EFFECTS OF ELECTRICAL STIMULATION, MIXER TYPE, PROCESSING TIME AND MIXING TEMPERATURE ON AMOUNT OF PROTEIN EXTRACTION IN MIXED LONGISSIMUS PORK MUSCLE

Efectos de estimulación eléctrica, tipo de mezclador, tiempo de elaboración y temperatura de mezclado sobre la cantidad de proteína extraída del músculo longissimus en cerdos

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ABSTRACT

Loins from 14 pig carcasses were randomly assigned to Electrical Stimulation (ES) treatments (sixteen loins stimulated and 11 loins not stimulated) and processed either 2 h (hot processing), 48 h (conventional processing) or after 19 days (processing after aging) postslaughter. Twenty-five chunks of porcine longissimus dorsi samples, each providing constant volume and surface, were mixed one hour at 24°C vs 4°C using two mixer types (models K-5A vs K-45 Kitchen-aid food preparers). Protein exudate formed on meat surfaces as a result of mixing muscle in the presence of 1% salt, 0.3% sodium tripolyphosphate and 5% water was collected to determine myofibrillar and sarcoplasmic protein extraction efficiency in relation to four different processing variables. Mixer type exerted a significant (P<.05) effect on exudate development and myofibrillar protein extractability. The model K-45, equipped with a smaller bowl capacity than the K-5A, developed a larger amount of exudate and extracted more myofibrillar protein (P<.05) probably due to more frictional energy and agitation intensity. Sarcoplasmic protein concentration was lower (P<.05) in the exudate recovered from non-stimulated samples and also from aged samples (P<.01). Mixing temperature did not affect (P<.05) protein extractibility. Processing time x mixer type and mixer type x mixing temperature interactions were significant (P<.05) for sarcoplasmic protein concentration in the exudate. Also, two-way (processing time x mixing temperature) and three-way interactions (ES x mixer type x mixing temperature) for the amount (mg) of myofibrillar protein extracted per gram of muscle were significant (P<.05) indicating the interdependence of all these treatments on the protein extractability variables. Some recommendations for similar studies are given in terms of the experimental procedure and protein extraction measurements.

Key words: Electrical stimulation, myofibrillar protein, mixer type, pork, Sarcoplasmic protein.

RESUMEN

Se utilizaron 14 lomos de cerdos asignados aleatoriamente a los siguientes tratamientos: Estimulación Eléctrica (ES): 16 lomos estimulados y 11 no estimulados; Tiempo de Elaboración (TiempE): 2 horas (proceso en caliente), 48 horas (proceso convencional) y 19 días después del sacrificio. Se mezclaron las 25 muestras por una hora a dos Temperaturas de Mezclado (TempM): 24°C vs 4°C usando 2 Tipos de Mezclador (TipM): modelos K-5A y K-45. El exudado de proteína en la superficie de la carne al mezclar el músculo con 1% de sal, 0.3% de tripolifosfato de sodio y 5% de agua fue recogido para determinar la eficiencia de extracción de la proteína sarcoplasmática y miofibrilar para los 4 tratamientos. El análisis reveló efectos significativos (P<.05) de TipM sobre el desarrollo de exudado y la extracción de proteína miofibrilar. El K-45, que es un equipo con un recipiente de menor capacidad que el K-5A, logró una mayor cantidad de exudado y más extracto de proteína miofibrilar (P<.05) probablemente

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debido a una mayor energía de fricción e intensidad de agitación. La concentración de proteína sarcoplasmática fue menor (P<.05) en el exudado obtenido de muestras no estimuladas. TempM no afectó (P>.05) la extracción de proteína. Las interacciones TempM x TipM y TiempE x TipM fueron significativas (P<.05) para la concentración de proteína sarcoplasmática en el exudado. Las interacciones TiempE x TempM y Es x TipM x TempM para la cantidad de proteína miofibrilar extraída (mg por gramo de músculo) fueron significativas (P<.05) indicando la interdependencia de todos estos tratamientos sobre las variables de extracción de proteína.

Palabras claves: Estimulación eléctrica, proteína miofibrilar, proteína sarcoplasmática, cerdos.

