, such as pre- and

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post-harvest conditions [Winiarska-Mieczan *et al*. 2016]. Poultry meat is considerably leaner and has more protein than lamb and mutton, goat meat, pork, veal or beef [Apata *et al*. 2013, Attia *et al*. 2016]. Meat with a greater nutritional value is associated with greater consumption levels [Marangoni *et al*. 2015, Nasr *et al*. 2017]; moreover, broiler meat is nutritious, delicious and easy to digest, thanks to many nutrients that meet the RDA [WHO, 2007, Silva *et al*. 2016, Kim *et al.* 2017].

Meat is a perfect protein/AAs source, its different types contain the same basic nutrients, but the nutritional values vary depending on the type of meat [Zhao *et al.* 2011, Attia *et al.* 2016, 2017]. Meat proteins contain all AAs, which are readily digested, absorbed and effectively utilised [Kim *et al*. 2017, Attia *et al*. 2016]. Protein and/or AAs have a great impact on meat quality [Wang *et al.* 2017, Sohaiba *et al.* 2017].

From a nutritional point of view, proteins/AAs, minerals, vitamins and unsaturated fatty acids of poultry meat are its main benefits [Grashorn 2007, Bogosavljevi-Boškovic *et al*. 2011]. The quality of poultry meat is dependent on pre- and post-harvest factors [Attia *et al*. 2016]. Moreover, gender, age and genotype are genetic aspects that influence meat quality [Bogosavljevi-Boškovic *et al*. 2010; Zhao *et al*. 2011, Nasr *et al*. 2017]. Nutrition as well as the macro- and microenvironments also significantly influence meat quality [Andersen *et al*. 2005, Grashorn 2007, Kim *et al.* 2017]. Meat quality may also be affected by slaughter practices, storage and handling [Fanatico *et al*. 2007], whether it is fresh or frozen [Bianchi *et al*. 2007, Attia *et al.* 2016], the applied cooking method [Sliva *et al*. 2016] and the type of cut [Kim *et al.* 2017]. The dietary composition may influence the nutritional profile of meat [Haščík *et al.* 2016, Wang *et al.* 2017]. In addition, the rearing system of broiler meats is an important consideration [Andersen *et al*. 2005, Bogosavljevi-Boškovic *et al*. 2011] as the market is dramatically affected by competition both in terms of price and quality [Bogosavljevi-Boškovic *et al*. 2010].

Proteins/AAs of poultry products are essential nutrients for maintaining the health and welfare of humans [Marangoniet *et al*. 2015, Kim *et al*. 2017]. Proteins are structural and principal components of muscle, while also affecting body functions, as well as meat quality, flavour and antioxidants [Damgaard *et al*. 2015, Wang *et al.* 2017]. However, the increasing selection pressure for greater body weight and more advantageous carcass composition may lead to a deterioration in the quality of poultry meat [Zerehdaran *et al.* 2012; Nasr *et al*. 2017]. Therefore, studying the variability of proteins/AA profiles, quality, non-essential amino acids [NEAAs], flavour-related amino acids [FRAAs] and antioxidant AAs is essential to develop quality control aspects and to mentor variation in meat quality of different brands of frozen and fresh broiler meat in the retail market in relation to the RDA for adults.

Material and methods

Whole broiler carcasses (n=126) from commercial broiler brands were randomly selected from six sources in the Hyper Panda retail market in Jeddah, Saudi Arabia.

The samples were selected on a monthly basis during June- August, 2017. Two types of carcasses were selected: fresh and frozen. The frozen carcasses (A, B and C) were imported brands available in the retail market. The fresh carcasses (D, E and F) were locally produced. Seven carcasses were selected from each meat source (commercial brand) per meat type each month, therefore, 42 carcasses were selected each month. The carcasses were chosen from A grade weight (approximately 1 kg) and each had a similar production and expiration date. Carcasses were divided into two halves; the right side was skinned and deboned, and the meat was minced using a meat mincer (Moulinex-HV8, France). The samples of each source were pooled over time, mixed together and homogenised to represent seven samples per source, per type. Meat samples were immediately frozen at -20˚C until the protein and AAs analysis was performed.

