Biochemical changes during production of *ogiri*, a fermented melon (*Citrullus vulgaris* Schrad) product

S. AYO ODUNFA

Department of Botany, University of Ibadan, Ibadan, Nigeria

(*Received 17 March 1981; in revised form 25 June 1982*)

Key words: *Citullus vulgaris*, fermentation, *ogiri*, biochemical changes, enzymes

Abstract. The changes in the principal constituents of melon during *ogiri* production by fermentation were investigated. The total nitrogen decreased in the fermented *ogiri*. The activities of proteinases increased during the fermentation as well as the amounts of amino acids. The amylase activities also increased with fermentation, but the soluble sugars showed a remarkable fluctuation culminating in a peak at 120 h of fermentation. Lipase activity was minimal in the fermenting mash. The results of the enzymatic activities in *ogiri* are compared with the fermentation of similar vegetable proteins.

Introduction

Ogiri is a product of fermentation of melon (*Citrullus vulgaris* Schrad); it is a popular food condiment in southern Nigeria. During its production, melon seeds are boiled till they are very soft. These are wrapped tightly in leaves and left to ferment for about five days. Various organisms are known to occur in the fermenting mash [5]. No information, however, is available on the biochemical changes that occur during the fermentation. In the present paper, information is provided on some of the biochemical changes in the food constituents of melon and the extracellular enzyme activities during the course of the fermentation.

Materials and methods

Preparation of extracts

Ground unfermented melon and fermenting mash at 24, 48, 72, 96 and 120 h were used as samples for analyses. For chemical analysis, approximately 5 g of sample was weighed into a 100-ml conical flask and 50 ml ethanol–water mixture (50:50 vol/vol) was added. The suspension was then washed with 5 ml petroleum ether to extract the oil, centrifuged at 5000 rpm and the clear supernatant used for analyses. To extract the enzymes, the mash was ground in an appropriate buffer, after which the suspension was centrifuged at 5000 rpm in an MSE high-speed refrigerated centrifuge at 5 °C for 30 min. The supernatant was stored in a deep-freezer

Qual Plant Plant Foods Hum Nutr 32 (1983) 11–18. 0377-3205/83/01/0011-08 $0.20

at −20 °C. Assays and analyses were carried out on duplicate fermentation and, for each sample, three determinations were made.

Determination of total sugars

The total free sugars were determined using the anthrone regent method [4]: 1 ml of the washed extract was diluted tenfold; 1 ml of the resultant solution was added to 9 ml anthrone reagent. After 10 min in a boiling water bath, the optical density was measured with Pye Unicam SP6 250 spectrophotometer at 600 nm. The total concentration was determined from a standard curve by using the known concentration of glucose.

Determination of free amino acids

The total (free) amino acids were determined by the ninhydrin colorimetric analyses method of Rosen [9]. The extract was suitably diluted and to 1 ml of this was added ½ ml cyanide–acetate buffer and ½ ml of 3% ninhydrin solution in Methyl Cellosolve. The mixture was heated for 15 min in a 100 °C water bath. Thereafter 5 ml isopropyl-alcohol–water mixture was added and shaken vigorously. After cooling, the colour was read in a colorimeter at 570 nm. The concentration of amino acids was calculated from a standard curve based on known concentrations of leucine. Each determination was done in triplicate.

Determination of total nitrogen content

The total nitrogen contents of unfermented and 120-h fermented samples were determined by the Kjeldahl method. Protein content was obtained by multiplying the nitrogen content by 6.25 [3].

Qualitative determination of sugars

The type of sugars present in the fermenting pulp was determined by paper chromatography. Extracts were prepared as above (5 of sample in 20 ml of 80% alcohol). An aliquot of 50 µl aqueous solution along with a reference standard mixture was spotted on Whatman no. 4 chromatography paper. The standard reference mixture contained glucose, fructose, maltose, galactose, xylose and sucrose, each at a concentration of 0.1%.

One-dimensional descending chromatography was done. The spotted paper was run in a mixture of *n*-butanol–acetic acid–water (4:1:1). After 18 h, the paper was removed from the tank and left to dry in air after which the chromatogram was developed with silver nitrate, following the method of Trevelyan et al. [11]. The chromatogram was left for 10 min and the colour allowed to develop. The excess silver nitrate background was removed by washing through 20% sodium thiosulphate. Identifications were done by comparison with the position of the standard substance (singly or in mixtures run simultaneously).

