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FILE	1-DEVELOPMENT_OF_LOW_.PDF (160.83M)	WORD COUNT	4525
TIME SUBMITTED	03-JAN-2019 02:36PM (UTC+0700)	CHARACTER COUNT	23323
SUBMISSION ID	1061281475		



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# DEVELOPMENT OF LOW AFLATOXIN SOYCORN MILK: OPTIMISATION OF SOYBEAN AND SWEET CORN RATIO AND ITS STABILITY DURING STORAGE

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**Abstract:** A nutritious soycorn milk can be produced by combining soybean and corn. The beverage offers dual nutritional advantages for public health in combating protein and Vitamin A deficiency. However, many studies have shown high level aflatoxin contamination in corn and corn foods. The high content of aflatoxin in food become a public health problem throughout the world. In this research, soybean and sweet corn blend have been used to produce low level aflatoxin soycorn milk. Ratio of soybean and sweet corn affected the physicochemical and sensory properties of the soycorn milk. Ratio of soybean and sweet corn of 70:30 produced soycorn milk with the highest consumer acceptance, total protein of 1.12%, total solid of 11.5%, viscosity of 179.10 cP and colour of 0.60 Orange/4.63 Yellow. Aflatoxin B<sub>1</sub> was detected in soycorn milk at a low level of 0.24 ng/ml, while aflatoxin B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> were not detected by High Performance Liquid Chromatography (HPLC). The aflatoxin content in the soycorn milk was below the maximum limit regulated by European Union and USA Food and Drug Administration. Based on the tolerable daily intake set by WHO, it could be calculated the maximum daily consumption of the soycorn milk to control the aflatoxin exposure concerning

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public health. Further researches to produce soycorn milk with lower content of aflatoxin, even no aflatoxin, are needed. Bacteria growth was found in the soycorn milk during cold storage in refrigerator, with containing bacteria of  $4.07 \times 10^2$  cfu/ml after three days. Slightly declining of colloidal stability was found during storage. Efforts to extend the shelf life of the soycorn milk are also needed.

**Keywords:** development; soycorn milk; soybean; sweet corn; soymilk; aflatoxin; aflatoxin B<sub>1</sub>; high performance liquid chromatography; stability; microbial growth; colloidal stability; cold storage; refrigerator.

## INTRODUCTION

Soymilk is a popular nutritious beverage in almost all over the world. The increasing popularity of soymilk as a beverage worldwide is credited to health benefits, for example, low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevent and reduce of heart diseases and certain cancer. Combining soybean and dried field corn (Kolapo and Oladimeji, 2008), corn flour (Azima and Yani, 2007) or fresh green field maize (Omueti and Ajomale, 2005) to create a nutritious soycorn milk has been done. Corn has fair amount of sulphur-containing amino acids (methionine and cystine), whereas soybean is lack of those amino acids. In developing countries, the use of yellow maize offering dual nutritional advantages in combating protein and vitamin A deficiency (Omueti and Ajomale, 2005). Addition of corn to soymilk improved the overall acceptability by both adults and children and it has high digestibility (Kolapo and Oladimeji, 2008; Omueti et al., 2000).

On the other hand, many studies on contamination of aflatoxins and infection of aflatoxin producing fungi in corn, corn flour and corn foods have been done (Abbas et al., 2006; Ali et al., 1998; Flach, 1987; Kumar et al., 2008; Medina-Martinez and Martines, 2000; Muthomi et al., 2009; Park et al., 2007; Sangare-Tigori et al., 2006). Aflatoxins are a group of extremely toxic metabolites produced

