AN ABSTRACT OF THE THESIS OF

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MEASUREMENT OF PHYSICAL PARAMETERS FOR SHRIMP DIETS PRODUCED WITH A SERIES OF BINDERS

Abstract approved: ______________________
Michael T. Morrissey

Shrimp diets were produced in a hand-driven pelletizer without heat or pressure. Diet formulas contained fish meal, soybean flour, soft wheat clears and native starches. Native starches (corn, potato, tapioca and wheat) were evaluated as binders at levels of 5, 10, 15, 20, and 25 percent. The water stability of the pellets was tested by placing samples in a mesh ball in 500 ml of water at 25°C with intermittent stirring. The pellets were measured for weight loss every 2 hr over an 8 hr period. Density ($g/cm^3$), sinking rate (cm/sec), and absorbance of test water were evaluated and compared to diet formulations.

Diets made with 5% starch had higher water stability than diets with higher starch concentration. Diets made with wheat starch had slightly better values for physical parameters measured.

The drying method was an important factor in the water stability of the pellets. Diets dried at ambient temperature for 20 hr to 10-12% moisture or oven dried at 30°C for 2 hr were the most stable. Rapid drying at higher temperatures (60-80°C) caused pellets to be less stable in water.

The water testing temperature and salinity were inversely related to water stability of the diets. Implications for this low cost technology for shrimp aquaculture in developing countries are discussed.

# MEASUREMENT OF PHYSICAL PARAMETERS FOR SHRIMP DIETS

# PRODUCED WITH A SERIES OF BINDERS

BY

Manuel Ulises Solis

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Typed by Manuel Ulises Solis

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# MEASUREMENT OF PHYSICAL PARAMETERS FOR SHRIMP DIETS PRODUCED WITH A SERIES OF BINDERS

## INTRODUCTION

Shrimp aquaculture has increased dramatically over the past decade. In 1991, farmed shrimp production rose 9 percent to an estimated 690,000 metric tons which represents 28 percent of the global shrimp harvest (Anonymous, 1992). The ten leading countries in farmed shrimp production are developing countries that are located in tropical or sub-tropical areas (FAO, 1991). Presently, China, Indonesia, Thailand, Ecuador and India are responsible for 75 percent of the farmed shrimp in the world market. Shrimp aquaculture represents a growing industry that can have a significant socio-economic impact in several countries. It is estimated that, by the turn of the century, worldwide farmed production of shrimp could double depending on the advances made in certain areas of aquaculture sciences.

An important area of shrimp diet research is providing better information on dietary requirements and diet formulations. In developing countries, a substantial number of shrimp ponds and hatcheries use algal blooms to feed the shrimp at different life-stages (Lasso de la Vega and Villarreal, 1985). Although this method is economical and can provide adequate nutrition, there is an increasing demand for developing artificial feeds providing better nutrition and faster growth. An essential aspect of artificial diets is the physical parameters of the pellet. A good pellet needs to have an acceptable shape, density, texture, and proper scent as well as a balanced nutritional composition. Good water stability is also very important for shrimp feeds (Lovell, 1989). Shrimp are slow feeders, finding their feed on the bottom of ponds by chemosensory mechanisms. The pellets are taken by the shrimp and reduced to edible size through action of the mandibles and mouth parts. This is very different from finfish which swallow their feed whole. Shrimp pellets need to remain stable in the water for several hours for proper feeding to occur.

Binders are important components in shrimp diets enabling pellets to maintain their water stability. Increased water stability means increased availability of the pellet to the organism and decreased loss of nutrients and organic compounds to the water. A good binder for shrimp diets should function well under a number of environmental conditions, be non-toxic to the host organism, be easy to use, maintain its binding properties over several months, and be economical. The list of compounds that can act as binders is extensive and includes: alginates, gums, celluloses, molasses, whey, gluten and starches (New, 1987). The efficacy of the binder depends on the amount used and the manufacturing process of the pellet. Steam pelleting, heat extrusion and simple extrusion will affect the binding properties of these compounds differently.

With the growth of aquaculture in developing countries, there is a need to develop good diet formulations with low cost technology. Steam pellet mills and cooker-extruders can be a substantial investment for an aquaculture operation. The purpose of this research was to evaluate a simple method for producing water stable shrimp diets using ingredients that are readily available. Several native starches, corn, potato, tapioca, and wheat, were investigated as binders in this study. The objectives were to: 1) determine the effect of increasing concentrations of starches on the physical parameters of shrimp pellets 2) evaluate the effects of different drying regimes of shrimp diets on their water stability, and 3) determine the effects of water temperature and salinity on the pelleted diets.

# LITERATURE REVIEW

## Biology of Shrimp

Shrimp life cycles have several stages including, fertilized eggs, nauplius, protozoea, mysis, megalopa, juveniles, subadult and adult. The developmental stages from the egg to postlarvae have the same characteristics in all species of the penaeid shrimp. Courtship and mating generally occur in the water column. The first larval stage, the nauplius, hatches from the egg membrane and molts 5 or 6 times before becoming a protozoea (Balley and Moss, 1992). The larval cycle lasts from 2 to 3 weeks depending on the species and ecological conditions. In the postlarval stage, the shrimp already presents the morphologic characteristics of an adult. In this stage, they start to approach the brackish water of the estuaries and coastal wetlands where there is good availability of food, low salinity, high temperature and protection against predators. These inside waters are considered "breeding areas" because the postlarvae utilize the benthic substrate and change to the juvenile stage, taking advantage of the rich substrate and aquatic vegetation. The postlarvae enter the estuaries at a size of approximately 7 mm. In this stage, they need the help of the tides in order to colonize all the estuarine zone. The distribution of the organism in the estuarine zone depends on several factors, such as, the nature of the bottom of the pond, turbidity of the water, salinity, temperature and food availability (Pretto, 1984).

In the estuaries, shrimp develop very fast and remain for a period of 3 or 4 months depending on the species and ecological conditions. After they reach a size of 10-13 cm, they start to migrate off-shore, closing the cycle.

## Shrimp Aquaculture

The tremendous growth in shrimp aquaculture over the past two decades has been due to research and development in the nutritional aspects of artificial diets and the success of hatcheries in raising young shrimp through several larval stages (Rosenberry, 1990). The standard process requires the capture of wild, gravid shrimp which are transported to the hatchery for spawning. The eggs are hatched into nauplii which feed on the yolk sac. They undergo several larval stages during which they feed on zooplankton and algae. In the postlarval stage, they take on the physical characteristics of an adult shrimp. After 1 to 4 weeks, undergoing daily molts, the postlarvae are transferred to growout facilities.

Growout operations are classified as extensive, semi-intensive and intensive (Hirono, 1983). The extensive farms have low density stocking and usually consist of low-lying impoundments in bays and along tidal rivers. Extensive shrimp farming ponds can range in size from one hectare to several hundred hectares. Natural feeds and water exchange (5-10% daily) are dependent on the tidal flow. Natural feeds within the ponds are increased by the use of commercial fertilizer.

Semi-intensive shrimp ponds are usually constructed above the high-tide line and the exchange of water is undertaken with pumps. Stocking densities are higher ranging from 25,000-250,000 per hectare. There is more competition for natural shrimp feeds and farmers enhance the natural diet with artificial feeds. Semi-intensive shrimp farms may have two types of ponds. A nursery pond stocked at high density for juveniles, and larger growout ponds for finishing growth.

Intensive shrimp farming is characterized by high stocking densities, heavy feeding and close management of the ponds. Aeration and waste removal are essential to maintain high survival and growth rates. Thailand has recently introduced "super-intensive" farming which can produce up to 100,000 kg of shrimp per hectare per year (Rosenberry 1992). However, these farms are very susceptible to disease and management problems.

## Shrimp Diet Ingredients

The most important protein source for shrimp diets is fish meal (McCoy, 1990). Fish meal made from whole fish will vary between 60-80% protein, 12-30% ash and 7-10% lipids depending on the species of fish utilized (Borgstrom, 1965). Fish meal, made from fish wastes, has higher ash and lipid contents while the protein content is decreased (Babbitt, 1990). The protein content can be increased, if the bones are screened out during processing and lipids are partially removed by centrifugation. Fish meal is usually high in lysine and methionine which are often lacking in plant feed components. Marine fish meal contains 1-2.5% omega n-3 fatty acids which are essential for growth and survival of all shrimp species (Lovell, 1989). Fish meal is rich in minerals and is found to be both highly digestible and palatable for shrimp aquaculture. For commercial diets, fish meal concentrations vary between 10-50%.

Shrimp meal is commonly used for artificial shrimp diets. It provides a number of essential dietary components, is readily available near the majority of shrimp farms, and is considered a low-cost processing waste (Schoemaker, 1991). Shrimp meal is made from dried composite shrimp wastes and/or whole shrimp. Well processed shrimp meal can have an amino acid profile similar to fish meal. A significant part of shrimp waste is the exoskeleton which is primarily chitin and can be as high as 20% of the shrimp meal. It is unclear whether or not chitin can be broken down by digestive enzymes and if it has nutritional value for shrimp. Shrimp meal is considered a valuable source of n-3 fatty acids, cholesterol and carotenoids. Shrimp meal is used in concentrations as high as 30% in some shrimp feeds.

Soybean meal is often used in shrimp diet formulations due to its nutritional value, low cost and availability. Soybean meal has one of the better amino acid profiles for plant proteins, however, it has low levels of methionine and cystine. It is readily accepted by shrimp and has high digestability but is recommended for use at levels below 32% (Lim and Dominy, 1990).

