FREE AMINO-ACIDS AND DIPEPTIDES IN PORCINE MUSCLES

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SUMMARY: A reverse-phase high performance liquid chromatographic technique (with phenylisothiocyanate derivatization) was used to quantify free amino-acids and dipeptides in aqueous extracts of four porcine muscles (*longissimus thoracis, masseter, psoas major* and *trapezius* muscles) considered at two times of storage (30 min and 48 h after slaughter).

Analyses of the variation of the amount of 26 measured compounds were made by using the multivariate analysis of centered data and Wilcoxon's test. The different muscles can be easily separated by their composition in the amino-acids and dipeptides known to exhibit a meat-like taste.

Samples stored at 4°C during 48 h did not show significant differences with samples taken 30 min after slaughter.

Results show that free amino-acids and dipeptides can partly explain differences between taste of muscles in the same species.

INTRODUCTION: Meat flavors result from precursors which are among the constituent components of muscle tissues. Literature has noted the effects of low molecular weight compounds of the aqueous meat extracts in food tastes (Mabrouk *et al.*, 1969). Among these related molecules, free amino-acids play an important role.

Amounts of free amino-acids were described in several meat species (Gardner *et al.*, 1969, Blum *et al.*, 1966, Koga *et al.*, 1988) but little is known about variations of these compounds in different muscles within the same species and the same animal.

In this work, free amino-acids and dipeptides analyses were performed on porcine muscles to study the variation between muscles of different anatomical origin for some amino-acids which are known as important precursors of flavor. Futhermore the effects of the time of storage on these compounds are also considered.

MATERIALS AND METHODS: Six pigs were used in this study. Analyses were made on 4 muscles : *longissimus thoracis, masseter, psoas major* and *trapezius* muscles. Two series of samples were removed from the carcasses : one serie from the right side, 30 min after slaughter a second one from the left side, after 48 h *post mortem* storage at 4°C.

Muscle samples (about 2 g) were minced and homogenized in 2 ml of high purity water. Centrifugation at 38 000 g for 1 h, at 4°C, and removal of proteins by precipitation with trifluoroacetic acid to a final concentration of 4.8 % yielded clear supernatants which were directly derivatized with the phenylisothiocyanate according to the method described by Bidlingmeyer *et al.* (1984). The resulting phenylthio-carbamyl-amino-acids and dipeptides (PTC-AA) were separated in 100 min by reverse-phase high-performance liquid chromatography at 54°C on octadecyl (C18) column, 25 cm in length. Components were eluted by a serie of linear gradients. Solvents A and B contained 0.07 sodium acetate, both at pH 6.5, solvent B having 40 % acetonitrite and 10 % methanol. The PTC-AA were detected at 254 nm.

The statistical treatment consisted of :

- calculations of means and coefficients of variation of the amount (in millimoles/100 g of fresh muscle) of the different amino-acids and dipeptides ;
- analysis of the relationships between the measured amino-acids and dipeptides in the various muscle samples by using the multivariate analysis of centered data (Lefebvre, 1976) ;
- comparisons of groups of amino-acids according to Wilcoxon's test.

RESULTS AND DISCUSSION: Twenty four free amino-acids and two dipeptides were identified and quantified in 48 muscle samples (6 pigs x 4 muscles x 2 times of storage). Tables 1 and 2 present results of these measurements as means of pooled amounts of free amino-acids and dipeptides for the four muscles, taken at the two times of storage. The degree of variability is indicated by the coefficient of variation and for some amino-acids, like lysine (K) in the *masseter* muscle, it is very high. That means it exists a great variability between the animals.

