ANALYSIS OF MYOGLOBIN DERIVATIVES AT THE SURFACE OF FRESH BEEF

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On basis of reflectance spectra analysis the method of measuring the proportions of myoglobin derivatives at the surface of beef was developed. The reflectance of meat sample was recorded at three isobestic points /473,525,572 nm/ and at 730 nm. This last value was assumed to represent the background reflectancy, independent of myo - globin, oxymyoglobin and metmyoglobin concentrations. It was subtracted from reflectancy measured at isobestic points. Limiting values for ratios R_{572}/R_{525} and R_{525}/R_{473} were established for meat samples containing at the surface 100 % of one of three myoglobin derivatives. Assuming linear relation between changes of these ratios with pigment concentration formulae for calculating proportions of myoglobin forms were derived. The method can be also applied to meat packed in transparent plastic bags.

ANALISE DES DERIVÉES DE MYOGLOBIN À LA SURFACE DE VIANDE DE BOEUF FRAIS

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Sur la base de spectra reflectance analise la methode pour mesurer les proportions de myoglobin derivées sur la surface de la viande de boeuf fus élaborée. La reflectance de viande a eté mesurée à trois isobestiques points /473, 525, 572 nm/ et à 730 nm. Cette dornier valeur est independant de la concentration de myoglobin, oxymyoglobin et metmyoglobin et représente la reflectance du fond /viande sans pigment/. On calculait valours limitées aux relations R_{473}/R_{525} et R_{572}/R_{525} pour les echantillons de viande contenant à leur surface 100 % de un entre les trois de derivées de myoglobin. Supposant la relation lineàr entre les changes des relations et la concentration du pigment, les formules pour la calculation des proportions de formes de la myoglobin on été calculées. Cette methode peut être appliquée pour la viande paquée aux folie transparentes.

K5:2

ANALYSE DER DERIVATE VON MYOGLOBIN AUF DER OBERFLÄCHE FRISCHEN RINDFLEISCHES

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Auf dem Grund die Reflexionsspektrumanalyse gelang es die Methode der Zahlwert Bezeichnung von Myoglobin Derivate auf Rindfleischoberfläche zu bearbeiten. Die Lichtabsorption wurde bei drei isobestischen Punkten /473, 525, 572 nm/ und 730 nm gemessen. Die Absorption gemessen bei 730 nm ist unabhängig von der Konzentration des Myoglobin, Oxymyoglobin und Metmyoglobin, und representiert die Absorption des Hintergrundes /Fleisch ohne Farbstoff/. Es wurden die Grenzwerte für Verhältnisse R_{473}/R_{525} und R_{572}/R_{525} berechnet für Fleischproben, deren Oberfläche in 100 % durch eine von den dreien Myoglobin Derivate bedeckt waren. Annehmend die lineare Abhängigkeit dieser Verhältnisse hingegen Veränderungen der Farbstoff-Konzentration, fuhrte man Formel zur Berechnung prozentigen Inhalts der Formen des Myoglobin auf Fleischoberfläche aus. Diese Methode kann ebenfalls für Fleisch in durchsiechtiger Folieverpackung Verwendung finden.

ОПРЕДЕЛЕНИЕ ПРОИЗВОДНЫХ МИОГЛОБИНА НА ПОВЕРХНОСТИ СВЕЖЕЙ ГОВЯДИНЫ

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На основании анализа спектров отражения разработан метод определения количества производных миоглобина на поверхности говядины. Измерено абсорбцию света в 3 изобестических пунктах / 473, 525, 572 нм / и прп 730 нм. Абсорпция при 730 нм не зависит от концентрации миоглобина, оксимиоглобина и метмиоглобина, а представляет абсорбцию основания / мясной ткани без красителей /. Определено предельные величины для соотношений P_{473} / P_{525} и P_{572} / P_{525} для образцов мяса, покрытых на поверхности в 100 % одной из трёх производных миоглобина. Принимая линейную зависимость этих соотношений от изменений концентрации красителей выведены формулы для определения процентного содержания отдельных форм миоглобина на поверхности мяса. Метод можна применять также для мяса завернутого в прозрачную фольгу.

