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PARAMETERS AFFECTING THE LIBERATION OF SKINS FROM DIFFERENT EGYPTIAN FISH SPECIES COMMONLY USED FOR FILLET PROCESSING.

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ABSTRACT

The solubility of skins from different fish species: Bolti (Talipia) fish, Wakar (Grouper) and Kishr-Bayad (Nile perch) were investigated using the proteolytic enzymes from their viscera.

It was found that by careful control of the pretreatment solution, fish viscera enzymes and skin substrates concentrations, fish skins from different species are readily to become solubilized. Concentration of 5.0% NacL. 3.0% HcL and 3% Acetic acid in addition to the proper concentration of viscera enzymes were found to be efficient in solubilizing skins from all the studied species. Increasing the level of NacL more than 5% had an adverse effect of the conversion rate of the skins; due to its inhibitory effect on fish viscera enzymes.

Logestic regression analysis of both the viscera enzymes and skin concentrations were also employed to stimulate the results. From these equations the optimum concentrations of both fish skin substrates and enzyme concentrations to obtain maximum conversions of the fish skins could to predicted.

Key Words : Skin solublization- fish Skin- fish viscera enzymes.

INTRODUCTION

The importance of enzymes in fish processing has, until recently, mainly been restricted to endogenous enzymes naturally present in the fish tissues. The deliberate use of added enzymes in fish processing is not common. However in the last several years; interest in controlling or aiding traditional processes that rely on endogenous enzymes by using added enzymes has increased. The use of enzymes as “specific” tools for fish processing operations has appeared also. Deskining of fish, membrane removal and roe purification are examples of such processes . (Stefansson 1993).

Digestive proteolytic enzymes from fish viscera posses unique properties when compared to mammalian proteases. It has been demonstrated that advantages may be taken of the; thermal instability of these enzymes in certain food process operation, where residual protease activity is undesirable. Also their high molecular activity at low temperature may be used to advantage in low-temperature, where proteolysis is desirable; (Haard 1998).

A number of inventions were applied to use the protease to remove skin from fish. Tuna skin removal is facilitated with a complex mixture of protease and carbohydrases (Fehmerling 1973). Herring skin removal is accomplished with cod pepsin isolated from stomach (Joakimsson, 1984).

Squid is skinned with papain; Hemple, 1983 and skate skin is removed with an unspecified mixture of proteases and carbohydrases (Wray, 1988). Also it is possible to loosen shrimp shells from the meat using enzyme (Raa, 1990). The objective of this present paper was to determine the factors which affected the solubilization of skin tissues from different Egyptian fish species like Bolti (Talipia), (Wakar) Grouper and (Kishr-Bayad) Nile perch commonly used for fillet processing with the aid of viscera enzymes. This is create a basis for a discussion of the enzymology of skin tissue solubilization. Furthermore the experiment indicate simple, reliable and practical method to selectively let fish skinned by enzymes.

MATERIALS AND METHODS

1- Materials

All the fish samples under study, Bolti (Talipia) fish (*S. Nilotica*), (Wakar) Grouper (*Epinephellus guaza*) and (Kishr-Bayad) Nile perch (*Lates niloticus*) were obtained from the local market. Abdominal viscera and lateral skin were separated and stored at –20^{o}C.

2- Methods

2-1- Preparation of the skin as substrate :-

The side skin from all the species under study, were removed immediately after thawing the skin and the remaining muscle fragments thoroughly scraped off. The skin was lyophilized and scales were removed and cut into small pieces using a scissors; the skin preparation were lyophilized and stored at –18^{o}C. The lyophilized tissue used in this study may be criticized as representative model substrates of intact tissues.