Cereals and cereal by-products are the main carbohydrate sources in shrimp diets (Lovell, 1989). The starch component can contribute to the water stability of the feed especially in feed manufacturing processes that require heat. Corn and wheat gluten are high in protein and are often used specifically for their binding properties (Akiyama et al., 1992). Flours and starches contribute significant amounts of polysaccharides to the diet and are readily digested by shrimp. Wheat clears are a by-product of cake flour production and a good source of polysaccharides for shrimp diet formulations (Matheson, 1992).

Special ingredients such as vitamin mixes and lipids are normally added for essential nutrients (New, 1987). Commercial vitamin mixes are added at concentrations between 1-2%. Fish oils, with high n-3 fatty acid contents, are added to the diets either in the initial mixing of the ingredients or allowed to soak into the dried pellet after processing. Squid meal, clam meal or other meals based in aquatic organisms may be added as attractants.

## Binders

Binders are important ingredients in crustacean feeds as they maintain the water stability of the diet. Aquatic crustaceans are slow feeders and manipulate their food extensively before ingesting it. Therefore, diets prepared to feed these animals should be firmly bound, remain intact in water for prolonged periods of time, resist disintegration during feeding manipulations, and minimize leaching of essential dietary components to the water (Heinen, 1981). There are a number of compounds that are used in shrimp diets as binders (Meyers and Zein-Eldin, 1972; New, 1987). Hydrocolloids such as carrageenan, furcellaran, agar and alginates are extracted from a variety of seaweeds. Other hydrocolloids such as gum arabic and locust bean gum are derived from land based plants. Both native starches and pregelatinized starches have been used as binders. Modified starches and celluloses with specific water binding properties have been shown to have binding properties for artifical diets. Proteins such as casein, gelatin, whey, and gluten have been found to have some binding capacity. Sulfonates and phosphates have been studied for their synergistic action with other binders. Compounds such as carbamides are effective as binders but appear to affect the palatability of the diet (Lovell, 1989). There are a number of compounds, such as cereal hulls, that are antagonistic to water stability and should be avoided.

Hastings (1971) showed that standard hard pellets lost 10% of their mass every ten minutes in moving water. Water stability experiments by Meyers et al. (1972) demonstrated that alginates were effective binders. Their results showed that calcium alginate and propylene glycol alginate gave good water stability for 24 hr, after drying the pellets for 2 hr at 65°C. Pascual and Tabbu (1979) used fish fins, fish bones and skin combined with crude agar in shrimp diet formulations and demonstrated acceptable water stability over 24 hr. Pascual et al. (1978) evaluated the effect of a series of binders (corn starch, gelatin, sargo palm starch, agar and wheat flour) for prawn feeds. Their results showed agar had the best binding properties for their experiments. Murai et al. (1981) studied a series of binding agents and determined that alginate gave the best results.

Farmanfarmaian et al. (1982) investigated several commercial Kelco algins for their binding efficiency. Pellets made with 2% Kelco HV algin binder had 80% survival after 22 hr of testing. Heinen (1981) determined the binding properties of more than 12 binders for both moist and dry pellets. His results showed that corn starch, carboxymethyl cellulose, guar gum and collagen were not suited for moist or dry pellets as survival in water was less than 6 hr. Agar and alginate based compounds were the only binders found suitable for both dry and moist pellets. Sago palm starch, various types of carrageenan and gum arabic were evaluated as shrimp diet binders by Pascual and Sumalangcay (1982). Their results showed that carrageenan at 3-5% concentration in the diet gave the best water stability over a 12 hour soaking period.

The feed manufacturing method will affect the binding capacity of these compounds. In processes that generate heat, such as cooker-extruders, gelatinization of starches will improve the binding properties of these compounds. In addition, the reduction of particle size by milling of various ingredients and elimination of coarse materials through sieving will have an impact on the water stability of the pellet.

The cost of binders represent a wide range of market prices (e.g. \$7/lb. for alginates to \$0.12/lb. for starches). These costs need to be taken into account when choosing a binder. Information regarding the degree of effectiveness, concentration needed, ease of utilization, and availability of products are important factors to consider in choosing a binder.

The standard measurement for determining the efficacy of a binder is to determine the weight loss of the pellet in water over time. The percentage of remaining pellet weight compared to the original weight over a test period is referred to as water stability (Pascual et al., 1978). There are several other physical characteristics that are important for shrimp diets. These include the size and shape of the pellet, its density, sinking rate in water, and texture (Meyers and Zein-Eldin; 1972 Hanson and Goodwin, 1977). Size and shape of the pellet will depend on the size of the shrimp to be fed. Recommended pellet diameter varies from 1 mm for 0.5 g shrimp to 4 mm for shrimp larger than 10 g (Lovell, 1989). Since shrimp are benthic feeders, the density must be high enough to allow the pellet to sink to the bottom of the pond or tank. The water activity needs to be low enough to preclude mold growth during storage. The pellets should be attractive to the shrimp and maintain their integrity when placed in water.

## Shrimp Diet Manufacturing

As mentioned previously, the type of processing will have a significant effect on the water stability of the pellet. Production of shrimp diets can be divided into three categories: pelleting, cooker-extrusion, and simple extrusion. Pelleting involves compression with minimum heat generation. The pellets produced are dense and have a good sinking rate. Pelleting machines can be simple mechanical devices such as the California Pellet Mill, which is used for producing a number of finfish diets. A disadvantage to this process is that the ingredients have to be in the dried state for the pelleting process and there is little opportunity to use hot water or steam to facilitate gelatinization. More sophisticated pelleters allow the use of steam which will increase the water stability of the diet. Steam pelleting involves the use of moisture, heat and pressure to facilitate the interaction of diet ingredients and gelatinization of the starches (Lovell, 1989).

Cooker-extruders are becoming increasingly utilized for shrimp diet manufacturing because of the increased water stability of the diet produced (Lovell, 1989). The extrusion process allows the use of more moisture and higher levels of heat than steam pelleting. The feed mixture is usually conditioned or precooked with steam and water before entering the extruder. In the extruder, the mixture is subjected to pressure and temperatures between 100-150°C (Mercier et al., 1989). This causes complete gelatinization of the starches and increased water stability. As the material is extruded through the die holes, the pellet will expand due to the rapid decrease in external pressure. This expansion needs to be controlled or the majority of the pellets will be "floaters" and not serve as shrimp feed.

Simple extruders or mincers are commonly used for producing diets in developing countries (New, 1987). The process involves extruding a moist mixture of ingredients into spaghetti-like strands. The diameter of the pellet is controlled by the die hole size and the pellets are cut into the desired length. This process can be used for forming moist feeds or the pellets can be dried for an extended shelf-life. They are simple to operate, have little maintenance, and are low in cost.

# EXPERIMENTAL

## Diet Formulation

The formulation utilized to study the effects of different starches on the water-stability of shrimp diets was based on preliminary work undertaken at the OSU Seafood Laboratory. The general formula is shown in Table 1.

Table 1. Formula for shrimp diets.

| Ingredients | % Dry Component |
|---|---|
| Fish meal | 50 |
| Wheat clears | 35 |
| Soybean flour | 10 |
| Native starch | 5 |
| | 100 |

Decrease of 5% wheat clears and increase of 5% native starches were performed stepwise in order to test native starches at different levels. E.g. 10% potato starch would reduce wheat clears to 30% in the formula; 15% potato starch would reduce wheat clears to 25%.
water added = 131.5 ml/400 g of sample.

All ingredients for these formulations were obtained from regional companies in the United States as well as from local markets. Fish meal made from anchovy (*Engraulis ringens*) was obtained from Bioproducts Inc. Warrenton, OR. Soybean flour was purchased from Darigold Feed, Astoria, OR. Soft wheat clears were donated by Advanced Hydrolyzing

Systems, Inc, Astoria, OR. Wheat starch was obtained from Madsen Mill, Yamhill, OR, potato starch was donated by Alpha Biochemical Corp. Richland WA, tapioca starch was purchased at a local grocery store distribuited by Energy, Co. Seattle, WA, and cornstarch was obtained from A.E. Staley Mfg. Co. Decatur, IL.

## Preparation of the Diets

The steps and drying treatments for processing shrimp diets is described in Figure 1. The ingredients were mixed in a Hobart mixer (Model N-50G, The Hobart M.F.G. Co, Troy OH). Tap water was slowly added together with starch to avoid formation of aggregated particles. After complete addition of these ingredients, agitation was continued for 10 min. Soft wheat clears and soybean flour were added and agitated for another 10 min. The last ingredient added was fish meal and mixing continued for an additional 10 min. Due to the granular nature of the mixture, it was easily packed and transferred to a manual lab pelletizer. The dough was extruded from an 18" string hand-driven pelletizer with a plate of 0.125". The long pellets were placed in a mesh tray and separated from each other by approximately 0.5 cm and dried to 10-12% moisture. The drying temperatures were monitored by using a Micrologger (Model 21x, Campbell Sci. Inc.). Moisture analyses of the diets, at different

