AN ABSTRACT OF THE THESIS OF

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Title: Comparison of Physical, Thermal and Chemical Methods to Measure Protein Denaturation in Frozen Pacific Whiting (*Merluccius productus*)

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To investigate the potential of using a torsion rheological test and differential scanning calorimetry (DSC) to determine the effect of frozen storage on protein denaturation on Pacific whiting, fillets were stored for 12 weeks at three temperature conditions: $-20^{\circ}C$, $-8^{\circ}C$, and at a level varying between 0 and $-8^{\circ}C$. Salt soluble protein (SSP) extractability and Ca^{++}-ATPase activity were used to evaluate the torsion test and DSC.

The shear strain value of the torsion test provided a good correlation with SSP extractability, Ca^{++}-ATPase activity, and myosin transition enthalpy as measured by DSC. Therefore, shear strain can be considered as a useful tool for the determination of protein denaturation in Pacific whiting during periods of frozen storage. Since Ca^{++}-ATPase activity, shear stress and shear strain, myosin transition

enthalpy decreased within one week, protein deterioration in frozen Pacific whiting appears to be rapid, with no significant differences between vacuum and non-vacuum packaging treatments.

Comparison of Physical, Thermal and Chemical Methods
to Measure Protein Denaturation in Frozen
Pacific Whiting (*Merluccius productus*)

by

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INTRODUCTION

The U.S. fishing industry is characterized by seasonal
production, fluctuating catch rates, and unsteady demand.
One solution to these problems has been to preserve the
quality of fish by freezing, a solution which has been
found to improve the shelf-life of seafoods. Depending
upon the species in question, the quality of frozen seafood
has been found acceptable by consumers even after more than
six months have following the time the product was frozen.
Therefore, freezing has been recognized as an excellent
method for maintaining marketable fish quality.

However, decreases in such functional properties of
the fish such as emulsifying capacity, lipid-binding
capacity, water-holding capacity, and gel-forming ability
have been observed in frozen fish. The losses of
functional properties in fish meats during frozen storage
were attributed principally to protein denaturation,
especially in the case of myofibrillar protein. These
changes in frozen fish meats were highly significant after
long-term periods of storage. Thus, it has been suggested
that changes of the quality of frozen seafoods should be
examined to facilitate the ability to process these foods

into healthy and tasty products. The mechanism of protein
denaturation in fish meats during periods of frozen storage
remains unclear. Several hypotheses have been proposed,
including damage caused by ice crystals, changes in ionic
strength and pH, the effects of lipid-protein interactions,
and reactions with formaldehyde (Suzuki, 1981). However,
it has been difficult to demonstrate that protein dena-
turation is the result of any single one of these mechani-
sms.

Several methods have been developed to test quality
changes in frozen seafoods. Salt soluble protein (SSP) and
Ca^{++}-ATPase activity have been developed as traditional mea-
sures for the determination of protein denaturation in
frozen fish. SSP activity has been used to measure the
changes of protein solubility in fish meats during periods
of frozen storage, whereas Ca^{++}-ATPase activity has been
used to determine myosin status, an indicator of protein
denaturation. Other measurements, including levels of
trimethylamine oxide (TMAO), trimethylamine (TMA),
dimethylamine (DMA), and formaldehyde (FA), have also been
used widely to test fish quality (Babbitt et al., 1972).

Most methods in use for the determination of quality
deterioration in frozen fish meats have been based upon
chemical assays, but these are methods which provide little
information about physical and thermal properties of the
fish meats. Physical properties are closely related to the
functional properties of proteins and it is of considerable

importance that these properties affect consumers' accep-
tance of the products through the sensory properties of
texture. The thermal properties of fish meat are also very
important to food engineers and processors since they gov-
ern changes in the temperature of food processing, involv-
ing such heat transfer properties as heating, cooling, dry-
ing, and freezing.

Recently, torsion tests and differential scanning
calorimetry (DSC) have been used to determine, respective-
ly, protein gel-forming ability and protein thermal sta-
bility. However, the potential of using the torsion test
or DSC to determine protein denaturation in frozen fish re-
mains an area in need of evaluation. In addition, it would
be desirable to demonstrate the correlation of conventional
testing methods with torsion and DSC testing.

The torsion test was originally developed to measure
the texture of vegetables and fruits and subsequently used
to test the gel-forming ability of surimi, a washed, minced
fish that is commonly heated and gelled to produce various
analog seafood products. Hamann and Lanier (1988) reported
the torsion test to be a good measurement for the represen-
tation of the texture quality of surimi. Torsion test
results are presented as shear stress and shear strain,
indicating, respectively, the gel strength and the cohe-
siveness of surimi gels. The correlation between torsion
and sensory testing was studied by Montejano et al. (1985),
who determined that there was a good correlation between

shear strain and most sensory notes. Kim et al. (1986)
demonstrated that freeze-thaw cycles significantly decrease
the shear stress and strain of protein gels composed of
Sand trout and Alaska pollock surimi. During frozen stor-
age, a decrease in shear stress and strain was found in
protein gels made from both pollock surimi (Park et al.,
1988) and hoki (MacDonald et al., 1992).

The advantages of using a torsion test to determine
protein gel-forming ability as an indication of protein
denaturation include: 1) torsion testing offers informa-
tion on the gel-forming ability of the muscle proteins
which are important to meat processing; 2) torsion testing
procedures are easy and simple, and are thus suitable for
use in the food industry; and 3) the sample size (i.e., 800
—1200 g) requires larger quantities than used for tradi-
tional chemical assays (i.e., usually from 5—20 g), and the
results are thus more representable.

A number of factors, including the setting/heating
procedures for making protein gels, the protein quality,
ingredients, mixers, and vacuum treatments, affect torsion
testing results. Documented effects of these factors are
summarized in Appendix A. In the Food Science and Tech-
nology laboratories at Oregon State University (OSU) and
North Carolina State University (NCSU), the effects of dif-
ferent kinds of mixers upon torsion testing were
investigated and the results are represented in Appendix C.

Differential scanning calorimetry is a technique in which difference in energy inputs into a substance and a reference material are measured as a function of temperature, while the substance and the reference material are subjected to a controlled temperature program (Wright, 1984). DSC has been used to determine the thermal stability of animal proteins by measuring their heat transition temperature (Wright et al., 1977; Stabursvik and Martens, 1980). DSC has also been applied to study the denaturation of fish proteins (Hastings et al., 1985; Poulter et al., 1985; Davies et al., 1988).

It has been reported that fish muscle exhibits two major endotherm peaks, representing the transitions of myosin and actin (Hasting et al., 1985). It is generally accepted that myosin is unstable during storage and that the denaturation of myosin during storage results in changes in the functional properties of fish meat (Suzuki et al., 1981). By examining changes of peak temperature and/or peak area of myosin transition, researchers have been able to monitor the myosin denaturation which takes place during processing and storage. Transition temperatures and the transition enthalpy are two of the important parameters indicated by a DSC thermogram. Most research has concentrated upon T_{max}, the peak temperatures indicating the maximum rate of heat input, for the description of protein thermal stability. However, changes in transition enthalpy have been found to relate to protein denaturation

during frozen storage (Hasting et al., 1985). They
reported that myosin transition enthalpy measured from DSC
thermograms of cod muscle, decreased following two weeks of
storage at -10°C. Reid et al. (1988) demonstrated a
decline of myosin transition enthalpy in rockfish muscle
following five months of storage at -5°C. A reduction of
myosin transition enthalpy was also reported by Kim et al.
(1986), who examined the effects of freezing and thawing
cycles on the heat stability of surimi. More detailed
information about DSC testing is reviewed in Appendix B.

With respect to the correlations of torsion testing
and the DSC with conventional methods, findings can be used
for the evaluation of the potential for the application of
these techniques to determine protein denaturation. In
pollock surimi, Katoh et al. (1979) reported a good
correlation between the gel properties and Ca^{++}-ATPase
activity. Wagner and Anon (1986) showed a good correlation
between the transition enthalpy measured by DSC and Ca^{++}-
ATPase activity in bovine muscle. The relationships
between gel properties and DSC measurements were estab-
lished by Akahane et al. (1981), who used DSC to determine
the amount of captured water in cooked Alaska pollock gels.
It was determined that the content of the captured water in
fish gels was highly correlated with gel strength. For the
current investigation, the intent was to use DSC to deter-
mine changes in the transition enthalpy of myosin, a tran-

sition which indicates the denaturation of myosin which results in the decrease of gel properties.

Evaluating the ability of torsion testing and DSC to determine the effects of frozen storage upon protein denaturation was the principal objective of this study. Pacific whiting was the subject fish used for the experimental procedure. Thus, protein denaturation in Pacific whiting has been investigated subject to different frozen storage conditions. The torsion test and DSC were used to determine changes, respectively, in the gel-forming ability and in the transition enthalpy of Pacific whiting fillets during frozen storage. Comparisons of the results of torsion testing and DSC with salt-soluble protein extractability and Ca^{++}-ATPase activity were also investigated in part to establish authenticity of torsion tests and DSC.

