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IQ-95-17

Santiago, May 11, 1995

Dr. John Daly, Team Leader
Office of Policy and Programs
Agency for International Development
Washington, DC 20523-1814
USA

Dear Dr. Daly:

In reference to your letter RE: **PSTC Project No. 7.334 "Bases for the Separation of Light and Dark Tissue from Pelagic Fish"**, I would like to inform:

1. Dr. Rutman resigned to the University in 1989 and he is now in private practice. I took over the project.

2. The project was completed very satisfactorily. As a result there were Thesis by Mr. G. Bisso, Mr. C. Nakousi, O. Bono and a publication in Journal of Food Science (copy enclosed), where due credit was given to AID.

3. Financial aspects of the project were significantly delayed by the AID office in Washington. Upon Dr. Rutman's resignation I contacted directly the AID office in Washington (Mr. Lassine). This delay (2 years) in funding costed the University an extra amount of US$ 2,200 which were invoiced to AID but never paid.

Please accept the enclosed documents as our final report.

You may contact me if needed via fax (562-552-4054) or e-mail (jmaguile@ing.puc.cl).

Sincerely,

José Miguel Aguilera
Associate Dean

JMA/cp.

Encl.

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A Research Note

Properties of Dark and Light Tissue from Horse Mackerel (*Trachurus murphyi*)

M. RUTMAN, J.M. AGUILERA, and G. BISSO

ABSTRACT

Mechanical and physical properties of light and dark tissues from horse mackerel (*Trachurus murphyi*) were determined for tissue separation. Raw dark tissue was 1.8 times harder than raw white (1.4 and 0.79 kg-f/g, respectively). Hardness after heating to 20°C decreased slightly but it increased sharply to a value of about 2.4 kg-f/g at 40°C. Tensile strength of the skin was highest at 30°C and a large reduction in the shearing force of the interfibrillar matrix (connective tissue) was also attained at that temperature. Pieces of light muscle were denser (d = 1.082) than those of dark tissue (d = 1.059) and relative separation was attained based on terminal velocities. Heating at 30°C appeared adequate to implement future mechanical disruption processes.

INTRODUCTION

SKELETAL MUSCLE is composed of long, narrow, multinucleated cells or fibers which differ in pigmentation depending on physiological functions. In fish, the dark muscle is located superficially, particularly around the lateral line, and concentrates towards the tail region. A thin layer of intermediate fibers usually separates the dark from the light fibers, compartmentalizing both types of muscle.

Horse mackerel or jurel is a pelagic fish important for the Chilean fishing industry, that ranked third in the world in annual catches in 1984. Over 90% of the four million tons of fish landed that year went into meal production, making Chile the largest exporter in the world. A recent trend in low-quality fish utilization is to separate valuable components and restructure them into human foods (Rutman and Bitran, 1984), surimi being an excellent example (Lee, 1984). In a typical adult specimen of horse mackerel the yield in filleting is around 45% with a proportion of 12% dark and 88% light tissue. Light muscle has an average of 1.8% lipids (4.4 times less than the dark tissue), a higher content of saturated fatty acids (IFOP, 1983), and is also more stable and appealing to consumers than dark tissue (Love, 1974).

The objective of this work was to study some technological bases to develop industrial processes which effectively separate light and dark tissues of horse mackerel.

MATERIALS & METHODS

Fish

Horse mackerel, *Trachurus murphyi* (35–45 cm length) were caught off the port of San Antonio in central Chile by artisan fishermen. Less that 10 hr elapsed from the time the fish were caught until they were frozen to −24°C. Headed and gutted fish were kept in a freezer at that temperature until needed (less than 2 months). Thawing was performed at 5°C for 17 hr. For histological studies (not presented here), only refrigerated samples were used.

Authors Rutman, Aguilera, and Bisso are with the Dept. of Chemical Engineering, Universidad Católica de Chile, P.O. Box 6177, Santiago, Chile.

Effect of temperature

Small pieces of light and dark tissue sampled from each third along the main axis were incubated for 20 min in test tubes with water at 15°, 20°, 30°, 35° and/or 40°C. The incubated pieces were removed from the tubes, blotted in paper towel to remove excess moisture and centrifuged at 5000xg for 20 min. Water-holding capacity (WHC) of the incubated portions was calculated as the percent total weight released as liquid after centrifugation. Thin sections (7–10 μm) from representative samples were treated with picrofucsin for collagen staining and optical microscopy (van Gieson, 1889).

Textural characteristics

Portions (15–20g) of whole fish (including light, dark tissue and skin), as well as light and dark muscle, were incubated in a 1% (w/v) NaCl solution at 20°, 30°, and 40°C. These samples along with an unheated control were assayed for hardness in a 10 cm² OTMS standard cell attached to an Instron Universal Testing Machine under the following conditions: crosshead speed: 5 cm/min; paper speed: 2cm/min; force range: 0–200 kg-f.

Portions of white muscle were tested for intercellular softening by shearing a piece of muscle (with the fibers perpendicular to the shearing force) between a moving and a fixed flat surface. The force required for failure divided by the weight and contact area of the sample was expressed as a shear force (g-f/g cm²). Pieces of skin cut into dumb-bell shapes were assayed for breaking strength in the Instron under the following conditions: Crosshead and paper speed: 10 cm/min; force range: 0–10 kg-f.

Physical and chemical characterization

Proximate analyses of both types of muscle were performed according to AOCS (1970); protein was expressed as Nx6.25. Muscle density was determined by flotation according to Matsumoto et al. (1980). Determination of terminal settling velocity as a function of size and shape was based on the experimental arrangement proposed by Hottovy and Sylvester (1979). Particles cut into different shapes settled in a 1.4 m long, 7.5 cm diameter glass tube filled with water at 20°C.

RESULTS & DISCUSSION

Tissue characterization

The diameter of most white muscle fibers varied between 30 and 80 μm while dark muscle had appreciably smaller fibers, mostly in the 10–30 μm range as evident from light microscopy studies. Individual muscle fibers were separated by sheets of connective tissue or myocommata which were apparently more abundant in dark than in light tissue. Similar structural characteristics are common to a wide range of vertebrate fish species (Howgate, 1979). Also, the fatty layer under the skin appeared to be thicker in areas surrounding the dark portions.

Chemical composition of the light tissue was different from that of dark portions and more similar to that of a lean fish such as halibut. Lipid content in dark tissue was over four times that of light muscle (26.9% and 5.9% d.b., respectively) and protein lower (67% versus 83%, d.b., respectively).

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Effect of heating on tissue disruption

Raw dark tissue was 1.8 times harder than raw white (1.4 and 0.79 kg-f/g, respectively; Fig 1). Hardness decreased slightly at 20°C but increased sharply until a value of about 2.4 kg-f/g was reached for both samples at 40°C. Total hardness determined with the OTMS cell measured the simultaneous effect of compression, shear and extrusion on a composite biological material formed by muscle fibers enshrouded in connective tissue. Fish collagen is less heat stable and more soluble than mammalian collagen, so thermal shrinkage and denaturation of fish collagen occurs at lower temperatures. Heating causes a progressive softening of collagen in the connective tissue and a simultaneous toughening of the myofibrils (Dunajski, 1979; Eide et al., 1982). Eventually, at 40°C most of the collagen had softened and the muscle fibers remained as the sole structural elements in both tissues, explaining the similar hardness value. Denaturation of myofibrillar proteins was related to a decrease in the WHC of the tissue, expressed as water loss, which was 2% at 20°C, 5% at 30°C and 9% at 40°C.

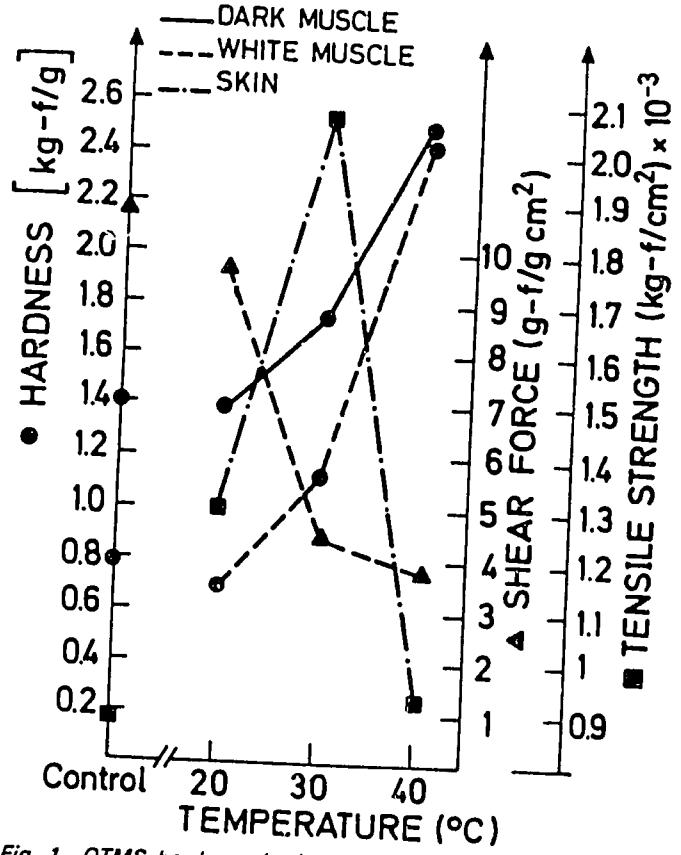

Fig. 1—OTMS hardness (●) of dark and white muscle, shear force of white tissue (▲) and tensile strength of the skin (■) of horse mackerel as a function of temperature.

