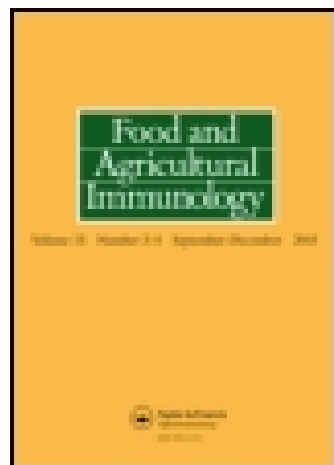


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SHORT COMMUNICATION

Simple and Rapid Determination of Papain in Beer by Polymer-enhanced Immunonephelometry

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By exploiting the enhancing effect of 4% polyethylene glycol (MW 6000) on the immunochemical reaction, papain can be rapidly and simply quantified in beer using an immunonephelometric measurement. Reaction time is only 10 min, and the assay has a detection limit of 0.5 µg/ml. The method was applied to the determination of papain in beer samples originating from several countries.

The addition of proteolytic chill-proofing enzyme preparations to beer is one of the technological treatments which prevent chill-haze formation and increase storage stability. Regulations in some countries forbid the use of non-traditional raw materials in beer processing—including the use of proteolytic enzymes in beer for chill-haze prevention. In those countries where such additives are permitted, concentrations of chill-proofing enzyme preparations in beer reach about 20 µg/ml. The majority of these preparations contains crude papain as an active component (Jones & Mercier, 1974). The analysis of such low papain concentrations is not easy. Two principles are involved in the assessment of papain in beer—the measurement of proteolytic activity and the measurement of protein concentration by immunochemical techniques.

The determination of proteolytic activity in beer by means of certain substrates can be sufficiently sensitive for the purpose, though insufficiently specific (Scriban & Stienne, 1970; Scriban *et al.*, 1976; Fukal & Kasafirek, 1988). On the other hand, immunochemical methods (Donhauser, 1979) can be very specific and enable the determination of added enzyme preparation with the same or even higher sensitivity than is possible with the commonly used enzyme activity assays. An additional benefit of the immunochemical approach is that thermal treatment taking place during beer pasteurization and the presence of free radicals affect the immunoreactivity of papain rather less than its proteolytic activity (Fukal *et al.*, 1983, 1984b). Immunochemical methods are, therefore, more advantageous for papain determination than assays of proteolytic activity.

Although radio- and enzyme-immunoassays for papain have been described (Rauch *et al.*, 1984; Donhauser *et al.*, 1985) which are eminently suitable for batch processing of samples, an immunonephelometric assay could be more useful for analysis of

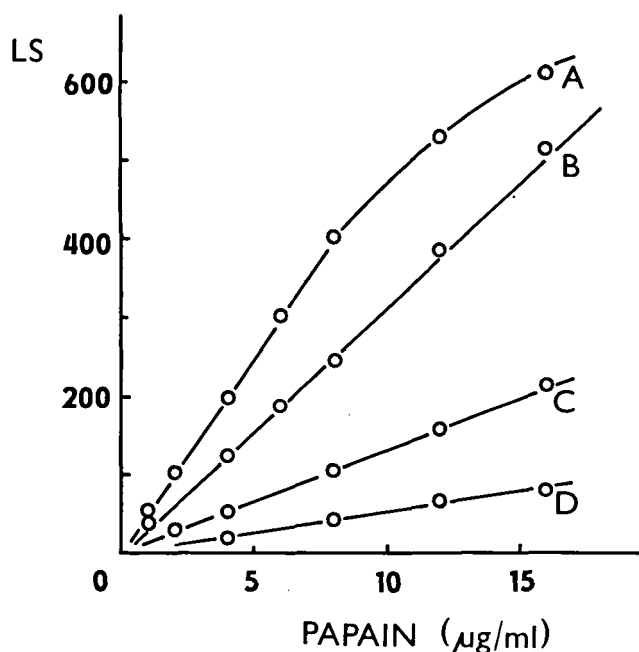


FIG. 1. Calibration curves for immunonephelometric assay of papain. LS, intensity of light scattering $\times 10^3$ (value of 100 corresponds to BaSO_4 concentration $31 \mu\text{g/ml}$). A, incubation mixture contained 2% antiserum and 5% PEG; B, 2% antiserum and 4% PEG; C, 0.4% antiserum and 4% PEG; D, 2% antiserum and 0% PEG.

