J. Korean Fish. Soc. 29(6), 920~922, 1996 한수지, 29(6), 920~922, 1996

〈TECHNICAL NOTE〉

Development of Microparticle Feed using Protein Microencapsulation Technique

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Key words : microparticle feed, protein microencapsulation

There have been many attemps to develop artificial diets for marine fish larvae (Teshima et al., 1982; Kanazawa et al., 1983, 1989; Kanazawa, 1986) and marine suspension feeders (Langdon, 1982; Langdon and Bolton, 1984; Langdon and Siegfried, 1984). These attempts are concentrated on digestibility and capsule or particle size that the marine suspesion feeder and fish larvae can take easily.

Microencapsulation technology provides an useful approach for delivering nutrients to marine fish and the reduction of water pollution in aquaculture. Therefore, a variety of materials have been used for microencapsulated feed production. In this study, the methods for the preparation of protein microparticles using marterials as shown in Table 1 were evaluated. Those were alginate microgel particle, protein walled microcapsule, nylon protein walled capsule and solidified blood microparticle.

Alginate microgel particles were prepared with 35 % (w/w) skim milk, 5% sodium alginate and 20% soluble starch. Those were mixed by a homogenizer. Five g of mixture were emulsified in 15 ml of chloroform with 20% soy lecithin. Three ml of 5 M $CaCl_2$ and 0.5 ml of 10 N NaOH were added to the emulsion to prepare protein gel. The size of protein microparticle in the range of 25~100 μm was obtained as shown in Fig. 1 (A).

The preparation of protein walled microcapsule was prepared with following procedure. A solution of 15% skim milk and 3% carboxymethyl cellulose (CMC) was prepared in 1M NaOH. Two ml of the mixture were emulsified in 20 ml of cyclohexane with 2% w/v soy lecithin using homogenizer. The emulsion was poured into 50 ml beaker and agit-

Table 1. Major particle size and components of protein microparticle

Capsule Type	Components	Major Capsule Size (μm)
Alginate Microgel Particle	Skim Milk Sodium Alginate Soluble Starch	25~100
Protein Walled Microcapsule	Skim Milk CMC PVA	10~15
Nylon Protein Walled Capsule	Skim Milk Ethylenediamine BTC	5~10
Solidified Blood Particles	Soy Bean Oil Fish Blood Meal	50~200

