Journal of Food Technology 3 (1): 87-91, 2005
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Soaking and Cooking of Soybean for Soy-*daddawa Production*: Bacteria Profile and Proximate Composition.

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Department of Microbiology,Obafemi Awolowo University,Ile-Ife, Osun State, Nigeria.[1]Soaking and Cooking of Soybean for Soy-*daddawa* Production: Bacteria Profile and Proximate Composition.

Abstract: The bacteria profile and proximate composition of soybean soaking and cooking for soy-*daddawa* production were investigated. Soybean soaked (in tap water at 30°C) for up to 24h underwent natural acid fermentation with a reduction in pH from 7.10 to 5.40. The fermentation was characterized by a total aerobic mesophilic bacteria (TAMB) count ranging from 4.18 $\log_{10}$ cfu/ml at the onset to 11.00 $\log_{10}$ cfu/ml. at the end of the soaking period. The bacteria associated with the soaking included species of *Staphylococcus, Enterobacter, Citrobacter, Micrococcus, Bacillus, Streptococcus, Flavobacterium, Proteus and Klebsiella.*
Crude protein and fibre increased while ether extract, ash and total carbohydrate decreased slightly with soaking. Dehulling and cooking of soaked soybean resulted in reduction of ash, crude fibre, and total carbohydrate but increased crude protein and ether extract. The total soluble sugar in soybean seeds decreased with soaking and cooking but increased in the soak water (soybean soak) while the excess water drained from the cooked seeds (cook water) contain appreciable amount.

Keywords: Soybean, soaking, cooking, bacteria, soluble sugars, proximate

Introduction

Soy-*daddawa* is one of the important fermented products from soybean (*Glycine max* (L) Merrill) consumed in the major soybean producing areas of Nigeria (Omafuvbe *et al.*, 2000). The first and most important step in making traditional soy-*daddawa* is to wash thoroughly and soak the dry beans in tap water at room temperature overnight (12h or more), dehulled and cooked for 2 hours (Popoola and Akueshi, 1985). The second stage is the fermentation of the cooked dehulled seeds to produce soy-*daddawa*. The dehulled cooked soybean seeds have been reported to undergo an alkaline fermentation involving mainly *Bacillus* species to produce soy-*daddawa* (Ogbadu and Okagbue, 1988; Omafuvbe *et al.*, 2000). Of the *Bacillus* species associated with the traditional fermentation process, only *Bacillus subtilis* has been reported responsible for the production of organoleptically acceptable soy-*daddawa* in starter culture fermentation of sterile soybean (Omafuvbe *et al.*, 2002). The biochemical and nutritional changes associated with the second stage (fermentation) of soy-*daddawa* production has also been studied (Omafuvbe *et al.*, 2000; Omafuvbe and Awowole, 2003).
Soaking of soybean is considered important because it is belief to reduce cooking time and improve the quality of the product (Wang *et al.*, 1979). However, it is not clear whether the improvement in the quality produced by soaking is in the nutritional value, texture or the flavour of the product. Unfortunately, soaking as the first step in the processing of soybean for soybean foods has not been given much attention. There are few reports on the microbial ecology (Mulyowidarso *et al.*, 1989) and solid losses associated with soybean soaking (Wang *et al.*, 1979). The purpose of this study is to identify the bacteria associated with the soaking of soybean and the effect of soaking and cooking process on the proximate composition of soybean for *daddawa* production.

Materials and Methods

Samples: Soybean (*Glycine max* (L) Merrill) seeds were purchased from a local market in Ile- Ife, Osun State, Nigeria.

Soaking and cooking Process: Soybean seeds were sorted to eliminate broken ones and those with damaged seed coats. The sorted seeds were thereafter distributed in 50g portions in to 250ml capacity conical flasks. The content of each flask was rinsed thrice with 200ml of tap water and drained properly. The washed seeds were then soaked in tap water (1:4, w/v) at 30 ± 2 °C for up to 24 during which period duplicate flask were removed at six hour intervals for analysis. At each sampling period, the liquid from soaking was decanted and designated as "soybean soak " while the soaked seeds were designated as " soaked soybean". Soybean soaked for 12 and 24 hours were divided into two portions, one of which was dehulled and boiled in tap water (1:4, w/v) for 2 hours on a Gallenkamp hot plate to obtain "cooked soybean". The excess liquid obtained from cooking was removed and designated as "cook water".

Table 1:Changes in total aerobic mesophilic bacteria (TAMB) count, pH and moisture during soybean soaking for soy-*daddawa* production.

Soaking period(h)	TAMB count*	pH	Moisture**
0	4.18	7.10	17.52
6	4.93	6.10	56.50
12	8.69	5.70	60.50
18	10.83	5.50	61.30
24	11.00	5.40	62.60

Values are means of three determinations.
* TAMB count expressed as $\log_{10}$ of colony forming unit (cfu)/ ml. of soybean soak.
** Moisture content expressed as g/100g of soaked soybean.

Table 2: Bacteria profile at different stages of soybean soaking for soy-*daddawa* production.

