

EFFECT OF SEASONAL CHANGES ON MUSCLE CATHEPSIN B ACTIVITY AND pH_{24h} OF ITALIAN HEAVY PIGR. Virgili*, T. Toscani*, C. Schivazappa*, L. Mazzotta*, R. Manfredini[§], F. Portaluppi[§]*Stazione Sperimentale per l'industria delle Conserve Alimentari, 43100 Parma, Italy; [†]Consorzio del Prosciutto di Parma, 43100, Parma Italia; [‡]Food Chemistry Laboratory and [§]First Internal Medicine, University of Ferrara, Ferrara, Italy

Background

The influence of seasonal effects (change in photoperiod, temperature, rainfall) on meat quality has been known for a long time (Briskey, E. J., 1964). There is evidence that seasonal-type heat stress can contribute to development of the PSE-like condition in swine and fast-growing turkeys (Owens et al., 2000; McKee et al., 1998). Kuechenmeister et al. (2000) reported that seasonality had significant effects on the ability of sarcoplasmic reticulum to regulate Ca⁺⁺ concentration in muscle and thereby on meat quality. Season-related changes were reported for backfat thickness for swine, lamb, deer and cattle (Anon, 1996; Parker et al. 1993; Laurenz et al., 1992), body growth, acid and neutral proteases in fish (Matsumiya et al., 1990). In domestic boars and sows the seasonal variations of gonadal functions and fertility, proved to be affected by the photoperiod and can be influenced by light schedule (Claus et al. 1983; Claus and Weller 1985). pH_{24h} and post-mortem activity of cathepsin B in pork muscle were found to be related to proteolysis of Italian dry-cured hams (Schivazappa et al., 2002). A high degree of proteolysis leads to muscle softness and pastiness, surface white film development, onset of metallic and bitter taste (Guerrero et al., 1996; Virgili et al., 1998). Sources of variability for muscle proteolytic enzymes were found in genetics (Rosell and Toldrà, 1998; Armero et al., 1999; Russo et al., 2000), weight and age (Sarraga et al., 1993; Toldrà et al., 1996) and feeding (Van den Hemel-Grooten et al., 1997) of animals, while no literature is available for possible seasonal influence on endogenous proteolytic activity in pork.

Objectives

The aim of the work is to look for the presence of a time period regulating the variability of muscle cathepsin B activity and pH_{24h} of Italian heavy pigs.

Methods

A total of 4638, 9 to 13 mo-old pigs were monitored for cathepsin B activity and pH_{24h}. Data were recorded over a period of 4 (1997-2001) and 2 years (1999-2001) for cathepsin B and pH_{24h} respectively. A pre-fixed number of samples was taken from the 29 breeding houses giving the highest number of pigs/year among the domestic breeding houses allowed to provide raw matters for Parma ham manufacturing. Samples were taken from each breeding house once a season. Pigs were slaughtered in different slaughter houses. Monthly sample distribution through the years is displayed in table 1.

Muscle (M. *Semimembranosus*) cathepsin B activity was assayed 48h p.m., with N-CBZ-Phe-Arg-AMC as described by Barrett (1980). Activities are expressed as nmol substrate hydrolysed · min⁻¹ · g meat⁻¹.

The muscle (M. *Semimembranosus*) pH was determined 24h p. m. using a Hamilton glass electrode probe and portable pHmeter (WTW pH330).

Analysis of rhythmicity was performed by applying a partial Fourier series to the data, using the Chronolab software (Mojon et al. 1992). With the program, estimates of the amplitude (half of peak to trough of rhythmic change) and acrophase (peak time of the rhythmic change) of each harmonic were calculated. The program permits the selection of the harmonic or the combination of harmonics that best explains the variance of the data. As a result of such preliminary testing, a Fourier model with two harmonics (periods of 2922 and 876.6 hours, corresponding to 4 months and 36.5 days, respectively) was chosen as best fit for data of pH_{24h}, whereas the data of muscle cathepsin B activity were best fitted by a single harmonic with a period of 8766 hours (corresponding to 12 months). For each fitted curve, the midline estimated statistic of rhythm (mesor, the rhythm-adjusted mean over the time period analyzed) was determined. The percentage of rhythm (percentage of overall variability of data about the arithmetic mean attributable to the fitted rhythmic function) and the P (probability) value resulting from the F statistic (used to test the hypothesis of zero amplitude) were also calculated and were reported in the results as representative parameters of goodness of fit and statistical significance of the fitted function, respectively.

Results and discussion

Descriptive statistics of recorded data is reported in Table 2.