MATERIAL AND METHODS

Sampling of Pacific Whiting

Pacific whiting were stored on ice for about 14 hours
following the catch, were filleted at Seafood Sales, Inc.
in Astoria, Oregon, and then immediately transported on ice
to the OSU Seafood Laboratory in Astoria. Four to five
fillets, weighing a total of about 750 g, were randomly se-
lected, placed in a plastic bag, and packaged both with and
without a vacuum seal. The packages were then frozen in a
blast freezer in which the core temperature reached -25°C
within 11 hours. Frozen fillets were stored at the two
uniform temperatures of -20°C and -8°C, and at one "abuse
condition" of -8°C. The abuse condition was conducted by
removing the samples from the -8°C storage freezer to a 5°C
room for 16 hours, then returning them into the -8°C freez-
er, twice each week. While stored in the 5°C room, the
abused samples were completely thawed, as indicated when
the central temperature of the fillet package reached 0°C
after 16 hours. A temperature controller (Goldline TD-SP,
Independent Energy Inc.) was used to override the controls
on conventional chest freezers and to maintain temperatures
within a 1°C range. Temperature fluctuation was monitored

using a datalogger (Electronic Controls Design INC., Model 3020T).

The K-value, a measure of the nucleotide component breakdown, was determined for serval samples prior to freezing to determine freshness. Sampling at week 0 used fresh unfrozen fillets weighing about 1500 g. At weeks 1, 2, 3, 4, 6, 8, and 12, two packages of frozen fillets having a total weight of about 1500 g were removed from each storage temperature condition, then partially thawed at room temperature for one hour. For all samples, a torsion test, the DSC, and both salt-soluble protein (SSP) extractability and Ca^{++}-ATPase activity measures were used to test protein denaturation. Thiobarbituric acid (TBA) was also measured to examine comparative lipid oxidation for both vacuum- and nonvacuum-packaged frozen samples.

Measurement of K-Values

The measurement of the K-values followed the procedure described by Uchiyama and Kakuda (1984). Mince (5 g) was extracted with cold perchloric acid and then neutralized to around pH 6.5—6.8 with caustic potassium solution. A column (0.7 × 5.0 cm) of ion exchange resin (Bio-Rad, AG 1-X4, 100-200 mesh, Cl type) was used to separate inosine (HxR) and hypoxanthine (Hx) with inosine monophosphate (IMP), adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). K-value was

calculated as the ratio of the sum of HxR and Hx to the sum of all nucleotide component.

Salt Soluble Protein Extractability

Salt-soluble protein (SSP) was extracted according to a modified method of Jiang et al. (1985). To reduce foam formation during extraction, an Osterizer cycle blender (Pulse-A-Matic, Model 860-61K) was used in place of the Waring blender recommended in the published procedure. Protein concentrations were determined by the Biuret method, as modified by Umemoto (1966). Bovine serum albumin was used as a protein standard.

Ca^{++}-ATPase Activity

Actomyosin was extracted according to the method described by Noguchi (1974). Concentrations of the actomyosin solution were determined by the Biuret method, as modified by Umemoto (1966). Bovine serum albumin was used as a protein standard. The rate of release of inorganic phosphate at 25°C from the Na_2-ATP was determined according to Arai (1974).

Torsion Test

A Stephan vacuum mixer (Stephan Machinery Corporation, UM5 universal) was used to mix 1 Kg of muscle tissue with 2.5% NaCl and 1.5% beef plasma protein (a protease enzyme inhibitor) for 4 min. Ice was added during mixing to

adjust the final moisture content to 79%. From the mixing process, a paste was produced. A sausage stuffer (5-lbs capacity, The Sausage Maker, Buffalo, NY) was used to extrude the paste into stainless steel cooking tubes (inside diameter 2.2 cm, length 17.8 cm). Protein gels were produced by heating in a 90°C water bath for 15 min., followed by cooling in ice water for 10 min. The torsion test system involved cutting the protein gels into hourglass shapes, then twisting them to the point of failure in a viscometer, as described by Lanier (1991). The results of the torsion test were presented as shear stress and shear strain at failure, calculated from the equations provided by Hamann (1983).

Differential Scanning Calorimetry

At least four muscle tissue samples, each weighing about 5—8 mg, were taken at random from the fresh, or partially-thawed fillets. Samples were sealed in aluminum sample pans and scanned from 20 to 90°C at a heating rate of 10°C per min., using a DuPont 910 DSC (DuPont Instruments, Wilmington, DE). Water was used in the reference pan. Indium was used as a calibration material. To enhance sensitivity, 40 ml/min. of helium gas was bled through the purging port to facilitate heat transfer to the sample. The transition enthalpy and the T_{max} of the myosin heat transition were used as indicators of protein denaturation. The enthalpy of the myosin transition was calcu-

lated by peak integrationing the area under the peak; using DuPont DSC standard software and assuming a sigmoidal base-line.

Thiobarbituric Acid Test

The lipid oxidation of the vacuum and non-vacuum frozen samples was assessed by 2-thiobarbituric acid analysis, using the procedure of Yu and Sinnhuber (1975).

RESULTS AND DISCUSSION

K-Values of Pacific Whiting

The K-values for the fresh Pacific whiting fillets were averaged 17%, indicating that the samples were very fresh.

Salt Soluble Protein Extractability

The SSP extractability is presented in Fig. 1. After three weeks, SSP extractability started to decrease rapidly for both the abuse and the -8°C conditions. There were no significant differences between these two conditions after 12 weeks of storage, and no significant decline in SSP extractability was found for the uniform -20°C case during the 12-week frozen storage period. The decrease in SSP extractability during the periods of frozen storage was mainly due to the denaturation of myofibrillar proteins, which tend to aggregate during frozen storage and thus to lose solubility in salt solutions.

The results for the -20°C case differed from those obtained by Babbitt et al. (1972), who reported that the total extractable protein in Pacific whiting stored at -20°C rapidly decreased within one month. This difference in results could have been due to the difference in the

freshness of the fish, to harvest/handling methods, and/or to the extent of fluctuation of storage temperatures. For the experiments considered in the present investigation, temperatures were controlled within 1°C. The degree to which temperature fluctuations influence SSP extractability should be the subject of further investigation.

Ca^{++}-ATPase Activity

The Ca^{++}-ATPase activity of extracted actomyosin is presented in Fig. 2. Similar to results for salt-soluble protein extractability, there was a significant difference (p < 0.01) between the abuse and uniform -8°C conditions during 11 weeks of frozen storage, but on significant differences at week 12. Ca^{++}-ATPase activity also decreased in samples held at the uniform -20°C condition. Compared to the activity among the fresh fillets (at week 0), there was approximately 40% activity left after following 12 weeks of storage at a uniform -20°C. A decrease in Ca^{++}-ATPase activity at this temperature, in the absence of a significant decline in SSP extractability, may have been due to the fact that Ca^{++}-ATPase activity was a more sensitive measurement. It is generally accepted that the head of the myosin heavy chain has the ability to hydrolyze ATP and to release energy for muscle contractions (Suzuki, 1981). A small change in the head region of the myosin may serve to decrease Ca^{++}-ATPase activity, but without effect extractability of SSP. Again, the results showed that both

storage temperatures and abuse temperature had a significant effect on the Ca^{++}-ATPase activity of actomyosin extracted from Pacific whiting.

Torsion Test

The results of shear stress for all three storage conditions demonstrated a significant decrease ($p < 0.01$) within one week, and then continued at the same level subject to some degree of variation (Fig. 3). There were no significant differences in shear stress among the three storage conditions. A sudden increase in shear stress at 12 weeks of storage for the abuse case indicated that the protein had lost some water holding capacity and thus the protein gels had become relatively tough. Water tended to be released in the sausage stuffer while extruding the fish paste into the cooking tubes. This decrease of water holding capacity was a further demonstration that the protein had been severely denatured.

The results of shear strain are shown in Fig. 4. Shear strain significantly decreased ($p < 0.01$) for all three conditions by week 1. Following week 1, the shear strain values decreased more slowly for all three conditions. No significant differences between abuse and uniform -8°C conditions were observed during 12 weeks of storage, while the samples held at -20°C tended to experience higher values of shear strain.

The significant decrease in shear stress and strain within one week indicates that protein denaturation took place during frozen storage as indicated by a decrease in gel-forming ability. Since the change in gel-forming ability occurred among all three frozen conditions within one week, it is reasonable to hypothesize that the reduction of gel-forming ability in whiting fillets may be due to the effects of the freezing (vs. frozen storage) process.

Differential Scanning Calorimetry

A representative DSC thermogram for whiting whole muscle is shown in Fig. 5, representing two transition peaks: The first peak, occurring between 35 and 55°C, represents myosin heat transition; the second peak, an actin heat transition occurring between 65 and 80°C. The maximum heat transition temperatures (T_{max}) for myosin and actin were, respectively, approximately 45.5 and 75.0°C. Similar T_{max} values have been reported by several other investigations. Beas et al. (1990) reported that the DSC thermogram for the whole muscle of fresh hake (*Merluccius hubbsi*) revealed two endothermic transitions, with T_{max} values of 46.5 and 75.3°C. Martens and Vold (1976) and Hastings et al. (1985) also found two maximal transitions for DSC thermograms for whole cod muscle at T_{max} of approximately 45 and 75°C. The T_{max} for the myosin of whiting muscle did not change with storage time for a period up to three months under conditions of -8 and -20°C.

