

**MICROBIOLOGICAL AND PHYSIOCHEMICAL ASSESSMENT OF STREET
VENDED FRIED SOYABEAN CHEESE SOLD WITHIN
ILORIN METROPOLIS, NIGERIA.**

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Abstract

*The microbiological and physicochemical quality of street vended fried soyabean cheese products from five different locations was assessed. Standard pour plate technique was used to examine the microbial load of the soyabean cheese. The pH ranged from 6.48 ± 0.241 – 6.58 ± 0.192 , Moisture content ranged from $13.94\% \pm 0.152$ – $14.22\% \pm 0.179$. The viable bacterial counts ranged from 7.0 to 61×10^5 cfu/g, while the fungal load ranged from 1 to 40×10^3 cfu/g. Eight bacteria species were isolated and identified as *Bacillus subtilis*, *Escherichia coli*, *Micrococcus* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Similarly, four fungal species were isolated and identified as *Mucor racemosus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Rhizopus stolonifer*. The results suggest that the soyabean cheese samples were contaminated with pathogenic microorganisms and could cause health hazard to the consumers. There is the need to improve personal hygiene and environmental sanitation during and after production of the soyabean cheese.*

Keywords: Soyabean cheese, Quality, Bacteria, Fungi, microbial quality.

Introduction

Food is any substance taken or consumed to provide nutritional support for the body and can be either of plant or animal origin which contains the entire necessary nutrient for growth such as protein, carbohydrate, vitamins and minerals, fat and oils (Davidson, 2006). Foods (such as soyabean cheese) are not only of nutritional value to those who consume them but are often ideal culture medium for microbial growth. Since food is capable of supplying man with nutrients, it is equally capable of supporting the growth of microorganisms (Pelczar *et al.*, 1993)

Soyabean (*Glycine max* merrile) of the family Leguminosea is native of China and is one of the world's oldest crops. The major areas of soyabean production in Nigeria are: Benue, Bauchi, Plateau and Kaduna States (Ikuomola & Eniola, 2010). Soyabean, on dry weight basis, contains about 40% Protein, 21% Oil, 34% Carbohydrate and 4.9% Ash. The amino acid profile of soyabean is close to that recommended by Food and Agricultural Organization (FAO) of United Nations for human nutrition in terms of essential and non essential amino acids. Investigation of the quality of soyabean products has been the subject of intense research for several decades due to increasing importance of soyabeans in human diet.

The seed is used to produce soy-oil and other products such as soy-milk, soy-cheese, soy-flour, soy-meat, soy-ogi, soy-garri, beske and tempeh. Soy flour has several uses in feed, baked products (bread, cake and chin chin) and infant feed formulation. It is also used as condiments in stew, sauce and in vegetable soup preparation, while the cake obtained after oil extraction is utilized to formulate diet for animals and human being.

Soyabean cheese, is an indigenous, non-fermented fried soyabean snack widely consumed owing to its high protein quality and content. The product is usually consumed either alone or along with soaked garri. It is a cheap product because the grains and other ingredients used for its production and packaging are readily available. Furthermore, the processing methods are simple and cheap as no sophisticated equipment and expertise are required. The snack is largely produced on small scale, usually as a family venture, using an indigenous method with unquantified amounts of ingredients (Ikuomola & Eniola, 2010). To make soyabean cheese, soya beans is collected and cleaned, the cleaned grains are then soaked overnight in big uncovered bowls. The soaked grains are then washed in cleaned water and then wet milled into fine particle. The mash is then mixed with large amount of water and then sieved using a muslin cloth. The slurry is then placed in a clean pot and cooked for 30 minutes with continuous stirring to prevent burning. While boiling some amount of 'ogi' whey is added which serves as a coagulant. The resulting paste is poured in a jute bag and pressed manually, the paste is poured into a neat muslin cloth and a heavy clean stone is placed to drain out all water. With the aid of a clean sharp knife, the compressed paste is then cut into desired shapes and sizes. The pieces are then boiled in water with the addition of magi, pepper and salt. After boiling, the cubes are the fried. It is cooled and soyabean cheese is ready for sale. (Ikuomola & Eniola, 2010).

