Evaluation of Several Methods for Analysis of Sweet Potato Phenolics

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The phenolic content of the sweet potato cultivars, Australian Canner, Pelican Processor, Porto Rico, Julian, Jewel, Centennial, and the selection NC-319 was measured using several methods. These included bonding to insoluble polymers (Rexyn and polyvinylpyrrolidone), reaction with the Folin–Dennis reagent, computation from the absorbance at 323 nm, coupling with diazotized *p*-phenylazoaniline and with 3-methyl-2-benzothiazolinone, and measurement by a high-pressure liquid chromatographic procedure. Measurement of changes in absorbance at 323 nm caused by treatment of the sample with Rexyn-201($-Cl^-$) gave the most rapid and reasonably accurate assay of total phenolic content.

Sweet potato phenolics have been of interest to a number of workers since Rudkin and Nelson (1947) isolated chlorogenic acid (3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid) and other closely related compounds. In 1955 Uritani and Masateru reported that chlorogenic acid and its isomers accumulated in sweet potato tissue which had been attacked by the black rot fungus *C. fimbriata*. Since then, the phenolics of sweet potato have been investigated with respect to their mode of formation (Kojima and Uritani, 1973), and to their role in metabolic changes caused by stress conditions (Porter et al., 1976; Buescher et al., 1975) including mechanical injury (Tanaka et al., 1974).

Phenolics have also been postulated to play a role in the darkening of canned sweet potatoes (Scott et al., 1944) as well as off-color development in precooked dehydrated sweet potato flakes (Hoover, 1963). Another undesirable characteristic attributed to phenolics in cereals and vegetables (Maga and Lorenz, 1973; Arai et al., 1966) is that of imparting a bitter flavor. However, no information is available relative to the effect of phenols on the flavor of sweet potatoes.

Phenolic concentration in sweet potato has been measured in several ways. Lieberman et al. (1959) assayed the phenolics as chlorogenic acid by measuring the absorbance of a tissue extract and of a series of chlorogenic acid standards at the λ max of chlorogenic acid. This method could lead to an overestimation of the concentrations because of other materials which contribute to the absorbance at that wavelength.

Buescher et al. (1975) used a Folin-type reagent to measure phenols in sweet potatoes. Phenols are oxidized with a complex molybdotungstophosphoric reagent, followed by colorimetric measurement of the blue color formed when the mixture is made alkaline with sodium carbonate. Modifications have been proposed for the method (Slinkard and Singleton, 1977) but it is still nonspecific. The reagent can react with a variety of reducing compounds present in plants, such as ascorbic acid, glutathione, and various amino acids thus causing erroneously high results (Andersen and Todd, 1968). Reagent nonspecificity is a serious problem in sweet potatoes because of their high levels of ascorbic acid.

To circumvent the problem of nonspecificity, Andersen and Todd (1968) measured the intensity of the blue Folin color on samples before and after treatment with insoluble polyvinylpyrrolidone (PVP). Thus, phenols were adsorbed by PVP while many impurities which could be oxidized by the reagent were not. The phenol levels were calculated from the difference in color between treated and untreated samples.

The principle of using a water-insoluble polymer to remove phenols from solution has since been employed by Lam and Shaw (1970) who treated homogenized plant material with strong anion-exchange resin and thus avoided phenolic inactivation of enzymes. Whether one uses ion-exchange resin or PVP, it is assumed that all of the phenols and only phenols are removed and that, therefore, the difference in apparent phenolic concentration between treated and untreated samples is a true measure of the phenolic content. Although a wide range of compounds are not adsorbed onto PVP or anion-exchange resin, it is possible that some interfering substances can be removed and thus contribute to the apparent phenol concentration.

The procedure of Zucker and Ahrens (1958) which has been used to quantitate phenols in sweet potatoes involves adsorption of the phenolics onto an alumina column, treatment with nitrous acid, elution with sodium hydroxide solution, and spectrophotometric assay of the resulting red solution. The disadvantages of this method are nonspecificity of the nitrosation and the time required by the chromatographic step.

