Assessment of Preservative Ability of Aqueous and Ethanolic Extracts of Onion (Allium cepa L.) on Soybean Daddawa- A Condiment

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ARTICLE INFO

ABSTRACT

Research Article

Received: 07/06/2019
Accepted: 21/02/2020

Keywords:
Antioxidant
Onion
NaCl
Peroxidation preservation
Soybean daddawa

Reports have indicated that lipid peroxidation is a key factor in soybean daddawa deterioration and Alliums are rich in antioxidants. The present study was carried out to investigate the preservative abilities of aqueous and ethanolic extracts of Onion (Allium cepa L.) on stored soybean daddawa compared with Sodium Chloride treated samples. Soybean (Glycine max (L). Merr.) seeds fermented into soybean daddawa was preserved with both extracts (at 3, 5 and 7% concentration) and NaCl (at the concentrations of 0, 3 and 5%). The samples stored at 30±2°C for up to 14 days were analyzed for peroxide value (POV), free fatty acid (FFA), pH, titratable acidity (TTA), water absorption capacity (WAC) and fat absorption capacity (FAC). Type of solvent used in extraction did not have significant effect on all the storage indices. Extracts of Onion significantly lowered the POV, FFA and TTA generated in stored soybean daddawa. The pH of stored soybean daddawa samples treated with the NaCl and aqueous extract was not significantly affected while the ethanolic extract significantly reduced the pH from 8.26 – 8.34 to 5.72 – 5.89. The tested extracts lowered the WAC significantly while the FAC of treated soybean daddawa was not significantly affected compared to those treated with NaCl. Results of this study indicate that both extracts of Onion are more effective in lowering peroxidation in stored soybean daddawa and thus could be exploited for soybean daddawa preservation.

Introduction

In many West African countries, traditional fermented foods of legumes and oilseeds origin are used as condiments and sources of dietary protein and minerals. Prominent among these is Soybean daddawa which is a product of spontaneous fermentation of soybean (Glycine max L.) seeds (Popoola and Akueshi, 1985; Omafuvbe et al., 2000). In Nigeria, the use of soybean seed as an alternative to locust bean (Parkia biglobosa) seed is also seen as a way of incorporating soybean protein into local diets. However, the popularity of soybean daddawa is marred by the perception that it is prone to faster deterioration compared to locust bean daddawa at the end of the fermentation period (Obatolu et al., 1998; Kolapo and Sanni, 2006; Popoola et al., 2007). Reports have indicated that higher level of lipid peroxidation in soybean daddawa compared to locust bean daddawa is most probably responsible for faster deterioration of soybean daddawa (Kolapo and Sanni, 2007; Kolapo et al., 2007a).

Addition of food grade sodium chloride salt to food for preservation purpose is an ancient practice. This is done with a view of enhancing human health by killing or limiting growth of foodborne pathogens and spoilage organisms. Incidentally, extension of soybean daddawa’s shelf stability using sodium chloride salt is a common practice among Nigerian soybean daddawa consumers. However, the effect of this salting practice on the lipid peroxidation, which is a key biochemical index of soybean daddawa spoilage, has not been documented.

Meanwhile, recent data from around the world show that over 95% of men and over 75% of women exceed the recommended daily tolerable upper intake (2.3g/day) of sodium (Doyle and Glass, 2010). These authors further submitted that, since these high levels of dietary sodium are associated with a high prevalence of hypertension, prehypertension and, possibly, other adverse effects on health, many national and international health
organizations recommend that sodium intake be significantly decreased. For instance, the American Public Health Association and the American Medical Association called for a 50% reduction in sodium in processed and restaurant foods (Dickinson and Havas, 2007).

Consequently, preservation of soybean daddawa using a more health friendly approach which exploits the antimicrobial and antioxidant potentials of plant extract is urgently needed.

Reports have shown that some plant extracts have been recognised as antimicrobials as well as antioxidants in foods (Ahn et al., 2007; Gottardi et al., 2016), thus the recent growing interest in the substitution of synthetic food antioxidants by natural antioxidants of such plant extracts due to health consideration (Kela and Anantharayanan, 2004). In this regard, dichloromethane extract of ginger (Zingiber officinale) has been shown to be effective in arresting lipid peroxidation and microbial proliferation in stored soybean daddawa, thus extending its shelf life (Kolapo et al., 2007b).

