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Emulsifying Capacity of Collagenous Material from the Muscle and Skin of Hake (*Merluccius merluccius* L.) and Trout (*Salmo irideus* Gibb): Effect of pH and NaCl Concentration

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ABSTRACT

The relationship between collagen concentration and emulsifying capacity as well as the effect of pH and sodium chloride concentration on the development of this functional property were studied. Due to what is known as the dilution effect, emulsifying capacity, when expressed in terms of the quantity of soluble protein, decreased as collagen concentration increased. When expressed in terms of total protein, emulsifying capacity decreased as the NaCl concentration increased and was highest at pH levels of between 1 and 3. A power function that described the behaviour of this functional property in terms of soluble protein independently of the factors considered, i.e., concentration, pH, and percentage sodium chloride, was found. Generally speaking, emulsifying capacity, expressed in terms of the quantity of soluble protein, can be regarded as higher in the collagenous material from the hake than in that from the trout, and higher in the muscle connective tissue than in the dermal connective tissue.

INTRODUCTION

Emulsifying capacity (EC) is an extremely important functional property in food processing, and it has been studied extensively in such food systems as myofibrillar proteins (Carpenter & Saffle, 1965; Neelakantan & Froning, 1971; Dawood, 1980) yet only slightly in collagen, particularly fish collagen.

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In one of the few references on the subject, all dealing with food animals, Schalk (1980) replaced a portion of muscle with connective tissue and found hardly any differences in such parameters as emulsion stability and texture, with only colour being affected. Gielissen (1981) prepared bologna containing 5, 10 and 15% collagen which were in all cases comparable to the control sausages.

Perhaps the most important impediment to the use of collagen is that, because it is a highly insoluble protein, it has been presumed to have little functional activity. As early as 1973, however, Satterlee *et al.* (1973) had pointed out that, when adequately processed, the use of collagen afforded advantages with respect to milk proteins.

Today's fish processing industry is largely based on more intensive processing, leading to the accumulation of large amounts of waste, much of which is still of high nutritional and functional value, as is the case of skin and muscle connective tissue. The utilization of such products in human nutrition instead of in the manufacture of fish meal for animal feeds could therefore be of great importance.

The object of the present study was to examine the emulsifying capacity of muscle and skin connective tissue from two species of fish that are frequently used in Spain, hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb) in solutions with differing pH levels and varying percentage proportions of NaCl. Only the acid range of pH was tested, because antinutritional effects have been described for the basic range of pH. Basic treatment results in the deamination and decomposition of the essential amino acids, primarily lysine, arginine, histidine, and threonine (Asghar & Henrickson, 1982). Furthermore, basic preparation of gelatine causes other alterations such as racemization of the amino acids and the formation of lysino-alanine (Friedman *et al.*, 1981).

MATERIALS AND METHODS

Two fish species, hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb), were employed. The hake were caught by long-lining over the continental shelf off Galicia in Spain during the month of November, with specimens making up a homogeneous batch 15 kg in weight. The trout were farm-reared and made up a homogeneous batch 10 kg in weight. Mean individual size was 60·7 cm for the hake, 24·5 cm for the trout; mean individual weight was 1600 g for the hake, 250 g for the trout.

Specimens were chilled in ice from capture to the start of the experiment some 24 h later.

The fish were headed and gutted, and fillets were skinned. To separate the

collagenous material, i.e. the connective tissue, from the skin, the epidermis was removed manually, along with any remnants of muscle tissue and fat adhering to the dermis. The connective tissue was separated and cleaned as described by Borderías and Montero (1985).

When clean the connective tissue was pressed between sheets of absorbent paper in order to remove as much water as possible without giving rise to any chemical changes. The collagenous material was then collected in 1 g batches in Eppendorf vials and stored frozen at −24°C for the duration of the study period (no more than 15 days).

Moisture, ash, and crude protein were analyzed following AOAC procedures (1975). Crude fat was determined according to the method of Bligh and Dyer as modified by Knudsen (pers. comm.). Three replicates were performed for all determinations.

The emulsifying capacity of the connective tissue was measured using a modified version of the method set out by Tejada *et al.* (1987). Samples were prepared by homogenizing the collagenous material in 0·5M acetic acid in an Omnimixer operated at setting 9 for 1 min. The mixture was then magnetically stirred at 3°C for 24 h. After stirring the solution was filtered through thick cheesecloth and centrifuged at 1000*g* at 3°C for 10 min in order to eliminate any air bubbles produced by filtration. The extract thus obtained was used to prepare an emulsion. In order to do this, 5 g of extract were added to 20·0 ml of H_2O and 4·2 g of oil and blended in an Osterizer homogenizer in which two electrodes had been placed such that they were in contact with the emulsion. During blending, oil was added continuously until the emulsion was unable to hold more and ruptured. Such rupture of the emulsion was determined as the cessation of conductivity, measured by a polymeter connected to the electrodes. The amount of oil added was then quantified. Emulsifying capacity was expressed as g of oil/100 g of soluble protein and as g of oil/g of extract.

