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Full Length Research Paper

Amylolytic potentiality of fungi isolated from some Nigerian agricultural wastes

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Five common Nigerian agricultural wastes: cassava peels, yam peels, banana peels, plantain peels, and Brewery Spent Grains (BSG) were formulated into media on which amylolytic fungal strains were isolated. These isolates were subsequently identified, characterized using standard mycological keys and were thereafter employed in production of α-amylase using submerged and solid-state cultivation regimens. Amount of α-amylase in enzyme unit (E.U.) liberated by each mould was quantified by estimating the amount of reducing sugars produced when specified quantity of starch was hydrolyzed after incubation at 40°C with known concentration of enzyme solution. Results showed the amylolytic isolates to be *Helminthosporium oxysporium, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus,* and *Penicillium frequestans.* The mould *Helminthosporium oxysporium* liberated 10.77 and 10.42 E.U of α-amylase on cassava and yam peels media respectively using submerged cultivation method while *A. flavus* produced 11.94 E.U. of the enzyme on cassava peels with submerged cultivation method. Production of α-amylase on cassava, yam and plantain peels was facilitated by submerged cultivation method. With exception of banana peels substrate, other investigated Nigerian agricultural wastes favoured α-amylase production at different rates.

Key words: Agricultural wastes, submerged cultivation, solid-state cultivation, α-amylase, fungal isolates.

INTRODUCTION

Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries. They are mainly employed for starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup, and maltotetraose syrup. In detergents production, they are applied to improve cleaning effect and are also used for starch de-sizing in textile industry (Radley, 1976; Aiyer, 2005). α-Amylase also known as α-1,4-glucan-4-glucanohydrolase, dextrinogenic, endoamylase or liquefying enzyme, is an important class of amylases applied industrially; others being β-amylase and amyloglucosidase. α-Amylase is characterized by its random hydrolysis of α–1,4–glucosidic bonds in amylose and amylopectin molecules of starch while α–1,6– links in amylopectin molecules are resistant to attack (Whitaker, 1972).

The enzyme can be sourced from porcine pancreas, human saliva, rat pancreas and malted grains; however, a great proportion of large-scale amylase enzyme production relies on microbial origin. This is because of the possibility of increasing the levels of enzyme protein synthesized by classical genetic techniques (Dixon and Postgate, 1972), by continuous culture selection (Rigby et al., 1974), by induction (Jacob and Monod, 1961) or even by optimizing the growth conditions for the enzymes of interest (Ali et al., 1989).

Cost of substrates on which amylases producing microbes can be cultivated has always been an important factor in production cost (Aunstrup, 1977). Agrarian nations have abundant supply of agricultural wastes that are generated from their rice mills, cassava processing plants, breweries, plantain and banana chips producing outfits, yam flour processing units, and other cottage industries annually. At present, these wastes constitute a nuisance to the environment as they are dumped at dumpsites indiscriminately. Exploitation of such wastes as substrates for amylases production can be a viable option. Earlier studies focussed on rice bran, cassava peels and sugar cane straw (Ali et al., 1989; Sani et al.,

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1992; Ortega et al., 1993; Akinyosoye et al., 2004).

This study investigated five agricultural wastes that are common in most Nigerian dumpsites as sources of amylolytic fungal strains for production of α-amylase. Fungal isolates that colonized media prepared from the cassava, yam, banana, and plantain peels, and Brewery spent grains (BSG) were also screened for amylolytic potentiality and their potentialities for α-amylase production compared on the investigated wastes that served as substrates.

MATERIALS AND METHODS

Yam, cassava, banana, and plantain peels were sourced from domestic sources while BSG was sourced from a brewery (International Breweries Plc, Ilesa) in Osun State, Nigeria. These samples were dried separately at 60°C to a moisture level of about 10%. After the samples had been allowed to cool, they were subsequently milled in a 'warring' commercial blender before being packaged in cellophane bags and kept in desiccators until required (Sani et al., 1992).

Isolation of fungi

The waste media were treated with 1% streptomycin sulphate before being poured onto sterile plates. After setting, the plates were exposed to air outside for 1 h. The plates were incubated aerobically at 29 ± 1°C for 72 h. The organisms on the waste media were thereafter sub-cultured repeatedly on Potato Dextrose Agar (PDA) plates to obtain pure cultures for further studies.

