A Research Note

Detection of Low Molecular Weight Reduced Zein Polypeptides Separated by Polyacrylamide Gel Electrophoresis

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ABSTRACT

Several bands including some low molecular weight (MW) peptides [9 and 11 kd (Kilo-Dalton)] were visualized in reduced zeins after separation by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). It was concluded that 40% isopropanol-10% acetic acid was effective in fixing these fast moving polypeptides. The demonstration of higher MW (40–45 Kd) polypeptides may have also resulted from this treatment.

INTRODUCTION

THE ELECTROPHORETIC ANALYSIS of reduced protein fractions obtained from five samples of South African maize by the modified Landry-Moureaux (1970) and Soave et al. (1976a, b) procedures was reported recently by Gorinstein et al. (1983). The reduced polypeptides of zein fraction extracted from the same maize samples by the procedure of Paulis et al. (1969) have also been examined by SDS-PAGE, but in this case the gels were fixed by treating them with isopropanol-acetic acid-water (40:10:50) prior to staining. This study was undertaken to check this fixation effect in detection of low MW reduced zein polypeptides.

MATERIALS AND METHODS

ZEIN FRACTIONS of five types of maize: normal white dent(N), high-lysine(HL), drought-damaged(D), waxy(W), and bread (a floury type) were previously extracted with 70% (v/v) isopropanol containing 0.5% sodium acetate at 20°C for 3 hr according to the procedures of Landry-Moureaux (1970) and Soave et al. (1976a, b) and also prepared from the saline extracted defatted meals by the procedure of Paulis et al. (1969) involving extraction for 3 hr at 20°C with ethanol water (70:30) containing 0.5% sodium acetate. The two sets of zein preparations will be referred to as isopropanol-extracted and ethanol-extracted zeins, respectively.

All samples were subjected to SDS-PAGE separation in 10% gels, stained with Coomassie brilliant blue R250 (0.5% in 10% isopropanol-10% acetic acid; overnight) and destained (10% isopropanol-10% acetic acid; 4-6 hrs) as previously described by Gorinstein et al. (1983). Two sets of gels were examined. In the first, the isopropanol-extracted zein preparations were used with and without prior fixing. The second set referred to ethanol-extracted preparations with and without fixing. In the two sets the gels were fixed by immersion in isopropanol-acetic acid-water (40:10:50) for 1 hr prior to staining.

RESULTS & DISCUSSION

THE ELECTROPHOREGRAMS obtained with the reduced zeins from each of the five maize types are presented in Fig. 1. The isopropanol-extracted zein gels (Fig. 1a) show the usual 2-banded pattern of reduced zein polypeptides (mean MWs:24.8 and 22.3 Kd), the slower moving band being considerably reduced in the case of high-lysine maize (Gorinstein et al., 1983). Although not visible in Fig. 1a, a very faint band corresponding to a polypeptide of MW ca. 10 Kd was observed in the first set of gels indicating either that low MW polypeptides were present in low concentrations in these reduced zein preparations of that polypeptides may have diffused out of the gels during staining and destaining. The gels from the same set, but with prior fixing (Fig. 1b) showed two fast moving polypeptide bands with MWs of 11 and 9 Kd respectively.

Gels in Fig. 1d (ethanol-extracted zeins) show the same two major bands of reduced polypeptides (mean MWs:25.2 and 22.2 Kd) but the electrophoregrams are complex. Particularly noteworthy are the two fast moving polypeptide bands with approximate mean MWs of 11 and 9 Kd respectively, the former being very faint in the case of the drought-damaged maize (D), in spite of the fact that unfixed gels didn't show these last two polypeptide bands (Fig. 1c).

Low MW polypeptide (11.0 and 10.4 Kd) were observed by Lee et al. (1976) in the reduced zeins from two normal maize inbreds and their opaque-2 counterparts while Misra et al. (1970) detected a 10 Kd polypeptide as the major component in the reduced zein from a brittle-2/opaque-2(bt_2/O_2) double mutant. Absence of this band in the gels obtained with a number of other maize types led these authors to suggest that the 10 Kd polypeptide is unique to bt_2/O_2 double mutants. While there is no doubt as to the dominance of the 10 Kd polypeptide in the double mutant, its uniqueness to this type of mutant must now be questioned particularly if allowance is made for an accuracy of only 18% in the estimation of MWs of less than 20 Kd by SDS-PAGE (Swank and Murkes, 1971).