The concentrations of collagenous material studied were 1/25, 1/50, 1/60, 1/75, 1/100, 1/150, 1/175, 1/200, 1/300 and 1/1000 (w/v) in the hake, corresponding to 9·6, 4·8, 4·0, 3·2, 2·4, 1·9, 1·6, 1·4, 1·0, 0·8 and 0·2 g/ml of muscle connective tissue and 13·4, 6·7, 5·6, 4·5, 3·3, 2·7, 2·2, 1·9, 1·7, 1·1 and 0·3 g/ml of skin connective tissue. These same concentrations, except 1/1000, were used in the case of the trout, corresponding to 9·8, 4·9, 4·1, 3·3, 2·5, 1·8, 1·6, 1·4, 1·2 and 0·8 g/ml of muscle connective tissue, and 13·3, 6·6, 5·5, 4·4, 3·3, 2·7, 2·2, 1·9, 1·7 and 1·1 g/ml of skin connective tissue.

In order to study emulsifying capacity in response to changes in the pH and in the percentage proportion of NaCl, a dilution of collagenous material/solvent of 1/25 (w/v) was used, corresponding to 9·6 g/ml of the collagenous material from the hake muscle and 13·4 g/ml of the collagenous material from the hake skin and 9·8 g/ml of the collagenous material from

the trout muscle and 13·3 g/ml of the collagenous material from the trout skin. The proportions of NaCl tested were 0·0, 1·0, 1·5, 2·0 and 3·0% for the hake and 0·0, 1·0, 1·5, 2·0, 3·0 and 6·0% for the trout. The pH values studied were 1, 2, 3, 4, 5, 6 and 7 for both species. Four replications were performed for all determinations.

Statistical analysis consisted of analysis of variance using Tukey's test to determine the level of significance of the differences between the mean values of emulsifying capacity recorded as the concentration of collagenous material, pH, and percentage proportion of NaCl were varied.

The coefficients of correlation and the level of significance of the correlations ($P \leq 0{\cdot}05$) were determined for the collagenous material from the two species and for the two types of collagenous material (skin and muscle) within each species.

RESULTS AND DISCUSSION

Table 1 gives the proximate composition of the collagenous material from the skin and muscle used in this study.

Collagen yield with respect to total protein was 95·7% for the muscle connective tissue and 95·0% for the skin connective tissue; that is, nearly all the protein in the collagenous material was collagen.

Emulsifying capacity of the collagenous material from the hake, expressed in terms of the weight of the homogenate, increased as collagen concentration increased (Fig. 1(a); Fig. 1(b)), in contrast, depicts the opposite effect when emulsifying capacity is expressed in terms of the weight of the soluble protein. The reason for this was that, while the quantity of

TABLE 1
Proximate Analysis (%), Crude Protein (with respect to dry matter) and Initial pH of Muscle and Skin Connective Tissue

	Hake		*Trout*	
	Muscle connective tissue	*Skin connective tissue*	*Muscle connective tissue*	*Skin connective tissue*
Moisture	72·02 ± 1·0	64·46 ± 0·2	72·48 ± 0·4	63·99 ± 0·3
Ash	1·69 ± 0·0	1·12 ± 0·1	1·47 ± 0·0	1·27 ± 0·1
Crude fat	1·20 ± 0·1	2·00 ± 0·3	3·64 ± 0·0	1·47 ± 0·0
Crude protein	24·1 ± 1·7	33·5 ± 1·3	24·6 ± 0·2	33·3 ± 0·1
Crude protein	86·1 ± 1	94·3 ± 2	89·5 ± 0·6	92·4 ± 1
pH	7·47 ± 0·0	7·19 ± 0·0	7·54 ± 0·1	7·06 ± 0·0

