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Calorific Losses in Maize in Relation to Colonisation by Isolates of *Aspergillus ochraceus* Under Different Environmental Conditions

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ABSTRACT

The effect of water activity (a_w, 0·85–0·95), temperature (15–30 °C) and incubation period (2–4 weeks) on growth and calorific losses in relation to colonisation by three mycotoxigenic isolates of *Aspergillus ochraceus* was studied on maize-based substrates. The calorific losses (kJ) of maize flour due to fungal growth in relation to temperature and a_w were quantified and found to be maximal at 20–30 °C (10·52–16·18%, after 4 weeks at 0·95 a_w), with only slight losses at 0·85 a_w (0–7·14%), at both 15 and 20 °C. This suggests that growth of mycotoxigenic *Aspergillus* spp. can significantly contribute to nutritional losses of this staple agricultural food. Calorific values correlated inversely with fungal biomass, with the latter being greatest at 0·95 a_w after both two and four weeks, and maximal at 30 °C. Most of the single, two-, three- and four-way interactions of a_w, temperature, time and isolate had a statistically significant ($p<0·01$) influence on calorific losses and fungal biomass.

Keywords: maize, *Aspergillus ochraceus*, calorific value, fungal spoilage, water activity, temperature.

INTRODUCTION

Poor post-harvest practices in many parts of the world often result in significant (up to 15%) losses of grain due to mould spoilage and insect damage[1,2]. Thus, consumption of nutritionally poor quality staple grains and flour are often a fact of life in many tropical and subtropical regions of the world. However, few attempts have been made to quantify the nutritional losses in terms of calorific value in such deteriorated foodstuffs[3]. On the other hand, some elegant studies have been made on the bioenergetics of insects feeding on different grains to quantify losses under different storage conditions[4,5]. Changes in the calorific value of *Linum usitatissimum* due to seed-borne infection by spoilage fungi have been reported but conditions of storage were not accurately determined or controlled[6], and there have been no determinations of calorific losses of staple cereals by spoilage fungi, particularly *Aspergillus* and *Penicillium* species.

Aspergillus ochraceus Wilhelm is a storage fungus that has been frequently isolated from grains, oilseeds and different vegetables[7,8] and is commonly isolated from Spanish corn[9]. The development of *A. ochraceus* on different grains

ABBREVIATIONS USED: ATP = adenosine triphosphate; a_w = water activity; diam = diameter; DF = degrees of freedom; kcal = kilocalories; kJ = kilojoules; m.c. = moisture content; NRRL = Northern Regional Research Laboratory; Q_c = kJ released by combustion of cotton; Q_{sample} = kJ released by combustion of sample; S = *A. ochraceus* isolate factor; SS = sum of squares; t = incubation period factor; T = incubation temperature factor; T_f = final water bath temperature; T_i = initial water bath temperature.

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including maize, barley, wheat, and other oilseeds has been followed[10–12]. However, except in a few reports[10,11,13], little attention has been paid to the influence of the most important environmental factors influencing fungal deterioration of grain such as water availability (water activity, a_w), storage temperature and gas composition.

The objectives of this work were to determine the effects of a_w (0·85–0·95), temperature (15–30 °C), time (2–4 weeks) and their interactions on (a) calorific losses of coarse maize flour and (b) *in vitro* growth on a maize extract agar due to colonisation by *A. ochraceus*.

EXPERIMENTAL

Fungal isolates

Three isolates of *A. ochraceus* (NRRL 3174, 3·38 and 3·113) were used in all experiments. Isolates 3·38 and 3·113 are ochratoxigenic strains isolated from Spanish corn held in the Food Technology Department of the University of Lleida[14].

Maize

Spanish maize grain with an initial water content (wet weight basis) of 13·9% (= 0·71 a_w) was ground and sieved to a uniform particle size (0·45 mm mean diam).

Ground samples were weighed in flasks and rehydrated to the desired a_w treatment levels (0·85, 0·90, 0·95) by the addition of distilled water with reference to a moisture adsorption curve. The treated maize was allowed to equilibrate at 4 °C for 48 h, with periodic shaking. The flasks were sealed and autoclaved for 20 min at 121 °C. The a_w values were confirmed using a Novasina Humidat IC I Thermoconstanter (Novasina, Switzerland).

Inoculation and incubation of ground maize

Sterile humidified ground maize was placed in thin layers in sterile Petri dishes (12 g/plate). Uniform inoculation of the dishes for each isolate was made by 17 applications of 8 µL each of a 10^7 spores/mL suspension. Spore suspensions were prepared in glycerol-water solutions at the treatment a_w levels.

