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Comparative study of the effect of dry and wet ginger (*Zingiber officinale Roscoe*) spice on the proximate and microbial safety of soybean beverage

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Summary

Soybean beverage, most common nutritious local beverage in Nigeria and in the world, is a high protein beverage used as a dairy milk substitute with the limited utilization due to natural or ambient conditions that serve as growth medium for microorganisms. Hence, it has a short shelf life. This study examines the shelf life of soybean beverage preserved with the ginger spice (dried at 70 ˚C, 80 ˚C, 90 ˚C and 100 ˚C, and 2 g and 4 g of fresh/wet ginger respectively) over 7-week period. The samples were (A: plain soybean beverage; B: 200 ml soybean beverage + 2 g of ginger dried at 100 ˚C; C: 200 ml soybean beverage + 2 g ginger dried at 90 ˚C; D: 200 ml soybean beverage + 2 g ginger dried at 80 ˚C; E: 200 ml soybean beverage + 2 g ginger dried at 70 ˚C; F: 200 ml soybean beverage + 2 g fresh ginger; and G: 200 ml soybean beverage + 4 g fresh ginger respectively). The proximate, pH, microbial and sensory analyses of samples ranged as follows: 87.35% - 90.83% for the moisture content; 0.58% - 0.65% ash content; 4.65% - 4.96% protein; 0.10%-0.26% fibre content; 2.06% - 2.98% crude fat and 1.68% - 4.17% carbohydrate, and pH values ranged from 6.2 - 6.5. Microbiological analysis over storage period showed that the control sample ranged from 0.4×10^6 -8.3×10^6 cfu/ml, and treated samples from 0.4×10^6 to 2.4×10^6 cfu/ml. Low values of the samples treated with dry ginger spice were preserved better than others, probably due to preservative and anti-microbial properties of the spice. Sensory evaluation, carried out by twenty-eight persons, showed that the sample E: (200 ml soymilk+ 2 g ginger dried at 70 ˚C) was most preferred (with respect to taste, aroma and overall acceptability), while there was a significant difference in the appearance of the samples.

Keywords: Soymilk, ginger (*Zingiber officinale Roscoe*), proximate analysis, microbiological analysis, safety, shelf life, preservation

Introduction

Soybean beverage has been given considerable attention as an economically high protein beverage that can help to eradicate or overcome protein deficiencies found in animal milk. It is a water extract of soybean, a grain legume and one of the world's oldest known food sources for human beings. It contains ingredients of a good-quality for food, feed and pharmaceuticals, and has other industrial applications (Messina, 2000; Tripathi et al., 2010). Phytochemicals present in soybean are health promoting. Soybean is nutritious, economical and ecofriendly, as it can be cultivated in many places. The edible portion of soybean contains about 40% protein, 27% complex carbohydrates, 20% oil, 8% moisture, and 5% minerals. Soy protein is the best plant protein and its quality is close to that of livestock proteins in terms of protein digestibility-corrected amino acids score (PDCAAS), and it is least expensive (Rs 100 – 150/kg of soy protein), whereas pulse proteins cost Rs 300 – 350/kg of protein and the cost of that from livestock sources is Rs 500 – 1800/kg of protein (Henkel, 2000; Tripathi and Mangaraji, 2011). As of now, the world soybean production is about 240 MT (million tonnes), and that of India is about 13 MT. The five major soybean producing countries of the world are USA, Brazil, Argentina, China and India (Mangari, 2011; Mangari et al., 2011). Rs is the nation's unit of currency. The edible portion of soybean contains about 40% protein, 27% complex carbohydrate, 20% oil, 8% moisture, and 5% minerals.

