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# Effect of Water Activity on the Heat-Induced Deterioration in the Protein Digestibility of Corn (*Z. mays*) and Cowpeas (*V. unguiculata*)

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*ABSTRACT*

*Flour of corn (*Z. mays*) adjusted to 0·35, 0·56 and 0·75 water activity ($a_w$) and flour of cowpea (*V. unguiculata*) adjusted to 0·33, 0·55 and 0·75 $a_w$ were, respectively, stored at 80°C, 100°C and 120°C for 6 h. Analysis of the samples at 1 h intervals showed that the protein digestibility (PD) increased to a maximum ($PD_{max}$) during the first 60 min, followed by progressive decrease with time. The PD was more affected by temperature and time, than $a_w$ ($P = 0·01$). Both the activation energy (EA) and temperature coefficient ($Q_{10}$) tend to increase with increased $a_w$. The EA (kJ/mol) ranged from 14·2 to 19·7 and 18·8 to 29·1, while the $Q_{10}$ ranged from 1·14 to 1·20 and 1·19 to 1·32 for corn and cowpea, respectively. The net percentage decrease in PD and the long half lives (27–133 h) showed that 6 h was too short for first order kinetics to be confirmed. The maximum decrease of 9·3% PD in cowpea and 11·5% PD in corn (0·33 $a_w$; 120°C) could be of nutritional significance.*

## INTRODUCTION

Heat treatment of proteinaceous foods results in the denaturation of the protein which explains the improvement in their protein digestibility (Molina *et al.*, 1975; Boonvisut & Whitaker, 1976). The protein quality of food deteriorates during high temperature–short time treatment and

prolonged storage under mild temperature conditions. The loss in protein quality has been attributed to the formation of enzyme-resistant cross linkages (Horn *et al.*, 1968; Erbersdobler, 1976), the premelanoidic inhibition of the proteolytic enzymes (Adrian & Fragne, 1973) and the unavailability of lysine involved in the Maillard linkages (Tsao *et al.*, 1978).

Non-enzymatic browning and available lysine have been widely used as indices of protein quality deterioration in many food systems. The decrease in the protein digestibility of heat-damaged foods has not been as thoroughly investigated. The report of Phillips *et al.* (1983) in this regard covered a time interval observed to be too short for measurable decrease in protein digestibility of cowpea to have occurred at intermediate moisture range. This study was, therefore, designed to further investigate the decrease in protein digestibility of cowpea as affected by temperature and water activity over a longer period. The inclusion of corn in this study follows our observation on the storage changes in corn (Onigbinde & Akinyele, 1988) and the complementary role of corn and cowpea as sources of dietary protein, especially among the low income group of Nigerians.

## MATERIALS AND METHODS

### Samples

Clean grains of cowpea (WIT 812–716) and corn (8339–17) obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria, were milled to pass a 0·5 mm sieve (Wiley Mill, Model 4). Samples of cowpea flour were equilibrated over saturated slurries of $MgCl_2$, $Mg(NO_3)_2$ and NaCl (Labuza, 1984) to water activity values of 0·33, 0·55 and 0·75 (Lufft $a_w$-value meter, Model 5803) and corn flour was similarly equilibrated to 0·35, 0·56 and 0·75, respectively. A small beaker of toluene was placed in the desiccators containing NaCl to prevent moulding.

### Storage

Glass vials (16 mm × 45 mm; Kimble) were each filled with the flour (about 4 g) at each of the water activity levels, sealed with a layer of parafilm, screw-capped tightly and weighed. The vials were incubated at 80°, 100° and 120°C, respectively, in a Gallenkamp air oven (Mizrahi *et al.*, 1970) for 6 h in a completely randomised design. The vials were sampled at 1 h intervals, cooled to room temperature and weighed. The weights before and after incubation were used to detect moisture leakage and packages with weight differences greater than 5% of the initial moisture content were rejected and

repeated. The experiment was performed thrice for each sample at each of the treatment combinations.

### Protein digestibility

The protein digestibility was estimated, *in vitro*, by the multienzyme procedure of Satterlee *et al.* (1979) after estimating the per cent nitrogen contents by the macro-Kjeldahl procedure (AOAC, 1980). The four enzyme combination was chosen to ensure optimum digestibility.

### Statistical analysis

The significance of the effect of temperature, water activity, time and replication was calculated by ANOVA and the rate of decrease in protein digestibility was equated to the slope of the regression of natural logarithim of digestibility against time (Geoffrey, 1980).