The different experimental parameters were: a_w; 0·85, 0·90 and 0·95, incubation temperatures; 15, 20 and 30 °C, and incubation periods; 2 and 4 weeks. All treatments were repeated three times.

After inoculation, Petri dishes with the same water activity were placed in sealed containers together with two beakers containing 100 mL of a glycerol-water solution providing the same relative humidity as the enclosed Petri dishes[15] and incubated. At the end of the experiment, maize samples were carefully homogenised, transferred to sealed polyethylene bags, and stored frozen until analysed.

Calorific value determination

A Gallenkamp Autobomb calorimeter was used for this purpose. This apparatus measures the heat released by the combustion of a known weight of sample with pressured oxygen.

Samples were freeze-dried prior to calorific value determination. Samples contained both maize and fungal mycelium, so that in practice there was a slight underestimation of the actual calorific losses in maize. Samples in the calorimetric bomb undergo complete combustion which results in the heating of the water that surrounds the bomb core. From the increase in temperature of the water, the calorific value of the sample can be calculated as follows:

$$Q_{sample} = \{(T_f - T_i)\cdot K\} - Q_c$$

where Q_{sample}, kJ released by combustion of sample; T_f, final temperature of the water bath; T_i, initial temperature of the water bath; K, 10·43 kJ/°C (2·494 kcal/°C), constant reference value previously calculated from combustion of benzoic acid; Q_c, kJ released by the combustion of the cotton thread used as ignition starter.

Growth studies

Studies for quantification of fungal biomass were carried out on maize extract agar with water activity modified with glycerol[16,17]. Three replicates each of the same treatments as for the ground maize experiments were used. Maize extract agar was inoculated with 136 µL of a 10^7 spores/mL suspension and spread with a bent glass spreader. Petri dishes of the same a_w were enclosed in polyethylene bags and incubated. The agar cultures were heated and the mycelium separated from the melted agar. Mycelial mats were freeze-dried and weighed.

Statistical treatment of results

Analysis of variance for the different sets of results, as well as analysis of correlation (Pearson correlation coefficient), were calculated out using the SAS package (version 6·11, SAS Institute Inc.).

RESULTS

Effect of water activity, temperature and time on calorific losses of maize

Initial calorific value of ground maize was $19{\cdot}69 \pm 0{\cdot}13$ kJ g^{-1} (4·70 kcal) dry matter of maize flour. Figure 1 compares the effect of time, a_w and temperature treatments on the calorific losses in kJ g^{-1} dry weight maize. This shows that there were significant losses at 30 °C, particularly at 0·90 and 0·95 a_w after both 2 and 4 weeks incubation. Losses of 5·55–14·85% (1·09–2·92 kJ g^{-1}) in the initial calorific value were found under these conditions. There were slight losses in calorific value at 0·85 a_w (0–1·41 kJ g^{-1}), at both 15 and 20 °C. Decrease of caloric value of maize was much more evident after 4 weeks of fungal degradation.

Temperature, a_w and time of incubation had a significant influence on results ($p<0{\cdot}01$). There were no significant differences between the isolates tested. In addition, almost all the two-, three- and four-way interactions were statistically significant ($p<0{\cdot}01$), except for $a_w \times S$. Thus, for example, the pattern for a_w and temperature was different at 4 weeks compared with 2 weeks; while after 2 weeks the highest calorific losses were at 30 °C regardless of a_w, and after 4 weeks, there was a significant decrease at 20 °C/0·95 a_w and 15 °C/0·90 a_w. Moreover, trends were dependent on the strain tested.

Effect of water activity, temperature and time on biomass of *A. ochraceus*

In vitro studies of the growth of the three isolates showed that fungal biomass correlated inversely with the results obtained for calorific loss (Fig. 2). Biomass was greatest in the 0·95 a_w treatment after both 2 and 4 weeks growth, and optimum at 30 °C (0·12–0·73 g dry mycelium). The biomass of isolate 3·113 was considerably smaller than that produced by the other isolates under all conditions tested. No appreciable mycelium was developed by this isolate at 0·85 a_w under any of the temperatures examined after 2 and 4 weeks incubation. Isolates NRRL 3174 and 3·38 grew well, resulting in a measurable amount of mycelium at 0·85 a_w, but only at 30 °C.

All single factors were significant ($p<0{\cdot}01$). Most two-, three- and four-way interactions were also significant (except for $a_w \times T$, $a_w \times S \times t$). Clearly, the effect of temperature was similar under the different a_w levels. There was a significant ($p<0{\cdot}01$) inverse correlation between maize calorific value and increases in biomass (Pearson correlation coefficient $= -0{\cdot}4827$).