Soybean products are rich in proteins, fats, carbohydrates, and other mineral elements. High-quality soy protein is considered equal in quality to that of poultry and milk. For example, the soybean beverage and bean curd contain the highest concentration of protein among all the legumes, about 40% protein by volume compared to 20% for other beans. The protein is very important in our body and helps in avoiding serious diseases. In the last decades, soybean foods have generated a lot of interest due to the fact thattheir consumption may alleviate menopausal symptoms (Messina, 2000), reduce the risk of osteoporosis and some chronic diseases, most notably coronary heart disease and cancer (McGee, 2004).

The quality of the food is also determined by its nutrient contents. Nowadays, customers are well educated and knowledgeable, as they are more concerned with the nutritional contents of the food they take. For this reason, it is important to develop new nutritional food, maximize the nutritional contents of, both processed and/or stored food, by extending its shelf-life to meet market requirements.

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Ginger, a herbaceous perennial *zingiber officinale roscoe*, belonging to the family zingiberaceae is grown commercially in most tropical regions (Ghayur et al., 2005). The plant is native to tropical South East Asia from where it was later introduced to Jamaica, Africa and other tropical regions of the world (Ghayur et al., 2005). The family is cultivated widely in the tropics for its showy flowers and useful products, derived mostly from the rhizomes. These products include the flavouring ginger; east Indian arrowroot, a food starch; and turmeric, an important ingredient in the curry powder. The rhizome, which is valued for its flavour, contains two classes of constituents: the essential oils and oleoresins (Rhode et al., 2007).

The essential oil consists of mono-terpenes and sesquiterpenes, which contribute to the characteristic flavour of ginger, and the more volatile oleoresin is responsible for the pungent flavour of ginger, which is also a source of antioxidants (Bartley and Jacobs, 2000). Essential oil and oleoresin are internationally commercialized for use in the food and pharmaceutical industries. Moreover, ginger is well known all over the world, especially as a remedy for the disorders of gastrointestinal tract such as constipation, dyspepsia, diarrhea, nausea and vomiting (Ghayur, 2005). Ginger is also recommended by the traditional healers in South Asia, because it reduces the risk ofcardiovascular diseases, high blood pressure and palpitations, and also serves as a vasodilator (Ghayur, 2005).

In this research, a comparative study of the preservative potentials of both dry and wet ginger spice on the soybean beverage was performed. It is aimed at emphasizing the safety of the product for consumption, making it attractive to consumers and potential consumers, but also at providing means of reducing the level of malnutrition that is prevalent in most third world countries by using a plant product with cheaper high protein content (e.g soybean).

Materials and methods

Soybeans and ginger spice were purchased at a popular market in Ilorin metropolis in Kwara State, Nigeria. Other materials and equipment used for the research work were obtained at the food processing laboratory of the Department of Home Economics and Food Science, University of Ilorin, Ilorin, Kwara State. There were seven (7) samples in all (A-G). Sample A was a plain soybean beverage, samples B-E had soybean beverage treated with equal quantities of ginger dried at temperatures of 100-70 °C respectively, while samples F and G had soybean beverage treated with 2 g and 4 g of fresh ginger respectively.

Production of Soymilk

The soymilk was processed from the soybeans by the method described by Iwe (2003).

Drying of ginger

Ginger was divided into 6 different portions. Four portions were dried at different temperatures, namely those of 70 ˚C, 80 ˚C, 90 ˚C and 100 ˚C in the oven, while the remaining two portions were used as fresh samples.

Sensory evaluation

Sensory evaluation of both treated and untreated samples of the soymilk with respect to the colour, flavour, taste and general acceptability was carried out by twenty eight (28) untrained, but regular consumers of juice and soybean beverage. A nine-point hedonic scale, varying from “dislike extremely” (score 1) to “like extremely” was used (score 9), according to method of Stone and Sidel (1992).

Chemical analysis

Proximate composition of the soymilk samples was determined by the methods of AOAC, 2005, where the carbohydrate content was determined by estimating the difference: %Total carbohydrate = 100-(% water + % protein + % fat + % ash + % crude fibre). The moisture content of the ginger used for the preservation was determined, but the other parameters of the composition were not done.