Determination of Amylolytic potentiality of Fungal isolates

A modified medium of Ali et al. (1989) consisting of soluble starch; 2%, peptone; 0.2%, ammonium sulphate; 0.3%, potassium dihydrogen phosphate; 0.1%, magnesium sulphate heptahydrate; 0.03% and calcium chloride; 0.03% (w/v) was sterilized and employed as the substrate for fungal isolates. Spore suspension containing 10^5 spores/ml of 7 day old culture of each isolate was aseptically introduced into each tubes of fermentation medium (Metwally, 1998). After 5 days of incubation at room temperature (30 ± 2°C), a set of each isolate was incubated further at room temperature and the other at 60 – 65°C. At intervals of 5 min, samples were drawn and tested with 0.2% w/v iodine solution to determine hydrolytic action of the amylase on the soluble starch (Lim et al., 1987). Time at which iodine failed to form a blue coloration was used as the point at which each organism exhibited amylolytic property (Alli et al., 1998). In addition, each pure isolate was transferred with sterile inoculating pin on sterile starch agar plate and incubated for 5 - 7 days at 29 ± 1°C. Each colony was checked for halo-forming zone whose diameter was measured after which Gram's iodine solution was added to each plate to test for presence or otherwise of starch around each colony.

Identification of amylolytic fungal isolates

Isolates obtained were characterized and identified the basis on morphological and microscopic features. Among the characteristics used were colonial characteristics such as size, surface appearance, texture and colour of the colonies. In addition, microscopy revealed vegetative mycelium including presence or absence of cross-walls, diameter of hyphae, and types of asexual and sexual reproductive structures. Slide culture method that minimized serious distortion of sporing structures was used. Appropriate references were then made using mycological identification keys and taxonomic description. In cases where the structures were not well elucidated, agar block slide cultures were used (Harrigan and McCance, 1976; Samson and Reenen-Hoekstra, 1988).

Submerged cultivation method for α-amylase production

The cultivation medium containing dry waste; 2%, peptone; 0.2%, ammonium sulphate 0.3%, potassium dihydrogen phosphate; 0.1%, magnesium sulphate heptahydrate; 0.03%, and calcium chloride. 0.03% (w/v) in distilled water was employed (Ali et al., 1989). The medium was sterilized, allowed to cool, and inoculated with 7day old fungal suspension containing 10^5 spores/ml (Metwally, 1998). This was followed by incubation at room temperature (29 ± 1°C) for 6 days. The flasks of inoculated cultures were incubated as stationary cultures.

Preparation of crude α-amylase

Harvested cultures were cooled at 4°C for 30 min, centrifuged at 12,000 X g, and the supernatant separated and used as crude enzyme as described by Ali et al. (1989).

Solid-state cultivation method for α-amylase production

Modified cultivation medium containing 2 g dry agricultural wastes, and 3 g distilled water was used. Dissolved in the distilled water were peptone; 0.2%, ammonium sulphate; 0.3%, potassium dihydrogen phosphate; 0.1%, magnesium sulphate heptahydrate; 0.03%, calcium chloride; 0.03% (w/w) (Ali et al., 1989). The wet medium was then sterilized, cooled and inoculated with a spore suspension of approximately 10^5 spores/ml prepared from 7 day old fungal cultures.

Preparation of crude α-amylase

Following incubation, each waste medium was mixed with distilled water in the ratio of 1:9 (w/v) and agitated at 30°C for 30 min as used by Kuhad and Singh (1993) and Ramadas et al. (1996). The mixture was subsequently cooled, centrifuged and supernatant assayed for enzyme activities.