Table 1.- Composition* in amino-acids and dipeptides of four porcine muscles 30 min. after slaughter

Muscles	*masseter*		*trapezius*		*psoas major*		*longissimus thoracis*	
Amino acids**	mean	cv%	mean	cv%	mean	cv%	mean	cv%
A	536.3	49.9	396.9	20.0	258.5	30.7	349.2	28.3
D	23.2	48.0	10.8	66.9	12.6	111.8	31.5	121.5
E	58.9	86.9	61.8	28.2	48.3	38.5	72.3	61.6
F	10.4	36.8	10.8	39.6	9.3	30.8	8.2	51.2
G	133.3	38.4	131.6	15.0	104.0	15.2	174.0	28.9
I	15.1	54.3	13.6	33.9	11.5	34.4	14.6	37.9
K	33.2	70.1	31.6	38.5	17.1	53.2	29.6	54.8
L	26.3	34.2	26.1	27.9	20.8	29.3	24.9	30.6
M	5.3	66.9	5.6	44.9	2.6	35.4	3.4	60.0
N	11.8	54.5	9.2	45.2	5.4	42.2	9.2	45.2
P	54.8	52.8	45.3	29.6	35.7	9.1	69.1	34.7
Q	930.5	52.2	519.3	35.7	217.1	27.2	393.7	46.3
S	42.6	47.3	32.8	37.3	23.5	31.7	34.5	40.9
T	20.1	38.9	19.8	14.8	15.9	28.5	24.6	35.6
V	38.8	40.4	38.9	28.3	41.0	51.6	45.7	30.4
W	1.9	57.8	1.7	44.9	1.6	44.4	1.9	62.3
Y	8.8	30.9	10.5	53.2	10.3	53.3	9.4	51.6
AAA	*11.7*	*65.9*	*11.9*	*59.5*	*22.0*	*70.5*	*15.7*	*56.1*
ANS	*61.4*	*36.2*	*67.3*	*23.3*	*125.6*	*18.7*	*87.2*	*17.7*
βALA	*14.2*	*50.6*	*17.4*	*44.6*	*24.1*	*29.7*	*44.8*	*33.6*
CAR	*518.3*	*29.5*	*1527.4*	*33.7*	*2882.1*	*16.6*	*2823.9*	*18.8*
HPRO	*20.3*	*53.0*	*17.1*	*37.2*	*15.0*	*22.5*	*21.3*	*40.3*
ORN	*7.5*	*51.5*	*8.4*	*39.2*	*7.4*	*25.8*	*9.8*	*37.7*
PEA	*15.7*	*46.6*	*12.6*	*44.1*	*7.1*	*54.7*	*8.8*	*17.5*
PSER	*78.7*	*45.0*	*96.9*	*32.4*	*148.5*	*24.0*	*133.4*	*42.7*
TAU	*2336.7*	*46.2*	*916.3*	*38.9*	*675.4*	*16.9*	*307.9*	*30.3*

* Results given as means (n=6) expressed in millimoles per 100 g of muscle
** Amino-acids are named by their usual code with one letter, except for the following ones : *AAA* = α amino adipic acid ; *ANS* = anserine ; *βALA* = βalanine ; *CAR* = carnosine ; *HPRO* = hydroxyproline ; *ORN* = ornithine ; *PEA* = phosphoethanolamine ; *PSER* = phosphoserine ; *TAU* = taurine.

Table 2. -Composition* in amino-acids and dipeptides of four porcine muscles 48 h *post mortem*