Cathepsin B: analysis of the data shows a clear seasonal effect for muscle proteolytic activity (table 3 and figure 1): a highly significant yearly rhythm was demonstrated, with significantly higher occurrence of increased cathepsin B activity during the cold winter months (maximum predicted value at the half of January).

pH_{24h}: variability found for pH_{24h} over the 24 months (table 2) was not influenced by season, as demonstrated by the lack of any significant rhythmicity with a period of 12 months; instead, rhythm analysis (table 3 and figure 2) showed cycles of 4-month duration with shorter oscillations of less than 40 days superimposed on them. This complex temporal pattern has no correspondence in the rhythmic changes of environmental factors commonly believed to determine seasonal variations. Based on our findings, it seems possible that specific determinants of temporal variation may exist for pH_{24h}, only occasionally coinciding with, but possibly unrelated to, any monthly, seasonal or yearly rhythm. The nature of such determinants remains unknown.

Table 1. Monthly sample distribution (average of 4 and 2 years for cathepsin B and pH_{24h} respectively).

	J	F	M	A	M	J	J	A	S	O	N	D
Cathepsin B	334	429	383	397	386	372	379	397	387	352	459	363
pH	145	174	163	162	161	148	163	169	150	134	204	154

Table 2. Descriptive analysis of time series data.

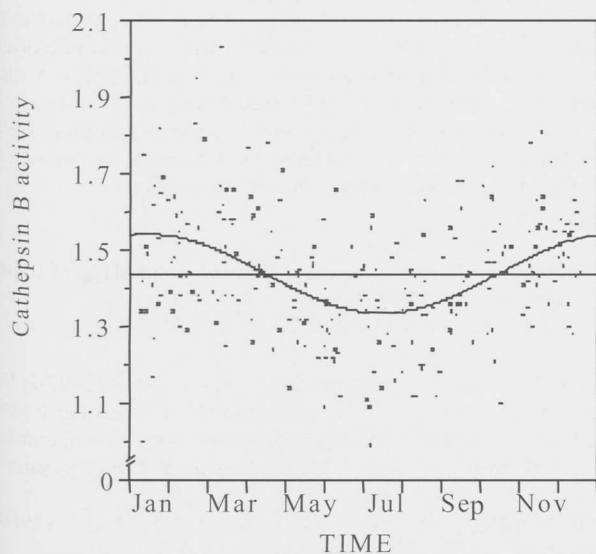
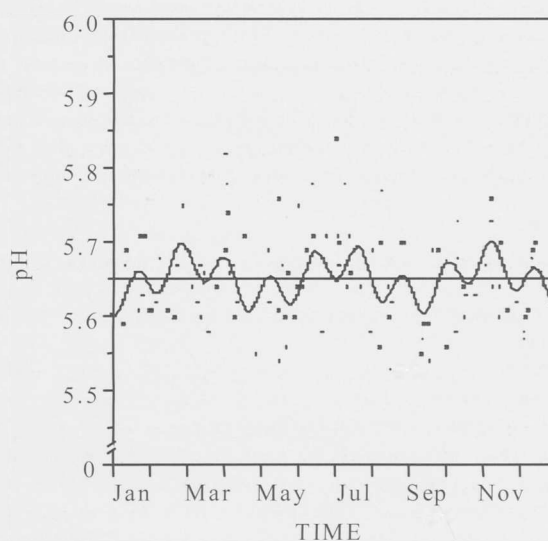
item	n°	mean	std. dev.	Skewness	Kurtosis
cathepsin B	4638	1.43	0.298	1.27	4.93
pH _{24h}	1927	5.65	0.134	2.00	7.83

Table 3. Analysis of rhythmicity of time series data

Item	Best fitting components	PR	P	Mesor \pm s.e.m.	Amplitude \pm s.e.m.	Peak Time
cathepsin B	12 mo	19.2	<0.001	1.44 \pm 0.01	0.10 \pm 0.01	January
pH _{24h}	4 mo + 36.5 days	14.9	<0.001	5.65 \pm 0.01	0.05 \pm 0.01	November

PR: percentage of rhythm; P: probability value to test the hypothesis of zero amplitude

Figure 1. Best-fitting curve for the data of muscle cathepsin B activity

Figure 2. Best-fitting curve for the data of pH_{24h}

Conclusion

Further studies are needed to understand and explain seasonal changes of muscle cathepsin B activity. Before formulating any hypothesis to explain this finding, further researches are needed to assess if this variability is dependent or not on known seasonal changes affecting parameters like body growth, fat deposition or fertility.

For the present, dry-cured ham manufacturers should be aware of the fact that hams undergoing processing during the cold winter months (max cathepsin B activity, min pH_{24h}) have a higher probability to give texture and taste drawbacks as final outcome, due to increased proteolysis. A possible suggestion is to slightly increase salt adjuncts in this period and to protract the cold drying period to further decrease a_w and inhibit proteolytic activity during the following maturation steps at higher temperature.

Pertinent literature

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