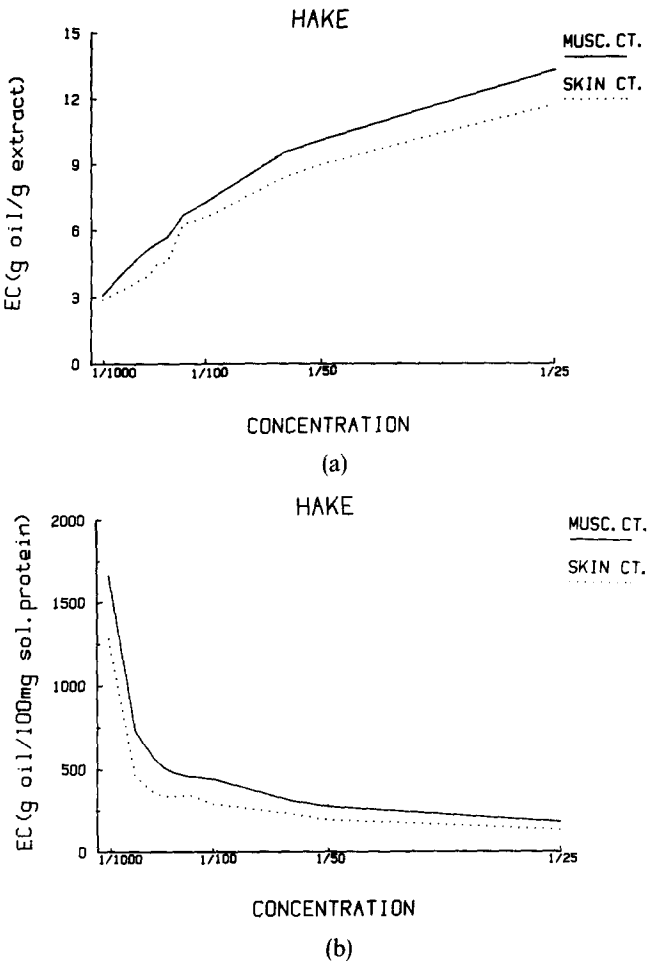

Fig. 1. Emulsifying capacity (EC) of differing concentrations of the collagenous material from the hake.

total protein remained constant, augmenting the dilution reduced the number of intermolecular crosslinks, resulting in greater unfolding of the polypeptides, thereby exposing the hydrophobic associations. This phenomenon is known as the dilution effect, and has been reported by certain other workers in the case of other proteins (Kinsella, 1976; Bello *et al.*, 1978; Borderías *et al.*, 1985).

The behaviour of the collagenous material from the muscle was similar to that of the collagenous material from the skin, although the values for the latter were lower, even when emulsion capacity was expressed in terms of the quantity of soluble protein. This behaviour was not in conformity with that for hydration properties observed by Montero (1988). Although solubility is an important factor affecting the distribution of the proteins at the interface,

other factors, e.g. the proportion of the hydrophobic groups, also play a role in determining the emulsifying capacity (Schut, 1976; Kinsella, 1976).

In the hake there were significant differences at the 95% level between the emulsifying capacity values at varying concentrations of the collagenous material from both the muscle and the skin (Table 2). Although the differences between the emulsifying capacity values for the collagenous material from the muscle and for that from the skin were quite small, they were significant at the 95% level in practically all cases.

In the trout (Fig. 2), the behaviour of the emulsifying capacity values was

TABLE 2

Analysis of Variance among Mean Values of Emulsifying Capacity for Differing Concentrations of the Different Collagenous Materials

Concentration	*Emulsifying capacity (g of oil/g of homogenate)*		*Emulsifying capacity (g of oil/100 g of soluble protein)*	
	Muscle connective tissue	*Skin connective tissue*	*Muscle connective tissue*	*Skin connective tissue*
Hake				
1/1 000	*a/x*	*a/y*	*a/x*	*a/x*
1/300	*b/x*	*b/y*	*b/x*	*b/y*
1/200	*c/x*	*c/y*	*c/x*	*c/y*
1/175	*c/x*	*d/y*	*ec/x*	*c/y*
1/150	*c/x*	*d/y*	*ed/x*	*c/y*
1/125	*d/x*	*e/y*	*fe/x*	*cd/x*
1/100	*d/x*	*f/y*	*f/x*	*c/y*
1/60	*e/x*	*g/y*	*g/x*	*d/y*
1/50	*f/x*	*h/y*	*g/x*	*e/y*
1/25	*g/x*	*i/y*	*h/x*	*f/y*
Trout				
1/300	*a/x*	*a/y*	*a/x*	*a/y*
1/200	*bc/x*	*ab/y*	*a/x*	*a/y*
1/175	*b/x*	*bc/y*	*b/x*	*b/y*
1/150	*bc/x*	*bc/y*	*bc/x*	*c/y*
1/125	*cd/x*	*c/y*	*c/x*	*a/y*
1/100	*cd/x*	*d/y*	*d/x*	*ed/y*
1/75	*d/x*	*e/y*	*e/x*	*f/y*
1/60	*e/x*	*f/y*	*fe/x*	*fe/y*
1/50	*f/x*	*g/y*	*e/x*	*f/y*
1/25	*g/x*	*h/y*	*f/x*	*g/y*

Different letters indicate significant differences ($P \leq 0{\cdot}05$): *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i* for columns, *x*, *y* for rows. Separate analyses of variance were carried out for g of oil/g of homogenate and g of oil/100 g of soluble protein in both the hake and the trout.

similar to that observed in the hake. Significant differences at the 95% level were recorded between the emulsifying capacity values for differing concentrations of the collagenous material from both the muscle and the skin (Table 2). The differences observed between the collagenous material from the muscle and that from the skin in the trout were significant ($P \leq 0{\cdot}05$) in most cases.

