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Effect of boiling and roasting on the fermentation of soybeans into dawadawa (soy-dawadawa)

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Abstract

Soybeans which had initially been dehulled by either boiling (boiled/dehulled) or roasting (roasted/dehulled) before peeling, were cooked and fermented into dawadawa, a traditional food condiment. The micropopulation, enzymatic activities, proximate composition, amino acid, and aroma profiles of the two types of soybean dawadawa were evaluated during fermentation. Only minor differences were found in the microbial profiles of the two types of soy-dawadawa. Although boiled/dehulled soy-dawadawa initially had lower microbial counts, it recorded higher counts at the advanced stages of fermentation. Proteolytic and amylolytic *Bacillus* species including *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus cereus*, and *Bacillus firmus* dominated the micropopulation of the two types of soy-dawadawa with *Bacillus subtilis* accounting for about 50% of the *Bacillus* species in all samples. Lactic acid bacteria and yeasts occurred in low numbers in the two types of soy-dawadawa. The proximate composition of the two types of soy-dawadawa were similar, and their contents of moisture and protein increased whilst fat and ash decreased during fermentation. Both types of fermenting soy-dawadawa recorded similar levels of α-amylase activity, but boiled/dehulled soy-dawadawa showed slightly higher protease activity. The levels of isoleucine, leucine, lysine, phenylalanine, arginine and proline increased significantly with fermentation time in both types of soy-dawadawa. With respect to differences in their aroma profiles, hexanodecanol, octadecyl acetate, 1,2-dimethyl benzene, tetradecene, (*E*)-5-eicosene, cyclohexadecane, and hexacosane were found only in the roasted/dehulled samples, whilst 1,2-ethanediol, ethyl acetate, dimethyl disulfide, cyclotetradecane, decene, indole, 2 butyl-octenal, acetophenone, and toluene were found only in the boiled/dehulled samples. A market focus group showed preference for roasted/dehulled soy-dawadawa over boiled/dehulled soy-dawadawa. Apart from the volatile aroma compounds, the biochemical and microbiological profiles of the two types of soy-dawadawa showed only minor differences and were also similar to the profiles reported for African locust bean dawadawa.

Keywords: Fermentation; Soybeans; Dawadawa; *Bacillus* species; Soy-dawadawa

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1. Introduction

The diet of a majority of people in some parts of West Africa are based largely on starchy staples such as maize, sorghum, rice, cassava, yams and plantains. In these areas, animal protein is relatively scarce and expensive. To combat problems of protein malnutrition that may result from such food consumption patterns, the use of new and unconventional low cost high quality protein foods are being encouraged. One such product is dawadawa, also called soumbala, iru, afitin or netetu in different West and Central African countries. Dawadawa is a high protein traditional food condiment, which is used to flavour soups and stews, and is produced by the alkaline fermentation of African locust bean seeds (*Parkia biglobosa*). Low income families often use it generously as a low-cost meat substitute and in such instances it contributes to the protein and calorific content of the diet.

Dawadawa is traditionally prepared by initially dehulling the seeds of the African locust beans involving cleaning, boiling, pounding and separating the seed coat from the cotyledons by floating in water. The cotyledons are re-boiled and packed into baskets or perforated pots and allowed to ferment spontaneously for about 48 h. Before fermentation, ash, maize, or millet flour is sprinkled on the cotyledons. The fermented cotyledons, which have an appearance of a dark brown sticky mass with a strong ammoniacal smell, are sundried and moulded into balls and sold in the local markets.

Several constraints are encountered during the processing of locust bean seeds into dawadawa and this has given rise to the use of alternative raw materials for making dawadawa. African locust bean trees grow in the wild and long hours are spent hunting for seeds that are only produced seasonally. Processing the seeds into dawadawa is labour intensive and time consuming due to the hard seed coat, which must be removed by dehulling before the cotyledons are fermented. Currently, soybeans are being promoted in some West African countries as an alternative raw material for making dawadawa. Soybeans in comparison to locust bean seeds are also rich in proteins, fats, vitamins and minerals, and make acceptable dawadawa. Soybeans are easy to grow, can be obtained all year round and require easier dehulling procedures, which are relatively less labour intensive, requiring less energy. Two methods are being used for the treatment of soybeans prior to fermentation. In the first method, the soybeans are boiled and then rubbed between the palms or are pounded to remove the seed coat. In the other method, the soybeans are roasted and then rubbed between the palms or pounded to remove the seed coat. The dehulled soybeans are re-boiled, fermented and sundried just as the African locust beans. Both methods of dehulling soybeans make acceptable dawadawa, and soybean dawadawa is sold in some of the local markets.

Although traditional African locust bean dawadawa has been the subject of scientific investigations for a number of years, only few studies have been carried out on soy-dawadawa including Ogbadu and Okagbue (1988), Barimalaa et al. (1994) and Omafuvbe et al. (2000).

This study was carried out to confirm the dominant microbial species responsible for the fermentation of soybeans into dawadawa, and also to study the effect of the initial pretreatments of boiling and roasting on the microbiological and biochemical changes which occur during the subsequent fermentation of the dehulled soybeans.

2. Materials and methods

2.1. Brief field study

A brief field study was conducted in Accra involving visits to three of the local markets to investigate the different types of dawadawa available on the market. The study involved informal interviews of traders selling dawadawa to find out the different types of dawadawa available, raw materials used for processing, production methods, and consumer preferences.

