THE EFFECT OF BLANCHING METHOD, STORAGE AND TEMPERATURE TO THE CHARCTERISTICS OF COWPEA TEMPEH SUBTITUTED by 40% SOYBEAN

Mery Tambaria Damanik Ambarita[1], C.C. Nurwitri[2] and Bertha Florensia Yaputra[1]
[1] Dept. of Food Technology, Universitas Pelita Harapan, Jakarta
[2] Dept. of Food Science & Technology, Bogor Agricultural University

ABSTRACT

Cowpea is mainly used as vegetable and animal fodder. Tempeh is highly perishable food that has to be consumed shortly after it reaches acceptable degree of fermentation. This study aims were to observe the methods for inhibiting tempeh spoilage, methods of blanching and its effect to tempeh. methods and optimum temperature of four weeks tempeh storage and characteristics of cowpea-soybean tempeh after four weeks storage in freezer. Vacuum packaging combined with frozen storage could retain tempeh quality after four weeks storage. The result showed that after storage, tempeh with steam blanching method has higher carbohydrate (18.20%) and isoflavone content (66.20%), lower protein (28.99%) and water content (49.15%), fewer total microbes; lactic acid bacteria and coliform compare to before storage. *In* addition, tempeh with hot water blanching method, after storage, has lower protein content (16.27%), higher water (70.25%), carbohydrate content (10.53%), and isoflavone content (75.28%) compare to before storage. Before storage, tempeh either with steam or hot water blanching method has *linoleic* acid, palmitic acid, β-tocopherol, ergost 5,7,22 trien-3-ol, and stigmast 5,22, dien3-ol whereas after storage, tempeh with both of blanching method has γ-tocopherol and stearic acid.

INTRODUCTION

Cowpea is one kind of legumes that can grow well in Indonesia and had been produced 5.12-8.90 ton/ha/year in 1979-1980 (Rusastra *et al.,* 2004). Cowpea mainly is used as vegetable and animal fodder (Kabas et *al.,* 2007). Soy products are considered to have potential role in preventing chronic diseases such as atherosclerosis, cancer, osteoporosis, and menopausal dissorder (Shimakawa *et al.*, 2002). Cowpea tempeh (40% soybean substitution) is better due to its pro-vitamin D_2 and anti cholesterol component (Noviany, 2008). Tempeh is perishable product therefore it should be consumed shortly after it is processed (e.g. frying). Blanching and vacuum packaging in optimum storage temperature can inhibit tempeh spoilage. Blanching, a preliminary treatment before freezing, drying, and storage, can inhibit enzymatic spoilage and reduce the amount of microbes. Vacuum packaging can minimize aerobic microbes' growth during storage (Forsythe, 2000).

METHODS

Materials

Materials, used to produce and store tempeh, were soybeans, cowpeas, vinegar (Dixi), *ragi* tempeh (Raprima), vacuum plastics from PT. Siwani Makmur Tbk., frying oil (Tropical), and banana leaves. Materials, used for microbiology analysis, were Plate Count Agar (Merck), Brilliant Green Lactose Bile Broth (Merck), Man Rogosa Sharpe Agar (Merck), Vogel Johnson Agar (Merck), Eosin Methylene Blue Agar (Merck), NaCl (Merck-Pa), disposable petri dish, filter paper, and aquadest (Bonanza). Materials, used for chemical analysis, were sunflower oil (Orosol), K_2SO_4 (Merck-Pa), H_2SO_4 (Merck-Pa), H_2O_2 (Merck-Pa), Selenium (Merck-Pa), boric acid (Merck-Pa), mixed indicator (Merck- Pa), NaOH (Merck-Pa), HCl (Merck-Pa), tween-20 (Merck, Pa), phosphate-oxalate buffer, ascorbic acid (Merck-Pa), and 2,6-dicholoroindophenol (Merck-Pa).

Factors and Experimental Design

Factors in this research were blanching methods (A) which consist of steam method (A1) and dip method (A_2) and storage temperatures (B) which were three levels (freezer temperature ±-24°C (B_1); refrigerator temperature ± 4°C (B_2); and room temperature ± 28°C (B_3)). This experimental design was two factors completely randomized design with four times repetition.