## RESULTS AND DISCUSSION

Figures 1 to 4 are the plots of per cent protein digestibility (*PD*) against time (h). The figures show that *PD* increased to a maximum ($PD_{max}$) during the first hour of incubation; followed by a progressive decrease. The increase

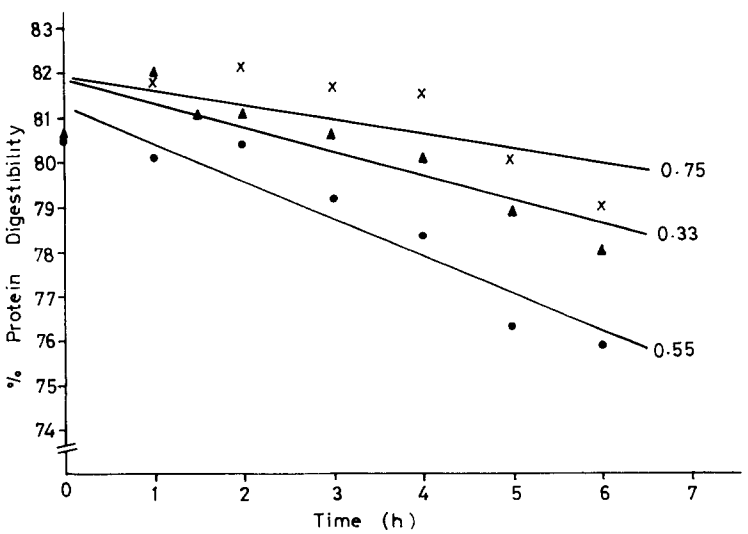


**Fig. 1.** Trend of protein digestibility of cowpea (*V. unguiculata*) at 80°C and three levels of water activity.

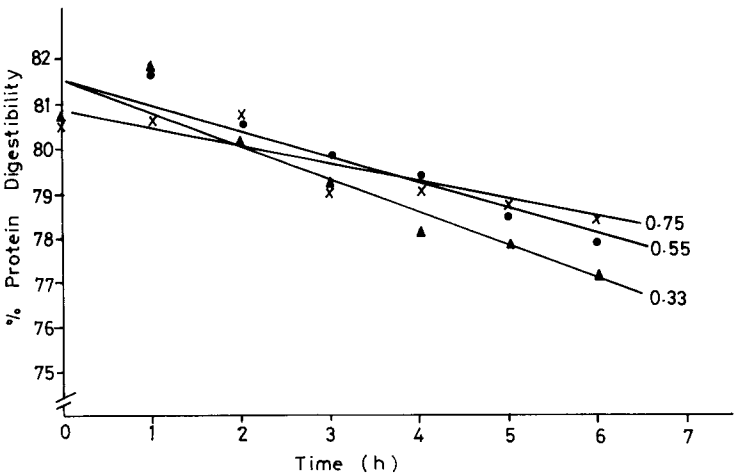


**Fig. 2.** Trend of protein digestibility of cowpea (*V. unguiculata*) at 100°C and three levels of water activity.

during the first 60 min was consistent with the role of water molecules during cooking. The prevention of the renaturation of the heat-denatured proteins by the hydrogen and electrostatic linkages between water molecules and the protein side chains increased the accessibility of the peptide bonds to the proteolytic enzymes. The $PD_{max}$ attained by cowpea (82–85%) agreed with the data of Phillips *et al.* (1983). There is a dearth of similar information for corn in the literature.

The ANOVA (Table 1) shows that the *PD* of the samples were

**TABLE 1**
Analysis of Variance (ANOVA) Table for the Effects of Temperature, Water Activity and Time on the Protein Digestibility of Corn and Cowpea

| *Sources of variation* | *Cowpea* | | | *Corn* | | |
|---|---|---|---|---|---|---|
| | *DF* | *MS* | *F-ratio* | *DF* | *MS* | *F-ratio* |
| Temperature ($T$) | 2 | 81·88 | 46·3** | 2 | 312·82 | 106·0** |
| Time ($t$) | 6 | 98·91 | 55·9** | 6 | 150·76 | 51·1** |
| Water activity ($a_w$) | 2 | 49·65 | 28·1** | 2 | 29·45 | 10·0** |
| $T \times t$ | 12 | 5·12 | 2·9** | 12 | 21·75 | 7·4** |
| $T \times a_w$ | | | | 4 | 10·84 | 3·7** |
| $T \times t \times a_w$ | 24 | 5·09 | 2·9** | | | |
| Residual | 142 | 1·77 | | 162 | 2·95 | |
| Total | 188 | | | 188 | | |