Several workers (Walter et al., 1979; Uritani and Masateru, 1955) have confirmed that only chlorogenic acid isomers are present in those cultivars which have been analyzed to date. Walter et al. (1979) were able to quantitate phenolics using high-pressure liquid chromatography (LC). While LC gives accurate, specific results, it is slow relative to total phenol assay procedures, requires expensive equipment and specialized skills. Moreover, in many cases, the details provided by this method (i.e., relative concentrations of each isomer) are not needed.

The purpose of this study was to compare some methods of measuring phenolics from sweet potato and to determine which of them is the most rapid and accurate. The standard of comparison was the LC method.

MATERIALS AND METHODS

About 20 lb each of Jewel, Centennial, Pelican Processor, Australian Canner, Julian, and Porto Rico-198 cultivars and the selection, NC-319, was harvested from the North Carolina Agricultural Experiment Station in Johnston County and transported immediately to the laboratory for analysis. Three sound roots of each cultivar or selection were selected, washed, carefully hand-peeled, and quartered. Slices, approximately 5 mm thick, were prepared and a 100-g composite blended in a Waring Blendor for 3 min with 400 mL of boiling 95% ethanol. The slurry was cooled to room temperature and filtered and the mat was

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extracted twice with 200 mL of hot ethanol. Alcoholic filtrates were combined and evaporated in vacuo at 35 °C. The syrup remaining was taken up in water and diluted to 25 mL in a volumetric flask. The aqueous phase was extracted three times with equal volumes of hexane to remove lipids, centrifuged at 40000 *g* and filtered through a 0.2-μm membrane filter. The filtrate was stored at −10 °C until needed.

Phenolic Assays. Each analytical method was calibrated against a standard curve prepared with a series of chlorogenic acid (CA) standards. After setting up a CA standard curve, a series of six solutions which encompassed the usable concentration range of the method to be tested was prepared from an extract obtained from Jewel sweet potatoes. The phenolic content was then determined (as CA) using each method. From these data the method coefficient of variability was calculated. Each of the methods was then used to determine the phenolic content for the remainder of the sweet potato cultivars and selection. The analyses were performed in duplicate at two concentration levels.

Bonding to Insoluble Polymers. Rexyn 201 (Fisher Scientific Co.) strong anion-exchange resin (200–400 mesh, medium porosity, Cl^- form) was purified before use by washing with 0.1 N NaOH solution, distilled deionized water, 0.1 N hydrochloric acid, and, finally, with distilled water. Polyvinylpyrrolidone (PVP) (Sigma Chemical Co.) was purified according to Andersen and Todd (1968). Both Rexyn and PVP were air-dried and stored in tightly capped brown bottles.

Replicate samples from each cultivar were diluted in volumetric flasks such that the absorbance at 323 nm was less than 1.5. For bonding to Rexyn, the sample was made up to volume with 0.1 M phosphate buffer (pH 6.5); for bonding to PVP, the sample was diluted with water–methanol (80:20 v:v) and adjusted to pH 3.5 with glacial acetic acid.

Ten-milliliter aliquots of the sample in phosphate buffer were mixed with 0.2 g of Rexyn and an equal volume of the water–methanol sample was mixed with 0.25 g of PVP. Both mixtures were agitated at 200 rpm on a rotary shaker for 30 min and then centrifuged. Absorbance at 323 nm (A_{323}) was measured both before and after treatment of the original sample with either Rexyn or PVP. The change in absorbance caused by the treatment was then compared with changes measured for a series of chlorogenic acid standards treated in the same way as the sample. The phenolic content (mg/100 g of tissue) was calculated as the quantity of phenolics removed by the treatment multiplied by the appropriate dilution factor.