Procedure

Cowpea ternpeh with 40% soybean substitution was blanched for five minutes, vacuum packed, and stored in three storage temperatures. To know which storage temperature can be used to retain tempeh quality after four weeks storage, analysis was done by comparing their quality to fresh tempeh quality.

Parameter

Parameters consist of texture, cohesiveness, and hedonic test. Organoleptic tests were done using scoring test for textures, cohesiveness, and hedonic parameter. Microbiology analysis consists of total microbes (Yousef dan Carlstorm, 2003), total lactic acid bacteria (Yousef and Carlstorm, 2003), coliform bacteria presume test (Vanderzant and Splittstoesser, 1992), *ragi* tempeh analysis (Johnston, 2008) and total *S. aureus* (Yousef and Carlstorm, 2003). Chemical analysis consists of pH (AOAC, 2000), water content (oven method, AOAC 1995), ash content (dry ash method, AOAC 2005), protein content (Kjeldahl method, AOAC 2005), fat content (Soxhlet method, AOAC 1995), carbohydrate content (by *difference*), blanching adequacy test (Gokmen *et al.*, 2005 and AOAC, 2005), component identification, and total isoflavone in tempeh (Pawiroharsono, 1995).

RESULT AND DISCUSSION

Nutrition content: and size of legume, used to make tempeh, will affect mold growth and its adaptation time to penetrate legumes to produce good tempeh (done by organoleptic test with cohesiveness, texture, and hedonic parameter). The data was analyzed using one way ANOVA with Tukey simultaneous test.

Proximate Analysis for Raw Material

Cowpea has high carbohydrate content causing incresing glucose after 60 hours fermentation, therefore, longer fermentation time is needed. (Miskiewicz *et al.*, 2004) Fat and protein were used by mold as energy source for their growth, that makes tempeh has lower fat and protein content than ternpeh raw material.

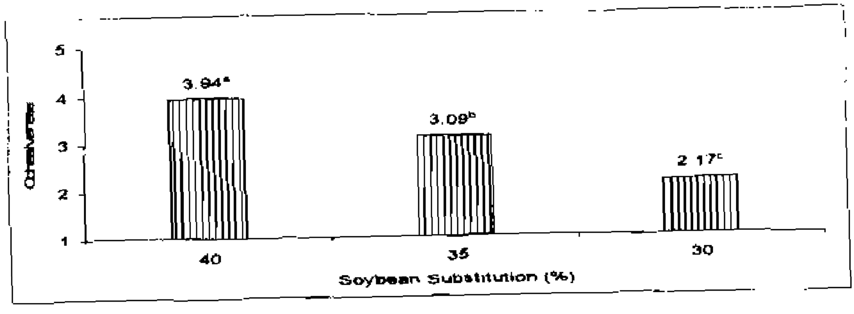
Table 1. Proximate **Analysis** for Cowpea and Soybean

Composition	Cowpea (%)	Soybean (%)
Water	11.5753	**9.3429**
Ash	**3.3457**	5.0694
Protein	**22.31**	40.45
Fat	1.3745	18.3405
Carbohydrate *[by difference)*	**61.39**	26.80

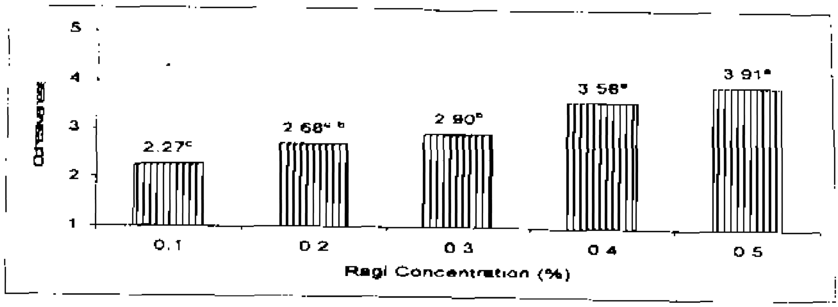
Lipoxygenase test results showed that the steaming and boiling **process had** effectively **inactivated peroxidase.**

