Method for estimating marrow content of mechanically separated meat

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Mechanical separation of meat and bone often results in the incorporation of red bone marrow into the meat. Red bone marrow added to muscle in large amounts can change functional properties of muscle protein as well as color, texture, flavor and nutritional value of meat products. Therefore, a method for determining amount of red marrow in mechanically separated meat is needed. Muscle adjacent to the cervical vertebrae of 18-month-old steers and red marrow removed from the cervical vertebrae by centrifugation were used to obtain marrow/muscle mixtures containing 50, 60, 70, 80, 90 and 100% muscle. Total pigment in each muscle/marrow mixture was replicated six times and the myoglobin and hemoglobin fractions were determined.

Simple correlations between percent muscle in the sample and mg hemoglobin, hemoglobin:myoglobin ratio, percent myoglobin, percent hemoglobin and total pigment were -.99, -.96, .92, -.90 and -.99 respectively. Regression equations developed from the values for total pigment and percent muscle in muscle/marrow mixtures were used to estimate amount of muscle in different samples of mechanically separated meat. Mechanically separated meat ranged from 64 to 81% muscle. The remaining portion of the mechanically separated meat ranged from 16 to 30% marrow and from 3 to 5% bone powder. Estimating amount of marrow in mechanically separated meat from total pigment content is a faster and more accurate method for estimating marrow content than other methods we have investigated. Equations must be developed within anatomical location, age and species because of variability in pigment content for muscle and marrow.

Methode für das Berechnen des Knochenmarkinhalts von mechanisch getrenntem Fleisch

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Mechanische Trennung von Fleisch und Knochen hat oft als Folge die Einverleibung des roten Knochenmarks ins Fleisch. Die Beifügung von größeren Mengen des roten Knochenmarks ins Muskelfleisch kann die Funktionseigenschaften des Muskelproteins wie auch die Farbe, die Beschaffenheit, den Geschmack und den Nahrungswert der Fleischprodukte ändern. Daher braucht man eine Methode für das Berechnen des Inhalts von rotem Knochenmark in mechanisch getrennten Fleischprodukten. Neben den Nackenwirbeln von anderthalbjährigen Ochsen liegendes Muskelfleisch und rotes Knochenmark, das von den Nackenwirbeln durch Schleudern entnommen wurde, wurden gebraucht, um Mischungen von Mark und Muskelfleisch zu erhalten, die 50, 60, 70, 80, 90 und 100% Muskelfleisch enthielten. Bestimmung des gesamten Farbstoffs in jeder Muskelfleisch-Knochenmarkmischung wurde sechsfach durchgeführt und die Fraktionen von Myohämatin und Hämoglobin festgestellt.

Einfache Wechselbeziehungen zwischen dem Prozentsatz Muskelfleisch in der Probe und mg von Hämoglobin, dem Verhältnis Hämoglobin:Myohämatin, dem Prozentsatz Myohämatin, dem Prozentsatz Hämoglobin und dem gesamten Farbstoff waren der Reihe nach: -,99; -,96; ,92; -,90 und -,99. Rückfallgleichnisse wurden gebraucht, um den Muskelfleischinhalt von verschiedenen Proben des mechanisch getrennten Fleisches zu bestimmen. Muskelfleischinhaltswerte des mechanisch getrennten Fleisches lagen zwischen 64 und 81%. Die übrigen Teile des mechanisch getrennten Fleisches hatten Markinhaltswerte zwischen 16 und 30% und Knochenpulverinhaltswerte zwischen 3 und 5%. Das Berechnen des Knochenmarkinhalts in mechanisch getrenntem Fleisch aufgrund des gesamten Farbstoffinhalts ist schneller und genauer als andere Methoden, die wir untersucht haben. Gleichungen müssen wegen der Veränderlichkeit der Farbstoffinhaltswerte für Muskelfleisch und Knochenmark je nach anatomischer Lage, Alter und Spezies entwickelt werden.

II.2

Méthodes pour estimer le contenu en moelle de la viande obtenue par séparation mecanique

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La séparation de la viande des os par procédé mecanique entraîne souvent un mélange de la moelle rouge des os avec la viande. De grandes quantités de moelle rouge des os ajoutées aux fibres musculaires peuvent transformer les propriétés fonctionnelles des protéines des muscles, ainse que la couleur, la texture, le goût et la valeur nutritive de la viande. On a donc besoin d'une méthode propre à déterminer la quantité de moelle rouge qui se trouve dans la viande obtenue à partir de la séparation mécanique. Nous nous sommes servis de tissus musculaires adjacents aux vertèbres cervicales de bouvillons de 18 mois, ainsi que de moelle rouge prélevée sur les vertèbres cervicales par centrifugation, cela afin d'obtenir des mélanges muscle/moelle contenant 50, 60, 70, 80, 90 et 100% de tissu musculaire. Le pigment total dans chaque mélange muscle/moelle a été répété six fois et les fractions de myoglobine et d'hémoglobine ont été déterminées.