In addition to the spectrophotometric assay above, the Folin–Dennis (FD) reagent (Swain and Hillis, 1959) was also used to measure phenol content. This determination was performed on both the original (untreated samples) and the samples after shaking with Rexyn or PVP. A measured volume of sample and sufficient buffer to total 2.0 mL were mixed. To the mixture exactly 1.0 mL of the FD reagent (Swain and Hillis, 1959) was added and then mixed. After 3 min, 2.0 mL of a saturated solution of sodium carbonate was added and the mixture again agitated. One hour later the solution was centrifuged, the absorbance at 725 nm measured, and the phenolic content calculated.

Diazotized p-Phenylazoaniline. A modification of the method of Whitlock et al. (1972) was used in this study. The sample to be analyzed was diluted with 0.1 M phosphate buffer, pH 6.85. A 5-mL aliquot was removed and mixed with 5 mL of redistilled tetrahydrofuran and 0.5 mL of a diazotized *p*-phenylazoaniline (DPAA) solution. After exactly 3 min the absorbance of the mixture was measured at 470 nm. Sample blanks were run with each set of samples and the value corrected for reagent absorbance before phenolic content was computed.

The DPAA solution was prepared by mixing 0.099 g of *p*-phenylazoaniline (Aldrich Chemical Co.), 10 mL of acetone, 5 mL of 2 N HCl and 30 mL of distilled water and cooling at 14 °C. A solution containing 0.035 g of sodium nitrite in 20 mL of distilled water was added to the stirred, chilled mixture over a period of 15 min. The temperature was maintained at 14 °C during the addition. The final solution was then made up to 100 mL with chilled (14 °C) water and stored on ice at 5 °C in the dark.

Oxidative Coupling with 3-Methyl-2-benzothiazolinone Hydrazone (MBTH). This method is a modification of the MBTH coupling procedure of Friestad et al. (1969). A 1-mL aliquot of the sample was mixed with 1 mL of 0.1% MBTH·HCl (Aldrich Chemical Co.) and held for 5 min. One milliliter of a solution consisting of 0.3% Cerric ammonium sulfate in 0.49% H_2SO_4 was added. After 5 min, 2 mL of buffer (4.8 g of NaOH, 2.0 g of disodium EDTA, 8.0 g of boric acid in 500 mL of distilled water and diluted 1:1 with ethanol daily before use) was added, mixed, and allowed to stand 15 min. The absorbance of the solution was then measured at 450 nm and corrected for reagent absorbance and the amount of phenolic present was determined.

High-Pressure Liquid Chromatographic Analysis. The procedure used was essentially that of Walter et al. (1979).

RESULTS AND DISCUSSION

Characteristics of the Analytical Methods. The most sensitive method among those evaluated was the direct spectrophotometric determination using A_{323} and the least sensitive was the DPAA procedure (Table I). The low coefficients of variability (C.V.) indicated that the precision of the methods was fairly high within the concentration ranges studied. However, the DPAA method was >2 times as variable as any of the other methods. All of the methods except MBTH coupling obeyed Beer's law. The MBTH procedure gave a slightly curvilinear relationship between concentration and absorbance. However, when a standardized concentration–absorbance curve was used to obtain sample concentrations, reasonable precision was obtained as indicated by a C.V. of 2.76% (Table I).

Table I. Concentration Ranges and Standard Deviations for Phenol Assay Methods

analytical method	concn[a,b] range	coeff.[b] of var.
A_{323}		
A_{323}	12.3-28.7	0.48
Rexyn/A_{323}	12.3-28.7	1.65
PVP/A_{323}	12.3-28.7	2.13
Folin-Dennis		
Folin-Dennis (FD)	21.6-41.5	0.45
Rexyn/FD	21.6-41.5	1.62
PVP/FD	21.6-41.5	2.08
DPAA (A_{470})	40.5-147.0	5.95
MBTH (A_{450})	28.6-78.0	2.76

[a] Micrograms in assay solution (as CA). [b] Six concentration levels of an extract prepared from Jewel cultivar.