Changes in myosin transition enthalpy are shown in Fig. 6. The thermogram for samples stored in the abuse condition were subject to extremely large variations, thus these results were not considered for purposes of discussion. These variations may have been caused by heat-stable protease enzymes, which are able to digest muscle protein at the relatively high temperatures of the abuse condition. In one week, there was a significant decrease ($p < 0.01$) of myosin enthalpy for both the -8 and -20°C cases. However, following the first week of storage there were no further significant changes for either case during the remaining 12-week storage period. These results were in agreement with those obtained by Hastings et al. (1985), who reported that the myosin of cod muscle underwent some degree of partial denaturation within two weeks when stored at -10°C, and then showed a negligible degree of subsequent change in myosin transition for the period following two weeks. Again, the initial reduction of myosin enthalpy may have been due to the freezing process; the effects of subsequent storage could then have been relatively minor.

Difficulties were experienced when measuring myosin transition enthalpy by DSC. The variation attributed to the sampling process was the source of one problem. It was difficult to obtain a representative sample when the sample size for DSC was only in the range from 5—8 mg. Interference by lipids constituted an additional and significant sampling problem. Lipid transition occurred at about 40°C

(i.e., at a temperature range close to that of myosin), and the enthalpy of the lipid transition was very large when compared to that for the myosin heat transition. It was possible to identify the presence of a fat globule since the peak was reversible while that indicating myosin transition, was not. A second scan of a suspect sample would then demonstrate a different transition peak.

An additional problem was variation related to the location and definition of the baseline used to integrate the peak area of myosin transition (Fig. 5). Moreover, the calibration of the system using indium created a problem. The transition enthalpy of indium is very large (i.e., 28.4 J/g at 156.6°C) compared to that for myosin (i.e., approximately 0.8 J/g at 45°C). It thus became difficult to quantitatively determine accurate and valid myosin transition enthalpy values.

Correlations between Various Measurements

Correlations between the different experimental measurements are summarized in Table 1 and Fig. 7. The greatest degree of correlation was found between SSP extractability and Ca^{++}-ATPase activity ($p < 0.001$). Shear strain tended to correlate well with other methods, including SSP extractability, Ca^{++}-ATPase activity, and DSC. This correlation indicated that shear strain could be a useful tool for the determination of the protein denaturation of fish fillets during frozen storage. This result was also in

agreement with Hamann (1988) who suggested that shear strain at failure is a fairly stable measure of protein functional quality. However, the results of shear stress showed a much greater degree of variation than those for shear strain, and there was no significant correlation between shear stress and either SSP extractability or Ca^{++}-ATPase activity. Hamann (1988) suggested that shear stress is strongly influenced by protein concentration, processing conditions, and ingredient variables.

The transition enthalpy of myosin as measured by DSC was found to correlate significantly ($p < 0.01$) with both shear stress and shear strain, but not with either SSP extractability or Ca^{++}-ATPase activity. Due to the fact that myosin is the main component which contributes to the gel properties of fish gels, it could be suggested that there is some degree of relationship between thermal behavior and the heat-induced gelation of myosin.

Effect of Vacuum Packaging

The effects of vacuum packaging on the rate of protein denaturation in whiting fillets were also investigated by sampling nonvacuum-packaged fillets three times during the three-month experimental period. No significant differences in SSP extractability, Ca^{++}-ATPase activity, shear stress, or shear strain between the vacuum- and nonvacuum-packaged cases were determined. The results of lipid oxidation determination by TBA testing for vacuum- and

