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## **EFFECT OF PRESSURE AND SAMPLE WEIGHT ON FREE WATER CONTENT IN BEEF ESTIMATED ACCORDING TO GRAU-HAMM METHOD USING COMPUTER IMAGE ANALYSIS**

**Summary.** The aim of the study was to evaluate the influence of sample weight and pressure on free water content (FWC) values of beef as well as the usefulness of computer image analysis program Nikon NIS-Elements BR 2.20 for measurement of areas of pressed meat sample and liquid stains. The FWC was determined in samples weighted 0.3, 0.5, 0.7 and 1.0 g pressed for 5 min by 1, 2, 5 kg weights. Application of the program enabled fast and easy measurements of stains. Statistical analysis showed that pressure affected FWC values stronger than sample weight. FWC values ranged from 12.02 to 25.61%. Newman-Keuls test distinguished six homogenous groups of mean values of FWC, among which the most numerous group contained mean values of FWC obtained when weight 1 kg and sample weight 0.5, 0.7, 1.0 g and weight 2 kg and sample 0.7 and 1.0 g were used. Thus, it is possible to compare the same results obtained with the use of different pressure and sample weight.

**Key words:** free water, beef, Grau-Hamm method, computer image analysis

### **Introduction**

Computer image analysis (CIA) is a consistent, rapid and economic technique which can be used in scientific research and food industry (BROSNAN and SUN 2002). CIA was used for a non-destructive monitoring of Iberian ham maturation (ANTEQUERA et AL. 2007), measurement of visible fat in muscles (ALBRECHT et AL. 2006, FAUCITANO et AL. 2005), identification of meat origin and assessment of the quality of meat and meat products (BASSET et AL. 2000, CHANDRARATNE et AL. 2006) or salt distribution in dry-cured hams (VESTERGAARD et AL. 2005). Also the structure or even micro-structure of meat and meat products can be investigated by CIA (DU and SUN 2006, HULLBERG and

BALLERINI 2003, RINGKOB et AL. 2004, WEGNER et AL. 2000). CIA programs might provide information about quality and fat content based on colour evaluation of meat (LU et AL. 2000) and fish (MARTY-MAHÉ et AL. 2004, MISIMI et AL. 2007). CIA technique is also useful in free water estimation in meat. One of the common methods used for free water content (FWC) or water-holding capacity (WHC) determination is proposed by Grau-Hamm (IRIE et AL. 1996). FWC is calculated from areas of pressed meat sample and liquid. Traditionally areas of meat sample and liquid were measured by planimetry, but the method was not very precise, laborious and time-consuming. IRIE et AL. (1996) showed that video image analysis is more efficient method of area measurement than planimetry. Also PIPEK et AL. (2005) successfully applied computer image analysis program for area measurement in Grau-Hamm method.

The study was designed to investigate the influence of a sample weight and pressure on free water content values of beef. Also the usefulness of the computer image analysis program Nikon NIS-Elements BR 2.20 for measurement of the pressed meat sample and liquid areas in Grau-Hamm method was evaluated.

## Material and methods

Free water content was estimated according to Grau-Hamm method (HAMM 1986). Beef (*muscles semitendinosus*) was minced twice (net size 3 mm). Then meat samples weighting 0.3, 0.5, 0.7 and 1.0 g were placed on a filter paper (POCH S.A., Poland) between two glass plates and pressed with 1, 2 and 5 kg weights for 5 min. The paper surface with pressed meat sample and liquid areas was photographed with a digital camera (Fujifilm Fine Pix M603, Japan) mounted on a photographic bench 34 cm above the paper illuminated with two lamps. The angle between a paper and lighting source was 30°. Pictures saved as \*.jpgs were analysed with Nikon NIS-Elements BR 2.20 program (Nikon, Tokyo, Japan), which enabled direct measurement of areas of pressed meat sample and liquid. For each variant (sample weight – pressure) 15 repetitions were performed. In order to determine water absorbability of the filter paper on five pieces of the paper 0.1 cm<sup>3</sup> of distilled water were poured. Water stains were measured and amount of water per 1 cm<sup>2</sup> of paper ( $W$ ) was calculated according to the formula:

$$W = \frac{a \cdot b}{P} \text{ (cm}^3\text{)}$$

where:  $a$  – amount of poured water (cm<sup>3</sup>),  $b$  – 1 cm<sup>2</sup>,  $P$  – mean area of liquid stain (cm<sup>2</sup>).

Then a coefficient necessary to convert the liquid area to water content per cent in a meat sample was estimated with the formula:

$$K = \frac{W}{C} \cdot 100$$

where:  $C$  – meat sample weight (g).

With known liquid ( $L$ ) and meat sample ( $M$ ) areas, free water content in meat sample was calculated according to the formula:

$$FW = K \cdot (L - M) (\%)$$

The statistical analysis was conducted using two-ways ANOVA (Statistica 7.1., StatSoft, Inc. 2005). In order to distinguish homogenous groups of means Newman-Keuls test was applied at significance level  $P = 0.05$ .