Cohesiveness [Scoring test)

The best cowpea tempeh **was** made of 40% soybean substitution. Cohesiveness is affected by **the** mold ability to penetrate inside the **raw** materials. **The** amount of spores in *ragi*, which used in this research **was** 3.9×10^8 spores/gram, the **higher** ragi's concentration **was used,** the more **cohesive tempeh was produced.** *Ragi's* concentration that is **usually** used is 0.1%-0.3% of soybean's **wet** weight (Rahman, **1992).** *Ragi's* concentration, **used** in this research, was **0.5%.**



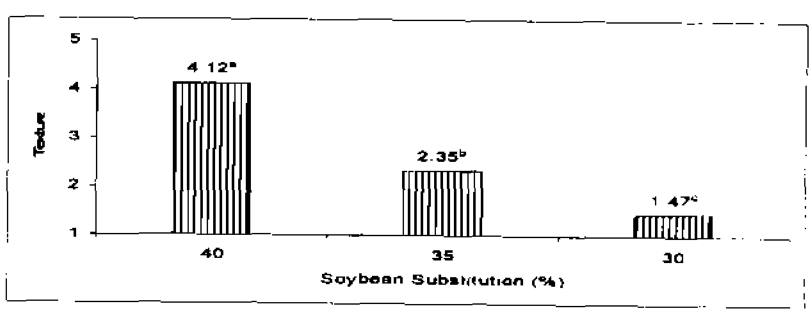
Picture 1. Effect of **soybean** substitution to **tempeh's cohesiveness** (α=5%)



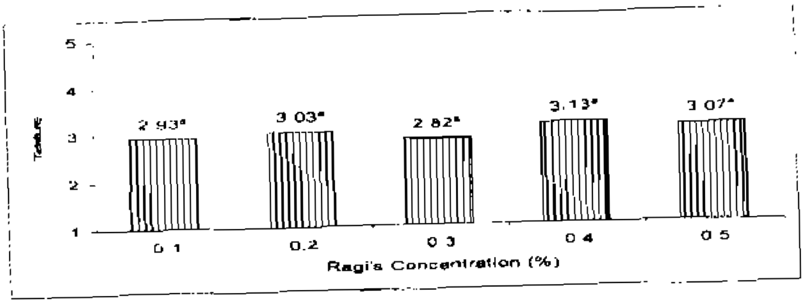
Picture 2. Effect of *ragi*'s concentration to tempeh's cohesiveness (α=5%)

Texture (Scoring test)

The best tempeh's texture was produced by tempeh with 40% soybean substitution. *Ragi*'s concentration doesn't make any differences in tempeh's texture. The higher carbohydrate content in tempeh the softer tempeh's texture because the carbohydrate will be hydrolyzed into simple sugar (Hidayat *et al.*, 2006) which makes the tempeh's texture soften. Tempeh's texture is affected by cohesiveness. The most cohesive tempeh was made of 40% soybean substitution and 0.5% *ragi*'s concentration.



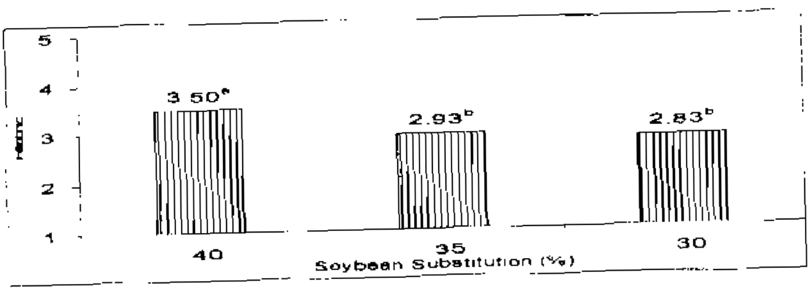
Picture 3. Effect of soybean substitution to tempeh's texture (α=5%)