In the trout, when emulsifying capacity was expressed as g of oil per g of homogenate, EC values for the collagenous material from the skin were higher than those for the collagenous material from the muscle (Fig. 2(a)). However, this was because the former homogenate contained more soluble protein; and consequently, when emulsifying capacity was expressed in units of soluble protein, the EC values for the collagenous material from the muscle were higher than those for the collagenous material from the skin (Fig. 2(b)).

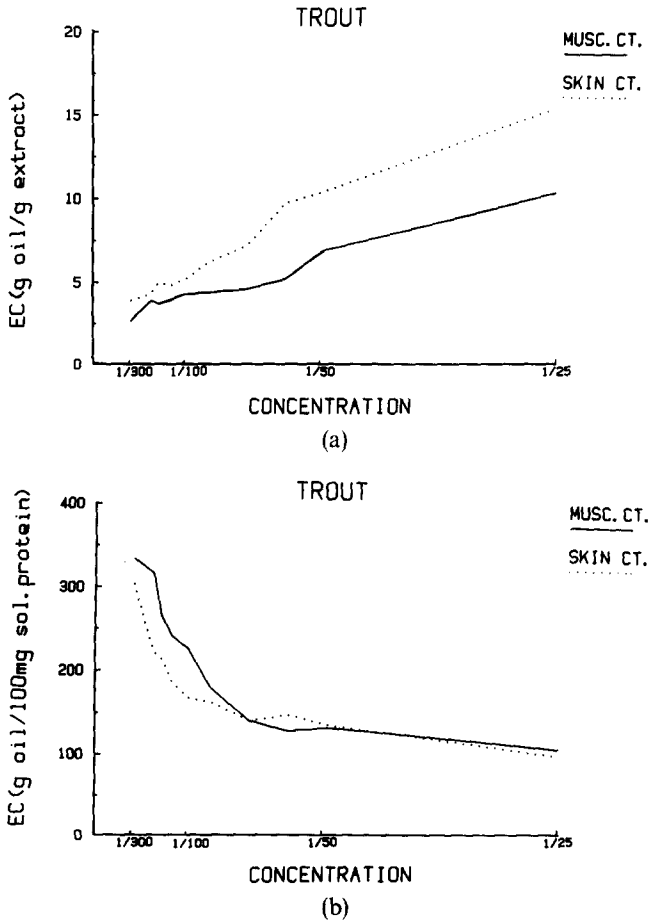

Fig. 2. Emulsifying capacity (EC) of differing concentrations of the collagenous material from the trout.

As Figs 1(b) and 2(b) make clear, for equal quantities of soluble protein, the soluble protein from the hake was more effective at forming emulsions. Similar findings have been reported with respect to viscosity (Montero, 1988).

Figure 3(a) shows that adding NaCl in amounts of up to 2% of the solubilized collagenous material from the skin had no effect on the emulsifying capacity of the collagenous material in the case of the hake; in contrast, the emulsifying capacity of the collagenous material from the hake muscle decreased for all quantities of NaCl added. One explanation for this might be that, at amounts greater than 2%, the collagen became insolubilized (Montero, 1988), thereby reducing the amount of protein available for emulsification. When emulsifying capacity was expressed in