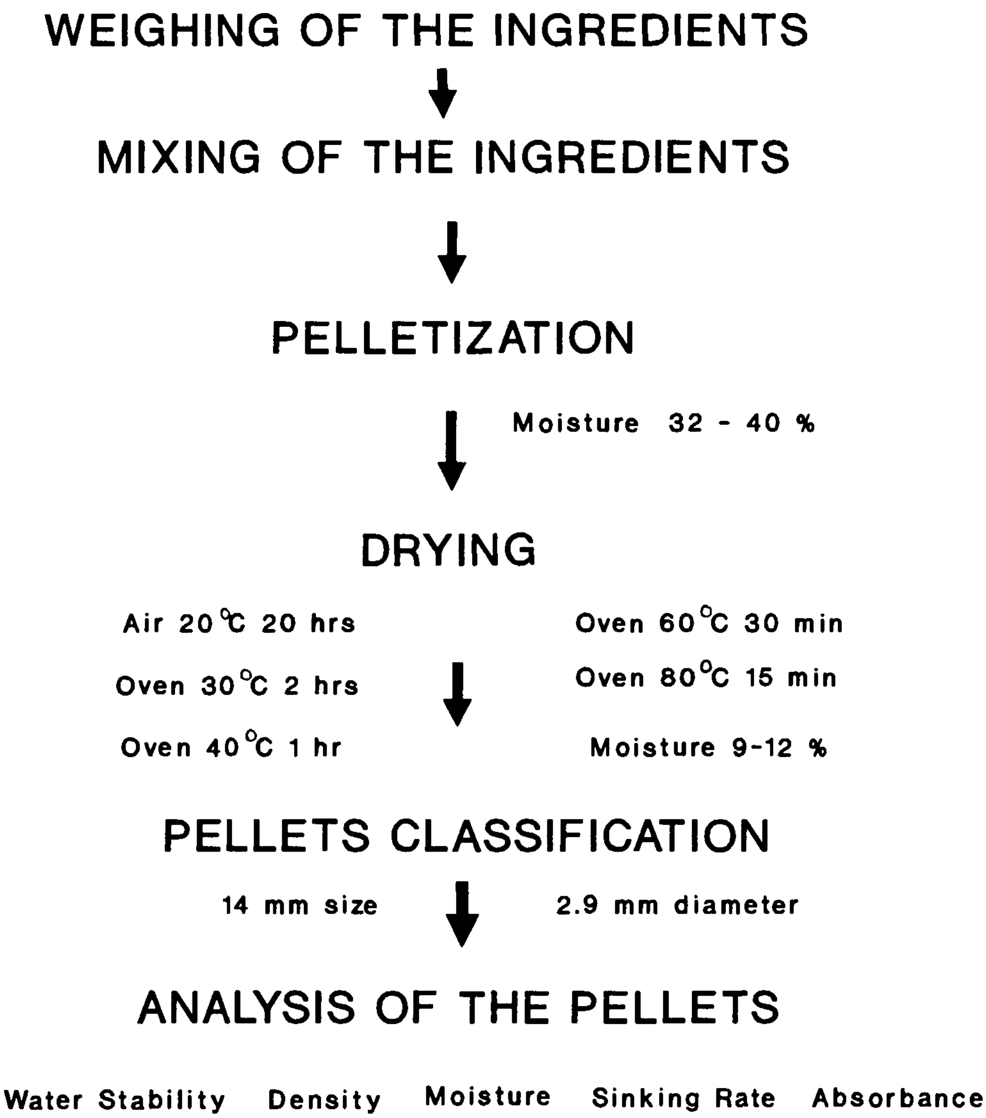


Figure 1. Steps for processing shrimp diets

stages, was determined using an Ohaus Moisture Balance (Model No. 6010, Ohaus Scale Corp. Union, N.J). The moisture content of initial dough was in the range of 30-40%. The moisture content of the dried pellets varied from 10-12%.

## Evaluation of Drying Temperature

The diets made with different starch levels (Table 1) were dried at ambient temperatures (18-21°C). Previous experiments by Kantt (1991) and Solis (1992) demonstrated that lower drying temperatures improved the water stability of the pellets. After selecting a standard diet, further drying and water stability analyses were undertaken. The following drying temperatures were used for testing the best diet; ambient temperature (18-21°C) for 20 hr, 30°C for 2 hr, 40 °C for 1 hr, 60°C for 30 min, and 80°C for 15 min. Two ovens were used for drying the pellets. A National Laboratory Incubator (Model 3211, National Appliance Co. Portland OR.) was used at 30, 40 and 60°C and a Despatch Laboratory Oven (Model Leb-1-75, Despatch Oven Co. Minneapolis, MN.) was used at 80°C.

## Analysis of Physical Parameters

### Water Stability

The procedure of Pascual and Sumalangcay (1982) were used for water stability analysis of the diets with the following modifications. Ten dried pellets were placed in a mesh ball (63.7 mm of circular diameter, 0.7 x 0.7 mm of mesh). Eight mesh balls, with pellets, were then weighed in a Mettler Analytical Balance (Model BB244, Instrument Corp. Hightstown, N.J). The mesh balls were transferred to eight beakers containing 500 ml of tap water at 25°C with one stir bar inside each one. Pellets were soaked for 2 hr and then stirred for 1 min with a Pyro multi magnestir (Model 1263, Lab Line, Inst, Melrose Park, IL.). The speed of the stir bar was approximately 90 rpm and controlled by a Powerstat (Model 3PN116B, The Superior Electric Co. Bristol CN). This procedure was repeated at 2 hr intervals for 8 hr. After each 2 hr interval, pellets were evaluated. Water stability was determined by placing each mesh ball with pellets, after each interval, into a drying oven at 100°C overnight. The samples were then cooled until constant weight was reached. Water stability is calculated using the following formulation:

$$WS = FW/IW(100 - \%\ moisture\ content)100$$

Where: WS = water stability

FW = final weight

IW = initial weight

Pellet density

Density was calculated by taking the average volume and weight of several dried pellets after splitting them into a length of 14 mm. Since the pellets were cylinder shaped, the density was calculated using the following formulation: $\rho = m/v$, where $\rho$ = density, m = mass and v = volume of the cylindrical pellet.

Sinking rate

Sinking rate was determined using a Polivinyl chloride tube filled with tap water and attached to a graduated cylinder of 95 cm in length. The pellets were placed on the surface of the water at the top of the tube and allowed to sink. A timer was used to measure the time that the pellets took to reach the bottom of the tube.

Absorbance

Absorbance of the water in which the diets were tested was determined using a Beckman Spectrophotometer (Model DU 640, Beckman Instruments Inc. Redmond, WA.). A wavelength scan for the diet formula made with 25% corn starch and tested for 8 hr was undertaken. A wavelength of 460 nm was chosen to determine the absorbance of the test water. Tap water was used as a blank. The absorbance of the water was determined at 2 hr intervals in an 8 hr test period.

## Testing of Environmental Conditions

Water stability and absorbance analyses for the chosen diet made under different drying regimes were tested at 4 different environmental temperatures (20, 24, 28 and 32°C). These diets were also evaluated at 3 different salinity levels (15, 20 and 25 ppt) at a water temperature of 27°C. A Thelco Bath Water (Model 83, Precission Scient. Co. Chicago IL.) was used to maintain the desired temperature of the water.

## Proximate Analyses of the Ingredients

Moisture content of the ingredients and diets was determined using an OHAUS Moisture Balance Determination (Model No 6010). The fat content of the ingredients was determined using the acid hydrolysis method adapted from the Official Methods of Analysis (AOAC, 1984). Protein content and determination of ash were done using the Kjeldahl Method adapted from the Official Methods of Analysis (AOAC 1984).

## Statistical Analyses

Analyses of variance were conducted on the different levels of starch concentrations using one-way analysis of variance (ANOVA) at 95% confidence interval. Multiple range analysis for water stability of the different starch concentrations were done by the Tukey Test. Regression analyses of absorbance against time for different diets were derived by the least squares method. The computer program Statgraphics (Statistics Graphics Corp.) was utilized for all analyses.

# RESULTS

## Preparation of Diets

The method chosen for processing shrimp diets is shown in Figure 1. Controlled mixing was one of the keys to successful shrimp diet production. The mixing order of the ingredients needed to be controlled to avoid the formation of aggregated particles at the end of the process. A portion of the water was added first, followed by the ingredients with potential binding properties, and finally the soybean flour and fish meal. The firmness of the dough was tested using the method of New (1987) in which a ball of dough was squeezed between thumb and forefinger. If the dough stuck together and did not crumble, it was considered a good mixture. The diets were pelletized into spaghetti-like strands that were later cut into a uniform size. Diets made with high concentrations of starch often needed extra care to prevent premature breakage of the strands before cutting to size.

The die hole size chosen for pelletizing of the dough was similar to that of Meyers and Zein-Eldin (1972). They found that pellets of 0.15 to 0.3 cm in diameter by 1.25 cm in length appeared suitable for shrimp feed. Sick et al. (1972) observed that penaeid shrimp were able to feed more readily on pellets 0.3 cm x 1.5 cm. In our study the

Table 2. Water stability for diets made with different types and amounts of starches and tested at 2, 4, 6 and 8 hr.

| Starch Type | Conc. (%) | Water Stability (%) | | | |
|---|---|---|---|---|---|
| | | Soaking Time (hr) | | | |
| | | 2 | 4 | 6 | 8 |
| Control | 0 | 84.48 | 82.79 | 80.48 | 79.40 |
| Corn | 5 | 91.63 | 84.12 | 80.82 | 79.87 |
| | 10 | 84.48 | 81.90 | 79.93 | 79.25 |
| | 15 | 83.75 | 81.40 | 79.88 | 79.20 |
| | 20 | 83.19 | 80.25 | 79.85 | 79.04 |
| | 25 | 80.64 | 80.16 | 79.82 | 76.80 |
| Wheat | 5 | 92.62 | 84.42 | 81.87 | 81.10 |
| | 10 | 85.62 | 83.44 | 81.31 | 81.01 |
| | 15 | 85.33 | 82.14 | 81.20 | 80.58 |
| | 20 | 84.92 | 81.71 | 81.12 | 79.96 |
| | 25 | 84.02 | 81.66 | 80.20 | 79.60 |
| Tapioca | 5 | 91.66 | 84.29 | 81.80 | 80.14 |
| | 10 | 84.71 | 82.62 | 81.26 | 79.81 |
| | 15 | 84.48 | 81.68 | 81.14 | 79.59 |
| | 20 | 84.46 | 81.54 | 80.96 | 79.57 |
| | 25 | 83.68 | 81.39 | 80.48 | 79.50 |
| Potato | 5 | 90.53 | 84.17 | 81.12 | 79.96 |
| | 10 | 84.53 | 81.83 | 81.06 | 79.92 |
| | 15 | 84.28 | 81.61 | 80.40 | 79.40 |
| | 20 | 83.81 | 80.57 | 80.06 | 79.12 |
| | 25 | 82.54 | 80.44 | 79.96 | 78.04 |

Diets dried at ambient temperatures and tested at 25$^{o}$C average diameter, after drying the pellets, was approximately 2.9 mm x 14 mm. Separation of the pellets at approximately 0.5 cm, before drying, gave a homogenous moisture content for all the drying temperatures tested.

