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Changes in enzyme activities during germination of cowpeas (*Vigna unguiculata*, cv. California blackeye)

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Abstract

Cowpeas (*Vigna unguiculata,* cv. California blackeye), steeped at 25°C for 20 min, were germinated at room temperature for 0, 1, 2 and 3 days. The samples were then dried at 60°C for either 2.5, 5, 7.5 or 10 h. α-Amylase, β-amylase and endo-(1,3)(1,4)-β-D-glucanase activities were determined in the cowpea samples. The results obtained showed β-amylase activity was not present in any of the experimental samples. Germination had a highly significantly positive effect on α-amylase activity ($P < 0.05$), where activities ranged from 85.6 to 720.9 μmoles maltose/ml/min at day 0 to day 3 of germination. The effect of germination and drying time on α-amylase activity was significant ($P < 0.05$). Compared to α-amylase activity, the activity of endo-(1,3)(1,4)-β-D-glucanase was low. Further analysis, using Duncan's Multiple Range Test, indicated that there was no significant difference in α-amylase activity in samples dried for 5–10 h at 60°C. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Cowpeas (*Vigna unguiculata*), also known as black-eye beans, are a good source of protein, energy and other nutrients in developing countries (Uzogara & Ofuya, 1992). Cowpeas are grown extensively in 16 African countries, with Niger and Nigeria producing 49.3% of the annual world crop (Rachie, 1985). The carbohydrate content of cowpeas ranges between 30 to 50%. Carbohydrate digestibility is increased by germination. Akinlosotu and Akinyele (1991) reported that germination increased and decreased monosaccharide and disaccharide contents of cowpeas, respectively. Monosaccharides can be utilized for energy and will also increase palatability of cowpeas.

Considerable interest has developed in expanding the usage of cowpeas in other forms, as a low cost, high quality protein supplement that would impart desirable functional properties in products such as weaning foods. One such form is germinated cowpea meal, produced by the malting process.

The malting process consists of steeping, germination and drying. Germination triggers the production of enzymes, which hydrolyze starch into oligosaccharides. Drying times, reported in the literature, have exceeded 10 h (Almeida-Dominguez, Serna-Saldivar, Gomez, & Rooney, 1993; Hansen, Perdersen, Munck, & Eggum, 1989; Kulkarni, Kulkarni, & Ingle, 1991; Palmer, 1992). A possible disadvantage of such lengthy drying regimes is the inactivation of endogenous enzymes. Thus, the present study was undertaken to study the effect of germination and drying times on the development of α-amylase, β-amylase and endo-(1,3)(1,4)-β-D-glucanase, in cowpeas.

2. Materials and methods

2.1. Materials

Corn-starch, barley-β-glucan, *p*-nitrophenyl-maltopentaose (PNPG-5), yeast α-glucosidase [E.C.3.2.1.20] Type 1, α-amylase Type II [E.C. 3.2.1.1] from Bacillus species and sweet potato β-amylase [E.C. 3.2.1.2] Type 1-B were from Sigma Chemical Co., St. Louis, Missouri. Cowpeas (*Vigna unguiculata,* cv. California blackeye) were purchased from Kroger Co. (Blacksburg, VA).

2.2. Methods

2.2.1. Germination procedure

Cowpea samples (100 g) were immersed in 1% sodium hypochlorite solution (150 ml) for 20 min at room temperature (Aisien, Palmer, & Stark, 1983). After sterilization,

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the seeds were steeped in distilled water (400 ml) for 6 h at 25°C (Marero, Payumo, Librando, Lainez, Gopez, & Homma, 1988). On termination of steeping, the seeds were spread evenly on trays (11×8×2 in) lined with moist filter paper and incubated at 25°C for 0, 24, 48 and 72 h, to allow germination to occur. The seeds were rinsed every 8 h with distilled water during germination. After each germination period, cowpea seeds were dried in a Blue-M Constant Temperature Cabinet air-oven (Blue Island, IL) at 60°C for 2.5, 5, 7.5 or 10 h. The dried seeds were roasted in an 80°C oven for 30 min (Malleshi, Daodu, & Chandrasekhar, 1989). The roasted seeds were cooled for 10–15 min and ground in a Regal™ coffee grinder for 1 min. Ground samples were stored desiccated, in labelled Ziplock™ bags at 7°C. Replicates of each germination and drying time were done.

2.2.2. Proximate composition

Moisture content, crude protein (N×6.25), fat and ash contents were determined by standard AACC (1983) procedures. Carbohydrate content was determined by difference. Results were expressed on a dry weight basis.

2.2.3. Enzyme assays

Enzyme extract was obtained by extracting 0.8 g of cowpea flour with 50 mM sodium maleate buffer (8 ml, pH 5.2). The extract was centrifuged in a Dynac centrifuge (Persipanny, NJ) at 1000 ***g*** for 10 min and decanted into new labelled test tubes for determination of α-amylase and endo-(1,3)(1,4)-β-D-glucacanase activity.