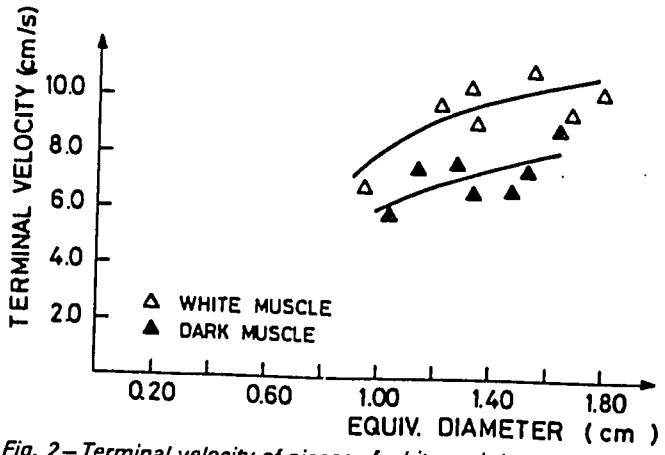

Fig. 2—Terminal velocity of pieces of white and dark tissue from horse mackerel in water at 20°C.

Disintegration of tissue into intact fibers was favored by degradation of the cementing collagen. Softening of the interfibrillar matrix by heating was easily verified qualitatively by rubbing a piece of tissue between the thumb and the index. The top-to-bottom shearing induced by the apparatus previously described simulated this action satisfactorily as the piece always disintegrated along the interfibrillar matrix. Shearing force for white muscle as a function of temperature is presented in Fig. 1. White muscle rapidly lost resistance to shear in the temperature range 20–30°C probably due to the following effects: (1) denaturation of collagen (Sikorski et al., 1984); (2) breakdown of connective material by lyosomal enzymes that exist in high concentration in this tissue (Steiner et al., 1984); (3) some structural breakdown brought about during freezing.

The effect of temperature on the tensile strength of the skin is also shown in Fig. 1. Maximum strength was achieved at 30°C, which is in accordance with Love et al. (1972) who report that the skin of *Chionodraco kathleenae*, a cold water fish, was stronger between 23–29°C. Based on the present findings and the need to preserve as much functionality of the fish protein as possible, heating at 30°C appeared to be adequate to effect satisfactory modifications in the physical properties of the main tissues of horse mackerel to implement future mechanical disruption processes.

Separation

After disruption, separation of the different components, mainly light from dark tissue, must be effected. Density of pieces of light and dark muscles were 1.082 ± 0.002 and 1.059 ± 0.002 g/cc, respectively, which paralleled the fat content. The density of the light tissue was slightly higher than that reported by Japanese workers while that for dark tissue was similar (Matsumoto et al., 1980). Based on this density difference a water classification system was used and the relationship between the terminal velocity and particle diameter for both tissues is shown in Fig. 2. All particles of white muscle larger than 1.20 cm were separated from dark pieces ranging in equivalent diameters from 1.0 to 1.80 cm. Although classification in water based solely on density differences may be a crude pre-separation step, more refined techniques could be used to accomplish further fractionation, among them, classification in a medium of intermediate density, centrifugation and/or color sorting techniques.

CONCLUSIONS

THERE WERE important mechanical and physical differences between different tissues in horse mackerel which may be further exploited to recover intact pieces and/or fibers. Further work should be done on the effect of other variables (pH, salt concentration, use of enzymes) on tissue separation and on the functionality of separated muscle.

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Pontificia Universidad Católica de Chile
Escuela de Ingeniería

PROCESOS DE SEPARACION DE TEJIDOS EN ESPECIES PELAGICAS

Memoria para optar al título de Ingeniero Civil de Industrias Mención Química

Carlos F. Nakousi Salas

Profesores Guía
José M. Aguilera R.
Andrés Hohlberg R.
Max Rutman S.

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Santiago de Chile, 1988

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Memoria para optar al título de Ingeniero Civil de Industrias Mención Química

Giuseppe Bisso Capurro

Profesores Guía
Max Rutman
José Miguel Aguilera.

Santiago de Chile, 1985

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA
DEPARTAMENTO DE INGENIERIA QUIMICA

7.334

ALIMENTOS COMPUESTOS: REESTRUCTURACION DE FIBRAS DE PESCADO

Ornella Bono Ferrando

Memoria preparada bajo la supervisión de la Comisión integrada por los profesores:

José Miguel Aguilera R.

Andrés Francke Sch.

Andrés Hohlberg R.

Quienes recomiendan que sea aceptada para completar las exigencias del título de Ingeniero Civil de Industrias mención Química

Santiago de Chile, 1989

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Mechanical properties of the muscle of Horse Mackerel *(Trachurus murphyi)*

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Submitted for Publication

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MAY 2 2 1995

INTRODUCTION

Horse mackerel (*Trachurus murphyi*) is an abundant pelagic fish of the Pacific coast of South America. The annual catch of approximately 2.4 million tonnes constitutes about 40% of all fish landed in Chile, and nearly 92% is processed into fish meal.

Several alternatives have been proposed to better utilize fish by-catch and pelagic fish presently used for meal production, including: salting (IDRC,1982) mincing (Grantham, 1981; IDRC, 1982), mincing-salting (Nair and Gopakumar, 1986) and mince preserved by combined methods (Aguilera et al., 1991). Recently, the alternative of utilizing these resources in surimi production has also been advocated (Min et al., 1982) but results do not look too promising at present due to low yields and poor quality products (Suzuki, 1986). Another approach, is to fractionate the tissue into fiber bundles or individual muscle fibers and use them in restructured products (Rutman et al., 1988). This latter approach requires a sound scientific basis regarding the effect of processing variables, such as pH and temperature, on the separation of individual fibers in the tissue.

The flesh of a typical bony fish is constructed of adjacent muscle blocks called myotomes, separated by thin membranes known as myocommata, which are composed of connective tissue. The myotomes contain muscle fibers which run approximately parallel to the long axis of the fish, each being surrounded by a membrane containing thin collagenous fibrils. The connective tissue of the myocommata fibers is to a large

extent responsible for the integrity of fish tissue. If the myocommata has been weakened (e.g., by handling or freezing) the myotomes separate giving rise to a textural defect known as gaping (Sikorsky et al 1984) which depends on temperature, pH and salt concentration (Dunajski, 1979). Love et al. (1972) showed that the breaking strength of cod myocommata decreased significantly with an increase in temperature in the range of 20-35°C. Heating is also known to partly dissolve the interfibrillar collagen in muscle (Asghar and Henrickson (1982) which in the case of fish is less abundant than in beef and appreciably less heat stable (Sikorski et al., 1984).

Muscle tissue can be physically modeled as a composite of parallel semi-rigid rods (fibers) bound together by an gel-like interfibrillar matrix of connective tissue. As discussed above, the low connective tissue content of fish leads to easy disintegration of fish flesh on heating, leaving the muscle fibers as the main textural elements in heated fish tissue. Because of this, most popular texture testing instruments are not adequate to study the mechanical properties of fish muscle (Dunajski,1979). One alternative to study changes in the composite connective tissue/ fibers would be to measure the force necessary to induce the flow in the interfibrillar gel matrix, with minimal disruption of the fibers. A back extrusion assay consists of a solid rod which is thrusted downward into a cylindrical cup containing the sample, inducing material to flow in the direction opposite to the movement of the descending rod. In the case of gels, there is an abrupt change in the slope of the force deformation curve caused by the initial rupture of the material (Steffe and Osorio, 1987). If the tip of the plunger is designed as a cylindrical rod

pressing down on a flat sample, significant flow can be induced (Aguilera et al., 1985).

The objective of the present work was to assess the effect of time, temperature and pH on the mechanical properties of horse mackerel tissue. To accomplish this objective a texture cell had to be developed which adequately accounted for the simultaneous softening of the matrix and toughening of the fibers.

MATERIALS AND METHODS

Materials

Bled headed and gutted horse mackerel (*Trachurus murphyi*) were obtained from day boats in San Antonio (lat. 33°40' south) and transported to the laboratory on ice (aprox. 120 km) within 6 - 8 hr from being caught. The size of 18 fish ranged from 35 -40 cm and the weight from 690 - 780 g. In the laboratory they were washed with cold water, placed in polyethylene bags and kept refrigerated at 4°C for 24 hr.

Each assay consisted of three replicates of light or light/dark (mixed) tissue (without skin) cut with a template from the same zone, as illustrated in Figure 1. Dark tissue alone was not evaluated due to the small size and irregular shape of the muscle. The average weight of samples was 8.4 g.

Experiments on softening of interfibrillar matrix

Tissue samples were incubated in 250 mL beakers with 150 mL of a saline solution (1% NaCl w/w) at pH 5.7 and 30°C in a Kotterman

controlled-temperature bath for 15, 30, 60 and 120 min. Contents were rapidly cooled in a ice/water bath and the beakers kept for approximately 1 hr in a 22°C before texture measurement. Tissue samples to study the effect of temperature on softening of interfibrillar matrix were incubated in the same experimental set-up for 30 min at constant temperatures of 20, 30, 40 and 50°C. Similarly, the effect of pH was assessed at a temperature of 30°C in a 0.2 M phosphate-citric acid buffer at the following average pH values: 2.9, 3.8, 4.5, 6.0, 7.0, and 8.1.

Texture cell

The cell designed to assess the effect of time, temperature and pH on softening of the interfibrillar matrix is presented in Figure 2. It was constructed in transparent (poly)-methyl methacrylate and was based on the principle of compression and flow. The descending plunger compresses the sample to a point where flow starts (yield point) due to failure of the interfibrillar matrix. The annulus available for extrusion or flow was 3.5 mm and the cross section of the cavity 26x30 mm. The cell was attached to an Instron Universal Testing Machine model 1122 (Instron Corp., Canton, MA).