Microbial analysis

The bacterial count of the samples was determined by the methods of Adegoke (2000). Total viable counts of the samples were determined using a pour plate technique. Approximately 1 ml of the dilution was placed on the nutrient agar plates and potatoes dextrose agar. The plates were incubated at 27 °C and 37 °C for 72 h and 24 h respectively for nutrient and potatoes dextrose agars as colony forming unit per sample (CFU/g). The 28 g of nutrient agar was weighed and dissolved in 1 liter of distilled water. It was shaken in order to mix properly, and heated for the powder to be dissolved completely. After heating, the mouth of the flask was plugged with the cotton wool and wrapped with the aluminum foil. The medium was then sterilized in the autoclave at 121 °C for 15 min and then allowed to cool to about 45 °C before pouring aseptically into petri-dishes.

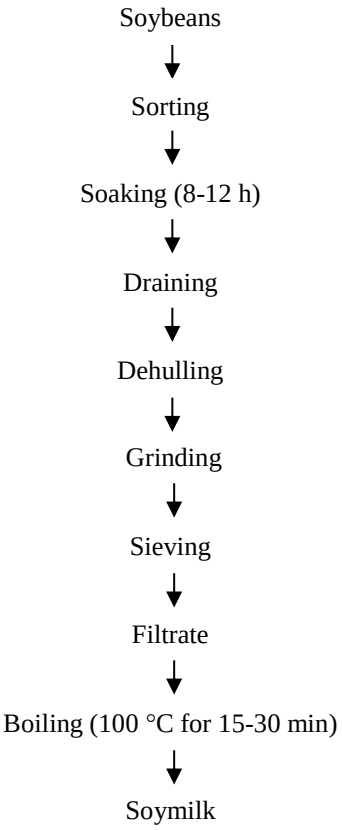

Fig. 1. Flowchart of the soybean beverage production (Adapted from Iwe (2003))

The 1 g of the sample was pipetted aseptically into 9 ml of sterile, distilled water in a test-tube and a serial dilution was used by pipetting 1ml of the dilution into a 9 ml of sterile, distilled water. The dilution was prepared up to 10^{-6} dilutions. The dilutions were plated by transferring 1ml of the dilution into a separate petridish (in duplicate), and sterile molten nutrient agar was added and mixed by swirling the plate before allowing it to solidify. The plates were incubated at 37 °C for 24 hours and were examined for growth and colonies, counted and recorded as a total bacterial count.

Statistical analysis

Data obtained from the proximate composition and sensory overall quality attributes were analysed statistically using the Analysis of variance (ANOVA). The mean scores were computed and the significant difference among the mean was determined using the Least Significant Difference (LSD) using Statistical Package for Social Sciences (SPSS) Version 16.0 (SPSS Inc., Chicago, IL USA). The microbial count was illustrated using the line chart. The soymilk samples were subjected to the qualitative physicochemical analysis.

Results and discussions

Proximate composition of the soymilk samples

The results of the proximate composition of the samples are presented in Table 1. The pH of the samples ranged from 6.2 to 6.5, showing that the medium is in the basic range and as such, liable to spoilage faster. The sample A (plain soybean beverage) had the highest pH value of 6.5. The spice in the other samples could have caused the slight reduction in the pH values of the treated samples, while 6.75 was reported for raw soybean beverage, as similarly reported by Ezekiel and Fapohunda (2012). The moisture content of the soybean beverage samples ranged from 87.35% to 90.77%. The untreated soymilk had a moisture content of 90.58%. The slight differences noticed in the moisture content of the treated samples could be due to the addition of the spice which gave the sample a slight solid content. There was a significant difference between the samples at $p<0.05$. All the samples had high moisture content

similar to 89.60% and 89.40% reported in the work of Salim-ur-Rehman et al. (2007), and Isanga and Zhang (2008).

