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DETERMINATION OF BLACK SPOT INHIBITOR (4-HR) BY HPLC

By

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Introduction

Shrimp quality is depended on biochemical changes, may be determined by light coloni of hall body tissue, tenderness or odour as an indications of freshness. Bacterial degrada-tion may cause chemical and physical changes such as "black spot", odour, spoilage etc, also protein denaturation can also occur.

To prevent the biological degradation on shrimp, sometime can be done by adding chemical additive or antibiotic to inhibit the formation of black spot. In this report we determine the chemical additive 4-HR as a black spot inhibitor in shrimp (black tiger) by HPLC.

Many methods have been developed for black spot determination on shrimp and a microbiological procedure is the most addaptive one. Based on the speed of analize, the used of HPLC method has excellent measuring and result: high resolution, more sensitive and more accurate. So the HPLC method has been chosen for this determination.

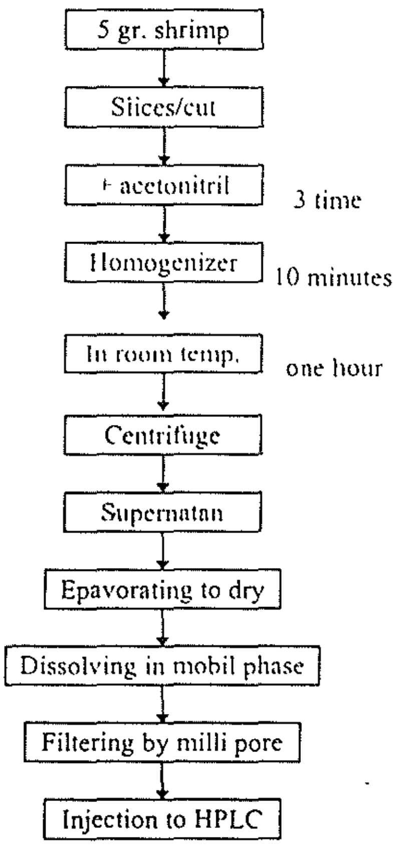
Materials and Method

Materials: TCA

Mobil phase
Instrument: Shimazu HPLC Model
Collum: Chromacil 100-5C18
Rotary shacker
Rotary evaporator
Milli pore
Shellings.

Methods

Flow chart for determination of 4-HR



Preparation for standardization of 4-HR

10.01 mg of 4-HR in 100 ml mobil phase it means the 100 ppm standard solution is dilluted to: 2,4,6,8,10 ppm.

Sample Work-Up

Romove Shell
Homogenize
Extract with Acetonitrile (3X)
Rotovap
Redissolve in mobile phase
Filter
Inject

Preparation for mobil phase

Dissolve 19.21 gram of citric acid and 5 ml of triethylamine in water to 1 liter Olace 14.42 of sodium dodecyl sulfate in a 2 liter's beaker. Add the citric acid/triethylamine solution and stil until clear. Adjust the pH to 1.9 with perchloric acid. Add 1 liter of acetonitrile. Filter through a 0.45 micro nylon filter and degas.

Analitycal Condition

Filter 0.45 micro meter, nylon filter and degas.

HPLC Condition

Column Kromasil 100-5 micro C 18, length 15 cm, Id 4.6 mm. Detector UV at 275 nn meter. Flow rate 1.00 ml/min. Injector volume 100 micro lit.

Results

1.Standard curve: point absolute calibration
2.Control sample: not 4-HR was detected
3.Treated sample: 0.93262 mg/kg (1 ppm)
1 ppm from 100 ppm ----1% after treated.
Rf standard 12.174 min-reference
Rf standard 11.28 min-standard
Rf sample 11.54 min - sample treated.
4. Microbiological test (TPC)
Total colony; 236 colonies for sample treated.
428 colonies for control.

Conclusion

The 4-HR is a good chemical additive to prevent the black spot reaction on shrimp, espeially for Frozen product. As much as 100 ppm of especially frozen 4-HR was used as a standard additive concentration. Washing treatment after thawing was enough to reduce 99% of 4-HR. So the residu of 4-HR after washing just 1% (1 ppm).

The specific bacteria can also stimulate the black spot reaction, we founds that the sample treated by 4-HR has a flore bacteria 50%.

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