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REPORT TO THE PROTEIN RESEARCH COMMITTEE

ON

DETERMINATION OF THE PRESENCE OF GIZZEROSINE IN FISH MEAL WITH THE USE OF PTC, FMOC AND OPA DERIVATIVES

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EXECUTIVE SUMMARY

SAMPLE PREPARATION

Various experiments were carried out to simultaneously clean up fish meal hydrolysate and concentrate gizzerosine (gz). Reversed phase Sep-paks were initially used, but had little selectivity and efforts centred around removal of most of the lysine and other amino acids. Since this could not be achieved, strong cation exchange resin cartridges were used. Although selectivity was greatly increased, gz was found not to be stable to some of the sample treatment steps, which led to poor reproducibility.

PTC DERIVATIVE

A method was developed for the sensitive determination of gz by HPLC, as the phenylthiocarbamate (PTC) derivative. Lysine co-eluted with gz and a chromatographic method had to be developed to separate these two compounds. Although this derivative worked perfectly with gz standard, an unknown compound from gz-free fish meal co-eluted with gz, forcing us to investigate alternative derivatives.

OPA AND FMOC DERIVATIVES

A combination of these two derivatives were used to increase the selectivity of the separation system and detection for gz. Ortho-phthalaldehyde (OPA) was used to first derivatize the primary amino groups. Secondary amino groups were subsequently derivatized with 9-fluorenylmethylchloroformate (FMOC). The latter derivative is fluorescent and would greatly increase sensitivity and selectivity. Since the formed gz-derivative could not be separated by HPLC, method development continued without the OPA. Good separation was achieved after method development with the FMOC derivative, and gz was well separated from ordinary amino acids present in an acid hydrolysate. However, because FMOC reacted with more than one amino group of gz, three peaks formed which we considered would not provide us with a rigorous method to be easily implemented in other laboratories.

DIRECT CHROMATOGRAPHIC SEPARATION

The previous methods dealt with pre-column derivatization. Ion pair chromatography at different pHs was used to increase retention of this polar compound with reversed phase chromatography. Eluent was collected, concentrated, derivatized and injected on another column system. When elution of the underivatized gz could not be retarded, an ion exchange resin based column was used. Gz could, however, not be eluted from this column because of its high polarity.

Despite many attempts which included various different approaches, we have been unsuccessful in developing a fast, sensitive method for the determination of gz in fish meal.