Contamination of soyabean cheese by microorganisms occurs during and after processing. The ingredients, crude processing and retailing conditions predispose the product to microbial contamination. Other sources of contamination include water, utensils, soil dust, food handlers, packaging materials (Frazier & Westhoff, 2004). Contamination of soyabean cheese poses a serious health problem since it is a ready to eat snack and as such they might not be cooked before eating. This study was therefore conducted to determine the quality of ready to eat soyabean cheese retailed on the streets of Ilorin metropolis. The objectives of this research were to determine the bacterial and fungal loads, and their identities and to also determine some physicochemical characteristics of soyabean cheese that may predispose it to microbial contamination and proffer ways of preventing such.

Materials and Methods

Collection of Soyabean Cheese Samples

Freshly prepared fried soyabean cheese samples were collected from five locations within Ilorin metropolis. These were Production point (Tanke), Oja-oba, Ipata market, unity road, Oja-tuntun, and Yoruba road market. Samples from each location were collected separately into clean sterile sampling bags and taken to the microbiology laboratory, University of Ilorin for immediate analysis.

Physiochemical Analyses

pH Determination

The pH of the samples was taken using pH meter (Denver instrument model 20) which was first standardized with buffer solutions at pH 4, 7, and 9. Five gram (5g) of the macerated samples was weighed into 20ml of distilled water. It was mixed together and the mixture was filtered into a clean conical flask. The pH of the suspension was then determined by inserting the electrode of the pH meter into the solution and the pH value read (Daniyan *et al.*, 2011).

Determination of Titratable acidity

The whey of the samples was used to determine the titratable acidity, this was used for titratable acidity. From the whey, 25ml was pipetted into conical flask and 2 drops of phenolphthalein indicator was added. Each of the experiment was repeated 3 times with the

when titrated against 0.1N of Sodium Hydroxide (NaOH). The titratable acidity was expressed as the volume (in ml) of 0.1N NaOH used per 25ml of the filtrate (AOAC, 2006).

Determination of Moisture Content

One gram of each macerated sample was weighed into a crucible of known weight and then dried in an oven at temperature of 170°C, after 1 hour the samples were taken out of the oven and allowed to cool down. The weight of the crucible was subtracted from the weight of the dried sample leaving only the weight of the dried sample (AOAC, 2006). The percentage moisture content was then calculated thus:

$$\text{Moisture (\%)} = \frac{(W_1 - W_2) \times 100}{W_1}$$

Where: W_1 = weight (g) of sample before drying
 W_2 = weight (g) of sample after drying

Determination of Protein Content

Using the Kjeldahl method, two grams of the macerated sample was measured into a Kjeldahl flask and 5 grams of sodium sulphate, 1 gram of copper sulphate and 25 ml of concentrated tetra oxosulphate (vi) acid was then added. The flask was then carefully heated to boiling for 30 minutes and then allowed to digest. It was then allowed to cool and then diluted to 250ml with distilled water. Five milliliter of the resulting solution was then pipetted into a conical flask and excess concentration sodium hydroxide was added to make a strong alkaline solution so that all ammoniacal nitrogen changed to ammonium sulphate. Two percent (2%) boric acid was added and was then titrated against 0.01M hydrochloric acid (Kadash & Kusulum, 1992).

$$\text{Total nitrogen (g/100cm}^3\text{)} = \frac{14 \times 100 \text{ volume of acid}}{100}$$

$$\text{Protein/100 cm}^3 = \text{total nitrogen} \times 6.25$$

$$\% \text{ Crude protein} = \frac{14 \times 0.01 \times a \times b \times 6.25 \times 100}{C \times d}$$

Where a = volume of acid used
 b = total volume of distilled = 250ml
 c = volume of distilled use for titration = 5ml
 d = weight of sample = 2g

Determination of Ash Content

This was determined according to the method described by Kirk and Sawyer (1991). The crucible with the 5g of sample was intensely heated on the Bunsen flame until smoke ceased, and then transferred into a muffle furnace where it was ashed for 24 hours at 600°C to white ashes. The crucible and its contents were then removed and placed in a desiccator to cool after which it was weighed to a constant weight and the amount of ash calculated using the following relation:

$$\text{Ash content (\%)} = 100 \frac{A - B}{C}$$

Where:

A = weight of crucible with sample (g)
 B = weight of crucible with ash (g)
 C = weight of sample (g)

Determination of Fat Content

Determination of fat was carried out by Werner-Schmid process (Kirk & Sawyer, 1991). Proteins are digested with conc. hydrochloric acid. Five gram of samples was weighed in extraction thimble and place in extraction unit. Liberated fat was extracted using alcohol, ethyl ether and petroleum ether. Ethers are evaporated and residue left behind is weighed to calculate the fat content.

$$\text{Fat (\%)} = \frac{B - A}{C} \times 100$$

Where:

A = weight of clean dry flask (g)

B = weight of flask with fat (g)

C = weight of sample (g)

Determination of Crude Fibre content

The crude fibre content was determined using the method described by Kirk and Sawyer (1991). Four gram of sample was defatted using petroleum ether. The defatted sample was boiled under reflux for 30 min with 200 ml (1.25%) H₂SO₄. It was further filtered and washed with boiling water until the wash in water was no longer acidic. The residue was boiled at 100°C in a round bottom flask with 200 ml (1.25%) NaOH for another 30 min and then filtered and washed with boiling water until the wash water was no longer alkaline. The residue was scraped into a previously weighed crucible and dried at 100°C for 12 hours. It was left in desiccators to cool and weighed. It was thereafter ashed in a muffle furnace at about 600°C for 24 hours, left in a desiccator to cool and then weighed and calculated using the following relation:

$$\text{Crude fibre content (\%)} = \frac{100(A-B)}{C}$$

Where A = Weight of crucible with dry residue (g).

B = Weight of crucible with ash (g)

C = Weight of sample (g)

Determination of Carbohydrate Content

A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in foods. The carbohydrate content of the sample was determined by calculating the percent remaining after all the other components have been measured: %carbohydrates = 100 - %moisture - %protein - %crude fibre - %ash - %fat (Chinma & Igor, 2007).

Microbiological Analyses**Total Bacterial Counts**

In order to isolate and enumerate bacteria, 1g of the soyabean cheese sample was macerated and serially diluted up to 10⁻⁵. Nutrient agar was used for the isolation using the pour plate technique (Fawole & Oso, 2004).

Total and Faecal Coliform Counts

Plating of 0.5ml of the aliquot was done at 10⁻¹ and 10⁻² dilutions using MacConkey agar and Eosin methylene blue agar medium for the isolation of total and faecal coliforms respectively using spread plate technique. Typical coliforms gave pink or red colonies on MacConkey agar, while faecal coliform (*Escherichia coli*) produced colonies with Greenish sheen on Eosin methylene blue agar medium (Willey *et al.*, 2011).

Fungal Counts

Spread plate method was used to isolate and enumerate fungal population. The medium of choice was potato dextrose agar. Plating of the sample was done at 10^{-2} dilution (Fawole & Oso, 2004).

Preparation of Pure Culture of the Isolates

Subculturing was done in order to obtain pure culture of each isolate from the mixed cultures. The pure culture obtained was then preserved in a slant and kept in the refrigerator until required for identification (Fawole & Oso, 2004).

Characterization of Isolates

The bacterial isolates were characterized on the basis of their colonial, cellular, and biochemical characteristics (Buchanan & Gibson, 1974), while the fungal isolates were identified based on their cultural, macroscopic and microscopic characteristics (Onion *et al.*, 1981). The biochemical tests conducted were: catalase, motility, citrate utilization, coagulase, starch hydrolysis, oxidase, Indole, Methyl Red Voges Proskauer (MRVP), Nitrate reduction, Urease and fermentation of various sugars.

Statistical Analyses

The statistical analysis package, SPSS 16.0 was used to determine the mean, range, and standard error of mean of the data, while means were then separated using Duncan's Multiple Range Test (SPSS, 2010).