by the common fungi *Aspergillus flavus* and *Aspergillus parasiticus*, and they are suspected to be human carcinogens. Above 17 aflatoxins have been isolated, but only four, called B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, are significant contaminants of food. High level aflatoxin contamination in corn and corn foods have been reported. Ali et al. (1998) reported that 11(69%) of 16 field corn samples collected in Indonesia, were detected containing aflatoxin at a mean level of 119 ng/g (maximum 487 ng/g) and all of aflatoxin contaminated-samples were infected by *Aspergillus flavus*/*Aspergillus parasiticus*. Abbas et al. (2006) also reported that aflatoxin contamination of field corn samples harvested in Arkansas in 1998 and 1999–2001 up to 19 µg/kg and 352.5 µg/kg, respectively. Outbreaks of acute aflatoxin poisoning are recurrent public health problem. Consecutive outbreaks of acute aflatoxicosis reported in Kenya in 2001–2005 responsible for over 150 deaths. These outbreaks emphasise the need to quantify and control aflatoxin exposure in developing countries and highlight the potential role of public health (World Health Organization, 2006). The regulatory limit for aflatoxin in crops and foodstuffs has been established by USA Food and Drug Administration and The European Union (Abbas et al., 2006; Sangare-Tigori et al., 2006). Foods for human consumption in the industrialised world (including export from developing countries) are enforced with regulatory limits varying from 4 to 20 ng based on

limited information from risk assessments of hepatocellular carcinoma (World Health Organization, 2006). Tolerable daily intake of aflatoxin has also been set by WHO (Sangare-Tigori et al., 2006).

Post-harvest handling and processing are critical factors of aflatoxin contamination, otherwise preharvest such as timing of planting, genotype<sup>3</sup> of seed planted and timing of harvest. Unlike field corn varieties, which are harvested when the kernels are dry and mature (dent stage), sweet corn picked when immature (milk stage). Sweet corn have recessive genes like *brittle-1* and *brunken-2* which minimise converting sugar into starch (Brewbaker, 2008). Sweet corn stores poorly and must be eaten fresh, canned or frozen before the kernels become tough and starchy. Corn on the cob does not seem to be affected by the<sup>10</sup> contamination problem. No aflatoxin was found in 263 samples<sup>10</sup> of canned and frozen sweet corn (Stoloff and Francis, 1980). Aflatoxins were only detected in 8 of 27 samples of sweet corn at a level below 4 ng/g and 1 sample contained of 5-10 ng/g (Candlish et al., 2000).

There are no studies on aflatoxin detection in soycorn milk and partially substitution of soybean by sweet corn to produce low level aflatoxin soycorn milk. The goal of our research was to develop a nutritious soycorn milk with low level aflatoxin using sweet corn that do not cause problems for public health. The specific objectives of this research were

- 1 to optimise the soybean and sweet corn ratio to produce low level aflatoxin soycorn milk with good physicochemical properties and the highest consumer acceptance
- 2 to study the stability of soycorn beverage during storage in refrigerator.

## MATERIALS AND METHODS

### Materials

Soybean and yellow sweet corn on the cob were obtained from local market in Surabaya, East Java, Indonesia. All chemicals and media were purchased from local distributor.

### Soycorn milk processing and storage

A 1 kg of sorted soybean was soaked in NaHCO<sub>3</sub> solution of 0.375% with ratio of soybean: solution = 1:2 for 30 min, then boiled in the solution for 30 min. A 1 kg of sweet corn was steamed for 30 min then the grains were threshed out. Boiled soybean cotyledone and steamed sweet corn grain were mixed at ratio of 90:10; 80:20; 70:30; 60:40 and 50:50, then added with water at ratio of 1:5 (w/v). Extraction were done by grinding, boiling 20 min and filtering. The filtrate was added by sugar at concentration of 7%. A 200 ml of the soycorn milk were packed in polyethylene cup of 220 ml volume. The soycorn milk were then analysed of the physicochemical properties (protein content, total solids, viscosity, colour) and sensory properties (taste, aroma and colour). Aflatoxin analysis of soycorn milk at the optimum soybean and sweet corn ratio was done. Soycorn milk at the optimum ratio were studied its stability during storage at refrigerator (4°C) for four days by analysing the total bacteria, colloidal stability and pH daily.

### Protein content analysis

Protein content of soycorn milk was analysed according to AOAC method (1990). A 5 ml of soycorn milk was digested by 25 ml of concentrated H<sub>2</sub>SO<sub>4</sub> and Kjeldahl tablet (1.5% CuSO<sub>4</sub>, 2% Selenium and 96.5% Na<sub>2</sub>SO<sub>4</sub>), then neutralised by 100 ml of NaOH 10 N and 100 ml of distilled water.