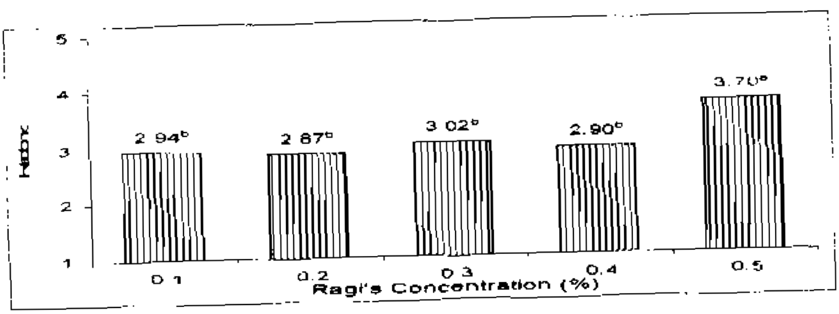
Picture 4. Effect of ***ragi's*** concentration to **tempeh's** texture (α=5%)

Hedonic

The highest **hedonic value** found in **tempeh** made of **40%** soybean substition and 0.5% **ragi's** concentration. The reasons are **the** higher ***ragi's*** concentration was **used** the better tempeh's **appearance and** the more chowpeas **were used** the lesser attractive tempeh colour.



Picture 5. Effect of soybean substitution to tempeh's hedonic (α=5%)



Picture 6. Effect **of** *ragi's* concentration **to tempeh's hedonic** (α=5%)

Storage Temperature Treatment

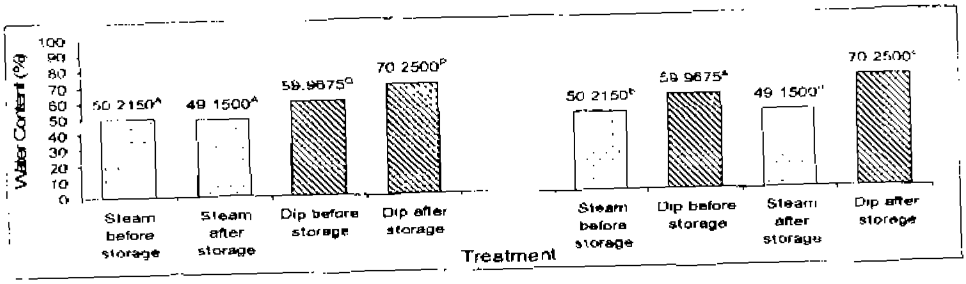
Room temperature storage could retain tempeh quality for three days (based on sour aroma). Dip blanched method decreased pH value (from 6.42 to 4.83) but increased microbes and total lactic acid bacteria (from < $2.5x10^2$ cfu/g to $3.5x10^5$ cfu/g and $2.6x10^5$ cfu/g) in tempeh. Steam blanched method decreased pH value (from 6.48 to 5.48) but increased total microbes and total lactic acid bacteria (from < $2.5x10^2$ cfu/g to $3.2x10^5$ cfu/g and $2.3x10^5$ cfu/g) in ternpeh.

Storage at refrigerator temperature could retain tempeh's quality for three weeks (based on sour aroma). Dip blanched method decreased pH value (from 6.42 to 4.53) but increased total microbes and total lactic acid bacteria (from < $2.5x10^2$cfu/g to $6.6x10^7$cfu/g). Steam blanched method decreased pH value (from 6.43 to 4.84) but increased total microbes and total lactic acid bacteria (from < $2.5x10^2$ cfu/g to $5.9x10^7$ cfu/g and $4.5x10^7$ cfu/g).

Freezer temperatur storage could retain the tempeh's quality which is comparable to the fresh tempeh. Therefore, this research compared the water, ash, protein, fat, carbohydrate content, amount of isoflavone, and component identification between fresh tempeh and tempeh after four weeks storage.

Water Content

There is no water content difference between tempeh that had been blanched with steam and dip method. On the other hand, the water content decreased after storage due to freezing process that makes the water inside the cell move out (Fellows, 2000). The water content of tempeh blanched with dip method also decreased after storage.