De simples corrélations entre le pourcentage de tissu musculaire dans l'échantillon et l'hémoglobine (mg), le rapport hemoglobine: myoglobine, le pourcentage de myoglobine, le pourcentage d'hemoglobine et le pigment total étaient -.99, -.96, .92, -.90 et -.99 respectivement. Nous nous sommes servis d'équations régressives pour estimer, à partir du pigment total, la quantité de tissu musculaire de differents echantillons de viande obtenue par séparation mécanique. La viande séparée par procédé mécanique contenait de 64 à 81% de tissu musculaire. Le restant comportait de 16 à 30% de moelle et de 3 à 5% de poudre d'os. La méthode qui consiste à estimer la quantite de moelle contenue dans la viande separee mecaniquement a partir de la teneur totale en pigment est une methode plus rapide et plus exacte que d'autres methodes que nous avons etudiees. Des équations doivent être établies en tenant compte des régions anatomiques, de l'âge et de l'espèce, cela en raison des variations de la teneur en pigment des tissus musculaires et de la moelle.

МЕТОД ВЫЧИСЛЕНИЯ СОДЕРЖАНИЯ КОСТНОГО МОЗГА ПРИ МЕХАНИЧЕСКОЙ ОБРАБОТКЕ МЯСА

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В результате механической обработки мяса и костей, часто красный костный мозг смешивается с мясом. Добавка большого количества красного костного мозга может изменить функциональные свойсва белка мышц, цвет, текстуру, вкус и питательную ценность мясных продуктов. Поэтому, необходим метод для определения количества красного костного мозга в мясных продуктах при их механической обработке.

Для смеси костного мозга с мясом содержащую 50, 60, 70, 80, 90, 100% мяса мышц было использовано мясо мышц смежанных с шейными позвонками от 18-ти месячного кастрированного бычка и красный костный мозг получен из шейных позвонков путём центрифугирования. Тотальный пигмент в каждой смеси мышцы с костным мозгом повторялся шесть раз: определились частицы миоглобина и гемоглобина.

Простые корреляции между процентом мышцы в пробе и в мг гемоглобина, коофициент гемоглобин: миоглобин, процент миоглобина, процент гемоглобина и тотальный пигмент были соответственно: -.99, -.96, -.92, -.90 и -.99.

Регрессивные уравнения были использованы для вычисления количества мышцы в разных пробах механически обработанного мяса. Содержание мяса мышц было в пределах 64-81%. Остальная часть механически обработанного мяса состояла из 16 до30% костного мозга и от 3 до 5% молотой кости.

Вычисление количества костного мозга в механически обработанном мясе из тотального содержания пигмента является более быстрым и более точным методом чем другие изученные нами методы. Необходимо найти уравнения учитывающие анатомию, возраст и породу животного в виду изменчивости содержания пигмента в мышцах и в костном мозгу.

Method for estimating marrow content of mechanically separated meat

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Introduction

A brighter color and a finer, more uniform texture in products containing mechanically deboned meat (MDM) is due to the addition of red bone marrow and to the elimination of connective tissue which is devoid of red pigments (Field, 1976). Although red marrow may enhance the appearance of meat products, potential problems do exist. A flavor distinctive of bone marrow is present in products made from meat which is high in marrow. In addition, Chang and Field (1977) believe that much of the variation in protein quality of MDM is a result of variable amounts of collagen and marrow present. Color variation in the finished product, due to variability in the amount of red marrow in MDM, can also be a problem for meat processors.

Color, flavor and nutritional value of products containing MDM, could be better controlled if a rapid, inexpensive method for determining the amount of red marrow in MDM was available. Marrow is high in hemoglobin and devoid of myoglobin (Field et al., 1978) whereas the major pigment in muscle is myoglobin (Warriss and Rhodes, 1977). The purpose of this investigation was to determine hemoglobin, myoglobin, hemoglobin:myoglobin ratios and total heme pigments in mixtures containing known amounts of marrow and muscle. Heme pigment concentration was then used to estimate the amount of muscle or marrow in MDM.