Muscles	*masseter*		*trapezius*		*psoas major*		*longissimus thoracis*	
Amino acids**	mean	cv%	mean	cv%	mean	cv%	mean	cv%
A	447.7	43.3	428.6	18.5	210.0	52.4	328.2	33.6
D	15.0	79.0	11.9	55.4	13.5	31.6	13.9	95.3
E	50.4	43.4	55.7	25.3	80.1	37.7	67.4	59.4
F	9.7	34.8	10.7	16.0	11.5	24.7	11.9	28.6
G	100.6	37.3	102.5	56.8	84.4	35.9	143.2	31.7
I	13.1	38.8	13.9	20.6	13.7	23.2	15.3	25.0
K	40.0	40.8	48.8	30.4	30.9	45.9	46.8	34.7
L	23.1	31.7	19.6	45.1	23.0	18.7	26.8	32.5
M	3.5	67.4	4.4	39.2	5.4	36.8	4.8	50.1
N	10.6	53.6	8.6	23.9	5.1	17.6	9.6	37.9
P	44.2	39.6	44.5	47.0	33.1	57.4	58.8	43.3
Q	694.0	54.8	502.0	65.1	202.3	35.1	322.4	49.9
S	31.5	38.7	34.1	35.3	21.2	31.9	30.9	34.2
T	17.2	44.5	21.9	42.0	13.5	46.9	21.4	26.7
V	28.7	41.3	31.1	32.7	33.5	46.5	36.1	63.5
W	2.6	33.3	3.2	23.1	3.0	24.7	3.3	21.4
Y	7.7	39.5	10.2	59.9	11.2	48.2	10.4	60.1
AAA	*6.9*	*84.9*	*10.6*	*78.3*	*10.1*	*102.8*	*11.4*	*104.3*
ANS	*66.5*	*31.5*	*83.5*	*34.5*	*119.1*	*49.6*	*116.6*	*41.4*
bALA	*11.0*	*24.4*	*15.9*	*54.0*	*20.1*	*42.7*	*36.7*	*54.8*
CAR	*457.8*	*38.0*	*1861.6*	*52.2*	*3021.2*	*48.2*	*3421.9*	*47.6*
HPRO	*16.6*	*52.6*	*17.8*	*64.5*	*18.1*	*39.8*	*16.4*	*79.8*
ORN	*15.0*	*143.3*	*7.3*	*45.9*	*6.2*	*49.9*	*8.0*	*50.9*
PEA	*8.8*	*58.1*	*7.35*	*60.6*	*5.0*	*75.1*	*4.6*	*98.9*
PSER	*136.3*	*30.3*	*132.7*	*26.8*	*116.3*	*29.0*	*148.3*	*34.4*
TAU	*1668.9*	*37.7*	*775.2*	*25.5*	*350.7*	*38.2*	*355.2*	*39.4*

* Results given as means (n=6) expressed in millimoles per 100 g of muscle

** Amino-acids are named by their usual code with one letter, except for the following ones : *AAA* = α amino adipic acid ; *ANS* = anserine ;β *ALA* = β alanine ; *CAR* = carnosine ; *HPRO* = hydroxyproline ; *ORN* = ornithine ; *PEA* = phosphoethanolamine ; *PSER* = phosphoserine ; *TAU* = taurine.

The results of the multivariate analysis of centered data achieved on the 26 amino-acids or dipeptides as measured variates of the 48 muscles samples are shown in fig. 1. The first axis explains 27 % of the variation, the second 17 %, and for the four first axes together, only 65 % of the variation was explained. This relatively low amount of explanation reflects the very complex relation that exists between the amount of each free amino-acids or dipeptides within the four muscles. However, along the first axis, muscle samples are grouped into areas corresponding to their anatomical origin. Furthermore, muscle samples were associated with specific amino-acids and dipeptides. It thus exists an opposition between the variates taurine and glutamine on one hand and carnosine,β alanine and anserine on the other hand. Along the second axis, the variation is explained by the opposition existing between aspartic acid and ornithine.