Several edible Allium including onion (Allium cepa L.), garlic (Allium sativum L.) and leek (Allium ampeloprasum L.) are among the oldest cultivated plants with wide culinary applications. It has been reported that onion may be used to reduce pathogenic microorganisms during unhygienic food productions (Degirmencioğlu and Irkin, 2009). Similarly, many yeast species and several gram positive bacteria have been reported to be sensitive to extracts of onion (Benkeblia, 2004, Ghahtarokhi-Shams et al., 2006) while the antioxidant activity of Onion has also been documented (Slimestad et al., 2007; Abdel-Salam et al., 2014).

As part of efforts to finding panacea to storage problem of soybean daddawa using substances that pose no health threat, the present study therefore assessed the preservative abilities of aqueous and ethanolic extracts of Onion (Allium cepa L) to arrest lipid peroxidation in stored soybean daddawa. It also documents, for the first time, the effect of NaCl preservative on lipid peroxidation in soybean daddawa. In addition, the impact of the addition of both aqueous and ethanolic extracts on functional properties of stored soybean daddawa was also evaluated.

Materials and Methods

Collection of Seeds and Soybean Daddawa Preparation

The Soybean seeds of variety TGX 1440-2E used for the present work were obtained from the Institute of Agricultural Research and Training, Ibadan. Nigeria. Soybean daddawa was prepared according to the method of Popoola and Akueshi (1985) as described by Omafuvbe et al. (2000).

Onion Samples and Preparation of Extracts

Red Onion (A. sativum L.) samples were purchased from a local market in Ibadan, Nigeria. The samples were sorted to remove stones and dirt, washed and air dried. Both ethanolic and aqueous extract of the samples were obtained using the method reported by Irkin and Korukluoglu (2007).

Application of Onion Extracts to Soybean Daddawa.

Soybean daddawa samples (10g) containing 3, 5 and 7% (v/w) of both ethanolic and aqueous extracts of Onion were prepared separately in sterile container. Positive controls containing 3 and 5% common salt (NaCl) (w/w) and a negative control containing 0% salt and extract were equally set up. All samples of each treatment were prepared in triplicates and stored at ambient temperature (30±2°C) for two weeks and the various analyses were done at two days’ interval.

Measurement of Antioxidant Activity

The effectiveness of the extracts of Onion as an antioxidant in stored soybean daddawa was tested by the determination of Peroxide value (POV) and Free Fatty Acid (FFA) contents of soybean daddawa kept at ambient temperature. The POV (meqkg⁻¹) of samples was measured by titration with 0.1M sodium thiosulphate solution using starch as indicator (AOAC, 1990) while the FFA content (expressed as % oleic acid) was estimated using an alkali titration method (AOAC, 1990). All determinations were carried out in triplicates and mean values were calculated. The experiments were repeated three times and the average of the various determinations were reported as the final values.

pH and Titratable Acidity Determination

The pH and titratable acidity (expressed as lactic acid equivalent) of the preserved, stored soybean daddawa were determined as previously described by Ikenebomeh (1989).

Measurement of Functional Properties

The fat and water absorption capacity of the samples were determined using the centrifugation method previously described by Sosulki et al. (1976). All determinations were carried out in triplicates and mean values were calculated.

Statistical Analysis

Data obtained were expressed as means. The statistical significance of differences was assessed using analysis of variance. A two-tailed P value of less than 0.05 was considered to be statistically significant. Values that were significantly different were separated using Duncan Multiple Range test using SPSS for windows Version 11.0 statistical package.

Results and discussion

A progressive increase in peroxide value (POV) was observed in both positive (NaCl treated) and negative (no preservative) controls as the storage time increased. In this regard, values for negative control were significantly (P<0.05) higher than those of the positive control. However, the POV of soybean daddawa preserved with both aqueous and ethanolic onion extracts was significantly (P<0.05) lower than those of positive and negative controls (Table 1). This initial trend of POV increase in both positive and negative control samples is similar to previous observations made in stored soymilk by Iwe (1991) and stored soybean daddawa by Kolapo and Sanni (2007), Kolapo et al. (2007a) and Popoola et al. (2007), thus underscoring peroxidation as a key biochemical factor in soybean daddawa deterioration.
Table 1. Effect of aqueous and ethanolic extracts of Onion on Peroxide value of stored soybean daddawa

| ST | Common salt (NaCl) | Onion extract (Aqueous) | Onion extract (Ethanolic) |
|----|-------------------|-------------------------|--------------------------|
| 0  | 3.92±0.28c       | 3.70±0.14g               | 3.20±0.35a               |
| 2  | 5.20±0.00e       | 5.60±0.01f               | 4.45±0.13f               |
| 4  | 5.00±0.85c       | 5.40±0.70d               | 4.20±0.14c               |
| 6  | 4.50±0.23d       | 4.60±0.28e               | 4.00±0.24d               |
| 8  | 7.00±0.43c       | 7.20±0.57d               | 6.40±0.15f               |
| 10 | 10.30±0.13e      | 10.50±0.56d              | 9.40±0.10d               |
| 12 | 12.22±0.23f      | 12.35±0.26g              | 9.20±0.02f               |