The ash content was in the range from 0.58% to 0.68%. There were slight differences between the samples with a significant difference at $P<0.05$. The untreated soybean beverage had the lowest ash content of 0.58%, which coincided with the result of Salim-ur-Rehman et al. (2007), while the sample C (200 ml soybean beverage + 2 g ginger dried at 90 °C) had the highest ash content of 0.68%. The ginger could have contributed to the increase in the ash content of the soybean beverage samples. Ash is a source of minerals, and a large, substantial amount of mineral element depends on the chemical composition of the soil, cultural practices, time of planting, and the amount of water available to the plant (Hymowitz, 2003). The protein content ranged from 4.65% to 4.96%. The protein content of the untreated sample was the highest (4.96%). There were slight differences in the protein content of the samples and they were significant. The variation in the protein content could be attributed to variety in cultivar and species linked to factors such as climate, maturity at harvest, and length of storage or time.

Crude fibre, consisting principally of cellulose, and lignin together with the small amounts of hemicelluloses had values ranging from 0.10% to 0.26%. The untreated sample had no fibre content, which conforms to the report of Salim-ur-Rehman et al. (2007).

The fat content ranged from 2.06% to 2.98%. The sample E (200 ml soybean beverage + 2 g ginger dried at 70 °C) had the highest value of fat, while the sample F (200 ml soybean beverage + 2 g wet ginger) had the lowest value of fat. The high fat content noticed in the sample E could be due to the concentrated nature of the components of the ginger that was dried. The samples were significantly different at $p>0.05$. The results were comparable to the results obtained by Salim-ur-Rehman et al. (2007).

Crude carbohydrate ranged from 1.68% to 4.17%. The untreated sample had the lowest carbohydrate value, while the sample E (200 ml soymilk + 2 g ginger dried at 70 °C) had the highest value of 4.17%. The results were comparable to the results obtained by Ezekiel and Fapohunda (2012). The high content noticed in the sample E could be due to the concentrated nature of the components of the ginger that was dried.

The moisture content of the ginger used for the treatment and dried at temperatures between 70 and 100 °C ranged from 12.4 to 14.3%, which provided the high concentration of the gingerol that disrupted the growth of bacteria.

Microbial analysis of the soybean beverage samples

It was reported that the milk sample after storage at 5 °C, should have a count that was less than $1x\ 10^5$ cfu/ml (Ihekoronye and Ngoddy, 1985). It was established that the milk sample containing 5.0×10^3 cfu/ml of bacteria is classified as good for consumption. About 1.0×10^4 to 4.0×10^5 cfu/ml is fairly good. Counts of about 2.0×10^6 are acceptable, while over 2.0×10^7 cfu/ml are not good (i.e harmful) for consumption. It is also a fact that soybean beverage is a high protein beverage used as a dairy milk substitute, but its utilization is limited because at natural ambient, most especially in the tropics,, the conditions serve as a growth medium for microorganisms, and confer a very short life span of the soybean beverage product.

It can be noticed in Table 2 that the microbial load of the plain soymilk product was much higher than that of the treated samples. Initially, the microbial load noticed in the prepared samples was 0.4×10^6 cfu/ml. The high value recorded could have been a result of the poor hygienic handling coupled with the environmental factors. For about 5 weeks of storage, no microbial load was observable in the treated samples, while the microbial load of the control samples kept increasing, since the product was kept in storage. Fitri and Rina (2016) reached a conclusion that green tea and ginger are effective in preventing bacteria growth due to their active ingredients gingerol and shogaol, which inhibited the growth of bacteria in the soybean beverage.