Texture measurements

Samples were placed at the bottom of the cavity with fibers oriented along the main axis and the anatomical upper part of the muscle facing upward. This arrangement minimized shearing of fibers by the descending plunger. In light/dark tissue the dark part was first to contact the plunger. Test conditions were: Crosshead speed, 5 cm/min; chart speed, 2 cm/min; force scale, 15 kg; plunger return: 3 mm from the bottom of cavity; temperature, 22°C. A typical force-deformation curve is represented in Figure 2.

RESULTS AND DISCUSSION

A typical force-deformation curve for a texture assay is presented in the inset of Fig. 1. A yield point was clearly identifiable by a brake in the initial slope of the curve and represented the onset of extrusion (Bourne,

1982; Borresen and Alsted, 1983). This phenomenon was corroborated by visual observation of initiation of the upward flow of material. Hence, the force at the yield point was considered as a good indication of the breaking strength of the interfibrillar matrix. No major disruption of the fiber arrangement was observed to occur at this point.

Yield point values as a function of the weight of sample for light and mixed tissue are presented in Figure 3. The weight of samples of mixed tissue and the force values were generally higher than those of light tissue. Szczesniak et al. (1970) reported that the maximum force (F) for different foods tested in texture cell had a different dependence on sample weight (W) depending on whether compression, extrusion or shear was the predominant mechanism. A model of the type $F = B W^n$ (B,n constants) was developed for a cell where compression and shear was important, with n=0, 1, or 2 for extrusion, shear and compression, respectively. Results reported in Figure 3 for 24 points of light and dark/light tissue followed the relationship $F = 288.0 W^{1.2109}$ ($R = 0.95$). Hence, in testing to the yield point the predominant mechanisms appear to be compression and shear of the interfibrillar matrix.

Figure 4 shows the dependence of the yield point for mixed and light tissue on incubation time at 30°C. The purpose of this set of experiments was to determine an appropriate time for the temperature and pH experiments. Yield point values for the mixed muscle were higher than those of light muscle, a result which is in agreement with Rutman et al. (1988). This result is also in accordance with the histological characteristic of the dark muscle which is made up of fibers with smaller

diameter (10-25 µm) than light tissue (50-70 µm), resulting in a more compact structure having a higher proportion of connective tissue (Love, 1980), typical of "slow" or dark muscle (Kovanen et al., 1980). Both curves showed maximum yield points after 60 min of incubation time. The difference in yield points of light and mixed tissue increased up to 60 min incubation time due to gradual softening of the connective tissue. However, the difference was smaller after 2 hr of incubation at 30°C due to solubilization of most of the collagen in the dark tissue resulting in a final strength similar to that of light tissue. (Sainclivier, 1983). Another mechanism of softening may be the hydrolysis of connective tissue by endogenous proteases which act optimally at around 25°C (Gildberg and Raa, 1979). It is also expected that even at 30°C, long term incubation would induce toughening of muscle fibers (Dunajski, 1979).

The effect of incubation at different temperatures on the yield point are presented in Fig. 5. Here the phenomena of collagen solubilization, denaturation and softening on the strength of the interfibrillar matrix are clearly noticed. An abrupt transition occurs in the range 30-40°C. Above 40°C all the collagen has been solubilized and both tissues show similar mechanical properties, which are about 70% of the control. At this point the fibers are the main structural elements left in both tissues, hence there are no differences in yield points. Based on this diagram a temperature of 30°C was chosen for the incubation procedure leading to fiber separation.

Drastic changes in yield point were found when pH was the chosen variable, as shown in Fig. 6. A large shift in yield point value is noticed

between pH 4.5 and 6.0. Probable mechanisms contributing to these large effects of pH are: changes in the water holding capacity of fibers, solubilization of collagen and changes in proteolytic activity of endogenous enzymes. The isoelectric point of actomyosin is 5.0-5.1 (Sainclivier, 1983) and water holding capacity is minimal at this point. Below pH 4 lipids and pigments were removed during incubation, a result similar to that reported by Eide et al., (1982) in studies with capelin (*Mallotus villosus*).

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FIGURE LEGENDS

Figure 1. Location and size of samples in horse mackerel

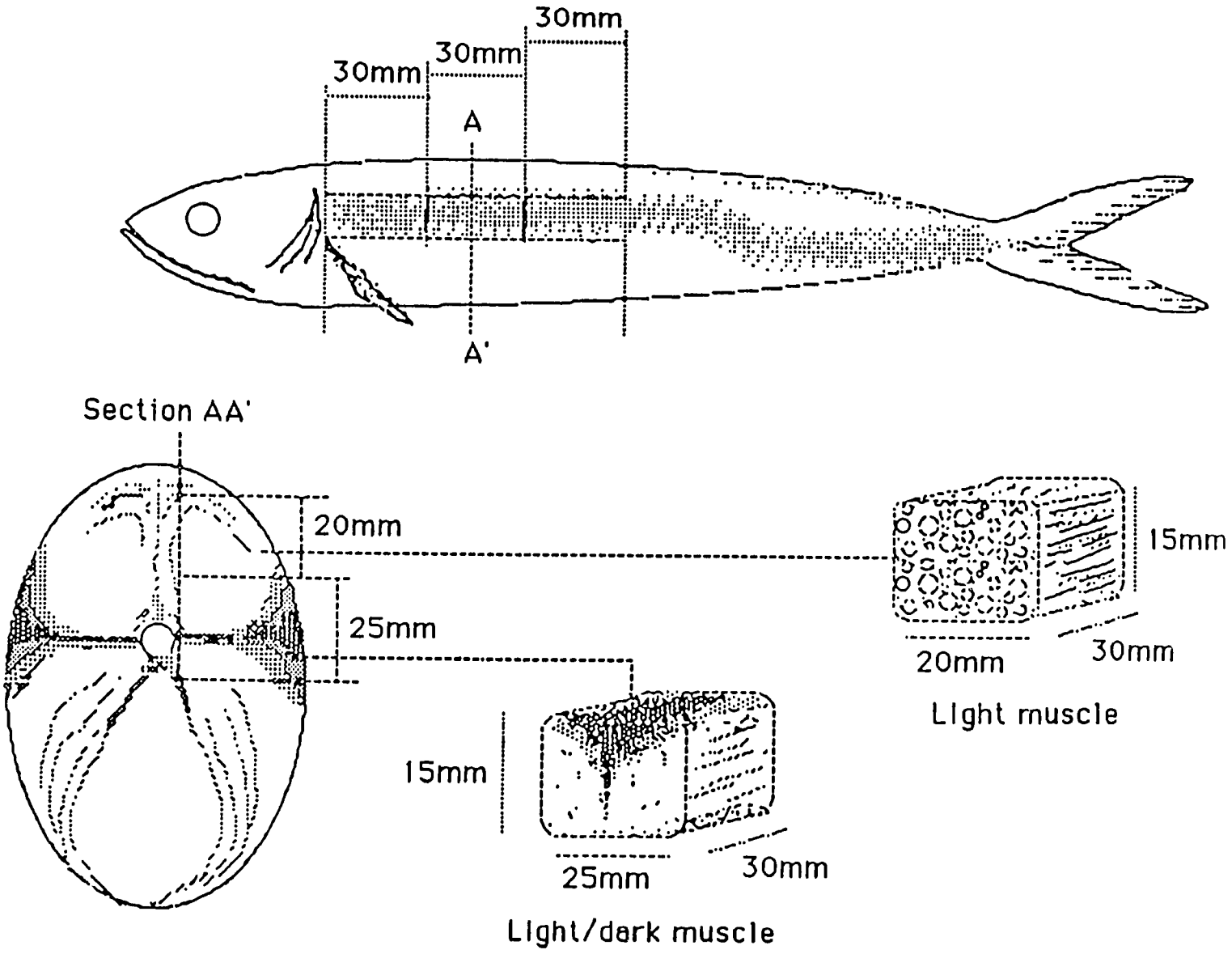
Figure 2. Testing cell and typical force-deformation curve

Figure 3. Relationship between force at yield point and weight of sample for light and mixed tissues.

Figure 4. Effect of time of incubation at 30°C on yield point of light and mixed tissue.

Figure 5. Effect of temperature on yield point of light and mixed tissue incubated in saline solution (buffer pH 7.1) for 30 min.