The neutralised solution was distilled with 100 ml of HCl 0.1 N as receiving solution. Remaining HCl was measured by titration with standardised NaOH 0.1 N. Protein content was calculated by converting total nitrogen to protein using factor of 5.75.

#### Total solids, viscosity, colour, pH and colloidal stability measurement

Total solids of soycorn milk was measured by refractometer (Abbe). A 0.1 ml of soycorn milk was spread on sample dish, then measured the total solids. The viscosity was measured using Viscometer (Brookfield model DV-E). A 250 ml of soycorn milk in beaker glass was measured the viscosity using spindle 1 with minimum accuracy of 95%. Colour of soycorn milk was measured by tintometer (Lovibond) by putting the sample into optical glass cell, then measured the colour. pH was measured by pH metre. The colloidal stability of soycorn milk was measured according to Omueti and Ajomale (2022) with modification. A 10 ml of soycorn milk samples were placed in graduated tubes held in racks in the refrigerator undisturbed at 4°C overnight. Changes in colloidal stability were indicated by separation into two layers. Level of visible line of demarcation between the settled and remaining portion of the milk solution was measured.

#### Aflatoxin analysis

Aflatoxin analysis was done by High Performance Liquid Chromatography (HPLC) according to Setyabudi et al. (2007). Soycorn milk was extracted and degreased by mixing 50 ml of sample with 50 ml of methanol and 1 g of NaCl, then filtered. The filtrate was added by 25 ml of hexane, followed by collecting the methanol phase. A 4 ml of the methanol phase was diluted with 16 ml of Buffer Phosphate Saline

and loaded into Immunoaffinity Column (IAC), then washed by passing 10 ml of distilled water and completed by flushing air into IAC to remove remaining water. After that, elution through IAC with 1 ml of methanol (first, incubated for 5 min) following with 1 ml of methanol at flowrate one drop per second. After that, 2 ml of obtained eluate was mixed with 50 µl of trifluoroacetic acid and 200 µl of n-hexane for derivatisation, then evaporated with stream of nitrogen gas, then reconstituted with 200 µl of mobile phase. A 20 µl of aliquot was injected into HPLC column with below condition: HPLC column Octadesyl reverse-phase column (250 mm length × 4.6 mm i.d, 5 µm), mobile phase acetonitrile/methanol/water:11/26/63 with flowrate of 0.75 ml/min and fluorometer detector ( $\lambda_{\text{ext}}$  365 nm,  $\lambda_{\text{em}}$  435 nm). Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> were used as standard.

#### Sensory evaluation

The sensory evaluation of the soycorn milk were done by 90 panelists who are familiar with the soymilk. Hedonic method was used with scale of one represent dislike extremely to nine represent like extremely. The panelists were requested to evaluate the taste, aroma and colour of the soycorn milk. The test was conducted in sensory evaluation room.

#### Microbiological analysis

1 ml of soycorn milk was mixed with 9 ml of sterile peptone water 0.1%. Transfer 1 ml of homogenised mixture aseptically into 9 ml of sterile peptone water 0.1% in glass be. Using separate sterile pipet, prepare decimal dilutions 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup> of sample homogenate by transferring 10 ml of previous dilution to 90 ml of phosphate buffer. Pipette 1 ml of each dilution into separate, duplicate, appropriately marked

petri dishes. Add 12–15 ml of Nutrient agar (cooled to  $50 \pm 1^\circ\text{C}$ ) into each plate within 5 min of original dilution and also 6 negative control plate. Mix immediately sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify and then incubate promptly for 48 hr at  $37^\circ\text{C}$ . Count the colonies which grow on the medium. The colony was examine macroscopically and microscopically.

### Statistical analysis

The obtained data were analysed using Analysis 18 Varians (ANOVA) with  $\alpha = 5\%$  and Duncan's Multiple Range Test (DMRT) test with  $\alpha = 5\%$ .

## RESULTS AND DISCUSSION

### Optimisation of soybean and sweet corn ratio

Table 1 showed the physicochemical properties of soycorn milk at different ratio of soybean and sweet corn.

