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College of Engineering and Technology

Food Process Engineering Department

Title: - Effect of Preservatives on the Quality of Soymilk produced

A thesis submitted to Food Process Engineering, Wolkite University in partial fulfillment of the requirements for the degree of Bachelor of Science in Food Process Engineering

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Declaration

We the undersigns declare that this thesis is our original work and has not been done for a degree in any of other universities.

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Abstract

the study was conducted in order to identify or evaluate the effect of preservatives on soymilk. This evaluation was done through laboratory tests and sensory evaluation with 10 semi trend panelists. 5 samples were taken for the laboratory tests to evaluate the physical properties (pH, titrable acidity and TSS) of the soymilk and microbial enumeration to determine the shelf life of the soymilk. The sample were taken by adding two preservatives in different concentration. The preservatives used were benzoic acid and citric acid. The samples were A1(citric acid with a concentration of 0.3ml/L), A2(citric acid with a concentration of 0.6ml/L), B1(benzoic acid with a concentration of 0.02g/100ml), B2(benzoic acid with a concentration of 0.05g/100ml) and C (control or sample without preservative). The experimental design used for this study was 2x2 factorial. In the experiment, the sample with a maximum pH value was sample C (5.24 ± 0.09) and the sample with a minimum pH value was sample B2(4.40 ± 0.02). according to the result sample B2 is the most acidic from the five samples and sample C is the least acidic from the five samples. The sample with a maximum titrable acidity was sample C (0.55% ± 0.03) from the five samples and the minimum was sample B2(0.36% ± 0.04). according to this result less percent of lactic acid was present in sample C and more percent of lactic acid was present in sample B2. The maximum value of TSS observed from the five sample was sample C (6.63 ± 0.21) and the minimum value was sample B2(6.58 ± 0.23). according to this, there is no significant difference between the five samples. In the sensory evaluation, sample A1 has the highest overall acceptability (6.80 ± 0.63) from the five samples. In the microbial enumeration, sample B2 has the least CFU/g ($0.9*10^{3}$ in 10^{-2} dilution, $1.3*10^{5}$ in 10^{-4} dilution and $1.7*10^{7}$ in 10^{-6} dilution) from the five sample on day seven. According to this result sample B2 has the minimum of LAB count and it is preferable because, it is not favorable for the growth of microorganisms. So benzoic acid with the concentration of 0.05g/100ml was the best preservative to increase the shelf life of soymilk when compared with citric acid.

Key words: Preservative, Concentration and soymilk.

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Acronyms

PPM……... parts per million

AMC……. Aerobic Mesophilic count

LAB……... Lactic Acid Bacteria

PCA…… Plate count agar

ANOVA……. Analysis of variance

Chapter One

Introduction

1.1 Background

Soymilk is an aqueous, white, creamy extract produced from soybeans which is similar to cow milk in appearance and consistency. It is because of this nutritious value and comparative low cost, that soymilk plays an important role in the dietary pattern of people in most developing countries (Purdue and Illiors, 2000). The nutrients content in eight ounces of plain soymilk are 140gm calories, 10gm protein, 4gm fat, 14gm carbohydrate, 120mg sodium, 1.8mg iron, 0.1mg riboflavin and 80mg calcium (Cruz N., 2007).

The increasing popularity of soymilk as a beverage worldwide is credited to health benefits e.g. low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention and reduction of heart diseases and certain cancers (Erdman Jr. *et al*., 1998.). As this drink is cholesterol free and low in energy, it could enhance health benefits in terms of reducing body weight and blood lipids. With its unique nutty flavor and rich nutrition, soymilk can be used as supplementary way of dairy milk. It is available as a plain, unflavored beverage or in a variety of flavored beverage including chocolate, vanilla and almond. Soy-based diets can reduce blood pressure in spontaneously hypertensive rats but apparently not in hypertensive humans (Khodke SU. *et.al*. 2015).