INTRODUCTION

The increasing success of restructured meat products has been based on the efficient use of mixing or tumbling meat with salt and phosphates for extraction of salt-soluble proteins. These proteins bind together chunks of meat in order to produce a texture similar to the more desirable steaks and chops [13]. Studies have indicated that salt, phosphates and agitation affect physical properties and the histological structure of meat [2,3,9,11,12,14,20,21]. Most of these studies have been based on massaging as the selected mechanical agitation process to enhance myofibrillar protein extractability. In view of the commercial existence of small food processors with different mixing actions, it would be beneficial to conduct studies on alternative mechanical agitation processes and to compare the effectiveness of equipment and devices for extracting myofibrillar proteins.

Tissue condition exerts a tremendous influence on the physical and chemical state of muscle proteins. There were more salt-soluble proteins in pre-rigor than post-rigor beef [16]. The best time post slaughter for processing pork for restructuring purposes remains unknown.

Temperature of processing and their effect on meat protein extraction has received considerable attention from several workers [1,6,10].

The use of ES* has been combined with hot processing in beef in order to improve acceptability [5], but ES does not appear to improve quality attributes in pork [19]. However, histological changes such as contraction banding, intracellular edema and disintegration of the myofibril after 1 hour post mortem, should have some effect on meat protein extractability. The present study was designed to determine whether ES, processing time, mixing temperature and mixer type influence the amount of protein extracted from pork muscle.

* Electrical stimulation.

** No stimulation.

EXPERIMENTAL PROCEDURE

Fourteen pigs (average weight = 100 Kg) randomly selected from the Texas A & M Swine Center were conventionally slaughtered and used in this study. The principal effects were a comparison of ES vs NS** in combination with three processing times (hot processing, conventional processing and processing after ageing), two mixer types (K-5A vs. K-45 Kitchen Aid food preparers) and two temperatures of mixing (24°C and 4°C).

Carcasses were assigned to one the following stimulation treatments: 1) ES of undressed carcasses (n=8). 2) untreated NS carcasses (n=6). Within 10 min after exsanguination ES carcasses received 17 electrical impulses of 250 V, 2-6 Amp. alternating current of 1.5 sec in duration followed by 1.5 sec current using a Koch-Britton Stimulator 350 unit.

The loins from each carcass were removed within 2 hour post-slaughter. Seven loins were packaged, identified and stored at 1.8°C for 19 days, representing "aged" samples. Twenty loins from 10 carcasses were assigned either to hot processing or conventional processing treatments. The hot processed loins were treated immediately after their removal from the carcass. Excised loins were trimmed of external fat and connective tissue. The longissimus dorsi was diced into 25 chunks and mixed to extract salt soluble muscle proteins. Loins to be conventionally processed were vacuum packaged, identified and stored at 1.8°C for 48 hours at wich time they were prepared in a like manner as the hot processed and aged processed loins.

Muscle protein extraction

Chunking was accomplished by hand cutting in a plexiglass slotted mold container and sample sized into pieces approximately 3 cm x 3.8 cm x 2.5 cm. Twenty five chunks of muscle tissue, weighing 800 to 1000 g each and providing 66.28 cm^2 of muscle surface and 36.1 cm^3 of constant volume were mixed in one of the two types of mixers. Samples were randomly either model No. K-45 or model No. K-5A.

Differences in mixer types mainly refers to bowl size and consequently to intensity of agitation and friction of meat chunks. The agitation speed and the paddles which provided the mechanical energy were equal in both models.

Two temperatures of mixing were used. The samples were randomly assigned either to room temperature (24°C) or cooler temperature (4°C). The exudate that developed during mixing was collected by scrapping (with a spatula) muscles surfaces of individual meat chunks, from the walls of the bowl, from the dough hook agitator and from the hands of the operator. The amount of surface exudate was recorded in plastic containers, and frozen at -12°C until further analysis tray containing crushed ice and using a modified method of Hargus *et al.* [10].

TABLE I

EFFECT OF MIXER TYPE ON EXUDATE AND MUSCLE PROTEIN EXTRACTION

Variable	Mixer type	
	K-5A (n=13)	K-45 (n=14)
Amount of exudate developed, g	20.100[a]	31.100[b]
Amount of exudate g/g muscle	0.021[a]	0.034[b]
Amount of exudate g/cm^2	0.012[a]	0.019b
Myofibrillar protein concentration in exudate, mg/g	48.200[a]	47.700[a]
Total myofibrillar protein extracted, mg	862.800[a]	1,319.900[b]
Total sarcoplasmic protein extracted, mg	971.300[a]	1,501.300[a]
Myofibrillar protein, mg/g muscle	0.900[a]	1.450[b]
Myofibrillar protein, mg/cm^2	0.520[a]	0.800[b]
Sarcoplasmic protein, mg/g muscle	1.030[a]	1.650[b]
Sarcoplasmic protein, mg/cm^2	0.590[a]	0.910a

[a,b] Means in the same row bearing different letters differ (P<.05).