## Evaluation of Physical Parameters of Diets

Shrimp diets were formulated at 0, 5, 10, 15, 20 and 25% starch using potato, wheat, tapioca, and corn starches. These diets were air dried at approximately 20°C to a moisture level between 10-12% and tested for water stability in 25°C tap water at 2 hr intervals. The diets underwent weight loss and showed a decrease in water stability during the testing period (Table 2). All the pellets demonstrated similar behaviors during testing. The largest decrease in water stability ocurred over the initial 4 hr period, followed by stabilization, and a slower weight loss over the final 4 hr test period. At the end of the test period (8 hr), water stability of the pellets ranged from 76.80% to 81.10% for all the diets. The control diet (0% starch) compared favorably with the other diet formulations, except for the diets made with 5% starch. The highest water stability values are shown for 5% starch concentrations at the 2 hr test period.

Higher starch levels in the diet appeared to cause a decrease in the water stability of the pellets for all the starches. Although diets made with wheat starch gave slightly higher values, the water stability tests were not rigid enough to discriminate among the starches.

Absorbance of the soak water for the diets tested at 2, 4, 6, and 8 hr intervals is presented in Tables 3-7. Absorbance increased with increasing desintegration of the pellets in the water. There was an increase in absorbance of the soak water as the diets were tested over time, $P < 0.01$. There was also an increase in absorbance with increased starch concentrations for each time interval, showing significant differences between starch levels, $P < 0.001$. In Fig. 2 the absorbance of the soak water from diets made with different starches (5% level) was plotted versus test time. Diets with 5% wheat starch showed less increase in absorbance over time.

Table 3. Absorbance (460 nm) for a diet made with (0%) of native starch at different soaking time

| | Soaking time (hr) | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| control | 0.007 ± 0.001 | 0.013 ± 0.002 | 0.023 ± 0.001 | 0.030 ± 0.001 |

± = standard error based on 3 observations

Table 4. Absorbance (460 nm) for diets made with native corn starch at different soaking times and different starch levels.

| Level (%) | Soaking time (hr) | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 5 | 0.007 ± 0.001 | 0.014 ± 0.001 | 0.019 ± 0.001 | 0.026 ± 0.001 |
| 10 | 0.007 ± 0.001 | 0.014 ± 0.000 | 0.019 ± 0.001 | 0.028 ± 0.000 |
| 15 | 0.006 ± 0.001 | 0.014 ± 0.001 | 0.020 ± 0.001 | 0.029 ± 0.001 |
| 20 | 0.011 ± 0.001 | 0.021 ± 0.000 | 0.024 ± 0.001 | 0.030 ± 0.001 |
| 25 | 0.014 ± 0.001 | 0.021 ± 0.001 | 0.025 ± 0.001 | 0.031 ± 0.001 |

± = standard error based on 3 observations

Table 5. Absorbance (460 nm) for diets made with native wheat starch at different soaking times and different starch levels.

| Level (%) | Soaking time (hr) | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 5 | 0.003 ± 0.001 | 0.004 ± 0.001 | 0.005 ± 0.001 | 0.014 ± 0.001 |
| 10 | 0.003 ± 0.001 | 0.004 ± 0.001 | 0.006 ± 0.001 | 0.020 ± 0.001 |
| 15 | 0.004 ± 0.001 | 0.006 ± 0.001 | 0.013 ± 0.001 | 0.022 ± 0.001 |
| 20 | 0.010 ± 0.001 | 0.011 ± 0.001 | 0.014 ± 0.001 | 0.020 ± 0.001 |
| 25 | 0.012 ± 0.001 | 0.014 ± 0.001 | 0.017 ± 0.001 | 0.022 ± 0.001 |

± = standard error based on 3 observations

Table 6. Absorbance (460 nm) for diets made with native tapioca starch at different soaking times and different starch levels.

| Level (%) | Soaking time (hr) | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 5 | 0.005 ± 0.001 | 0.015 ± 0.001 | 0.019 ± 0.001 | 0.026 ± 0.001 |
| 10 | 0.010 ± 0.001 | 0.016 ± 0.001 | 0.019 ± 0.001 | 0.027 ± 0.001 |
| 15 | 0.013 ± 0.001 | 0.016 ± 0.001 | 0.021 ± 0.001 | 0.028 ± 0.001 |
| 20 | 0.013 ± 0.001 | 0.018 ± 0.001 | 0.021 ± 0.001 | 0.029 ± 0.001 |
| 25 | 0.015 ± 0.001 | 0.020 ± 0.001 | 0.025 ± 0.001 | 0.029 ± 0.001 |

± = standard error based on 3 observations

Table 7. Absorbance (460 nm) for diets made with native potato starch at different soaking time and different starch levels.

| Level (%) | Soaking time (hr) | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 5 | 0.004 ± 0.001 | 0.017 ± 0.001 | 0.017 ± 0.001 | 0.026 ± 0.001 |
| 10 | 0.006 ± 0.001 | 0.010 ± 0.001 | 0.020 ± 0.001 | 0.027 ± 0.001 |
| 15 | 0.006 ± 0.001 | 0.012 ± 0.001 | 0.021 ± 0.001 | 0.028 ± 0.001 |
| 20 | 0.007 ± 0.001 | 0.014 ± 0.001 | 0.023 ± 0.001 | 0.029 ± 0.001 |
| 25 | 0.011 ± 0.001 | 0.019 ± 0.001 | 0.022 ± 0.001 | 0.030 ± 0.001 |

± = standard error based on 3 observations.

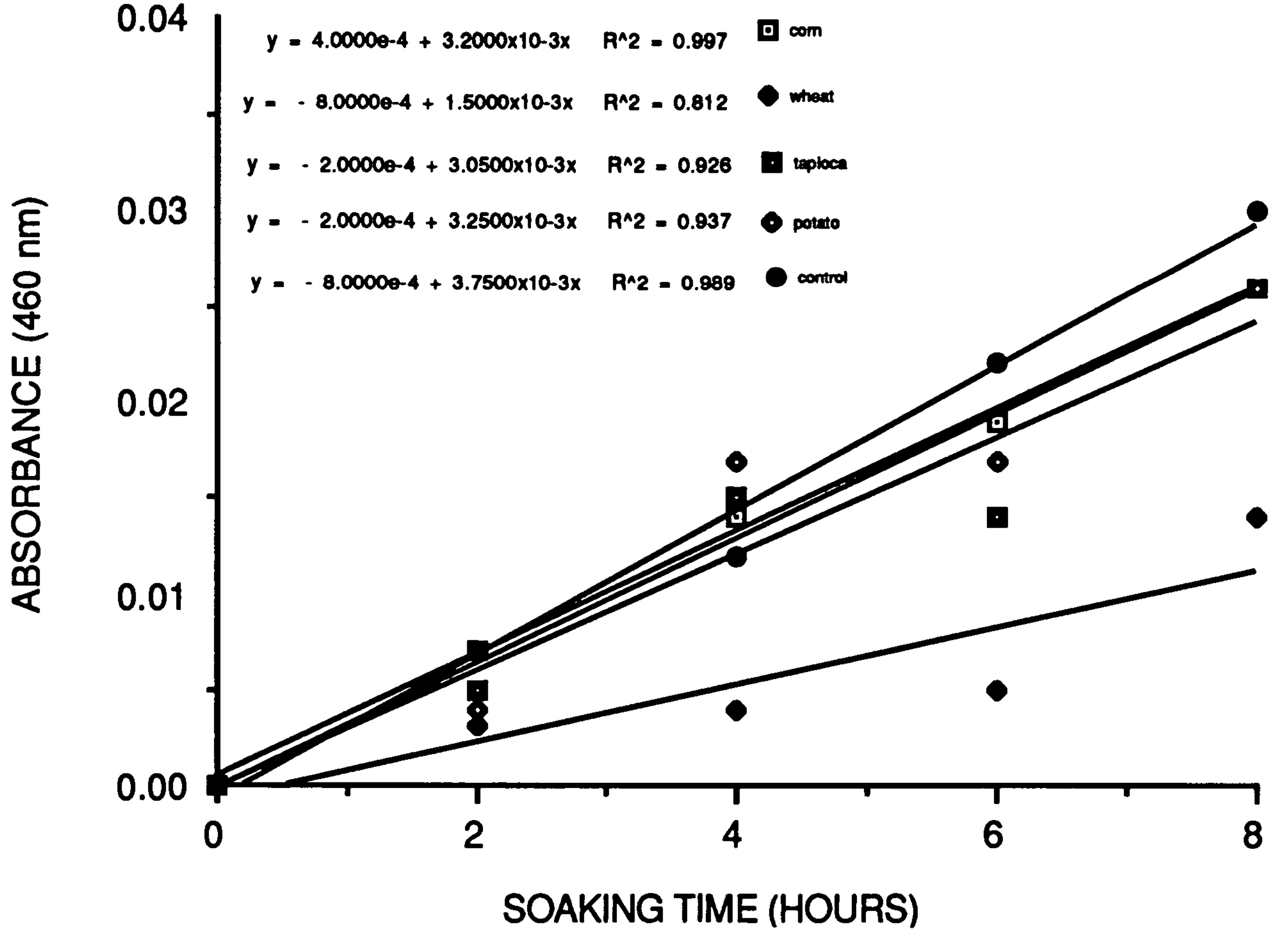


Figure 2. Regressions of absorbance of the test water (25°C) on soaking time for diets made with different types of starch at a 5% level.