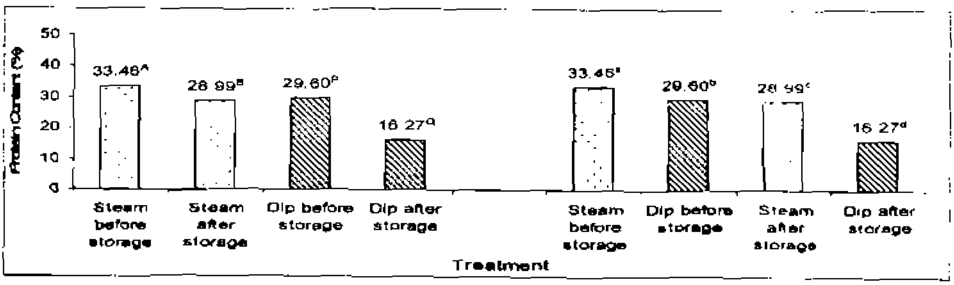


Notation Explanation :
A,B : Showed significant level at α=5% between steam and dip blanch method before and after storage
P,Q : Showed significant level at α=5% between steam and dip blanch method before and after storage
a,b : Showed significant level at α=5% between steam and dip blanch method before and after storage
c,d : Showed significant level at α=5% between steam and dip blanch method before and after storage

Picture 7. Tempeh's water content before and after storage

Protein Content

Tempeh has high protein content. Tempeh's protein contents either with steam or dip blanched method decreased after storage due to protein denaturation during freezing process and thawing (Hui, 2004) and lactic acid bacteria. Lactic acid bacterias need amino acid to be changed into protein for their growth. (Todar[1], 2008). Steamed blanched tempeh samples had lactic acid bacterias $9.4x10^4$ cfu/g but latic bacterias were $1.4x10^5$ cfu/g in dip blanched tempeh samples.



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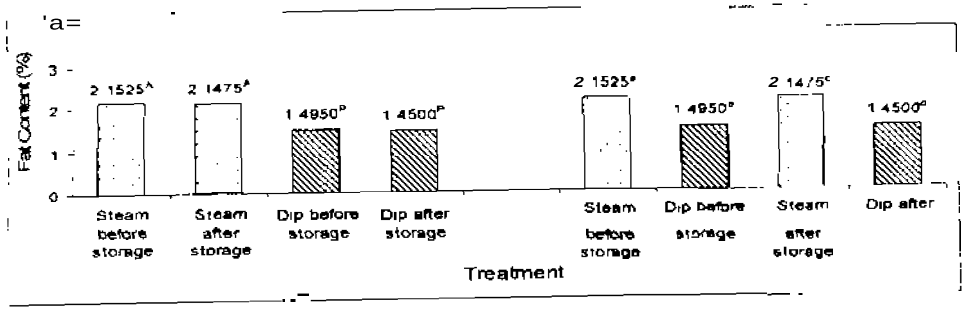
Picture 8. Tempeh's protein content before and after storage

Fat Content

There is no significant changing in fat content in tempeh either with steam or dip blanched and iether before or after storage. This low fat content (± 1-2%) is caused by hydrolysis and oxidation. Fat content in tempeh blanched with steam method was higher than tempeh blanched with dip method. This phenomenon agreed with Inyang and Ike research (1998) which blanching process caused a slightly decreased fat and carbohydrate content, color component, and ascorbic acid.