ST: Storage Time (Day); Values are means ± SD (n=3). Values in the same column with different superscripts are significantly different (P<0.05).

Table 2. Effect of aqueous and ethanolic extracts of Onion on Free Fatty Acid of stored soybean daddawa

| ST | Common salt (NaCl) | Onion extract (Aqueous) | Onion extract (Ethanolic) |
|----|-------------------|-------------------------|--------------------------|
| 0  | 0.019±0.004f      | 0.022±0.001f             | 0.010±0.000f             |
| 2  | 0.024±0.004f      | 0.028±0.002f             | 0.010±0.000f             |
| 4  | 0.036±0.005d      | 0.029±0.004f             | 0.010±0.000f             |
| 6  | 0.39±0.000e       | 0.032±0.004e             | 0.010±0.000f             |
| 8  | 0.045±0.001d      | 0.038±0.004e             | 0.010±0.001d             |
| 10 | 0.058±0.006e      | 0.041±0.003d             | 0.010±0.000d             |
| 12 | 0.061±0.003d      | 0.042±0.002d             | 0.010±0.000d             |

ST: Storage Time (Day); Values are means ± SD (n=3). Values in the same column with different superscripts are significantly different (P<0.05).

Table 3: Effect of aqueous and ethanolic extracts of Onion on pH of stored soybean daddawa

| ST | Common salt (NaCl) | Onion extract (Aqueous) | Onion extract (Ethanolic) |
|----|-------------------|-------------------------|--------------------------|
| 0  | 8.26±0.11d       | 8.04±0.00g               | 8.29±0.01c               |
| 2  | 8.45±0.01c       | 8.39±0.01d               | 8.50±0.01e               |
| 4  | 8.45±0.01c       | 8.46±0.01c               | 8.50±0.01c               |
| 6  | 8.50±0.00c       | 8.51±0.01c               | 8.55±0.06d               |
| 8  | 8.59±0.04b       | 8.52±0.02c               | 8.60±0.01c               |
| 10 | 8.56±0.01c       | 8.59±0.02c               | 8.60±0.01c               |
| 12 | 8.64±0.09b       | 8.67±0.01b               | 8.66±0.04b               |

ST: Storage Time (Day); Values are means ± SD (n=3). Values in the same column with different superscripts are significantly different (P<0.05).

Zia-ur-Rehman et al. (2003) opined that increase in peroxide value is a good predictor of fat deterioration. Consequently, reduced POV observed in stored soybean daddawa samples treated with onion extracts in the present study is possibly indicating arrest of peroxidation by the extracts used in the preservation. It may be therefore inferred that treatment of stored soybean daddawa sample with extracts of onion was responsible for the lowered POV of treated samples at the end of 14 days of storage. This is plausible on the understanding that the antioxidant activity of Onion has been documented (Slimestad et al., 2007; Abdel-Salam et al., 2014).

Free Fatty Acid (FFA) content of untreated sample and positive control increased progressively with time of storage while a corresponding decrease was recorded for samples treated with onion extracts. The FFA values of samples treated with natural preservatives were significantly (P<0.05) lower compared to the values obtained for both positive and negative controls. Solvent used in extraction did not have any significant effect (P<0.05) on the antioxidant activity of the extracts (Table 2). Lipid oxidation is an important factor influencing quality and acceptability of food products (Hunt et al., 1999). The formation of organic acids and free fatty acid is an initial step in fat oxidation/deterioration, development of rancidness and off-flavour in fatty foods (Sattar and Demen, 1973). In this connection, the increase in the amount of products of fat deterioration with storage in the control samples observed in the present study is in agreement with previous reports on stored soybean daddawa (Popoola et al., 2007; Kolapo and Sanni, 2007) and stored canned tuna in brine (fish product) (Siriamorupim et al., 2008).