Approximately 3 g of frozen exudate were placed in a pre-cooled (2°C) mortar with the same amount of silica and 5 volumes (15 ml) of cold 2°C) isolating medium (0.1 M sodium chloride, 0.03 M sodium phosphate buffer pH 7.4, 0.001 M ethylenediaminotetraacetate, EDTA, and 0.001 M sodium azide). This was ground to a fine slurry and transferred to a centrifuge tube. The solution was centrifuged at 2,500 x g for 15 min in a refrigerated centrifuge (Beckman model J-21). The supernatant was decanted, the residue resuspended in 5 volumes of isolating medium and centrifuged under the same conditions. The supernatants were combined and total sarcoplasmic protein was determined by the biuret procedure of Gornall [8]. The residue was suspended in 5 volumes (15 ml) of deionized water, centrifuged and the supernatant discarded.

The residue was suspended in 3 volumes (9 ml) of solubilizing solution (23 mM sodium phosphate buffer pH 7.4, 2% sodium dodecyl sulfate, SDS) was centrifuged, the supernatant was poured off and total myofibrillar protein was determined using the biuret procedure of Gornall [8].

Data from the amount of exudate and protein determinations were subjected to Analysis of Variance [15] and Duncan's multiple range test was used as the mean separation technique when main effects were significant (P<.05).

RESULTS AND DISCUSSION

The effects of mixer type on exudate development and muscle protein extraction are shown in TABLE I. The Kitchen-Aid model K-45, equipped with a smaller bowl capacity than the K-5A produced a larger amount of exudate and extracted more myofibrillar protein, expressed either as total, per gram of muscle or per unit of muscle surface (P<.05). A similar trend was observed for the total amount of sarcoplasmic protein. However, the only significant differences were noted when expressed as mg/g of muscle (P<.05). In model K-45, the smaller bowl capacity allows for a higher degree of "planetary action" on the same volume and surface area of meat chunks. These results indicate the importance of mechanical agitation intensity and load effect on protein extraction effectiveness. Other types of mechanical agitation processes such as massaging, appear to aid primarily in the extraction and distribution of myofibrillar proteins from myofibrils locates at the muscle surfaces; the surface tissue destruction caused by massaging would aid in myofibrillar protein extraction, with the amount of such extraction being determined mainly by the ionic strength of the salt solution [18]. These authors suggest that a high ionic strengths such as those present on muscles surfaces when 2% or 3% salt was used, all the myofibrillar protein were solubilized into the exudate before massaging was initiated. In our case, however, at a relatively low ionic strength (1% salt) the role of the mixing action proved to be of significant importance to protein extraction.

Mixing temperature did not affect (P>.05) the variables studied, TABLE II, but it should be noted that exudate development and sarcoplasmic protein extraction appear to be favored by higher mixing temperature, while myofibrillar protein extractability tends to be higher at a cooler temperature (4°C). However, mixing temperature was not significant and only approached statistical significance (P<.10) in the case of sarco-

TABLE II

EFFECT OF TEMPERATURE OF MIXING ON EXUDATE AND MUSCLE PROTEIN EXTRACTION

Variable	Mixing Temperature*	
	24°C (n=15)	4°C (n=12)
Amount of exudate developed, g	27.700	23.400
Amount of exudate g/g muscle	0.031	0.025
Amount of exudate g/cm^2	0.016	0.014
Myofibrillar protein concentration in exudate, mg/g	37.100	49.500
Total myofibrillar protein extracted, mg	1,021.500	1,197.600
Total sarcoplasmic protein extracted, mg	1,402.600	1,050.500
Myofibrillar protein, mg/g muscle	1.120	1.280
Myofibrillar protein, mg/cm^2	0.620	0.720
Sarcoplasmic protein, mg/g muscle	1.550	1.110
Sarcoplasmic protein, mg/cm^2	0.850	0.630

* The effect of mixing temperature was not significant (P<.05).

plasmic protein concentration in the exudate. There were, in fact, a significant mixer type x mixing temperature interaction (P>.05) for sarcoplasmic protein concentration in the developed exudate. While there appeared to be no differences between temperatures under K-5A mixing, the trend is toward a higher concentration of sarcoplasmic protein at 24°C when the smaller bowl (K-45) mixer was used, FIG. 1.