Notation Explanation :
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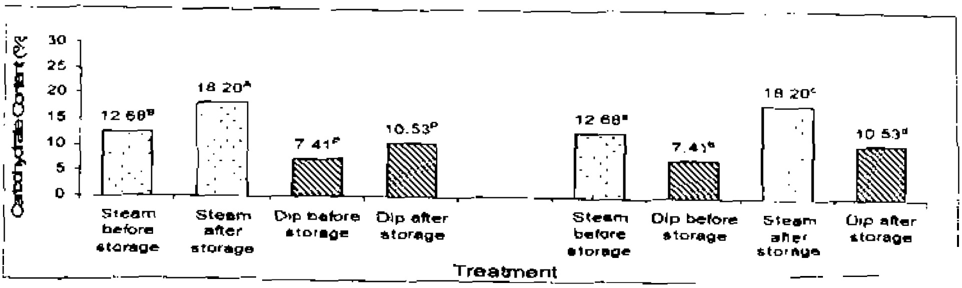
a,b : **Showed** significant **level** at α=5% **between steam and dip** blanch method **before and after** storage
c,d : Showed significant level at α=5% **between steam and dip** blanch method before **and after** storage

Picture 9. Tempeh's **fat** content **before** and after **storage**

Carbohydrate Content

Increasing carbohydrate content after **storage** was caused by increasing simple sugar like fructose, **glucose,** and sucrose because of hydrolysis (Hui *et al.,* 2004). Cowpea has high carbohydrate content, therefore alpha-amylase enzyme could degrade starch become oligosaccharides that will be hydrolyzed into maltose and glucose. Glucose can solve **in** the water **especially due** to **dip** blanched method (Winarno, **2004). Therefore, carbohydrate** content **of dip-blanched** tempeh was lower **before** or **after storage** compare to steamed-blanched tempeh.



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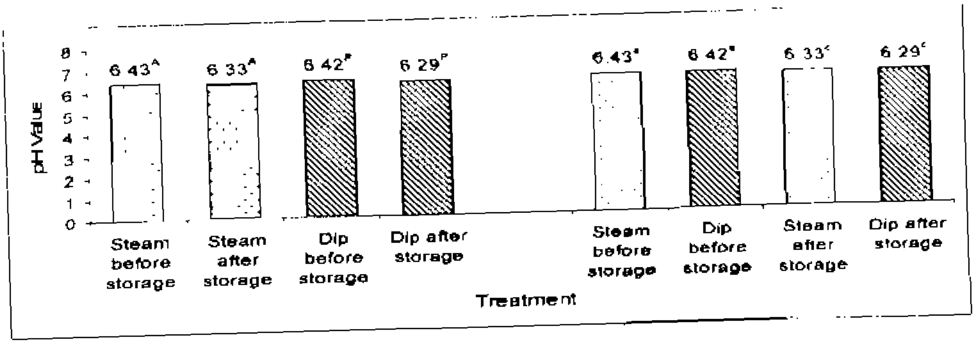
c,d : **Showed significant level** at α=5% between **steam and dip** blanch **method before and** after **storage**

Picture 10. Tempeh's carbohydrate content **before** and after **storage**

pH

The **value** of **pH** is one *of* the **parameter** to determine tempeh's quality. **Dood tempeh has pH value** from 6.3-6.5 (Hidayat *et al.*, 2006). Tempeh's **pH was affected** by total lactic **acid bacteria.** If the amount of lactic **acid** bacteria **has** reached 10^7 cfu/g then the concentration **of lactic** acid **will** start **to rise** (Blackburn and Clive, 2006). **If pH value does** not **significantly decrease,** it **means** lactic acid concentration and **lactic** acid bacterias in **samples** are **still** low.



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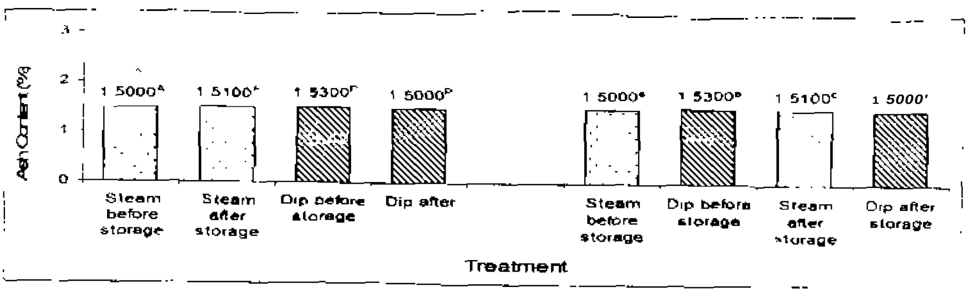
c,d : Showed significant level at α=5% between steam and dip blanch method before and after storage