** Significant ($P < 0·01$).

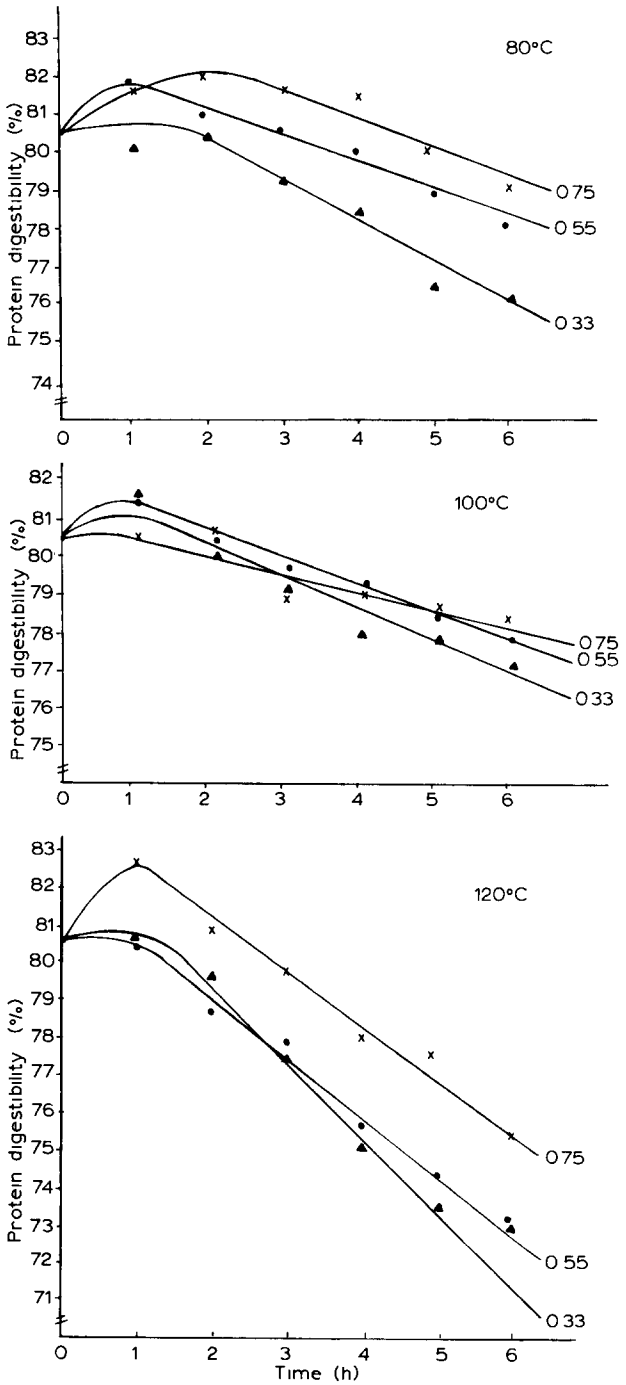


**Fig. 3.** Protein digestibility changes in cowpea (*V. unguiculata*) during heating at 80°, 100° and 120°C and 0·75 (×), 0·55(●) and 0·33 (▲) water activity levels.