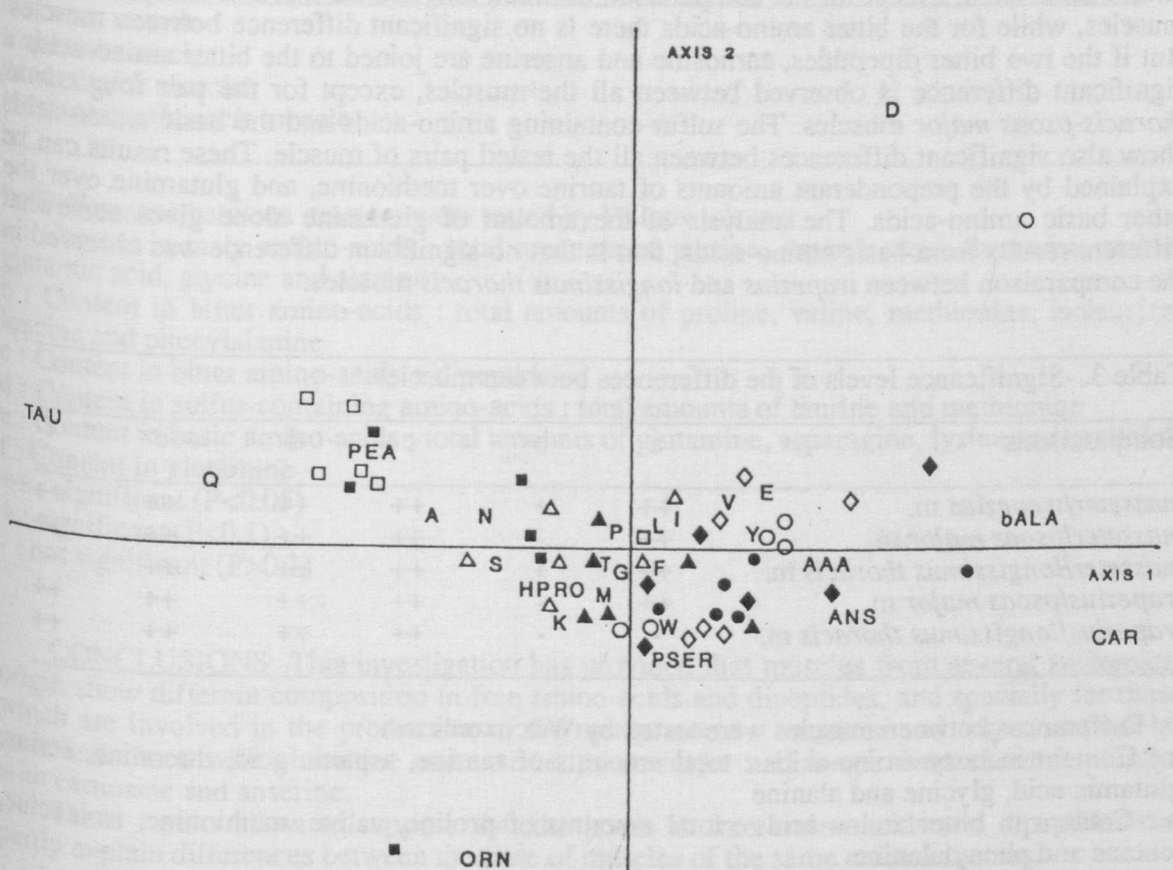

Fig.1 : Multivariate analysis of centered data. Only axes 1 and 2 are represented. The code for amino-acids is the same as that for Table 1 or 2.

30 min after slaughter :

□ *masseter* muscle
△ *trapezius* muscle
◇ *psoas major* muscle
○ *longissimus thoracis* muscle

48 h after slaughter :

■ *masseter* muscle
▲ *trapezius* muscle
◆ *psoas major* muscle
● *longissimus thoracis* muscle

These noticeable amino-acids or dipeptides are mostly described as precursors of flavors (Jarboe *et al.*, 1974). Taurine is a sulfur-containing amino-acid which is implicated in the production of meat-like taste. Glutamine presence is interesting because monosodium glutamate enhances the flavors of food. The bitterness of the two histidine-containing dipeptides, carnosine and anserine, is well known (Spanier *et al.*, 1987). Ornithine is a basic amino-acid. In many patents concerning the production of meat flavor, basic amino-acids are used to produce meaty aroma.