Assay for α-amylase [EC 3.2.1.1]

The method used involved estimating the amount of reducing sugar produced by the activity of each enzyme on buffered starch. A-amylase was assayed as reported by Demoraes et al. (1999). The substrate for assay was 0.5 ml of 0.5% soluble starch, buffered with 0.2 ml of 0.1 M sodium acetate (pH 5.6). Crude enzyme extract (0.3 ml) was added to the mixture, mixed and incubated at 40°C for 30 min in a water-bath. DNSA (colorimetric) method as used by Miller (1959) was thereafter employed for estimation of reducing sugars produced. One ml of DNSA solution was added to the mixture and boiled for 5 min. Four ml of distilled water was introduced after cooling before absorbance is read at 540 nm in "CECIL 3041" spectrophotometer. Blank that consisted of 0.3 ml distilled water, 0.5 ml of 0.5% soluble starch, 0.2 ml of buffer was subjected to similar treatments. The amount of reducing sugars already present in the enzyme extract was also determined for appropriate corrections. One unit of α-amylase was the amount of enzyme that produced one micromole of reducing sugar measured as glucose

Table 1. Screening of fungal isolates for amylolytic property.

Isolate	Width of halo-zone (cm)	Iodine test on broth	Amylolytic potentiality	Identity of isolate
BFA	2.23	+	Amylolytic	*Helminthosporium oxysporium*
BFB	0.45	-	Non amylolytic	Not identified
BFC	2.09	+	Amylolytic	*Penicillium frequestans*
BFD	2.17	+	Amylolytic	*Aspergillus fumigatus*
BFE	0.55	-	Non amylolytic	Not identified
CFA	2.04	+	Amylolytic	*Aspergillus flavus*
CFB	2.12	+	Amylolytic	*Aspergillus fumigatus*
CFC	0.45	-	Non amylolytic	Not Identified
PFA	2.14	+	Amylolytic	*Aspergillus niger*
GFA	0.35	-	Non amylolytic	Not identified
PFB	2.21	+	Amylolytic	*Aspergillus flavus*
YFA	2.05	+	Amylolytic	*Aspergillus fumigatus*

+ = Iodine colour retained (starch in substrate hydrolysed); - = blue-black reaction (starch in substrate not hydrolysed).
BFA, BFB, BFC, BFD and BFE, five isolates on banana peels; CFA, CFB and CFC, three isolates on cassava peels; GFA, isolate on BSG; PFA and PFB, two isolates on plantain peels; YFA, isolate on yam peels.

under experimental conditions.

All experiments were carried out in triplicates and mean data were subjected to analysis of variance, Duncan's multiple range tests and correlation analysis with SPSS software package (SPSS Inc., Chicago, Illinois) before being presented.

RESULTS AND DISCUSSION

Identification of amylolytic fungal isolates

The result of Table 1 shows that eight of the twelve fungal isolates obtained from the investigated waste media were amylolytic while four were non- amylolytic. Of the eight fungal isolates, six were different species of Aspergillus namely; *Aspergillus fumigatus, A. flavus* and *A. niger.* Three of the Aspergilli were *A. fumigatus;* two were *A. flavus* while one was *A. niger.* The remaining fungal isolates were identified as *Helminthosporium oxysporium* and *Penicillium frequestans* (Table 2). In an earlier study, *A. awamori* was figured to be responsible for the production of an amylolytic enzyme on rice flour medium (Pestana and Castillo, 1985). Ali et al. (1989) also reported *A. terreus* cultured on rice bran as a good producer of amyloglucosidase. Sani et al. (1992) equally employed *A. flavus* and *A. niger* in the production of amylase on cassava peels substrate. Previous study has reported *Rhizopus* stolonifer as capable of producing amylases (Akinyosoye et al., 2004). Gomes et al. (2005) extracted a thermostable glucoamylase from *A. flavus*. In a recent study, Xu et al. (2008) also established α-amylase production capacity of *A. oryzae* on spent grains. Common agricultural wastes that are littering dumping sites of many developing countries can serve as substrates for production of biologically useful materials like α and β-amylases and amyloglucosidase. *H. oxysporium* has not been reported on as capable of producing α-amylase on agricultural wastes.