Figure 6. Effect of pH on yield point of light and mixed tissue incubated in saline solution at 30°C for 30 min.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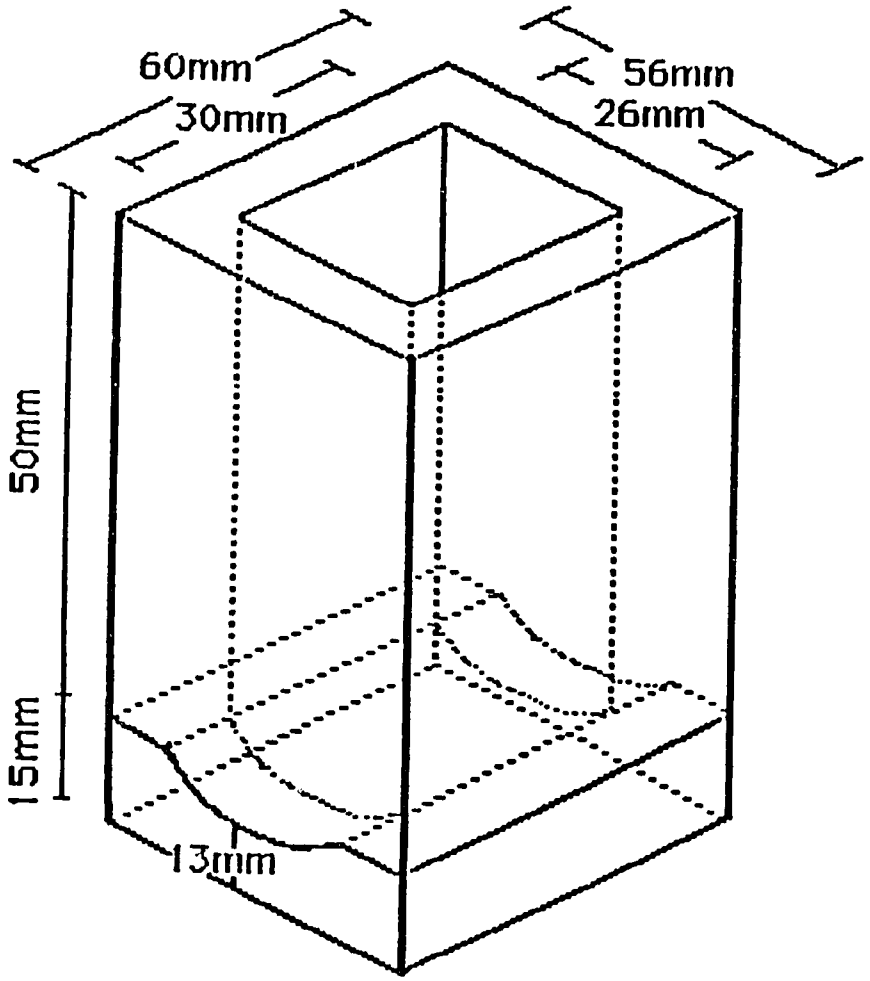
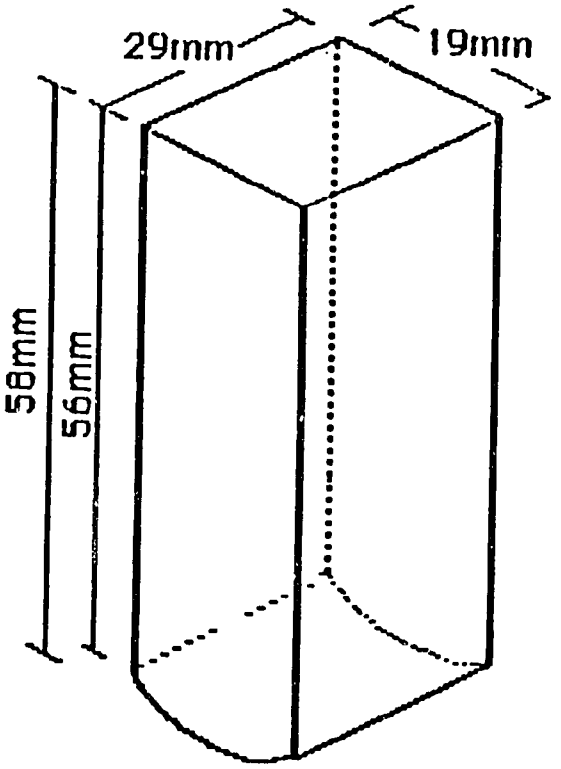
Light/dark muscle