2-2- Preparation of the viscera silage and recovery of the crude enzyme

This was carried out according to the process described by Reece 1988. Thawed viscera were minced and 0.85% formic acid and 0.5% sulphuric acid were well mixed and then stored at room temperature. Proteolytic enzymes were extracted from the ensiled offal at noted time intervals; it was found that the enzymes are relatively stable at 5 days. By adding an equal volume of warm water and blending the mince for 30 minutes. During this time the temperature was held at 40^{o}C; before extracting proteolytic enzymes; the pH of the silage was held at pH 3.0; which facilitated the extraction of acidic proteases.

2-3- Solubilization of the skin:

Solubilization of the skin by the fish enzymes was carried out according to the procedure described by Gildberg and Raa 1979. The reaction was terminated by the addition of the same amount of TCA mixture (0.11M TCA, 0.22M Sodium acetate and 0.33M acetic acid; After 24hrs incubation period at room temperature and the contents were allowed to stand for 15 mins at the same temperature, filtered through whatmann filter paper No. 1. Aliquot was processed as per the methodology of Lowery et al

(1951) and the absorbance was measured at 660 nm as described by Ramana Murthy et al (1997).
The following equation was carried out to calculate the conversion percentage of the skin (Mannheim and Cheryan 1992).

$$Y = (p-p_o) / (S_o-P_o)\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where :
Y= Fractional conversion (percent conversion is 100x)
P= Product concentration (Nitrogen in TCA-soluble fraction of skin hydrolyzate)
P_o = Initial product concentration (Nitrogen in TCA-soluble fraction of unhydralyzate skin substrate)
S_o= Initial substrate concentration (nitrogen in unhydrolyzed samples)

2-4-: Experimental design
The following parameters were varied in the skin solubilization:-

2-4-1 Effect of pretreatment solutions :
To illustrate the effect of different solutions that is used in combination with the viscera enzymes; NacL was added with a concentration ranged from 5.0 to 15.0% with 1 unit intervals; HcL with a concentration ranged form 0.5 to 3.0% with 1 unit intervals and Acetic Acid with a concentration ranged from 1.0 to 3.0 % with 1 unit intervals.

2-4-2 Effect of crude enzyme: Solutions ratios (v/v):
The following ratios were used 1:1, 1:2, 1:3 and 1:4 (v/v).

2-4-3 Effect of skin substrate
The following quantities were used 0.25, 0.50, 0.75 and 1 gm.

2-5- Statistical analysis
All the data were replicated three times. The data were expressed in terms of logisitc regression analysis as follows:

$$Y = c \div (1+a.\ e^{-bx}) \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

where
X: Variables under study (enzyme concentration substrate concentration).
Y: is the conversion % of the skin substrate. hr^{-1}
c,a,b are constants
Also; statistical analysis systems were also applied (SAS 1985) to test the significance of the resulted data at ($P<0.05$) and ($P<0.01$).

RESULTS AND DISCUSSION

This research was carried out to find how careful control pretreatment solutions, fish viscera enzyme concentration and skin fish substrate concentration could lead to complete solubilization of the skin, Hence initiating a biological process could be applied on industrial scale for the removal of skin of fish tissues.

Effect of pretreatment solutions:-

In order to find the optimum conditions for skin substrate hydrolysis; different concentrations from the NacL, HcL and acetic acid solutions were applied as a pretreatment and tested for its effect after and before the addition of fish viscera enzymes. Fish viscera proteolytic enzymes were added to the skin belonging to the same species. The data were illustrated in table (1) for NacL%; table (2) for HcL % and Table (3) for acetic acid. As seen from these tables and in all the studied skin tissues obtained from Bolti fish, Grouper and Nile perch; that concentrations up to 5.0% NacL, 3% HcL and 3% Acetic acids were completely satisfactory for enhancing the conversion rate %. hr^{-1} after viscera enzymes were added. Increasing the level of NacL % more than 5% had an adverse effect on the activity of the enzymes added since the conversion rate %.hr^{-1} was lowered to 0.679, 0.850 and 0.440%.hr^{-1} in case of using the skins of dusky grouper, Nile perch and Bolti fish, respectively at 15% NacL. It seems that increasing the level of NacL had an inhibitory effect on fish enzymes, in contrast to HcL and Acetic acid. Treatments with dilute acids caused quick solubilization of the skin. The myocommata also dissolved whereas the muscle contracted and got a tough and dry appearance. Thus created an excellent media to facilitate the enzyme penetration (Gildberg and Raa 1979). Since the action of these enzymes is on denatured collagen (Yoshinaka et al 1978).