Bonding to Insoluble Polymers. Initial studies with Rexyn indicated that the resin lowered the pH of the sample and thereby lowered the amount of phenolics extracted. When the pH was adjusted to pH 6.5 with 0.1 M phosphate buffer, optimum amounts were removed. Shaking 0.2 g of Rexyn for 30 min removed 90% of the CA from a series of standard solutions containing up to

Table II. Recovery of Chlorogenic Acid (CA) Added to Sweet Potato Extract as Determined by Rexyn/A_{323} and Rexyn/Folin Dennis Methods

analysis method					
Rexyn/A_{323}[a]			Rexyn/Folin Dennis		
CA added, μg	CA recov., μg	corrected CA[b] recov., μg	CA added, μg	CA recov., μg	corrected CA[b] recov., μg
41.5	36.5	41.0	47.5	43.3	48.1
100.2	90.0	101.0	99.5	90.8	100.9
164.7	146.5	162.8	160.1	142.8	158.6
214.4	194.7	216.3	212.0	199.0	221.1

[a] Absorbance at λ max of CA 323 nm. [b] CA recovered ÷ 0.90.

0.4 mg. In order to determine if similar efficiency was attained in sweet potato samples, an extract from Jewel sweet potatoes was spiked with several levels of CA and the analysis run. The recoveries were always 90% regardless of whether the concentrations were determined by the Rexyn/A_{323} method or by the Rexyn/FD method (Table II). Therefore, a correction factor of 10/9 was applied to the actual recoveries. Similarly, when PVP was used as the phenol adsorbing polymer, the actual recoveries of CA were 76.4%. Our recovery values for the PVP/A_{323} method were similar to those reported by Andersen and Todd (1968).

The PVP-treated samples were much harder to clarify than were Rexyn-treated samples. Apparently, the density of PVP was close to that of the methanol–water medium; and, consequently, centrifugation was not an effective means of polymer removal. On the other hand, Rexyn tended to settle very rapidly, and centrifugation was not essential. This property of the ion-exchange resin allowed analyses to be performed with fewer manipulations and in a shorter period of time than when PVP was used.

Diazotized *p*-Phenylazoaniline (DPAA). This method is based on the reaction of DPAA with substituted aromatic compounds (e.g., phenolics) to yield colored azo dyes. Fieldes and Tyson (1973) used diazotized sulfanilic acid (DSA) (Pauley's Reagent) to measure phenolic levels in flax. However, the calibration curve using CA as the standard was found to be linear only within the range of 30–70 μg at 420 nm (λ_{max}). Whitlock et al. (1972) investigated several diazotized aromatic amines for the assay of phenols including DSA and DPAA. They suggested that DPAA was the preferred compound because the extended system of conjugation gave high molar adsorptivities and consequently was more sensitive than DSA. Moreover, the coupling rate of DSA with different phenols was pH sensitive while that of DPAA was not. Thus, DPAA was used.

Whitlock et al. (1972) used 0.1 M sodium bicarbonate to adjust the pH of the coupling reaction to the optimum value of 7.6. However, this method gave erratic results. Upon further examination, it was noted that the pH of the water–tetrahydrofuran (THF) mixture changed over time. This problem was avoided by making up the sample in 0.1 M phosphate buffer and then mixing it with THF.

The absorbance spectrum of the CA–DPAA mixture (Figure 1) contained a broad maximum centered at about 418 nm. Attempts to use this maximum to establish a calibration curve with a series of CA standards were not successful, since at high concentrations, molar absorptivity decreased. The decrease indicates absorbance due to other reaction products. When the analyses were performed at 470 nm, Beer's law was followed over the entire concentration range. The molar absorptivity at this wavelength was 10 469.