Figure 1


60mm
30mm
56mm
26mm
50mm
15mm
13mm


29mm
19mm
58mm
56mm

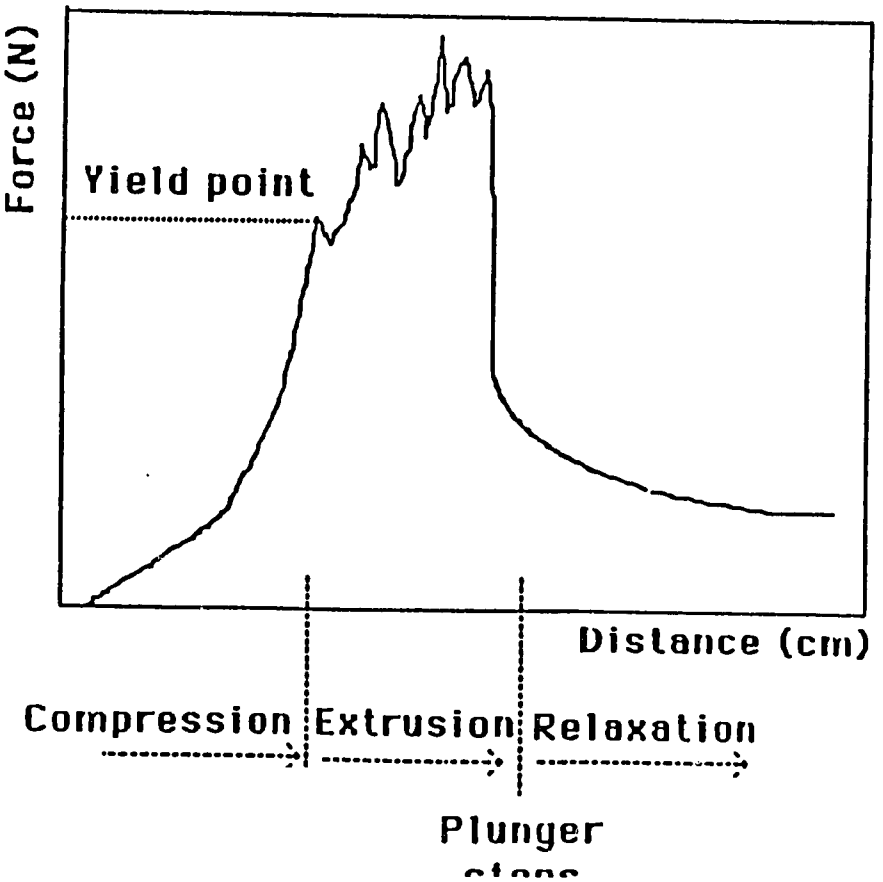

Force (N)
Yield point
Distance (cm)
Compression
Extrusion
Relaxation
Plunger

Figure 2

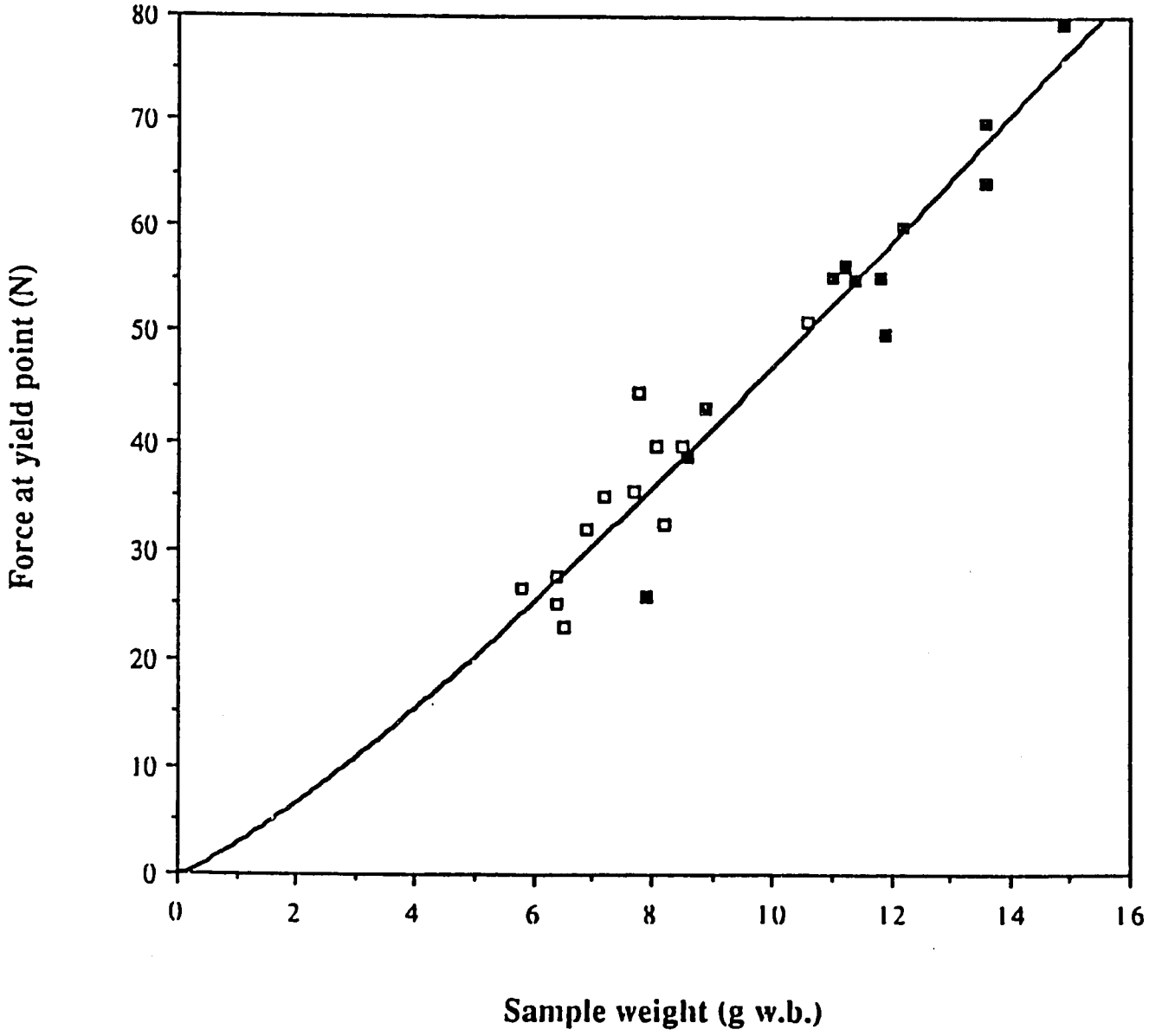
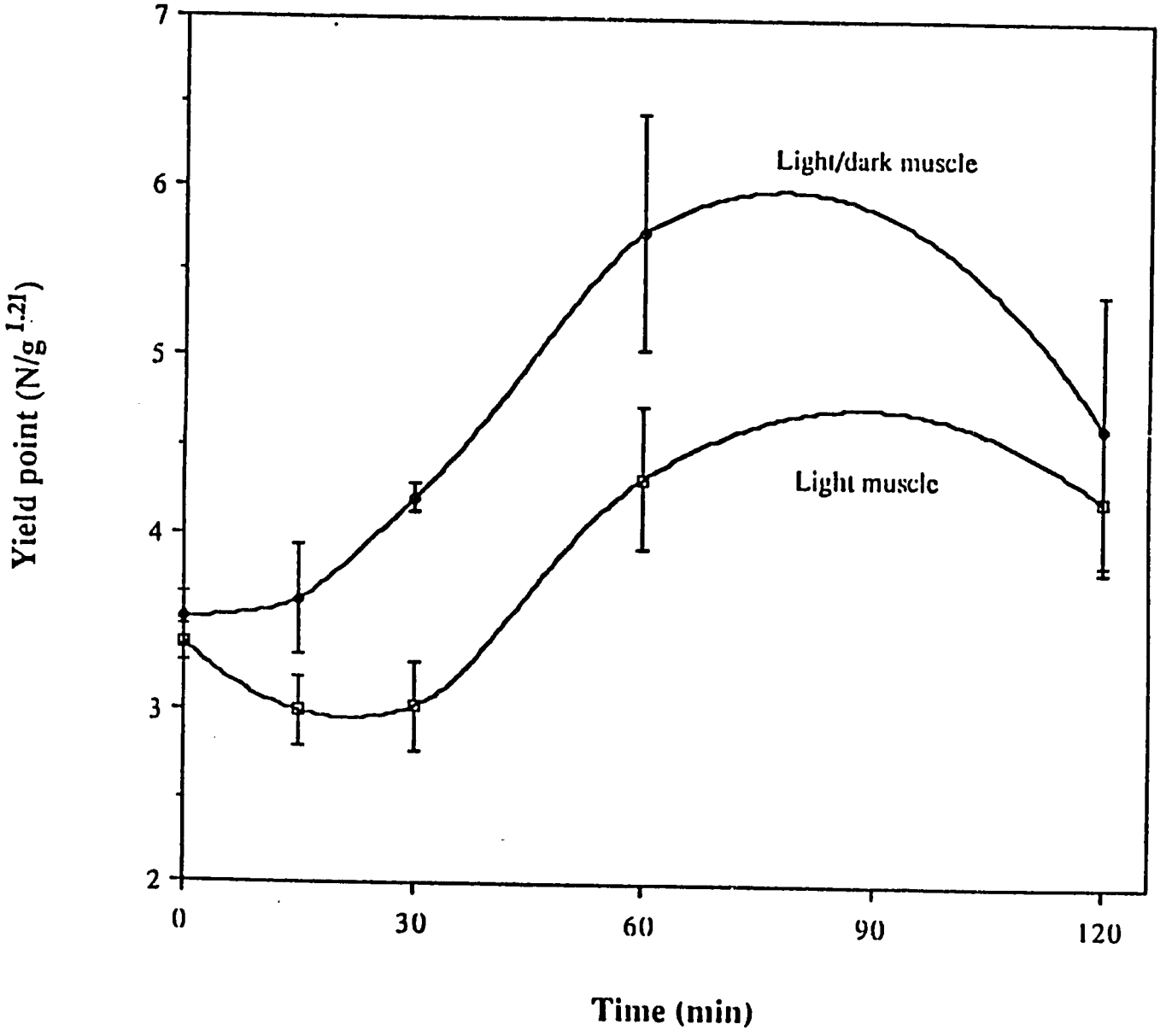
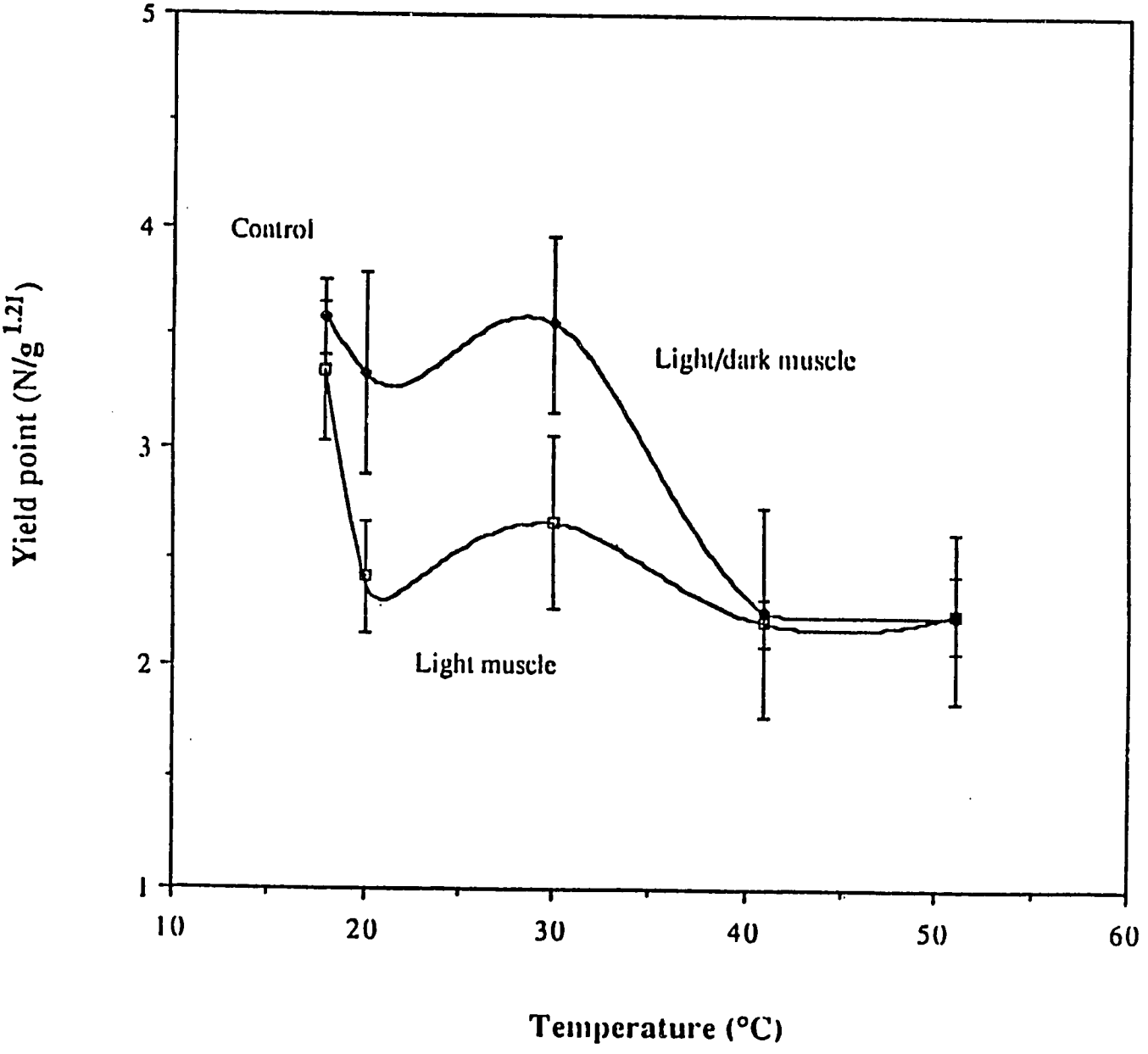

Force at yield point (N)
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
Sample weight (g w.b.)