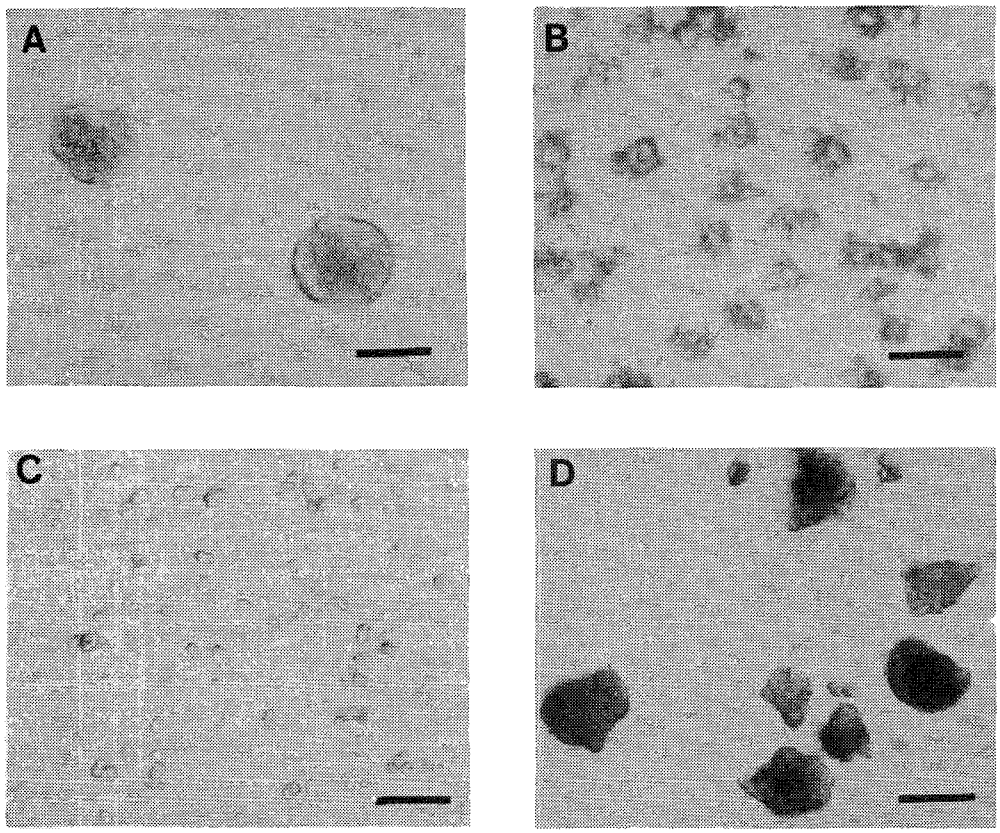

Fig.1. The protein microcapsule (scale bar : 50 μ).
A : alginate microgel particles **B : protein walled microcapsule**
C : nylon protein walled capsule **D : solidified blood particle**

ated on a magenetic stirrer. Ten ml of chloroform with 2% soy lecithin were then added to the stirred emulsion, followed by adding 200 ml of cross-linking agent, BTC (1,3,5-benzenetricarbonly trichloride). After 20 min, the emulsion was added to 150 ml of cyclohexane and the suspension was left for 24 h to set the capsules. The set capsules were washed twice by 150 ml cyclohexane and freeze-dried. Two mg of freeze-dried capsule were dissolved in the solution of 1% polyvinyl alcohol (PVA) and 0.33 M $CaCl_2$. Thirty five ml of 1% PVA dissolved in 0.5 M NaCl were added to suspend the capsules. The capsules were sonicated for 1 min using a sonicator bath (C.J. Langdon et al., 1990). As shown in Fig. 1 (B), reaction residue and microcapsule were aggregated and the capsule size was around 10~15 μm.

Nylon protein walled capsule was prepared with 25 % skim milk dissolved in ethylenediamine. Hundred ml skim milk solution was added to the 100 ml of chloroform with 2% soy lecithin and homogenized for 1 min at room temperature. The emulsion was agitated with magnetic stirrer and 20 ml of cyclohexane with 2% soy lecithin was added to the emulsion. The mixture was added to 400 ml BTC and agitated for 20 min (C.J. Langdon 1989). The capsule suspension was washed three times with cyclohexane and then freeze-dried. The prepared capsule size (5~10μm) was smaller than other types of capsule as shown in Fig. 1 (C). This was due to the fact that the ethylendiamine was proper solvent to emulsify skim milk to

make small capsules.

In order to prepare solidified blood particles, twenty ml of 40% fish blood meal was added to 200 ml of soybean oil with 2% soy lecithin and emulsified by homogenizer. The emulsion was heated in hot water bath for 20 min at 80℃. After heating, the capsule were allowed to settle out from the suspension for 24 hours. The settled particles were then washed twice by cyclohexane. Cyclohexane was removed from by vacuum evaporator and the particles had the size range of 50~200 μm as shown in Fig. 1 (D). Solidified blood particles seem to contribute to the recycling of the waste fish blood. The solidified blood particles can be used as the substitute for the fish blood meal to prepare the fish feed.

Further studies are carried out to simplify the processes to prepare these protein microparticles.

Acknowledgement

This study was supported by the research fund (96 K3-1506-01-03-1) from Research Center for Ocean Industrial Development (RCOID) of Pukyong National University.

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Received October 5, 1996
Accepted November 7, 1996