There exists some discrepancy about which is the best temperature of myofibrillar protein extraction. Studies have indicated that temperature above or below 7.2°C do not enhance extraction [6] while that in other study [1] found optimum extraction at a lower temperature range -5°C to 2°C. These divergent conclusions are probably due to different mixing procedures in terms of salt concentrations and stirring intervals used in those studies. At higher chopping temperatures the protein solubility, measured as total extractable protein (%), has been found to decrease, indicating protein denaturation [10]. A decrease in myofibrillar proteins in the exudate as meat temperature increased has also been reported [2].

TABLE III shows the effect of processing time on extraction variables. With the exception of sarcoplasmic protein concentration in the developed exudate, all other variables were not affected (P>.05). The concentration (mg\g) of sarcoplasmic protein in the exudate was lower (P<.01) in pork loins that were processed after 19 days of aging. No significant differences were observed between hot processing and conventional processing (P>.05).

Sarcoplasmic protein solubility is highest after death and either remains unchanged or decreases by some variable

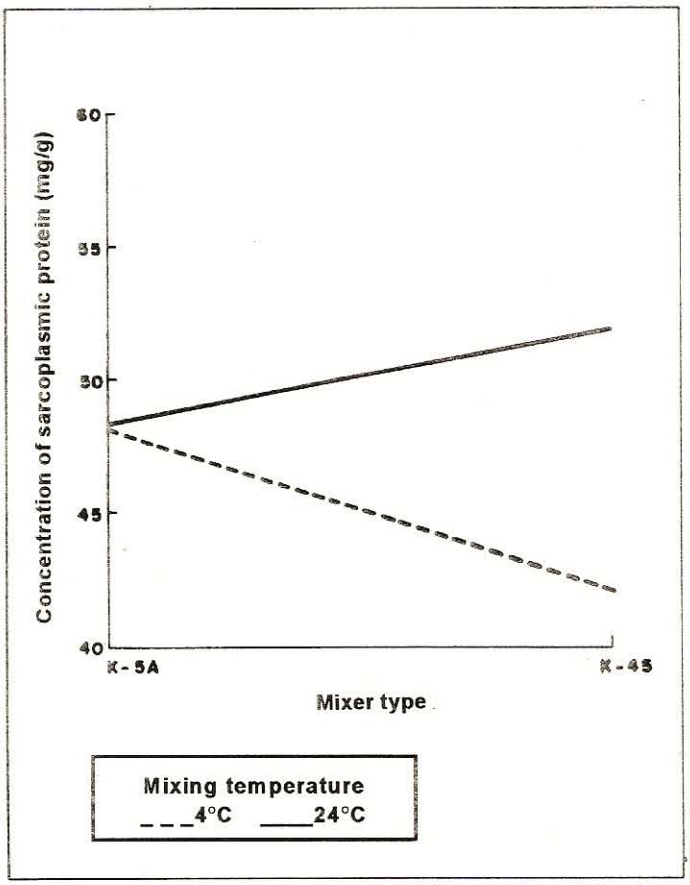

FIGURE 1. EFFECT OF MIXER TYPE ON THE CONCENTRATION OF SARCOPLASMIC PROTEIN IN THE EXUDATE AT TWO MIXING TEMPERATURES (mg/g).

TABLE III

EFFECT OF PROCESSING TIME* ON EXUDATE AND MUSCLE PROTEIN EXTRACTION

Variable	Hot Processing (n=10)	Conventional Processing (n=10)	Processing after ageing (n=7)
Amount of exudate developed, g	28.20	25.00	23.40
Amount of exudate g/g muscle	0.30	0.027	0.025
Amount of exudate g/cm^2	0.017	0.015	0.014
Myofibrillar protein in exudate, mg/g	36.10	46.50	46.40
Sarcoplasmic protein in exudate, mg/g	49.80^a	51.00^a	40.80^b
Total myofibrillar protein, mg	1,030.00	1,169.70	1,099.70
Total sarcoplasmic protein, mg	1,435.90	1,283.50	921.60
Myofibrillar protein, mg/g muscle	1.08	1.29	1.18
Myofibrillar protein, mg/cm^2 muscle	0.62	0.71	0.66
Sarcoplasmic protein, mg/g muscle	1.55	1.43	0.98
Sarcoplasmic protein, mg/cm^2	0.87	0.77	0.55

* Time at which mixing process was initiated. Within 2 h postmortem (hot processing), 48 h postmortem (conventional processing) and 19 days postmortem (processing after ageing).

