Rapid Estimation of Muscle Proteins in Beef–Vegetable Protein Mixtures

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Two different methods for measuring the amount of beef proteins were developed as a means of determining the amount of vegetable proteins added to meat products. These methods are based on estimating creatine and on the buffer-extractable protein content of the mixture. Phosphocreatine-breakdown products determined as creatine were present in fairly constant amounts in lean beef, but were absent in vegetable proteins. The content of protein extractable in a potassium iodide–phosphate buffer was fairly high in beef, but very low in textured-vegetable proteins. These two parameters were significantly correlated to the muscle protein content of the mixture and appear suitable for routine analysis.

The increasing demand for protein has resulted in the use of vegetable protein as a supplement to or a partial replacement of muscle protein in food. Current development has shown that minced meat and hamburger can be extended with vegetable protein to yield a product acceptable to consumers and up to 30% of the meat is being replaced by hydrated soy products in some blends (Shelef and Morton, 1976). Since the use of less expensive vegetable or microbial protein could lead to the replacement of expensive muscle protein as well as lower the nutritive value of the product, a regulatory limit on amounts added may be required. As yet, however, a practical and simple method for analyzing extended products has not been developed. Methods proposed to date are based on such techniques as acrylamide gel electrophoresis (Lee et al., 1975), chromatography (Rangeley and Lawrie, 1976; Zarkadas, 1975), histochemical staining (Flint and Lewin, 1976), microscopy (Jewell, 1974), and serology (Hargreaves et al., 1974) and thus require special equipment and tedious and lengthy procedures. They are not satisfactory, therefore, for rapid and routine analysis of a large number of samples. Additionally, most of these methods are qualitative and not suitable for quantitative work.

Since the processing conditions, such as heat, pressure, alkali, or acid treatments, used in the manufacture of textured nonmeat proteins cause denaturation, and since the nature and degree of this denaturation can vary with the processing conditions as well as with the nature of the product, a method based on the identification of a specific nonmeat protein will have limited application (Rangeley and Lawrie, 1976). On the other hand, a method based on the direct estimation of meat proteins in these products would considerably simplify this problem and would be highly feasible. In the present study, therefore, tests were made to develop a simple method based on the presence in meat of phosphocreatine (PC), a high-energy phosphate reserve, and myofibrillar proteins for the determination of beef protein in uncooked beef–vegetable protein mixtures. The presence of PC is restricted to animal tissue, while the presence of myofibrillar proteins in plant tissue has not been shown.

EXPERIMENTAL SECTION

Minced meat samples for analyses were prepared from Adductor, Biceps femoris, Gastrocnemius, Gluteus medium, Gracilis, Longissimus dorsi, Obliquus abdominis internus, Psoas major, Rectus femoris, Semimembranosus, Semitendinosus, and Vastus lateralis muscles from the round and short loin cuts of three different carcasses (choice steers, A-1 carcass weight 250–300 kg). Four different textured-vegetable protein samples were used: (a) Pepplus, soy–wheat protein (Industrial Grain Products Limited, Montreal, Quebec, Canada), (b) Heat-Saver (La Petite Bouchère Foods, Montreal, Quebec, Canada), (c) Promate-III, and (d) GL-301 Soya-Protein Concentrate (The Griffith Laboratories Limited, Scarborough, Ontario, Canada). For preparing beef–vegetable protein mixtures, textured-vegetable protein samples were hydrated with twice their weight of water and mixed with minced beef to replace 0, 10, 20, 30, 40, and 50% (w/w) of the mixture. They were mixed thoroughly and made into patties (50 g each) and analyzed immediately or after freezing. For freezing, each patty was vacuum packaged in a Cry-o-Vac bag, frozen in an air blast (8–13 m/min) at −40 °C, and kept at −40 °C until analyzed. For analysis, frozen patties were cut up into small pieces before thawing and ground in a mortar with a pestle in order to prevent loss of creatine via the drip. A total number of 96 patties of meat–vegetable mixtures were analyzed. All analyses were made in duplicate for each patty.

For measuring the total soluble protein content, 2-g samples of the patties were ground with a pestle and sand (5 g) and made up to about 75 ml with 1.1 M potassium iodide–0.1 M potassium phosphate buffer (pH 7.4). The extract was allowed to stand at 2 °C for a few hours and then centrifuged (3000*g*, 20 min). After the collection of supernatant, the residue was resuspended in 20 ml of the same buffer and recentrifuged. The two supernatants were pooled and made up to 100-ml volume with buffer. The above buffer was selected for this work because (a) the presence of KI caused the dissociation of actomyosin and led to a more complete extraction of soluble proteins from the muscle tissue and (b) textured-vegetable proteins were least extractable in this buffer. Additionally, it has been shown that the extractability of muscle proteins in this buffer does not vary with tenderness or the maturity of beef (Dikeman et al., 1971). Total and extractable nitrogen estimations were made by the standard micro-Kjeldahl method as well as by the Biuret reaction (Robson et al., 1968).