Wall and Paulis (1976) suggested that the low MW polypeptides observed by Lee et al. (1976) may have resulted from extraction of zeins with 70% ethanol at 60°C or from the higher concentration of the gels (15%) used in their experiments. The former explanation is ruled out by the present demonstration of low MW reduced polypeptides in the ethanol-extracted zeins (Fig. 1d). Mirsa et al. (1970) also carried out the extraction of zein at 20°C, although they used 70% isopropanol as solvent, and Fornasari et al. (1975) used the same solvent but the extraction was done at 60°C. It is concluded, therefore, that higher gel concentration may have facilitated the trapping of 10 to 11 Kd polypeptides in the experiments of Lee et al. (1976), while pretreatment of the gels with 40% isopropanol-10% acetic acid was responsible for detection of these components in the present study. Isopropanol (25% was pinpointed by Fairbanks et al. (1971) and Olden and Yamada (1977) as the primary fixing agent in their SDS-PAGE analysis of the polypeptides of human erythrocytes. By contrast Wilson (1979) and Scott et al. (1979) gave preference to trichloroacetic acid. The gels (Fig. 1d) obtained with reduced zeins from the normal (N), high-lysine (HL), drought-damaged (D), and waxy (W) kernels showed one or two lightly stained bands in the 18.6–19.9 Kd range. The electrophoregrams (Fig. 1d) also showed slowly moving polypeptides 45.2 and 43.0 Kd in the preparation of waxy maize (W), and the 47.3 and 44.7 Kd polypeptide bands in that of drought-damaged maize (D). A single

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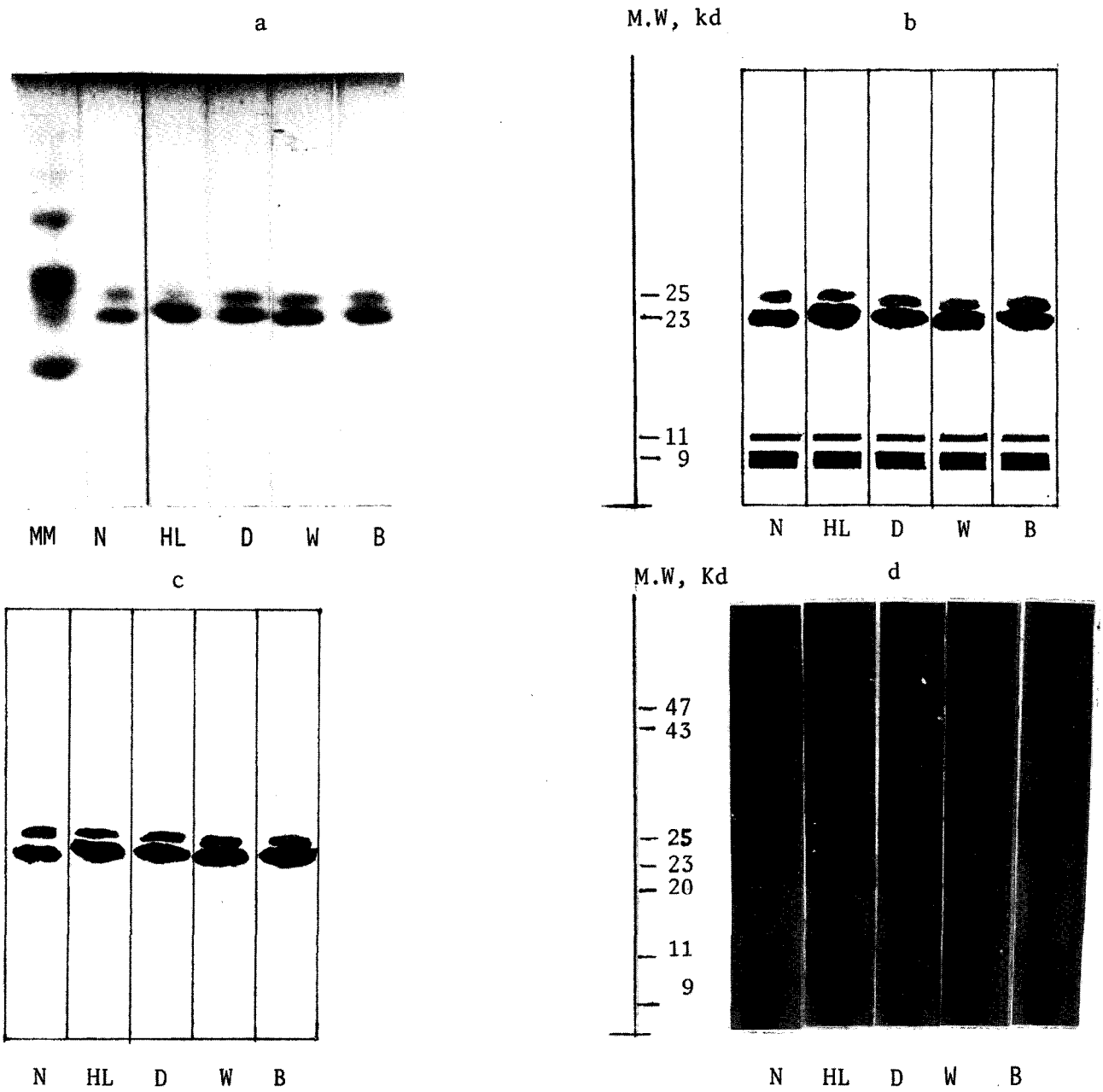