nonvacuum-packaged frozen Pacific whiting fillets are shown at Table 2. The TBA values did not undergo significant change with storage temperature, storage time, or vacuum treatment.
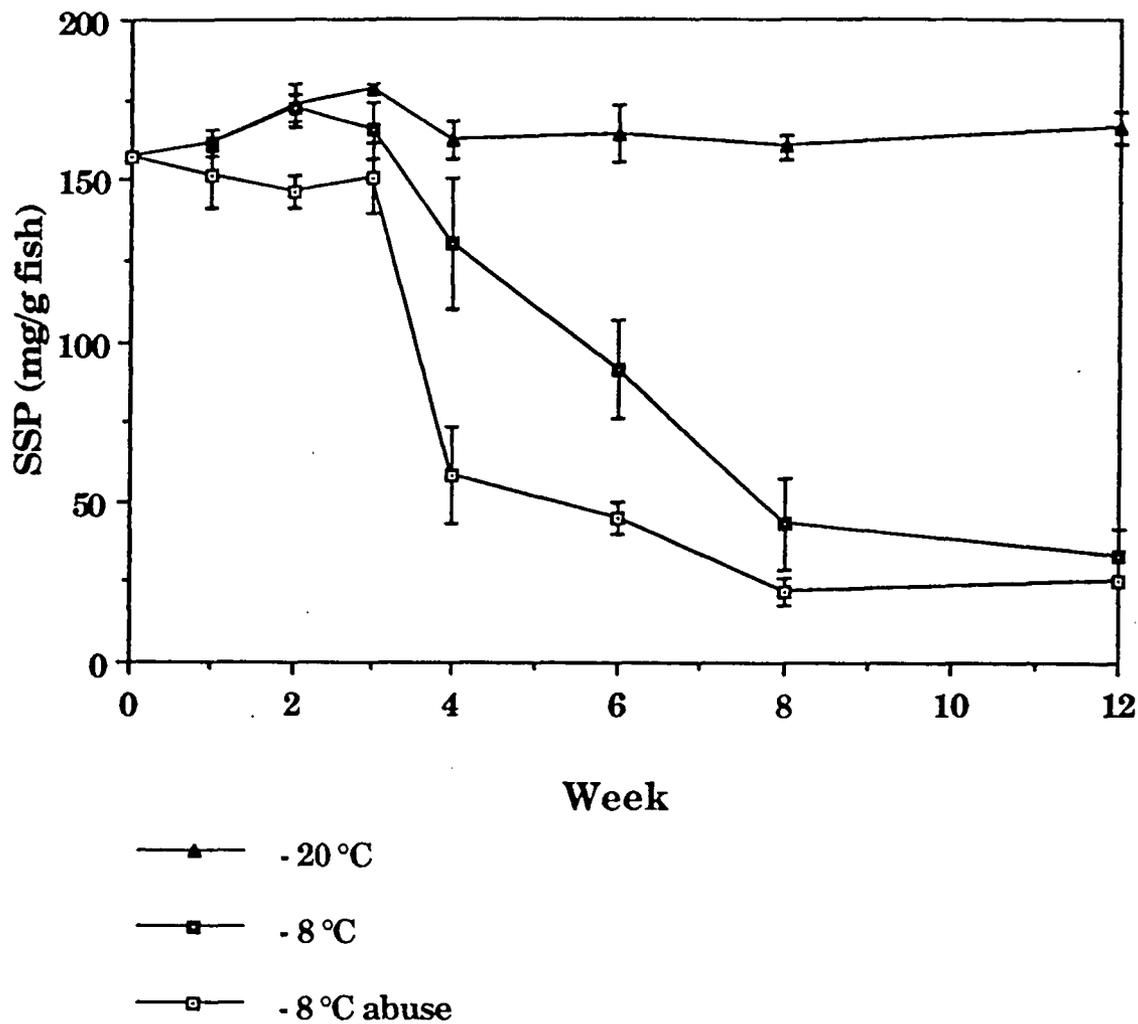
Fig. 1. Salt soluble protein extractability in Pacific whiting fillets during frozen storage
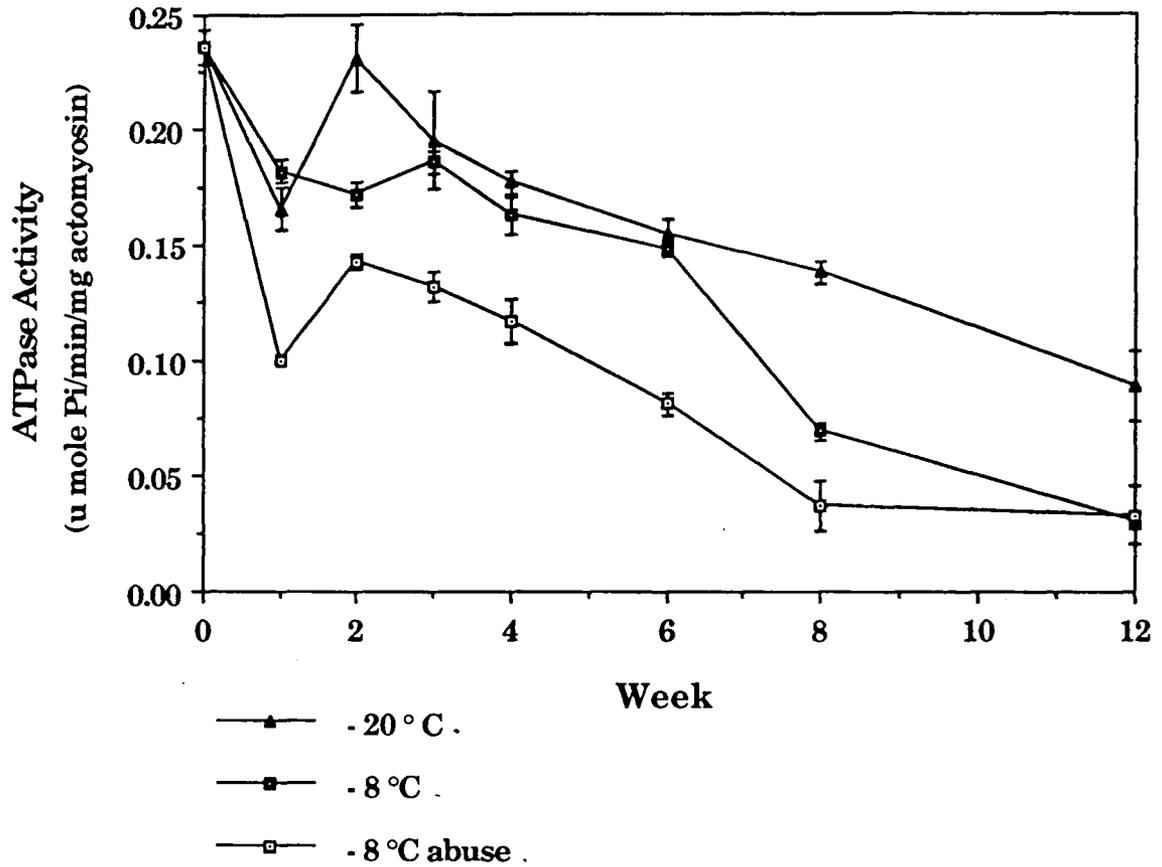
Fig. 2. Ca^{++}-ATPase activity in Pacific whiting
fillets during frozen storage
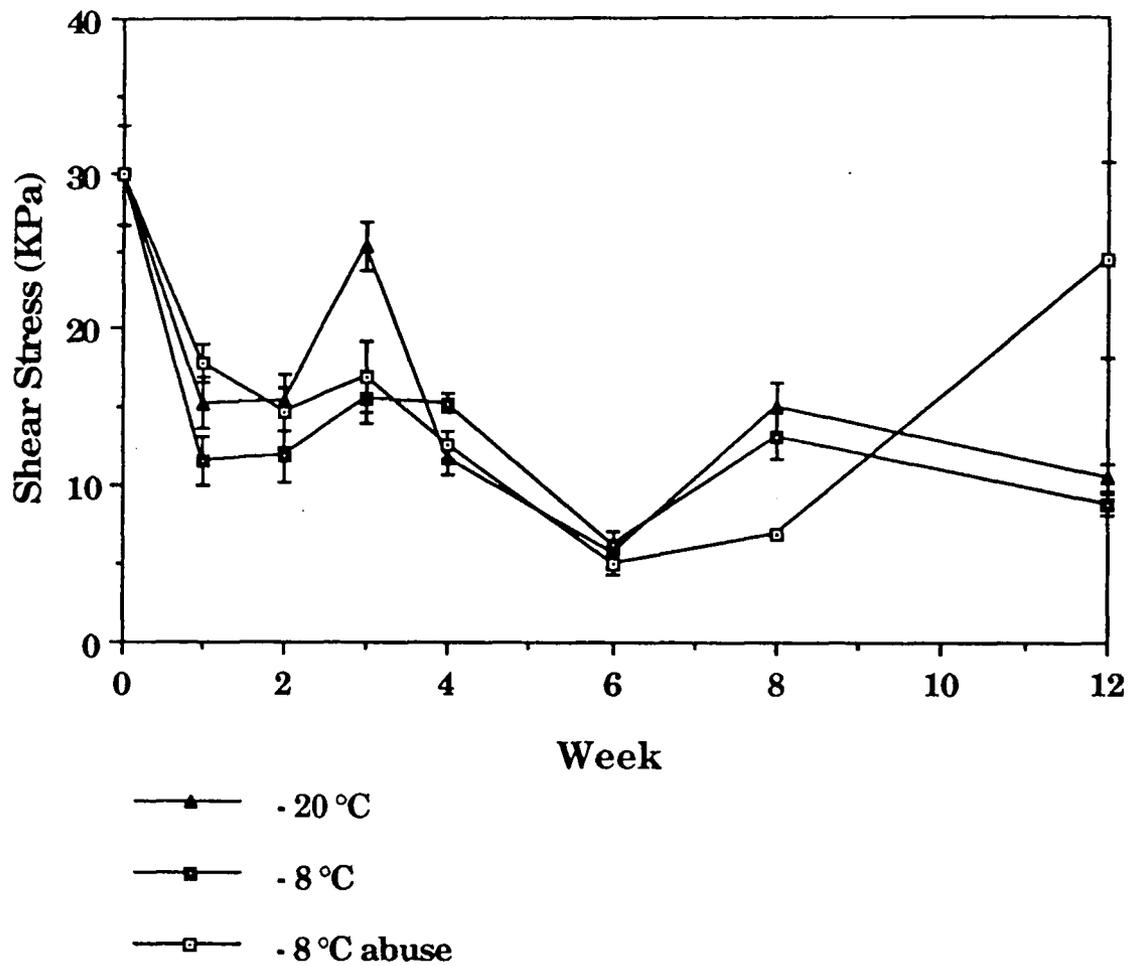
Fig. 3. Shear stress in Pacific whiting during frozen storage