^{a,b} Means in the same row bearing different letters differ (P<.05).

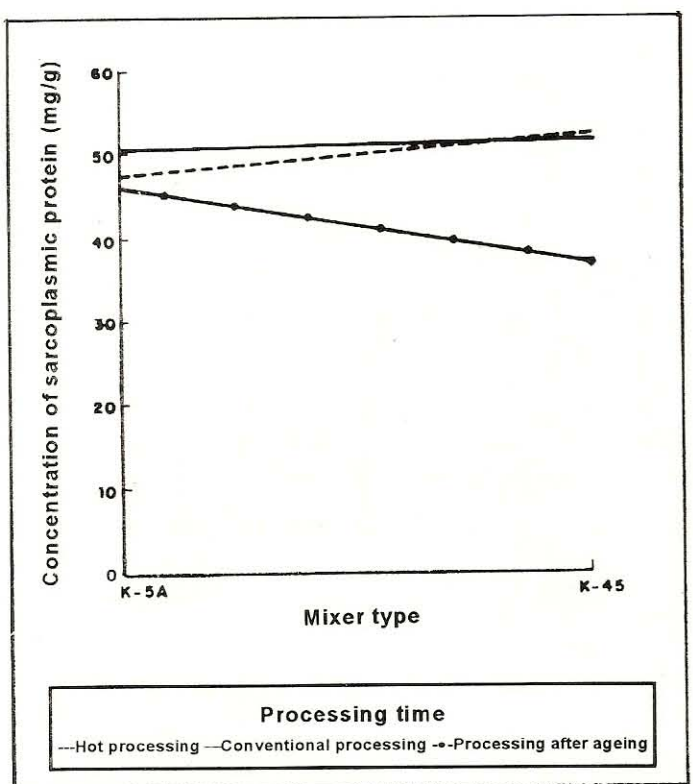

FIGURE 2. EFFECT OF PROCESSING TIME ON THE TOTAL AMOUNT (mg) OF SARCOPLASMIC PROTEIN EXTRACTED WITH TWO TYPES OF MIXERS.

amount during post-mortem storage [7]. Thus, it is possible to hypothesize that slight decreases in protein solubility combined with the higher water holding capacity expected in aged loin samples and unavoidable purge losses occurring during the long storage period of vacuum packaged loins did influence the trend toward a lower extractability of the water soluble proteins from the aged muscle.

The interaction between processing time x mixer type was found to be significant (P<.05) and FIG. 2 shows in inverse trend in the mixer type effectiveness. The K-5A model appeared to be more effective than K-45 for aged samples while the reverse was true for hot processed tissue. Conventionally processed tissue was not affected by mixer type.

There was also a significant (P<.05) processing time x mixing temperature interaction for the amount of myofibrillar protein either expressed as total, per gram of tissue or per cm^2 of muscle surface. In general, hot processing allowed for more myofibrillar protein extraction at the lower temperature while the conventional and aged pork yielded more myofibrillar protein at 24°C temperature, FIG. 3.

The effect of electrical stimulation on exudate development and protein extraction is shown in TABLE IV. With the exception of concentration of sarcoplasmic protein in the exudate, all other extraction variables were not affected by ES (P>.05). A higher (P<.05) concentration of sarcoplasmic protein in the

TABLE IV

EXUDATE AND MUSCLE PROTEIN EXTRACTION FROM ELECTRICALLY STIMULATED PORK

Variable	Non-stimulated (n=11)	Electrically stimulated (n=16)
Amount of exudate developed, g	23.600[a]	27.200[a]
Amount of exudate g/g muscle	0.026[a]	0.029a
Amount of exudate g/cm^2	0.014[a]	0.016[a]
Sarcoplasmic protein in exudate, mg/g	45.000[a]	50.000b
Total myofibrillar protein, mg	1,045.400[a]	1,137.200[a]
Total sarcoplasmic protein, mg	1,041.100[a]	1,387.100[a]
Myofibrillar protein, mg/g muscle	1.160[a]	1.210[a]
Myofibrillar protein, mg/cm^2	0.630[a]	0.690[a]
Sarcoplasmic protein, mg/g muscle	1.150[a]	1.500[a]
Sarcoplasmic protein, mg/cm^2	0.630[a]	0.840[a]

[a,b] Means in the same row different letters differ (P<.05).

exudate was found when ES was used and the same trend was observed for other sarcoplasmic protein extractability indicators and exudate development variables.

