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CHANGES IN FUNCTIONAL PROPERTIES AS A MEASURE OF BIOCHEMICAL DETERIORATION OF STORED SOYBEAN DADDAWA CONDIMENT

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Abstract. Soybean daddawa is a popular condiment made by fermenting dehulled seeds of soybean (*Glycine max*); it is commonly used in soups in the soybean growing areas of Nigeria. Soybean daddawa prepared using three different processing methods (traditionally fermented seeds, seeds fermented with salt (NaCl) and seeds fermented with starter cultures) was evaluated for biochemical changes in ambient storage conditions. The fermented product was assessed for pH, titratable acid, peroxide, free fatty acids, antioxidant capacities as well as water and fat absorption capacities as indices of deterioration. pH of stored samples increased in the alkaline range, titratable acidity of samples also increased with storage. Peroxide values of samples increased significantly from 3.87 meq/kg on first day of storage to 30.73 meq/kg in two weeks on naturally fermented soy daddawa samples. Values obtained for FFA also increased with storage period, while, antioxidant capacity, water and fat absorption capacities decreased drastically with storage. Although, the values obtained for the parameters monitored differed with the methods of processing, the differences were not significant ($p > 0.05$) however; values obtained at the start of the experiment and after 14 days of storage differed significantly. Most of the biochemical changes in monitored functional properties of the samples became noticeable on the 4th and 6th day of storage. The results are discussed with reference to food safety and shelf--life of the product.

Key words: soybean daddawa, condiment, deterioration, storage, shelf-life

INTRODUCTION

Soybean (*Glycine max* (L) Merr.) is valued in many parts of the world for its high protein content. Recently, in Nigeria, the use of soybean in diets has been receiving attention. A popular product that is common in the soybean producing areas of the

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country is soybean daddawa (also referred to as soy-daddawa). Daddawa is a condiment used in soups for taste enhancement and more importantly its usage is a way of incorporating soybean protein in diets.

The preparation of soybean daddawa from dehulled soybean seeds is done mainly by traditional art using rudimentary utensils, there are however few cases of commercial production on small-scale basis. Production is by fermentation of cleaned, dehulled soybean seeds; the end product is similar in its characteristic stickiness and pungent smell to that of *Iru* made from fermented locust bean (*Parkia biglobosa*) seeds [Odunfa 1985, Ogbadu and Okagbue 1988, Abiose et al. 1988]. Reports of Popoola and Akueshi [1985], Omafuvbe [1994] on the microbiology of soybean daddawa have implicated species of *Bacillus* sp. as microorganisms playing a major role involved in the fermentation process. In this respect, the product is similar to *Oso* in Nigeria [Popoola et al. 2006], *soumbala* in Burkina Faso [Ouoba et al. 2003], kinema [Sarkar et al. 1993], Natto [Ohta 1986] and meju [Kim et al. 1997], which are fermented products made from soybean that are popular in Asia.

There are reports on the biochemical changes accompanying fermentation of the seeds [Omafuvbe et al. 2000] and on the nutritive value [Popoola and Akueshi 1986] of soybean daddawa. Attempts have also been made to identify the specific roles of the bacterial species involved in the fermentation process. The use of starter culture has also been investigated [Omafuvbe et al. 2002]. This is with a view to optimize production process.

Fresh soybean daddawa is prone to quick deterioration at the end of the fermentation period. This poor storage factor has a limiting effect on the acceptability of the product. This problem is encouraged by the fact that the product has high moisture content and the organisms of fermentation are still present in the product at consumption, thus it is difficult to distinguish between the end of acceptable product fermentation and when deterioration in the quality of product sets in. This paper examines some of the biochemical changes in stored soybean daddawa after the usual 72 hours of fermentation for the production of soybean daddawa. This is with a view to providing food safety information and the potential risks associated with the consumption of stored unpreserved product. The study will also give an insight into ways of extending the shelf life of the product.

MATERIALS AND METHODS

Collection of seeds and preparation of daddawa

Soybean seeds (variety TGX 1440-2E) used for the study were obtained from the Institute of Agricultural Research and Training (IART), Moor Plantation, Ibadan, Nigeria. Soybean daddawa was prepared using the procedure described by Popoola and Akueshi [1985]. In another approach, traditional method of preparation was used but with salt added (1% w/w) to the product on the first day of fermentation. A third batch of fermented product was done in a controlled setting replacing the rudimentary equipment used in the traditional method with sterile glassware. Starter cultures were also introduced into the fermentation medium at the onset of fermentation.