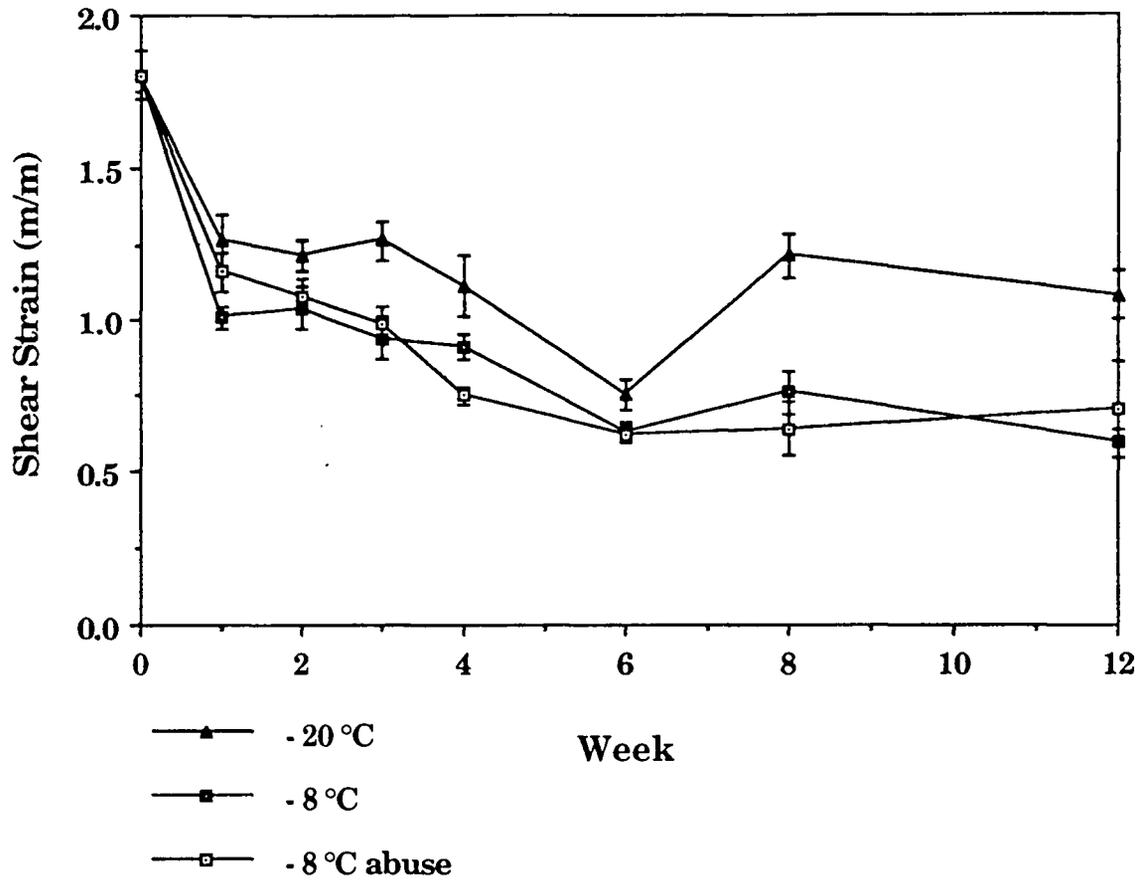
Fig. 4. Shear strain in Pacific Whiting
          during frozen storage
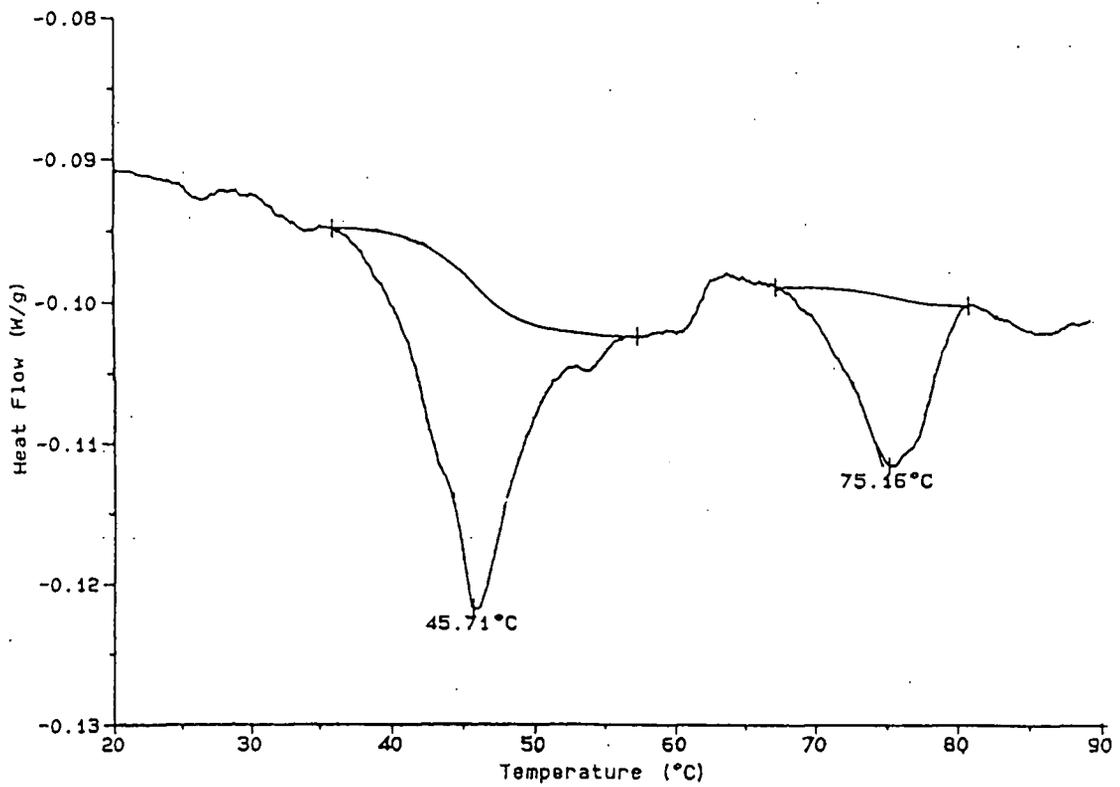
Fig. 5. DSC thermogram of Pacific whiting muscle
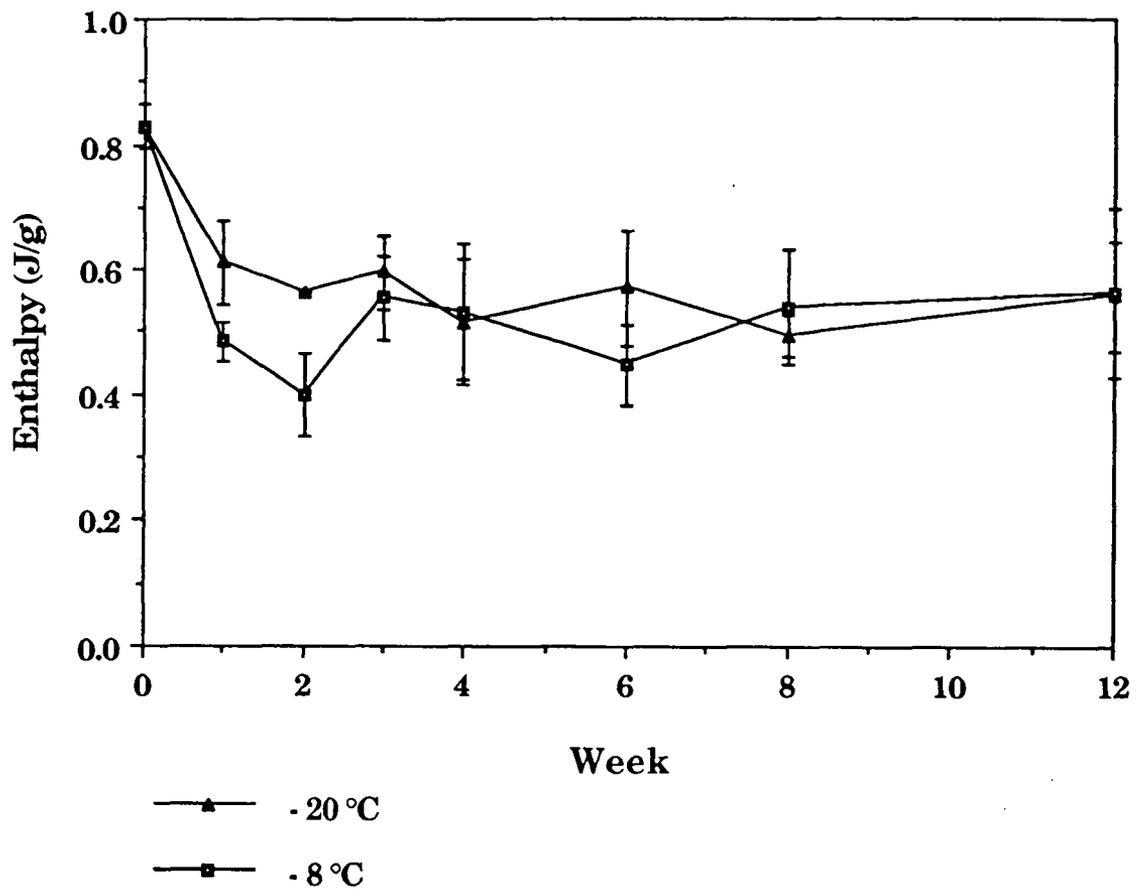
Fig. 6. Myosin thermal transition enthalpy in Pacific
        whiting fillets during frozen storage
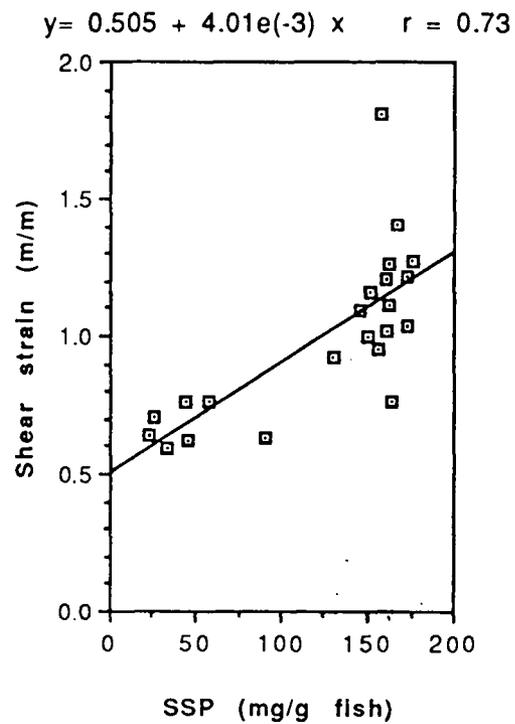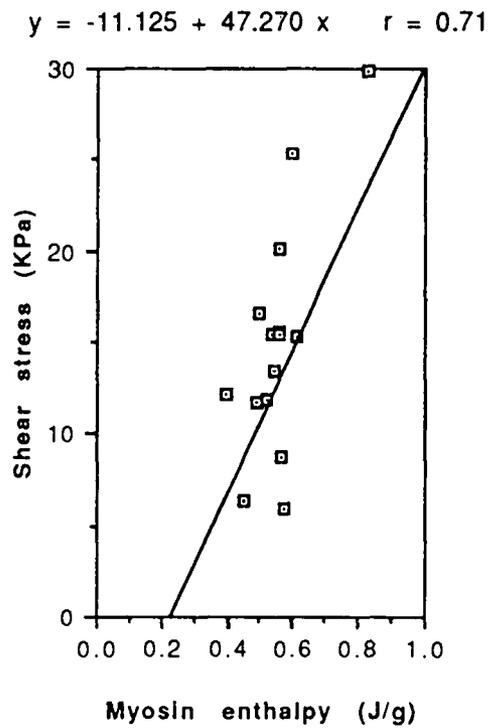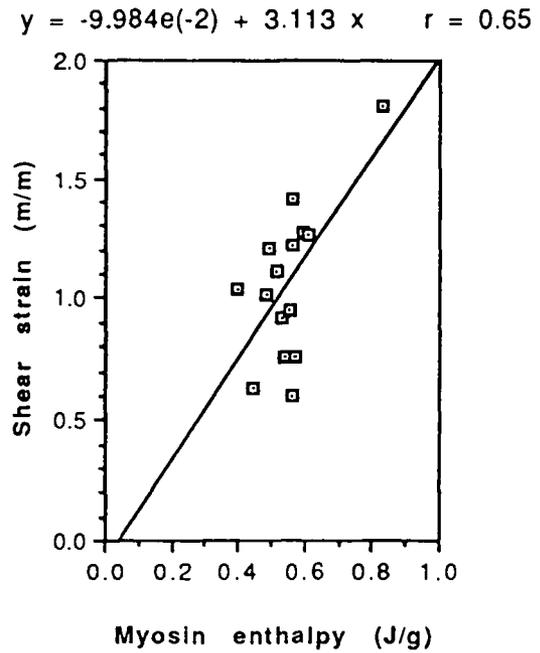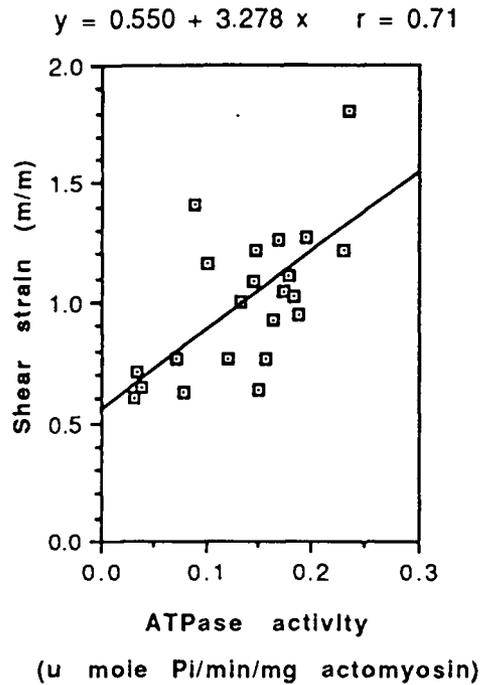
Fig. 7.   Correlations between the torsion test
and other measurements