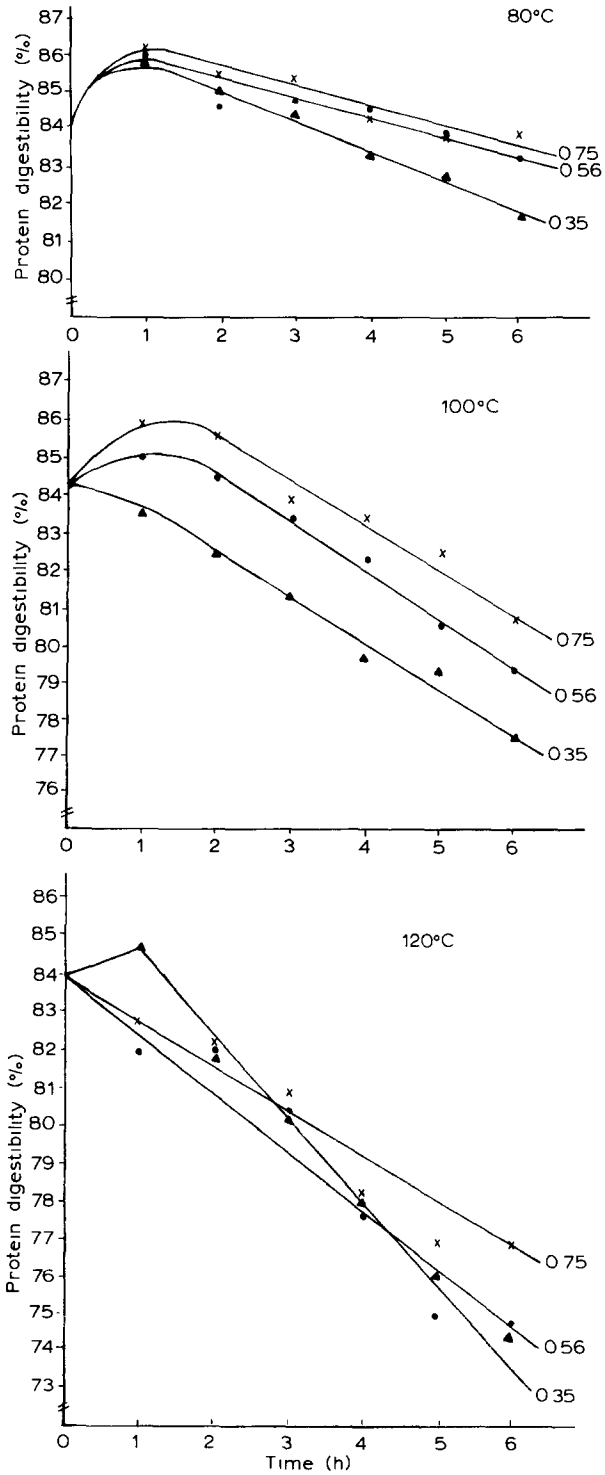


**Fig. 4.** Protein digestibility changes in corn (*Zea mays*) during heating at 80°, 100° and 120°C and 0·75 (×) 0·56 (●) and 0·35 (▲) water activity levels.

significantly affected by incubation temperature, water activity and time ($P < 0.01$). However, the effects of temperature and time were more significant than the effect of water activity. The decrease in *PD* with increasing temperature and time agreed with the reports of Erbersdobler (1976) and Knipfel (1981). Using the $PD_{max}$ as the origin for the onset of deterioration and determination of kinetics of the decrease in *PD*, the rate constants, the activation energy, *EA* (kcal/mol) and the $Q_{10}$ (the increase in reaction rate with 10°C rise in temperature) were calculated assuming first order kinetics (Table 2). The activation energy was calculated by fitting the rate constants into the Arrhenius model:

$$K = K_0 \exp(-EA/RT)$$

in the form

$$\ln K = \ln K_0 - EA/RT$$

where $R$ is the rate constant, $EA$ is the activation energy (kcal/mol), $R$ is the gas constant (1·987 cal/mol K), $T$ is the absolute temperature and $K_0$ is a frequency factor.

The rate constants increased with increasing water activity, irrespective of the water activity of the samples, and the high coefficients of determination

**TABLE 2**
Kinetic Data for the Effects of Temperature and Water Activity on the Decrease in Protein Digestibility of Corn and Cowpea

| *Samples* | *Water activity* | *Rate constants (% PD $h^{-1}$)*[a] | | | *EA (kJ/mol)* | $r^2$ | $Q_{10}$ |
|---|---|---|---|---|---|---|---|
| | | *80°C* | *100°C* | *120°C* | | | |
| Corn | 0·35 | 10·0 ± 0·6 (1·00)[b] | 14·5 ± 1·8 (0·98) | 25·9 ± 1·7 (1·00) | 14·2 | 0·95 | 1·14 |
| | 0·56 | 5·8 ± 0·8 (0·89) | 14·2 ± 1·3 (0·97) | 21·3 ± 1·4 (0·95) | 19·7 | 0·96 | 1·20 |
| | 0·75 | 5·2 ± 1·0 (0·95) | 13·7 ± 1·0 (0·98) | 16·8 ± 2·3 (0·95) | 17·8 | 0·89 | 1·18 |
| Cowpea | 0·33 | 11·8 ± 1·4 (0·96) | 14·7 ± 1·0 (0·91) | 22·3 ± 2·8 (0·98) | 18·8 | 0·95 | 1·19 |
| | 0·55 | 8·4 ± 0·5 (0·83) | 9·6 ± 1·9 (0·99) | 20·0 ± 0·4 (0·99) | 25·4 | 0·83 | 1·27 |
| | 0·75 | 6·6 ± 0·4 (0·85) | 9·9 ± 0·7 (0·92) | 17·6 ± 0·9 (0·98) | 29·1 | 0·98 | 1·32 |

[a] Rate constant $\times 10^3$.
[b] Coefficient of determination for the rate equations; $r^2$ = Coefficient of determination for the Arrhenius equations, and $Q_{10}$ = Temperature coefficient.