Physical parameters such as density ($g/cm^3$), sinking rate (cm/sec) and moisture content were measured and are reported in Table 8. Density values decreased with increased starch concentration. There was less than 6% difference in the density between the highest and lowest starch content. There was an optimum density for all the starches, and this optimum ocurred at 5% for all the starch levels investigated. As expected, the sinking rates were highly correlated to the density for all the starch levels $P < 0.05$ and $r^2 > 0.80$ (Table 8). Sinking rates were similar and close to 10 cm/sec.

Diets made with 5% wheat starch had the best values for the physical parameters measured. Consequently, experiments to determine the effect of drying temperatures on the water stability, were undertaken with this diet. Drying of the diet was done at ambient temperature, 30, 40, 60 and 80°C. Ambient temperature drying was undertaken in an enclosed space away from sunlight. The other temperature regimes were accomplished in a standard laboratory drying oven. The moisture content for the diets ranged between 10-12% at the end of the process. At ambient temperature, the majority of conditions occurred between 18 to 21°C and the time required for the diets to reach 10-12% moisture content was approximately 20 hr.

Table 8. Physical parameters values for different types and starch levels.

| Starch (%) | $\rho$ (g/cm$^3$) | s.r. (cm/sec) | H (%) |
|---|---|---|---|
| Corn | | | |
| 5 | 1.28 | 10.5 | 11.2 |
| 10 | 1.26 | 10.5 | 10.4 |
| 15 | 1.25 | 10.1 | 10.4 |
| 20 | 1.23 | 10.0 | 10.2 |
| 25 | 1.21 | 9.3 | 9.9 |
| Wheat | | | |
| 5 | 1.31 | 10.9 | 11.6 |
| 10 | 1.30 | 10.9 | 11.6 |
| 15 | 1.29 | 10.5 | 11.6 |
| 20 | 1.29 | 10.5 | 11.5 |
| 25 | 1.27 | 10.5 | 10.9 |
| Tapioca | | | |
| 5 | 1.29 | 10.5 | 11.5 |
| 10 | 1.27 | 10.5 | 10.9 |
| 15 | 1.26 | 10.2 | 10.6 |
| 20 | 1.26 | 10.2 | 10.6 |
| 25 | 1.26 | 10.2 | 10.6 |
| Potato | | | |
| 5 | 1.29 | 10.5 | 11.2 |
| 10 | 1.29 | 10.5 | 11.2 |
| 15 | 1.28 | 10.5 | 11.1 |
| 20 | 1.24 | 10.1 | 10.2 |
| 25 | 1.23 | 9.6 | 10.1 |
| Control | 1.26 | 10.2 | 10.4 |

$\rho$ = density calculated by taking the mean of 3 observations of mass and volume, s.r. = sinking rate is the mean of 3 observations, and H = moisture content represents, the mean of 4 observations.

As temperatures were increased (in the drying oven) to dry the selected diet, drying times were decreased, accordingly, to achieve the same moisture levels. The drying time at different temperatures for the selected diet was 2 hr at 30°C, 1 hr at 40°C, 30 min at 60°C, and 15 min at 80°C.

The effect of drying temperatures on water stability are shown in Figures 3-6. Each figure represents a different water temperature at which the diets were tested. In Figures 3-4, at the lowest test temperatures, the diets began to show divergence in water stability as the soak time approached 6 hr. At 8 hr soak time, the diets prepared at the lower drying temperatures of 20 and 30°C had increased water stability compared with the other diets. The differences in water stability values are greater in the diets tested at higher water temperatures. At water temperatures of 28°C and 32°C (Figures 5 and 6) there was considerable mass lost between diets even after 2 hr of soaking time. In Figure 6, at the 32°C test temperature, the water stability of the diets dried at lower temperatures (20°C and 30°C) was 70% after 8 hr of soak time. The diets dried for shorter time periods, but at higher temperatures (60 and 80°C) had less than 50% water stability. This confirmed earlier results showing that survival of the pellets in water was directly related to the drying regime.

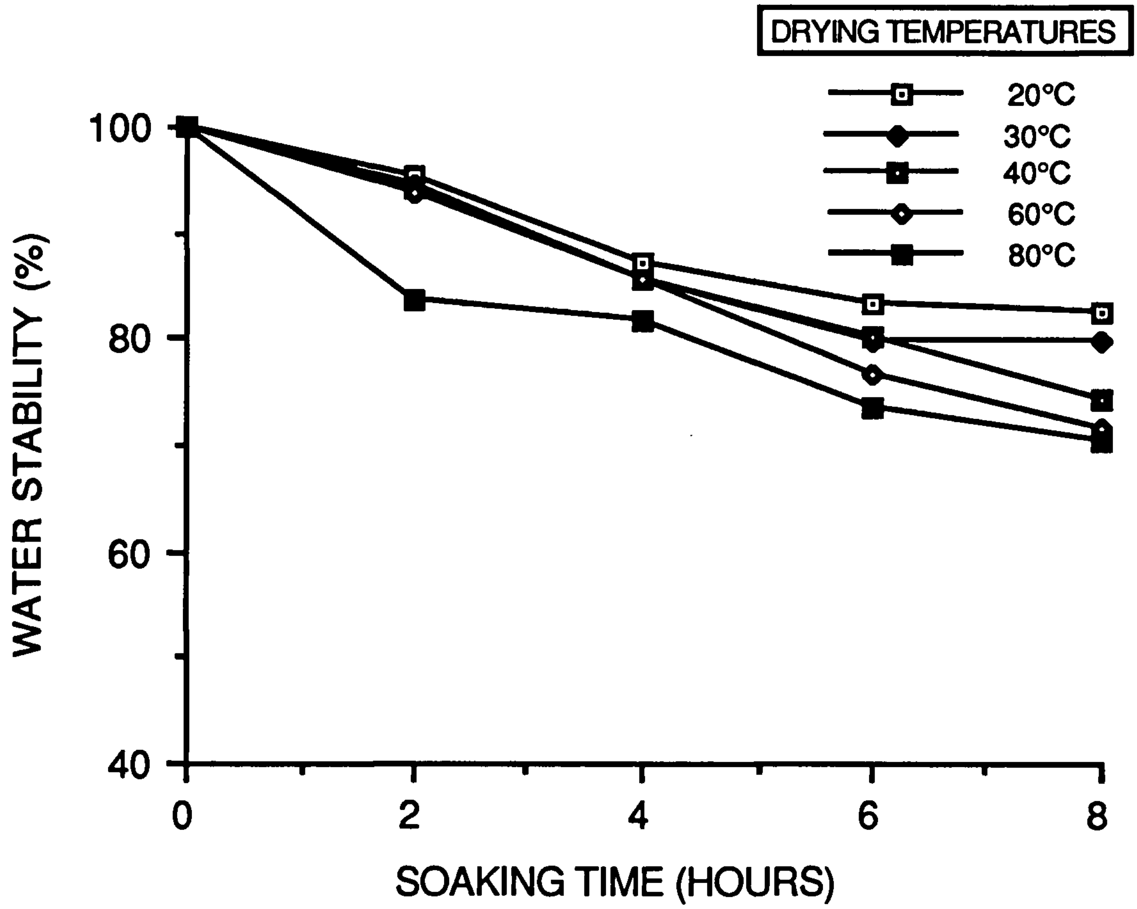


Figure 3. Water stability versus soaking time for a shrimp diet made with 5% wheat starch dried at different temperatures and tested at a water temperature of 20°C.