Oxidative Coupling with MBTH. This method has not been applied to the assay of plant phenolics. It involves the formation of a colored azo dye due to the reaction between the benzothiazolinone moiety and a phenol. Reaction conditions were very similar to those reported by Friestad et al. (1969) except that some changes were instituted in reagent concentratons.

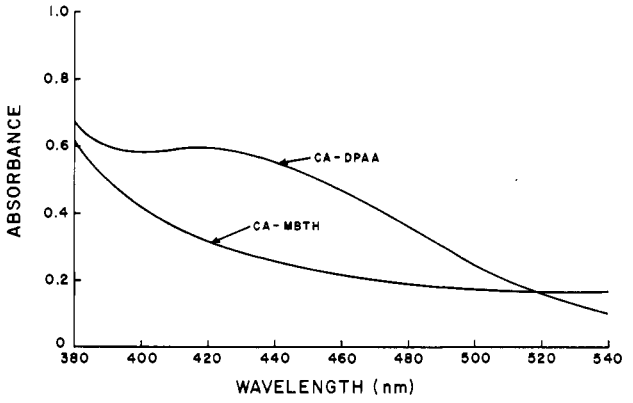

Figure 1. Absorbtion spectrum resulting from the reaction of chlorogenic acid (CA) with diazotized *p*-phenylazoaniline (DPAA) and 3-methyl-2-benzothiazolinone (MBTH).

The absorption spectrum (Figure 1) showed no distinct peaks for MBTH–CA, indicating formation of several reaction products. However, when a sample of Jewel cultivar was treated with MBTH, a very broad maximum centered at about 520 nm was observed. The absorbance of a series of CA standards (20–100 μg/mL) were analyzed at 450 and 520 nm. For both wavelengths, the relationship between concentration and absorbance was curvilinear, indicating that the reaction was nonstoichiometric. The phenolic levels in the dilution series of the Jewel extract were obtained by the MBTH procedure with the absorbance measured at 450 and 520 nm. At 450 nm the mean was 0.96 mg of CA/mL (2.76% C.V.) and at 520 nm, 1.45 mg of CA/mL (1.79% C.V.). The value was higher at 520 nm because the sample had a higher specific absorbance than CA at that wavelength. Since Jewel has been shown to contain only caffeoylquinic acid type phenolics (Walter et al., 1979) and since the sample exhibited a maximum at 520 nm, which was not present in the CA standard, it was apparent that material other than phenols caused the increased absorbance. It appears that the value calculated from the absorbance at 450 nm gives a more accurate measure of phenolic levels.

High-Pressure Liquid Chromatographic Analysis. The LC method is described in detail in the accompanying manuscript (Walter et al., 1979). This method separates the caffeoylquinic acid esters and permits the quantitation of each from its LC peak area as compared with the peak areas for a series of chlorogenic acid standards.

Results of Analyses. The phenolic levels as determined by all methods except the one involving only A_{323} were in the same order (Table III): Australian Canner > Pelican Processor > Porto Rico > Jewel > Julian >

Table III. Phenol Concentrations[a] of Several Sweet Potato Cultivars and a Selection Obtained by Several Analytical Methods

	analytical method[b]								
cultivars and selection	LC	Rexyn/A_{323}	A_{323}	PVP/A_{323}	Folin-Dennis	PVP/Folin-Dennis	Rexyn/Folin-Dennis	DPAA	MBTH
Australian Canner	46.7	56.7	66.9	68.0	75.0	61.8	64.9	70.0	74.5
Pelican Processor	34.1	39.8	47.8	49.8	51.9	59.5	50.4	47.3	54.1
Porto Rico	34.7	33.6	40.6	41.0	57.0	53.4	44.5	47.3	52.1
Jewel	25.2	28.2	29.1	32.8	46.0	48.7	38.9	45.8	44.6
Julian	23.0	23.4	31.2	30.7	44.6	45.9	34.6	45.8	41.0
NC-319	20.1	22.7	30.2	27.8	42.5	32.7	33.1	45.6	40.6
Centennial	11.7	14.0	16.8	16.3	31.5	19.6	21.6	25.4	23.0

[a] Milligrams of phenol per 100 g of fresh tissue (as chlorogenic acid). [b] Values for Jewel cultivar obtained from analysis in duplicate of six concentration levels. All others were obtained from duplicate analysis at two concentration levels.