Phosphocreatine-breakdown products were estimated colorimetrically as creatine by the α-naphthol–diacetyl reaction (Wong, 1971). For extraction, 10-g samples of the patties were homogenized in a blender with 50 ml of trichloroacetic acid solution (Cl_3CCOOH 10%, w/v) and centrifuged at 3000*g* for 20 min. The supernatant was collected and the residue washed twice with 50 ml of Cl_3CCOOH solution. The supernatant and the washings were combined and made up to 200 ml with Cl_3CCOOH solution, allowed to stand at room temperature for 2 h, filtered, and analyzed. The test solution contained 1 ml

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Table I. Total Nitrogen (TN), Extractable Nitrogen, and Creatine Content of Several Beef Muscles and Textured-Vegetable Protein

Protein source	Total nitrogen, mg/g	Extractable nitrogen		Creatine	
		mg/g	% of TN	mg/g	% of TN
Beef					
Adductor	34.7	29.1	84	4.06	11.7
B. femoris	33.6	26.1	78	3.48	10.4
Gastrocnemius	35.1	25.3	72	4.06	11.6
Gluteus medius	33.1	27.0	82	3.66	11.1
Gracilis	33.4	26.9	81	3.86	11.6
L. dorsi	32.2	24.6	76	3.55	11.0
O. abdominus int.	33.2	28.6	86	3.50	10.6
P. major	31.5	26.3	83	3.30	10.5
R. femoris	32.8	26.5	81	3.60	11.0
Semimembranosus	34.8	28.6	82	3.84	11.0
Semitendinosus	31.3	22.4	72	3.42	10.9
V. lateralis	32.7	25.9	78	3.86	11.8
Vegetable[a]					
GL-301	34.7	5.3	15	<0.01	<0.01
Meat Saver	27.3	2.6	10	<0.01	<0.01
Pepplus	30.3	2.6	9	<0.01	<0.01
Promate III	27.9	3.1	11	<0.01	<0.01

[a] Values are based on samples containing 75% water.

Table II. Creatine and Buffer-Soluble Nitrogen Content of Mixtures Containing Various Amounts of Beef and Vegetable Proteins (Values Are Averages for 16 Samples Containing Four Different Types of Textured-Vegetables Proteins)[a]

Vegetable protein added, %	As % of total nitrogen	
	Creatine	Buffer-soluble N
No addition	10.47 (10.30-11.04)	80 (76-83)
10	9.53 (9.24-9.94)	73 (71-74)
20	8.45 (8.11-8.94)	67 (62-69)
30	7.39 (7.10-7.78)	58 (55-60)
40	6.14 (6.05-6.96)	52 (50-53)
50	5.21 (4.57-5.69)	46 (44-48)

[a] Range is shown in parentheses.

of sample, 5 ml of NaOH–Na_2CO_3 solution (60 g of NaOH + 160 g of Na_2CO_3/l.), 3 ml of α-naphthol solution (freshly prepared, 1%, w/v, in NaOH–Na_2CO_3 solution), 2 ml of diacetyl solution (freshly prepared, 0.1%, v/v, in water), and water to a 25-ml total volume. This test solution along with appropriate blanks and standards were allowed to stand at room temperature for 20 min and read at 520 nm.

RESULTS AND DISCUSSION

Total nitrogen, extractable nitrogen, and creatine content of beef and textured-vegetable protein are shown in Table I. In beef muscles, creatine was present in fairly constant amounts, but it was absent in textured-vegetable protein. In postmortem muscle creatine originates from the breakdown of phosphocreatine during the onset of rigor, but is fairly stable once rigor is complete. The amount of nitrogen extracted in KI–phosphate buffer varied between 24.6 and 29.1 mg/g of sample or 72–84% of the total nitrogen in various beef muscles, and 2.6–5.3 mg/g of sample of 9–15% of the total nitrogen in textured-vegetable proteins.

The amount of creatine as well as nitrogen extracted in KI–phosphate buffer depended directly on the amount of meat proteins present in meat–vegetable protein mixtures (Figure 1, Table II). The amount of creatine (expressed as percent of total nitrogen) as well as the amount of nitrogen extracted (expressed as percent of total nitrogen) were significantly correlated ($\gamma = 0.97$, $n = 96$, SD = 0.48 and $\gamma = 0.96$, $n = 72$, SD = 3.3, respectively) to the amount of beef protein present in the mixture. Calculations on the basis of sample weight instead of total nitrogen were also significantly correlated ($\gamma = 0.96$, $n = 96$ and $\gamma = 0.97$, $n = 72$, respectively). Analysis of mixtures containing similar amounts of beef gave results reproducible within ±5% by the creatine method and within ±7% by the extractable nitrogen method. This variation was caused largely by samples containing fibrous or granulated types of textured-vegetable proteins, which were difficult to blend uniformly with meat. Between samples containing powdered vegetable proteins, the variation was much smaller. Assessment of proteins on a weight basis eliminates the need of determining total nitrogen. However, determination of the total nitrogen content is helpful in assessing the total protein content of the sample, as well as the quantity of fat and any filler present. Both Biuret and Kjeldahl procedures were found suitable for nitrogen determination.