Fig. 1—SDS-polyacrylamide gel (10%) electrophoresis at pH 7 of reduced polypeptides in extracted zeins (40 μg) from five types of maize: N = normal white dent; HL = high-lysine; D = drought-damaged; W = waxy; B = bread (a floury type) maize. MW = SDS Molecular marker. a,b - isopropanol-extracted zeins; c,d - ethanol-extracted zeins; a,c - gels were stained without prior treatment with 40% isopropanol-10% acetic acid solution; b,d - gels were immersed in a 40% isopropanol-10% acetic acid solution for 1 hr prior to staining.

faint band corresponding to polypeptides of about 45 Kd was visible in each of the other gels. These bands, which are not seen at all in the unfixed gels (Fig. 1c), may correspond to the 44 Kd component reported by Misra et al (1970) in the reduced zein from a floury-2 mutant and the suggested uniqueness of this component to floury-2 mutants is now also open to question. Relevant in this regard is the report of Abe et al. (1981) that native zein contained three relatively high MW (44.0, 45.5 and 48.0 Kd) components which were considered to be α-α, α-β and β-β dimers. It is possible, therefore, that the 43 to 47 Kd components seen in this study may reflect incomplete reduction of certain high MW species. Absence of the 44 Kd component in the other reduced zein preparations of Misra et al. (1970), in the gels prepared by Lee et al. (1976), and in the unfixed gels of the present study (Fig. 1c) may reflect more complete reduction of the relevant zeins. However the two sets of zeins examined here were reduced under the same conditions so that pretreatment of the second set of gels with 40% isopropanol-10% acetic acid may have contributed to the visualisation of these polypeptides.

The above findings illustrate the usefulness of 40% isopropanol-10% acetic acid as fixing agent in SDS-PAGE analysis of the polypeptides in reduced zein preparations and underline the need for caution in drawing conclusions from electrophoregrams obtained by only a single procedure.

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minutes. Each taster was presented two sets of samples at different sittings to avoid taste fatigue.

Following these tests the juices were centrifuged until about 75% of suspended matter had been removed. They were then tasted again and TLC analyses carried out for the compounds listed in Table 1, as described (Tatum and Berry, 1973, 1983).

RESULTS & DISCUSSION

THESE STUDIES showed these compounds were detectable and could lower taste quality at levels lower than had been found in some commercial products, with a significant difference at the 99% confidence level. When carried out in the manner described, there were 20 correct judgements and four incorrect. The original juice used for these taste tests had a °Brix/acid ratio of 9.6, a naringen level of 545 ppm, and a limonin level of 1.0 ppm. No coumarins or psoralens were detectable in the base juice as determined by TLC. It had received an "excellent" rating by the taste panel when tasted alone.

Taste-tests of grapefruit juice which had been found by taste panels to have a distinctive "green," "woody" flavor earlier, indicated it was improved in flavor when most of the pulp was removed by centrifugation. The centrifuged juice contained much lower levels of the undesirable flavor traits (Tatum and Berry, 1983). Our studies also indicated removal of pulp from orange juice resulted in reduction of 35% of the naringenin-7-beta-rutinoside and 80% of the hesperidin. Analyses of insoluble solids removed during clarification, using the TLC method of Tatum and Berry (1973), indicated over 50% of compounds 2, 3, 5, 6, and 8 were removed. About 25% of compounds 4, 7, 9, 10, and 11 were also removed with the pulp while about 15% of the limonin was removed. Also removed was 60% of the nootkatone, which gives grapefruit a distinctive odor but can be bitter at excessive levels, (Berry et al., 1967). This is a higher value than reported earlier by Radford et al. (1974).

We recommend that pulp from immature juice be reduced before being blended with other juices to greatly reduce the "green," "woody," astringent and bitter flavor. When original taste tests indicate a distinctive "immature or green" flavor, it can be improved if 65% or more of the pulp is removed by centrifugation and the remaining serum blended with a high quality juice. If the juice yield on such "green and immature" fruit is reduced by 5 to 10% and the pulp content reduced by 50–75% by lowering extraction and finishing pressures, our studies indicate the "green, "woody" astringency of the resulting juice will be greatly reduced.

CONCLUSION

IMMATURE FLAVOR, described as sourness, astringency and bitterness, was associated with pulp and rag and was due to coumarins and psoralens. The best control was to lower extraction and finishing pressures and remove pulp by centrifugation. Removed solids could be used in other drinks and formulations where bitterness or astringency are desirable. The resultant serum with small amounts of suspended pulp could be blended with higher quality juices.

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