DISCUSSION

This study has shown that mycotoxigenic strains of *A. ochraceus* can cause significant calorific losses of a staple grain substrate, maize, over a range of storage conditions. It was also notable that calorific losses and fungal biomass increased with incubation time (2 to 4 weeks). The maximum calorific losses observed were 10–17% at 0·95 a_w, the highest water availability treatment examined. Temperature also had a significant effect on calorific losses. There were significant effects overall of a_w, temperature, time and their interactions, and sometimes with isolate. Very few previous studies have attempted to examine the relationship between environmental factors of staple food substrates, growth of spoilage fungi and calorific losses.

In a parallel study on the effect of mycotoxigenic fusaria on maize, it was found that calorific losses increased from 0–9% to 17–64% at 0·92 to 0·98 a_w over periods of 4 weeks storage[17]. An inverse correlation was found between calorific value and fungal biomass and fumonisin production. Prasad and Prasad[6] studied the calorific losses caused by six different seed-borne fungi on linseed with time at 28 °C. They demonstrated a significant decrease from the initial calorific value (1·896 kJ; 7·9348 kcal) g^{-1} seed, with *Aspergillus niger* infection causing maximum calorific losses (25% within 15 days, and 49% within 30 days) on surface-sterilised seeds, whereas losses were higher on autoclaved seeds, especially due to *Fusarium oxysporum* (47% and 54%, respectively). Unfortunately, the water availability was not monitored or accurately controlled in this study and thus comparisons with our study are difficult. The calorific losses reported are higher than those with *A. ochraceus*, which were less than 17% for all conditions. However, a_w was only 0·85–0·95, and wetter, more unrealistic storage conditions were excluded.