The Michalis-Menten parameters for the fish skin fish viscera enzymes reaction (table 4) were determined from a lineweaver Burke analysis of data from kinetics experiments. The data showed that pretreatments by 5.0% NacL, 3.0% HcL and 3.0% acetic acid had a (statistically) significant effect on the hydrolysis rate and a maximum conversion level. In all the studied skins from different species; these pretreatments enhanced Vmax by almost 2-5 times and also the Km values. This large difference might be due to the greater steric hindrance of skin proteins (collagen). Covalent intermolecular crosslinked in collagen are responsible for the stability, physical strength and mechanical properties of connective tissues and other ECM components (extracellular matrix). (Bracho and Haard 1995). So degradation of these types of skin proteins by proteases caused remodeling, and consequently let the collagen be denaturated, so its physical strengths and stability be reduced.

Table 1: The contents of soluble proteins in extracts from different skin species after and before the addition of viscera proteolytic enzymes.

Skin from	Concentration of NacL in extraction solution [%]	Conversion rate %.hr^{-1}*	
		Pretreatment	After addition of fish enzymes**
Grouper	5.0	1.346	1.596
Grouper	10.0	1.041	1.554
Grouper	15.0	0.919	0.679
Nile perch	5.0	1.555	1.721
Nile perch	10.0	1.166	1.635
Nile perch	15.0	1.180	0.850
Bolti fish	5.0	1.722	1.860
Bolti fish	10.0	1.333	1.281
Bolti fish	15.0	0.885	0.442

*** Date were calculated as a mean value from 3 separate samples**
**** Fish viscera proteolyitc enzymes were added to the skin belonging to the same species. Enzymes were added to a ratio of 0.25% v/v .**

Table (2): The contents of soluble proteins in extracts from different skin species after and before the addition of viscera proteolytic enzymes

Skin from	Concentration of HcL in extracting solution [%]	Conversion rate %.hr^{-1}*	
		Pretreatment	After addition of fish enzymes**
Grouper	0.5	1.791	2.566
Grouper	1	1.860	2.607
Grouper	3	1.957	2.649
Nile perch	0.5	2.686	3.849
Nile perch	1	2.790	3.911
Nile perch	3	2.936	3.973
Bolti fish	0.5	2.081	2.776
Bolti fish	1	2.152	2.818
Bolti fish	3	2.234	2.859

*** Data were calculated as a mean value from 3 separate samples**
**** fish viscera proteolytic enzymes were added to the skin belonging to the same species . Enzymes were added at a ratio of 0.25% v/v.**

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Table (3): The contents of soluble proteins in extracts from different skin species after and before the addition of viscera proteolytic enzymes

Skin from	Concentration of acetic acid in extracting solution [%]	Conversion rate %.hr^{-1}*	
		Pretreatment	After addition of fish enzymes**
Grouper	1	1.692	1.776
Grouper	2	1.818	1.929
Grouper	3	1.916	2.054
Nile perch	1	1.790	1.884
Nile perch	2	1.810	1.949
Nile perch	3	2.010	2.100
Bolti fish	1	1.913	2.153
Bolti fish	2	2.096	2.411
Bolti fish	3	2.176	2.416

*** Data were calculated as a mean value from 3 separate samples**
**** fish viscera proteolytic enzymes were added to the skin belonging to the same species . Enzymes were added at a ratio of 0.25% v/v.**