2.2. Production and sampling of soy-dawadawa

Soy-dawadawa samples were prepared in the laboratory for analysis using the two common methods for dehulling soybeans. In the first method, about 5 kg of soybeans were boiled for 30 min, and dehulled by rubbing between the palms. In the second method, about 5 kg of soybeans were roasted in a pan over a fire for 30 min, and also dehulled by hand. In both cases

the dehulled beans were washed, boiled for 1 h, drained, cooled, and packed into a basket lined with a plastic sack, and allowed to ferment at ambient temperature (ca 32 °C) for 72 h. The fermenting beans were sampled at 0, 24, 48, and 72 h. After fermentation, the soy-dawadawa were sundried for 2 days, and also sampled for analysis. Each experiment was replicated.

2.3. Microbiological analysis

2.3.1. Enumeration and isolation of bacteria and yeasts

For all samples, 10 g were added to 90 ml sterile diluent containing 0.1% peptone (Difco 0118-17, Becton Dickinson and Co, Sparks, MD, USA), 0.85% NaCl, with pH adjusted to 7.0 and homogenized in a stomacher (Lab Blender, Model 4001, Seward Medical, London, England) for 120 s at high speed. From appropriate ten-fold dilutions, enumeration of aerobic mesophiles was carried out on Plate Count Agar (Difco 0479-17-3, Difco Laboratories, Detroit, MI, USA) incubated at 37 °C for 3 days. Lactic acid bacteria were isolated on DeMan, Rogosa and Sharpe Agar (MRS, Merck 10660, Merck, Darmstadt, Germany) incubated anaerobically in an anaerobic jar with anaerocult A (Merck) at 30 °C for 5 days and yeasts on Malt Extract Agar (Merck 5398) containing 100 mg chloramphenicol (Sigma C-0378, Sigma Chemical Co, St Louis., MO, USA) and 50 mg chlorotetracycline (Sigma C-4881) per litre and incubated at 25 °C for 5 days.

All colonies totalling to about 20 from a segment of the highest dilution or suitable PCA and MRS plates were subcultured on Nutrient Agar (Difco 0001-17, Difco Laboratories, Detroit, MI, USA) and MRS Agar (MRS, Merck 10660), respectively, and streaked successively until pure colonies were obtained. The purified isolates were initially examined by colony and cell morphology, Gram reaction, and catalase production.

2.3.2. Identification of Bacillus species

Gram-positive catalase-positive rods, some bearing phase bright spores, isolated aerobically on PCA plates, were examined according to Claus and Berkerley (1986) and Parry et al. (1983) for characterisation and confirmation of their genus as *Bacillus*. Tests carried out were anaerobic growth; acid production from D-glucose, L-arabinose, D-xylose and D-mannitol; hydrolysis of casein and starch; growth at pH 5.7, in 6.5% and 10% (w/v) NaCl; and at 37 and 65 °C. The species of *Bacillus* isolates were confirmed by examining them for their ability to ferment 49 different carbohydrates using the API 50 CHB kit (Bio Mérieux S.A., Marcy-l'Etoile, France).

2.3.3. Characterisation of lactic acid bacteria

Isolates on MRS which were Gram-positive, catalase-negative regular rods, coccoid and cocci were assumed to be lactic acid bacteria, and further examined by gas production in MRS broth with Durham tube and also in MRS broth in which glucose was replaced with gluconate as sole carbon source; growth at 10 °C and 45 °C; growth at pH 4.4 and 9.6; growth in 6.5% and 18% (w/v) NaCl; and Hugh and Leifson's (1953) test.

2.4. Enzymatic analysis

2.4.1. Screening of isolates for proteolytic and amylolytic activities

Cultures were streaked on surface dried plates of Skim Milk Agar containing g/l distilled water; 100, Skim Milk (Difco 0032-17-3) and 20, Agar (Merck 1.01614) for determination of proteolytic activity, and Starch Agar containing g/l distilled water; 10 Starch (Merck Art 1259) and 23, Nutrient Agar (Difco 0001-17) for determination of amylolytic activity, and incubated at 37 °C for 3 days. Enzymatic activity was indicated as clearing zones on the plates. For observation of amylolytic activity, the agar plates were first flooded with iodine solution (Aldrich 20,777-2, Aldrich Chemical Co. Inc., Milwaukee, WI, USA).

2.4.2. Determination of protease and amylase activities in fermenting soy-dawadawa

An enzyme extract of the fermenting soybeans was prepared by grinding 5 g of sample in 50 ml of 0.1 M sodium hydrogen phosphate buffer, pH 6.5 as the extracting buffer. The suspension was washed with petroleum ether (Aldrich 26,173-4) to extract the oil and centrifuged at 4000×*g* for 5 min (Yong and Wood, 1977). The supernatant constituting the crude enzyme extract, was stored at −20 °C.

Protease activity was determined by a modification of the assay method described by Abiose et al. (1988)

and Omafuvbe et al. (2000). 5 ml of enzyme extract were added to 10 ml of 2% light soluble casein solution (BDH, BDH Chemicals Ltd. Poole, England), and incubated at 35 °C for 30 min. The reaction was terminated by adding 10 ml of 10% trichloroacetic acid (Merck 100807). Undigested protein was removed by centrifugation at 4000×*g* for 15 min. The trichloroacetic acid soluble peptides in the supernatant was determined by the method of Lowry et al. (1951) using tyrosine (Aldrich T9,039-5) as standard solution. One unit of protease activity was defined as the amount which produced 1.0 μmol of tyrosine in 1.0 ml of the trichloroacetic acid-soluble peptides under assay conditions.