Moreover the means of amino-acids and dipeptides grouped following their roles in meat flavor were compared between muscles according to Wilcoxon's test. The significant levels of the test are given in Table 3. Significant differences between muscles in the levels of tasty amino-acids exist, except for the comparaison between *longissimus thoracis* and *psoas major* muscles, while for the bitter amino-acids there is no significant difference between muscles. But if the two bitter dipeptides, carnosine and anserine are joined to the bitter amino-acids a significant difference is observed between all the muscles, except for the pair *longissimus thoracis-psoas major* muscles. The sulfur-containing amino-acids and the basic amino-acids show also significant differences between all the tested pairs of muscle. These results can be explained by the preponderant amounts of taurine over methionine, and glutamine over the other basic amino-acids. The analysis of the amount of glutamine alone gives somewhat different results from basic amino-acids, that is that no significant difference was observed in the comparaison between *trapezius* and *longissimus thoracis* muscles.

Table 3. -Significance levels of the differences between muscles *

Comparaisons	a	b	c	d	e	f
masseter/trapezius m.	++	+	++	++	++	++
masseter/psoas major m.	++	-	++	++	++	++
masseter/longissimus thoracis m.	++	+	++	++	++	-
trapezius/psoas major m.	++	-	++	++	++	++
trapezius/longissimus thoracis m.	-	-	++	++	++	++

* : Differences between muscles were tested by Wilcoxon's test
a : Content in tasty amino-acids : total amounts of taurine, aspartic acid, threonine, serine, glutamic acid, glycine and alanine
b : Content in bitter amino-acids : total amounts of proline, valine, methionine, isoleucine, leucine and phenylalanine
c : Content in bitter amino-acids + dipeptides
d : Content in sulfur-containing amino-acids : total amounts of taurine and methionine
e : Content in basic amino-acids : total amounts of glutamine, asparagine, lysine and ornithine
f : Content in glutamine
++ : significant ($P<0.05$)
+ : significant ($P<0.1$)
- : not significant ($P>0.1$)

In most of the cases, muscles well differ from each other about amino-acids which are implicated as precursors of flavor. Nevertheless, none distinction can be made between *psoas* and *longissimus thoracis* muscles. The small sample (n=6) can explain this lack of difference.

Table 4 gives the results obtained in the comparaison of muscles composition at two times of storage. No significant difference was observed except for *longissimus thoracis* muscle in the group of basic and sulfur-containing amino-acids, and *psoas major* muscle in the group of tasty amino-acids. These results show that no large change occurs in the amino-acids composition of these muscles during a relatively short time of storage.

Table 4. -Significance levels of the differences between two times of storage*

Comparaisons at two times of storage	a	b	c	d	e	f
masseter muscle	-	-	-	-	-	+
trapezius muscle	-	-	-	-	-	-
psoas major muscle	++	-	-	+	-	-
longissimus thoracis muscle	-	-	-	++	++	-

* : Differences between muscles were tested by Wilcoxon's test
a : Content in tasty amino-acids : total amounts of taurine, aspartic acid, threonine, serine, glutamic acid, glycine and alanine
b : Content in bitter amino-acids : total amounts of proline, valine, methionine, isoleucine, leucine and phenylalanine
c : Content in bitter amino-acids + dipeptides
d : Content in sulfur-containing amino-acids : total amounts of taurine and methionine
e : Content in basic amino-acids : total amounts of glutamine, asparagine, lysine and ornithine
f : Content in glutamine
++ : significant ($P<0.05$)
+ : significant ($P<0.1$)
- : not significant ($P>0.1$)

CONCLUSIONS: This investigation has provided that muscles from several anatomical origin show different composition in free amino-acids and dipeptides, and specially for those which are involved in the production of the meaty aroma : *masseter* and *trapezius* muscles were associated with glutamine and taurine, *psoas major* and *longissimus thoracis* muscles with carnosine and anserine.

These results allow to suppose that the levels in free amino-acids and dipeptides can partly explain differences between the taste of muscles of the same animal species.

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