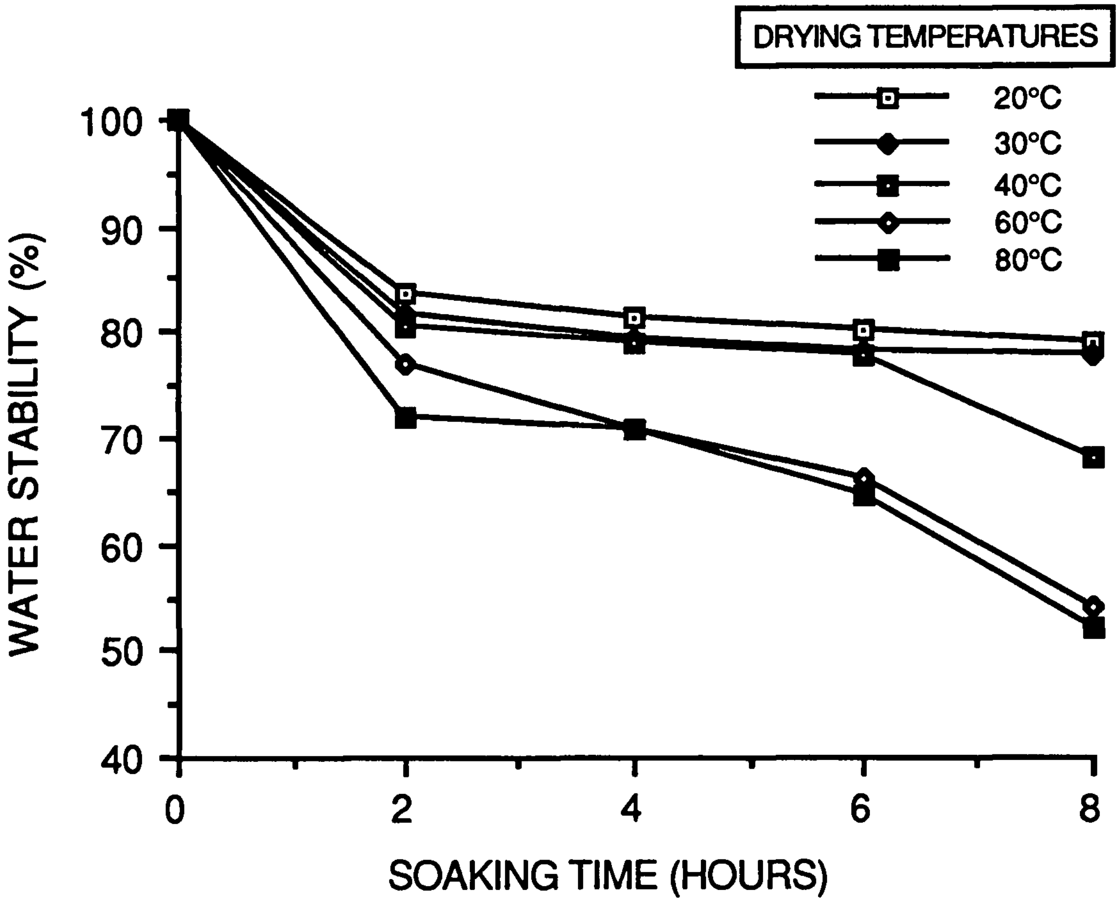


Figure 4. Water stability versus soaking time for a shrimp diet made with 5% wheat starch dried at different temperatures and tested at a water temperature of 24°C.

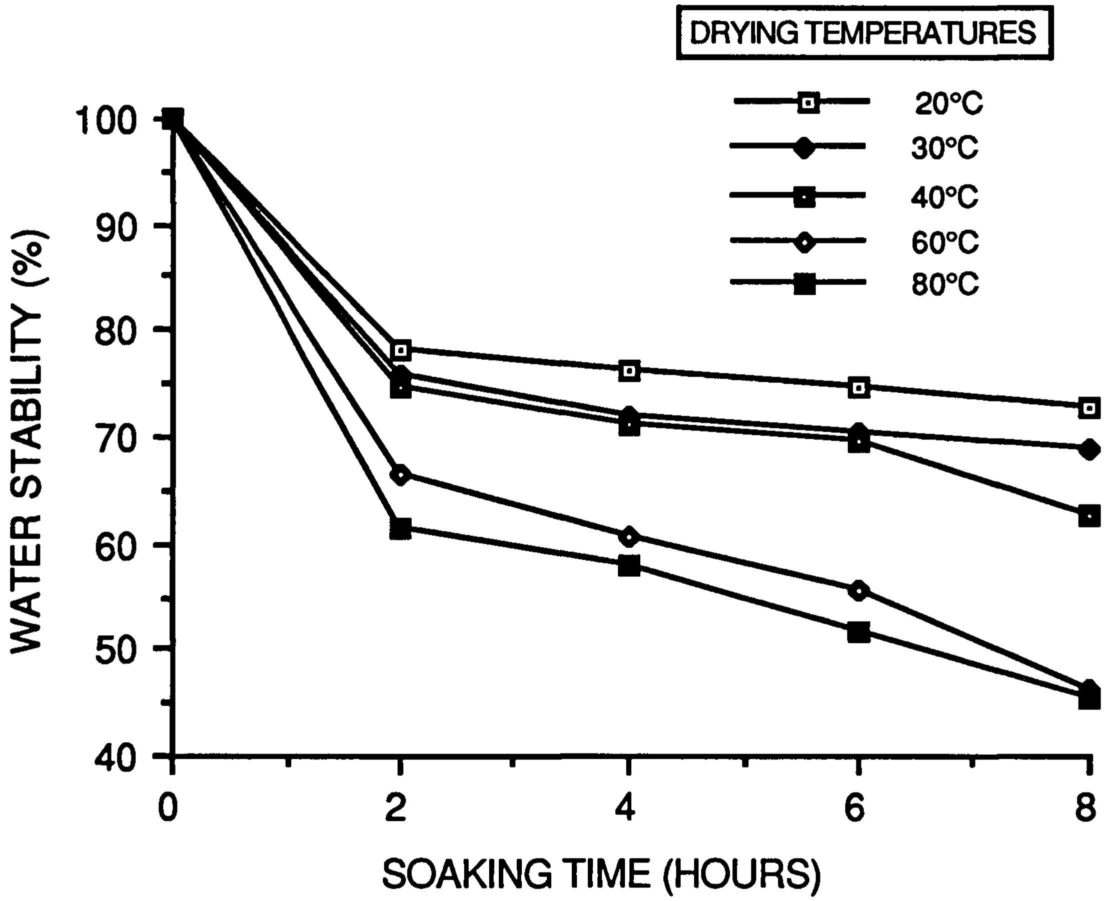


Figure 5. Water stability versus soaking time for a shrimp diet made with 5% wheat starch dried at different temperatures and tested at a water temperature of 28°C.

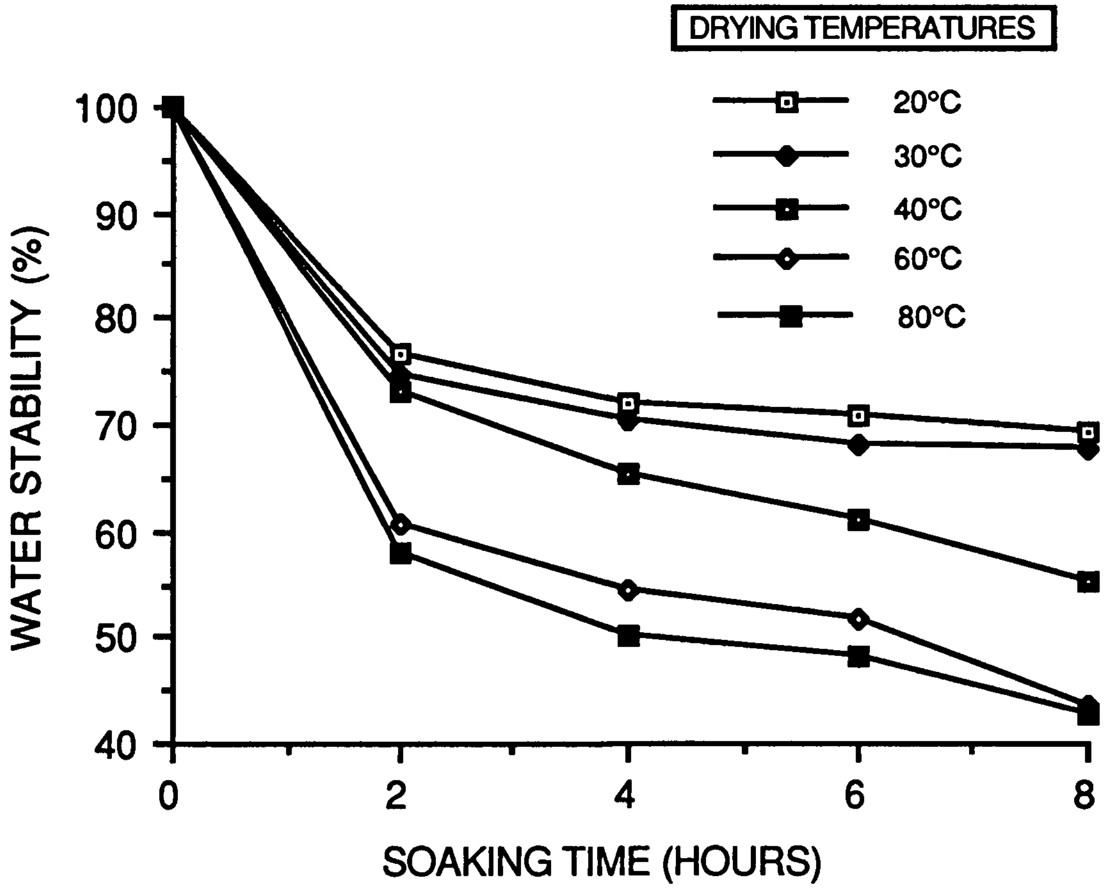


Figure 6. Water stability versus soaking time for a shrimp diet made with 5% wheat starch dried at different temperatures and tested at a water temperature of 32°C.

It was also observed, that faster drying times often left the center of the pellet hollow. This would often facilitate swelling of the pellet causing it to dissolve rapidly in water.

## Evaluation of the Diets under Different Environmental Conditions

Because shrimp diets are used under a variety of environmental conditions, a series of experiments were run to determine water stability at different water temperatures and at different salinities. The diets made with 5% wheat starch and dried at ambient temperature (18-20°C) were used for this experiment.

Water stability of the shrimp diets decreased as the test temperature was increased. The greatest variations in water stability appeared in the first 2 hr. The diets tested at 20°C had a 95% water stability, after a 2 hr interval, while diets tested at 32°C decreased in water stability to 75% (Figure 7). The loss of weight in the pellets was minor during the first 4 hr. After the 8 hr testing period, the lower test temperature had the least effect on the water stability of the diets while the highest test temperature decreased the water stability to 70%.