The husbandry practice in the case of broiler carcasses selected for this study followed the recommendations of the management guide for manufacturing companies, although the details are not available. Chickens were slaughtered according to the Islamic method as notified on the product labels.

The sample size used to determine crude protein and AA profiles was seven pooled samples per source, per type. Method number 920.39 of the Association of Official Analytical Chemists [AOAC, 2004] was used for this test. The profile of AAs in the samples was determined using the Automatic Amino Acid Analyser, Model AAA400 [Ingos Ltd, K Nouzovu 2090, 14316 Prague 4, Czech Republic]. The column used in the test was filled with resin material and a ninhydrin reagent. Ground samples with its fat removed (0.2 g) were hydrolysed with 6N HCl (10 ml) in sealed tubes and heated in the oven at 100°C for 24 hours. The resulting solution was added to 25 ml of deionised water. After filtration, 5 ml of hydrolysate were evaporated until completely free of HCl vapour. The residue was then dissolved in a diluted citrate buffer. The separation of AAs depends on various gradients of pH buffers. The AAs obtained were used to estimate the quality of protein in the protein isolates. The AAs were determined according to the protocol by Moore *et al.* [1958] and Csomos and Simon-Sarkadi [2002].

The ratio between AAs in the meat protein compared with the needed values for adults was used to measure the quality of protein [Oshodi *et al.* 1998]. The Essential Amino Acid Score [EAAS] was then estimated according to the Food and Agriculture Organisation [FAO]/ World Health Organization [WHO] [FAO/WHO, 1989].

The P-PER of the different meat sources was calculated from the AA profiles, according to the following equation by Alsmeyer *et al.* [1974]:

$$\text{P-PER} = -0.468 + 0.454\ (\text{leucine}) - 0.105\ (\text{tyrosine})$$

The following ratios were also calculated.

The essential AAs to total AAs ratio or the NEAAs ratio were calculated as indicators of protein quality. The FRAAs were calculated as absolute and relative values to total AAs, according to the sum of aspartic acid, arginine, glycine, proline, alanine, glutamic and cysteine [Wang *et al.* 2017]. Cysteine was not determined in this study; therefore, it was excluded from the equation. The TAAAs to total AAs ratio was calculated as an index of the antioxidant property of meat [Nimalaratne *et al*. 2011].

The antioxidant AAs were calculated as absolute and relative values in relation to total AAs, according to a study by Murad *et al.* [2013], using 2.2-Azino-Bis 3-ethylbenzothiazoline-6-Sulfonic acid [ABTS]. The study by Murad *et al.* [2013] showed that the ABTS was highly correlated (r = 0.705 – 0.976) to methionine, tyrosine, phenylalanine, histidine, cysteine and tryptophan. The two latter AAs were not determined in this study and thus, they were excluded from the analysis. Therefore, the ABTS in this study was the sum of methionine, tyrosine, phenylalanine and histidine. Other measurements of antioxidant AAs were calculated as absolute and relative values to total AAs, according to the study by Murad *et al*. [2013], using 2.2-Diphenyl-2-Picrylhydrazyl (DPPH). The DPPH was also found to be highly correlated (r = 0.640 – 0.927) to methionine, phenylalanine, cysteine and tryptophan. As the two latter AAs were not determined in this study, they were excluded from the analysis. Therefore, the DPPH in this study was the sum of methionine and phenylalanine.

Data were tested for empirical distribution before running the statistical analysis, which is based on the following linear model:

$$Y_{ijk} = \mu + C_i + B_{ij} + e_{ijk}$$

Y_{ijk} – the observed value;
μ – the overall mean;
C_i – fixed effect of the i-th meat source effect (frozen versus fresh);
B_{ij} – fixed effect of the ij-th type (brand) nested in the i-th source effect;
e_{ijk} – the random error connected with ijk-th observation.