Results

Physiochemical characteristics

The pH value of the snacks ranged between 6.48 to 6.58, titratable acidity ranged between 3.62 to 3.70 cm³, the ash content was 3.10 to 3.22%, moisture content ranged between 13.94 to 14.90 %, crude fibre ranged between 0.550 to 0.612 %, crude protein ranged between 15.32 to 16.48 %, fat content ranged between 13.74 to 15.30 %, while carbohydrate ranged between 50.16 to 51.76 g. The sample from Ipata Market had the highest pH of 6.58 while the lowest pH of 6.48 was recorded for both Oja tuntun and Obo Market. The highest protein content was found in samples from Obo Market while the lowest was found in samples from Oja-oba Market (Table 1).

Microbial counts of soyabean cheese samples for different markets

The bacterial count, total coliform and faecal coliform of the soyabean cheese samples ranged from $7.0 - 61 \times 10^5$ cfu/g, nil to 14×10^3 cfu/g, and nil to 3×10^3 cfu/g respectively. While the fungal count ranged from 1 to 33×10^3 cfu/g (Table 2).

Microbial isolates and their occurrence

Eight bacterial species and four fungal species were identified (Table 3) and their occurrences was as presented in Table 4. The bacteria comprise both Gram positive and Gram negative bacteria.

Table 1: Physico-Chemical Properties of the Beske samples collected within Ilorin metropolis

Chemical Properties	Tanke Market	Yoruba Market	Ojo Oba Market	Ipata Market	Oja Tuntun	Obo Market (Unity Road)
Ash(g)	3.16±0.152 ^b	3.20±0.158 ^b	3.10±0.100 ^b	3.20±0.158 ^b	3.18±0.148 ^b	3.22±0.148 ^b
Moisture content(%)	13.94±0.100 ^a	14.22±0.259 ^b	14.90±0.152 ^b	14.20±0.141 ^b	14.22±0.179 ^b	14.02±0.110 ^b
Crude Fibre (%)	0.550±0.500 ^b	0.540±0.198 ^b	0.588±0.075 ^b	0.612±0.081 ^b	0.610±0.061 ^b	0.526±0.026 ^b
Crude Protein(g)	16.35±0.537 ^a	16.45±0.249 ^a	15.32±0.874 ^a	15.60±0.719 ^a	15.79±0.884 ^a	16.48±0.192 ^a
Fat(g)	14.04±0.167 ^b	15.30±0.224 ^b	13.74±0.297 ^b	14.58±0.396 ^b	15.08±0.217 ^b	14.18±0.249 ^b
Carbohydrate(g)	51.76±0.643 ^a	50.16±0.207 ^a	53.26±1.226 ^a	51.82±0.522 ^a	51.28±0.779 ^a	51.60±0.224 ^a
pH	6.54±0.241 ^b	6.56±0.195 ^b	6.54±0.241 ^b	6.58±0.192 ^b	6.48±0.228 ^b	6.48±0.259 ^b
Titrateable Acidity(cm ³)	3.62±0.286 ^b	3.62±0.432 ^b	3.66±0.439 ^b	3.64±0.391 ^b	3.62±0.408 ^b	3.70±0.381 ^b

Each value is the mean of triplicate (n=3) ± standard deviation of different markets. The letters and b represent ranking of means across each groups of samples. Values with superscript A and B are difference between markets. Values not sharing the same superscript differ significantly at <0.05.

Table 2: Microbial Count of Fried Soyabean Snack from Different Sellers within the Sampled Market

Sampling site	Bacterial count (x10 ⁵ cfu/g)	Total Coliform count (x 10 ⁴ cfu/g)	Faecal coliform (x 10 ³ cfu/g)	Fungal count (x 10 ³ cfu/g)
A1	23	0	0	7
2	11	6	0	3
3	15	1	0	6
4	19	1	0	2
5	18	1	0	1
B1	19	2	1	20
2	20	5	2	13
3	22	10	1	19
4	18	14	1	16
5	16	3	3	12
C1	23	2	0	17
2	32	7	2	14
3	31	11	2	33
4	24	1	0	23
5	26	3	1	14
D1	20	2	0	20
2	7.0	0	0	15
3	21	11	4	13
4	11	2	0	15
5	24	4	1	19

E1	12	1	0	21
2	48	10	4	40
3	40	4	4	17
4	15	3	1	20
5	33	2	0	19
F1	16	3	1	30
2	33	2	2	16
3	19	1	0	14
4	60	10	6	31
5	61	12	7	20

Each value is the mean of triplicate (n=3)

KEY: A, Production point (Tanke); B, Yoruba market; C, Oja-oba; D, Ipata market; E, Oja tuntun Market; F, Unity market.