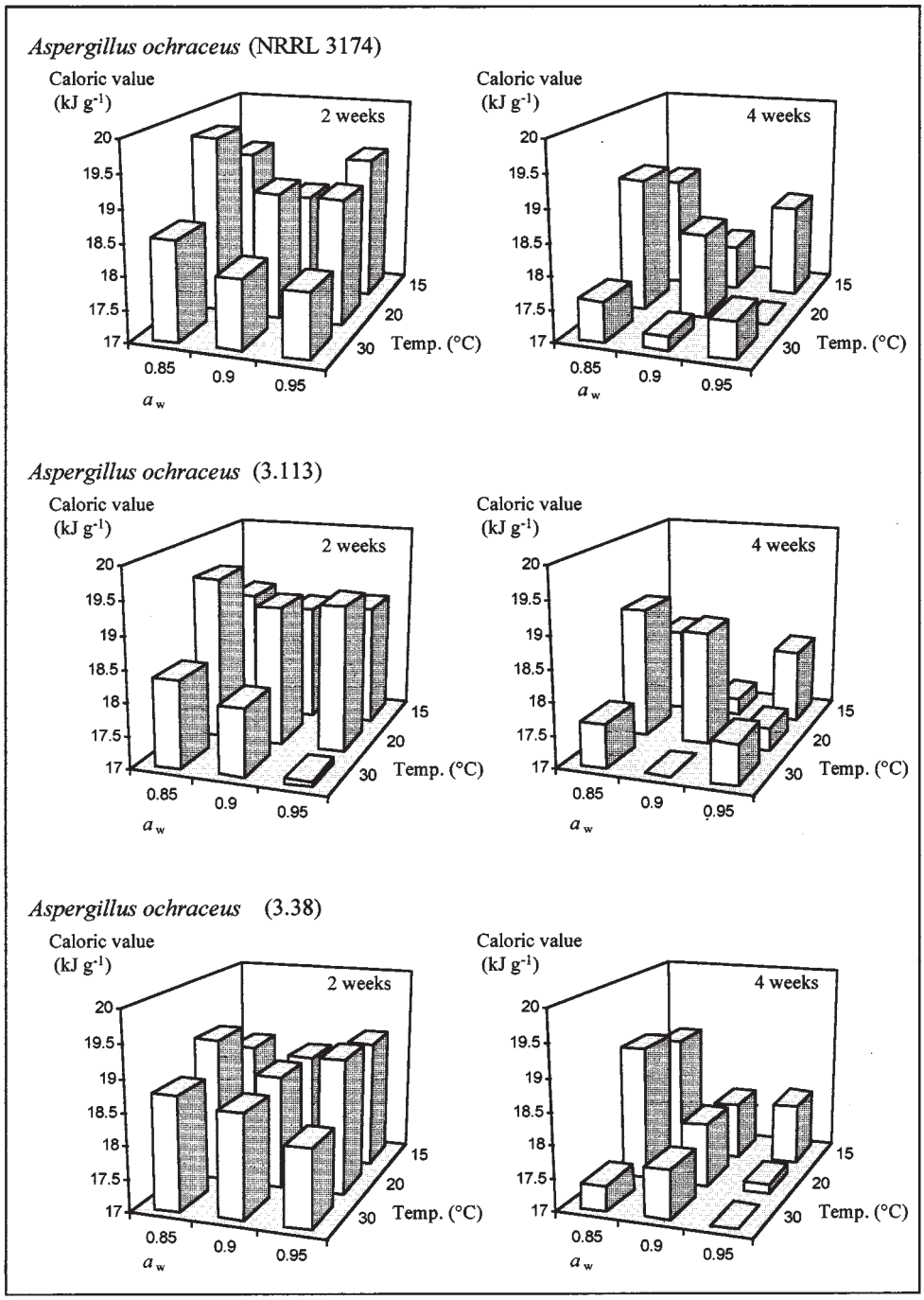

Figure 1 Effect of water activity, temperature and time on calorific value of maize due to growth of *A. ochraceus*.

Other detailed studies of the energy flow and calorific losses from grains have been made in relation to the different developmental stages of insect pests[5,18]. The insect components were separated from the grain to produce more comprehensive energy flow measurements and losses from grain during deterioration by insects. Such studies with fungi are more difficult because of the inherent difficulty of separating the fungal biomass from the substrate. In this study the data are presented on a dry weight basis so that the loss in actual dry matter through respiration, as well as