Table 1. Correlation coefficients for physical, ther-
mal, and chemical measurement in Pacific whiting
for different conditions of frozen storage

	SSP	ATPase Activity	Shear Stress	Shear Strain	Myosin Enthalpy
SSP	1	0.81**	—	0.73**	—
ATPase Activity		1	—	0.71**	---
Shear Stress			1	0.71**	0.71*
Shear Strain				1	0.65*
Myosin Enthalpy					1

* Significant at p < 0.01
** Significant at p < 0.001
--- Not Significant

Table 2. Lipid oxidation of Pacific whiting fillets
as determined by TBA test

| Month | -8°C | | -20°C | |
	Vacuum	Non-Vacuum	Vacuum	Non-Vacuum
0	0.37 (0.06)	0.37 (0.06)	0.37 (0.06)[a]	0.37 (0.06)[a,b]
1	0.24 (0.10)	0.64 (0.17)[a]	0.27 (0.11)[a,b]	0.49 (0.17)[a]
2	0.33 (0.09)	0.27 (0.03)	0.32 (0.16)[a,b]	0.29 (0.04)[b]
3	0.41 (0.22)	0.33 (0.16)	0.24 (0.07)[b]	0.43 (0.19)[a,b]

[a,b] Means (Mg malonaldehyde / Kg) in the same column followed by different letters
are significantly ($p < 0.05$) different..

CONCLUSION

Shear strain correlates significantly with traditional chemical assays for protein denaturation and with DSC measurement of myosin peak enthalpy. Thus, shear strain may be considered a potentially useful tool for the determination of protein denaturation in fish fillets during periods of frozen storage. Variations in measurement attributed to differences in the sampling procedures, interference from lipid heat transition, and the difficulties relevant to problems of quantitative measurement make the DSC less valuable as a tool for the measurement of protein denaturation in frozen whole muscle fish.

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APPENDICES

APPENDIX A

Literature Review of Torsion Testing

Torsion tests were originally developed for testing the texture of vegetables and fruits (Diehl et al., 1979; Diehl and Hamann, 1979; Hamann, 1983). Subsequently, they were applied to determine the textural quality of surimi (i.e., washed minced fish) made from hoki (Macdonald et al., 1990), Alaska pollock (Kim et al., 1986; Hamann et al. 1990; Park et al., 1988), Atlantic menhaden (Hamann et al., 1990) and sand trout (Kim et al., 1986), and also to test the protein gel-forming ability of egg white (Montejano et al., 1984b), turkey and pork (Montejano et al. 1985), and beef (Montejano et al. 1985; Park et al., 1987). Hamann and Lanier (1988) concluded that torsion testing is a good method for the representation of the textural quality of seafoods. Hamann (1988) stated that torsion testing is based upon well-developed procedures of mathematical analysis, producing results which bear strong relationships to a sensory panel.

The torsion testing system involves heating protein gels, cutting them into dumbbell shapes, and finally twisting the hourglass-shaped sample gels to the point of failure with a modified viscometer (Kim et al., 1986). The results of torsion tests are presented as shear stress and

strain indicating, respectively, the strength and cohesive-
ness of protein gels (Kim et al., 1986). The equations for
the calculation of shear stress and true shear strain were
developed by Hamann (1983).

The factors that affect on torsion tests have been
examined as follows: protein quality (Kim et al., 1986);
the addition of water (Lanier et al, 1985); starch (Wu et
al., 1985b); egg white (Hamann et al., 1990); beef plasma
protein (Hamann et al., 1990); cryoprotectant (Park et al.,
1988); differential setting/heating procedures (Hamann et
al., 1990; Wu et al., 1985b; Montejano et al, 1984a; Kim et
al., 1986); and mixers, vacuum, and stuffing horn size
(Babbitt and Reppond, 1988). Torsion shear stress and
strain values respond to most of these factors in a similar
fashion. However, responses differ between shear stress
and shear strain for certain factors, such as the addition
of starch and water.

Hamann (1988) stated that both shear stress and strain
at rupture are important parameters since they often differ
in response to ingredient and process variables. Kim
(1987) found that shear strain was a more consistent indi-
cator of protein functionality and that shear stress was an
even stronger indicator of how temperature histories influ-
ence the formation of gels. Hamann (1988) suggested that
shear strain at failure is a fairly stable measure of pro-
tein functional quality, whereas shear stress is strongly
influenced by protein concentration, processing conditions,

and ingredient variables. For surimi products to pass the
Japanese folding tests which are the measure of good quali-
ty surimi, shear strain must be approximately 1.9 m/m or
greater (Lanier et al., 1985).

Factors Which Affect Torsion Test Results

Quality of Protein

The quality of protein is a very important factor in
the determination of torsion test results. It is generally
accepted that the season, species, size, and storage param-
eters will have significant effects upon the quality of sea-
food. Park et al. (1990) reported that tilapia surimi
processed at a pre-rigor stage produced shear stress and
shear strain values that were higher than those at the
post-rigor stage. In terms of gel-forming ability, Park et
al. (1987) also demonstrated significant differences be-
tween pre-rigor and post-rigor beef.

In protein gels made from beef (Park et al., 1987) and
from pollock surimi (Park et al., 1988), a decrease of
shear stress and shear strain was found during periods of
frozen storage. Kim et al. (1986) demonstrated that the
freeze-thaw cycle significantly decreased shear stress and
the true shear strain of protein gels made from sand trout
and Alaska pollock surimi.

Concentration of Protein

Shear strain has been proven to be a stable measure of protein functional quality, whereas stress is strongly influenced by dilution (Hamann, 1988). The results obtained by Lanier et al. (1985) demonstrated that the addition to Alaska pollock surimi significantly decreased shear stress, but remained without significant effect upon shear strain.

Effect of Ingredients

1. Starch:

Starch is added during traditional surimi processing to increase the gel-strength of the product. The effects of adding starch to fish protein gels were studied by Wu et al. (1985b). The starches used in these experiments including modified and unmodified waxy maize starches, potato starch, and pre-gelatinized tapioca starch. Results indicated that all of the starches, with the exception of the pre-gelatinized tapioca, increased shear stress without significant effect upon shear strain. Hamann et al. (1990) also demonstrated that the addition of unmodified potato starch increased the shear stress of protein gel made from Atlantic menhaden surimi without serious effect upon shear strain values.

2. Egg White and Beef Plasma Protein:

Hamann et al. (1990) found that the addition of egg white or beef plasma protein to pollock surimi gels cooked at either 90°C for 15 min. or at 40°C for 30 min. followed by cooking at 90°C for 15 min., were not significant. However, the addition of egg white or beef plasma protein significantly increased the shear stress and strain of pollock and menhaden surimi gels cooked at 60°C for 30 min., followed by cooking at 90°C for 15 min. It was concluded that this effect was due to the inhibition of proteolytic enzymes activated at temperatures between 50—70°C. Hamann et al. (1990) also found that the addition of egg white or beef plasma protein to low-grade pollock surimi increased shear stress and shear strain values, but that the effect of adding the same substances to high-grade pollock surimi remained without significant effect.

3. Cryoprotectant:

The effects of adding cryoprotectant to surimi were measured by Park et al. (1988), who found that the addition of sucrose/sorbitol and polydextrose increased shear stress and strain (shear strain value > 1.9) and maintained good quality pollock surimi exposed to -28°C storage for periods up to 8 months. The addition of only phosphates produced low shear stress and strain values and failed to demonstrate a cryoprotective effect. However, in the maintenance of gelling qualities (i.e., increased strain values),

sodium tripolyphosphate appeared to have a synergistic effect with the carbohydrate additives.