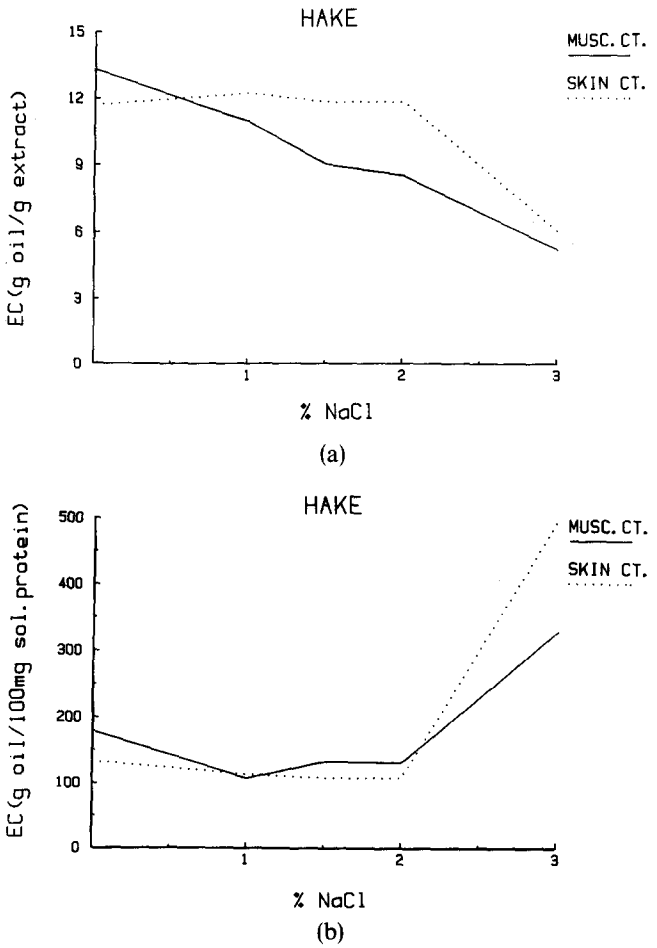

Fig. 3. Emulsifying capacity (EC) of the collagenous material from the hake at differing percentage proportions of NaCl.

terms of soluble protein (Fig. 3(b)), EC values remained stable at levels of up to 2% NaCl, after which they increased appreciably. This quantity of NaCl corresponded to the point at which protein insolubilization set in, suggesting that the dilution effect reduced the availability of the soluble protein and, as a consequence, this resulted in fewer protein–protein cross-links and greater unfolding of the polypeptides, thereby exposing the hydrophobic associations.

Comparing the emulsifying capacity values recorded at varying proportions of NaCl in all cases yielded significant differences at the 95% level between the collagenous material from the muscle and from the skin, although the trends were similar for both types of collagenous material.

As just discussed in the case of the hake, in the trout (Figs 4(a) and 4(b))

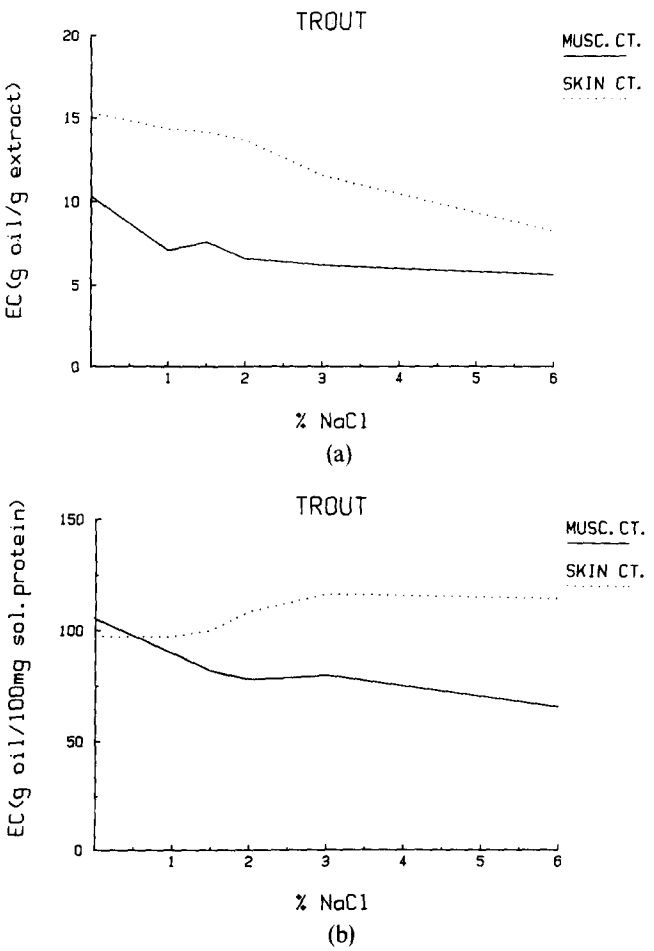

Fig. 4. Emulsifying capacity (EC) of the collagenous material from the trout at differing percentage proportions of NaCl.

emulsifying capacity at different proportions of NaCl was related to collagen solubility and presented higher values at very low or negligible proportions of NaCl, which was the point at which the solubility of the collagen was highest (Montero, 1988). Nevertheless, the addition of NaCl to the acid solution of collagenous material did not exert any marked effect on this functional property.

The EC values for the collagenous material from the muscle were significantly different ($P \leq 0{\cdot}05$) in practically all cases in both the hake and the trout, but this was not true for the collagenous material from the skin (Table 3). Thus, the addition of NaCl to the solution affected emulsifying capacity, even if only to a minor extent. The charging of the water–protein film interface may be the mechanism by which high concentrations of ions reduced the emulsifying capacity, with the interface then possibly repelling the protein, which was also highly charged in the acid solution.

The differences between the two types of collagenous material were always significant ($P \leq 0{\cdot}05$), with higher EC values for the collagenous material from the skin.