Enzyme activity was determined spectrophotometrically according to the method described previously by Uriyo and Eigel (1999). The substrate for determining β-amylase activity contained 5 mM of PNPG-5 and 100 U of α-glucosidase/ml distilled water. β-Amylase was assayed by following the method of McCleary and Codd (1989).

2.3. Statistical analysis

Means and standard deviations were calculated for moisture, protein, carbohydrate, fat and ash (triplicate determinations). Enzyme activity data was subjected to analysis of variance according to the procedures of the Statistical Analysis System (SAS, 1985). Significance among means was determined by the Duncan Multiple Range Test. Unless otherwise stated, $P < 0.05$ was used to establish significant differences.

3. Results and discussion

3.1. Proximate analysis

Ungerminated cowpeas had a mean protein and ash content of 20.6% and 3.2%, respectively (Table 1). The carbohydrate content was 57.2%. These values were slightly lower than those of Bressani (1985). Factors causing these variations in proximate values could arise from location in which the crops were grown as well as seed variation. For example, variation in protein content has been reported to be from 23.1 to 27.3% and is influenced by genotypes as well as by environmental factors such as planting seasons (Bliss, Barker, Franckowiak, & Hall, 1973).

3.2. Enzyme activity

Germination had a highly significant effect ($P < 0.05$) on cowpea α-amylase activity. α-Amylase levels increased from 85.6 to 720.9 μmoles maltose /ml of extract at 0 and 72 h germination time, respectively (Table 2). Similar behaviour was reported by Malleshi et al. (1989), whose investigations indicated that α-amylase activity had attained a maximum at 3 days germination and had begun to decline at 4 days.

The combined effect of germination and drying time on α-amylase activity was significant ($P < 0.05$; Table 3). Sumanthi, Malleshi, and Rao (1995) found an increase in α-amylase activity after 48 h of germination amongst several legumes that included cowpeas (*Vigna catjang*). They reported malting losses of 19.1% and higher at 48 h germination. Germination beyond 48 h resulted in excessive loss of dry matter and, consequently, a much lower yield of malted flour. Malting loss is attributed to dry matter loss occurring due to leaching of seed con-

Table 1
Nutrient content found in cowpeas[a]

Nutrient (%)	Experimental cowpea sample	Cowpea[b] reference	Range[c]
Moisture	9.6±0.08	ND	ND
Protein	20.6±0.55	24.8±0.48	24.1–25.4
Carbohydrate	57.2±0.63	63.6	60.8–66.4
Fat	0.7±0.03	1.9±0.62	1.1–3.0
Ash	3.2±0.002	3.60±0.17	3.4–3.9

[a] ± S.D., triplicate determinations. ND, not determined.
[b] Mean values from work by Bressani (1985).
[c] Range of nutrient content from 8 cowpea cultivars from work by Bressani (1985).

Table 2
Germination effect on cowpea enzyme activity(μmoles maltose/ml/min)[a]

Germination (days) at 25°C	α-Amylase	Endo-(1,3)(1,4)-β-D-Glucanase
0	85.6 d	4.5 b
1	138 c	9.3 b
2	528 b	16.3 b
3	72 a	62.5 a

[a] Values reported on a dry weight basis and are means of 48 observations. Means in the same column with different letters are significantly different ($P < 0.05$).

stituents during steeping, metabolic loss due to respiration and utilization of nutrients for the growth of root and shoot during germination. In this study, root growth increased noticeably as germination time increased. Rootlets, measuring approximately 3.46 and 5.18 cm, were observed for seeds germinated at room temperature for 48 and 72 h, respectively. Nnanna and Phillips (1988) reported rootlets shorter than 4 and longer than 12 cm at 25°C at the same germination times.

These roots and shoots are subsequently removed along with the seed coat. It is generally believed that the germination does not affect the seed coat but rather germination causes biochemical changes in the endosperm that result in dry matter loss in the endosperm only (Sumanthi et al., 1995).

β-Amylase activity could not be detected in either germinated or ungerminated cowpea samples. Few studies have been made on β-amylase activity in legumes. The substrate, PNPG5, was used in the experiment due to its specificity for β-amylase. PNPG5 contains α-glucosidase and cysteine. When PNPG5 is incubated with β-amylase, maltose is released and allows α-glucosidase to rapidly cleave the resulting product — *p*-nitrophenol maltotrioside — to glucose and *p*-nitrophenol (McCleary & Codd, 1989).

The extraction agents used in this experiment contained cysteine, which has the ability to split disulphide bonds and thus solubilize the insoluble β-amylase. Insolubility is due to crosslinking of sulphydryl groups of the enzyme to sulphydryl groups in other soluble proteins (McCleary & Codd, 1989).

The concept of "bound" and or "free" β-amylase has also been reported amongst cereal grains, such as barley (*Hordeum vulgare* L.), wheat (Evans, Wallace, Lance, & Macleod, 1997) and sorghum (Taylor, 1993). Evans et al. reported on a new latent β-amylase that is linked to the insoluble proteins in the endosperm protein matrix and the surface of starch granules in barley. The synthesis of β-amylase occurs in the endosperm during barley grain maturation, but little or no β-amylase is synthesized during germination. In wheat, β-amylase has been found in fractions containing glutenin (Evans et al.). The absence of β-amylase activity in cowpeas in this study could also be due to the synthesis of β-amylase in the cowpea endosperm during maturation and not during germination.