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INTRODUCTION

The colour of meat depends on proportion of myoglobin derivatives present in its surface layer. Several methods of meat pigment analysis has been already published / 1 - 4 / but none of them is fully satisfactory. The most promising attepmts are based on reflectance spectrophotometry and a calculation of K/S ratios. These however are significantly influenced by factors other than pigment concentration /e.g. pH, marbling/ and can lead to erroneous results.
The following method presents a new approach to the problem, which may help to overcome some of the difficulties met in the analysis of raw meat pigments.

MATERIAL AND METHODS

Samples for experiments were taken from fresh beef obtained from slaughterhouse after 48 hours of chilling. All of them had pH in the range from 5.6 to 5.8. For reflectance measurements slices of meat about 1 cm thick were cut.
It was assumed that fresh surface of sample, 30 minutes after cutting was covered in 100 % by oxymyoglobin. To obtain samples covered by myoglobin slices of beef were packed under vacuum and stored for 12 hours at 0 - 4°C. Metmyoglobin was formed by dipping meat samples in 1 % solution of $K_3Fe/CN/_6$ for 2 to 3 hours. The results of conversion were controlled by examining the reflectance spectra.
The reflectance of samples was measured with Pye-Unicam SP 1800 spectrophotometer with reflectance attachment SP 890. Spectra were recorded in the 360 - 760 nm region and absorbance units were used.

RESULTS AND DISCUSSION

The calculation method of myoglobin derivatives at the surface of beef is based on that given by Broumand /1/. However, since in reflectance spectrophotometry the thickness of absorbing layer is not known and light is also scattered employment of only absorption coefficients is not possible. Therefore the use of K/S values was introduced /2/ on the assumtion that S is reasonably constant in visible range and hence they should be proportional to the amount of pigment. In table 1 are given K and S coefficients determined by the method described by Judd and Wyszecki /5/. The results show that above assumption is not true as S coefficient varies significantly with a light wavelength.
The present method of calculation is based on the observation that each reflectance spectra curve has a minimum in the range of 720 - 740 nm. This minimum occurs always exactly in this range regardless of the myoglobin form present at the surface of meat, chemical treatment of sample /H_2O_2, $K_3Fe/CN/_6$ /, pH of meat and the amount of intermuscular fat /marbling/. It is suggested that a reflectance at 730 nm represents a background reflectance i.e. the reflectance of pigment-free meat. As it was observed its value is independent of myoglobin derivatives concentration but is related to pH of meat and amount of marbling.

Considering the additive properties of K and S coefficients /5/ one may regard a difference / $R - R_{730}$ / for $\lambda < 730$ nm as being proportional to a light absorption by myoglobin derivatives. At the isobestic point at 525 nm this difference is proportional to the total concentration of oxymyoglobin, myoglobin and metmyoglobin in the surface layer of meat /1,4/. In order to determine proportions of individual pigments to each other one has to measure the reflectance at two isobestic points more, namely, at 473 and 572 nm. At 473 nm oxymyoglobin and metmyoglobin have got the same molar absorption coefficient and at 572 similar situation occurs for myoglobin and oxymyoglobin /1/. Thus, the difference / $R_{473} - R_{730}$ / is not affected by changes in proportion of met - to oxymioglobin, the difference / $R_{572} - R_{730}$ / by changes in proportion of mio- to oxymyoglobin and / $R_{525} - R_{730}$ / is constant as long as the total concentration of all three myoglobin derivatives is not changed. When the first two differences are related to the third one, the obtained two ratios become independent of total pigment concentration:

$$a_1 = \frac{R_{473} - R_{730}}{R_{525} - R_{730}} \qquad /1/, \qquad a_2 = \frac{R_{572} - R_{730}}{R_{525} - R_{730}} \qquad /2/$$

The a_1 index reaches its minimum at 100 % of myoglobin and maximum when only metmyoglobin and/or oxymyoglobin are present at the surface of meat. The a_2 index reaches its minimum in a presence of 100 % metmyoglobin and maximum when only myoglobin and/or oxymyoglobin are present. Limiting values of these indices are given in table 2. They were calculated from measurements performed on samples covered predominantly by one of the myoglobin forms.