Soybean (*Glycine max*) is a rich oilseed crop with about 18% fat and 40–48% crude protein. It is particularly noted for its richness in lysine, arginine, cysteine, leucine, and methionine (Rastogi and Singh, 1989), minerals and some vitamins like calcium, iron, vitamin A, riboflavin and other trace elements (Cronan and Atwood, 1972). It can be conveniently consumed as powder, milk or tofu (LU., et al 1980). However, some anti nutritional factors like proteinase inhibitors, alkaloids and tannins may sometime weigh down the benefits of its whole unprocessed consumption, as these substances are not well digested by monogastrics like man. Various processing techniques generally are aimed at detoxification and the removal of flatus factors so that enhanced digestibility is achieved. Soybean, an attraction for vegetarians and health enthusiasts, is considered low in saturated fats and cholesterol which may reduce the risk of heart disease and diabetes. It also alkalizes the blood.

During processing into soymilk, which is a traditional nondairy protein food, certain microbes are resident and find nutritional support in the matrix (Scott and Aldrich, 1970). To our knowledge, not much has been done on the mycological profile of soymilk processing. The keeping quality and nutrient profile of the milk, and the cultural conditions of the resident LAB were studied.

1.2 Statement of problem

The consumption of soymilk has increased, but the greatest problem consumer's encounter with soy milk remains its very short shelf life due to microbial activity. A large number of microorganisms such as coliforms, mesophilic aerobic bacteria, yeasts and molds are responsible for the spoilage of soymilk and it can produce undesirable changes in the milk (Kohli D, *et.al.* 2017). So, the combination of different preservation methods may just be the solution to the problem. The synergistic effect of citric acid and benzoic acid preservatives at various concentrations within their maximum permissible levels along with pasteurization, on the microbial keeping quality of soy milk stored at ambient temperature will be therefore, studied.

1.3 Significance of the study

The significance of this work was improving or extending the shelf life of soy milk produced and to select better preservative technique.

1.4 Objectives

1.4.1 General objective

- To study the effect of preservatives on the quality of soymilk.

1.4.2 Specific objective

- To study physical properties of soymilk produced.
- To study the sensory quality of soy milk.
- To study microbial quality of final product

Chapter Two

Literature review

2.1 Soybean studies with recent trends

2.1.1 Soybean – The Physical parameters

Soybean is used in various forms in many parts of the world. Soybeans and products derived from them have served as an important source of protein in the diet of millions of oriental people for nearly 5,000 years (Hackler *et al.*, 1962). The diets of people in many developing countries comprise mainly starchy roots and cereals and few legumes.

2.1.2 Physio-chemical properties of soy milk

Soymilk which is a watery extract of whole soybean is rich in water soluble protein, carbohydrate and oil (Adebayo-Tayo *et al*, 2008). Soymilk is made by soaking soybeans in water before grinding and straining. The milk is a white or creamy emulsion which resembles cow milk (conventional milk) in both appearance and consistency (Kolapo and Oladimeji, 2008). It is commonly characterized as having a beany, grassy or soy flavor, which reportedly can be improved by lactic acid fermentation, as in yoghurt- like products (Jimoh and Kolpo, 2007). The increasing popularity of soymilk as a beverage worldwide is credited to health benefits e.g. low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention and reduction of heart diseases and certain cancers (Adebayo-Tayo *et al*, 2008).

2.2 Soybean milk

Soymilk is a water extract of soybeans, closely resembling dairy milk in appearance and composition. Soymilk contains total solids of 8 to 10 percent, depending on the water: bean ratio in its processing. Protein is about 3.6 percent; fat, 2 percent; carbohydrates, 2.9 percent and ash, 0.5 percent. Soymilk composition compares favourably with those of cow's milk and human milk. It contains the highest amount of protein, iron, unsaturated fatty acids and niacin, but the lowest amount of fat, carbohydrates and calcium. It is lactose and cholesterol free.

Soymilk provides proteins and other nutrients to people in regions where the supply of animal milk is inadequate. It is especially important for infants and children who exhibit allergic reactions to dairy milk or have a particular need for adequate protein in their diets (Liu, 1997). Progress in reducing beany flavour by researchers at Cornell University and the University of

Illinois, has made soymilk popular as a beverage in the Far East. It is steadily spreading to the Western world.

Soymilk is quite popular en China and Korea, but its consumption is insignificant in Japan (Saio, 1999). The United Kingdom is the main European market for soymilk, followed by Germany, France and Holland (Kerntke, 1992). In many other countries, soymilk is being produced and commercialized under different names, for example "A de S" in Mexico; soymilk
in India; "Soja drink" in Brazil.