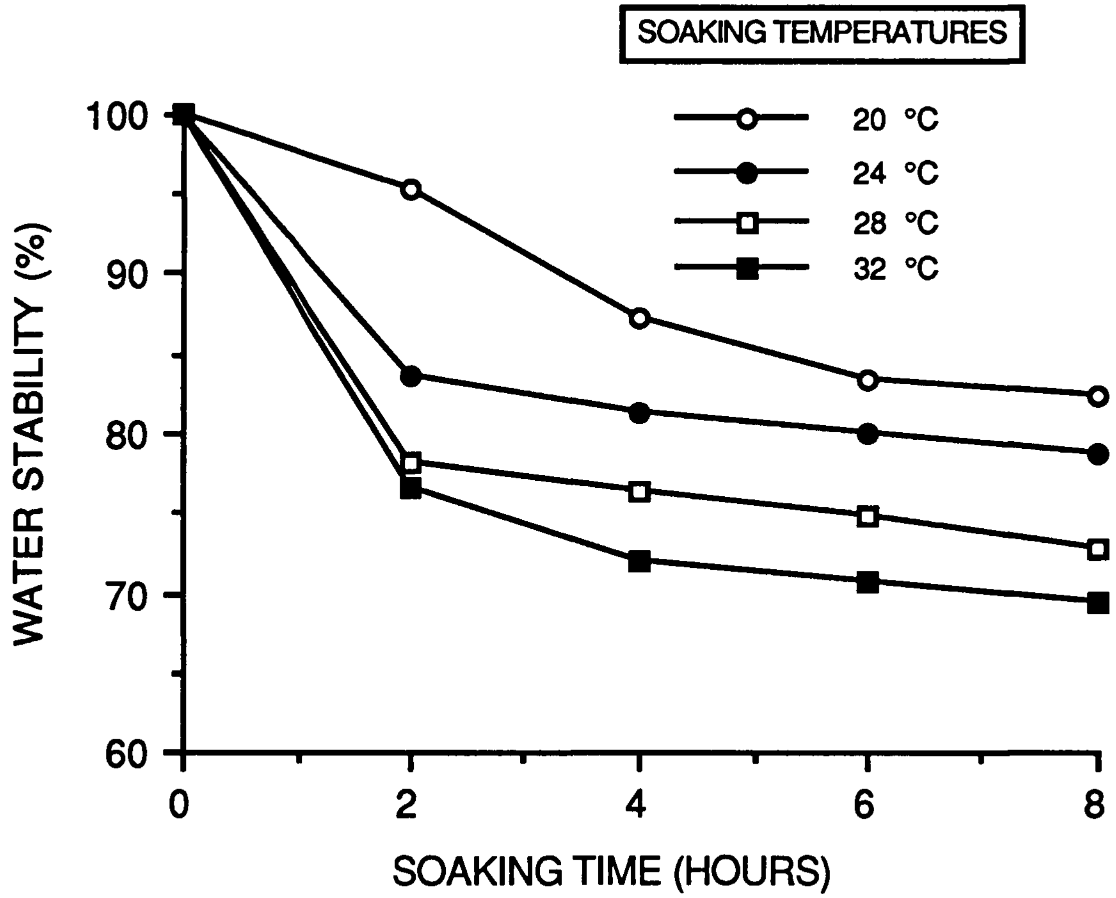


Figure 7. Water stability versus soaking time for a shrimp diet made with 5 % wheat starch dried at ambient temperature (18 - 21°C) and tested at different water temperatures.

The effect of different salinities on the water stability of the pellet is shown in Figure 8. Results showed that the higher the salinity, the greater the decrease in water stability. The largest differences in water stability values appeared during the first 4 hr of the water soak. There was little change in the shrimp diets soaked at 15 parts per thousand (ppt) salinity in the first stage of the experiment. These diets were less stable during the second 4 hr and decreased 15% in water stability during this time period. The higher salinities showed a gradual loss over all time intervals measured and decreased 30% water stability after 8 hr.

Water stabilities for diets made with different amounts of starch were compared through ANOVA at 2, 4, 6, and 8 hr soaking time. Because the behavior of the diets made with different types of starch were similar, it is valid to assume that there is no interaction effect among the starch types. Significant differences of water stability among the levels of starches were found $P < 0.001$ at 2 and 4 hr soaking time; meanwhile, at 6 and 8 hr soaking time, the water stability for different levels of starches is not significant $P > 0.05$. Multiple range analyses tests using the Tukey method at 95% confidence interval for soaking times and different amount of starches is shown in Table 9.

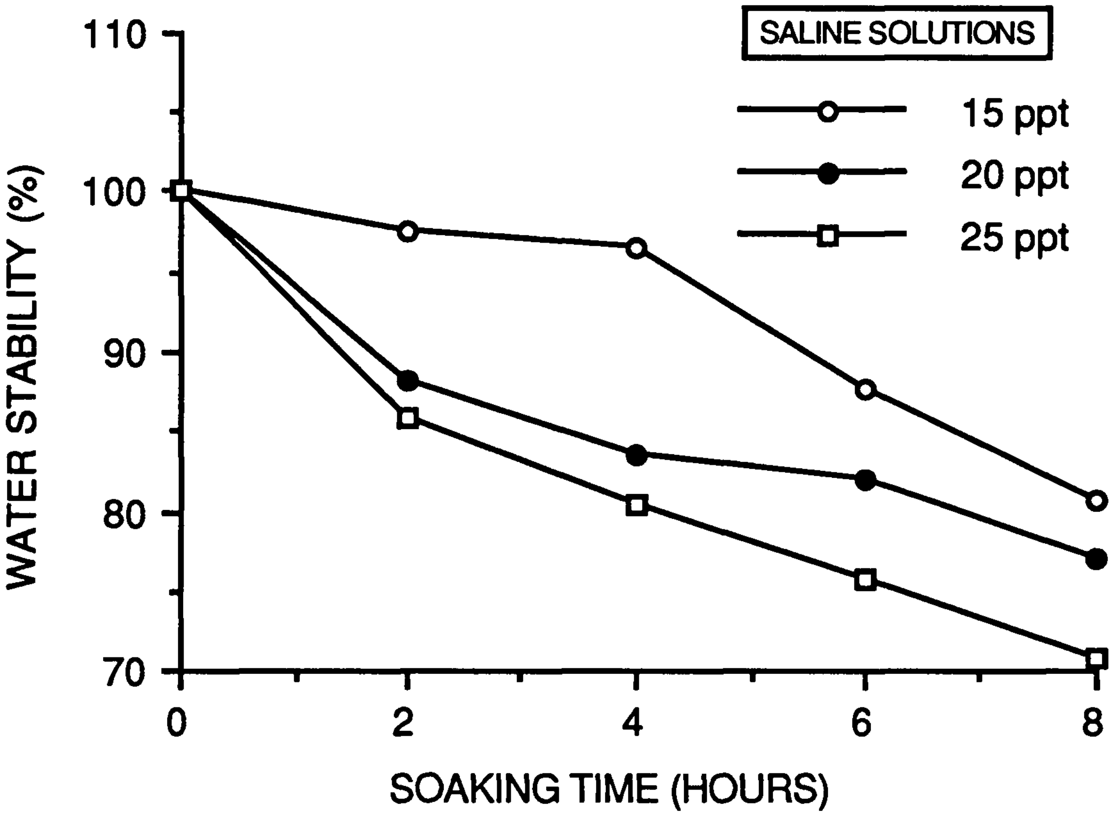


Figure 8. Water stability versus soaking time for a shrimp diet made with 5% wheat starch dried at ambient temperature (18 - 21°C) and tested at different saline solutions.

Table 9. Multiple range analyses for water stabilities among diets made with different amounts of native starches at different levels.

| Soaking Time (hr) | Level (%) | Water Stability Average | Comparison |
|---|---|---|---|
| 2 | 0 | 84.480 | X |
| | 5 | 91.610 | X |
| | 10 | 84.835 | X |
| | 15 | 84.320 | X |
| | 20 | 84.230 | X |
| | 25 | 82.730 | X |
| 4 | 0 | 82.720 | X |
| | 5 | 84.250 | X |
| | 10 | 82.455 | X |
| | 15 | 81.707 | X |
| | 20 | 81.017 | X |
| | 25 | 80.912 | X |
| 6 | 0 | 80.480 | X |
| | 5 | 81.400 | X |
| | 10 | 80.890 | X |
| | 15 | 80.650 | X |
| | 20 | 80.497 | X |
| | 25 | 80.250 | X |
| 8 | 0 | 79.400 | X |
| | 5 | 80.260 | X |
| | 10 | 79.990 | X |
| | 15 | 79.800 | X |
| | 20 | 79.420 | X |
| | 25 | 78.480 | X |

Data based in 96 observations. X not grouped vertically, indicates significant difference. Water stability of the different levels at 2 and 4 hr of soaking time are statistically different. $P < 0.001$, and for 6 and 8 hr they are not significant $P > 0.05$. F-value at 2 hr = 44.0810, at 4 hr = 17.5930, at 6 hr = 1.7630 and at 8 hr = 2.4480.

