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Biochemical changes in fermented melon (egusi) seeds (*Citrullis vulgaris*)

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SUMMARY

Biochemical and sensory changes of Nigerian melon seeds fermented with four *Bacillus* strains isolated from African locust beans were studied. In all fermentations, the reducing sugar content doubled from a starting value of 45 mg/g. The total free amino acid content decreased for the first 40 h and then increased. With *B. licheniformis*, *B. subtilis* and *B. pumilis*, there was a subsequent large increase in free amino acid content. The extracted oils in the fermentation product increased in saponification number and free acid content but decreased in iodine number. The sensory properties of the *B. licheniformis* product was similar to that of ogiri and that of *B. subtilis* to Iru.

INTRODUCTION

Bacteria and fungi have been used to cause desirable changes in texture, flavour and taste of foods in African countries. In Nigeria, products of such fermentations, which are widely consumed, range from non-alcoholic meals (gari, ogi and nono) to alcoholic beverages (pito and burukutu) and to food flavouring agents such as fermented African locust beans, castor oil beans, and melon seeds. These highly relished vegetable soup condiments are known to have high protein content [1]. Thus, fermented vegetable seeds serve the dual purpose of flavouring as well as sources of protein supplements.

Characterisation of the microorganisms responsible for the fermentation of most of these foods when done by traditional methods has been carried out [10,11]. Results from these studies show that such ferments usually contain mixed microbial flora but the extent to which each microbe contributes to the changes obtained in the final product is unknown. The study being reported below was therefore designed to investigate the possible changes occurring during in-vitro fermentation of melon seeds by pure cultures of singular microorganisms isolated from fermented oil beans.

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MATERIALS AND METHODS

Fermented African locust bean (*Parkia biglobosa*) and unfermented melon seeds were purchased from the Nigerian local markets. Brain heart infusion agar was obtained from Sigma Chemical Company, U.S.A. Sugars, polyols, solvents and other reagents all of analytical grade were purchased from BDH and Thomas Kerfoot, England.

Isolation of pure colonies from fermented locust beans. One gram of locally fermented locust bean was dissolved in 10 ml of sterilised distilled water and further diluted to approximately 10^{-2}–10^{-3} mg/ml of the solution. Samples of 0.1 ml from these were pipetted and spread on brain heart infusion agar medium. Plates were incubated at 37°C for 48 h during which time organisms which mostly appeared as white dry-surfaced and in some cases volcano-like protruding colonies were picked from the agar plates. The colonies were placed into five groups on the basis of their colonial morphology. Isolates from these morphologically distinguishable colonies were further purified to obtain single colonies and identified using the methods of Smith et al. [20], Gordon et al. [7] and Cowan and Steel [5].

Fermentation of melon seeds. Ground unfermented melon seeds previously stored at 4°C in an air-tight bottle were weighed into sterile erlenmeyer flasks and autoclaved at 15 psi of pressure for 10 min. Samples of the pure strains of microorganisms isolated were separately

and asceptically inoculated into each erlenmeyer flask. One of the flasks was inoculated with all five strains while another flask representing the control was not inoculated with any of the microorganisms. All inoculations were done in triplicate. The flasks were incubated for 7 days at 37°C.

Analysis of fermentation products. During the period of fermentation, the changes in pH were monitored periodically with a pH meter. The reducing sugars and the free amino acids were also monitored. Samples taken from the fermenting melon seeds were dried in vacuo for 48 h and then extracted in ethanol/water (1 : 1) mixture. The resulting suspension was washed with petroleum ether to extract the oil. The aqueous layer was centrifuged at 4000 × *g* to obtain a clear supernatant sample which was used for the analysis of the sugar and the free amino acids. The reducing sugars were determined by the dinitro-salicylic acid method of Rick and Stegbauer [17] and the free amino acids were determined by the ninhydrin method of Rosen [18].

At the end of fermentation, samples were similarly taken for the determination of reducing sugar, free amino acids, total lipids, saponification and iodine number, free fatty acid and peroxide value of the oil extracted. The lipid analyses were carried out by the methods outlined in the AOAC [2], including the rancidity value which was determined as the soluble volatile fatty acids (C_2–C_8) as measured by the Reinchert-Meissl values. The amount of 0.1 N sodium hydroxide required to titrate 100 ml of the resulting filtrate was used to calculate the value. The various protein fractions in the dried sample obtained from the final product of fermentation were sequentially extracted by a modified Osborne method [15,16] as carried out by Sodek and Wilson [20]. The fractions obtained from the above procedure were centrifuged at 4000 × *g* for 15 min to obtain supernatants. The protein content of these supernatants was determined by Lowry et al.'s method [9].

RESULTS

The predominant organism isolated from the fermented locust bean are bacteria of the *Bacillus* species. These are two strains of *B. licheniformis* and one of *B. subtilis* and *B. pumilis* strain. In addition however, an unidentified cocco-bacillary form was consistently the fifth strain found.