2.2.1 Benefit of soymilk

- ✓ Soymilk contains probiotic sugars such as stachyose and raffinose that boost immunity and help decrease toxic substances in the body.
- ✓ Fewer people are allergic to soymilk, only 0.5% of children are allergic to soymilk whereas 2.5% are allergic to cow milk.
- ✓ Soymilk contains isoflavones (mainly genistein and daidzein) while cow milk does not. isotiavones are antioxidants which protect our cells against oxidation and they also help to reduce cholesterol and the risk of prostate and breast cancer.
- ✓ Vegetable proteins have an advantage in that they cause less loss of calcium through kidneys and hence lower risk of osteoporosis compared to animal proteins.
- ✓ Cow milk also contains natural hormones which can influence the working of our body.
- ✓ Soymilk contains dietary fiber which is not available in cow milk.

2.3 Benzoic Acid

Benzoic acid and sodium benzoate are used as preservatives in beverages, dairy products, fruit products, chemically leavened baked goods, and condiments. A disadvantage is the off-flavour they may impart to foods (Chipley, 1983). Owing to their inhibitory effect on yeast, they cannot be used in yeast-leavened products (Friedman & Greenwald, 1994). Examples of concentrations allowed in food are up to 0.05g/100ml benzoic acid (USA) and between 0.06 and 0.07g/100ml (other countries) (Chipley, 1983). The European Commission limits for benzoic acid and sodium benzoate are 0.015–0.5% (EC, 1995).

2.4 Citric Acid

Citric acid is found naturally in citrus fruit. Due to its excellent acidulant, flavourant and preservative properties, citric acid (citrate) is found in a large number of natural and processed foods and beverages, such as fruit juice and other soft drinks, beer, milk, bread, candies, and dairy and meat products. This acid also finds many other applications, such as in paper manufacture or in the wine industry, where the presence of significant quantities indicates the use of citric acid as an acidulant, a practice with an allowable upper limit of just 1 g/L (final concentration) in the EU (Mollering, H., 1989).

Chapter Three

Material and Methods

3.1 Experimental location/site

Experiments were carried out in Wolkite University, Food Process Engineering Laboratory.

3.2 Sample collection, transportation and storage

The required material soybean, citric acid and benzoic acid were purchased from Wolkite town market and transported to Wolkite University and stored at Food Process Engineering Laboratory for further use.

3.3 Sample preparation

3.3.1 Preparation of Soy Milk

Soybean were sorted and cleaned to remove stones and damaged, deformed seeds. Then the dry soybean was washed and soaked in water (500g in 1 Liter) for 12 hours. It was rinsed and blanched in 1.25% $NaHCO_3$ for 30 minutes. The rehydrated soybean was washed, manually dehulled and rinsed. The soybean seeds were grounded in blender and expressed in the ratio of 3:1 (water to beans on a weight basis) to remove the okra. The soybean seeds were grinded and the milk was extracted by Muslin cloth to remove okra (fig 3.2). The obtained milk was then formulated by adding preservatives. The milk was pasteurized at the temperature 71^{o}C for 15 minute and subsequently bottled and stored in refrigerator.