Preparation of starter culture

Strains of *Bacillus subtilis*, *B. pumilus* and *B. licheniformis*, which were representatives of isolates obtained from naturally fermenting soybean daddawa, were used. Preparation and inoculation of cultures was as described by Omafuvbe et al. [2002].

Biochemical investigations

Antioxidant capacity, pH value, titratable acidity (TA), free fatty acid (FFA) and peroxide content of stored products were monitored for 14 days after the normal 72 hours of fermenting the seeds into daddawa. pH value and titratable acidity was assessed using the methods of Ikenebomeh [1989]. Fat and water absorption capacity of samples were determined using the centrifugation method previously described by Sosulki et al. [1978], while the procedures of Sanchez et al. [2003] was used for the determination of antioxidant capacity. The free fatty acid content (as percentage oleic acid) of stored daddawa and peroxide values were estimated using alkali titration method described in Pearson [1985].

Statistical analysis

Data obtained were expressed as the mean ± standard deviation (SD). The statistical significance of differences was tested using analysis of variance.

RESULTS AND DISCUSSIONS

The pH and the titratable acidity of stored soybean daddawa increased steadily with days of storage (Table 1). The observed pH trend is in accordance with the reports of Omafuvbe et al. [2000] during the fermentation of soybean seeds. Increasing pH during fermentation has been attributed to proteolytic activities and the release of ammonia by microorganisms involved in the fermentation. The released ammonia is responsible for the pungent smell that usually accompanies most vegetative protein fermentation [Sarkar et al. 1993]. The implication of the observed result is that there is likelihood of continued fermentation even in storage. Differences observed in pH with prolonged storage and with the different methods of preparation were not significantly different ($p > 0.05$).

Titratable acid (TA) values increased significantly with storage period. The increase was rather pronounced on the first two days of storage for all the samples. The general trend during fermentation of seeds into daddawa is that TA increases with days of fermentation [Omafuvbe et al. 2000]. However, simultaneous increases in TA and pH have been reported [Ikenebomeh et al. 1986, Wagenknecht et al. 1961].

The concurrent increase in pH and titratable acidity is an indication that both acid and alkaline producing activities were at work in the stored condiment. This may be due to the fact that while proteolytic activities were taking place on the protein components of the seeds; the carbohydrate components were also being hydrolyzed to sugars and organic acids. A significant reduction in crude protein and carbohydrate was reported to accompany the fermentation of the soybean seeds into daddawa [Popoola and Akueshi 1986].

Table 1. Changes in pH and titratable acidity of stored soy-daddawa, mg lactic acid/g^{-1}
Tabela 1. Zmiany pH i kwasowości miareczkowej w czasie przechowywania przyprawy sojowej daddawa, mg kwasu mlekowego/g^{-1}

Storage time days Czas przechowywania dni	Processing methods – Metody produkcji					
	natural fermentation naturalna fermentacja		natural fermentation + salt naturalna fermentacja + sól		fermentation with starter culture fermentacja z kulturami startowymi	
	pH	titratable acidity kwasowość miareczkowa	pH	titratable acidity kwasowość miareczkowa	pH	titratable acidity kwasowość miareczkowa
0	7.83^{e}	0.047^{g}	7.95^{e}	0.053^{e}	7.48^{f}	0.045^{c}
2	8.06^{d}	0.093^{f}	8.05^{d}	0.103^{d}	7.94^{d}	0.101^{b}
4	8.11^{c}	0.096^{ef}	8.08^{d}	0.104^{cd}	7.98^{d}	0.102^{b}
6	8.16^{b}	0.099^{de}	8.11^{c}	0.108^{c}	8.44^{a}	0.105^{b}
8	8.17^{b}	0.103^{d}	8.15^{b}	0.116^{b}	8.18^{bc}	0.106^{b}
10	8.17^{b}	0.112^{c}	8.18^{ab}	0.116^{b}	8.16^{b}	0.106^{b}
12	8.23^{a}	0.124^{b}	8.18^{ab}	0.124^{a}	8.14^{b}	0.107^{b}
14	8.24^{a}	0.134^{a}	8.20^{a}	0.125^{a}	8.12^{c}	0.135^{a}

Values are means of three replicates.
Means with different superscripts within columns are significantly different at $p < 0.05$.
Wartości średnie z trzech powtórzeń.
Średnie oznaczone różnymi literami alfabetu w kolumnach są statystycznie istotne dla $p < 0,05$.