Bacteria isolate	Soaking period (h)				
	0	6	12	18	24
Staphylococcus epidermis	+	+	+	+	+
Enterobacter cloacae	-	+	+	+	+
Citrobacter intermedius	-	+	+	-	-
Flavobacterium sp	-	-	-	+	+
Proteus sp	-	-	-	+	+
Klebsiella orzaenae	-	-	+	+	+
Bacillus subtilis	+	+	+	+	+
Bacillus brevis	+	+	+	+	+
Micrococcus luteus	-	-	+	+	+
Streptococcus sp	-	-	+	+	+

+ Present - Absent.

Analytical methods: Isolation and identification of bacteria
The content of each flask was shaken vigorously at each sampling period and 10ml of the soybean soak was aseptically transferred into sterile 90ml of 0.1% peptone water diluent in a conical flask. Further dilutions were made and 0.1ml of appropriate dilutions was plated out in triplicate on nutrient agar (NA oxiod). The NA plates were incubated at 30°C for up to 48h for total aerobic mesophilic bacteria (TAMB) count. The bacteria isolates were purified by repeated streaking on NA and identified according to the schemes of Harrigan and McCance (1976).

pH determination: Approximately 10ml of soybean soak was collected in triplicates at each sampling period. The pH of the suspension was determined using a Pye Unicam pH meter (Model 290 MK2).

Moisture content determination: The moisture content of soaked soybean sample collected at each sampling period was determined in accordance with AOAC (1990).

Determination of soluble sugars: The soluble sugars in the soaked and cooked soybean samples were extracted with 80% ethanol following the method described by Odibo *et al.* (1990). The ethanolic extract was appropriately diluted and the total soluble sugar content was estimated following the Dreywoods anthrone reagent method of Morris (1948). The total sugar concentration of each sample was estimated from a standard curve of known concentration of glucose. The reducing sugar of the sample extract was estimated by the colorimetric method (Somogyi, 1945) using glucose as standard solution. The soluble sugar in the soybean soak and the cook water was also estimated. The non-reducing sugar concentration of the samples was obtained by subtracting the reducing sugar value from the total sugar value.

Proximate analysis: Proximate composition was determined following the methods of the Association of Official Analytical Chemists (AOAC, 1990). Total carbohydrate content of the seeds was obtained by the difference method while organic matter was obtained by subtracting the weight of ash from weight of dry material (Pomeranz and Meloan, 1971).

Table 3:Changes in soluble sugar content of soybean soak, soaked and cooked soybean used for soy-*daddawa* production.

Sample type/ processing time (h)	Total soluble sugar	Reducing sugar	Non-reducing sugar
Soybean soak*			
0	0.02	0.00	0.02
6	3.74	1.56	2.18
12	4.45	2.95	1.50
18	5.18	4.63	0.55
24	5.23	4.21	1.02
Soaked soybean**			
0	99.20	7.03	92.17
6	88.40	3.17	85.23
12	72.20	3.10	69.10
18	68.00	3.52	64.48
24	61.40	4.07	57.33
Cooked soybean**[a]			
12	24.40	2.07	22.33
24	22.20	3.45	18.75
Cook water*[b]			
12	7.14	1.21	5.93
24	5.55	1.13	4.42

Values are means of three determinations.
* Sugar concentration expressed as mg glucose/ml.sample.
** Sugar concentration expressed as mg glucose/g dry wt of sample.
a, only soybean soaked for 12 and 24 hours were dehulled and cooked for 2h to obtain cooked soybean.
b, Represents excess water drained from the cooked soybean after cooking for 2h.

Table 4: Proximate composition * of soaked and cooked soybean for soy-*daddawa* production.

Sample type/ processing time (h)	Crude protein	Crude fibre	Ether extract	Ash	Total carbohydrate**	Organic matter***
Soaked soybean						
0	36.68	3.52	22.05	4.02	37.25	95.98
12	37.04	3.75	21.95	3.99	37.02	96.01
24	38.13	3.89	21.85	3.80	36.22	96.20
Cooked soybean[a]						
12	42.12	1.89	26.16	2.57	29.15	97.42
24	42.85	1.73	25.73	2.44	28.98	97.56

Values are means of three determinations.
* The proximate components are expressed as g/100g dry wt of sample.
** Total carbohydrate was obtained by difference (indirect) method.
*** Organic matter was obtained by subtracting the weight of ash from weight of dry matter.
a, Soybean samples soaked for 12 and 24 hours were dehulled and boiled for 2h to obtain cooked samples.

Results and Discussion

The changes in total aerobic mesophilic bacteria (TAMB) count; pH and moisture of soybean during soaking for soy-*daddawa* production are presented in Table 1. The TAMB count of the soybean soak increased with soaking from 4.18 $\log_{10}$ colony forming unit (cfu)/ml at the onset to 11.00 $\log_{10}$ cfu/ml at the end of the 24 hour soaking period. It is significant to note that there was a sharp increase in TAMB count between the 6th and 12th hour of soaking. This may be a result of the emergence of other bacteria species (Table 2). The observed increase in bacteria population in this study falls within the range earlier reported (Mulyowidarso *et al.*, 1989).