The effect of fermenting melon seeds with each of these organisms is shown in Table 1. The product fermented with *B. licheniformis* gave a pleasing smell and tasted like ogiri, while that of *B. subtilis* had the odor and taste of iru. *B. pumilis* fermentation had a sweet aroma and tasted like cheese. However, the cocco-bacillary form appears not to have fermented the melon. The fermented product brought about by all the organisms put together produced an objectionable smell and a strong unacceptable taste.

The effect of fermentation on the lipid content of melon seeds is shown in Table 2. As fermentation progressed, saponification number and free fatty acids increased. There is a decrease in iodine number which was not accompanied by increases in peroxide and rancidity values. All these, plus a decrease in pH as shown in

TABLE 1

Sensory characteristics of fermented melon seeds by the bacillus species

Bacteria isolates from fermented locust beans iru (*Parkia biglobosa*)	Effect on melon seeds				
	Occurrence of fermentation	Taste	Odour	Colour of ferment	Colour of oil extract
Bacillus licheniformis spore former, G(+)	Yes	Similar to ogiri flavour, is refined and pleasing	Very strong but neither pungent nor objectionable	Cream	Dull yellow
Bacillus subtilis spore former, G(+)	Yes	Similar to iru fermented from locust beans	Similar to iru	Light	Wine colour
Bacillus pumilis rods G(+)	Yes	Pleasing sharp, mildly sweet and sour taste	Mild and sweet aroma	Yellowish brown	Wine colour
Bacillus licheniformis strain (different from the first one)	Weakly so	Similar to *B. pumilis* but milder	Very mild aroma	Greyish	Dull yellow
Unidentified coccobacillary species	Not much	Almost like unfermented melon seed	Very weak smell	Cream	Yellow
Mixture of all organisms isolated	Yes	Exceedingly strong	Completely objectionable	Greyish	Not done
Unfermented control	None	Melony and bland	Melony	Ivory	Clear light yellow

TABLE 2

Effect of fermentation on the lipid content of melon seeds

Fermenting microorganisms	% Fat	Saponification number	% Free fatty acid	Iodine number	Peroxide value	Rancidity value
B. licheniformis	50.50	211	13.05	78	Nil	0.01
B. subtilis	51.20	207	6.50	94	Nil	0.01
B. pumilis	50.40	216	24.50	91	Nil	0.01
B. licheniformis	49.80	200	–	110	Nil	–
Control	49.00	200	1.00	115	Nil	0.13

TABLE 3

pH Changes during fermentation of melon seeds

Fermenting microorganism	Time in hours							
	0	25	40	75	92	145	166	190
B. licheniformis	6.4	5.8	5.7	5.7	5.4	5.5	5.4	5.6
B. subtilis	6.4	5.7	5.5	5.6	5.6	5.7	5.7	5.9
B. pumulis	6.4	5.4	5.4	5.3	5.3	5.4	5.3	5.4
B. licheniformis	6.4	6.2	6.6	6.8	6.8	6.6	6.5	6.4
Control	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4

Table 3 suggest an increase in saturated fatty acid content during fermentation. The increase in free fatty acids is highest where *B. pumilis* is used for fermentation whereas both strains of *B. licheniformis* produce the most saturated fatty acids.

Table 4 shows the effect of fermentation on the protein profile. All the fermented products have higher values for all protein fractions with the exception of globulins whose values did not show much change as compared to the control. The free amino acid profile is shown in Table 5. These increased in each of the fermented products to values which are more than four times those of the control. *B. pumilis* with a 13-fold increase showed the greatest change.

The change in reducing sugar content is shown in Table 6. The reducing sugar content doubled from a starting value of 45 mg/g in all cases, and *B. licheniformis* had the highest sugar content. The general increases in the reducing sugar during the first 40 h of fermentation is probably due to increased amylase activity which hydrolysed the starch content to sugars. The decrease in the sugar levels thereafter is possibly due to metabolism of the

TABLE 4

Effects of fermentation on the protein profile of melon (mg/%)

Fermenting microorganism	% Crude protein	Albumin	Globulin	Protamin	Glutelin	Total recovered
B. licheniformis	30.40	15.10	24.30	1.30	13.40	57.10
B. subtilis	30.20	13.40	20.20	1.50	18.20	56.50
B. pumulis	29.70	16.00	19.40	1.10	12.10	53.70
B. licheniformis	31.50	10.20	21.20	2.40	14.20	51.00
Control	32.40	10.90	24.30	0.80	10.80	48.00

TABLE 5

Changes in free amino acid content during fermentation (free amino acid in mg/g melon sample)

Fermenting microorganism	Time in hours						
	0	25	40	75	92	145	168
B. licheniformis	56	182	182	72	220	250	460
B. subtilis	56	90	138	60	50	180	402
B. pumulis	56	107	150	158	345	582	760
B. licheniformis	56	60	90	45	110	150	240

The uninoculated control value represented that of zero hour.

TABLE 6

Changes in reducing sugar content during fermentation (reducing sugar content in mg/g melon)

Fermenting microorganism	Time in hours							
	0	25	40	75	92	145	166	190
B. licheniformis	45	103	82	82	45	2	102	133
B. subtilis	45	82	120	0	43	45	80	102
B. pumulis	45	60	61	42	0	0	80	102
B. licheniformis	45	102	82	63	63	45	103	130

The uninoculated control value represented that of zero hour.

sugars to glycolytic end products such as lactic acid. Lactic acid values were not measured in this work, but in similar fermentations where these same organisms were used in fermentation of water extracts of melon seeds, in this laboratory, increases in lactic acid values have been noted. The need for more sugars as energy source for growth probably triggered another increase in amylase activity between 145 and 190 h of fermentation.