($r^2$) for the rate equations tend to support first order kinetics. The rate constants, however, showed some decrease with increasing water activity. The absolute values of the rate constants are higher than reported by Phillips *et al.* (1983). The *EA* values ranged from 3·38 (0·35 $a_w$) to 4·68 (0·55 $a_w$) for corn and 4·48 (0·33 $a_w$) to 6·92 kcal/mol (0·75 $a_w$) for cowpea. Similarly, the $Q_{10}$ ranged from 1·14 (0·35 $a_w$)to 1·20 (0·55 $a_w$) for corn and 1·19 (0·33 $a_w$) to 1·32 (0·75 $a_w$) for cowpea, although the *EA* and $Q_{10}$ values increased with increase in water activity. The difference was more significant for the increase from 0·33 to 0·55 than from 0·55 to 0·75 ($P = 0·5$). Table 2 shows differences of 5% and 1·7% in corn and 6·8% and 3·9% in cowpea for the successive 0·2 unit increases in $a_w$, respectively. The lower *EA* than reported by Phillips *et al.* (1983) could be associated with the very short sampling interval used by the authors, which resulted in high rate constants and consequent under-prediction of the stability of the samples to changes in *PD*. Considering the $Q_{10}$ values, it seems very unlikely that measurable decrease in *PD* could have occurred during the few minutes interval used by the authors.

The effect of water activity was further investigated by computing the half life, $t_{1/2}$ (the time required for the $PD_{max}$ to decrease by 50%), from the equation

$$t_{1/2} = \frac{\ln 2}{K} = \frac{0·693}{K}; \ (K = \text{rate constant})$$

The increase in half lives (Table 3) with increasing water activity was compatible with the inhibition of the formation of enzyme resistant linkages

**TABLE 3**
Effects of Temperature and Water Activity on the Decrease in Protein Digestibility of Corn and Cowpea during Heat Treatment

| *Sample* | $a_w$ | $PD_{max}$ (%)[a] | | | *DPD* (%)[b] | | | $t_{1/2}$ (*h*)[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *80°C* | *100°C* | *120°C* | *80°C* | *100°C* | *120°C* | *80°C* | *100°C* | *120°C* |
| Corn | 0·35 | 86·8 | 84·5 | 86·5 | 3·0 | 7·9 | 11·5 | 69·3 | 47·8 | 26·8 |
| | 0·56 | 86·1 | 86·7 | 84·5 | 1·0 | 5·9 | 11·3 | 119·5 | 48·8 | 32·5 |
| | 0·75 | 86·6 | 88·1 | 84·4 | 0·3 | 4·0 | 8·6 | 133·3 | 50·6 | 41·3 |
| Cowpea | 0·33 | 82·9 | 82·5 | 82·7 | 4·4 | 5·9 | 9·3 | 58·7 | 47·1 | 31·1 |
| | 0·55 | 82·8 | 82·6 | 82·4 | 3·3 | 3·5 | 9·1 | 82·5 | 72·2 | 34·7 |
| | 0·75 | 84·0 | 84·6 | 84·2 | 2·8 | 2·1 | 6·3 | 105·0 | 70·0 | 38·4 |

[a] Maximum digestibility before the onset of decrease.
[b] Net decrease in protein digestibility after 6 h.
[c] Half life (h).

associated with Maillard reactions (Loncin *et al.*, 1965). These observations also tend to suggest that the rate of decrease in *PD* may be a function of the protein concentration of the samples. This has been reported for heated model systems (Lea & Hannan, 1949; Loncin *et al.*, 1965). The concentration-dependence might, however, be specific to the food item studied. Corn contains about half as much protein as cowpea (g%) but recorded similar or higher rates of decrease in *PD* under the same conditions of temperature and water activity (Table 2). The differences in the heat-sensitivity of the two food grains could be attributed to differences in structure and composition of their proteins (Fragne, 1972) and the chemical properties of the non-protein components (Adrian, 1974).

## CONCLUSION

This study has provided information on the heat-deterioration of protein quality of corn and cowpea, using protein digestibility as an index. The temperature and moisture dependence of the food items followed the pattern reported for non-enzymatic browning and loss of lysine (Labuza & Riboh, 1982). The relative stability of the *PD* of the samples under conditions which resulted in significant losses in available lysine content and non-enzymatic browning in some foods (Finot *et al.*, 1982; Chen *et al.*, 1983; Hurrel & Nielsen, 1983) suggests that *PD* changes might not be a good index of protein quality deterioration in heated foods. However, a decrease of up to 10% in the protein digestibility is of nutritional significance and heat-processing, such as extrusion, should therefore be performed at sufficiently high water activity (or moisture content). Since the duration of 6 h used in this study was less than the half lives for *PD* loss by the samples (Table 3), it was impossible to confirm the first order behaviour of the reaction (Labuza, 1982).

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