The bacteria associated with the soaking of soybean for soy-*daddawa* production were identified as *Staphylococcus epidermidis, Enterobacter cloacae, Citrobacter intermedius, Flavobacterium sp., Proteus sp., Kliebsiella orzaenae, Bacillus subtilis, B. brevis, Micrococcus luteus and Streptococcus sp.* (Table 2). Of these bacteria species only *Micrococcus, Proteus* and *Flavobacterium* species were not isolated in a previous study (Mulyowidarso *et al.*, 1989). The bacteria associated with the soaking of soybean may have originated from the soybean and the tap water used. The majority of the bacteria presumably originated from the soybean since boiled beans did not

undergo acid fermentation (Omafuvbe *et al.*, 2000). It is important to note that of the bacteria species involved in the soaking process only *Bacillus* sp. play important role in the fermentation of soybean into soy-*daddawa* (Ogbadu and Okagbue, 1988; Omafuvbe *et al.*, 2000; 2002).

The pH of the soybean soak dropped from 7.10 to 5.40 at the end of the soaking period (Table 1). This result suggests that soybean underwent an acid fermentation during soaking for *daddawa* production. A similar drop in pH with soybean soaking has been reported during *tempe* production (Mulyowidarso *et al.*, 1989). The associated bacteria may have hydrolyzed the content of the seeds thereby producing acids that resulted in a reduction in pH. Although in this study, the ability of the individual isolates to cause pH reduction in pure culture fermentation of decontaminated seeds in sterile water was not studied, *Lactobacillus, Streptococcus* and *Staphylococcus* have been reported responsible for pH reduction in similar investigation (Mulyowidarso *et al.*, 1989). The moisture content of soybean seeds increased with soaking from 17.52% to 62.60% (Table 1). The present findings are in agreement with those in existing literature (Wang *et al.*, 1979; Ikenebomeh *et al.*, 1986).

The soluble sugar content of soybean soak, cook water, soaked and cooked soybean is presented in Table 3. The total soluble sugar in the soaked soybean decreased from 99.20mg/g to 61.40mg/g while the reducing sugar decreased from 7.03mg/g to 3.10mg/g in the first 12h and increased slightly (3.10mg/g to 4.07mg/g) thereafter till the end of the soaking period. The total soluble sugar of soaked soybean dropped because some sugars were leached out into the soak water (soybean soak). The concentration of the total sugar in the soybean soak increased from 0.02mg/ml to 5.23mg/ml. while the reducing sugar increased from 0.00mg/ml to 4.21mg/ml at the end of the soaking period (Table 3). In addition, the metabolic activities of the bacteria associated with the process may have contributed to the drop in the total sugar and the fluctuation observed in the reducing level of soaked soybean. The soluble sugar levels in the dehulled and cooked soaked soybean dropped further (Table 3). This may be a direct effect of the dehulling process since soybean is made up of 7.30% hull that contains about 86% carbohydrate (Weingartner, 1987). In addition, some sugars were leached out into the "cook water". In general, the total soluble sugar level of the soaked and cooked soybean sample is composed mainly of non-reducing sugar while soybean soak in composed mainly of reducing sugar. Our findings agree broadly with earlier reports (Ikenebomeh *et al.*, 1986; Abdel-Gawad, 1993; Sarkar *et al.*, 1997).

The proximate composition of soaked and cooked soybean for *daddawa* production is presented in Table 4. The crude protein, crude fibre and organic matter increased slightly while ether extract, ash and total carbohydrate decreased slightly in the soybean with soaking. Dehulling and cooking of the soaked soybean resulted in increased protein, ether extract and organic matter while crude fibre, ash and total carbohydrate decreased. The protein contents in the soaked and cooked soybean may have increased only because of the loss in carbohydrate during soaking, dehulling and cooking of the seeds. Our finding agrees with those of Ikenebomeh *et al.* (1986) and Omafuvbe *et al.* (2004) but contradicts those of Abd El-Hady and Habiba (2003). The slight increase in crude fibre with soaking may have been a result of some water-soluble chemical component of the seeds been leached out. The decrease in crude fibre and ether extract components of the cooked soybean seed is presumably due to the dehulling and the cooking of the soaked seeds.

On the basis of our data, we conclude that soybean underwent acid fermentation during soaking which resulted in a decrease in pH, total soluble sugar, ether extract and an increase in moisture, protein and crude fibre. The losses and gains in soybean during soaking and cooking may be associated with the metabolic activities of the bacteria involved and the leaching out of the water-soluble components of the seeds. Of significant note is the decrease in the total soluble sugars that may be related to the reduction in the flatus-forming oligosaccharides. Although, the flatus forming oligosaccharides were not directly measured in this study, a substantial amount of these oligosaccharides has been reported to be removed with soaking and boiling of soybean (Ku *et al.*, 1976).

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