Experimental

Muscle adjacent to the cervical vertebrae of Good grade steers and red marrow removed from cervical vertebrae by centrifugation, (Field et al., 1978) were used to obtain marrow/muscle mixtures containing 50, 60, 70, 80, 90 or 100% muscle. Muscle and marrow were analyzed for moisture and ash by standard methods (A.O.A.C., 1970). Calcium and iron were determined by atomic absorption spectrophotometry as outlined by the Perkin-Elmer Corp. (1964). Total pigment in muscle and in each marrow/muscle mixture was replicated six times spectrophotometrically and myoglobin and hemoglobin in each mixture were replicated six times using sephadex gel filtration. The method used was similar to that of Franke (1973) and Warriss (1976).

Ten g frozen meat chips were weighed into a Virtis homogenization cup and the cup was lowered into liquid nitrogen and held there for 60 seconds. After the meat was completely frozen, a small amount of liquid nitrogen was allowed to flow into the cup. The cup was then placed on the Virtis homogenizer and the sample ground for 30 seconds. The resulting powder was carefully transferred to a Waring Blendor cup and homogenized with 40 ml cold 0.5 M Tris buffer, pH 7.9. The resulting slurry was centrifuged at 4 C for 15 min. at 8800 x g. After centrifugation the extract was filtered through Whatman No. 4 filter paper. Three 0.2 ml aliquots of the filtrate were each diluted with a 10 ml volume of Tris buffer and the absorbance measured at 416 nm. An average of the three readings was used to calculate total pigment. For the separation of myoglobin and hemoglobin, 2 ml of the filtrate were added to 0.5 g sucrose, mixed thoroughly and 0.2 to 0.3 ml was carefully applied to a sephadex (G-50 superfine) column. Fractions of 1.1 ml were collected and the absorbance of each fraction measured at 416 nm. Fraction number vs absorbance was plotted. Calculations for total pigment, hemoglobin and myoglobin were performed according to Franke (1973) and Warriss (1976).

Simple correlations, regression equations and standard errors of estimate giving the relationships between percentage of muscle in the muscle:marrow mixtures with mg/g hemoglobin, hemoglobin:myoglobin ratio, percentage myoglobin, percentage hemoglobin and mg/g total pigment were determined. The regression equations were then used to estimate the percentage of muscle in seven different samples of mechanically deboned beef.

The mechanically deboned beef was obtained from the vertebral comumn and ribs of Good grade steers using a Beehive mechanical deboner with .46 mm holes in the cylinder. Moisture, ash, calcium, iron, total pigment, hemoglobin and myoglobin were determined on the mechanically deboned beef samples utilizing the same procedures outlined for the muscle/marrow mixtures.

Results and Discussion

Means and standard deviations for pigment concentrations in muscle and in muscle/marrow mixtures show that total pigment, hemoglobin and the hemoglobin:myoglobin ratio increased and myoglobin percentage decreased as the amount of muscle in the mixture decreased (table 1). These findings were expected since previous work (Field et al., 1978) has shown that marrow is high in hemoglobin and devoid of myoglobin and that the major pigment in muscle is myoglobin (Warriss and Rhodes, 1977; Bodwell and McClain, 1971). The standard deviations give an indication of the precision in the methodology since six pigment determinations were obtained on the same muscle/marrow mixture. Each pigment in each of the muscle/marrow mixtures has a low standard deviation indicating that variability in the data due to methodology was minimal.

Simple correlations, regression equations and standard errors of estimate are shown in table 2. The highest correlations and lowest standard errors of estimate for the relationship between muscle percentage and pigment concentration in the muscle/marrow mixtures were found for total pigment and for mg of hemoglobin. It appeared from plots of the data and from the data in table 1 that some curvilinear function might better describe the relationships between muscle percentage and hemoglobin percentage, myoglobin percentage or the hemoglobin:myoglobin ratio. Possible curvilinear relationships were not tested because the high linear correlations with total pigment and mg hemoglobin indicated that these determinations could be used to accurately estimate the amount of muscle in muscle/marrow mixtures. In addition, total pigment, even when run in triplicate as was done for each of the six total pigment determinations, is a much easier and faster determination and requires

less equipment than determination of myoglobin or hemoglobin.

As shown in table 3, seven MDM samples were also used for heme pigment determination. The MDM samples were similar in moisture, ash and calcium to others from our laboratory (Field, 1976).