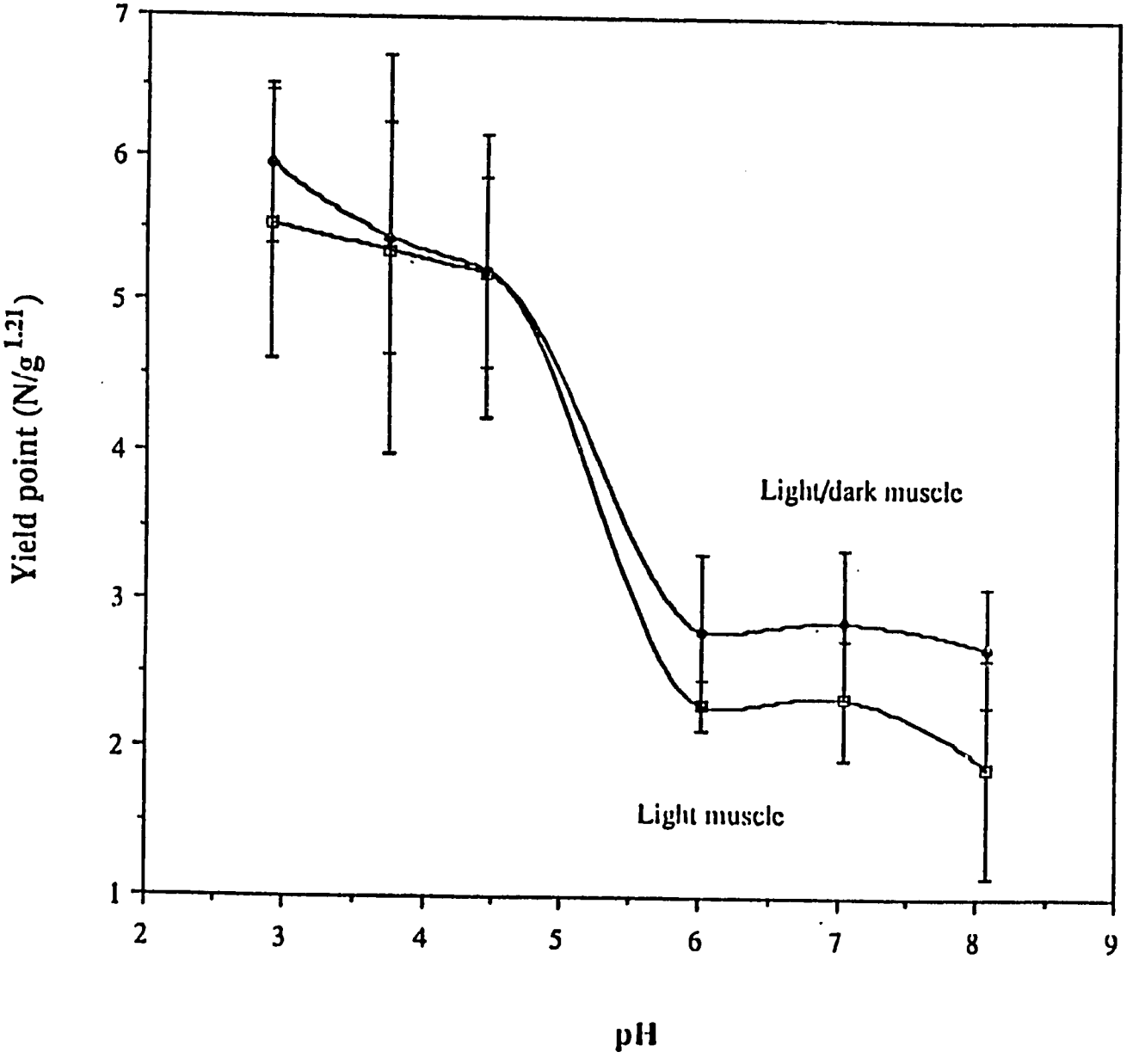
Figure 3


Yield point (N/g 1.21)
7
6
5
4
3
2
0
30
60
90
120
Time (min)
Light/dark muscle
Light muscle

22
Figure


Control
Light/dark muscle
Light muscle
Yield point (N/g 1.21)
Temperature (°C)
5
4
3
2
1
10
20
30
40
50
60

23
Figure



La cola es siempre más dura

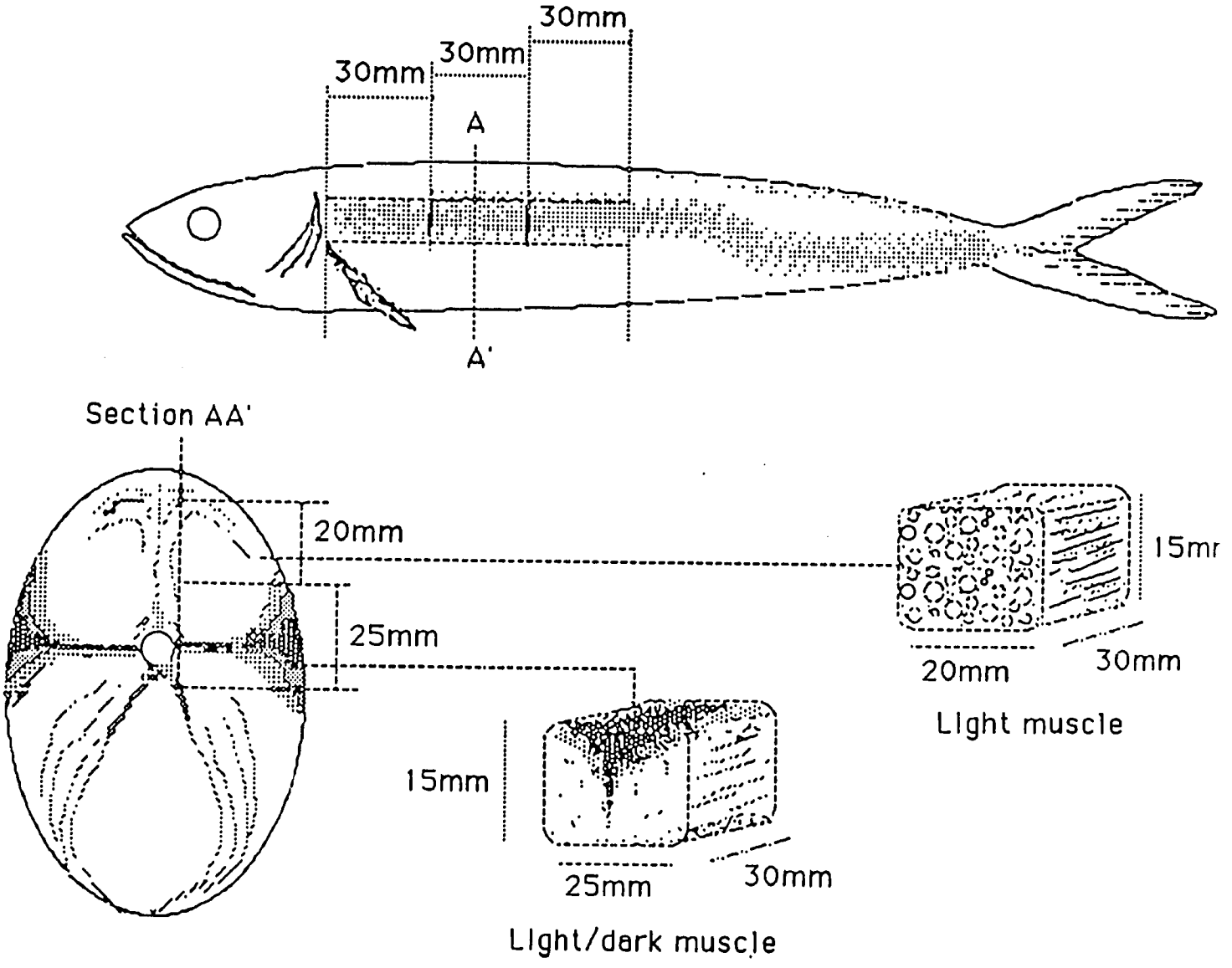

30mm
30mm
30mm
A
A'
Section AA'
20mm
25mm
15mm
25mm
30mm
Light/dark muscle
15mm
20mm
30mm
Light muscle

Figure 1

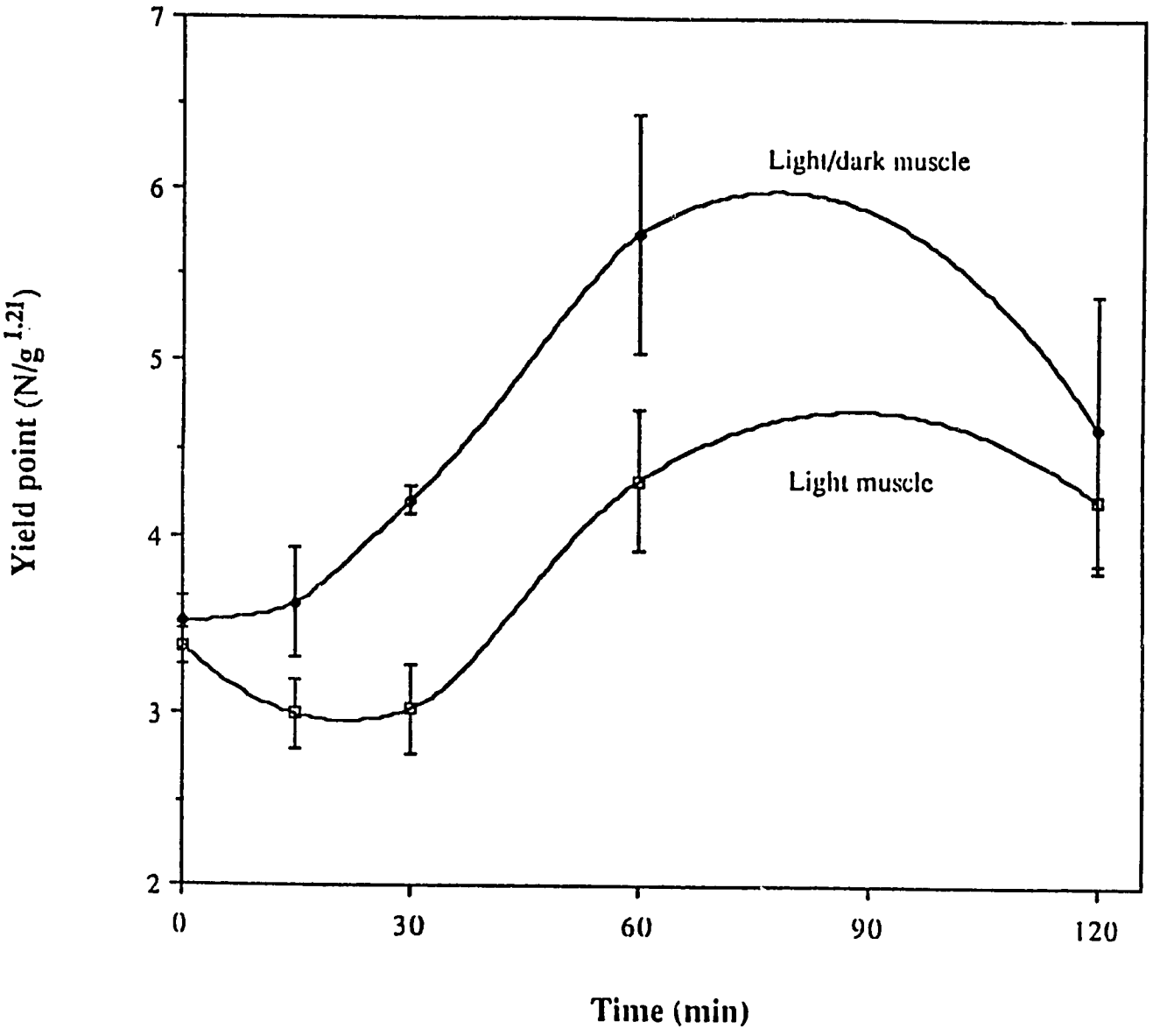

Yield point (N/g 1.2l)
7
6
5
4
3
2
0
30
60
90
120
Time (min)
Light/dark muscle
Light muscle