The peroxide content value of foods is an indication of the extent of peroxidation of the food. In this study, peroxide contents of the fermented seeds increased with storage, with a significant leap on the 4th day (Table 2). The values obtained were however less for product made with starter cultures. This observation corresponds with that of Iwe [1991] on soymilk even though the values obtained for soy daddawa were much higher.

Pearson [1985] opined that for fatty foods at peroxide value of 20-40 meq/kg rancidity sets in. In this study, for most of the samples, at the end of the fourth day of storage, peroxide value obtained was much higher (particularly for samples which were fermented naturally) than the lower range of suggested value at which rancidity start to manifest. If increasing peroxide value is a good predictor of deterioration as suggested by Zia-ur-Rehman et al. [2003] and a measure of oxidation of food product taken as an essential factor in food spoilage, it may then be inferred that deterioration of daddawa began after four days of storage.

The lower values observed for the product made with added salt may be due to the preservative effect of sodium chloride. Often, salt crystals are added to traditionally fermented soy-daddawa to prolong its shelf life. Ikenebomeh [1989] even though consumers have no knowledge of the principles involved. While the exclusion of unwanted organisms in the batch prepared using starter cultures may be responsible for the lower peroxide values observed within the first 10 days of storage.

Table 2. Peroxide values of stored soybean daddawa, meq/kg^{-1}
Tabela 2. Liczba nadtlenkowa w czasie przechowywania przyprawy sojowej daddawa, meq/kg^{-1}

Storage time days Czas przecho-wywania dni	Processing methods – Metody produkcji		
	natural fermentation fermentacja naturalna	natural fermentation + salt fermentacja naturalna + sól	controlled fermentation with starter culture fermentacja kontrolowana z kulturami startowymi
0	3.87 ± 0.25^{g}	3.87 ± 0.62^{g}	2.60 ± 0.49^{g}
2	5.20 ± 1.20^{f}	7.67 ± 0.34^{f}	4.40 ± 0.33^{f}
4	9.40 ± 0.49^{e}	10.80 ± 0.33^{e}	5.40 ± 0.59^{e}
6	21.40 ± 0.98^{d}	16.40 ± 0.59^{d}	13.53 ± 0.83^{d}
8	22.87 ± 0.25^{c}	18.93 ± 0.98^{c}	16.27 ± 0.19^{c}
10	29.27 ± 0.50^{b}	22.40 ± 0.59^{b}	19.73 ± 0.50^{b}
12	30.33 ± 0.34^{ab}	24.73 ± 0.34^{a}	21.40 ± 0.33^{a}
14	30.73 ± 0.25^{a}	25.40 ± 0.33^{a}	21.80 ± 0.16^{a}

Values are means ± standard deviation of three replicates.
Means with different superscript are significantly different at $p < 0.05$.
Wartości średnie ± odchylenie standardowe z trzech powtórzeń.
Średnie oznaczone różnymi literami alfabetu w kolumnach są statystycznie istotne dla $p < 0,05$.

The FFA contents in stored soy-daddawa increased progressively and significantly ($p < 0.05$) with days of storage (Table 3). Differences were also observed in the FFA content of samples prepared using the different methods.

Of the three methods of production the highest values of FFA were recorded for samples prepared with starter cultures. Soybean is an oilseed [Wolf and Cowan 1971], soybean daddawa contains 33.44% oil [Popoola and Akueshi 1986], the relevance of FFA in monitoring degradation cannot be ignored as the formation of organic acids and free fatty acids is an initial step in fat deterioration, development of rancidity and off flavours in fatty foods. With most fatty foods, rancidity becomes noticeable when FFA content (calculated as percentage oleic acid) is about 0.5-1.5% [Pearson 1985]. Although the values of FFA observed on the last day of the experiment are lower than this value, it is assumed that with prolonged storage, the FFA content of samples will increase well above this critical value. Traditionally, prepared soybean daddawa is often kept for periods longer than one month before it is consumed, by which time off flavour must have set in. However, the pungent smell associated with the product makes off flavour unnoticeable. Again, the lower values recorded for samples to which salt was added and samples prepared with starter cultures is an indication of the effectiveness of salt as a preservative acting by excluding unwanted organisms, retarding activities of others and therefore delaying oxidative degradation.