Alpha amylase activity was determined by the assay method of Bernfeld (1955). 2 ml of the enzyme extract were mixed with 1 ml of 1% starch solution (Merck Art 1259) and incubated for 1 h at 40 °C. The reaction was stopped by adding 3 ml dinitrosalicylic acid reagent (Aldrich 12,884-8), heated for 5 min, cooled, diluted with 18 ml distilled water, and the optical density measured at 550 nm using a spectrophotometer (Spectronic 21D, Milton Roy). A blank determination was carried out in which the dinitrosalicylic acid reagent was added before the starch solution. The amount of reducing sugars formed was calculated from a standard curve prepared with known concentrations of maltose (Merck 105912) according to Bernfeld (1955).

2.5. Chemical analyses

2.5.1. Determination of pH and titratable acidity

Fermenting soy-dawadawa sample weighing 10 g was homogenized in a blender with 90 ml distilled water, and the pH was determined with pH meter (Radiometer PHM 92, Radiometer Analytical A/S, Bagsvaerd, Denmark). Titratable acidity was determined by the titration of 80 ml of filtrate obtained from 10 g of soy-dawadawa sample dissolved in 250 ml distilled water against 0.1 N NaOH using phenolphthalein as indicator. 1 ml of 0.1 N NaOH was taken as equivalent to 9.008×10^{-3} g lactic acid.

2.5.2. Proximate composition

Moisture, crude protein, crude fat, and ash contents of soy-dawadawa samples were determined by standard AOAC (1990) methods.

2.6. Determination of amino acid profile

Amino acids in fermenting soy-dawadawa samples were separated using a modification of the Pico-Tag method (Cohen et al., 1989) by Anonymous (1995) and determined with an Amino Acid Analyzer comprising Waters 717 Plus Autosampler, Waters 510 HPLC Pump and Waters™ 486 Tunable Absorption Detection (Waters, Milford, MA, USA). A sample, corresponding to 30 mg of protein, was mixed with 60 ml of 6 M hydrochloric acid (Merck 1.00317), 300 μl of 0.1 M dithiothreitol (Sigma N6752) and 5 ml of 6.25 mM norleucine DL (Sigma N-6752) as internal standard, and hydrolysed at 110 °C for 22 h. It was made up to 100 ml and filtered through an 0.45 μm Millipore filter. 20 μl of 2.5 mM amino acid standard H (Pierce 20088, Pierce, Rockford, IL, USA) and 20 μl of 2.5 mM norleucine (Sigma N-6752) were added to 20 μl of the hydrolysed sample, and evacuated to dryness for 45 min in the vacuum work station. It was neutralised with 30 μl of redry solution containing methanol (Fisons M/4056/17, Fisons plc, Scientific Equipment Division, Loughborough, England), 0.2 M sodium acetate (Merck 6268) and trimethylamine (Aldrich 23,262-3) in a ratio of 2:2:1, and evacuated to dryness for 45 min. It was derivatised by adding 20 μl of derivatisation solution containing methanol (Fisons M/4056/17), distilled water, trimethylamine (Aldrich 23,262-3), and phenylisothiocyanate (Pierce 26922) in a ratio of 7:1:1:1. After standing for 10 min, it was evacuated to dryness for 15 min, 40 μl of methanol (Fisons M/4056/17) added and evacuated to dryness for 2 h. The dried sample was dissolved in 200 μl sample diluent containing per litre; 19 g, sodium acetate (Merck 6268), 0.5 ml, trimethylamine (Aldrich 23,262-3), 0.25 mg, EDTA (Sigma ED2SC), and 120 ml acetonitrile (Fisons A/0626/17) with pH adjusted to 6.1 using acetic acid (Aldrich 32,009-9), and filtered through Whatman no. 1 filter paper. The derivatives were analysed with reverse phase HPLC using gradient elution and UV detection at 254 nm.

2.7. Determination of aroma volatile compounds

2.7.1. Extraction of aroma volatile compounds

The aroma compounds present in the soy-dawadawa samples were extracted using the procedure described by Nickerson and Likens (1966) using a

micro-scale steam distillation low density solvent extraction device (micro-SDE, Chrompack, Middelburg, Holland). Soy-dawadawa sample weighing 18 g, was diluted with 400 ml of distilled water and 1 ml of 50 ppm 4-methyl-1-pentanol added as an internal standard in a 1 l Erlenmeyer flask. 6 ml of a 1:1 mixture of pentane (Fluka 76875, Fluka Chemie, Buchs, Switzerland) and diethyl ether (J.T. Baker, UN1153, Mallickrodt Baker B.V., Deventer, Holland) was measured into a 9-ml pear-shaped solvent flask, and both flasks connected to the distillation apparatus. Both flasks were brought to the boil, and extraction of the volatile compounds continued for 30 min from the beginning of condensation of vapours on the walls of the condenser. The collected solvent phase was purified by freezing out the water at −18 °C. The solvent extract was poured off and dried over 2 g of Na_2SO_4 and concentrated to about 100 mg by blowing nitrogen gas over the surface. The concentrated extract was analysed for volatile compounds using GC–MS. The experiment was replicated.

2.7.2. Determination of aroma volatile compounds by GC–MS

Separation and identification of volatile compounds in the extract was carried out in a Hewlett Packard G1800A GCD System (GC–MS, Hewlett-Packard, Palo, California, USA) equipped with a Hewlett-Packard DB-WAX column (30 m × 0.25 μm i.d., × 0.25 mm film thickness. 2 ml of the extract were injected into the GC–MS in a split ratio of 10:1 using a temperature programme of 10 min at 40 °C increased to 240 °C at 6 °C/min, and held constant for 30 min. Identification of compounds was carried out in the total ion scanning mode by matching spectra with reference from NBS75KD database of Enhanced Chemstation G1701AA Version A.03.00 software (Hewlett-Packard) (Annan et al., 2002).