Figure 26

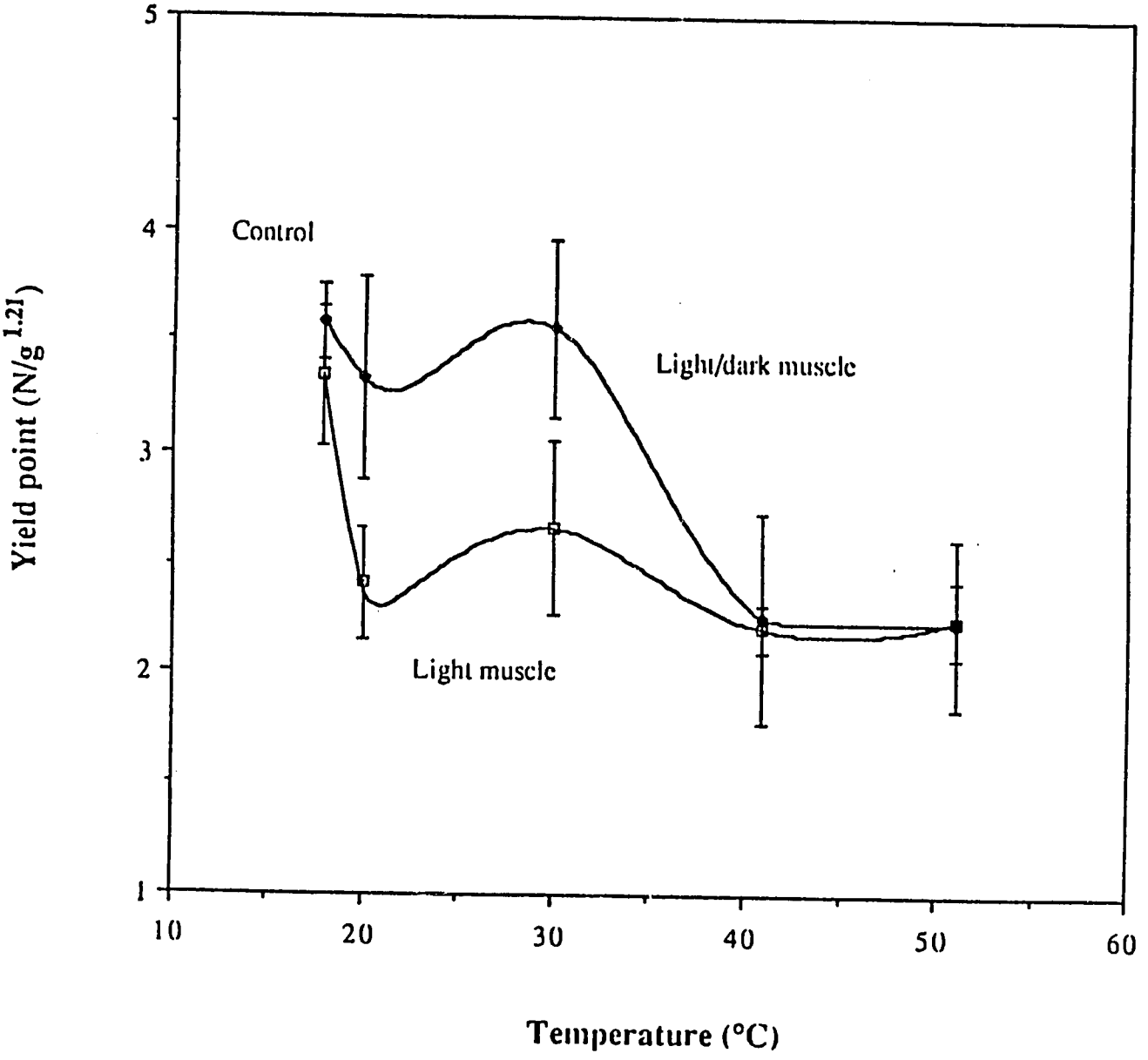
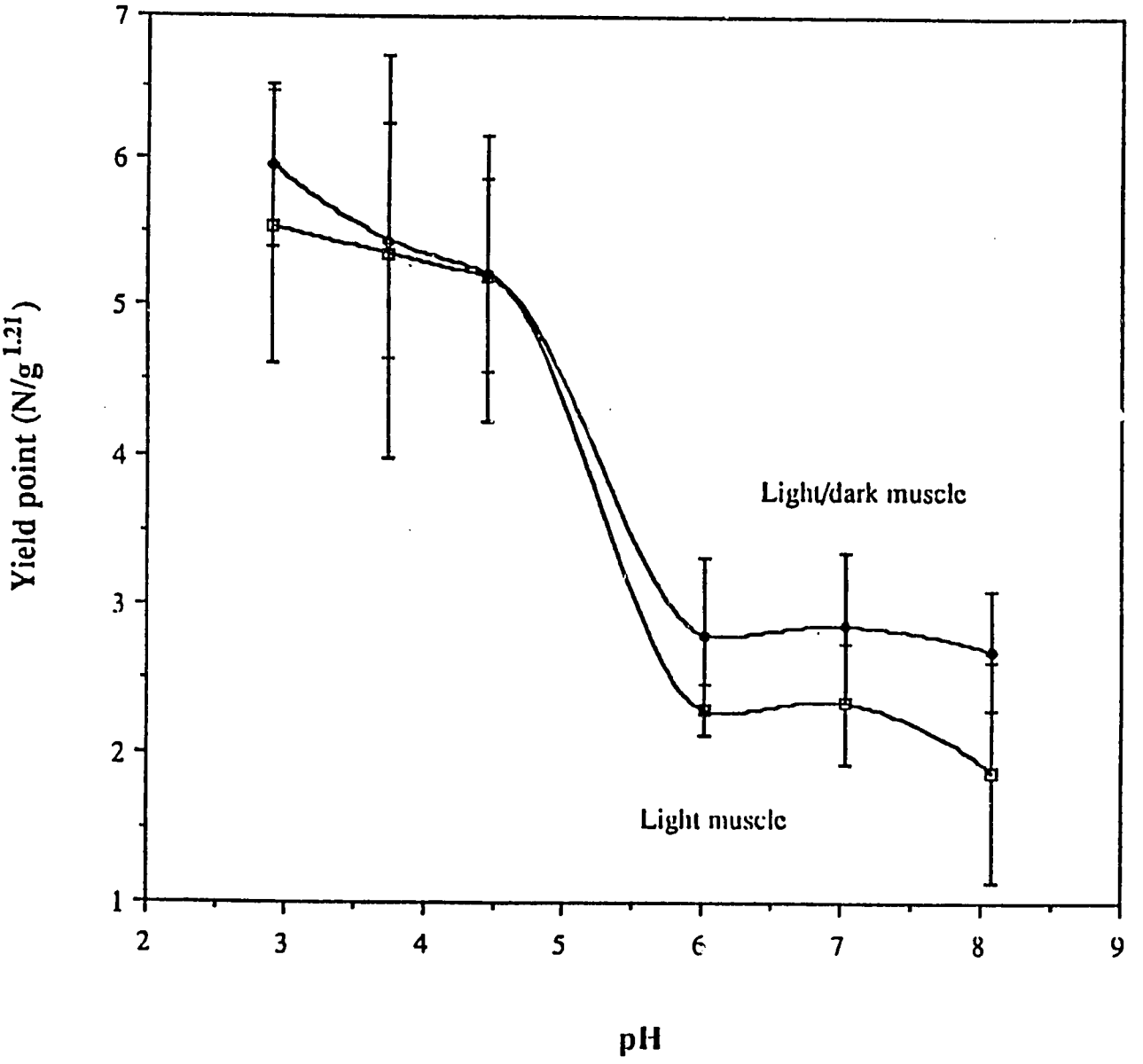
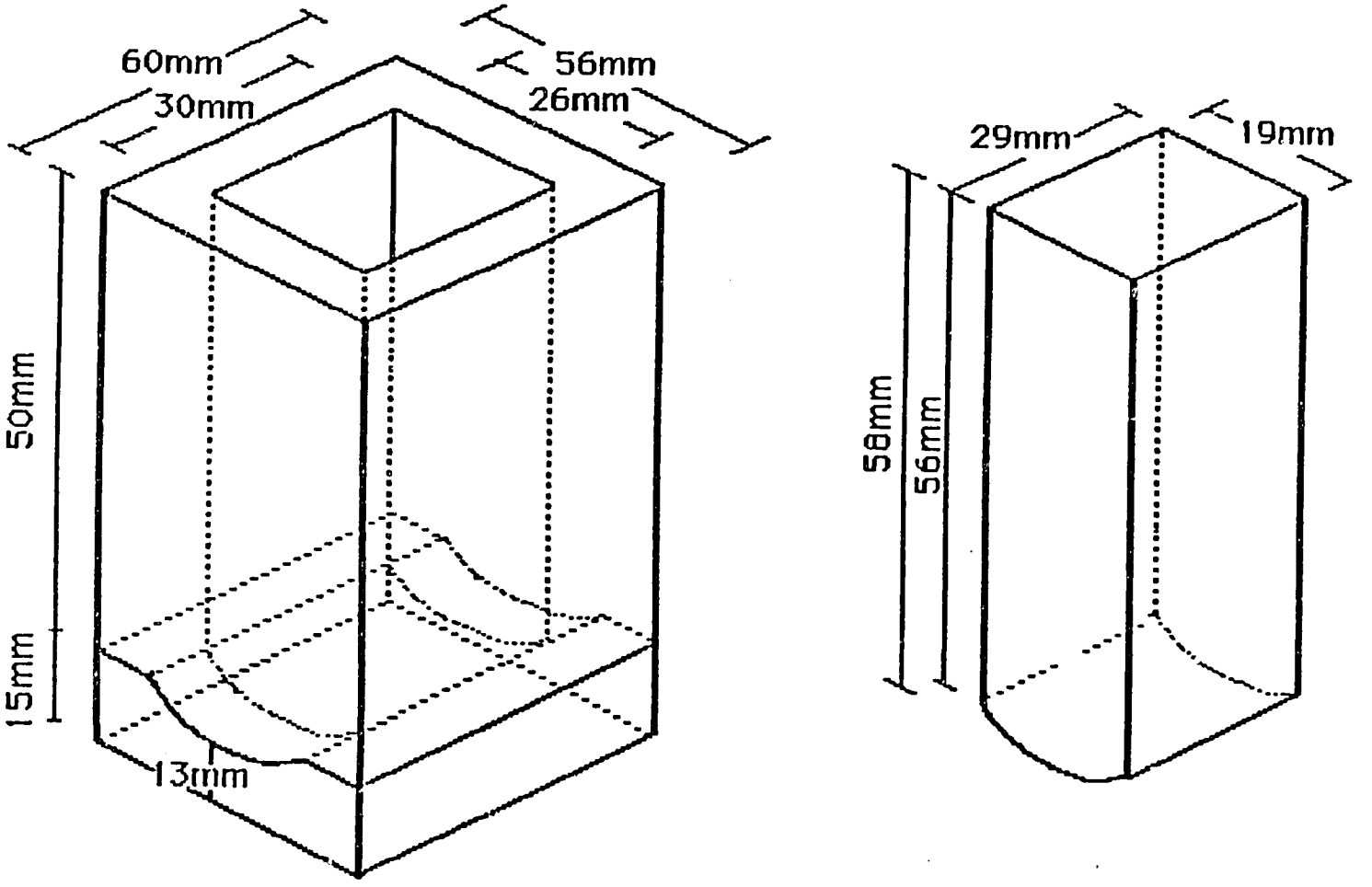