NC-319 > Centennial. For the A_{323} method the only difference was in the order of Jewel, Julian, and NC-319, which gave very close results by all methods.

Among the sweet potato cultivars we studied, caffeoylquinic acid esters were the only phenolics present; and all of these have a λ max at 323 nm. Calculation of phenolic content using the A at 323 nm would be high if nonphenols are present and contribute to the absorbance. Very few nonphenolic compounds have significant absorbance in this area of the spectrum without having color visible to the eye. Thus, measuring A_{323} would be a specific way to determine phenol concentration if only colorless phenolics were present. The purification procedure used in this study resulted in visibly clear, faintly yellow samples. Since the A_{323} method can only overestimate the phenol levels, those methods which indicate phenol levels greater than that given by this method must also be overestimates. Estimates would be more accurate if the phenolics are adsorbed onto PVP or Rexyn and then quantitated from the change in absorbance at 323 nm provided that the only 323 nm absorbing compounds removed are phenols. When the Rexyn/A_{323} method was used (Table III), the phenol content for all cultivars was 16.98% (SD 7.3) less than that obtained by the A_{323} method, indicating that there were some substances which were not absorbed by the resin contributing to the absorbance at 323 nm. The PVP/A_{323} method gave values similar to those obtained by the A_{323} method (Table III). This suggests that PVP adsorbed phenols as well as those nonphenolics which contributed to absorbance at 323 nm.

Use of the FD reagent without pretreatment gave phenol levels 28.74% (SD 11.44) higher than those obtained by the A_{323} method (Table III), indicating that nonphenolics were reacting, leading to overestimation of phenol concentrations. Removal of interfering substances with PVP followed by analysis with the FD reagent resulted in values 3.35% (SD 0.72) higher for Jewel, Julian, and Pelican Processor than when no PVP was used. For these cultivars, PVP removed nonphenolic, FD reactive substances; thus high values were observed. Comparison of phenolic levels measured by the FD method for samples which had been treated with Rexyn and PVP also illustrated that the two insoluble polymers removed different amounts of FD reactive materials depending upon the cultivar. Only NC-319 gave the same values by both methods. Of the methods based on the FD reagent, the PVP/FD method gave the lowest values for Australian Canner and Centennial; otherwise the Rexyn/FD method gave the lowest values. These results indicate that the FD method is influenced by nonphenolics and that neither PVP nor Rexyn consistently extract only phenolics from the samples. Apparently, depending upon the cultivar, a variety of nonphenolic, FD active substances are adsorbed by both polymers.

Analysis of the samples with DPAA gave a range of values in which Australian Canner and Centennial were widely separated but the other cultivars had apparently similar phenol content. The values were 35.2% (SD 14.1) higher than those obtained by the Rexyn/A_{323} procedure. Apparently, there are large cultivar differences in DPAA-active compounds. The MBTH method gave values which were similar to those from the FD method. These values, determined by the MBTH method also, were 35.5% (SD 7.74) higher than those obtained by the more specific Rexyn/A_{323} method. Possibly, the accuracy of both the DPAA and MBTH procedures could be improved if the phenols were adsorbed onto Rexyn and the change in levels measured.

The Rexyn/A_{323} procedure appears to be the best method for measuring phenolics in the cultivars that were investigated. The phenolic content determined by this method was compared with that obtained by the LC method. Table III shows that with the exception of Porto Rico, the Rexyn/A_{323} method overestimates the true phenolic levels. However, the values obtained by both methods are similar. Overestimation is likely due to 323 nm absorbing materials which are removed by Rexyn along with the phenols. Although no other phenolics were found by paper chromatography, it is possible that compounds resulting from phenylpropanoid metabolism other than chlorogenic acid isomer production might have been present individually at very low concentrations and collectively they could have caused the overestimation. The LC method is more accurate but is more time consuming. In those cases where concentration of individual phenols are not needed, the Rexyn/U.V. method would appear to be adequate and is the method of choice.