2.8. Sensory analysis

Boiled/dehulled and roasted/dehulled soy-dawadawa samples were evaluated for preference by a market focus group in the Makola market in Accra. This consisted of four groups of dawadawa retailers who were also regular dawadawa consumers. Each group was made up of six individuals, and the samples were assessed informally for preference based on colour, appearance, texture, odour, taste, and overall acceptability.

2.9. Statistical analysis

The effect of the method of sample preparation (boiling or roasting) and sampling/fermentation time on data obtained were subjected to analysis of variance (ANOVA). Significance of differences was defined at $P \leq 0.05$.

3. Results and discussion

3.1. Raw materials used for production of dawadawa

A brief field study of three markets in Accra confirmed that different raw materials are fermented into dawadawa with the preferred raw material varying from one ethnic group to another. Of the dawadawa found on the market, 60% were processed from the African locust beans, 25% from soybeans, 10% from a mixture of African locust beans and soybeans, and 5% from a mixture of African locust beans and groundnuts (*Arachis hypogaea*). In terms of product acceptance, consumers rated dawadawa processed from the African locust beans as the best product followed by soybeans, a mixture of African locust beans and soybeans, and lastly a mixture of African locust beans and groundnuts. Consumers also preferred roasted/dehulled soy-dawadawa to boiled/dehulled soy-dawadawa.

3.2. Alkaline fermentation of soy-dawadawa

The pH and titratable acidity values recorded for fermenting soybeans dehulled after either boiling or roasting of the legume, showed the typical pattern reported for the proteolytic alkaline fermentation of African locust bean seeds into dawadawa i.e. simultaneous rise in pH and acidity. The pH of soybeans dehulled after boiling rose from 6.96 (with standard deviation, $\sigma = 0.24$) to 8.25 ($\sigma = 0.16$) during 72 h of fermentation, whilst simultaneously the acidity increased from 0.07% to 0.42% on wet weight basis (data not shown). In soybeans dehulled after roasting, the pH rose from 6.41 ($\sigma = 0.06$) to 8.22 ($\sigma = 0.10$), whilst acidity increased from 0.12% to 0.42% on wet

weight basis (data not shown). The initial slightly higher pH of soybeans dehulled after boiling could be due to the samples taking up more water (with pH near neutral) than the roasted/dehulled samples as a result of boiling for a longer period i.e. a total of 1.5 h. Analysis of variance showed that both the method of pretreating the soybeans, i.e. boiling or roasting as well as the sampling time during fermentation, affected the pH at the 95% confidence level. With respect to acidity, although the fermentation period affected the acidity, the method of preparation of soy-dawadawa did not have a significant effect. The simultaneous rise in pH and acidity observed in the present work has been reported for fermentations involving legumes and seeds (Wagenknecht et al., 1961; Odunfa, 1985a). According to Hesseltine (1965) the simultaneous increase in pH and acidity during the fermentation of legumes, may be due to the high buffering capacity of the legume beans and microbial proteolytic activity leading to ammonia release characteristic of most vegetable protein fermentation.

3.3. The micropopulation of fermenting soy-dawadawa

The initial microbial population in soybean samples dehulled after boiling were lower than in the samples dehulled after roasting. This was attributed to the severer heat treatment during boiling as compared to roasting, resulting in a greater destruction of the resident micropopulation. However, microbial counts were higher in the boiled/dehulled samples at the advanced stages of fermentation and this was attributed to greater solubilisation of the soybean constitutes in the boiled/dehulled samples favouring microbial growth. In soybean samples dehulled after boiling, the population of aerobic mesophiles increased from 2.5×10^3 to 1.5×10^{11} cfu/g and the lactic acid bacteria from 6.1×10^3 to 1.4×10^6 cfu/g during 72 h of fermentation (data not shown). With respect to the yeast population, an increase was observed during the first 24 h of fermentation, after which yeasts were no longer detectable on the 10^{-2} dilution plates. In soybean samples dehulled after roasting, the micropopulation of aerobic mesophiles increased from 9.0×10^3 to 1.8×10^9 cfu/g and the lactic acid bacteria from 6.3×10^4 to 5.9×10^5 cfu/g during the 72 h of fermentation (data not shown). The yeast population increased during the first 48 h of fermentation but were subsequently not detected on the 10^{-2} dilution plates. In the final product i.e. the sundried fermented samples, the population of aerobic mesophiles and lactic acid bacteria in the samples dehulled after boiling were 6.2×10^8 and 5.4×10^3 cfu/g respectively, whilst in the sample dehulled after roasting they were 5.1×10^8 and 2.2×10^4 cfu/g, respectively. Yeasts were not detected in the final sundried products on the 10^{-2} dilution plates.

3.4. Bacillus species present in soy-dawadawa

The aerobic mesophiles in both types of fermenting soy-dawadawa were dominated by Gram-positive, catalase-positive rods, most bearing phase bright spores. Some Gram-negative bacteria were found in both types of soy-dawadawa but were usually present at levels of less than 10^3 cfu/g and were not found in the 72 h fermented products. The dominating Gram-positive, catalase-positive rods, most bearing phase bright spores, isolated aerobically on Plate Count Agar, were assumed to belong to the genus *Bacillus*. Further examination showed that most of these isolates produced acid from D-glucose, L-arabinose, D-xylose, and D-mannitol; hydrolysed casein and starch; reduced nitrate, grew at pH 5.7 and 6.8; and in 6.5% (w/v) NaCl.