Production of fungal α-amylase on agricultural residues

Among the fungal isolates investigated, *A. flavus* demonstrated the greatest potential in the production of α-amylase. A total of 43.33 E.U. of α-amylase was produced by the isolate on all the tested wastes under both cultivation techniques. It however performed better in submerged fermentation where a total of 31.80 E.U. in contrast to 11.53 E.U. of α-amylase produced on all the wastes in solid state fermentation regimen. Possibility of liberation of aflatoxin limits its application for commercial α-amylase production. *A. niger* produced the second highest amount of α-amylase with a total of 34.10 E.U. on all the tested wastes under both fermentation regimens. A total of 19.53 and 14.57 E.U. of α-amylase were produced on all the wastes under submerged and solid-state fermentation methods respectively. *A. niger* performed better than any other isolates under solid-state fermentation method as α-amylase producer.

Under submerged fermentation, *H. oxysporium* exhibited the greatest propensity for α-amylase production with a total of 33.29 E.U. from all the wastes. The mould was particularly weak under solid-state cultivation regimen in which it failed to produce any detectable α-amylase.

A. fumigatus and *P. frequestans* came fourth, and fifth as fungal α-amylase producers, each producing 23.70 and 21.73 E.U. respectively from all the wastes tested (Table 3).

Lim et al. (1985) showed that certain extracellular enzymes of some black aspergilli especially *A. niger* and

Table 2. Morphological and microscopic description of amylolytic fungal Isolates.

Isolate	Appearance of colony on PDA plates	Morphology of hyphae	Morphology of reproductive structure	Diagnostic features	Identity of isolate
BFA	Dirty greyish green colony that darkened the plate with advance age	Septate hyphae, short conidiophores arising from lateral hyphae	Septate conidia that were apically borne on conidiophores. Usually 3 conidia were borne on a conidiophore.	Conidia were septate, brownish and shaped like eggs of helminths.	*Helminthosporium oxysporium*
BFC	Dark green colony on PDA plate.	Septate hyphae that showed much branching.	Arrangement of conidiophores and conidiospores gave a brush-like structure.	Monoverticillate conidiophore that had brush-like structure.	*Penicillium frequestans*
BFD	Yellowish green compact colony on PDA plate	Septate hyphae from which conidiophores arose.	Conidiophores terminated in columnar vesicle from which uniseriate phiallides arose.	Presence of foot cell, columnar vesicle and biconvex spores.	*Aspergillus fumigatus*
CFA	Light, yellowish green compact colony on PDA plate.	Septate hyphae Conidiophore with foot cell arose from other vegetative hyphae.	Conidiophore ended in globose vesicle and arising from it were phiallides that were biserially arranged. Conidiospores were borne on the phiallides.	Presence of foot cell, globose vesicle, biseriate phiallides.	*Aspergillus flavus*
CFB	Yellowish green colony on plate. The green colour was more pronounced at the centre.	Septate hyphae. Erect conidiophore arising from vegetative hyphae.	Conidiophores terminated in columnar vesicles and from each vesicle, uniseriate phiallides arose. Conidia were borne on the phiallides.	Presence of foot cell, columnar vesicle, uniseriate arrangement of phiallides, colour of colony on PDA plate.	*Aspergillus fumigatus*
PFA	Dark brown compact colony on PDA plate. Colour was more intense at the centre.	Hyphae were eptate and branched. Arising from vegetatitive hyphae were conidiophores	Conidiophores arose from foot cells and each terminated in a globose vesicle. Arranged round the globose vesicle were phiallides in a uniseriate arrangement.	Dark brown colony on PDA plates, globose vesicles, presence of foot cells, phiallides that were arranged almost round the vesicle. Spiny spores.	*Aspergillus niger*
PFB	Yellowish green compact colony on PDA plates.	Hyhae were septate, Conidiophores arising from vegetative hyphae.	Conidiophores arising from foot cells and each terminated in a globose vesicle. Phiallides were arranged in a biseriate arrangement. Spores were borne on these phiallides.	Presence of foot cell, biseriate phiallides, globose vesicle, yellowish green colour of colony on PDA plate.	*Aspergillus flavus*
YFA	Yellowish green colony on PDA plate.	Septate, vegetative hyphae from which coniophores arose	Conidiophore had a columnar shaped vesicle from which uniseriate phiallides developed. The phiallides bore the conidiospores.	Presence of foot cell, columnar vesicle and uniseriate phiallides.	*Aspergillus fumigatus*

A. awamori isolated from rice grains, wheat grains, bread, rubber wood, air and soil in Singapore, exhibited amylolytic activity. Alli et al. (1998) had also reported *A. niger, A. fumigatus,* and *A. niger* among eight of amylolytic moulds isolated from Nigerian soil. The same study showed *Penicillium citrinum* to be amylolytic. There is, however, dearth of information about the ability of *H. oxysporium* to produce α-amylase, however, the present

Table 3. α-Amylase activity (E.U.) of fungal isolates in submerged and solid state agricultural wastes.