It is evident that no close relationship between the amount of calcium in MDM and the amount of heme pigment exists. Yield of mechanically separated meat, size of grinder plate through which the meat and bone is ground prior to deboning and design of the deboning equipment may influence calcium content but have very little relationship to amount of heme pigment. In contrast, age of animal, anatomical location of the bone, and amount of fat in the bone marrow may influence total heme pigment concentration in the bone marrow of mechanically separated meat but have little influence on calcium content. Therefore, the amount of red marrow present in MDM must be determined by a parameter such as heme pigment which is independent of calcium.

Estimated muscle percentages in MDM ranged from 64.3 to 80.7% in the seven lots of MDM when the equation using total heme pigments was used. Since dry, fat free bone which has had the marrow removed is approximately 25% calcium (Miller et al., 1977; Dickerson, 1962; Furagouri, 1976; and VanKempen et al., 1976), one can use the calcium percentages in table 3 to show that bone in the MDM samples ranged from 3 to 5% of the product leaving 16 to 30% as marrow. The figures for percentage marrow in MDM are in good agreement with other calculations. The U.S.D.A. (1977) reported that 16 MDM samples from beef averaged 4.09 mg/100 of iron. The figures in table 3 show that one composite sample of lean steer beef contained 1.81 mg/100g of iron while red marrow from the vertebrae of the same animals contained 12.48 mg/100g of iron. Therefore, 79% muscle (1.81 mg/100g of iron) and 21% marrow (12.48 mg/100g of iron) would be required to obtain a mixture of the two containing 4.09 mg/100g of iron. The muscle and marrow percentages would need some slight adjustment to account for the small amount of bone present in MDM. Nevertheless, it is evident that the percentage of muscle and marrow in MDM, when based upon iron content of muscle and marrow, is in the same range as that found in the present study using the equation for total heme pigments. Since the U.S.D.A. (1977) figure of 4.09 mg/100g iron in beef MDM is similar to that of Kruggel and Field (1977); since 1.81 mg/100g of iron for young steer beef muscle is similar to reported values (Watt and Merrill, 1963; Jenkins, 1977), and since 12.48 mg/100g of iron in red marrow agrees with other iron values for marrow (Blum and Zuber, 1975; Garcia, 1957; Seitz, 1969), we believe 21% marrow in MDM is a realistic figure. Previous work with other MDM samples using acrylamide gel electrophoresis also shows marrow percentages in MDM of about the same magnitude (Field et al., 1978).

Determination of marrow content of MDM from iron may not be accurate because: (1) some non-heme iron-containing compounds such as ferritin and hemosiderin are found in marrow in variable amounts; (2) some iron may come from the mechanical deboner and (3) iron content of muscle is variable (Jenkins, 1977; Blum and Zuber, 1975). Other potential methods of marrow determination in MDM such as acrylamide gel electrophoresis (Field, et al., 1978) and determination of porphyrins (Miller et al., 1978) have limitations in terms of accuracy. Total heme pigment concentration in muscle and marrow, like iron and the porphyrins, also varies by anatomical location, species and age of animal. In addition, variation in muscle pigments occur within animals of the same age

(Warriss and Rhodes, 1977; Rickansrud and Henrickson, 1967; Wilson, et al., 1959).

Variation in total pigment concentration in muscle and marrow can be minimized by determining total pigment per g of protein. Recent data from our laboratory (Sanchez, 1979) indicate that the total pigment concentration in red marrow from calves, steers and cows is almost constant when expressed as mg of total pigment per g of protein. Total pigment in muscle from calves, steers and cows increased (7.14, 15.47 and 29.54 mg total pigment/g protein respectively) with increases in age but much of the variation within animals of the same age is eliminated. Variation in total pigment content of muscles between individual animals would be minimized in MDM since bones from many animals are composited to produce each lot. Therefore, we believe that variation in total pigment per g of protein can be used to reflect variation in the amount of muscle or red marrow present in MDM. Equations such as the one developed for estimating muscle in MDM from cervical vertebrae of Good grade steers in this study would be most accurate if developed within meat processing plants using bone marrow and muscle from bones typical of those mechanically deboned within that plant. Accurate figures on marrow content of MDM would be useful in formulation of processed meat and in describing MDM to prospective buyers. Knowing the amount of marrow present in MDM should be useful because variation in the amount of marrow present can change functional properties of MDM as well as color, texture, flavor and nutritional value of meat products which contain MDM. Since pigment concentration remains constant with freezing rate (Nocito et al., 1973) and storage period (D. N. Rhodes, personal correspondence; Sanchez, 1979) regulatory control of the amount of red marrow present in MDM might be possible if equations based upon mg total pigment per g of protein were developed within anatomical location, age and species and if appropriate tolerance limits were established.