Figure 3.1: soymilk

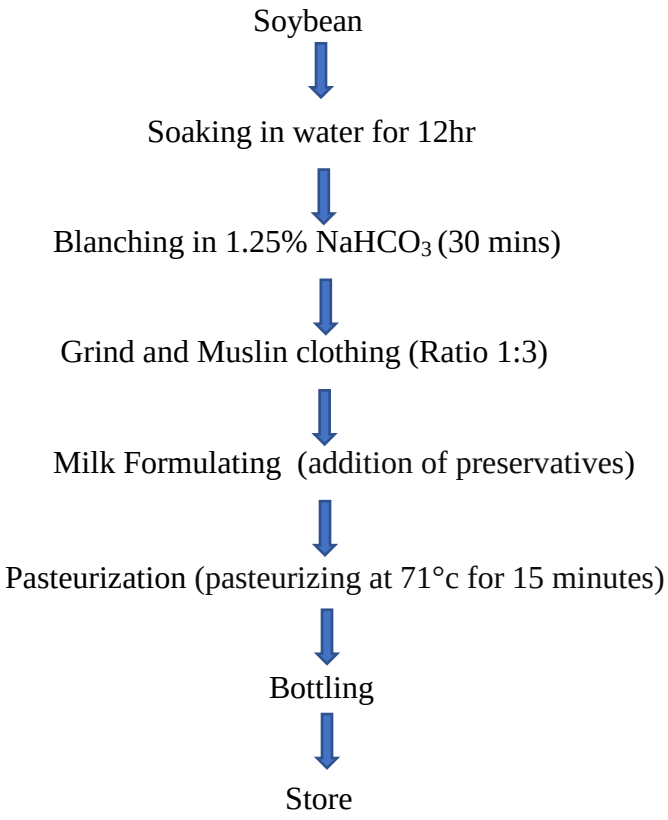

Figure 3.2: flow diagram of soymilk preparation using Muslin clothing, Extraction method (Kolapo A.L *et al*., 2008).

3.4 Laboratory Analysis

3.4.1 Titrable Acidity

The titrable acidity of samples was determined by titrating with 0.1 M NaOH standard solution by using phenolphthalein as an indicator. The result obtained was presented as percentage of the lactic acid produced (AOAC, 2000).

$$\% \text{ lactic acid} = \frac{\text{vol of NaOH} * \text{Mole of NaOH} * \text{Equivalent weight of lactic acid (0.09)} * (100)}{\text{Weight of Sample}}$$

3.4.2 pH Determination

pH was measured by using digital pH meter which was calibrated with buffer solution pH 4 and distilled water following AOAC, (2000) method.

3.4.3 Total Soluble Solid (TSS)

The total soluble solid of samples was measured by using digital refractometer meter.

3.4.4 Microbiological Enumeration

10ml sample from each treatment was taken aseptically and transferred separately to a blender with 90 ml sterile 0.85% peptone water and homogenized. 6-fold serial dilution was made from the homogenized samples and aliquots of 0.1 ml from the appropriate dilution was taken and spread plated by using glass rod in duplicate on pre- dried agar plates of MRS Colonies of lactic acid bacteria (LAB) was counted on MRS agar (De Man, Rogosa, Sharpe) after anaerobic incubation at 30 ^{0}C for 72 hrs. The number of LAB counted was reported as log CFU g^{-1} calculated from the arithmetic means (A.L. Kolapo, 2007).

3.4.5 Sensory Acceptability of Soymilk

The sensory analysis of soymilk samples were conducted in Food Process Engineering Laboratory. The sensory analysis was conducted using 10 panelists on the 5 soymilk samples. The panelists were evaluated the sensory attributes of soymilk samples according to color, flavor, sweetness and overall acceptability using 9-point hedonic scale from 1(extremely dislike) to 9 (extremely like).

3.5 Experimental Design and Stastical Data Analysis

A 2X2 factorial full experimental design is conducted by design expert with four run and one control with duplicate data. Preservatives with two level i.e. benzoic acid concentration with two levels (0.02g/100ml and 0.05g/100ml) and citric acid concentration with two levels (0.5 g/L and 1g/L) were analyzed.

The statistical analysis of the microbial and sensory quality at different concentration and sample type were compared by means one-way ANOVA ($P<0.05$). The experimental design was used for data analysis.

Table 3.1: two by two factorial

	Preservation			
	Citric acid		Benzoic acid	
concentration	0.5g/L or 0.3ml	1g/L or 0.6ml	0.02g/100ml	0.05g/100ml

Chapter Four

4.0 Result and Discussions

4.1 Physical properties of soymilk

The physical properties of soymilk (pH, titrable acidity and total soluble solid) were conducted as table below: -

All values are in the form of mean ± SD

Where A1: - citric acid preservative with a ratio of 0.3ml,

A2: - citric acid preservative with a ratio of 0.6ml,

B1: - benzoic acid preservative with a ratio of 0.02g/100ml,

B2: - benzoic acid preservative with a ratio of 0.05g/100ml,

C: - soymilk without preservative or control.

4.1.1 pH

The pH of the samples with preservatives was observed decrease, while the control's decrease. The result shows the addition of preservatives increases the acidity of the soymilk. the minimum pH observed was B2(benzoic acid with amount of 0.05g/100ml) and the maximum pH observed was C (soymilk without preservatives). the difference occurred between the control and the treated milk was addition of preservatives(citric acid and benzoic acid) decreases the pH value and without preservatives increases the pH value of the soymilk.