Picture 11. Tempeh's pH value before and after storage

Ash Content

Soybean tempeh contains Ca, P, and Fe (Steinkraus, 1996). Mineral content that might be found in cowpea tempeh with soybean substitution are P, K, and Ca from cowpea, and K, P, Ca, Fe, and Zn from soybean (Lam and Lumen, 2003). There is no changes in ash content in tempeh blanched either with steam or dip method and before or after storage. This phenomenon agreed with Lim *et al.* (2006) who said that ash content from fresh vegetables is the same as frozen vegetables.



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a,b : **Showed significant** level **at α=5% between steam and dip blanch method before and after storage**

c,d :**Showed significant level at α=5% between steam and dip blanch method before and after storage**

Picture 12. Tempeh's ash **content before and after storage**

Microbiology Analysis

Indicator of vacuum product spoilage is maximum total **microbes. The maximum total microbes to be allowed** in **product is** 10^6cfu/g (Forsythe, 2000). **Test result showed** that **total** microbes in **samples** are **still lower** than maximum total microbes.

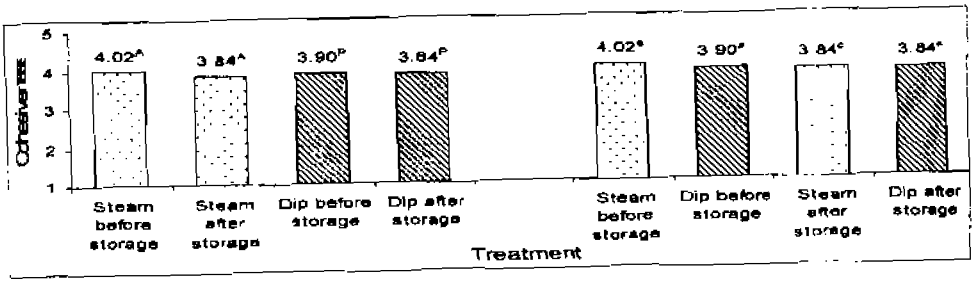
Total coliforrn bacterias increased because bacterias can grow **in anaerobic** facultative **condition** (Yousef **and** Carlstorm, **2003).** Test **result showed that coliform bacterias** that are **Found** in samples **were non-fecal** coliforms **which are safe for human health. In** addition, gram **staining result** showed that coliform bacterias in tempeh were **gram-negative. This means** coliforms **bacteria** *in* **tempeh** was not *E. coli* because *E. coli* **was a** gram-positive **bacteria. In** tempeh either before or after storage, ***staphylococcus aureus*** could not be found and no containation during tempeh **processing and storage.** Conclusively, tempeh could be **consumed safely.**