Protein content of soycorn milk varied between 1.06% and 1.17% (w/v), which were significantly affected by the ratio of soybean and sweet corn. It was decreased

with higher proportion of sweet corn, which due to protein content of soybean is higher than that of sweet corn. The protein content of the soycorn milk were lower than that was reported by other researchers (Kolapo and Oladimeji, 2008; Omueti and Ajomale, 2005). This might be due to different methods used to produce soycorn milk. In this research, soybean were soaked in sodium bicarbonate solution. Tunde-Akentunde and Souley (2009) reported that protein content of soymilk were affected by processing methods. Lower protein contents were found in soymilk produced by us 14 sodium bicarbonate because the potash reacts with protein to forms a complex, which reduces the protein availability.

Total solids of soycorn milk varied between 11.1% and 11.9%, which were lower than that produced using dried field corn about 12.20% (Kolapo and Oladimeji, 2008). This might be due to the lower content of protein, which contribute to the total solids. However, the total solid were higher than that of soycorn milk produced using freshly green harvested corn which within a range of 8.82–9.38% (Omueti and Ajomale, 2005). The sugar added to the this soycorn milk formula contributed to the higher total solids. Statistically, total solids of soycorn milk were significantly different at different ratio of soybean and sweet com.

**Table 1** Physicochemical properties of soycorn milk at different ratio of soybean and sweet corn

Soybean: sweet corn	Protein content (%)	Total solids (%)	Viscosity (cP)	Colour
100:0	1.19 <sup>f</sup>	11.0 <sup>a</sup>	176.55 <sup>a</sup>	0.50 O/9.48 Y
90:10	1.17 <sup>e</sup>	11.1 <sup>b</sup>	177.95 <sup>b</sup>	0.58 O/6.65 Y
80:20	1.14 <sup>d</sup>	11.3 <sup>c</sup>	178.60 <sup>bc</sup>	0.58 O/5.73 Y
70:30	1.12 <sup>c</sup>	11.5 <sup>d</sup>	179.10 <sup>cd</sup>	0.60 O/4.63 Y
60:40	1.09 <sup>b</sup>	11.7 <sup>e</sup>	179.80 <sup>de</sup>	0.68 O/3.58 Y
50:50	1.06 <sup>a</sup>	11.9 <sup>f</sup>	180.38 <sup>e</sup>	0.80 O/3.25 Y

Note: Values with different character in the column indicated significantly different at  $\alpha = 5\%$ .

Protein, starch, fibre and sugar are the major solids which contributed to the total solids. Starch content and natural sugar in sweet corn are higher than that of the soybean.

Viscosity of soycom milk varied within the range of 176.65–180.40 cP. Higher sweet corn proportion resulted the higher viscosity of soycom milk. This might be due to the gelatinisation of corn starch during boiling process. In the process, starch absorbed water of the system, hence resulted the higher viscosity. Colour of soycom milk was combination of orange and yellow. Colour measurement using tintometer showed the higher orange value at higher sweet corn proportion. It reflected that carotenoids of sweet corn contributed to the soycom milk colour.

Table 2 showed the result of sensory evaluation of soycom milk at different ratio of soybean and sweet corn. Hedonic score of panelists varied within the range of 4.6–6.4.

Statistically, hedonic score of taste, aroma and colour were significantly difference at different soybean and sweet corn ratio. Higher sweet corn proportion resulted the higher score of taste. This might be due to the flavour of sweet corn gave masking effect on the beany flavour of soybean. Soybean

and sweet corn ratio of 70:30 resulted the highest score of taste of soycom milk. The similar result was occurred to the aroma of soycom milk. Sweet corn contain volatile substance of acetaldehyde, acetone and ethanol which could contribute to the aroma of soycom milk. The higher orange value resulted higher hedonic score. However, more higher orange colour lowering score of preference.

Based on the physicochemical and sensory properties of soycom milk, soybean and sweet corn ratio of 70:30 was the optimum ratio. At the ratio, soycom milk contained protein of 1.12%, total solids of 10.5%, colloidal stability of 94.27%; viscosity of 179.10 cP; panelists preference score of taste, aroma and appearance of 6.40, 5.76 and 5.92.