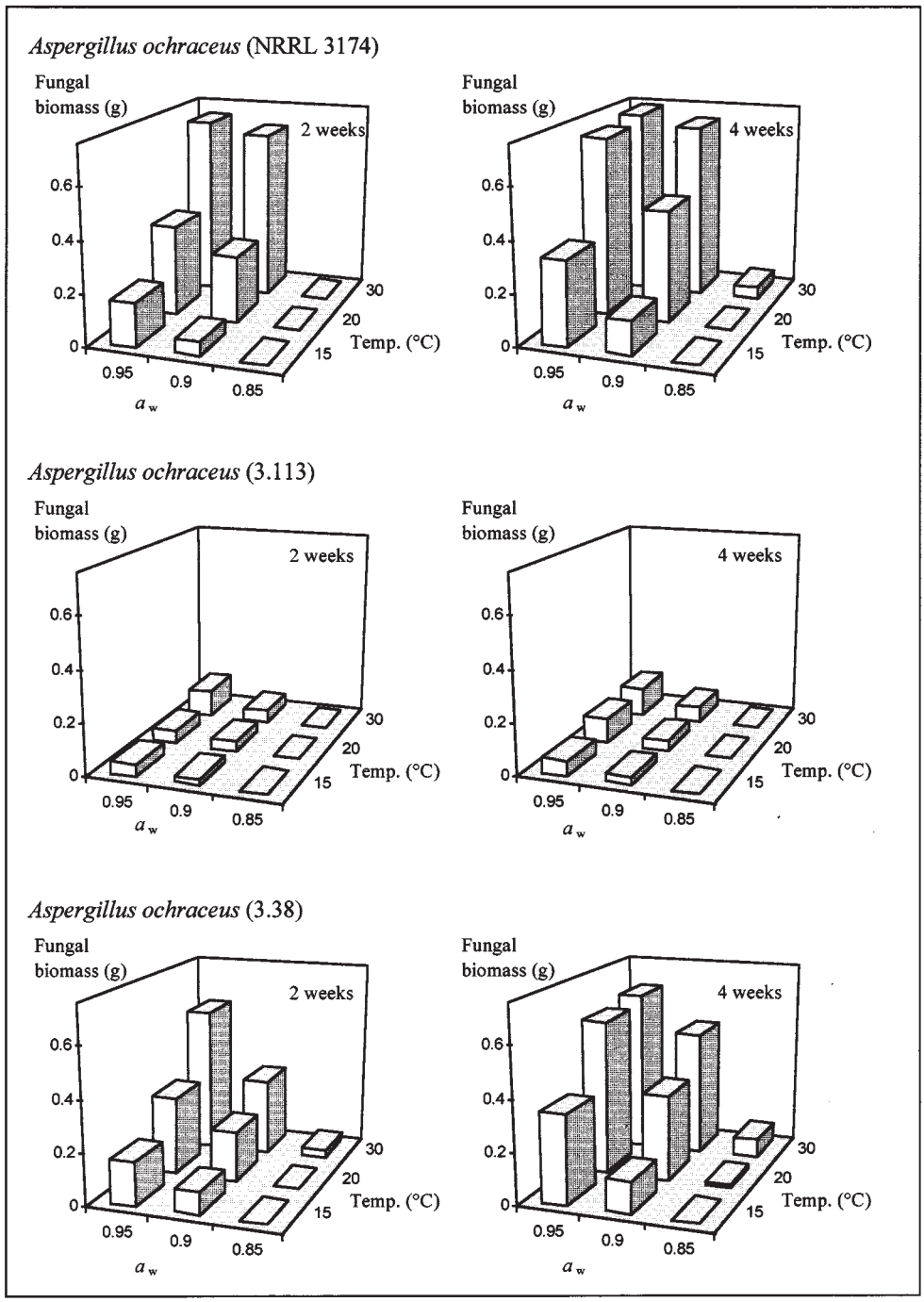

Figure 2 Effect of water activity, temperature and time on biomass of *A. ochraceus*.

the residual losses in nutritional components, are accounted for in the calculations. The only factor not included was the actual fungal biomass present in the maize substrate. Thus, the actual losses were probably slightly underestimated in our studies.

The maximum fungal biomass was always produced in the highest a_w and temperature treatment (0·95 a_w and 30 °C). Although, in general, biomass only increased slightly between 2 and 4 weeks incubation, a significant effect of incubation time was observed.

There were significant statistical effects on

fungal biomass of all the factors assayed, i.e., a_w, incubation time, temperature and isolate, and with all interacting factors. In general, as the fungal biomass increased, the caloric value of maize decreased, indicating a direct relationship between the colonisation of the maize substrate by *A. ochraceus*, and the caloric losses.

Previous studies on the ecological determinants for germination and growth of isolates of *A. ochraceus* showed that optimum growth was at 0·95 a_w, with minima at about 0·85 a_w[19]. In other reports the biomass of *A. ochraceus* has been quantified either in terms of both dry weight of mycelium (on agar medium), or using glucosamine concentration (on agar medium or seed samples)[20], and ergosterol biomass markers[21]. However, the concentration of the latter marker changes significantly with culture age and between fungal species so cannot be used as a comparative measure of fungal biomass. Using the glucosamine marker it has been demonstrated in wheat and rapeseed inoculated with *A. ochraceus* that biomass increased significantly with time of incubation (7, 15 and 30 days) and that maize supported growth of *A. ochraceus* better than other cereals and oilseeds[12].

Previously, studies have concentrated on dry matter losses of different cereals due to fungal activity[22–25]. Such losses result from the utilisation of carbohydrate during fungal metabolism and grain respiration. Estimates differ for the amounts of allowable dry matter losses before grain is rejected for human or animal consumption. In high moisture maize (25% m.c.) a loss of 0·5% dry matter can occur in 7 days, sometimes without any visible moulding, but the grain was unfit for use, and also contained aflatoxins. Some authors have considered grain to be fit for animal feed with dry matter losses of up to 2%[22]. However, it has been suggested that 1% dry matter loss is acceptable in grain for food use and that this could be applied to both wheat and maize[26]. However, implications for deterioration in nutritional value on a calorific basis were not considered. Different grain types all have an inherent but different calorific value[3]. A 1% dry matter loss in maize would represent a decrease of 0·197 kJ (0·047 kcal) g^{-1}. This must be an underestimate as we found much larger calorific losses after 2–4 weeks storage. Furthermore, nutritional losses from a maize meal flour may be greater than that from whole undamaged kernels.

Very few studies have examined the direct effect of individual fungi on dry matter losses of cereal and oilseed substrates[25,27]. The respiratory rates of fungus-free and maize infected predominantly with *A. flavus*, surface-sterilized maize (containing *Fusarium moniliforme*, *Cephalosporium acremonium* and *Penicillium* spp. internally), or untreated have been examined[24], but only over a narrow water availability range (21·7–24·9% m.c. $\approx$0·93–0·95 a_w). Dry matter losses were found to reach 0·5% within 5–8 days, and in many cases aflatoxins were already present. Studies on naturally contaminated maize at 19 and 22% m.c. also suggested that 0·5% dry matter was lost within 12 days storage due to fungal activity[28]. Thus infection of maize substrates by *A. ochraceus* could result in not only just calorific and dry matter losses but perhaps also ochratoxin production which would result in the substrate being downgraded and unacceptable for human consumption.

This work has demonstrated the important role that development of *A. ochraceus* plays on calorific loss of grains, specially at >0·90 a_w. More work is now needed to determine the influence of such fungal development on long-term storage, and on ochratoxin production, as well as on the interaction of *A. ochraceus* with other storage fungi on nutritional losses.

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