TABLE 1. Immunonephelometric determination of papain concentration in beer samples (obtained using a PEG level of 4%)

Country of producer	Number of beer brands analysed	Number of positive samples	Observed papain level ($\mu\text{g/ml}$)
Czechoslovakia	9	1	4.6
FRG	4	0	—
The Netherlands	4	1	3.2
Belgium	3	2	3.8, 4.7
UK	2	0	—
USA	3	2	4.2, 6.5
Japan	1	1	5.3

smaller sample numbers. Lizana & Hellsing (1974) have described the enhancement of the precipitin immunoreaction when it is performed in a medium containing polyethylene glycol. This principle was used in the present work to enhance an immunonephelometric assay for determination of papain in beer.

MATERIALS AND METHODS

The papain preparation (1.1 mAnson units per mg) was supplied by Enzymase (Brussels, Belgium). Polyethylene glycol 6000 (PEG) was from Serva (Heidelberg, FRG). Rabbit anti-papain antiserum was prepared as described elsewhere (Fukal *et al.*, 1984a). The antiserum does not cross-react with bromelain, ficin, malt extract,

brewer's yeast autolysate and beer prepared without chill-proofing enzymes, as determined by the double radial immunodiffusion technique. Other chemicals used were purchased from Lachema (Brno, Czechoslovakia). Beer samples for analysis were obtained from shops.

For immunonephelometric assay, papain was dissolved in 0.05 M-sodium phosphate buffer, pH 7.4, or in chill-proofing enzyme-free beer (pH value adjusted to 7.4 with concentrated NaOH solution). Into a test tube with 1 ml of standard papain solution or beer sample (adjusted to pH 7.4) 0.5 ml of 0.15 M-phosphate buffer, pH 7.4, containing 0–15% PEG and 0.02% Tween 20 was added. Then 1 ml of 0.05 M-phosphate buffer, pH 7.4, containing 0.9% NaCl, 1–5% (v/v) antiserum, 0–5% PEG and 0.02% Tween 20 was added. The mixture was incubated at 20°C. After a suitable incubation period, the light scattering of the antigen–antibody complexes was measured at a 90° angle in the Specol photometer with the corresponding accessory (Carl Zeiss, Jena, GDR). The instrument was set at zero using the reagent blank without papain.

Proteolytic activity was determined as described elsewhere (Fukal & Kasafirek, 1988).

RESULTS AND DISCUSSION

To estimate the amount of a non-specific precipitation at different polymer concentrations, we first measured the light scattering in solutions of PEG–antiserum and PEG–papain, respectively.

Figure 1 shows the effect of PEG (at levels between 0–6%) on the precipitin curve of a papain–anti-papain system with analyte concentrations ranging from 0.5 to 16 µg/ml. Experiments (not shown) showed PEG levels above 6% to be un-utilisable. Nephelometric response increased with increasing polymer concentration, maximal effect being seen at 5% PEG. In the presence of PEG, not only was the amount of immunoprecipitate increased but its formation was also more rapid. To obtain the maximum nephelometric effect, an incubation period of only 10 min was required, in comparison with 60 min needed for the system without PEG.

Standard samples containing papain at 0.5, 4.0 and 16.0 µg/ml gave intra-assay variation coefficient values of 22.7%, 8.4% and 10.8% at 5% PEG and 14.0%, 5.3% and 6.1% at 4% PEG; and inter-assay variation coefficient values of 24.2%, 9.1% and 12.3% at 5% PEG and 13.2%, 5.8% and 6.8% at 4% PEG. Standard curves for papain concentration in buffer and in beer were superimposable at a PEG concentration of 4%, giving a detection limit of 0.5 µg/ml. This PEG level was used for assaying 26 beer samples from different countries (Table 1). Papain was found in seven samples. Only these seven of all samples analysed also demonstrated proteolytic activity.

The immunonephelometric papain assay described, which uses 4% PEG to enhance the formation of antibody–analyte precipitate, is sufficiently sensitive, is unaffected by high reagent blanks and/or high absorbance values of the specimens and requires a minimum of equipment and less than one hour for analysis of several samples. Use of a laser nephelometer and of antiserum free from lipids and non-immunoglobulin proteins would, it is suggested, further increase sensitivity and reproducibility.

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