In the present study, the formation of fat deterioration products viz FFA and Peroxides were effectively controlled by the addition of aqueous and ethanolic extracts of onion. Details of the compounds of these extracts were not evaluated at this stage. However, previous reports have attributed antioxidant activity of onion extract to its phenolic and flavonoids contents (Slimestad et al., 2007; Abdel-Salam et al. 2014). These compounds would be most probably responsible for the observed reduction of lipid oxidation in soybean daddawa samples treated with onion extracts.

Studies have shown that increased salt concentration accelerated the rate of POV and FFA production in fish product stored at 4°C (Aubgbours and Ughano, 2002; Yarar et al., 2006; Ebob et al., 2006). In the present study,
though the amounts of peroxidation products increased with storage time, there was no significant difference between the quantities of peroxidation products generated in soybean daddawa preserved with 3 and 5% salt. It is obvious that peroxidative activity was significantly higher in NaCl treated samples compared with those treated with onion. The implication of this is that the traditional use of NaCl in prolonging the shelf life of daddawa does not guarantee oxidative stability of the innate lipid. However, the use of natural antioxidant such as the one investigated in the present study is potent enough to stem the lipid peroxidation tide in stored soybean daddawa.

In all the stored soybean daddawa samples, pH increased steadily with days of storage except in the samples that were treated with ethanolic extract whose pH decreased with storage days (Table 3). There was no significant difference (P>0.05) between the pH of stored soybean samples treated with aqueous extracts of onion and those of positive and negative controls. The pH increase in stored soybean daddawa samples observed in the present study is similar to the observed trend in the fermentation of soybean and locust bean daddawa (Ikenebomeh et al., 1986; Oyeyiola, 1988; Sarka et al., 1993; Omafuvbe et al., 2000). In those studies, increasing pH during fermentation was attributed to proteolytic activities and release of ammonia by microorganisms involved in fermentation. Therefore, similar pH increase in stored soybean daddawa with storage time also possibly indicates continuous, post processing activity of the fermenting microorganisms (Popoola et al., 2007; Kolapo et al., 2007a). The observed pH decrease in stored soybean daddawa treated with ethanol extracts of onion in the present study might be possibly indicating that continuous production of ammonia was inhibited in such samples, or that compounds that scavenge alkaline products were extracted with the use of ethanol.

The acidity of stored soybean daddawa (which was measured as titratable acidity (TTA)) increased progressively with days of storage in the positive and negative controls as well as in all the treated samples (Table 4). However, sodium chloride salt, a traditional daddawa preservative, as well as aqueous and ethanolic extracts of onion decreased the rate of TTA increase in stored soybean daddawa. In this connection, the extracts were more effective in the observed acidity reduction. The trend of an increased TTA with storage time observed in the present study compares favourably with those obtained in stored Nigerian packed fruit juices and local beverages (Dosumu et al., 2009) and soybean daddawa (Popoola et al., 2007; Kolapo et al., 2007a). These workers on soybean daddawa had opined that increased TTA in stored soybean daddawa reflects the post processing carbohydrate metabolism of storage microorganisms. Results from the present study may be further strengthening this position, as higher concentration of sodium chloride salt (an established antimicrobial) resulted to lower values of recorded TTA in samples so treated. Abdel-Salam et al. (2014) reported the antimicrobial potential of aqueous and ethanolic extracts of onion in sausage. This observed antimicrobial potential of onion might be consequent on the occurrence of significant quantities of glutamic, oxalic, pyruvic, malic, tartaric, citric and fumaric acids in A. cepa (Galdon et al., 2008). These innate compounds of onion may have contributed to subsequent slowing down of the carbohydrate metabolisms of the storage microorganisms thus resulting in relatively lower TTA increase in soybean daddawa treated with onion extracts.

Water absorption capacity (WAC) of stored soybean daddawa gradually decreased with storage days. In this regard, there was no significant difference (P>0.05) between daddawa sample that was treated with sodium chloride salt and onion extracts preservative. In addition, solvent used in extraction, as well as the preservative concentration had no significant (P>0.05) effect on the ability of various extracts to modify WAC of the treated samples (Table 5). WAC is an indication of a product to associate with water in conditions where water is limiting (Giami, 1993). Fermentation does not affect WAC in legumes (Giami and Bekahem, 1992) while an observed decrease WAC in stored soybean daddawa may be implying that even in wet form, less water is available for microbial activities (Popoola et al., 2007). The implication of the present result is that use of onion extract will not negatively impact on the functional properties of soybean daddawa preserved with it.