Fungus	Cultivation Regimen	Cassava peels medium	Yam peels medium	Banana peels medium	Plantain peels medium	BSG medium
H. oxysporium	Submerged	10.77 ± 0.10	10.42 ± 0.09	1.98 ± 0.02	5.05 ± 0.11	5.07 ± 0.12
	Solid state	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P. frequestans	Submerged	5.63 ± 0.21	5.90 ± 0.11	0.00 ± 0.00	4.65 ± 0.12	2.05 ± 0.02
	Solid state	0.03 ± 0.01	0.00 ± 0.00	3.47 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. fumigatus	Submerged	6.90 ± 0.17	7.74 ± 0.14	1.02 ± 0.04	4.46 ± 0.02	1.37 ± 0.03
	Solid state	1.05 ± 0.01	0.56 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.60 ± 0.00
A. niger	Submerged	8.36 ± 0.12	4.01 ± 0.06	1.50 ± 0.01	4.14 ± 0.02	1.52 ± 0.02
	Solid state	9.33 ± 0.05	2.64 ± 0.02	0.46 ± 0.00	0.00 ± 0.00	2.14 ± 0.01
A. flavus	Submerged	11.94 ± 0.04	9.37 ± 0.11	0.00 ± 0.00	5.85 ± 0.01	4.64 ± 0.02
	Solid state	6.44 ± 0.01	1.77 ± 0.02	0.19 ± 0.00	1.44 ± 0.02	1.69 ± 0.01

*Data represent the mean of three replicate determinations.

study has shown that it is a good producer of α-amylase under submerged fermentation method.

Effect of wastes on α-amylase production by fungal isolates

Cassava waste medium comparatively enhanced α-amylase production in *H. oxysporium* more than any of the other wastes. It supported liberation of 10.77 E.U of the enzyme in submerged fermentation while yam peels medium facilitated 10.42 E.U. by the mould with same cultivation regimen (Table 3). *A. flavus* surpassed *H. oxysporium* as α-amylase producer but cannot be regarded as "Generally Regarded As Safe" (GRAS) organism because it is often linked with aflatoxin production. The other tested agricultural residue gave a comparatively moderate support to α-amylase production. Sani et al. (1992) employed cassava peels as the substrate for production of fungal amylase.

Table 3 shows that α-amylase production on cassava, yam and plantain peels was significantly favoured by submerged cultivation method in all the studied fungal isolates ($P > 0.05$) while it was not in banana peels and BSG. Cassava waste medium gave the highest support for production of α-amylase production by the tested isolates. Total α-amylase activities of 43.60 and 16.85 E.U. were produced on the waste under submerged and solid state fermentations respectively. *A. flavus* (11.94 E.U.) and *H. oxysporium* (10.77 E.U) exhibited greatest ability to produce this enzyme under submerged cultivation regimen. Sani et al. (1992) has also reported cassava peels as a good substrate for amylase production.

Conclusion

This study has revealed *H. oxysporium* as capable of producing promising amount of α-amylase on cassava and yam peels under submerged cultivation. The mould, unlike *A. flavus,* has not been implicated as a source of aflatoxin, an indication of a safer and promising source of α-amylase with submerged cultivation regimen. In addition, it has provided information on the suitability of five agricultural wastes common in Nigeria as substrates on which amylolytic fungal species could be cultured for the production of α-amylase.