The rapid post-mortem glycolysis results in a reduction of both myofibrillar and sarcoplasmic protein extractability [17]. ES accelerates post-mortem glycolysis according to Cross [4]. However, a reduced extractability, as expected, did not occur in this study. ES has proven to be beneficial in enhancing migration and concentration of cure ingredients probably due to the disruption of muscle tissue in pork [11]. Nevertheless, the effects of ES in terms of protein extraction under model systems have not been previously evaluated.

CONCLUSIONS AND RECOMMENDATIONS

Based on protein extraction data obtained from KitchenAid food preparers, the meat "load" as related to bowl capacity is extremely important for muscle protein extraction. The smaller bowl provided for the better results obtained with the K-45 model, probably due to more frictional energy and agitation intensity of the constant muscle mass. Because myofibrillar proteins are of greater significance in the restructuring process, a higher concentration of sarcoplasmic protein in the exudate derived from both ES and aged samples becomes less important.In fact, it is supposed that the sarcoplasmic protein extraction was favored by the water content and the low ionic strength of the added solution.

For research purposes, the interdependence of the processing variables on the protein extractability measurements indicate that for elucidating effects of different treatments and

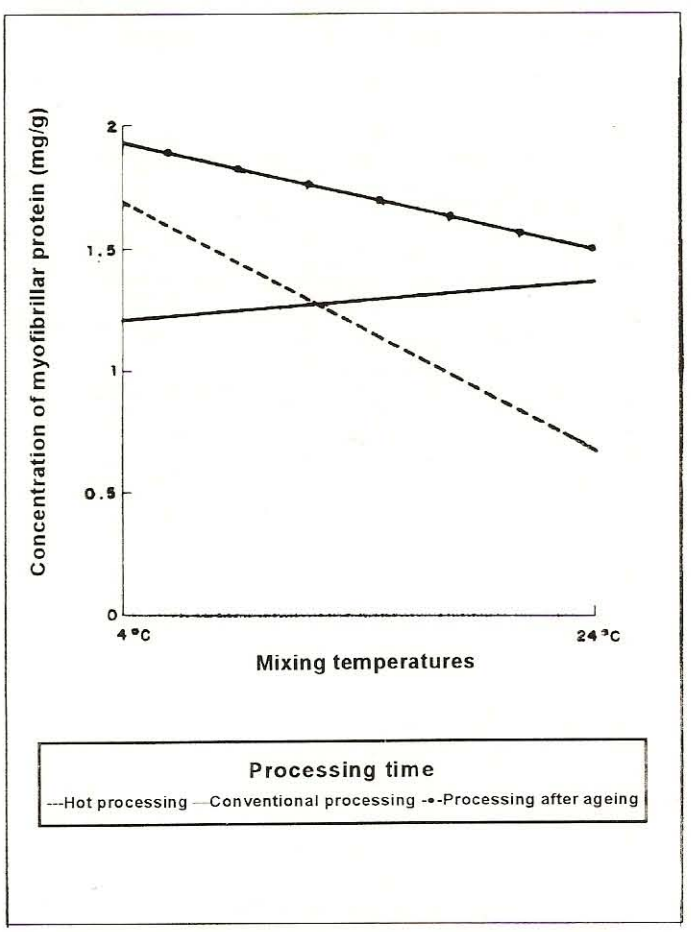

FIGURE 3. EFFECT OF PROCESSING TIME ON THE TOTAL AMOUNT (mg/g) OF SARCOPLASMIC PROTEIN EXTRACTED AT TWO MIXING TEMPERATURES.

better understanding of results it is necessary to simplify the experimental design. Due to de strong mixer type effect observed in this trial it is recommended to perform multiple and simultaneous extractions making use of identical mixer models with an adequate meat load.

Sample sizing by hand cutting is a time-consuming and inaccurate procedure for providing exact volumes of meat chunks. However, the corresponding experimental error must not only be involved in this manual procedure but also in any mechanical dicing because it is inherent to the variability of muscle firmness per se. Hence, an increase in replications to minimize the error is advised.

Expressing extracted proteins per unit of muscle surface is one of the most desirable approaches for monitoring effectiveness of extraction. Nevertheless, under mixing conditions it is practically impossible to accomplish this because during agitation meat chunks adhere to one another eliminating potentially available surfaces for extraction.

So the use of protein or exudate amounts expressed as per weight or volume unit of muscle is a more feasible and meaningful indicator of extractability.

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