The data included the meat source effect (frozen versus fresh) and the type (brand) within the source effect. Significant differences were tested at $p<0.05$ using the Student-Newman-Keuls test. These computations were performed with the use of the SAS package programs [SAS, 2009].

Results and discussion

Table 1 indicates the effect of different meat types and sources on EAAs and crude protein levels of broiler meat. There were no differences between frozen and fresh meat in terms of individual EAAs, such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine + tyrosine and valine ($P>0.083$-0.622). However, meat source of each type had a significant ($P<0.05$) effect on leucine, phenylalanine + tyrosine, valine and TEAAs. In particular, leucine ($P<0.012$), phenylalanine + tyrosine ($P<0.022$), and TEAAs ($P<0.03$) were higher in sources A and C of frozen meat and source F of fresh meat compared to source B of frozen and source E of fresh meat, whereas the levels were intermediately higher in source D of fresh meat. Valine content in source C of

Table 1. Means±SD of meat type and source in retail market on percentage RDA coverage by essential amino acid profiles and essential amino acids

Parameter	Percentage								
	His	Ile	Leu	Lys	Met	Phn+Try	Thr	Valine	TEAAs
Meat type									
frozen	2.341	1.020	4.347	3.422	1.098	8.471	1.481	2.599	24.9 (88.9%)
fresh	2.416	1.124	4.204	3.413	1.017	8.600	1.442	2.582	24.8 (88.6%)
Meat source									
frozen A	2.630	1.080	4.727^{a}	3.830	1.143	9.230^{a}	1.607	2.820^{ab}	27.1^{a} (96.8%)
frozen B	2.097	0.873	3.653^{b}	2.920	0.943	6.907^{b}	1.240	2.140^{c}	20.8^{b} (74.3%)
frozen C	2.567	1.067	4.660^{a}	3.516	1.207	9.277^{a}	1.597	2.837^{a}	26.8^{a} (95.7%)
fresh D	2.407	1.000	4.310^{ab}	3.510	1.037	8.467^{ab}	1.337	2.597^{abc}	24.7^{ab} (88.2%)
fresh E	2.143	1.073	3.543^{b}	3.010	0.950	7.503^{b}	1.340	2.250^{bc}	22.0^{b} (71.4%)
fresh F	2.697	1.100	4.760^{a}	3.720	1.063	9.930^{a}	1.650	2.900^{a}	27.7^{a} (98.9%)
RDA,% of protein	1.8	2.5	5.5	5.1	2.5[1]	4.7	2.7	3.2	28
SD	0.304	0.281	0.464	0.440	0.166	1.058	0.138	0.329	2.83
P-values									
meat type	0.915	0.445	0.528	0.967	0.321	0.800	0.562	0.916	0.948
meat source	0.088	0.622	0.012	0.083	0.355	0.022	0.007	0.037	0.030

All the values are means means±SD (standard division of means) of seven individual determinations.
Different superscripts in the same column indicate significant differences ($P<0.05$).
His – histidine; Ile – isoleucine; Leu – leucine; Lys – lysine; Met – methionine; Phn +Try – phenylalanine +Tyrosine; Thr – threonine; TEAAs – total essential amino acids; Numbers in parenthesis indicate the percentage coverage of recommended daily allowance (RDA) for amino acids (2002/2005).
[1] Methionine +cysteine.

frozen and source F of fresh meat was higher than in source B of frozen and source E of fresh meat, respectively ($P<0.037$). In addition, the level of valine in source A of frozen meat was higher only in comparison to source B of the same meat type.