Control
Light/dark muscle
Light muscle
Yield point (N/g^1.21)
Temperature (°C)
5
4
3
2
1
10
20
30
40
50
60

Figure 27


Yield point (N/g^1.21)
7
6
5
4
3
2
1
2
3
4
5
6
7
8
9
pH
Light/dark muscle
Light muscle


60mm
30mm
56mm
26mm
50mm
15mm
13mm
29mm
19mm
58mm
56mm

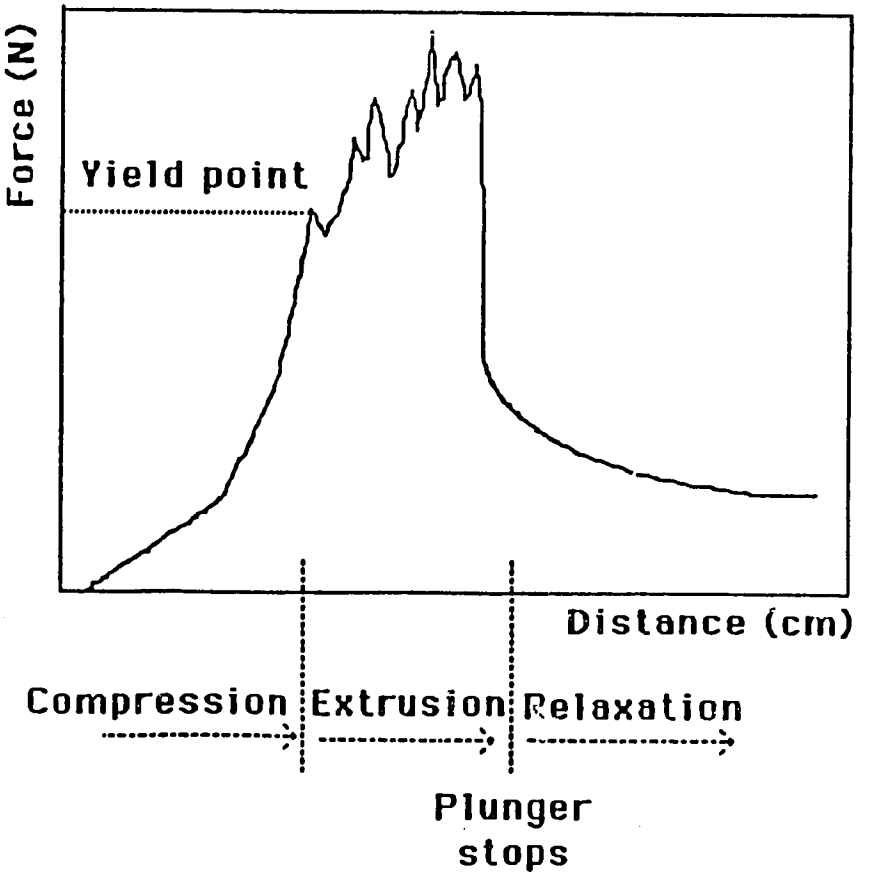

Force (N)
Yield point
Distance (cm)
Compression
Extrusion
Relaxation
Plunger stops

Figure 2

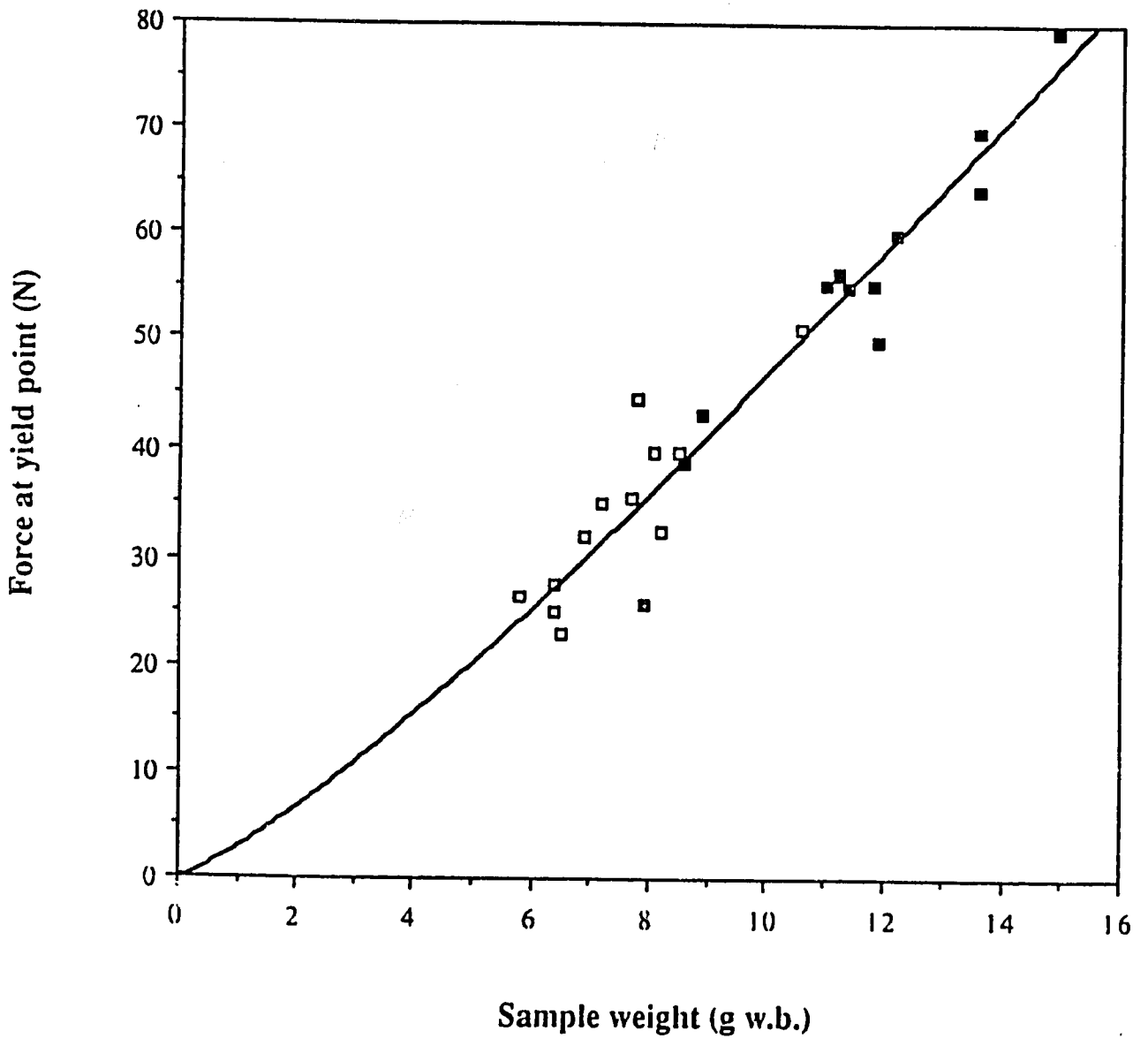

Force at yield point (N)
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
Sample weight (g w.b.)

Figure 3