Table (4): Kinetic parameters for skin solubilization [a] using viscera enzymes

Skin from	Concentration of HcL in extracting solution [%]	Km (%w/v)	Vmax (Conversion %. hr^{-1})
Grouper	Control	0.276^{b}	1.0304^{c}
Grouper	NacL 5.0	1.636^{d}	2.407^{e}
Grouper	HcL 3.0	1.666^{f}	5.097^{g}
Nile perch	Control	0.374^{h}	1.199^{i}
Nile perch	NacL 5.0	2.634^{j}	2.770^{k}
Nile perch	HcL 3.0	3.869^{l}	4.292^{m}
Nile perch	Acetic acid 3	2.439^{n}	3.438^{o}
Bolti fish	Control	1.775^{p}	1.461^{q}
Bolti fish	NacL 5.0	1.890^{r}	2.140^{s}
Bolti fish	HcL 3.0	4.189^{t}	4.873^{u}
Bolti fish	Acetic acid 3	2.654^{v}	3.750^{w}

a: parameters were obtained at Enzyme concentration = 0.33 v/v% at room temperature and substrate concentration = 2-10 w/v% at 24 hr.
(b-w) data with different letters were significantly different from each other at the 5% level.

Effect of enzyme concentration:

The graph of enzyme concentration vs conversion rate .% hr^{-1} of different fish skin substrates from different species (Fig 1) showed two kinetic zones was observed at enzyme concentration between 0-0.50 w/v%; and enzyme independent zone at concentration greater than 0.5 w/v% where enzyme level had little or no effect on conversion rate %. hr^{-1}. From the same figure and in all skins obtained from, Bolti, Grouper and Nile perch fish. The influence of dilute acids pretreatments of HcL and acetic acid in activating the fish viscera enzymes were clearly noticed; since their zymogens are easily activated either by acids or at room temperature (Squires 1986).

Effect of substrate concentration

The effect of substrate concentration is shown in fig (2) the data illustrated in all the studied fish skin species indicated that there was also two kinetic zones; at substrate concentration between 0-4% w/v. Followed by an

substrate-independent zone at concentrations greater than 4% w/v. Where the enzyme had little or no effect on conversion rate, the influence of acids pretreatments had also a positive effect in enhancing the enzyme-substrate binding affinity since acids induce partial solubilization of the skin.

All the data illustrating the effect of substrate and enzyme concentrations on the conversion rate %.hr^{-1} of fish skins can be modeled through logistic regression analysis. As it seemed, it is best applied for phenomena which there is a continual increase in one factor as another factor increases until a saturation point is reached as previously discussed. The results of this modeling were shown in table (5). From these models the maximum conversion rate can be achieved, if the suitable concentration of both enzyme and skin concentration can be applied.

Table (5) Prediction models for illustrating the effect of both skin substrates and enzyme concentration from different species on the conversion rate %. hr^{-1}.