## Results and discussion

Computer image analysis programs have already been used for measurement of stains area in Grau-Hamm method. IRIE et AL. (1996) who used video image analysis system (PIAS, LA-525) performed a several changes of stain's image such as converting the picture to binary image, skeletonizing of meat boundary image and flooding of image prior to measurements of areas. PIPEK et AL. (2005) who worked on Lucia 3.52b program (Laboratory Imaging Ltd., Praha, Czech Republic) estimated FWC in beef and pork. They reported that measurements were more precise when pressed meat sample was physically removed from the filter paper surface. They also proposed to use scanner to obtain images of higher sharpness and contrasts. The program used in the present study, NIS-Elements BR 2.20, was an efficient tool in measurements of liquid and meat sample areas. The measurement of both areas was performed with the use of simple commands such as erode, dilate, without converting images to binary ones or physical separation of meat and paper.

Values of FWC in beef depended on sample weight and pressure and ranged from 12.02 to 25.61% (Table 1). Generally the highest values of FWC were achieved when higher pressure was used, but a sample weight also affected free water values. These observation were confirmed by univariate significance tests (Table 2), where higher values of  $F$  were noted for "pressure" factor than for "sample weight" one.

Variation coefficients were the highest when the sample was pressed with 1 kg weight compared to the values obtained when meat was pressed with 2 and 5 kg (Table 1). These finding suggested that usage of 1 kg weight gives more diverse results than usage of 2 and 5 kg weights.

Table 1. Free water content (%)  
Tabela 1. Zawartość wody wolnej (%)

Sample weight (g)	Weight (kg)								
	1			2			5		
	mean	SD	C	mean	SD	C	mean	SD	C
0.3	18.09	4.96	0.27	19.69	5.85	0.30	25.61	4.08	0.16
0.5	12.02	2.40	0.20	20.48	3.03	0.15	24.54	4.59	0.19
0.7	13.87	3.91	0.28	16.27	3.50	0.22	23.90	5.09	0.21
1.0	12.82	4.78	0.37	15.25	3.12	0.20	23.66	4.72	0.20

SD – standard deviation, C – variation coefficient (%).

Table 2. Univariate significance tests for free water content

Tabela 2. Jednowymiarowe testy istotności dla zawartości wody wolnej

Effect	F	P
Sample weight	6.964	0.000191
Pressure	87.905	0.000000
Sample weight × pressure	2.484	0.024998

Table 3 shows homogenous groups of mean values of free water content obtained with different sample weights and pressure. It was noted that results obtained when 1 kg weight and sample 0.5, 0.7, 1.0 g and 2 kg weight and 0.7, 1.0 g sample were used did not differ statistically. All results obtained with the use of 5 kg weight can be compared to each other regardless the sample weight.

Table 3. Newman-Keuls test,  $\alpha = 0.05$

Tabela 3. Test Newmana-Keulsa,  $\alpha = 0,05$

Sample weight (g)	Pressure (kg)	Mean free water content (%)	Homogenous groups						
			1	2	3	4	5	6	
0.5	1	12.02	***						
1.0	1	12.82	***						
0.7	1	13.87	***						
1.0	2	15.25	***		***				
0.7	2	16.27	***		***	***			
0.3	1	18.09			***	***	***		
0.3	2	19.69				***	***		
0.5	2	20.48					***	***	
1.0	5	23.66		***					***
0.7	5	23.90		***					***
0.5	5	24.54		***					***
0.3	5	25.61		***					***

## Conclusions

1. Both sample weight and used pressure influenced free water content values, however as the statistical analysis showed it was possible to compare values obtained under different conditions.

2. The computer image analysis program Nikon NIS-Elements BR 2.20 is an efficient tool, which may be used to measure stain areas in assay of free water content according to Grau-Hamm method.

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## WPLYW WIELKOŚCI NACISKU I MASY PRÓBKII NA WYNIK OZNACZANIA ZAWARTOŚCI WODY WOLNEJ W WOŁOWINIE METODĄ GRAUA-HAMMA Z UŻYCIEM KOMPUTEROWEJ ANALIZY OBRAZU

**Streszczenie.** Celem pracy było zbadanie wpływu takich czynników, jak wielkość nacisku i masa próbki, na wynik zawartości wody wolnej w mięsie wołowym oraz określenie przydatności programu do komputerowej analizy obrazu Nikon NIS-Elements BR 2.20 do przeprowadzania pomiarów powierzchni nacieku i sprasowanej próbki mięsa. Zawartość wody wolnej w wołowinie

oznaczano, stosując próbki o masie 0,3, 0,5, 0,7 i 1,0 g poddane naciskowi odważników o masie 1, 2, 5 kg. Dzięki zastosowaniu programu do komputerowej analizy obrazu możliwe było dokonanie pomiarów powierzchni otrzymanych plam w sposób szybki i łatwy. Analiza statystyczna wyników wykazała, że silniejszy wpływ na otrzymane wyniki wywierał nacisk niż masa próbki. Otrzymane wartości zawartości wody wolnej kształtowały się w przedziale od 12,02 do 25,61%. Za pomocą testu Newmana-Keulsa wyróżniono sześć grup wyników nie różniących się pomiędzy sobą ( $P > 0,05$ ). Najbardziej liczna grupa zawierała wyniki otrzymane przy nacisku 1 kg próbek o masie 0,5, 0,7, 1,0 g oraz przy nacisku 2 kg próbek o masie 0,7 i 1,0 g. Stwierdzono tym samym, że możliwe jest porównywanie wyników uzyskanych dla różnych wielkości próbek i zastosowanego nacisku.

**Słowa kluczowe:** woda wolna, wołowina, metoda Graua-Hamma, komputerowa analiza obrazu

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