## Proximate Analyses of Diet Components

Proximate analyses of the dietary components are shown in Table 10. Fish meal has the highest protein content (62.14%), followed by soybean flour (34.38%), and wheat clears (10.49%). All the starches had no measurable protein. Soybean flour had the highest fat content (17.5%) followed by fish meal (7.95%) and wheat clears (2.13%). The protein and fat level of starches was less than 0.1%. Ash content was highest in the fish meal (16.74) followed by soybean flour (4.82%). Other than tapioca starch at 1.92% ash, the wheat clears and other starches had less than 1% ash. The moisture content ranged from a low of 8.96% for soybean flour to a high of 12.36% for wheat clears. For the different starch diets described in Table 1, the protein content can vary from 38.71% to 36.08% for the diets with 25% starch.

Table 10. Chemical analyses of the ingredients used to produce shrimp diets.

| Ingred. | Moist (%) | Fat (%) | Ash (%) | Prot (%) |
|---|---|---|---|---|
| F M | 10.2 ± 0.71 | 7.9 ± 0.07 | 16.7 ± 0.02 | 62.1 ± 0.23 |
| W C | 12.3 ± 0.38 | 2.1 ± 0.76 | 0.5 ± 0.05 | 10.4 ± 0.11 |
| S B F | 8.9 ± 0.04 | 17.5 ± 0.28 | 4.8 ± 0.13 | 34.3 ± 0.31 |
| P-S | 10.5 ± 0.38 | < 0.1 | 0.5 ± 0.03 | < 0.1 |
| W-S | 10.6 ± 0.59 | < 0.1 | 0.4 ± 0.03 | < 0.1 |
| T-S | 10.0 ± 0.08 | < 0.1 | 1.9 ± 0.16 | < 0.1 |
| C-S | 10.3 ± 0.62 | < 0.1 | 0.5 ± 0.04 | < 0.1 |

F M = fish meal, W C = wheat clears, S B F = soybean flour, P-S = potato starch W-S = wheat starch T-S = tapioca starch. C-S = cornstarch. Samples are based on 3 observations. ± = standard error.

# DISCUSSION

Determination of the physical parameters of shrimp diets is an important aspect of diet formulation. Understanding how long dietary components are available for aquaculture organisms, and what the losses are to the aquaculture system is critical for a successful operation. Although there have been a number of research efforts on the nutritional components of shrimp diets, relatively few studies have investigated how ingredients or drying methods affect the integrity of the pellet in water.

In this study, the water stability of the pellets was affected by the starch levels in the diets. Water stability decreased with increasing concentrations of the native starches. Increased starch concentration also decreased the density and sinking rate of the pellets. Higher drying temperatures (60-80°C) caused non-uniform texture across the interior of the pellet. In addition to the volume expansion observed, a stress crack was often formed across the center axis of the pellet. Water stability decreased noticeably in these diets as the pellets were tested over several time periods.

Slow uniform drying proved to be the optimum method for producing pellets with good water stability. This was discovered through a series of trials and errors while testing commercial binders (Kantt, 1991; Solis, 1992). It was observed that the drying method, rather than diet ingredients, had the greatest effect on water stability of extruded pellets made without heat or pressure. Water stability increased several fold when the pellets were air dried overnight. These drying experiments were repeated for diets with 5% wheat starch and similar results were found. It also appeared that diets dried under controlled conditions at low temperatures (30°C for 2 hr) produced pellets of comparable water stability as pellets dried at ambient temperatures (18-21°C).

This study showed the importance of drying methods used in production of shrimp diets resulting in a number of implications for the industry. First, it is possible to manufacture shrimp pellets with good water stability through small-scale operations and artisanal conditions. A hand-pelletizer is a simple device that can be manufactured in most countries. Although our testing was done on a very small scale, it would not be difficult to scale up the operation to produce larger quantities of diets. The air drying process is also a simple procedure and a method that is commonly used in a number of countries with shrimp aquaculture. Most shrimp farming operations in developing countries occur near the coast or in estuaries where there has been a long history of artisanal fisheries. These fisheries have depended on the drying of fish and other seafoods for preservation of the catch. The drying of diets would, in all likelihood, require a transfer of information rather than technology for these areas.

Oven drying at low temperatures (30°C) would allow scale-up operations that would supply diets in larger quantities. A number of developing countries are now depending on heat-extruded shrimp diets made in developed countries. These large-scale extruders are very expensive, require a degree of operating expertise, and replacement parts are very difficult to obtain in many countries with shrimp farming operations. In contrast, our results show that diets can be produced without heat extrusion, dried at ambient temperature or in low temperature ovens. Although we have not tried to compare production costs, the potential of producing diets using a simple methodology with raw materials available locally should provide a number of advantages over imported diets for developing countries.

Low temperature drying of the pellets also decreased excessive leaching of dietary components. Absorbance measurements of pellets immersed in water have been reported to have strong correlation with water stability (Pascual and Tabbu, 1979). Results demonstrated that increasing the starch concentrations of the diets raised the absorbance values over the test period. This increase in absorbance was slightly dependent on the type of starch used. Wheat starch appeared to have slightly better binder qualities than the other starches tested.

Starch, when used as a binder in shrimp diet formulations, appears to marginally improve the physical properties and water stability at 5% concentrations. Diets made with high starch levels were less stable and tended to break up after an 8 hr soak period. Shrimp diets prepared without starch held up well in several tests and gave comparable results after the 8 hr testing period.

Temperature of the water also affected the water stability of the diets. Native starches are insoluble in cold water, but soluble in hot water. This increased solubility could decrease the water stability of the pellet. The starch component may be strongly affected by water temperatures higher than 28°C; thereby, decreasing the water stability of the diet. Temperatures of shrimp aquaculture ponds range between 20 and 32°C depending on the location, time of the day during feeding, and season. These factors have to be considered during the formulation and production of aquaculture feeds.

Water stability was also affected by salinity conditions. The lower the salinity (15 ppt), the higher the water stability for diets manufactured under our conditions. At a salinity of 25 ppt, weight loss of the pellets may be promoted by penetration of salt particles into the pellets causing increased disintegration. In tropical countries, aquaculture operations face serious problems during the dry season where elevated salinities in water have been reported

(NMFS, 1992). As shown in this study, water stability of the diets is seriously affected by increased saline concentrations. The salinity, in our tests, was higher than that reported by Heinen (1981) who used 2% salinity to test diets made with several binding agents such as cornstarch, collagen and agar, and others.

The ingredients used to make shrimp diets in our formulations are ready available, and its processing did not require a high cost operation. This study demonstrated a potential for using soft wheat clears for shrimp diets. Its property as a filler, in this study, demonstrated that it was not antagonistic to the water stability of the diets. The use of soft wheat clears and its role as an ingredient in shrimp diets is worth future investigations. Soft wheat clears are considered a by-product of cake flour production. Its use in aquaculture diets has not been reported although it is used in preparing feeds for domestic animals (Glenn, 1992).

Although the method of measuring water stability has not been standardized, the results from our study compared favorably with several other studies. Heinen (1981) compared several binders. Among them were alginates, agar, corn starch, carboxymethyl cellulose, chitosan, guar gum, and collagen. He found that cornstarch, chitosan, collagen and guar gum were not suitable for either moist or dry pellets under their test conditions. This differs from our results which showed that diets made with cornstarch, could be considered water stable under our test conditions. Water stability, for even the highest level of cornstarch, was maintained at a level of 76.8% after 8 hr of soaking.

Pascual and Tabbu (1979) found that cornstarch demonstrated good water stability of 60 to 70% under their testing conditions. Meyer and Zein-Elding (1972) proposed that stability of the diets increased with decreased water temperatures. This assumption was validated by our research as diets tested at 20°C gave better water stability than at higher soaking temperatures. Sago palm starch and other binders were evaluated by Pascual and Sumalangcay (1982). In their formulations, they included wheat flour, soybean meal and fish meal. Water stability at 8 hr was between 70-80% for the majority of the diets. The water stability of our diets had a similar range for this period of soaking time.

# CONCLUSIONS

Shrimp diet pellets produced by a simple pelletizing method, without heat or pressure, demonstrated good physical properties and water stability. More than 70% of the pellet mass was retained after 8 hr of testing.

Low concentrations of starch demonstrated positive characteristics as a binder for shrimp diets. A concentration of 5% starch was the optimum level to improve the integrity of the pellet during the testing period. Wheat starch was marginally better than tapioca corn and potato starch as a binder in our testing conditions. Density and sinking rates appear to be related to the water stability of the pellets and are dependent on the concentration of starch. The use of wheat clears also showed promise as an ingredient that may contribute to the stability of the diet.

The drying method had the greatest influence on the survival of the pellets in water. Diets dried between a temperature range of 20-30°C and a final moisture of 10-12% gave the best results. The 20°C drying temperature was done at ambient temperature over a 20 hr period. The 30°C drying method was performed in a drying oven over 2 hr. Shorter drying times at higher temperatures often caused uneven drying of the pellet and lower water stability values. At the highest oven drying temperatures (60-80°C) the pellets often had cracks which decreased their integrity in water.

Environmental factors such as water temperature and salinity greatly affected the water stability of the pellets. Higher water temperatures increased the disintegration rate of the pellets in water. The shrimp diets had their lowest water stability values at 25 ppt salinity. The majority of shrimp aquaculture ponds are in tropical areas and water temperature of the ponds can reach 30°C and operate between 15 and 25 ppt salinity. Temperature and salinity should be taken into, consideration for determining physical parameters of shrimp aquaculture diets.

These results demonstrate that a simple extrusion method, can produce a shrimp diet with good physical parameters. Pellets, utilizing native starches as binders and wheat clears as filler, were produced using a manual pelletizer and dried for 20 hr at approximately 20°C. This low cost technology would permit the production of shrimp diets in a number of developing countries where shrimp aquaculture is a growing enterprise.

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