Assuming linearity of a_1 and a_2 changes with the changes of pigment proportions one can calculate sums /myoglobin + oxymyoglobin/ and /metmyoglobin + oxymyoglobin/ and finally the percentage of individual constituents:

$$\%/OXY + MET/ = /a_1 - a_1^{min}/ \frac{100}{a_1^{max} - a_1^{min}} \qquad /3/$$

$$\%/OXY + MIO/ = /a_2 - a_2^{min}/ \frac{100}{a_2^{max} - a_2^{min}} \qquad /4/$$

$$\%/MIO/ = 100 - /OXY + MET/ \qquad /5/$$

$$\%/MET/ = 100 - /OXY + MIO/ \qquad /6/$$

$$\%/OXY/ = /OXY + MIO/ - /MIO/ \qquad /7/$$

After substituting into equations /3/ and /4/ values from table 2 we obtain:

$$\%/OXY + MET/ = 294\ /a_1 - 0.66/ \qquad /8/$$

$$\%/OXY + MIO/ = 213\ /a_2 - 0.74/ \qquad /9/$$

Precision of the method can only be estimated at 0 and 100 % points. Standard errors and confidence limits are given in table 2. The lowest precision was observed in a case of samples covered in 100 % by metmyoglobin. The precision of limiting values calculated from measurements performed on samples covered by myoglobin and oxymyoglobin is higher. The confidence limits in this case are $\pm$ 5 % /n = 4, P = 0.95/. Similar precision would be expected for estimation of pigments on fresh beef without a visible discolourations. Results of calculation can be affected to some extent by changes of background reflectance within visible range of spectrum. This however cannot be corrected as there is no method avaiable yet to prepare meat pigment-free without

destroying its structure.
To illustrate an application of the method in table 3 are given results of pigment analysis on the surface of vacuum packed meat and stored at 0°C during 6 weeks period. The reflectance measurements were made on slices of meat removed from a bag, 30 minutes after cutting a sample. There is remarkable difference in proportion of oxymyoglobin to myoglobin in samples with low /5.5 - 5.8/ and high pH /over 6.3/.
Full account of the experiments will be published later.

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Table 1 Results of calculation of K and S coefficients

Sample number	Estimated parameter	Wavelength, nm 730	572	525	473
1	K	0.8010	1.7387	1.9666	1.8566
	S	0.6222	0.2809	0.3617	0.5507
	K/S	1.2873	6.1897	5.4371	3.3713
	R_∞	0.2302	0.0699	0.0782	0.1133
2	K	0.9891	1.7020	0.8720	2.1213
	S	0.5977	0.1980	0.1374	0.5327
	K/S	1.6548	8.5959	6.3464	3.9821
	R_∞	0.1955	0.0519	0.0684	0.1014
3	K	0.8075	1.5628	1.7932	1.6798
	S	0.5331	0.1432	0.2189	0.3506
	K/S	1.5147	10.9134	8.1918	4.7912
	R_∞	0.2074	0.0419	0.0546	0.0870
4	K	0.7129	2.090	2.190	2.2146
	S	1.3830	0.450	0.6948	1.200
	K/S	0.5154	4.6444	3.1519	1.8455
	R_∞	0.3768	0.0893	0.1222	0.1815
5	K	1.099	1.560	1.790	0.1671
	S	0.7195	0.142	0.2195	0.0361
	K/S	1.5274	10.9859	8.1548	4.6288
	R_∞	0.2062	0.0417	0.0547	0.0895

Table 2 Values of a_1 and a_2 indices and their statistical parameters

	a_1 OXY + MET		a_2 OXY + MIO	
	0 %	100 %	0 %	100 %
Mean value	0.66 /24/	1.00 /32/	0.74 /13/	1.21 /26/
Standard error	0.02	0.04	0.04	0.02
Confidence limits /n = 4, P = 0.95/	0.02	0.04	0.05	0.02
As above, in % of pigment	5	12	10	4

Table 3 Results of myoglobin derivatives analysis in vacuum packed meat stored at 0°C

Days of storage	pH 5.5 - 5.8			pH over 6.3		
	OXY	MIO	MET	OXY	MIO	MET
1	79	13	8			
8	88	1	11	59	18	18
21	76	7	17	50	47	13
33	88	5	7	64	27	9
42	79	13	8	47	45	8