#### Aflatoxin content in soycom milk

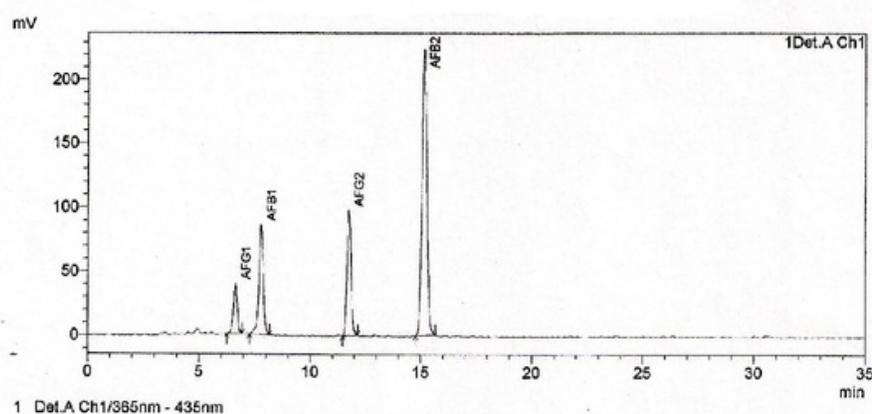
Figures 1 and 2 showed the chromatograms of standard and soycom milk sample, respectively. Figure 1 showed peaks of aflatoxin G<sub>1</sub>, B<sub>1</sub>, G<sub>2</sub> and B<sub>2</sub> appeared at retention time of 6.627; 7.781; 11.738 and 15.151 min, respectively. Figure 2 showed that only aflatoxin B<sub>1</sub> was detected in the soycom milk. The level of aflatoxin B<sub>1</sub> in the soycom milk was 0.24 ng/ml.

**Table 2** Sensory properties of soycom milk at the different ratio of soybean and sweet corn

Soybean: sweet corn	Taste	Aroma	Colour
100:0	4.55 <sup>a</sup>	4.45 <sup>a</sup>	4.65 <sup>a</sup>
90:10	5.13 <sup>b</sup>	5.02 <sup>ab</sup>	5.22 <sup>ab</sup>
80:20	5.52 <sup>b</sup>	4.99 <sup>ab</sup>	5.57 <sup>bc</sup>
70:30	6.40 <sup>d</sup>	5.76 <sup>c</sup>	5.91 <sup>c</sup>
60:40	5.79 <sup>bc</sup>	5.31 <sup>bc</sup>	5.57 <sup>ab</sup>
50:50	6.06 <sup>cd</sup>	5.48 <sup>bc</sup>	5.14 <sup>a</sup>

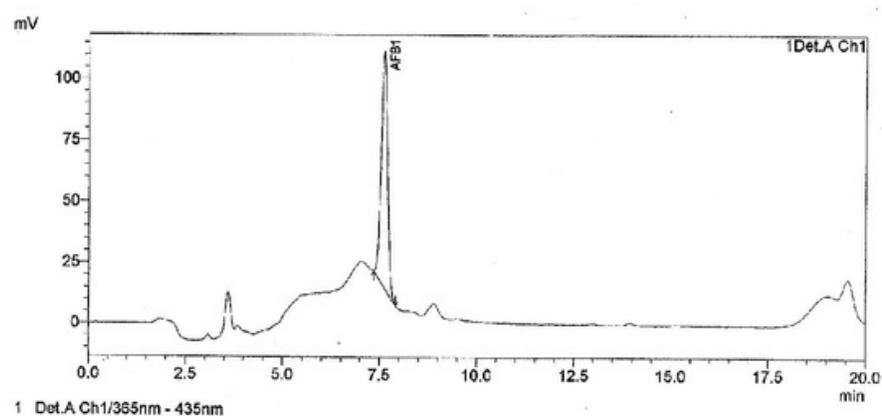
Note: Values with different character in the column indicated significantly different at  $\alpha = 5\%$ .