Table 1. Changes in the nitrogen content during melon fermentation for *ogiri* production

Melon samples	Total nitrogen (%)	Protein (%)
Sample 1: Unfermented	4.59	28.68
Fermented	3.91	
Sample 2: Unfermented	5.42	33.87
Fermented	4.63	
Sample 3: Unfermented	5.27	32.93
Fermented	4.87	

Enzyme assays

x-Amylase. The extracting buffer was 1 *M* potassium hydrogen phosphate, pH 6.5. The assay procedure described by Bernfeld [1] was used: 2 ml of the extract was mixed with 1 ml of 1% starch solution and incubated for 1 h at 40 °C. The reaction was stopped by adding 3 ml dinitrosalicylic acid reagent (DNS). The mixture was boiled in a water bath for 5 min, cooled in cold water, and then diluted with 18 ml water. The optical density of the resultant solution was measured at 550 nm, using an SP6 250 spectrophotometer. The blank was similarly treated except that the DNS was added before adding the starch solution. The amount of the reducing sugars formed was calculated from a standard curve prepared with known concentrations of maltose.

Proteinase. The extracting buffer was 0.1 *M* sodium hydrogen phosphate, pH 6.5. The assay method used was that of Yong and Wood [15]. It has been found useful for analysing proteinases in the presence of reducing sugars normally found in food substances: 5 ml of the extract was added to 10 ml of 2% solution of casein and incubated at 35 °C for 30 min. The reaction was terminated by adding 10 ml of 10% trichloroacetic acid solution. The mixture was filtered through Whatman no. 1 filter paper. The optical density of the filtrate was measured at 275 nm.

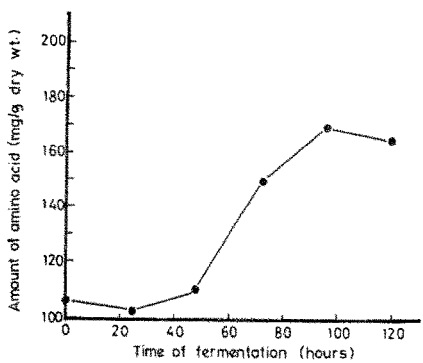

Figure 1. Changes in total amino acids during *ogiri* fermentation.

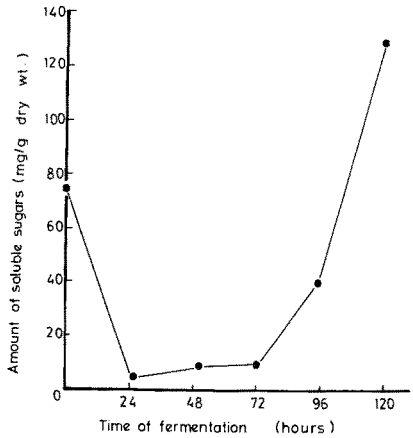

Figure 2. Changes in soluble sugar levels in *ogiri* fermentation.

Enzyme activity was expressed in terms of an arbitrary unit called an XS unit, and defined thus: 'An enzyme, a solution of which containing 1.5 g per litre, which, under the stated experimental conditions produced a filtrate with an optical density of 0.500 when measured in a 10 mm path length cell, had a strength of 36 XS units per gram.'

Lipase. The extracting buffer was 0.1 *M* sodium acetate–acetic acid mixture (pH 5.5). Five ml of the extract was added to a reaction mixture containing olive oil, 1 ml; sodium tauracholate, 0.4 g; $CaCl_2$, 1 ml of 0.1 *M* solution; acetate buffer, 6 ml. The mixture was incubated at 35 °C for 1 h. The reaction was terminated by adding 40 ml absolute alcohol. The mixture was then titrated with 0.02 *M* potassium hydroxide, using phenolphalein as an indicator.

The blank was a mixture of the assay medium and 5 ml distilled water. The difference between the titre of the blank and that of the reaction mixture gave the amount of alkali required to neutralise the liberated fatty acids and is expressed as oleic acid.

Results

The total nitrogen was decreased in the fermented end product *ogiri* (Table 1). However, the quantity of free amino acids was found to increase with the length of fermentation (Figure 1). The sugar levels fluctuated during the fermentation (Figure 2). There was an initial decrease in the first 24 h, during which time the level dropped close to zero. Thereafter there was a gradual increase till 72 h, after which there was a dramatic increase up to 120 h. Chromatographic analyses showed that the unfermented melon contained