Lipase activity generally is low in soybean fermentation [Omafuvbe 2000]. Although *Bacillus* sp. is not known to be good producers of lipase [Aderibigbe and Odunfa 1990], rancidity of the product is not considered as a major problem associated with the product fermentation but may be important in spoilage as the possibility of invasion of the modified environment by lipase producing organisms cannot be completely ignored.

Table 3. Changes in free fatty acids (FFA) of stored soybean daddawa
Tabela 3. Zmiany zawartości wolnych kwasów tłuszczowych (FFA) w czasie przechowywania przyprawy sojowej daddawa

Storage time days Czas przecho-wywania dni	Processing methods – Metody produkcji		
	natural fermentation fermentacja naturalna	natural fermentation + salt fermentacja naturalna + sól	controlled fermentation with starter culture fermentacja kontrolowana z kulturami startowymi
0	0.021 ± 0.001^{e}	0.017 ± 0.002^{e}	0.018 ± 0.001^{e}
2	0.024 ± 0.001^{de}	0.018 ± 0.001^{e}	0.025 ± 0.002^{d}
4	0.024 ± 0.001^{de}	0.021 ± 0.003^{de}	0.028 ± 0.002^{d}
6	0.026 ± 0.004^{d}	0.024 ± 0.003^{d}	0.030 ± 0.003^{d}
8	0.048 ± 0.002^{c}	0.039 ± 0.003^{c}	0.052 ± 0.001^{c}
10	0.055 ± 0.004^{b}	0.048 ± 0.002^{b}	0.066 ± 0.003^{b}
12	0.059 ± 0.002^{b}	0.055 ± 0.001^{a}	0.069 ± 0.001^{b}
14	0.069 ± 0.001^{a}	0.056 ± 0.002^{a}	0.075 ± 0.001^{a}

FFA recorded as percentage oleic acid in sample.
Values are means ± standard deviation of three replicates.
Means with different superscripts are significantly different at $p < 0.05$.
FFA jako procent kwasu oleinowego w próbie.
Wartości średnie ± odchylenie standardowe z trzech powtórzeń.
Średnie oznaczone różnymi literami alfabetu w kolumnach są statystycznie istotne dla $p < 0,05$.

Generally, antioxidant capacity decreased significantly by the 14th day of storage irrespective of the method of production (Table 4). The decrease is an indication of oxidative degradation. The implication of this is that the product has lost its ability to combat oxidative degradation, therefore exposing the food to uncontrolled decomposition and oxidative processes. This has nutritional implications, as most of the composite nutrients would have been degraded.

A gradual decrease was observed in water absorption capacity of stored soy-daddawa irrespective of the method of production. A somewhat similar trend was observed with values recorded for fat absorption capacities of the samples except that there were no significant differences in samples with the duration of storage (Table 5). The observed trend was similar to those reported by Obatolu et al. [1998] on soybean seeds fermented into daddawa.

Water absorption capacity (WAC) is an indication of a product to associate with water in conditions where water is limiting [Giami 1993] while fat absorption capacity (FAC) could be attributed to the physical entrapment of oils (in the seeds) which is related to number of non-polar side chains on the proteins that bind hydrocarbon chains of the fatty acids. These two functional attributes of foods could be important in monitoring spoilage as the ability of the proteins to bind fats is important since fat acts as flavour retainer and increases mouth feel of foods [Kinsella 1976].

Fat absorption capacity was not seriously affected by storage as minimal changes (decrease) were observed, this might be related to the slow rate at which fat is degraded in stored daddawa.