TABLE 3

Analysis of Variance among Mean Values of Emulsifying Capacity for the Different Collagenous Materials in NaCl Solutions of Differing Concentrations

% NaCl	*Emulsifying capacity (g of oil/g of homogenate)*		*Emulsifying capacity (g of oil/100 g of soluble protein)*	
	Muscle connective tissue	*Skin connective tissue*	*Muscle connective tissue*	*Skin connective tissue*
Hake				
0·0	*a/x*	*a/y*	*a/x*	*a/y*
1·0	*b/x*	*b/y*	*b/x*	*ab/y*
1·5	*c/x*	*c/y*	*c/x*	*b/y*
2·0	*c/x*	*c/y*	*c/x*	*b/y*
3·0	*d/x*	*d/y*	*d/x*	*c/y*
Trout				
0·0	*a/x*	*a/y*	*a/x*	*a/y*
1·0	*b/x*	*a/y*	*b/x*	*ab/y*
1·5	*c/x*	*ab/y*	*c/x*	*ab/y*
2·0	*d/x*	*bc/y*	*d/x*	*b/y*
3·0	*e/x*	*c/y*	*e/x*	*c/y*
6·0	*f/x*	*c/y*	*f/x*	*d/y*

Different letters indicate significant differences ($P \leq 0{\cdot}05$): *a, b, c, d, e, f* for columns, *x, y* for rows. Separate analyses of variance were carried out for g of oil/g of homogenate and g of oil/100 g of soluble protein in both the hake and the trout.

Figure 5(a) shows that emulsifying capacity, expressed in terms of the weight of the homogenate, remained stable at very low pH values, then decreased sharply at pH 3 (muscle connective tissue) and pH 4 (skin connective tissue), probably because protein solubility was high at very acid pH levels (Montero, 1988), with protein solubility falling and lowering the availability of protein for emulsification only when the pH approached the isoelectric point. On the other hand, when emulsifying capacity was expressed in terms of the weight of the soluble protein (Fig. 5(b)), EC values rose considerably after pH 5. The cause of this increase in the EC at pH levels close to the isoelectric point may have been a decrease in solubility at those levels, thereby reducing the number of intermolecular cross-links in the soluble proteins and favouring the formation of cross-links with the

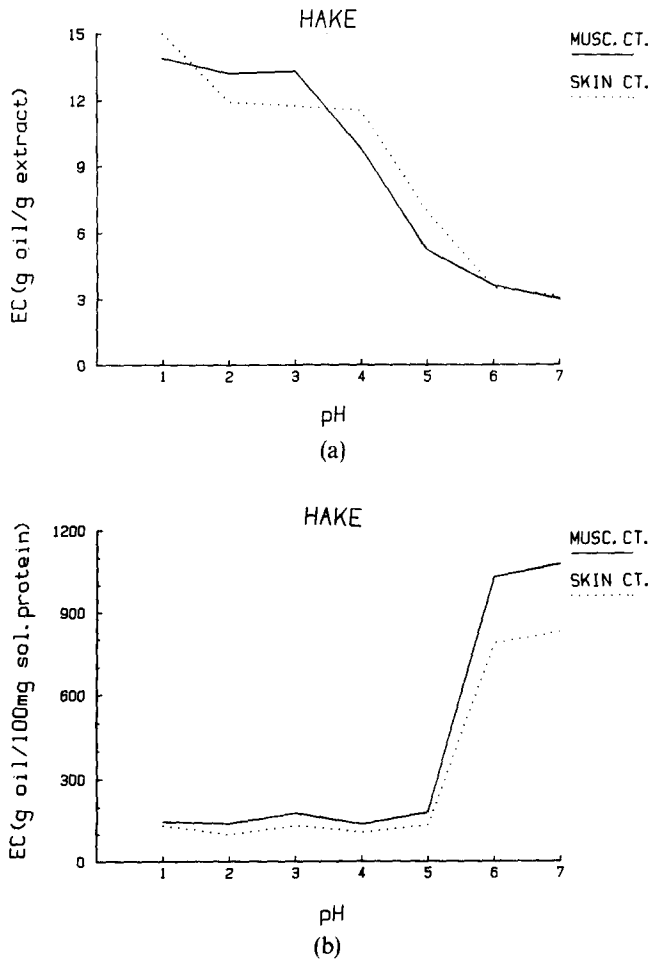

Fig. 5. Emulsifying capacity (EC) of the collagenous material from the hake at differing pH levels.

medium (dilution effect). In addition, as the isoelectric point is approached, the number of hydrophobic bonds increases, as pointed out by Cheftel *et al.* (1985) for certain proteins.

There were practically no significant differences ($P \leq 0{\cdot}05$) in EC values at varying levels of pH over the range between 1 and 5 when emulsifying capacity was expressed in terms of g of soluble protein. The differences found were small in comparison with those recorded between pH 5 and pH 6 (Table 4). The differences between the EC values for the collagenous material from the muscle and from the skin were significant at the 95% level in most cases, and the values for the collagenous material from the muscle were higher.