A total of 224 *Bacillus* cultures were isolated from two sets of samples of each type of soybean dawadawa during fermentation and also from the final sundried products. The most frequently isolated cultures from both types of soy-dawadawa had colonies with irregular margins and rough ridged or ringed surfaces often producing exudate. They had circular to oval centrally placed spores and in the API galleries, generally fermented glycerol, L-arabinose, ribose, D-xylose, D-glucose, D-fructose, D-mannose, inositiol, sorbitol, mannitol, α-methyl-D-glucoside, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, melibiose, saccharose, trehalose, inuline, D-raffinose, amidon, glycogen, β-gentiobiose and D-turanose. They were tentatively identified as *Bacillus subtilis* using the API database. Other *Bacillus* species tentatively identified using the API 50 CHB kit were *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus cereus*, and *Bacillus firmus*. Similar *Bacillus* species occurred in similar proportions in both types of soy-dawadawa with *Bacillus subtilis* accounting for 50%

and 48%, respectively of the *Bacillus* population in the boiled/dehulled and roasted/dehulled samples. *Bacillus pumilus* accounted for 20% and *Bacillus cereus* for 16% of the *Bacillus* population in both types of soybean dawadawa. *Bacillus firmus* accounted for 8% and 9% and *Bacillus licheniformis* for 6% and 7% of the *Bacillus* species respectively in boiled/dehulled and roasted/dehulled soy-dawadawa. The differences found between the micropopulation of *Bacillus* species in boiled/dehulled and roasted/dehulled soy-dawadawa were therefore minor.

The results of the present work is in agreement with the findings of Ogbadu and Okagbue (1988) and Omafuvbe et al. (2000) who reported *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus licheniformis* as the predominant microorganisms in fermenting soybeans during dawadawa production in Nigeria. *Bacillus* spp. mainly *Bacillus subtilis* have also been reported to be responsible for the traditional alkaline fermentation of several legumes and seeds in West Africa including the African locust bean seeds (Campbell-Platt, 1980; Odunfa, 1981a; Antai and Ibrahim, 1986; Ouoba et al., 2004), melon seeds, *Citrullus vulgaris* Schrad (Odunfa, 1981b), sesame seeds, *Sesamun indicum* (Odunfa, 1985b), castor oil seeds, *Ricinus communis* (Odunfa, 1985c), cotton seeds (Sanni and Ogbonna, 1990), fluted pumpkin bean, *Telfaria occidentale* (Odunfa, 1985b), and African oil bean seeds, *Pentaclethra macrophylla* Bentham (Obeta, 1983; Odunfa and Oyeyioye, 1985; Njoku et al., 1990). Campbell-Platt (1980) found about 31% of microorganisms isolated from numerous African locust bean dawadawa samples collected from different countries in West Africa to be *B. subtilis*. In some samples, Campbell-Platt found *B. subtilis* to constitute 61–69% of all isolates, whilst all *Bacillus* spp. constituted 83–93% of all isolates. The fermentation of soybeans into natto, thua-nao, and kinema in Asia are also reported to be due to *Bacillus subtilis* (Steinkraus, 1991). The micropopulation of *Bacillus* species in the two types of soy-dawadawa found in the present work and that reported for African locust bean dawadawa are very similar (Campbell-Platt, 1980; Odunfa, 1981a).

3.5. Lactic acid bacteria present in soy-dawadawa

Gram-positive, catalase-negative rods, coccoid and cocci, isolated anaerobically on MRS Agar from both types of soy-dawadawa, were assumed to be lactic acid bacteria. They were assumed to be responsible for increases recorded in titratable acidity despite rise in pH during fermentation. Over half of the lactic acid bacteria isolated from both types of soy-dawadawa were facultatively heterofermentative lactobacilli i.e. rods which were able to produce CO_2 from both glucose and gluconate. The population also included homofermentative lactobacilli. The lactobacilli were mainly non-oxidative and metabolised glucose fermentatively in Hugh and Leifson medium; grew at pH 4.7 and 9.6, but not in 6.5% and 18% (w/v) NaCl. The species of the lactic acid bacteria were not determined.

3.6. Yeasts present in soy-dawadawa

Yeasts were isolated in low numbers on Malt Extract Agar in both samples during the initial stages of fermentation but not at the advanced stages of fermentation. Due to their low numbers and subsequent drop in population, yeasts were not considered to play any significant role in the fermentation of soy-dawadawa. In African locust bean dawadawa Ikenebomeh (1982) did not isolate any yeasts on acidified Potato Dextrose Agar.

3.7. Microbial enzymatic activities during the fermentation of soy-dawadawa

According to Odunfa (1985a), the most significant biochemical change which occurs during the fermentation of African locust bean seeds into dawadawa is the hydrolysis of proteins into amino acids. This is in addition to the breakdown of carbohydrates. In the present work, the amylase and protease activities in the two types of soybean dawadawa were determined during fermentation and sundrying, and are presented in Fig. 1. The level of protease activity was similar in both samples at the start of fermentation. However, after 24 h and also during sundrying, higher protease activities were recorded in the soybeans dehulled after boiling. The highest protease activity of 82 units/ml was recorded in the boiled/dehulled sample at 72 h where one unit of protease activity was the amount of enzyme which produced 1.0 μmol of tyrosine in 1.0 ml of trichloroacetic acid soluble peptides under the assay condi-