Increasing EAAs in meat are mainly associated with improved quality and consumer desire to purchase the product [Zhao *et al.* 2011, Marangoni *et al.* 2015, Nasr *et al.* 2017]. The EAAs percentage in meat protein in this study was similar to the levels in a previous study by Nedkov [2004] for threonine (4%), methionine + cysteine (3.5%), valine (5.5%), isoleucine (4%), leucine (7%) and phenylalanine + tyrosine (6%). This indicates that broiler meats in the retail market are suitable sources of EAAs and meet the standard protein requirements according to the WHO [2007].

Methionine was found in adequate levels in frozen and fresh meat at 1.71 and 1.48%, respectively. The EAAs have an important role in human body functions, for example, methionine acts as a ‘methyl donor’; it builds blocks of protein and antioxidants that speed up or maintain chemical reactions within the body [Verhoef et al. 2005]. In addition, methionine is required in animals to form choline from lecithin and phospholipids [Adeyeye *et al.* 2016].

The phenylalanine + tyrosine complex is a dominant EAA in broiler meat. It is a precursor of specific hormones and the pigment melanin in suntanned skin, in hair and eyes [Aremu *et al.* 2013]. The average lysine content in frozen and fresh meat types was 5.4 g/100 g and 5.0 g/100 g, respectively; this indicates that broiler meat is a rich source of lysine and leucine. In fact, leucine is the most dominant branched chain

AA, followed by valine and isoleucine, and they are all fundamental AAs for protein formation [Marangoni *et al.* 2015].

It is worth noting that the variation between frozen and fresh meats in meeting the RDA of TEAAs was nil (88.9 vs. 88.6%; Tab. 1). However, the difference between various sources of meat within each type ranged from 71.4 to 98.9% of the RDA for sources E and F of fresh meat, respectively. This considerable difference (27.8%) indicates that selecting trusted sources of meat and the assessment of quality control are essential to meeting RDA of EAAs levels for humans.

Table 2 presents the effect of different meat types and sources on EEA scores of broiler meat. There were no effects of the different meat types (P>0.22-0.922), nor did the source have an influence on individual EAA scores (P>0.36-0.891). The results reveal that EAA scores of both frozen and fresh meat were similar. The EAA values of broiler meat found in this study are similar to those of broilers and quails reported in other studies [Zhao *et al.* 2011, Apata *et al.* 2013, Kim *et al.* 2017, Nasr *et al.* 2017, Wang *et al.* 2017].

Table 2. Means±SD of meat type and source in retail market on essential amino acid levels in frozen and fresh meat

Parameter	Percentage								
	His	Ile	Leu	Lys	Met	Phn+Try	Thr	Valine	TEAAs (%)
Meat type									
frozen	0.714	0.149	0.326	0.334	0.214	0.994	0.288	0.293	0.414
fresh	0.727	0.179	0.377	0.381	0.212	1.12	0.299	0.326	0.453
Meat source									
frozen A	0.747	0.153	0.344	0.362	0.217	1.049	0.304	0.309	0.436
frozen B	0.636	0.131	0.281	0.293	0.191	0.837	0.248	0.248	0.358
frozen C	0.757	0.162	0.351	0.345	0.235	1.094	0.313	0.321	0.448
fresh D	0.641	0.158	0.451	0.428	0.202	1.154	0.262	0.345	0.455
fresh E	0.717	0.209	0.306	0.338	0.213	1.008	0.299	0.290	0.423
fresh F	0.822	0.168	0.373	0.378	0.220	1.203	0.336	0.343	0.481
SD	0.189	0.048	0.091	0.083	0.057	0.233	0.064	0.069	0.091
P-values									
meat type	0.883	0.220	0.257	0.245	0.922	0.265	0.731	0.325	0.383
meat source	0.709	0.662	0.360	0.586	0.891	0.545	0.470	0.556	0.684

All the values are means means±SD (standard division of means) of seven individual determinatio
Different superscripts in the same column indicate significant differences (P<0.05).
His – histidine; Ile – isoleucine; Leu – leucine; Lys – lysine; Met – methionine; Phn +Try – phenylalanine +Tyrosine; Thr – threonine; TNEAAs – total essential amino acids.