Table 3: Characterization and identification of bacterial isolates

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Cellular Characteristics			Biochemical test																			Probable identity	
Bacterial isolates	Shape	Cell Arrangement	Gram Staining	Catalase	Motility	Citrate	Coagulase	Starch hydrolysis	Oxidase	Indole	Glucose	Sucrose	Lactose	Fructose	Maltose	Methyl red	Voges-Proskauer	Nitrate reduction	Urease	OF	Spore formation		H ₂ S
1	Cocci	Pairs	+	+	-	+	+	-	-	-	A	AG	AG	AG	AG	-	+	-	+	Fe	-	+	<i>Staphylococcus aureus</i>
2	Rod	Single	-	+	+	+	-	+	-	-	A	AG	AG	AG	A	-	+	+	-	Fe	-	+	<i>Enterobacter aerogenes</i>
3	Rod	Single	-	+	+	-	-	+	-	+	AG	AG	AG	AG	AG	+	-	+	-	Fe	-	+	<i>Escherichia coli</i>
4	Rod	Single	-	+	+	+	-	+	-	+	AG	A	A	AG	AG	+	-	+	+	Fe	-	-	<i>Proteus vulgaris</i>
5	Rod	Single	-	+	-	+	-	-	-	-	AG	A	AG	AG	A	-	-	+	+	Fe	-	-	<i>Klebsiella pneumoniae</i>
6	Rod	Single	-	+	+	+	-	-	+	-	AG	A	AG	AG	A	-	-	+	+	Fe	-	+	<i>Pseudomonas aeruginosa</i>
7	Rod	Pairs	+	+	+	+	-	+	-	-	AG	AG	A	AG	AG	-	+	+	-	Fe	+	-	<i>Bacillus subtilis</i>
8	Cocci	Clusters	+	+	-	+	+	-	+	-	A	AG	A	AG	A	+	+	-	+	Fe	-	-	<i>Micrococcus</i> spp.

KEY: + = positive, - = negative FE = Fermentative, OF = Oxidation- Fermentation A = Acid only AG = Acid and gas

Table 4: Occurrence of Bacterial and Fungal species from different locations

Microbial Isolates	A	B	C	D	E	F
<i>Staphylococcus aureus</i>	+	+	+	+	+	+
<i>Enterobacter aerogenes</i>	-	-	-	-	+	+
<i>Escherichia coli</i>	-	+	-	+	+	+
<i>Proteus vulgaris</i>	+	+	+	+	+	+
<i>Klebsiella pneumonia</i>	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	-
<i>Bacillus subtilis</i>	+	+	+	+	+	+
<i>Micrococcus spp.</i>	-	-	-	+	+	+
<i>Mucor mucedo</i>	+	+	+	+	+	+
<i>Aspergillus fumigatus</i>	-	-	-	+	+	+
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+
<i>Rhizopus stolonifer</i>	-	+	+	-	+	+

KEY: + = Isolated; - = Not isolated; A, Production point (Tanke market); B, Yoruba market; C, Oja-oba; D, Ipata market; E, Oja-tuntun; F, Unity market.

Discussion

The soyabean cheese samples were found to harbour *Staphylococcus aureus*, *Micrococcus sp.*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Bacillus subtilis*. The pattern of distribution showed that all the isolates were found in snacks obtained from the hawkers. This is in agreement with the research carried out by Ikuomola and Eniola (2010) who also recorded the presence of similar bacteria. All the bacteria species encountered in the soyabean cheese samples can affect the keeping quality and the health of the consumers (Willey *et al.*, 2011). The samples could also have been contaminated due to exposure and handling by intending buyers, either by touching or talking during bargaining.