Equipment and Procedures for Processing Protein Gels

1. Mixer, Vacuum and Horn Size:

A comparison of products when using a Hobart Silent Cutter (Model 84181D), a Stephen Vacuum Mixer (Model UM12), a National Food Processor (Model MK-5050W), and a Kitchen-Aid Mixer (Model K5SS) was conducted by Babbitt and Reppond (1988). Results indicated few differences among the different mixers used to process fish gels. It was suggested that in lieu of using a Stephen vacuum mixer, as recommended by Lanier et al. (1985), the application of a vacuum treatment prior to stuffing may provide an adequate preparation procedure. However, in experiments conducted for the current investigation, significant differences were found among different mixers. Our results indicated that the use of the Stephen vacuum mixer (Model UM5) resulted in higher shear stress and strain values than found with other mixers in the lab; this is further discussed in Appendix C. Babbitt and Reppond (1988) also determined that decreasing the stuffing horn size lowered shear stress without having significant effect upon shear strain.

2. Different Setting/Heating Procedures:

Traditional heating processes for making fish protein gels involve cooking at 90°C for 15 min. Wu et al. (1985b)

demonstrated that a cooking temperature range from 60—90°C
did not significantly affect shear stress in fish gels.
The addition of starches (with the exception of pregelatin-
ized tapioca starch) into fish gels resulted in an increase
of shear stress coincident with an increase in cooking tem-
peratures. For shear strain, a variation of cooking
temperatures in the range 60—90°C was not significant.

Preheating at 40°C for 30 min., followed by cooking at
90°C for 15 min., increased shear stress and shear strain
in sand trout and pollock surimi (Kim et al., 1986), and in
menhaden surimi gels (Hamann et al., 1990). Montejano et
al. (1984a) also reported that preheating at 40°C for 1
hour, followed by cooking at 90°C for 15 min., increased
shear stress and strain for a combination of Atlantic
croaker and sand trout surimi. These increases in shear
stress and strain, when exposed to the 40°C preheating
treatment, could have been due to exposure to hydrophobic
amino acid residues, thus leading to intermolecular
hydrophobic interactions (Lanier, 1986).

However, preheating at 60°C for 30 min., followed by
cooking at 90°C for 15 min., decreased shear stress and
strain in menhaden (Hamann et al., 1990), sand trout and
pollock (Kim et al., 1986) surimi gels cooked only at 90°C
for 15 min. This decrease was attributed to the activity
of heat-stable proteolytic enzymes.

A low temperature setting at 4°C for 24 hours prior to
cooking at 90°C for 15 min. significantly increased shear

stress and true shear strain in pollock surimi gels, but
did not have the same effect upon sand trout surimi gels
(Kim et al., 1986). It was suggested that the response to
different setting/heating processes is species dependent.

Correlations Between Torsion and Sensory Testing

Correlations between torsion and sensory testing was
studied by Montejano et al. (1985), who found high correla-
tions between shear strain and such sensory parameters as
firmness, chewiness, springiness, cohesiveness, coarseness,
denseness and graininess (correlation coefficients $\geq$ 0.8,
p < 0.01). Shear strain and sensory cohesiveness reflected
the highest correlation coefficient (r = 0.87, p < 0.01).
The correlations between shear stress and the sensory notes
were lower than the shear strain and sensory note correla-
tions. Shear stress and sensory firmness reflected the
highest correlation coefficient (r = 0.72, p < 0.01).

Comparison of Torsion and Punch Testing

The punch test, a traditional method of measuring the
gel strength of protein gels, is conducted with a 5 mm
spherical probe, wherein probe penetration force and dis-
tance is recorded to the point of failure. Hamann (1988)
stated that for surimi gels with shear strains below ap-
proximately 3 m/m, punch penetration force correlated well
with torsion shear stress (r = 0.97). However, the corre-
lation between punch penetration distance and torsion true

shear strain was not as good (r = 0.69). Hamann and Lanier (1988) stated that the torsion test produced results which were superior to those of the punch test since changes in specimen shape and size were minor considerations in torsion testing. Prior to penetration, punch testing changes the shape of the sample cylinder. Thus, stresses and strains cannot be accurately computed. Two experiments, described in detail by Hamann and Lanier (1988) and by Lanier et al. (1985), have been conducted to demonstrate the measurement superiority of torsion testing relative to the punch test.

APPENDIX B

Literature Review of Differential Scanning

Calorimetry of Food Proteins

Differential scanning calorimetry (DSC) is a technique in which the difference in energy inputs into a substance and a reference material is measured as a function of temperature while the substance and the reference material are subjected to a controlled temperature program. DSC has been widely used in polymer science for a variety of analyses. It was found to be a particularly suitable technique for the study of food proteins (Wright et al., 1977). DSC has been used to determine thermal stability of animal proteins (Wright et al., 1977; Stabursvik and Martens, 1980) and fish proteins (Hastings et al., 1985; Poulter et al., 1985; Davies et al., 1988).

Use of the DSC on food proteins allows the study of thermal properties, denaturation kinetics, the fundamental interests of protein, and also the determination of protein-protein interactions. At present, the DSC has gained wide acceptance for the study of the effects of processing on the thermal stability of food proteins. The processing factors which have been examined include salt, sugar, pH, ionic strength, alcohol concentration, moisture, purification, freezing, and storage.

The DSC is able to measure the thermal transitions associated with the denaturation of protein upon heating. Transition temperatures and transition enthalpy are two important parameters that can be determined from the DSC thermogram. The temperature which has the maximum rate of heat input, or T_{max}, is used to describe protein transitions (Wright et al, 1977). T_{max} is influenced by heating rate (Ruegg et al., 1977; Wright et al., 1977) and by protein concentration (Wright, 1984) but under controlled conditions it can provide direct comparisons of the thermal stability of different proteins. Transition enthalpy can be determined by calculating the area under the DSC transition curve. The response of transition enthalpy to food processing factors can differ from that determined by the transition temperatures. For example, Hastings et al. (1985) reported that myosin transition enthalpy taken from DSC thermograms of cod muscle decreased after two weeks of storage at -10°C, without significant changes of T_{max}. Instrumental factors, including heating rate, sample weight, and the calibration of materials and procedures, must also be considered. It is important to control those factors to obtain representable results.

DSC Applications for Food Proteins

Fundamental Protein Studies

1. Cooperative Nature of Protein Unfolding:

Protein denaturation by heating involves the disruption of intramolecular bonding, followed by the unfolding of polypeptide chains. The initial unfolding is usually a highly cooperative phenomenon and is accompanied by a significant uptake of heat (Wright et al.,1977). The sharpness of the transition peak can be measured as the width at half-peak height, providing an index of the cooperative nature of the transition from a native to a denatured state. If denaturation occurs within a narrow temperature range, then the transition is considered to be highly cooperative (Wright et al, 1977).

2. Folding Domain of Protein:

Protein denaturation is generally represented as a transition from a native (folded state) to a denatured (unfolded, disordered state) structure. It is also assumed that protein denaturation is a highly cooperative transition between two states, with no intermediate states. This two-state theory works very well for small globular proteins (Privalov, 1979). However, Privalov (1982) concluded that the two-state theory is not true for multidomain proteins such as myosin, tropomyosin, and troponin-C.

DSC is one of the principal techniques used to demonstrate that many proteins have more than one folding domain. For the denaturation of myosin, Potekhin et al. (1979) found six cooperative domains.

3. Differentiated Proteins:

The DSC thermogram profile may serve as a fingerprint to differentiate muscle proteins. Different DSC profiles have been found among rabbit (Wright et al., 1977), beef (Quinn et al., 1980), fish (Park and Lanier, 1989), and poultry (Acton and Dick, 1986; Kijowski and Mast, 1988a). At the same time, within the same species, muscle proteins at different positions may show different DSC profiles. Kijowski and Mast (1988a) reported that DSC thermal profiles for chicken thigh and breast meat exhibited, respectively, three and five endotherm peaks, and that the transition temperatures were different between these two types of muscle.

4. Protein Thermal Stability:

DSC allows the direct measurement of protein thermal stability by simply measuring the transition temperatures of proteins. The protein transition temperatures reported in the literature include those for conalbumin (65°C) (Donovan et al. 1975), ovalbumin (84°C) (Donovan et al. 1975), B-lactoglobulin (86°C) (Park and Lund, 1984), cod myosin (45°C) (Martens and Vold, 1976; Hasting et al., 1985; Beas et al., 1990), cod actin (75°C) (Martens and Vold, 1976; Hasting et al., 1985; Beas et al., 1990), mammalian myosin (57—60°C) (Wright et al., 1977; Wagner and Anon, 1986), and mammalian actin (74—-80°C) (Wright et al., 1977; Findlay and Stanley, 1984). Thus: the higher the

transition temperature, the greater the thermal stability of the protein.

Protein-Protein Interactions

DSC is also a useful technique for the study of interactions between two different proteins. Donovan and Beardslee (1975) observed the stabilization of bovine trypsin when associated with both soybean trypsin inhibitor and egg white ovomucoid. Paulsson and Dejmek (1990) showed that a-casein decreased the thermal stability of a-lactalbumin, B-lactoglobulin, and bovine serum albumin (BSA). The destabilization of myoglobin in the presence of BSA was reported by Ledward (1978).

Thermal Properties of Protein

DSC has been used to measure the thermal properties of surimi (Wang and Kolbe, 1991). The thermal properties measured included initial freezing point, apparent specific heat, unfreezable water, unfrozen water weight fraction, and enthalpy. It was concluded that DSC provides a potential tool for the investigation of the thermal properties of frozen foods.