Analysis of variance indicated endo-(1,3)(1,4)-β-glucanase activity was not significantly increased by germination ($P < 0.05$) from day 0 to day 2; however, significance occurred at day 3 (Table 2). Significance amongst means was determined by the Duncan Multiple Range Test, which indicated that endo-(1,3)(1,4)-β-glucanase activity was significantly higher ($P < 0.05$) at day 3 than at day 0 to day 2 (Table 2). The low activity of endo-(1,3)(1,4)-β-glucanase is due to the endosperm being reduced to a thin layer surrounding the cotyledons upon maturity. Another reason could be the removal of the endosperm with the testa upon soaking (Kadam, Deshpande, & Jambhale, 1989). The slight increase in endo-(1,3)(1,4)-β-glucanase activity in cowpeas (Table 2) during germination could be due to the growing acrospires. β-Glucan is found in leaves, stems, husks, coleoptiles and roots of cereals and other monocotyledons (Anderson, Cook, & Stone, 1978).

The modification of the starchy endosperm of cowpeas needs further exploration. The major component of the endosperm cell wall is endo-(1,3)(1,4)-β-D-glucan. Extensive work has been done on the effect of germination on sorghum cell wall degradation by Palmer (1992) and Etokakpan (1992).

Low temperature kilning allows greater enzyme survival. Elevated temperatures also allow greater enzyme survival when the malt is less moist (Owuama & Asheno, 1994).

Table 4 shows that drying significantly affected α-amylase activity ($P < 0.05$). No significant differences ($P < 0.05$) were obtained between 5 and 10 h of drying (Table 4). The only significance difference occurred at 2.5 h, where lower α-amylase activity was observed (Table 4).

After 2.5 h drying, the average moisture content of cowpeas was 29.8% (Table 5). At 10 h, the moisture content had dropped to 6.7% (Table 5). The combined action of germination and drying had a significant effect on cowpea α-amylase activity (Table 3), indicating enzyme activity was retained during prolonged dehydration. Owuama and Asheno (1994) reported that malts, with over 10% moisture when subjected to elevated temperature treatments, suffered accelerated inactivation

Table 3
Effect of drying and germination time on cowpea α-amylase activity (μmol maltose/ml/min)[a]

Drying time (h)	G = 0	G = 1	G = 2	G = 3
2.5	78.6	124.6	473	509
5	99.5	21.7	523	820
7.5	76.4	98.2	633	728
10	87.8	108	484	826

[a] Values are means of 24 observations. G, germination (days).

Table 4
Drying effect on cowpea enzyme activity (μmol maltose/ml/min)[a]

Drying effect at 60°C (h)	α-Amylase	Endo-(1,3)(1,4)-β-D-glucanase
2.5	298 b	21.2 a b
5	415 a	34.7 a
7.5	384 a	17.0 b
10	377 a	19.7 b

[a] Values reported on a dry weight basis and are means of 48 observations. Means in the same column with different letters are significantly different ($P < 0.05$).

Table 5
Moisture (%) content in germinated and dried cowpea samples[a]

Drying time (h)	G = 0	G = 1	G = 2	G = 3
2.5	27.6	30.3	20.3	40.3
5	7.3	7.0	8.9	17.6
7.5	5.9	4.4	5.8	7.6
10	4.9	3.8	4.2	5.6

[a] Values are means of two samples. G, germination (days). $P < 0.05$ for row values.

of enzymes. Greater enzyme survival can occur at higher temperatures if the malt is less moist.

Retention of enzyme activity could be attributed to the low kilning temperature (60°C) used or to continued germination during drying. For example, Bathgate (1973) reported malt dried at 35–55°C for over 24 h to a moisture content of 8% still retained 75% of its germinative power. He also reported that when moisture content was less than 30%, some enzymic activity continued as long as the temperature did not exceed 60°C (Bathgate).

4. Conclusion

The influences of both germination and drying time were significant ($P < 0.05$) for α-amylase and endo-(1,3)(1,4)-β-D-glucanase activity. β-Amylase activity was not present in ungerminated and germinated samples. Drying time and germination time main effects were significant ($P < 0.05$) for α-amylase. Based on results obtained from Duncan's Multiple Range Test, a drying period of 5 h and germination period of 48 h would be most effective in producing higher levels of enzyme activity in malted cowpea flour. The results indicate α-amylase is stable during drying times of 5 to 10 h. Thus shorter drying times could be used in making malt. Only cowpea endo-(1,3)(1,4)-β-D-glucanase showed a significant germination effect ($P < 0.01$). The results obtained from this study reveal the importance of determining conditions for optimal carbohydrase activity in starch-based food systems for developing countries. Optimum conditions will provide malts that can be used to minimize factors common to weaning foods in developing countries, such as high viscosity, low caloric density and poor sensory characteristics.

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