Table 1. Proximate composition of the soymilk samples

Samples	pH	Moisture Content (%)	Fibre (%)	Ash (%)	Protein (%)	Fat (%)	Carbo hydrate (%)
A	6.5	$90.58^{a} \pm 0.11$	-	$0.58^{c} \pm 0.007$	$4.96^{a} \pm 0.007$	$2.20^{b} \pm 0.02$	$1.68^{f} \pm 0.01$
B	6.2	$89.81^{b} \pm 0.08$	$0.18^{c} \pm 0.00$	$0.65^{ab} \pm 0.014$	$4.83^{b} \pm 0.014$	$2.14^{c} \pm 0.00$	$2.39^{e} \pm 0.03$
C	6.3	$89.62^{b} \pm 0.03$	$0.16^{cd} \pm 0.00$	$0.68^{a} \pm 0.014$	$4.87^{ab} \pm 0.014$	$2.18^{b} \pm 0.03$	$2.49^{d} \pm 0.04$
D	6.3	$89.69^{b} \pm 0.16$	$0.14^{cd} \pm 0.02$	$0.61^{b} \pm 0.007$	$4.75^{c} \pm 0.007$	$2.16^{bc} \pm 0.04$	$2.65^{c} \pm 0.06$
E	6.4	$87.35^{c} \pm 0.21$	$0.20^{b} \pm 0.00$	$0.65^{ab} \pm 0.014$	$4.65^{d} \pm 0.035$	$2.98^{a} \pm 0.02$	$4.17^{a} \pm 0.06$
F	6.4	$89.47^{b} \pm 0.25$	$0.26^{a} \pm 0.00$	$0.62^{b} \pm 0.014$	$4.84^{ab} \pm 0.007$	$2.06^{d} \pm 0.04$	$3.35^{b} \pm 0.04$
G	6.4	$90.77^{a} \pm 0.17$	$0.10^{d} \pm 0.04$	$0.63^{b} \pm 0.014$	$4.68^{d} \pm 0.014$	$2.12^{c} \pm 0.03$	$1.70^{f} \pm 0.02$

Values in the same column with different superscript are significantly different ($p>0.05$). A-pure soymilk; B-soymilk + ginger dried at 100 ˚C; C-soymilk + ginger dried at 90 ˚C; D-soymilk + ginger dried at 80 ˚C; E- soymilk + ginger dried at 70 ˚C; F- soymilk + 2 g fresh ginger; G- soymilk + 4 g fresh ginger

Table 2. Results of the microbial analyses of the soymilk samples (X 10^6 cfu/ml)

WK/Sample	A	B	C	D	E	F	G
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4
1	2.0	NG	NG	NG	NG	NG	NG
2	2.8	NG	NG	NG	NG	NG	0.1
3	3.1	NG	NG	NG	NG	0.1	0.4
4	3.5	NG	NG	NG	0.2	0.4	0.8
5	4.2	0.1	NG	NG	0.2	0.3	0.5
6	6.3	0.6	1.2	0.4	0.6	0.8	1.0
7	8.3	1.3	1.3	1.1	1.6	1.9	2.4

A-pure soymilk; B-soymilk + ginger dried at 100 ˚C; C-soymilk + ginger dried at 90 ˚C; D-soymilk + ginger dried at 80 ˚C; E- soymilk + ginger dried at 70 ˚C; F- soymilk + 2 g fresh ginger; G- soymilk + 4 g fresh ginger. NG: No growth

Table 3. Results of the sensory evaluation of the soymilk samples

Samples	Taste	Aroma	Flavour	Appearance	General acceptability
A	$6.90^{ab} \pm 1.19$	$7.10^{ab} \pm 1.97$	$6.70^{ab} \pm 1.83$	$7.40^{a} \pm 2.41$	$7.10^{b} \pm 1.73$
B	$5.60^{c} \pm 1.84$	$6.10^{b} \pm 1.79$	$5.90^{ab} \pm 2.18$	$7.80^{a} \pm 1.23$	$6.70^{b} \pm 1.16$
C	$6.60^{abc} \pm 1.17$	$5.70^{b} \pm 1.64$	$6.00^{ab} \pm 1.89$	$6.90^{a} \pm 1.59$	$6.70^{b} \pm 0.95$
D	$7.80^{a} \pm 0.92$	$6.90^{ab} \pm 1.19$	$6.40^{ab} \pm 1.35$	$7.90^{a} \pm 1.10$	$7.40^{ab} \pm 0.70$
E	$7.40^{a} \pm 1.08$	$8.10a \pm 1.10$	$7.30^{a} \pm 1.70$	$7.90^{a} \pm 1.29$	$8.40^{a} \pm 0.70$
F	$5.80^{bc} \pm 2.25$	$5.40^{b} \pm 2.41$	$5.90^{ab} \pm 2.13$	$7.50^{a} \pm 1.50$	$7.00^{b} \pm 1.16$
G	$7.20^{a} \pm 0.92$	$6.50^{ab} \pm 1.95$	$7.00^{ab} \pm 1.41$	$7.00^{a} \pm 2.36$	$6.90^{b} \pm 1.60$