Table 4. Antioxidant capacity (measured as ascorbic acid equivalent) in stored soybean daddawa
Tabela 4. Pojemność antyoksydacyjna (jako ekwiwalent kwasu askorbinowego) w czasie przechowywania przyprawy sojowej daddawa

Storage time, days Czas przechowywania dni	Processing methods – Metody produkcji		
	natural fermentation fermentacja naturalna	natural fermentation + salt fermentacja naturalna + sól	controlled fermentation with starter culture fermentacja kontrolowana z kulturami startowymi
0	423.33 + 2.36[a]	398.33 ± 3.12[a]	386.67 ± 3.12[a]
2	390.00 + 5.80[ab]	393.33 ± 2.36[ab]	373.33 ± 4.71[ab]
4	350.00 + 4.08[b]	365.24 ± 4.08[ab]	348.33 ± 2.36[b]
6	351.67 + 2.36[b]	355.01 ± 4.08[b]	343.33 ± 2.36[b]
8	120.00 + 5.14[c]	128.33 ± 4.48[c]	115.00 ± 3.14[c]
10	108.33 + 4.43[c]	90.00 ± 3.51[cd]	110.00 ± 5.60[c]
12	80.00 + 4.42[cd]	90.00 ± 3.51[cd]	100.02 ± 5.49[cd]
14	60.02 + 3.14[d]	60.00 ± 3.14[d]	70.00 ± 4.59[d]

Values are means ± standard deviation of three replicates.
Means with different superscript are significantly different at $p < 0.05$.
Wartości średnie ± odchylenie standardowe z trzech powtórzeń.
Średnie oznaczone różnymi literami alfabetu w kolumnach są statystycznie istotne dla $p < 0,05$.

Table 5. Water and fat absorption capacities in stored soybean daddawa
Tabela 5. Zmiany absorpcji wody (WAC) i tłuszczu (FAC) w czasie przechowywania przyprawy sojowej daddawa

Storage time, days Czas przechowywania dni	Processing methods – Metody produkcji					
	natural fermentation fermentacja naturalna		natural fermentation + salt fermentacja naturalna + sól		fermentation with starter culture fermentacja z kulturami startowymi	
	WAC %	FAC ml/g	WAC %	FAC ml/g	WAC %	FAC ml/g
0	190	0.9	180	0.8	150	0.4
2	153	0.9	140	0.8	137	0.5
4	140	0.8	140	0.7	120	0.5
6	137	0.8	130	0.7	113	0.6
8	133	0.7	120	0.7	113	0.6
10	130	0.7	117	0.6	113	0.6
12	120	0.6	107	0.5.	103	0.6
14	117	0.6	103	0.5	100	0.7

WAC – water absorption capacity, FAC – fat absorption capacity.
Values are means ± standard deviation of three replicates.
WAC – absorpcja wody, FAC – absorpcja tłuszczu.
Wartości średnie ± odchylenie standardowe z trzech powtórzeń.

With respect to WAC, Giami and Bekeham [1992] reported that fermentation does not affect WAC in legumes. The observed decrease in WAC may imply that even in wet form of daddawa, less water is available for microbial activities.

CONCLUSIONS

The results of this study has shown that the use of some of the biochemical indices examined particularly peroxide values, free fatty acids and antioxidant capacity could be a good measure of monitoring spoilage of soybean daddawa. Although, traditionally prepared daddawa is often kept for up to a month before consumption (after 72 h of fermentation), results of this study showed that after 4-6 days of storage, the product begin to deteriorate biochemically. The consumption of product stored beyond this period may have deleterious health implications.

The addition of 1% (w/w) sodium chloride to the fermenting product could be a simple and practical way of extending the shelf-life of soybean daddawa, however, this may slightly affect the organoleptic properties of the product. Ikenebomeh [1989] made a similar observation on African locust bean seeds fermented into a condiment 'Iru'. Further studies on the microflora of the product after the appropriate period of storage are going on.

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WŁAŚCIWOŚCI FIZYKOCHEMICZNE JAKO MIERNIK PRZECHOWALNICZYCH ZMIAN JAKOŚCI SOJOWEJ PRZYPRAWY SMAKOWEJ DADDAWA

Streszczenie. Daddawa jest popularną przyprawą smakową otrzymywaną w procesie fermentacji soi. Otrzymuje się ją z użyciem trzech głównych metod: tradycyjnej naturalnej fermentacji ziaren soi, fermentacji w obecności soli (NaCl) oraz z wykorzystaniem kultur startowych. W pracy zbadano wpływ zastosowanej metody na wyniki ocen podstawowych wyróżników fizycznych i chemicznych pozwalających na ocenę jakości wyrobu. Dodatek 1% soli (NaCl) jest prostą metodą przedłużenia trwałości wyrobu, ale powoduje nieznaczne pogorszenie jego właściwości sensorycznych.

Słowa kluczowe: soja, daddawa, przyprawa, trwałość, przechowywanie

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