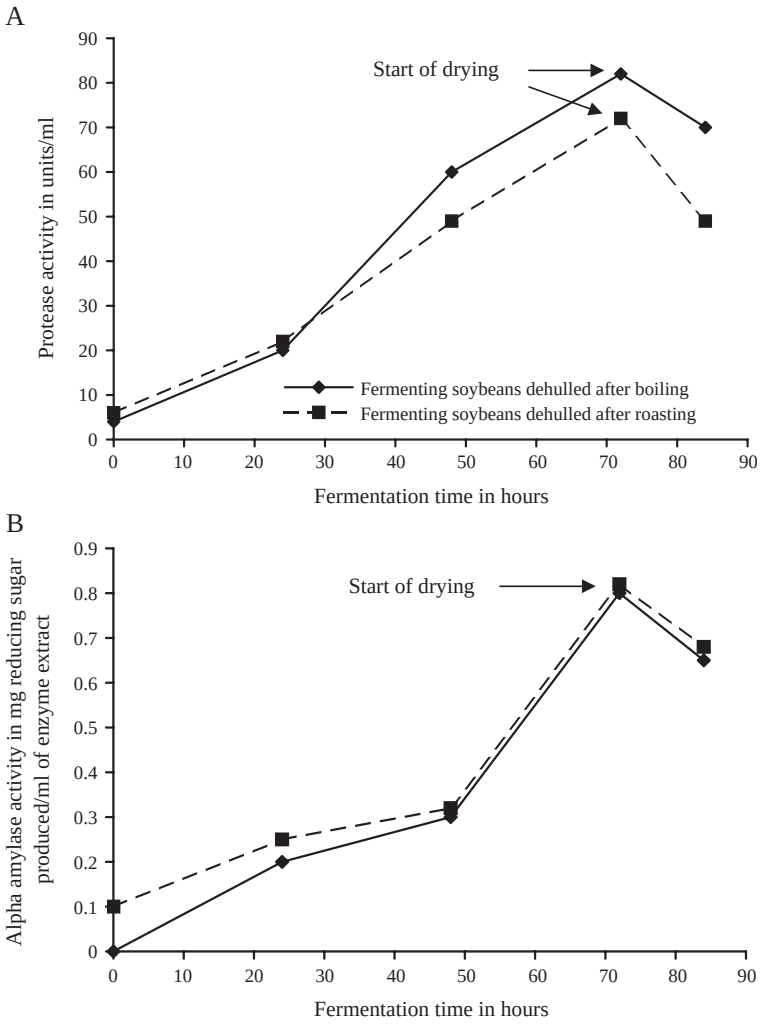

Fig. 1. Enzymatic activities in fermenting soybean dawadawa. (A) Protease activity; one unit of protease activity is the amount of enzyme which produces 1.0 μmol of tyrosine per 1.0 ml of trichloroacetic acid-soluble peptides under assay conditions. (B) α-Amylase activity expressed as mg of reducing sugar produced per ml of enzyme extract.

tions. This corresponded to a seven-fold increase in the initial protease activity of 4 units/ml. Similarly, the highest protease activity of 72 units/ml was recorded in the roasted/dehulled sample at 72 h. This corresponded to a six-fold increase in the initial protease activity of 6 units/ml. In the case of the α-amylase activity, a lower level was recorded in the soybeans dehulled after boiling up to 24 h of fermentation, after which similar values were obtained in both types of soy-dawadawa. Analysis of variance showed that both production method and fermentation time significantly affected protease activity. In the case of the α-amylase activity, only fermentation time had a significant effect on the enzyme activity. Increases in the enzymatic activities were attributed to extracellular enzymes produced by the microbial population and rise in temperature during fermentation. The maximum temperature attained during fermentation was 45 °C (data not shown) and this would facilitate enzymatic activities.

Representative cultures of *Bacillus* species isolated from both types of soy-dawadawa were examined for proteolytic and amylolytic activities. All *Bacillus* isolates had clearing zones around their colonies when cultured on Skim Milk Agar, thus each demonstrating proteolytic activity. The largest diameter of clearing zones were exhibited by the ten isolates of *Bacillus subtilis* and two isolates of *Bacillus firmus* examined, and ranged between 40 and 65 mm. Isolates of *Bacillus cereus*, *Bacillus licheniformis*, and *Bacillus pumilus* had smaller diameter of clearing zones, ranging from 12 to 30 mm. Based on the diameter of the clearing zones, it was concluded that the isolates of *Bacillus subtilis* and *Bacillus firmus* had higher proteolytic activities than the other *Bacillus* species present in soy-dawadawa. With respect to amylolytic activity, all isolates of *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus licheniformis* had clearing zones exceeding 30 mm around their cultures when grown on Starch Agar, thus exhibiting considerable amylolytic activities. Each of the isolates of *Bacillus firmus* and *Bacillus pumilus* showed very little amylolytic activity with clearing zones that did not exceed 5 mm. Ouoba et al. (2003a) demonstrated the ability of isolates of *Bacillus subtilis* and *Bacillus pumilus* to degrade African locust bean proteins during dawadawa/soumbala fermentation leading to a profile of soluble proteins and free amino acids specific for each isolate.

3.8. Proximate composition and amino acid profile of soy-dawadawa

Increases were recorded in the protein content of soybeans after fermentation into soy-dawadawa. In the sample dehulled after boiling, the protein content increased from 42.8% in the raw soybeans to 44.5% (dwb) in the fermented product and from 42.8% to 43.2% (dwb) in the roasted/dehulled sample. Increase in protein level during soybean fermentation has been reported by Barimalaa et al. (1994) and in African locust bean by Obizoba and Atu (1993).