Kinetic Studies of Protein Denaturation

By examining time-dependent changes in DSC thermograms for proteins held at different storage temperatures, DSC

can be used to study the kinetics of protein denaturation (Wright, 1984). The denaturation kinetics of bovine B-lactoglobulin were examined by De Wit and Swinkels (1980) and by Park and Lund (1984). The apparent reaction order was 2.0 over a pH range of 4.0 to 9.0 (Park and Lund, 1984). Donovan et al. (1975), Donovan and Ross (1973), and Donovan and Beardslee (1975) studied the denaturation kinetics of soybean trypsin inhibitor and egg white ovomucoid. It was determined that the Arrhenius plot was a straight line for soybean trypsin inhibitor, but that there was a significant deviation from linearity for ovomucoid with increasing temperatures.

<div align="center">

DSC Determinations of Food Processing

Effects on Proteins

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Salt

The effects of adding salt to food proteins has been widely studied. The addition of sodium-chloride to the storage proteins of plants, including legumes, oilseeds, and cereals, and to milk proteins, especially B-lactoglobu-lin, has resulted in an increase in T_{max} (Itoh et al., 1976; Hermansson, 1978; Bikbov et al., 1983; Danilenko et al., 1985; Arntfield et al., 1986; Ismond et al., 1986; Harwalkar and Ma, 1987). However, Goodno and Swenson (1975) reported that meat proteins such as myosin and actin were destabilized by increasing salt concentrations. Add-

ing NaCl was also found to destabilize poultry breast meat (Kijowski and Mast, 1988b), croaker surimi (Wu et al., 1985a), and beef muscle (Barbut and Findlay, 1991; Quinn et al., 1980; Stabursvik and Martens, 1980). Stabursvik and Martens (1980) showed that KCl destabilized actin in bovine muscle, and that the degree of destabilization was dependent upon the KCl concentration. Barbut and Findlay (1991) found that the effect of KCl addition closely resembled NaCl for most cases.

Sugar

Sucrose and/or glucose were found to increase the thermal stability of B-lactoglobulin (Itoh et al., 1976; De Wit and Klarenbeck, 1981; Harwalkar and Ma, 1989) and egg white proteins (Back et al., 1979; Donovan, 1977; Donovan et al., 1975). Ismond et al. (1988) found that sucrose caused a decrease in surface hydrophobicity and suggested that the influence of sugars on protein conformation primarily took place through their indirect effect on hydrophobic interactions. Murray et al. (1985) reported that the addition of hexane did not significantly change either the transition temperature or the enthalpy of soybean and canola proteins. It was suggested that this effect could have been due to the low moisture content of these proteins.

pH

The transition temperatures and enthalpy of muscle proteins were found to be sensitive to changes in pH (Wright and Wilding, 1984; Wright et al., 1977; Samejima et al., 1983). In addition, the numbers of transitions for isolated myofibrillar proteins were found to be dependent upon pH (Wright et al., 1977; Stabursvik and Martens, 1980). Xiong and Brekke (1991) used DSC to scan the salt-soluble protein (SSP) extracted from breast and leg of chicken and determined that the SSP T_{max} increased with changes in pH from 5.5 to 6.5. Stabursvik and Martens (1980) demonstrated that T_{max} of actin decreased slightly as pH values exceeded 6.5. Goodno and Swenson (1975) reported a decline in myosin transition temperatures from 43 to 37°C when the pH was decreased from 7.0 to 5.4.

Ionic Strength

Ionic strengths have been found to affect the transitions of purified myofibrillar proteins (Samejima et al., 1983; Wright et al., 1977). The numbers of myosin endotherm peaks varied from one at 0.1 M KCl (Samejima et al., 1983), to two at an ionic strength of 0.05 M KCl (Wright et al., 1977), and to three at 0.6–1.0 M KCl (Wright et al., 1977; Samejuma et al., 1983).

Alcohol

Arntfield et al. (1990) observed that the treatment of faba bean protein by several alcohols did not affect T_{max}, whereas the enthalpy values were significantly reduced. It was suggested that this effect was probably due to the ability of the alcohols to penetrate the protein molecules.

Purification Process

The purification process can affect the stability of food proteins (Park and Lanier, 1989; Wright et al., 1977). Wright et al. (1977) reported that the transition temperatures of actin and actomyosin in rabbit myofibrils were about 10°C lower than that in rabbit muscles. It was suggested that this effect may have been due to the presence of specific ions such as Ca^{++} or ADP in the muscle samples and to the absence of those ions in the myofibrillar or actomyosin samples.

Freezing

DSC was also used to measure the effects of freezing on fish muscle and egg white proteins. Hastings et al. (1985) demonstrated that periods of freezing followed by immediate thawing had little effect upon the characteristic thermal transitions of cod muscle. However, Wootton et al. (1981) observed that freezing caused a decrease in the enthalpy of egg white proteins, and that the magnitude of

the reduction was dependent upon the freezing rate and thawing conditions.

Storage

The effects of storage upon fish proteins has been studied by use of the DSC. Kim et al. (1986) examined the effects of freezing and thawing cycles upon sand trout and Alaska pollock surimi. It was found that the heat stability of fish proteins was not affected by freezing and thawing, at least not in the sense that the transition peaks were subject to shifts to different temperatures. However, the areas under the endothermic peaks decreased with additional freezing and thawing cycles. Reid et al. (1988) observed a decline in myosin transition enthalpy in rockfish muscle after 5 months of storage at -5°C. Hastings et al. (1985) reported that the myosin transition enthalpy taken from DSC thermograms of cod muscle decreased after two weeks of storage at -10°C. These results indicated that the changes in myosin transition enthalpy were related to the denaturation of myosin during periods of frozen storage.

DSC was also used to examine changes in egg proteins during storage. Ledward (1978) found that the storage of egg white at -19°C led to a 41% reduction in enthalpy after 84 days, with no change in T_{max}. Arntfield et al. (1990) also observed an 18% reduction in the enthalpy of egg white powder stored at 54°C for a single week. Donovan and Mapes

(1976) reported that ovalbumin was converted to the more stable S-ovalbumin during storage as indicated by an increase in T_{max} from 84.5 to 92.5°C. It was stated that S-ovalbumin could be detected easily by DSC, thus providing a rapid method for the assessment of the quality of eggs and egg products held in storage.

APPENDIX C

Comparison of the Effects of Mixers

Used for Torsion Tests

To select the appropriate mixer for producing fish
protein gels, the effects of different type mixers were
investigated in two separate experiments. The first exper-
iment involved the comparison of a KitchenAid mixer (model
K5-A) with a Cuisinart food processor (model DLC-7M).
Controlling final temperatures to 12°C, rockfish fillets
(800 g) were mixed with 2% salt for periods of 20 min. and
2 min., respectively, in the KitchenAid and Cuisinart
mixers. After mixing, the fish paste was extruded into
stainless steel cooking tubes (inside diameter 2.2 cm,
length 17.8 cm) with a sausage stuffer (5-lbs capacity, The
Sausage Maker, Buffalo, NY). Fish protein gels were pro-
duced by heating in a 90°C water bath for 15 min., followed
by cooling in ice water for 10 min. The torsion test was
then performed to determine the shear stress and shear
strain of the fish protein gels. The results demonstrated
that the use of the Cuisinart food processor significantly
increased shear strain, but without effect upon shear
stress.

The second experiment was a comparison between the
Cuisinart food processor in our labortory and a Stephan

vacuum mixer in the Food Science and Technology labortory at North Caloria State University (NCSU). Frozen rockfish fillets were put in a insolated box with dry ice and ship to NCSU by air freight. The rockfish (1200 g) fillets were mixed with 2% salt for 8.5 min. and 2 min., respectively, in the Stephan vacuum mixer and the Cuisinart food processor. The same procedure for processing fish protein gels described above was used for this experiment. In addition, the effect of a vacuum-bagging treatment on the fish protein gels was also investigated by transferring the fish paste into vacuum-sealed plastic bags prior to stuffing. The results indicated that the use of the Stephan vacuum mixer tended to provide higher shear strain values without significant differences in shear stress values. Vacuum treatment prior to stuffing had no effect upon either the shear strain or shear stress values.

Comparison Between a Cuisinart Food Processor and a KitchenAid Mixer		
Test item	Shear stress (KPa)	Shear strain (m/m)
Cuisinart	35.1 (2.3)[a]	1.84 (0.12)[a]
KitchenAid	32.0 (2.0)[a]	1.57 (0.08)[b]
[a,b] Means in the same column followed by different letters are significantly different ($p < 0.05$).		

Comparison Between Cuisinart Food Processor and a Stephan Vacuum Mixer		
Test item	Shear stress (KPa)	Shear strain (m/m)
Cuisinart	29.7 (2.1)[a]	1.90 (0.10)[a]
Stephan mixer (vacuum)	38.1 (2.5)[b]	2.32 (0.11)[b]
Stephan mixer (no vacuum)	34.0 (2.8)[a,b]	2.26 (0.08)[b]
[a,b] Means in the same column followed by different letters are significantly different ($p < 0.05$).		