Values in the same column with different superscript are significantly different ($p>0.05$). A-pure soymilk; B-soymilk + ginger dried at 100 ˚C; C-soymilk + ginger dried at 90 ˚C; D-soymilk + ginger dried at 80 ˚C; E- soymilk + ginger dried at 70 ˚C; F- soymilk + 2 g fresh ginger; G- soymilk + 4 g fresh ginger

They also reported that the compounds damage the membrane and the cell wall, resulting in a change in the permeability and the release of intra cellular constituents such as ribose transport, nutrient uptake, synthesis of nucleic acids and proteins, and enzyme activity, which invariably inhibits the activity of the pathogenic bacteria. The potency of the spice was shown when the initial load of the samples was destroyed and the sample was kept without microorganism for up to five weeks (samples C and D). It was equally noticed that samples treated with ginger spice dried at 70 and 80 degree centigrade preserve the product better than other treatments, most especially, samples treated with the wet ginger. Ghayur et al. (2005) reported that spices such as ginger have less antimicrobial effect on the microbial growth at lower concentrations, but more inhibitory at higher concentrations. Adegoke (2015) reported that the microorganisms found in products could be either positive or negative in nature. They could aid good production or produce chemicals that can lead to spoilage in soy or other products. *Bacillus cereus* is an example of the microorganism present and it can cause problems in the soybean beverage, as well as in in both raw and pasteurized milk, particularly during hot or warm periods of the year due to "Lecithinase activities" of the organism on fat globules present in milk. The microbial count of the samples treated with the dry spice over the period of storage was 1.6 x 10^6 cfu/ml (maximum), the samples treated with the wet ginger spice had 2.9 x 10^6 cfu/ml (maximum), while the untreated sample had 8.3 x 10^6 cfu/ml (maximum). Dried spices had a better preservative effect on the samples than the wet ones from the research, most probably because the wet spice had moisture which could have aided the growth of microorganisms in the product.

Sensory evaluation of soymilk samples

Table 3 show the results of the sensory evaluation carried on the fresh soybean beverage samples (both treated and untreated). There were significant differences in all the parameters measured except for the appearance at $p>0.05$. For the taste, the samples D and E were most preferred; for aroma and flavour, the sample E was most preferred; appearance had no significant differences, as all the products appeared good, while for general acceptability, the sample E was preferred. The acceptance of the sample E, the sample treated with spice dried at 70 degree centigrade, could be due to its less pungency nature because of the shorter time of drying.

Conclusions

This study looked at soymilk samples treated with ginger (dried and fresh), as well as their effect on the proximate composition, sensory characteristics and microbial load. It could be concluded that the sample E (200 ml soymilk + 2 g ginger dried at 70 °C) had the

best quality in terms of sensory evaluation. Furthermore, the spices (both wet and dry) were instrumental for the preservation observed in the treated samples by the reduction of the microbial load of the soymilk.

Use of the ginger spice in preserving soymilk should be intensified in order to obtain improved flavour, aroma, as well as to extend shelf life. For further improvement of the soymilk taste, Illinois method should be used in the production of the soymilk in order to remove the beany flavor.

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