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Comparative Nutritive Value of Fish Protein Concentrate (FPC) from Different Species of Fishes

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Fish protein concentrate (FPC) prepared from three different species of fishes [catfish, family Arridae (*Tachysurus arius, T. dussumieri, T. thalassinus*), picked ribbonfish, family Trichuiridae (*Trichiurus savala, Trichiurus lepturus*), milkfish, family (*Chanos chanos*)], available in plenty on the western coast of India, have been analyzed for their nutritive value, i.e., PER (protein efficiency ratio), NPR (net protein retention), amino acids, proximate composition, and organoleptic evaluation. The protein quality index based on PER and NPR at the 10% protein level was found to be highest in FPC made from picked ribbonfish (*T. savala*), followed by FPC from catfish (*T. arius*) and milkfish (*Chanos chanos*). PER value of *T. savala* was significantly higher than casein. Chemical score based on the essential amino acid content of FAO/WHO pattern (1973) and egg indicated the level of the first limiting amino acid methionine + cystine. The essential amino acid index and biological value were also calculated.

The nutritional needs of a significant part of the world population of developing countries are yet to be satisfied. In most cases the nutritional insufficiency is attributed to the unavailability of protein. Although vegetable protein is being used to increase the intake of protein quantity, the lack of an economic source of animal protein prevents the immediate improvement of protein quality of diet of these people.

An underutilized resource of animal protein is present in the world's oceans, but the main problem is to provide this resource of protein economically to the people of these developing countries. The perishability and lack of storage facilities in preventing spoilage of this valuable source add to the cost of marine products. Solvent extraction methods have been developed in defatting and dehydrating whole fish to fish protein concentrate (FPC) containing at least 70–90% protein (Snyder, 1967; Moorjani and Lahiry, 1970) in different countries. Taking into consideration these factors and potential for indigenous (FPC) production, the nutritional quality of (FPC) prepared from some of the important species of fishes available in plenty on the western coast of India and other parts of the world was taken in hand to find out which of the species give better product of higher nutritional value.

MATERIAL AND METHODS

The total yearly catch of fish in the Indian Ocean is about 2.8 million tons (FAO, 1970), out of which the Indian contribution is about 1.4 million tons yearly. This catch could be increased many fold. Some of the catch consists of certain varieties that are cheap and fairly abundant. Table I shows some of the cheap and abundant varieties of fish and the quantities likely to be available for FPC manufacture. Production of FPC in India seems both feasible and economical. FPC properly prepared can be stored for years without deterioration. The method developed for manufacture of FPC in India is described briefly.

Table I. Some of the Cheap and Abundant Varieties of Fish That Could Be Used for FPC Production

name of fish	family	quantity, Tons
catfish (marine)	Arridae[a] (*T. dussumieri, T. thalassinus*)[a]	57 000
picked ribbonfish	Trichuiridae[a,b] (*Trichiurus lepturus*,[a] *Lepturacanthus savala*)[a]	26 000 to 45 000
milkfish	*Chanos chanos*[a-c]	20 000

[a] Commercially important species. [b] Plate XIII, Figure 5, p 30, Jhingran (1970). [c] Plate III, Figure 3, p 20, Jhingran (1970).

Alcohol Extraction. The three species, catfish, picked ribbonfish, and milkfish, were chosen as the raw material because of their cheapness and availability on India's West Coast in abundance during a large part of the year.

Ethanol was chosen as solvent because of its availability, use in food processing, low toxicity, low boiling point, antibacterial properties, and low price. The method for

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