In the trout (Fig. 6) the behaviour of the emulsifying capacity of the collagenous material from the muscle and from the skin was similar to that

TABLE 4

Analysis of Variance among Mean Values of Emulsifying Capacity for the Different Collagenous Materials under Differing pH Conditions

pH	*Emulsifying capacity (g of oil/g of homogenate)*		*Emulsifying capacity (g of oil/100 g of soluble protein)*	
	Muscle connective tissue	*Skin connective tissue*	*Muscle connective tissue*	*Skin connective tissue*
Hake				
1	*a/x*	*a/y*	*a/x*	*a/y*
2	*a/x*	*b/y*	*a/x*	*b/y*
3	*a/x*	*b/y*	*a/x*	*a/y*
4	*b/x*	*b/y*	*a/x*	*b/y*
5	*c/x*	*c/y*	*a/x*	*c/y*
6	*d/x*	*d/y*	*b/x*	*d/y*
7	*d/x*	*d/y*	*b/x*	*d/y*
Trout				
1	*a/x*	*a/y*	*a/x*	*a/y*
2	*ab/x*	*b/y*	*a/x*	*b/y*
3	*b/x*	*b/y*	*a/x*	*c/y*
4	*a/x*	*c/y*	*a/x*	*c/y*
5	*c/x*	*c/y*	*a/x*	*d/y*
6	*d/x*	*d/y*	*b/x*	*e/y*
7	*cd/x*	*de/y*	—	—

Different letters indicate significant differences ($P \leq 0{\cdot}05$): *a*, *b*, *c*, *d*, *e* for columns, *x*, *y* for rows. Separate analyses of variance were carried out for g of oil/g of homogenate and g of oil/100 g of soluble protein in both the hake and the trout.

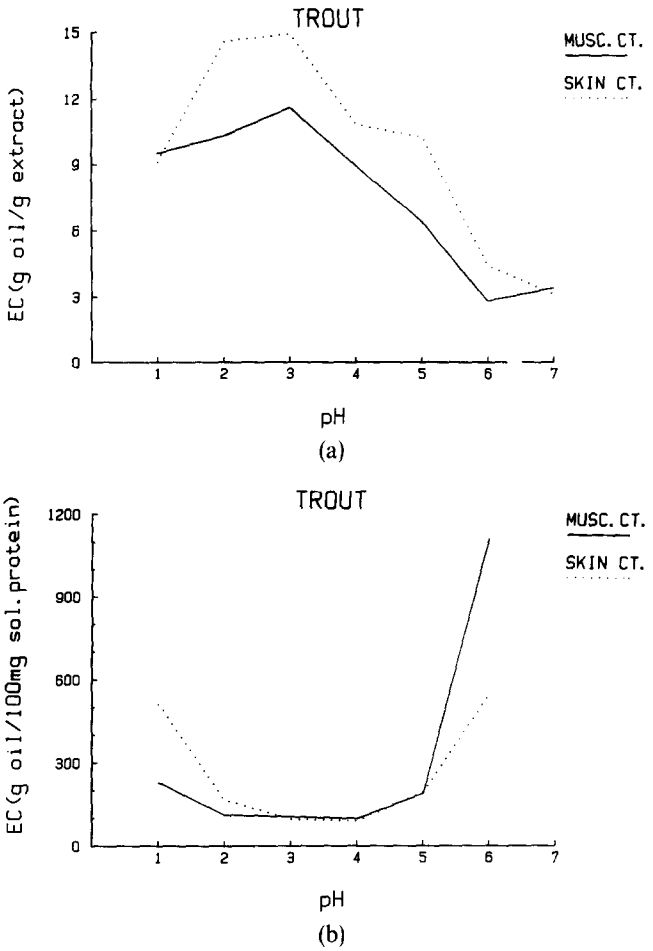

Fig. 6. Emulsifying capacity (EC) of collagenous material from the trout at differing pH levels.

in the hake. As in the hake, in the trout the differences ($P \leq 0{\cdot}05$) between the EC values at pH levels between 1 and 5 when emulsifying capacity was expressed in terms of g of soluble protein, though significant, were small when compared with those recorded between pH 5 and pH 6. The EC values stabilized after pH 6, and there were no significant differences ($P \leq 0{\cdot}05$) (Table 4).

As discussed above, emulsifying capacity values were generally higher for the collagenous material from the hake than for that from the trout when emulsifying capacity was expressed in terms of soluble protein.

Emulsifying capacity, expressed in terms of g of homogenate, increased in all cases as concentration increased; however, when emulsifying capacity was expressed in terms of g of soluble protein, the previously mentioned dilution effect came into play.