Table 3 summarises the influence of various meat types and source on NEAAs of broiler meat. There were no differences between frozen and fresh meat in most individual NEAAs (P>0.064-0.949), except for glycine, where a higher glycine level (P=0.064) was observed in frozen meat. The increased level of glycine in frozen meat may indicate poor meat quality. Glycine is found in skin, cartilage, connective tissue, bones, tendons and ligaments, and it is associated with low-quality meat [Marangoni *et al.* 2015].

Table 3. Means±SD of type and source in the meat of retail market on percentage nonessential amino acid profiles

Parameter	Percentage								
	Alanine	Arginine	Asp	Glu	Glycine	Serine	Proline	TNEAAs	NH_4
Meat type									
frozen	4.711	5.126	3.538	29.42	0.864	2.553	0.850	47.03	2.981
fresh	4.938	5.029	3.514	29.62	0.543	2.521	1.078	47.23	3.212
Meat source									
frozen A	4.937^{ab}	5.507	3.887^{a}	33.29^{a}	1.167^{a}	2.780^{a}	0.877	52.43^{a}	3.340
frozen B	3.810^{c}	4.420	2.870^{b}	25.43^{b}	0.910^{ab}	2.127^{b}	0.683	40.20^{c}	2.607
frozen C	5.387^{a}	5.450	3.830^{a}	29.54^{ab}	0.517^{bc}	2.753^{a}	0.990	48.47^{ab}	2.997
fresh D	4.397^{bc}	4.820	3.280^{ab}	28.27^{b}	0.997^{ab}	2.367^{ab}	0.903	45.00^{abc}	3.077
fresh E	4.977^{ab}	4.780	3.266^{ab}	27.35^{b}	0.000^{c}	2.377^{ab}	1.397	44.13^{bc}	3.083
fresh F	5.450^{a}	5.487	3.997^{a}	33.25^{a}	0.633^{ab}	2.820^{a}	0.933	52.57^{a}	3.477
SD	0.556	0.875	0.461	2.62	0.334	0.261	0.329	4.63	0.458
P-values									
meat type	0.404	0.819	0.949	0.872	0.064	0.798	0.168	0.928	0.305
meat source	0.018	0.426	0.039	0.009	0.014	0.019	0.295	0.023	0.312

All the values are means means±SD (standard division of means) of seven individual determinations. Different superscripts in the same column indicate significant differences ($P<0.05$).

Asp – aspartic acid; Glu – glutamic acid; TNEAAs – total nonessential amino acids; NH_4 – ammonium.

Most of the NEAAs were affected by the source within each type of meat, except for arginine and proline. The results revealed that source C of frozen and source F of fresh meat showed higher values of alanine compared to source B of frozen and source D of fresh meat ($P<0.018$). In addition, source A of frozen and source E of fresh meat displayed greater alanine levels than source B of frozen meat.

Aspartic acid and serine showed similar trends, revealing that sources A and C of frozen meat and source F of fresh meat exceeded those of source B of frozen meat. Glutamic acid, the dominant NEAA, was more abundant in source A of frozen and source F of fresh meat than it was in the other sources, with the exception of source C of frozen meat ($P<0.009$). The highest content of glycine was found in source A of frozen meat, while the lowest was identified in source E of fresh meat. The latter group was different from the other groups ($P<0.014$), except for source C of frozen meat ($P>0.05$).

Source A of frozen and source F of fresh meat exhibited significantly greater TNEAAs than source B of frozen and source E of fresh meat. Source C of frozen and source D of fresh meat displayed similar values, and they did not differ from most other meat sources. Several authors have reported similar TNEAA values for chicken and quail meat [Zhao *et al.* 2011, Kim *et a*l. 2017, Nasr *et al.* 2017, Wang *et al.* 2017]. Although NEAAs can be synthesised by the body in adequate amounts, arginine is indispensable in human infant growth, and it was found in reasonable amounts: 5.126% and 5.026% protein of frozen and fresh meat, respectively.