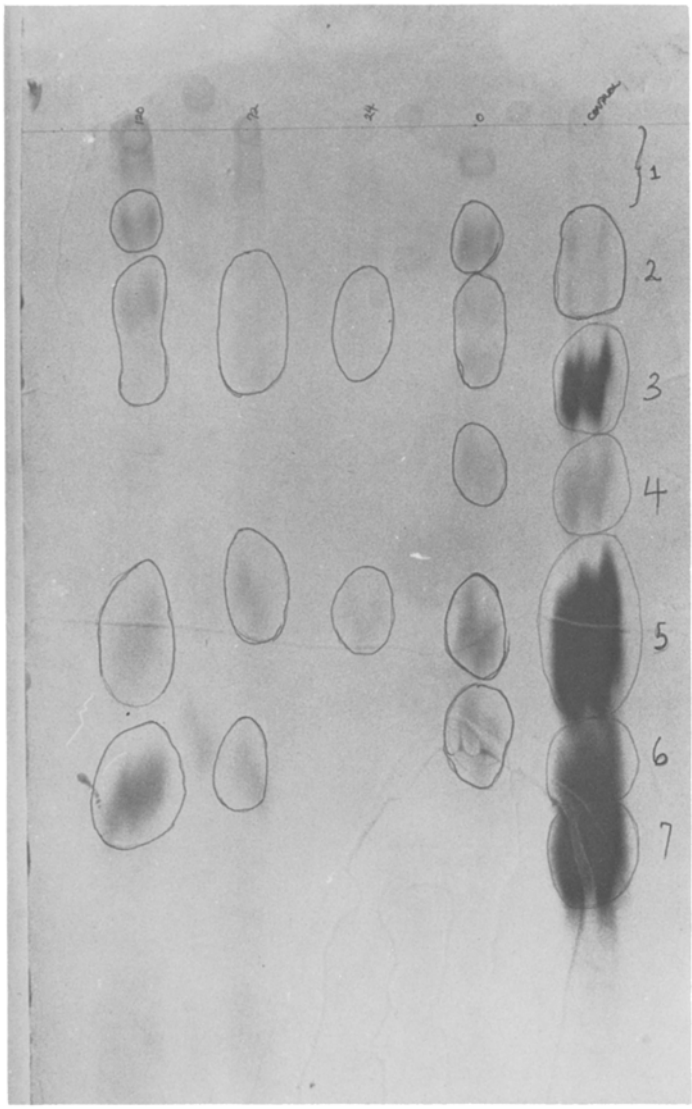

Figure 3. Chromatogram showing different types of soluble sugars present in fermenting melon. Samples chromatographed were (a) mixture of standard sugars, (b) unfermented melon and melon unfermented for 24 h (c), 96 h (d) and 120 h (e): 1, unidentified oligosaccharides; 2, maltose; 3, sucrose; 4, galactose; 5, glucose; 6, fructose; 7, xylose.

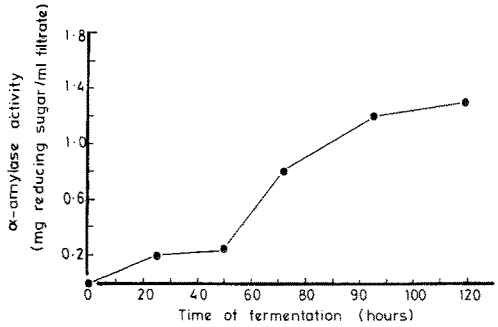

Figure 4. x-Amylase activities during *ogiri* fermentation.

maltose, sucrose, galactose, fructose, glucose and some oligosaccharides (Figure 3). Galactose was absent during the fermentation. The predominant sugars in the fermented melon were fructose, maltose, glucose and one unidentified sugar.

Of the enzymes, α-amylase and proteinase showed increases in activities with an increasing length of fermentation (Figures 4 and 5). The peak of the proteinase activity was at 72 h while that of α-amylase was at 120 h. The lipase activity did not increase significantly during the fermentation (Figure 6).

Discussion

The total nitrogen was decreased in the fermented food. A possible source of nitrogen loss is the high amount of ammonia given off during the fermentation.

Of the extracellular enzymes assayed, proteinases were the most im-

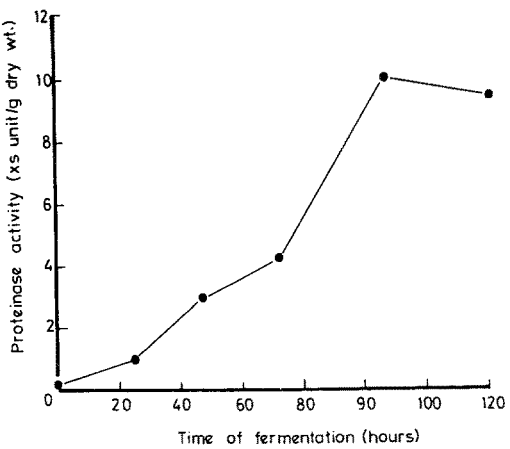

Figure 5. Proteinase activities during *ogiri* fermentation.