Skin species	Attributes	Pretreatment	Prediction equation
Grouper	Proteolytic	5.0% NacL	$Y=1.779 \div (1+23.677.e^{-21.889x_1})$
	Enzyme	3.0% HcL	$Y=2.438 \div (1+25.871.e^{-25.871x_1})$
	Concentration (x_1)	3.0% Acetic acid	$Y= 2.335 \div (1+24.817.e^{-20.817x_1})$
Nile perch	Proteolytic	5.0% NacL	$Y= 1.895 \div (1+27.995.e^{-19.235x_1})$
	Enzyme	3.0% HcL	$Y= 4.053 \div (1+13.063.e^{-26.807x_1})$
	Concentration (x_1)	3.0% Acetic acid	$Y= 3.642 \div (1+22.991.e^{-25.687x_1})$
Bolti fish	Proteolytic	5.0% NacL	$Y= 2.048 \div (1+23.291.e^{-22.363x_1})$
	Enzyme	3.0% HcL	$Y=2.927 \div (1+25.951.e^{-163.371x_1})$
	Concentration (x_1)	3.0% Acetic acid	$Y= 2.809 \div (1+25.857.e^{-20.847x_1})$
Dusky grouper	Skin substrate	Without pretreatment	$Y= 0.981 \div (1+23.446.e^{-2.854x_2})$
	Concentration (x_2)	5.0% NacL	$Y= 1.932 \div (1+36.019.e^{-2.145x_2})$
		3.0% HcL	$Y= 4.098 \div (1+35.919.e^{-2.149x_2})$
Nile perch	Skin substrate	Without pretreatment	$Y= 1.131 \div (1+24.905.e^{-2.740x_2})$
	Concentration (x_2)	5.0% NacL	$Y= 2.013 \div (1+11.813.e^{-1.3216x_2})$
		3.0% HcL	$Y= 2.741 \div (1+67.746.e^{-2.0466x_2})$
Table 5 continued			
		3.0% Acetic acid	$Y= 2.516 \div (1+42.2019.e^{-2.0659x_2})$
Bolti fish	Skin substrate	Without pretreatment	$Y= 1.151 \div (1+23.422.e^{-1.9282\ x_2})$
	Concentration (x_2)	5.0% NacL	$Y= 1.786 \div (1+34.039.e^{-2.2061x_2})$
		3.0% HcL	$Y= 2.995 \div (1+72.039.e^{-147x_2})$
		3.0% Acetic acid	$Y= 2.682 \div (1+43.929.e^{-2.054x_2})$

CONCLUSION

The skin and muscle of fish differ fundamentally in chemical structure and consequently in solubility as a function of pH and temperature and susceptibility to enzymatic degradation. Due to these differences it is possible to remove the skin biochemically depending on the conditions of pretreatments used and enzyme concentration .

Fish viscera silage is considered to be a suitable media for the recovery of valuable proteases suitable for lowest operations in the fish processing and food industries like the skinning of fish for the production of fish fillets, since these enzymes are extracted from viscera which are considered to be a waste material of no use. It should be important to notify that this study was carried out as a model system and will be continued to be applied on the fish itself.

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العوامل المؤثرة على درجة تحرر الجلد فى الأنواع المختلفة من الأسماك المصرية المستخدمة فى انتاج الفيلية

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قسم الصناعات الغذائية والألبان – المركز القومى للبحوث – الدقى

قد تم دراسة درجة تحلل جلود الأسماك الناتجة من سمك البلطى وقشر البياض والوقار باستخدام الإنزيمات المحللة للبروتين من الأحشاء الداخلية لهذه الأسماك. وقد وجد أنه بعملية التحكم الدقيقة ما بين تركيز المحاليل المستخدمة فى المعاملة المبدئية وتركيز مادة التفاعل (الجلد) وتركيز الإنزيم المستخدم من الأحشاء الداخلية انه يمكن زيادة درجة تحرر جلود الأسماك المختلفة تحت الدراسة.

استخدام تركيزات من 5% كلوريد الصوديوم،3% حامض الهيدروكلوريك ، 3% حامض خليك تكون فعالة فى إذابة الجلد مع إستخدام التركيز المناسب من الإنزيم . وزيادة تركيز كلوريد الصوديوم عن 5% كانت له تأثيرات عكسية فى معدل تحول وإذابة الجلد المستخدم وذلك لحدوث تثبيط للإنزيم الناتج.

وقد استخدمت بعض انواع معادلات تحليل الانحدار (Logestic regression) لكل من تركيزى إنزيمات الأحشاء الداخلية وتركيز مادة التفاعل (الجلد) وذلك لوصف النتائج. ومن هذه المعادلات يمكن التنبؤ بمعرفة التركيزات المثلى من تركيز الإنزيم وكمية الجلد اللازمة لحدوث أكبر قدر ممكن من الذوبان مقدرة كمعدل للتحول فى الساعة.

وستكمل هذه الدراسة على المستوى التطبيقى لإنتاج فيليه الأسماك بعد معاملتها لزيادة درجة تحرر نزع الجلد منه على الأنسجة اللحمية.