4.1.2 Titrable Acidity

The changes in titratable acidity (% lactic acid) of soymilk during storage (0 to 7 days) at room temperature of all soymilk increased with the increase in storage time as compared to the control (value at zero days).

This trend was same for all soymilk samples. Sample C had maximum titratable acidity at 7 days of storage and the increase was 39.73% as compared to titratable acidity at the beginning of the study.

Earlier studies reveal that the increase (about 80 to 90%) in acidity during storage of soymilk is mainly due to the lactic acid formed by a predominating lactic acid bacteria (True LC and Patel GR, 1973).

4.1.3 TSS

The TSS observed from each sample was shows a non-significant difference was observed in TSS in five samples. This is because of the TSS of each sample does not affected by addition of preservatives. This happens because of the samples were taken from same product of soymilk produced. The differences between these research work data and findings of Park (Park DJ (2005) can be explained by the factors such as differences in experimental parameters and natural qualitative and quantitative variability in the raw material. The yield and quality of soymilk is affected by soybean and soy milk characteristics and is not affected by the size of soybeans (Lim BT, et al., 1990).

Table: 5.1: physical properties of soymilk

Sample code	pH	Titrable acidity	TSS
C	5.27 ± 0.09^{a}	$0.55\% \pm 0.03^{a}$	6.63 ± 0.21^{a}
A1	4.89 ± 0.09^{b}	$0.49\% \pm 0.04^{b}$	6.62 ± 0.28^{ba}
A2	4.84 ± 0.01^{bc}	$0.45\% \pm 0.02^{cb}$	6.61 ± 0.09^{cba}
B1	4.43 ± 0.05^{d}	$0.43\% \pm 0.07^{dc}$	6.60 ± 0.78^{dcba}
B2	4.40 ± 0.02^{fd}	$0.36\% \pm 0.04^{e}$	6.58 ± 0.23^{edcba}

4.2 Sensory Analysis

Sensory property of food product is one of the most significant factor that affect the quality, acceptability and baying habit of individuals customers therefore the sensory quality of prepared soymilk was conducted to select best product from the other. These properties are Color, taste, flavor, sweetness, overall acceptability.

The color that was liked by the ten panelist from the five samples was sample B2 and A1, The taste that was liked by the ten panelist from the five samples was sample A1, B2 and B1, The flavor that was liked by the ten panelist from the five samples was A1 and B2, The sweetness that was liked by the ten panelist from the five samples was A1 and finally The overall acceptability observed shows that A1 was liked the most.

Table: 5.2: sensory evaluation

Sample code	Color	Taste	Flavor	Sweetness	Overall acceptability
A1	7.20 ± 0.63^{a}	6.20 ± 0.92^{a}	6.80 ± 1.13^{a}	6.20 ± 0.92^{a}	6.80 ± 0.63^{a}
B2	7.10 ± 1.64^{b}	6.18 ± 1.55^{ba}	5.60 ± 1.84^{b}	5.81 ± 1.50^{b}	5.50 ± 1.10^{b}
B1	6.88 ± 1.65^{c}	6.10 ± 2.02^{c}	5.54 ± 1.77^{c}	5.45 ± 2.10^{c}	5.48 ± 1.20^{cb}
A2	6.82 ± 0.85^{d}	6.04 ± 2.01^{d}	5.53 ± 1.50^{dc}	5.32 ± 1.93^{d}	5.43 ± 1.03^{dc}
C	6.80 ± 1.89^{ed}	5.97 ± 1.65^{e}	5.32 ± 1.50^{e}	4.92 ± 1.20^{e}	5.37 ± 1.64^{e}

4.3 Microbiological Enumeration

The standard of the microbial count states that if there is 30< lactic acid bacteria, the sample was accepted and if it ranges between 30-300 it was calculated and if it exceeds 300 bacteria, the sample was rejected.

According to the microbiological enumeration result of the five samples was found by knowing sample B2 (benzoic acid with amount of 0.05g/100ml) is preferable. Because the amount of the acid added was not favorable for the growth of microbes.

The preserved soymilk samples were recorded generally lower bacterial viable counts than the unpreserved samples of soymilk (Mita, B.K and Stemkrain, K.H. 1975). Thus, with adequate monitoring and adherence to quality control measures during production, acceptable standard is achievable in the production of soymilk.