DISCUSSION

Previous workers had identified various organisms associated with the fermentation of iru [10]. These organisms were mostly bacteria belonging to the *Bacillus* species and they include organisms that have been isolated in this work. The role of the organisms in the fermentation process has however not been defined. This work demonstrates that it is possible to ferment melon seeds with each of the organisms and obtain products defined by the biological characteristics of each organism. Therefore manipulation of each organism is possible for the purpose of product improvement as compared to mixed cultures which are more difficult to control.

The increase in free amino acids, free fatty acids and reducing sugars are indicative of the production of the various hydrolases, namely, proteases, lipases and amylases by the fermenting organisms. This is compatible with what is known about fermentation by the *Bacillus* species, however, variation among the organisms was observed. This is of interest since it should be possible to effect a meaningful product formulation by an appropriate combination of fermenting organisms in the presence of an appropriate substrate. This experiment demonstrates this clearly.

The results also indicate the possibility that unsaturated fatty acids are being fermented to saturated fatty acids. A similar observation has been made by previous workers [3,8]. This transformation is of particular interest since it would decrease the process of rancidity and thereby increase the shelf-life of the fermentation product.

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REFERENCES

1 Abaelu, A.M., M.A. Makinde and E.O. Akinrimisi. 1979. Melon (Egusi) seed protein: Study of amino acid composition of defatted meal. Nutr. Reports Internat., 20, 5: 607–613.
2 Association of Official Agricultural Chemists. 1975. Official Methods of Analysis, 12th Edn., Washington, DC.
3 Benchat, L.R. and R.E. Worthington. 1974. Lipid changes in fermented peanut seeds. J. Agr. Food Chem. 12: 509–512.
4 Campbell-Platt, G. 1980. African locust bean (Parkia species) and its West African fermented food product Dawadawa. Ecol. Food Nutr. 9, 122–132.
5 Cowan, S.T. and K.J. Steel. 1974. Identification of Medical Bacteria, 2nd Edn. Cambridge University Press, Cambridge.
6 Eka, O.U. 1983. A review of studies on changes in nutrient composition during fermentation of food. Conf. Nut. Soc. Nig. Nigerian. J. Nut. Science. 5, 1: 9–20.
7 Gordon, R.E., W.C. Haynes and C.H. Pang. 1973. The genus bacillus. United States Department of Agn. Handbook No. 427.
8 Kuishi, K., T. Ohta, H. Hiroshi, T. Tokuji and H. Ebire. 1976. Lipid composition of kinds of Natto obtainable from Soyabean. J. Agr. Food Chem. 24: 404–407.
9 Lowry, O.L., M.R. Rosenbrough, A.L. Far and J.R. Randall, 1951. Folin procedure for protein determination. J. Biol. Chem. 193: 265–275.
10 Odunfa, S.A., 1981. Micro-organisms associated with fermentation of African locust bean (*Parkia filicodiea*) during Iru preparation. J. Plant Foods 3: 245–250.
11 Odunfa, S.A. 1981. Microbiology and amino acid composition of Ogiri–A food condiment from fermented melon seeds. Die Nährung 25: 811–816.
12 Odunfa, S.A. and A.A. Adesomoju. 1985. Effects of fermentation of the free fatty acids of African locust beans during Iru production. J. Plant Foods 6: 111–115.
13 Odunfa, S.A. 1985. Production of Ogiri, a fermented melon (citrullus vulgaria schrad) product.
Qual. Plant Foods Hum. Nutr., The Hague, Netherlands.
14 Oke, O.L. and I.B. Umoh. 1978. Lesser known oilseeds. I. Chemical Composition. Nutr. Reports. Internat. 17: 293.
15 Osborne, T.B. 1912. The Vegetable proteins. Longmans, Green, New York.
16 Osborne, T.B. 1947. The Proteins of the Wheat Kernel. Carnegie Institute of Washington, DC Publ. 84.
17 Rick, W. and H.P. Stegbauer. 1974. Alpha-amylase, measurement of reducing groups. In: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), 2nd engl. edn., pp. 885–890. Verlag Chemie, Weinheim, and Academic Press, New York, San Fransisco, London. A subsidiary of Harcourt Brace Jovanovich Publ.
18 Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. 67: 10–15.
19 Shibasaki, K. and Hesseltine, C.W. 1962. Miso fermentation. Econ. Bot. 16: 180–195.
20 Smith, N.R., R.E. Gordon and F.E. Clarke. 1952. Aerobic Spore Forming Bacteria. U.S. Dept. Agric. Monograph No. 16.
21 Sodek, L. and C.N. Wilson. 1971. Amino acid composition of proteins isolated from normal, Opague-2 and Floury-2 Corn Endosperm by a modified Osborne procedure. J. Agr. Food Chem. 19: 6.