Summary

Mechanical separation of meat and bone often results in the incorporation of red bone marrow into the meat. Red bone marrow added to muscle in large amounts can change functional properties of the mixture as well as color, texture, flavor and nutritional value of meat products. Therefore, a method for determining amount of red marrow in mechanically separated meat is needed. Muscle adjacent to the cervical vertebrae of 18-month-old steers and red marrow removed from the cervical vertebrae by centrifugation were used to obtain marrow/muscle mixtures containing 50, 60, 70, 80, 90 and 100% muscle. Total pigment in each muscle/marrow mixture was replicated six times and the myoglobin and hemoglobin fractions were determined.

Simple correlations between percent muscle in the sample and mg hemoglobin, hemoglobin:myoglobin ratio, percent myoglobin, percent hemoglobin and total pigment were -.99, -.96, .92, -.90 and -.99 respectively. Regression equations developed from the values for total pigment and percent muscle in muscle/marrow mixtures were used to estimate amount of muscle in different samples of mechanically separated meat. Mechanically separated meat ranged from 64 to 81% muscle. The remaining portion of the mechanically separated meat ranged from 16 to 30% marrow and from 3 to 5% bone powder. Estimating amount of marrow in mechanically separated meat from total pigment is a faster and more accurate method for estimating marrow content than other methods we have investigated. Equations must be developed within anatomical location, age and species because of variability in pigment content for muscle and marrow.

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Table 1
Means and standard deviations for pigment concentration in muscle and in muscle/marrow mixtures[a]

Pigment	Percentage muscle in muscle/marrow mixtures: 100 Mean	100 SD	90 Mean	90 SD	80 Mean	80 SD	70 Mean	70 SD	60 Mean	60 SD	50 Mean	50 SD
Total pigments, mg/g	3.63	.16	5.32	.24	7.02	.29	9.23	.44	11.23	.46	13.14	.58
Hemoglobin, %	23.98	1.91	53.26	2.86	62.31	3.88	73.73	1.05	78.32	2.39	81.58	2.76
Myoglobin, %	76.01	1.91	46.74	2.87	36.01	.92	26.27	1.05	21.68	2.39	18.44	2.76
Mb/hb ratio	.32	.03	1.15	.14	1.78	.06	2.80	.16	3.66	.51	4.54	.87
Hemoglobin, mg/g	.87	.10	2.84	.25	4.49	.16	6.81	.41	8.80	.49	10.72	.69

[a] Each mean represents the average of six determinations

Table 2
Relationships between percentage muscle (Y) in muscle/marrow mixtures and pigment concentration (X)

Pigment	"r"*	Equation	Standard error of estimate
Total pigment, mg/g	-.99	Y=117.37 - 5.12 X	2.0
Hemoglobin, mg	-.99	Y=103.61 - 4.97 X	2.0
Hb/mb ratio	-.96	Y=101.14 - 10.98 X	4.5
Hemoglobin, %	-.90	Y=118.56 - .71 X	7.7
Myoglobin, %	+.92	Y= 44.97 + .80 X	6.7

* All significant ($P<.01$), N = 36

Table 3

Mechanically deboned meat composition and pigment concentration

Source	Yield, %	Moisture, %	Ash, %	Ca, %	Iron, mg/100g	Total heme pigment, mg/g	Hemoglobin, mg/g
Bullock flat bones[a]	40	46.09	2.50	.82	3.30	7.16 (80.7)[c]	4.77
Steer flat bones	40	38.92	3.61	1.33	4.27	7.90 (76.9)	5.96
Steer neck bones	48	36.89	2.36	.76	5.44	10.23 (65.0)	6.01
Steer neck bones	45	33.94	2.23	.74	4.12	8.68 (72.9)	4.73
Bullock flat bones	33	44.38	3.03	1.01	6.22	10.37 (64.3)	9.16
Steer flat bones	33	35.80	2.84	1.10	4.44	9.61 (68.2)	7.36
Bullock fat bones	30	46.80	2.43	.88	4.81	8.86 (72.0)	6.99
Lean muscle[b]		71.97	.85	.01	1.81	3.61	.69
Marrow[b]		55.56	1.56	.23	12.48	18.88	18.69

[a] Flat bones include all bones from the vertebral column plus the ribs.

[b] Lean muscle was obtained next to the vertebrae and red marrow was from within the vertebrae of Good grade steers.

[c] Figures in parenthesis represent the estimated percentage of muscle in MDM based upon the equation for total pigment in table 2. Percentage of marrow can be approximated by adding 4 times the calcium percentage to the muscle percentage and subtracting from 100.