Table: 5.3: microbial enumeration

Day three			
Sample code	Dilution		
	10^{-2}	10^{-4}	10^{-6}
A1	$0.6*10^{3}$	$0.8*10^{4}$	$1.1*10^{6}$
A2	$0.51*10^{2}$	$0.63*10^{4}$	$0.98*10^{6}$
B1	$0.49*10^{2}$	$0.54*10^{4}$	$0.69*10^{6}$
B2	$0.12*10^{2}$	$0.38*10^{4}$	$0.47*10^{6}$
C	$1.8*10^{3}$	$2.1*10^{5}$	$2.8*10^{7}$
Day seven			
A1	$2.3*10^{3}$	$3.1*10^{5}$	$3.9*10^{7}$
A2	$1.4*10^{3}$	$2.1*10^{5}$	$2.7*10^{7}$
B1	$1.8*10^{3}$	$2.1*10^{5}$	$2.5*10^{7}$
B2	$0.9*10^{3}$	$1.3*10^{5}$	$1.7*10^{7}$
C	$3.8*10^{3}$	$4.1*10^{5}$	$5.2*10^{7}$

Chapter Five

Conclusion and Recommendation

5.1 Conclusion

In this paper we have tried to test two techniques for the preservation of soya milk. Those are addition of acids like citric acid and benzoic acid. Laboratory tests like ph., TSS, titrable acidity and microbiological enumeration were studied for both methods. According to the result that the titrable acidity of the samples that were placed at room temperature increase more than the samples that are placed inside a refrigerator. The ph. of the samples with a preservative increase its acidity as the day increase from one to seven days rather the ph of the control sample with no preservative has highly increasing as compared with the samples contain preservatives. which means it becomes more alkaline in seven days. According to the sensory evaluation sample A1 (citric acid with amount of 0.3ml) gains over all acceptability. The TSS for the three samples doesn't vary meaning it is not significant. And with the microbiological enumeration conducted using MRS agar sample B2(benzoic acid with an amount of 0.05g/ml) was more favorable preservative as compared with citric acid.

5.2 Recommendation

In this section we have a recommendation for the users of the product i.e. soya milk and also, we have a recommendation for wolkite university food process laboratory. The recommendation for the users is that during purchase of this kind of products they have to check the expired date on the labeling properly and also during storage of the products it must be in a refrigerator. During transportation of the product a vehicle with full container shall be used in order to prevent direct contact with sun light which leads to oxidation that gives unwanted flavor and odor.

The recommendation for the laboratory is that it had been difficult for us to excute different laboratory tests because of shortage of laboratory facilities like chemicals and machineries. And that makes our research time consuming so we recommend that more facilities are added in the university laboratory.

We have studied the microbial count by using MRS agar to determine the LAB count of soymilk, but microbial counts like aerobic mesophic count and yeast and mould and other microbiological enumeration was recommended to be studied for better shelf life study of soymilk.

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Appendix

Sensory evaluation of ANOVA result

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Color	Between Groups	47.480	4	11.870	5.889	.001
	Within Groups	90.700	45	2.016		
	Total	138.180	49			
Taste	Between Groups	25.080	4	6.270	2.222	.082
	Within Groups	127.000	45	2.822		
	Total	152.080	49			
Flavor	Between Groups	25.080	4	6.270	2.558	.051
	Within Groups	110.300	45	2.451		
	Total	135.380	49			
sweetness	Between Groups	12.280	4	3.070	1.192	.327
	Within Groups	115.900	45	2.576		
	Total	128.180	49			
overall acceptability	Between Groups	24.680	4	6.170	4.574	.003
	Within Groups	60.700	45	1.349		
	Total	85.380	49			

Physio-chemical analysis of ANOVA result

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Ph	Between Groups	1.295	4	.324	1.884	.251
	Within Groups	.859	5	.172		
	Total	2.154	9			
titrable acidity	Between Groups	8.474	4	2.118	905.327	.000
	Within Groups	.012	5	.002		
	Total	8.486	9			
total soluble solid	Between Groups	9.678	4	2.419	62.373	.000
	Within Groups	.194	5	.039		
	Total	9.872	9			

Appendix figures

Figure 1: sensory evaluation

Figure 2: microbial enumeration

Figure 3: soymilk preparation