Results indicate that ammonium ions (NH_4), a waste product of protein metabolism, did not differ between various meat types ($P>0.05$); however, among different sources of meat the trend was higher in fresh than in frozen meat (Tab. 3). This may reflect the pattern of NEAAs in fresh and frozen meat, as the highest NEAAs were related to the highest NH$_4$ and vice versa. The lower ammonium level in frozen meat may indicate evaporation during meat storage.

Table 4 shows the impact of different meat types and sources on crude protein, TAAs, the TEAA/TNEAA ratio and P-PER in broiler meat. There were no differences in the AA indices in frozen and fresh meat (P>0.05), as indicated by the similar qualitative values between the two types of meat.

Table 4. Means±SD of meat type and source in retail market on total amino acids, indices of essential amino acids, predicted protein efficiency ratio and total aromatic amino acids

Parameters	Crude protein (%)	Total amino acids (%)	TEAAs/TNEA As ratio	Predicted protein efficiency ratio	Total aromatic amino acids (%)[1]
Meat type					
frozen	65.7^{b}	71.92	34.49	1.219	10.90
fresh	69.5^{a}	72.06	34.41	1.141	11.01
Meat source					
frozen A	68.2^{a}	79.50^{a}	34.03	1.370^{a}	11.86^{ab}
frozen B	63.2^{b}	61.03^{c}	33.90	0.946^{bc}	9.00^{c}
frozen C	65.6^{ab}	75.23^{ab}	35.53	1.340^{a}	11.84^{ab}
fresh D	70.3^{a}	69.70^{abc}	35.43	1.205^{ab}	10.87^{abc}
fresh E	70.1	66.17^{bc}	33.30	0.847^{c}	9.64^{bc}
fresh F	68.2	80.30^{a}	34.50	1.369^{a}	12.53^{a}
SD	7.21	7.22	1.53	0.187	1.35
P-values					
meat type	0.028	0.969	0.915	0.388	0.861
meat source	0.008	0.023	0.336	0.009	0.028

All the values are means±SD (standard division of means) of seven individual determinations.
Different superscripts in the same column indicate significant differences (P<0.05).
TEAAs/TNEAAs – total essential amino acids/total non-essential amino acids.
[1]As percentage of total amino acids.

Crude protein level was higher in fresh meat than in frozen meat (P<0.028), but the contents of TAAs were similar (P>0.969). In addition, sources D and E of fresh meat displayed higher crude protein levels than sources A and C of frozen meat (P<0.008). The pattern of crude protein reflected the change in TAAs, which might be because crude protein is composed of both EAAs and NEAAs. Similarly, Nasr et al. [2017] found a significant difference in EAAs and total protein contents of quail meat with different coloured plumage.

The TAAs were influenced by source within each type of meat (P<0.05), as source A of frozen meat and source F of fresh meat exhibited greater contents of TAAs than sources B of frozen and E of fresh meat. In addition, source C of frozen meat contained greater TAA levels than source B of frozen meat (P<0.05).

The P-PER was greater in sources A and C of frozen meat and source F of fresh meat than in source B of frozen meat and source E of fresh meat (P<0.05). In addition, source D of fresh meat showed higher P-PER values than source E of the same meat type (P<0.05).

The daily protein requirements for humans, assuming its high biological value, differed depending on gender, physiological status, stage of growth and age. It ranged from 0.66 g/kg body weight per day for adults to 1.12 g/kg body weight for children and 1.2-1.3 g/kg body weight per day for adults over 65-years old. The TAA contents of broiler meat recorded in this study ranged from 61.03% to 80.3% based on the type and source of meat. The NEAA to EAA ratio ranged between 1.82-2.01:1; this ratio is somewhat higher than the 1.35:1 ratio [Nedkov 2004].