TABLE 5
Correlations between Emulsifying Capacity Values for the Collagenous Material from Hake and Trout Muscle and for the Collagenous Material from Hake and Trout Skin

Functional property	*Muscle connective tissue*	*Skin connective tissue*
Emulsifying capacity/concentration	0·913*	0·986*
Emulsifying capacity/proportion of NaCl	−0·136	0·765*
Emulsifying capacity/pH	0·897*	0·657*

* $P \leq 0{\cdot}01$.

TABLE 6
Correlations ($P \leq 0{\cdot}01$) between Emulsifying Capacity Values for the Collagenous Material from the Muscle and the Skin Combined, in the Hake and in the Trout

Functional property	*Hake*	*Trout*
Emulsifying capacity/concentration	0·993	−0·397
Emulsifying capacity/proportion of NaCl	0·985	0·692
Emulsifying capacity/pH	0·959	−0·698

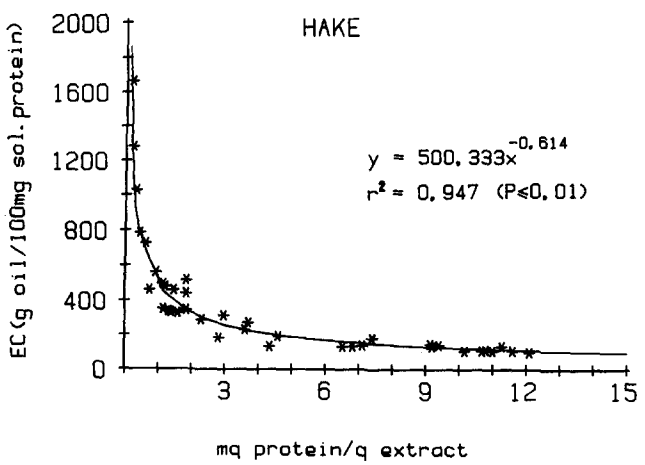

Fig. 7. Emulsifying capacity expressed as g of oil/100 mg of protein compared to g of protein/g of homogenate for the collagenous material from the hake at varying levels of concentration, proportions of NaCl, and pH.

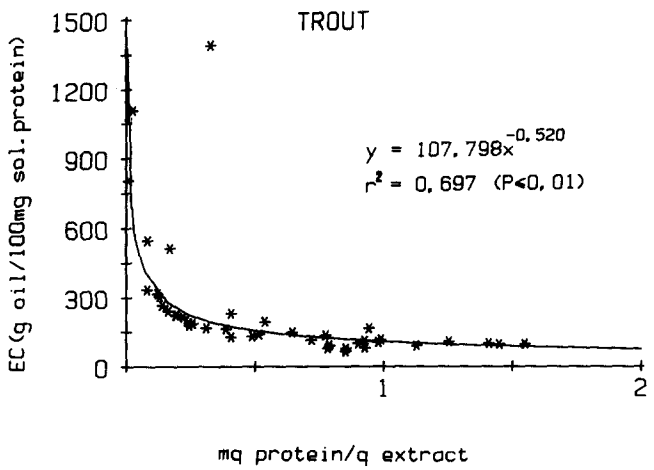

Fig. 8. Emulsifying capacity expressed as g of oil/100 mg of protein compared to g of protein/g of homogenate for the collagenous material from the trout at varying levels of concentration, proportion of NaCl, and pH.

The effect of adding NaCl and the effect of pH were dependent upon protein solubility, as has been reported by other workers for other kinds of protein (Frazen & Kinsella, 1976; Aoki *et al.*, 1980).

Tables 5 and 6 set out the degree of correlation obtained between emulsifying capacity values for the two types of collagenous material irrespective of species and between EC values for both types of collagenous material combined in each of the species separately, for different collagen concentrations, proportions of NaCl, and pH.

Correlations significant at the 99% level were obtained in practically all cases, both for the collagenous material from the same anatomical structures irrespective of species and for the collagenous material from the different anatomical structures combined for each species separately, which is indicative of a certain similarity in the behaviour of the emulsifying capacity of the collagenous material from the two different structures and from the two different species as the parameters under consideration were varied.

Emulsifying capacity is depicted in Figs 7 and 8 in terms of the quantity of soluble protein in relation to the factors collagen concentration, proportion of NaCl, and pH for the two types of collagenous material from each species, hake in Fig. 7 and trout in Fig. 8. An exponential function that describes the behaviour of emulsifying capacity in terms of soluble protein, independently of the factors studied, was derived. This means that, while protein solubility is certainly not the only important factor affecting emulsifying capacity, it is sufficiently important in itself to be capable of describing the behaviour of this functional property with a high degree of significance ($P \leq 0{\cdot}01$).

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