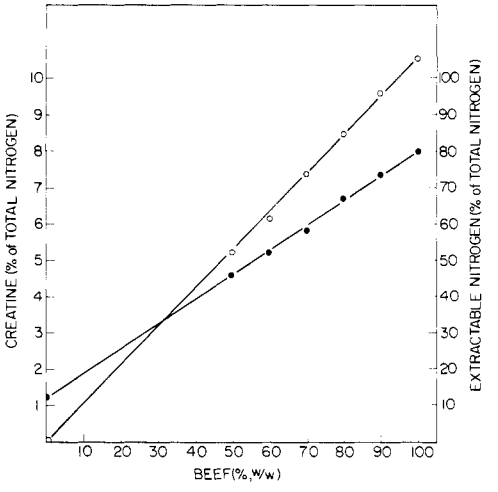

Figure 1. Relation between extractable nitrogen (●), creatine (○), and animal protein content of minced beef–textured vegetable protein mixtures. (Each point is an average of 16 samples. Results are significant at 1% level.)

The results show that parameters based on creatine and extractable-protein nitrogen content, either separately or in combination, are suitable indicators of the amount of beef proteins present in the mixture. Estimation of both of these parameters is fairly accurate yet sufficiently simple to permit the analysis of a large number of samples in a relatively short period of time. Neither requires sophisticated equipment or elaborate and cumbersome procedures like gel electrophoresis or column chromatography.

The method based on creatine determination is perhaps preferable because of its simplicity and speed. However, the use of the solubility method in conjunction with the creatine method would be helpful in verifying the amount of animal protein and in ruling out the possibility of adulteration of mixtures with exogenous creatine. For example, the ratio between extractable nitrogen (mg/g) and creatine (mg/g) is fairly constant in different beef muscles as well as in mixtures, varying between 7 and 9. A value lower than 7 would indicate adulteration of blends with exogenous creatine, while a value higher than 9 would indicate extensive loss of weep or drip as a result of mishandling of the meat samples. These parameters also would appear suitable for analyzing mixtures containing meat proteins other than beef. However, further work is required to determine the amount and variation in the content of PC breakdown products in poultry and pork muscles, and other edible animal tissues, as well as the effect of heating and cooking on this parameter.

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Reaction of Proteins with Formaldehyde in the Presence and Absence of Sodium Borohydride

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Lysozyme and ribonuclease reacted at approximately equal rates with small amounts of ^{14}C-labeled formaldehyde to give stable derivatives containing 1–2 equiv of label. After 6 h of incubation of lysozyme with formaldehyde at room temperature and pH 9, monomers, dimers, and trimers were identified, along with traces of higher polymers. The monomers retained nearly full enzymatic activity and contained slightly over 1 mol of irreversibly bound formaldehyde. In contrast, on reductive methylation of casein by formaldehyde and $NaBH_4$, no unreacted formaldehyde was detected by chromotropic acid in less than 4 s after addition of the aldehyde. Side reactions, which lead to polymers in the absence of the $NaBH_4$, have not been seen under the conditions of reductive methylation. Low rates of chymotryptic hydrolysis of reductively methylated casein and bovine serum albumin were not due to compounds formed from such side reactions, but were shown to be due to product inhibition by the peptides formed by hydrolysis of the proteins.

Formaldehyde (HCHO) has long been extensively used in large amounts in the tanning and related industries (Feeney et al., 1975). The pharmaceutical industry has also used it for nearly 50 years in the preparation of toxoids, which are injected into humans. Although the total usage of microbial toxoids produced with HCHO is extensive, toxoids are administered to any one human individual only in relatively small amounts. More recently, however, HCHO is being used for products fed animals that are eventually eaten by humans. The current investigations on use of HCHO treatment to encapsulate lipids inside protein shells for ruminant feeds (Scott et al., 1971; Hemsley et al., 1973; Reis and Tunks, 1973) raise the possibility that significant amounts of the reaction products between the protein and HCHO could find their way into humans via initial incorporation into ruminants. Sheep fed HCHO-treated casein had four- to sixfold higher serum levels of N^{ϵ}-methyllysine than did sheep fed casein which had not been treated with HCHO (Hemsley et al., 1973).

In spite of these uses of HCHO in feeding animals for human consumption, there is apparently only meager information on the nature of the compounds produced on treating proteins with HCHO (Feeney et al., 1975). Grisolia et al. (1975) have also recently focused attention on the possible role of aldehydes on the toxic effects of alcohols. Several alcohol dehydrogenases and a retinol dehydrogenase were rapidly inactivated by low concen-

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