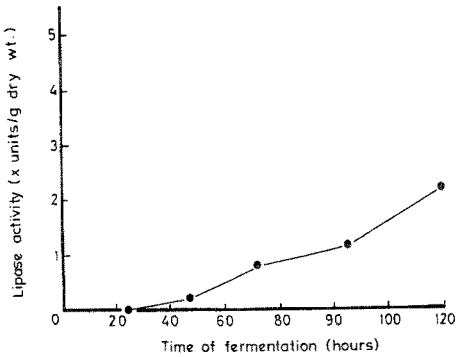

Figure 6. Lipase activities during *ogiri* fermentation.

portant in the *ogiri* fermentation. This is evident by the rapid increases in the amount of free amino acids during fermentation. The amylase is the next most active enzyme in the fermenting mash, causing increases in the amount of soluble sugars with increasing length of fermentation.

The high proteinase activity is probably due to the high protein content of melon seeds. Oyenuga [7] found that melon contained 38% protein. In the fermentation of other protein-rich seeds, proteinases have also been found to be abundant. This is true of the fermentation of soybean to produce the Japanese *miso* [10]; soy-sauce [16]; *tofu*, a Chinese soybean curd [13]; and *tempeh*, an Indonesian soybean fermented food [2, 12].

However, despite the fact that oil is the major component of melon, comprising up to 53% [7, 8], the lipase activity in *ogiri* fermentation is minimal. This is desirable since fatty acids produced might cause an objectionable taste and could cause rancidity to develop. Free fatty acids have also been found to be responsible for the trypsin-inhibiting activity in *tempeh*, a soybean product fermented by *Rhizopus oligosporus* [14]. However, in the fermentation of soybean to produce *miso*, lipases are most essential; the fatty acids produced react with some other components of the fermenting mash to form esters which produce the characteristic aroma of the food [10].

The sugar levels showed a remarkable fluctuation with the length of fermentation. The initial decrease in the sugar level may be due to the initial population of bacteria which preferentially utilise the soluble sugars in the melon (Oyolu [8] found that melon contains 2.5% soluble sugar and 11% starch by weight). After exhaustion of the sugars, the initial population is succeeded by amylolytic bacteria which hydrolyse the starch, thereby increasing the sugar level in the fermenting melon. Odusote (personal communication) [6] found that the later stage of *ogiri* fermentation was characterised by amylase-producing *Bacillus* species.

Perhaps the most significant aspect of *ogiri* fermentation is the increase

in the soluble products, thereby increasing the digestibility of the fermented food.

Acknowledgements. The author wishes to acknowledge the technical assistance of Miss Kike Odusote. The research is supported by funds from the International Foundation for Science, Sweden.

References

1. Bernfield P (1955) Amylases X and B. In: Colowicz SP, Kaplan NO (eds) Methods in enzymology, vol 1. New York: Academic Press, pp 149–158
2. Hesseltine CW (1965) A millenium of fungi, food and fermentation. Mycologia 62:149–197
3. Lillevik HA (1970) Methods of food analysis, 2nd edn. Joslyn MA (ed). London: Academic Press, p 601
4. Morris DL (1948) Quantitative determination of carbohydrate with Dreywoods anthrone reagent. Science 107:254–255
5. Odunfa SA (1981) Microbiology and amino composition of *ogiri* – a food condiment from fermented melon seeds. Die Nahrung 25:811–816
6. Odusote KO (1980) Microbiology of melon fermentation for *ogiri* production. Report in Department of Botany, University of Ibadan, Nigeria, 28 pp
7. Oyenuga VA (1968) Nigeria's food and feedstuffs: their chemistry and nutritive values, 3rd edn. Ibadan: Ibadan University Press
8. Oyolu C (1977) A quantitative and qualitative study of seed types in eugusi *Colocynthis citrullus* (L.). Trop Sci 19:55–62
9. Rosen H (1957) A modified ninhydrin colorimetric analysis for amino acids. Arch Biochem Biophys 67:10–15
10. Shibasaki K, Hesseltine CW (1962) Miso fermentation. Econ Bot 16:180–195
11. Trevelyan WE, Proctor DF, Harrison JS (1958) Detection of sugars on paper chromatogram. Nature 166:444–445
12. Wang HL, Hesseltine CW (1965) Studies on the extracellular proteolytic enzymes of *Rhizopus oligosporus.* Can J Microbiol 11
13. Wang HL, Hesseltine CW (1970) Sufu and Lao-Chao. J Agric Food Chem 18: 572–575
14. Wang HL, Swain EW, Wallen LL, Hesseltine CW (1975) Free fatty acids identified as antitryptic factor in soybeans. J Nutr 105:1351–1355
15. Yong FM, Wood BJB (1977) Biochemical changes in experimental soy sauce *koji.* J Food Technol 12:163–175
16. Yong FM, Wood BJB (1977) Biochemical changes in experimental soy sauce Moromi. J Food Technol 12:263–273