## &lt;Chromatogram&gt;



**Figure 1** Chromatogram of Standard: AFG<sub>1</sub>, Aflatoxin G<sub>1</sub>; AFB<sub>1</sub>, Aflatoxin B<sub>1</sub>; AFG<sub>2</sub>, Aflatoxin G<sub>2</sub> and AFB<sub>2</sub>: Aflatoxin B<sub>2</sub>

## &lt;Chromatogram&gt;



**Figure 2** Chromatogram of Soycorn milk, AFB<sub>1</sub>: Aflatoxin B<sub>1</sub>

**Table 3** Total bacteria, colloidal stability and pH of soycorn milk during storage in refrigerator

Storage duration	Total bacteria (cfu/ml)	Colloidal stability (%)	pH
0 day	1.32 × 10 <sup>1</sup>	100.00	6.96
1 day	3.80 × 10 <sup>1</sup>	100.00	6.99
2 days	1.55 × 10 <sup>2</sup>	98.53	7.01
3 days	4.07 × 10 <sup>2</sup>	97.45	7.04

Note: Different character indicated a significantly difference at  $\alpha = 5\%$ . O.

The level of aflatoxin in this product was below the maximum limit regulated by European Union (2 ng/g) and USA Food and Drug Administration (20 ng/g). In concerning the public health through control the aflatoxin exposure, maximum intake of soycorn milk can be calculated using the data. Person with 50 kg of body weight may safely consume 200 ml of the soycorn milk daily, which equal to 0.96 ng/13 bodyweight/day. This feature to be below 13 the tolerable daily intake set by WHO (<1 ng/kg body weight/day). It is recommended to person with body weight below 50 kg consume less than 200 ml of the soycorn milk daily.

#### Stability of soycorn milk during storage

Physicochemical and microbiological stability of soycorn milk packed in polyethylene cup during storage in refrigerator (4°C) were shown in Table 3.

Increasing of total bacteria indicated that bacteria have grown in the soycorn milk during storage in refrigerator. Based on the macroscopic and microscopic characteristics of colonies, the dominant bacteria was belong to *Bacillus* genus. The similar result has been reported by Kolapo and Oladimeji (2008) that *Bacillus subtilis* was found in soycorn milk. After three days storage, the soycorn milk contain bacteria of  $4.07 \times 10^2$  cfu/ml. The total bacteria was lower than that reported by Kolapo and Oladimeji (2008) which was  $3.00 \times 10^4$  cfu/ml. This value exceed the Indonesian standard for the total bacteria in soymilk that has been set of  $2.00 \times 10^2$  cfu/ml as the maximum limit. Increasing of pH during cold storage was found, which indicated that bacteria produce proteolytic enzymes which can hydrolyse proteins into peptides and free amino acids. Colloidal stability was slightly decline

during storage in the refrigerator. The soy-corn milk colloid was more stable than that reported by Omueti and Ajomale (2005). This might be due to the different chemical composition of both soycorn milk.

#### CONCLUSIONS

Sweet corn substitution to the soybean produced the soycorn milk with higher acceptability than the soymilk, which the optimum ratio of soybean and sweet corn was 70:30. Aflatoxin B1 was detected in the soycorn milk at low level of 0.24 ng/ml. The aflatoxin content in the soycorn milk was below the maximum limit regulated by European Union and USA Food and Drug Administration. Based on the tolerable daily intake set by WHO, it could be calculated the maximum daily consumption of the soycorn milk to control the aflatoxin exposure concerning public health. Further researches to develop soycorn milk with lower content of aflatoxin, even no aflatoxin, are needed. Since bacteria growth was found in the soycorn milk during storage in refrigerator, the further researches on inhibition of bacteria growth during storage are also needed.

#### BIOGRAPHY

Ignatius Srianta is Head of Center for Food and Nutrition Research, Widya Mandala Surabaya Catholic University from 2002 until now. He focuses on food safety and bioprocess researches. He published/presented his research papers in national journals/seminars and international seminars. Survey, consulting and training on food safety for school stakeholders, food industry practicists and society are his activities. He coordinated the food safety program for elementary school in Surabaya, Indonesia

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