Fat absorption capacity (FAC) of the stored samples decreased with storage time. There was no significant (P>0.05) difference between FAC values of positive and negative control and those samples treated with onion extracts. Both extracting solvent and preservative concentration did not have significant modifying effect on FAC values of preserved soybean daddawa (Table 6). Kinsella (1976) stated that FAC could be attributed to the physical entrapment of oils and it is related to a number of non-polar side chains on the proteins that bind hydrocarbon chains of fatty acids. Fat acts as flavour retainer and increase mouth feel of foods. Result from the present study is indicating that substituting onion extracts for sodium chloride salt to preserve soybean daddawa will not affect its FAC.

### Table 4. Effect of aqueous and ethanolic extract of Onion on titratable acidity of stored soybean daddawa

| ST         | Common salt (NaCl) | Onion extract (Aqueous) | Onion extract (Ethanolic) |
|------------|--------------------|-------------------------|---------------------------|
|            | 0%                 | 3% 5%                   | 3% 5%                     | 3% 5%                     |
| 0          | 0.017±0.002        | 0.030±0.005             | 0.024±0.001               | 0.017±0.008               |
| 2          | 0.025±0.014        | 0.038±0.001             | 0.025±0.009               | 0.019±0.009               |
| 4          | 0.029±0.005        | 0.051±0.023             | 0.026±0.009               | 0.031±0.001               |
| 6          | 0.042±0.012        | 0.052±0.004             | 0.035±0.004               | 0.037±0.001               |
| 8          | 0.045±0.012        | 0.052±0.004             | 0.038±0.006               | 0.039±0.013               |
| 10         | 0.057±0.004        | 0.058±0.003             | 0.041±0.004               | 0.035±0.003               |
| 12         | 0.076±0.018        | 0.073±0.000             | 0.055±0.001               | 0.040±0.000               |

ST: Storage Time (Day); Values are means ± SD (n=5); Values in the same column with different superscripts are significantly different (P<0.05).
Table 5. Effect of aqueous and ethanolic extracts of Onion on water absorption capacity of stored soybean daddawa

| ST  | Commonsalt (NaCl) | Onion extract (Aqueous) | Onion extract (Ethanolic) |
|-----|-------------------|-------------------------|--------------------------|
| 0   | 1.2±0.0           | 1.1±0.0                 | 1.0±0.0                  |
| 2   | 1.5±0.0           | 1.4±0.0                 | 1.3±0.0                  |
| 4   | 1.8±0.0           | 1.7±0.0                 | 1.6±0.0                  |
| 6   | 2.1±0.0           | 2.0±0.0                 | 2.0±0.0                  |
| 8   | 2.4±0.0           | 2.3±0.0                 | 2.3±0.0                  |
| 10  | 2.7±0.0           | 2.6±0.0                 | 2.6±0.0                  |
| 12  | 3.0±0.0           | 2.9±0.0                 | 2.9±0.0                  |

Table 6. Effect of aqueous and ethanolic extracts of Onion on Fat absorption capacity of stored soybean daddawa

| ST  | Commonsalt (NaCl) | Onion extract (Aqueous) | Onion extract (Ethanolic) |
|-----|-------------------|-------------------------|--------------------------|
| 0   | 2.5±0.0           | 2.4±0.0                 | 2.3±0.0                  |
| 2   | 2.8±0.0           | 2.7±0.0                 | 2.6±0.0                  |
| 4   | 3.1±0.0           | 3.0±0.0                 | 2.9±0.0                  |
| 6   | 3.4±0.0           | 3.3±0.0                 | 3.2±0.0                  |
| 8   | 3.7±0.0           | 3.6±0.0                 | 3.5±0.0                  |
| 10  | 4.0±0.0           | 3.9±0.0                 | 3.8±0.0                  |
| 12  | 4.3±0.0           | 4.2±0.0                 | 4.1±0.0                  |

Conclusion

Results of the present study showed that both aqueous and ethanolicionion extracts arrested lipid peroxidation in stored soybean daddawa while sodium chloride salt, which is commonly employed in soybean daddawa preservation did not. In addition, the use of onion extracts for soybean daddawa preservation in place of sodium chloride salt will not negatively impact on its functional properties. The observed potential of onion extracts coupled with the medicinal properties of onion will probably qualify it as a relevant alternative for soybean daddawa preservation especially now that there are great global concerns on regulating/reducing dietary salt intake.

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