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REFERENCES

Aiyer PV (2005) Amylases and their applications. Afr. J. Biotechnol. 4(13): 1525-1529

Akinyosoye FA, Adeniran HA, Oboh G (2004). Effects of Cultural changes on Amylolytic Activities of *Rhizopus stolonifer* Isolated from Decomposing Agro-Industrial Wastes. Niger. J. Biochem. Mol. Biol. 19(1): 41-46

Ali S, Mahmood S, Alan R, Hossain Z (1989). Culture conditions for production of glucoamylase from rice bran by *Aspergillus terreus"*. MIRCEN J. Appl. Microbiol. Biotechnol. 5: 525-532.

Alli AI, Ogbonna CIC, Rahman ATMF (1998). Hydrolysis of certain Nigerian starches using crude fungal amylase. Niger. J. Biotechnol. 9(1): 24-35.

Aunstrup K (1977). Production and purification of amylases In: Biotechnological pplication of proteins and Enzymes. Eds. Bohak Z, Shawn N. pp. 39-49, Academic Press Inc. New York.

DeMoraes LMP, Filho SA, Ulhoa CJ (1999) Purification and some properties of an α-amylase glucoamylase fusion protein from *Saccharomyces cerevisiae"*. World J. Microbiol. Biotechnol. 15: 561-564.

Dixon RA, Postgate JR (1972). Genetic transfer of nitrogen fixation from *K. pneumoniae* to *E. coli*. Nature London. 237: 102-103.

Gomes E, Regina de Souza S, Grandi RP, Dasilva R (2005). Production of thermostable glucoamylase by newly isolated *Aspergillus flavus* A1.1 and *Thermomyces lanuginosus* A 13.37. Braz. J. Microbiol. 36(1): 10-20.

Harrigan WF, McCance ME (1976). Laboratory Methods in Food and

Dairy Microbiology. pp. 101-452 Academic Press. London.
Jacob F, Monod J (1961). Genetic regulatory systems in the synthesis of proteins. J. Mol. Biol. 3: 318-358.
Kuhad RC, Singh A (1993). Enhanced Production of Cellulases by *Penicillium citrinium*in solid state fermentation of cellulosic residue. World J. Microbiol. Biotechnol. 9: 100-101.
Lim G, Tan TK, Rahim NA (1987). Variations in amylase and protease activities among *Rhizopus isolates*. MIRCEN J. 3: 319-322.
Metwally M (1998). Glucoamylase production in continuous cultures of *Aspergillus niger* with emphasis on growth parameters. World J. Microbiol. Biotechnol. 14: 113-118.
Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.
Ortega GM, Otero MA, Martinez EO, Gonzalez PC, Betancourt D (1993). Enzyme activitiesand substrate degradation during white rot fungi growth on sugar-cane straw in a solid-state fermentation. World J. Microbiol. Biotechnol. 9: 210-212.
Pestana F, Castillo FJ (1985). Glucoamylase production by *Aspergillus awamori* on rice flour medium and partial characterization of the enzyme. MIRCEN J. 1:225-237.
Radley JA (1976). Industrial Uses of Starch and Its Derivatives, pp. 51-115. Applied Science Publishers Ltd, London.
Ramadas M, Holst O, Maltiason B (1996). Production of amyloglucosidase by *Aspergillus niger* under different cultivation regimens. World J. Microbiol. Biotechnol. 12: 267-271. Rigby PWJ, Burleigh BD, Hartley BS (1974). Gene duplication in experimental enzyme evolution. Nature. London. 251: 220-224.
Samson AR, van Reenen-Hoekstra ES (1988). Introduction to Food-Borne Fungi.ThirdEdition.pp4-98.Centraalbureau voor Schimmel cultures. Baarn.
Sani A, Awe FA, Akinyanju JA (1992). Amylase synthesis in *Aspergillus flavus* and *Aspergillus niger* grown on cassava peel. J. Ind. Microbiol. 10: 55-59.
Whitaker JR (1972). Principles of Enzymology for Food Sciences. pp 442-454 Marcell Dekker. Inc. New York.
Xu H, Sun L, Zhao D, Zhang B, Shi Y, Wu Y (2008). Production of α-amylase by *Aspergillus oryzae* as 3951 in solid state fermentation using spent grains substrate. J. Sci. Food Agric. 88(3): 529-535.