The results in this study indicate that 125 g of broiler meat can meet ≈50% of the RDA for protein for adults, assuming a 20% crude protein content [Attia *et al.* 2016]. An approximate coverage of RDAs may be estimated, as previously mentioned, depending on variations in gender, physiological status, stage of growth and age.

Table 5 summarises the influence of various meat types and sources on FRAAs in broiler meat. AAs are major constituents of meat flavour, while protein influences various substances of smile [Jayasena *et al.* 2013]. Variations between frozen and fresh meat in most individual FRAAs were not significant, except for glycine, where a numerically higher value (P=0.064) was recorded in frozen meat than fresh one. This increase in glycine level may indicate poor quality of frozen meat and this is in agreement with previous research [Zhao *et al.* 2011, Wang *et al.* 2017].

Table 5. Means±SD of meat type and source in retail market on percentage flavour related amino acid profiles

Parameters	Percentage							
	Glycine	Aspartic	Arginine	Proline	Alanine	Glutamic	Total FRAAs	FRAAs (%)^{1}
Meat type								
frozen	0.864	3.528	5.126	0.850	4.711	29.42	44.49	61.96
fresh	0.543	3.514	5.029	1.078	4.938	29.62	44.72	62.10
Meat source								
frozen A	1.167^{a}	3.887^{a}	5.507	0.877	4.937^{ab}	33.29^{a}	49.65^{a}	62.47
frozen B	0.910^{ab}	2.870^{b}	4.420	0.683	3.810^{c}	25.43^{b}	38.12^{b}	62.60
frozen C	0.517^{bc}	3.830^{a}	5.450	0.990	5.387^{a}	29.54^{ab}	45.71^{ab}	66.80
fresh D	0.997^{ab}	3.280^{ab}	4.820	0.903	4.397^{bc}	28.27^{b}	42.66^{ab}	61.17
fresh E	0.000^{c}	3.266^{ab}	4.780	1.397	4.977^{ab}	27.35^{b}	41.76^{b}	63.13
fresh F	0.633^{ab}	3.997^{a}	5.487	0.933	5.450^{a}	33.25^{a}	49.75^{a}	62.00
SD	0.334	0.461	0.875	0.329	0.556	2.62	4.41	1.55
P-values								
meat type	0.064	0.949	0.819	0.168	0.404	0.872	0.915	0.847
meat source	0.014	0.039	0.426	0.295	0.018	0.009	0.025	0.347

All the values are means±SD (standard division of means) of seven individual determinations. Different superscripts in the same column indicate significant differences (P<0.05).
TFAA – total flavour related amino acids as an absolute value; FRAA – flavour related amino acids relative to total amino acids.

Individual FRAAs (alanine, arginine, aspartic acid, glycine, serine and proline), as affected by meat type and source, were previously discussed in the NEAA section (Tab. 3). The TFRAAs were not affected by the type of meat, reflecting the effect of individual FRAAs. Source of meat exhibited a significant (P<0.05) effect on

TFRAAs; higher values were found in source A of frozen meat and source F of fresh meat compared to source B of frozen meat and source E of fresh meat ($P<0.05$). Source C of frozen meat and source D of fresh meat displayed similar values to the other sources of meat.

Results for antioxidant AAs were calculated as TAAAs and are presented in Table 4, while the ABTS and DPPH are shown in Table 6. TAAAs, as an index of the antioxidant status [Nimalaratne *et al*. 2011] were not affected by the type of meat (Tab. 4). However, the source of meat had a significant effect. The results indicate that the levels of TAAAs were higher ($P<0.05$) in source F of fresh meat compared to source E of the same meat type and source B source of frozen meat (Tab. 4). Increasing the content of antioxidant AAs in broiler meat is beneficial, as it prolongs the shelf life of products [Damgaard *et al.* 2015, Attia *et al.* 2016, Sohaiba *et al*. 2017, Wang *et al.* 2017].