Most of the organisms encountered could be found in soil, dust and on bodies of insects, animals and humans (Frazier & Westhoff, 2004). The organisms are of particular interest because of their involvement in different infections. *Staphylococcus aureus* and *micrococcus sp.* are associated specifically with the hands and nasal cavity and an indicator of contamination from human sources. The deposition of these bacteria in soyabean cheese can occur if good sanitary practices are not followed by the food handlers. Growth of *Staphylococcus aureus* on food can lead to production of enterotoxin leading to staphylococcal food intoxication. *Enterobacter aerogenes* is an agent of food spoilage as it causes ropiness or sliminess of food. The presence of organisms such as: *E. coli* and species of *Klebsiella* which are indicators of faecal contamination could be attributed to the water source and the method used in the preparation of the food and it could also be as a result of the unhygienic activities of the handlers. The presence of *E. coli* is of public health concern considering its role in food borne infection. Species of *Pseudomonas* are motile organisms and are widely distributed in water, soil and sewage. *P. aeruginosa* inhabits the intestinal tract of humans and animal. The presence of this organism indicates contamination from any of the mentioned source and suggestive of possible post – production contamination (Simopoulos & Bhat, 2000). All of these suggest that the samples could serve as common vehicle for pathogens transmitted via the faecal oral route. The presence of species of *Bacillus* is an indicator of contamination from the soil as their spores are found in the soil. It produces endospores which help it to survive the heat treatment and is associated with miscellaneous problems (Drobniewski, 1993).

The fungal species encountered in the soyabean cheese samples (*Mucor racemosus*, *Saccharomyces cerevisiae*, *Rhizopus stolonifer*, *Geotrichum candidum* and *Aspergillus fumigatus*) could also come from the environment, their presence buttress the likelihood of post- production contamination. Tudor *et al.* (2009) reported that certain species of *Aspergillus* produce toxic metabolite, while *Mucor sp.* could degrade the biochemical structure of proteins and lipids thereby altering its organoleptic property. Frazier and Westhoff (2004) have implicated *Aspergillus* and *Mucor* in food poisoning and in the spoilage of cheese. *Saccharomyces cerevisiae* occurred in the soyabean cheese samples than the other fungi and this may be due to the fact that they are the dominant organisms found in soya milk. *R. stolonifer* has been reported to cause an elevation in pH beyond the safety value of 4.6 and makes the environment more conducive for the growth of pathogenic bacteria (Effiuwevwere & Akoma, 1997). None of the sampled soyabean cheese was mouldy as at the time of sampling suggesting that the fungi were yet to be established; hence contamination is likely due to fungal propagules in the air (Ikuomola & Eniola, 2010). If the moulds get established on the soyabean cheese, mycotoxic substances which would be hazardous to consumers could be produced.

The samples might have been contaminated with microorganisms as a result of unhygienic processing and packaging conditions. The number and type of microbes present on the products are major determinant of quality deterioration. The sampled products were found to contain bacteria and moulds, which under favourable conditions could multiply leading to spoilage of products and render them inedible or make them vehicles of food poisoning or food infection. Therefore, care should be taken to prevent contamination. The hawkers of these products should also handle them in such a way that they would be prevented from insects, especially houseflies which are known to transmit microbes (Ikuomola & Eniola, 2010). These products could also be packed in sealed transparent nylon bags after production, before being packed into buckets with a cover as against exposing them in bowls, trays or glass boxes.

Conclusion

It can be concluded from this study that there is the need to monitor the quality of fried soyabean cheese being sold in the metropolis since potential pathogens were isolated.

Recommendation

It is recommended that it is important to keep soyabean cheese fit for consumption by taking adequate measures to prevent contamination during and after production. In order to achieve this, the following measures should be strictly adhered to: there is the need to improve on the hygienic conditions surrounding soyabean cheese production. Pre- production and post – production operations should be adequately checked. Care should also be taken not to use contaminated water in the course of production; and contact with soil and dust should be avoided. It is also recommended that; If unwell, food should not be handled. Touching of the hair, nose and mouth should be discouraged during food preparation. Suitable light coloured protective clothing should be worn during food preparation. Cuts and abrasions should be covered with water proof bandages and if on the hands suitable gloves should be worn, ring and other jewellery especially those worn on the wrist and fingers should not be worn during food preparation as they can harbour dirt and bacteria, utensils used for cooking should be washed well after each use to prevent cross contamination from air. Lastly, soyabean cheese should not be exposed to contamination from air and flies while hawking.

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