The moisture content of the products increased from 8.8% to 25% and 24%, respectively, for the boiled/dehulled and roasted/dehulled soy-dawadawa samples but this was subject to the extent of sundrying. The fat content of soy-dawadawa decreased from 36.7% in the raw soybeans to 21.5% and 26.7% (dwb) for the boiled/dehulled and roasted/dehulled samples, respectively. The reduction in fat content was attributed to hydrolysis of fat as reported

Table 1
Amino acid profile of soybean dawadawa during fermentation and after sundrying (final product) in μg/g protein

Amino acid	Soybean	Fermenting soybeans dehulled after boiling					Fermenting soybeans dehulled after roasting				
		0 h	24 h	48 h	72 h	Final product	0 h	24 h	48 h	72 h	Final product
Isoleucine	230	228	227	232	280	276	225	223	230	272	269
Leucine	430	426	422	434	490	487	422	421	432	466	464
Lysine	340	338	336	342	400	398	332	331	341	392	390
Methionine	80	76	72	78	80	78	72	67	62	77	76
Cysteine	70	67	65	64	66	65	64	63	67	96	94
Phenylalanine	270	272	267	274	310	310	263	257	270	303	303
Tyrosine	210	207	205	213	200	198	203	200	192	196	193
Threonine	240	232	228	248	240	240	210	198	242	230	230
Tryptophane	90	89	86	89	80	78	85	83	79	77	75
Valine	310	302	300	312	300	300	280	276	335	289	288
Arginine	270	262	261	274	350	335	250	247	273	335	333
Histidine	150	147	144	153	160	157	132	120	154	158	157
Alanine	280	278	269	264	270	270	268	262	282	268	268
Aspartic	350	335	322	325	330	328	325	312	310	321	318
Glutamic	270	257	243	258	270	265	263	242	234	272	267
Glycine	240	233	229	246	260	256	238	235	242	252	250
Proline	240	229	218	338	340	335	229	226	234	339	336
Serine	330	324	318	316	320	320	298	296	312	318	316

Table 2
Aroma volatile compounds detected in roasted/dehulled and boiled/dehulled soybean dawadawa during fermentation

Aroma compounds*	Fermentation time in type of soy-dawadawa							
	0-h		24-h		48-h		72-h	
	Roasted/ dehulled	Boiled/ dehulled	Roasted/ dehulled	Boiled/ dehulled	Roasted/ dehulled	Boiled/ dehulled	Roasted/ dehulled	Boiled/ dehulled
Alcohols								
Ethanol	+++	–	+++	+	++	+	+++	++
3-methyl butan-1-ol	–	–	–	–	+	++	++	+++
2-methyl-1-propanol	–	–	–	–	–	–	+	++
2,3-Butandiol	+	–	++	–	–	++	+	+
Phenyl alcohol	–	–	–	–	+	–	+	+
Hexanodecanol	–	–	–	–	–	–	+	–
1,2-Ethanediol	+	–	–	–	–	–	–	–
Aldehydes								
(*E*,*E*)-2,4-Decadienal	–	–	–	–	–	–	+	+
3-methylbutyl aldehyde	–	–	–	–	–	–	+	+
Benzaldehyde	+	–	–	+	+	+	++	++
5-methyl-2-phenyl-2-hexenal	–	–	–	–	–	–	++	+
2-methylbutanal	+	–	–	–	–	–	–	–
Phenylethanal	+	–	–	–	–	–	–	–
2 butyl-octenal	–	++	–	+	–	–	–	–
Hexanal	–	+	–	–	–	–	–	–
Esters and acids								
3-methylbutyl pentanoate	–	–	–	–	–	–	+	++
Ethyl acetate	–	–	–	–	–	–	–	+
Octadecyl acetate	–	–	–	–	–	–	+	–
Hexadecanoic acid	++	+	–	+	–	–	++	++
Pyrazines								
Trimethyl pyrazine	++	–	++	–	++	+	+	+
Tetramethyl pyrazine	–	–	–	–	–	–	+	+
2-ethyl-5-methyl pyrazine	+	–	–	–	–	–	–	–
2-ethyl-6-methyl pyrazine	+	–	–	–	–	–	–	–
2-ethyl-3,5-dimethyl pyrazine	+	–	–	–	–	–	–	–
3-ethyl-2,5-dimethyl pyrazine	++	–	++	–	++	+	+	+
3,5-diethyl-2-dimethyl pyrazine	+	–	–	–	–	–	–	–
Sulphur compounds								
Dimethyl disulphide	–	–	–	–	–	–	–	+
Trimethyl disulphide	–	–	–	–	–	–	+	+
Aromatic compounds								
p-Xylene	++	+	++	+	+	+	+	+
D-Limonene	–	–	–	+	+	++	–	–
Chrorobenzene	+	+	+	+	+	+	+	+
1,2-dimethyl benzene	–	–	–	–	–	–	+	–
1,3-dimethyl benzene	+	+	+	+	+	–	–	–
Ketones								
Acetophenone	–	+	–	+	–	+	–	–

Table 2 (*continued*)

Aroma compounds*	Fermentation time in type of soy-dawadawa							
	0-h		24-h		48-h		72-h	
	Roasted/ dehulled	Boiled/ dehulled	Roasted/ dehulled	Boiled/ dehulled	Roasted/ dehulled	Boiled/ dehulled	Roasted/ dehulled	Boiled/ dehulled
Alkanes								
Cyclotetradecane	−	−	−	−	−	−	−	+
Hexadecane	+	−	−	−	−	−	−	−
Cyclohexadecane	+	−	+	−	−	−	−	−
Tetracosane	++	−	+	+	++	−	++	+
Hexacosane	+	−	+	−	++	−	++	−
Docosane	+	+	+	+	+	−	+	+
Nonal cyclopropane	+	−	−	−	−	−	−	−
Alkene								
Decene	−	−	−	−	−	−	−	+
Tetradecene	−	−	−	−	−	−	+	−
(*E*)-5-Eicosene	−	−	−	−	−	−	+	−
Miscellaneous								
Indole	−	−	−	−	−	−	−	+
2,4-bis(1,1-dimethyl ethyl) phenol	+	+	−	+	−	−	−	−