The variations between the two meat types in terms of AAs related to ABTS and DPPH were not significant (Tab. 6). However, phenylalanine was found to be affected by the source of meat ($P<0.05$), as sources A and C of frozen meat and F of fresh meat displayed greater values than source B of frozen meat and source E of fresh meat. There was no difference between meat sources and types in ABTS and DPPH as percentages of TAAs ($P>0.05$); however, there were differences in the total of ABTS and DPPH ($P<0.05$). The ABTS and DPPH values were higher in sources A and C of frozen meat and source F of fresh meat compared to source B of frozen meat and source E of fresh meat ($P<0.05$).

Table 6. Means ±SD of meat type and different sources of frozen and fresh meat in the retail market on percentage antioxidant related amino acid profiles of frozen and fresh meat

Parameters	ABTS related amino acids (%)						DPPH-related amino acids (%)			
	His	Met	Try	Phn	Total	% of TAAs[1]	Met	Phn	Total	% of TAAs[1]
Meat type										
frozen	2.341	1.098	2.719	5.752	11.99	16.63	1.098	5.752	6.85	9.47
fresh	2.416	1.017	2.861	5.740	12.03	16.68	1.017	5.740	6.76	9.35
Meat source										
frozen A	2.630	1.143	2.90	6.33^{a}	13.0^{a}	16.35	1.143	6.33^{a}	7.47^{a}	9.40
frozen B	2.097	0.943	2.33	4.58^{b}	9.94^{b}	16.21	0.943	4.58^{b}	5.52^{b}	9.00
frozen C	2.567	1.207	2.93	6.35^{a}	13.05^{a}	17.32	1.207	6.35^{a}	7.55^{a}	10.02
fresh D	2.407	1.037	2.70	5.77^{ab}	11.91^{ab}	17.09	1.037	5.77^{ab}	6.80^{ab}	9.76
fresh E	2.143	0.950	2.80	4.71^{b}	10.59^{b}	16.02	0.950	4.71^{b}	5.66^{b}	8.56
fresh F	2.697	1.063	3.09	6.75^{a}	13.59^{a}	16.91	1.063	6.75^{a}	7.81^{a}	9.72
SD	0.304	0.166	0.419	0.793	1.459	1.040	0.166	0.793	0.904	0.809
P-values										
meat type	0.915	0.321	0.486	0.974	0.963	0.924	0.321	0.974	0.830	0.746
meat source	0.088	0.355	0.313	0.014	0.032	0.467	0.355	0.014	0.018	0.219

All the values are means±SD (standard division of means) of seven individual determinations.
Different superscripts in the same column indicate significant differences ($P<0.05$).
ABTS, 2,2 – Azino-Bis 3-ethylbenzothiazoline-6-sulfonic acid; DPPH 2, 2 – Diphenyl-2-Picrylhydrazyl; His – histidine; Met – methionine; Try – tyrosine; Phn – phenylalanine.
[1] As a percentage of total amino acids.

Differences in histidine were close to being statistically significant (P=0.088); the highest values were evident in source F of fresh and source A of frozen meat, while the lowest value was found in source B of frozen meat. Antioxidants measured by different means of AAs demonstrated a similar trend, showing that the EAA rich sources, such as source F of fresh and sources A and C of frozen meat displayed a greater antioxidant status. Antioxidants have an important role in protecting cells from free radicals, thus AAs are an essential part of antioxidant enzymes that can prolong the shelf life of meat, such as glutathione [Attia *et al*. 2016]. Thus, higher levels of TAAs, ABTS and DPPH demonstrate better meat quality and superior preservation of meat.

In conclusion, broiler meat can cover 71.4-98.9% of the RDA of EAAs for adults and this depends particularly on the meat source. These variations suggest potential to enhance nutritional and qualitative value of broiler meat through husbandry and dietary manipulation.

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