− compound not detected. + compound detected in two replicates of sample. +, ++, +++ − shows relative increase or decrease in size of corrected peak area of compound during fermentation.

for African locust bean dawadawa (Ouoba et al., 2003b). The ash content decreased from 5.2% in the raw soybeans to 3.5% (dwb) in the boiled/dehulled soy-dawadawa but such a decrease was not observed in the roasted/dehulled sample which had an ash content of 5.0%. The loss in the mineral content of the boiled-dehulled soy-dawadawa sample was attributed to leaching during boiling.

The level of various amino acids in the two types of soybean dawadawa during fermentation and also in the final sundried products are shown in Table 1. Fermentation time had a significant effect on the levels of isoleucine, leucine, lysine, phenylalanine, arginine, and praline, increasing with time. Generally the level of methionine, cysteine, and tryptophane in both types of soy-dawadawa were low. Odunfa (1985a) reported increases in the levels of glutamic acid, valine, aspartic acid, and alanine during the fermentation of African locust bean seeds into dawadawa. The overall difference between the amino acid profiles of African locust bean dawadawa and soy-dawadawa appears to be minor, and so are the observed differences between the two types of soy-dawadawa.

3.9. Aroma volatile compounds in soy-dawadawa

The results of aroma analysis by GC–MS showed the presence of over 100 compounds in the two types of soy-dawadawa, however, only compounds detected in both replicates of the type of soy-dawadawa are shown in Table 2. Thus, sometimes, a compound was only detected in one of the replicate samples but these usually had very small relative peak areas and are not considered in the discussion. Although most of the compounds were detected in both types of soy-dawadawa at one stage or other of fermentation, some compounds were only detectable in one type of soy-dawadawa. For example, at the start of fermentation, six different types of pyrazines were detected in the roasted/dehulled samples, but none of these was detectable in the corresponding boiled/dehulled samples. Pyrazines are important aroma compounds in roasted products and are considered to contribute directly to the roasted type of flavour notes (Maga, 1982; Manley et al., 1999). According to Lindsay (1985), the most direct route to the formation of alkyl pyrazines result from the interaction of α-dicarbonyl compounds – which are

intermediary products in the Maillard reaction – with amino acids through the Strecker degradation reaction. In the course of the soy-dawadawa fermentation, some of the pyrazines were no longer detected in the roasted/dehulled samples, whilst some pyrazines were formed in the boiled/dehulled samples through microbial activity. Owens et al. (1997) reported the formation of pyrazines by microbial activity in fermenting autoclaved or roasted soybeans and attributed them to the metabolic activities of the *Bacillus* species generating various precursors and possibly other compounds that are converted to pyrazines by non-enzymatic chemical reactions as previously reported by Rizzi (1987).

Generally in analysing the aroma compounds in this work, one of either trend could be observed. Firstly, some aroma compounds present at the start of fermentation were no longer detectable as fermentation progressed suggesting that they had been broken down or converted to other compounds through microbial activity or chemical reactions. Secondly, some compounds not present at the start of fermentation were built up during fermentation with usually a steady increase in the concentration as deduced from the relative peak areas of the compounds in the chromatograms. These could also be attributed to microbial activity and chemical reactions with other compounds. In all, alcohols, aldehydes, esters, acids, pyrazines, aromatic compounds, ketones, alkanes, alkenes, sulphur compounds, and other compounds were found responsible for the strong aroma of dawadawa. More alcohols, aldehydes, alkenes, and sulphur compounds became detectable with the progress of fermentation, whilst with the aromatic compounds, higher concentrations and more compounds were found between 24 and 48 h after which there was a decline.

Generally the aroma volatile compounds found in the present work are in agreement with those found by Owens et al. (1997) in roasted or autoclaved soybeans fermented with *Bacillus subtilis*. The profiles are also in agreement with volatile aroma compounds found by Leejeerajumnean et al. (2001) in Asian *Bacillus*-fermented soybean products, thua-nao and natto.

Tetramethyl pyrazine, 3-methyl butan-1-ol, 2-methyl-1-propanol, phenyl alcohol, (*E*,*E*)-2,4-decadienal, 3-methylbutyl aldehyde, 3-methylbutyl pentanoate, and trimethyl disufide were found only at the advance stages of fermentation in both types of soy-dawadawa and were clearly the products of fermentation. Hexanodecanol, octadecyl acetate, 1,2-dimethyl benzene, tetradecene, and (*E*)-5-eicosene were found only in the roasted/dehulled samples and were detectable at the advance stages of fermentation, whilst 1,2-ethanediol, ethyl acetate, dimethyl disulfide, cyclotetradecane, decene, and indole were found only in the boiled/dehulled samples and were also detected only at the advance stages of fermentation. In addition to these, cyclohexadecane and hexacosane were found only in the roasted dehulled samples, whilst 2 butyl-octenal, acetophenone, and toluene were found only in the boiled/dehulled samples.

3.10. Preference of soybean dawadawa by a market focus group

A market focus group made up of dawadawa retailers who also consume dawadawa regularly, preferred soybean dawadawa prepared by roasting the beans before dehulling to soybean dawadawa prepared by boiling the soybeans before dehulling. The preference was with respect to colour, taste and overall acceptability (results not shown).

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