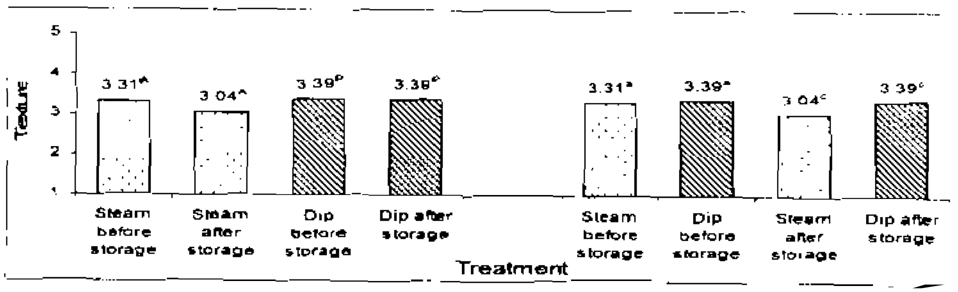
Table 2. Microbiology Test Result

Sample	Test	Before storage	After storage
Tempeh blanched with steam method	Total microbes	< 2.5×10^2 cfu/g	1.1×10^5 cfu/g
	Total lactic acid bacteria	< 2.5×10^2 cfu/g	9.4×10^4 cfu/g
	S. aureus	< 2.5×10^2 cfu/g	< 2.5×10^2 cfu/g
	Coliform presume test	< 3.0×10^0 MPN/g	3.6×10^0 MPN/g
Tempe blanched with dip method	Total microbes	< 2.5×10^2 cfu/g	1.5×10^5 cfu/g
	Total lactic acid bacteria	< 2.5×10^2 cfu/g	1.4×10^5 cfu/g
	S. aureus	< 2.5×10^2 cfu/g	< 2.5×10^2 cfu/g
	Coliform presume test	< 3.0×10^0 MPN/g	7.3×10^0 MPN/g

Organoleptic Test

Organoleptic test consist of triangle and scoring test. Picture 13 showed the range of tempeh's cohesiveness from rather cohesive to cohesive. However, there was no change in tempeh's cohesiveness after storage. This means that storage didn't affect tempeh's cohesiveness. Moreover, there was no change in texture and total acceptance after storage.





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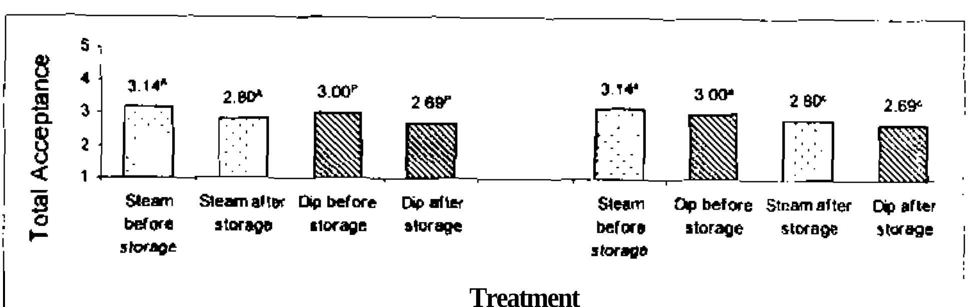
c,d : **Showed** *significant* level *at* α=5% **between** steam **and dip blanch method before and after storage**

Picture 13. Tempeh's cohesiveness and texture before **and** after **storage**



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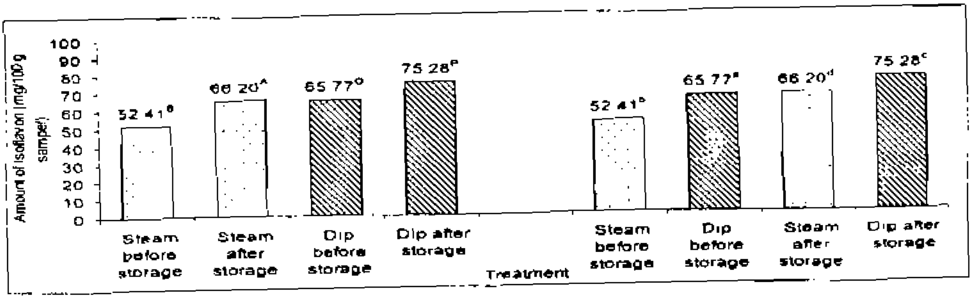
a,b : **Showed** significant **level** at α=5% **between steam and dip** blanch method **before and after storage**
c,d : Showed significant level **at** α=5% **between steam and dip blanch method** before **and after storage**

Picture 14. Tempeh's total **acceptance** before **and** after storage

Amount of Isoflavone in Tempeh

Isoflavone is abundant in soybean i.e. daidzein and genistein. The amount of isoflavone in steamed-blanched tempeh was lower than dip-blanched tempeh because daidzein is heat-labile isoflavone, therefore, daidzein lost during steam blanching (Stintzing *et al.*, 2006). The amount of isoflavone in tempeh blanched either with steam or dip method and after storage was higher than fresh tempeh. This phenomenon agreed with Kim *et al.* (2005) who said that three groups of isoflavone which consists of aglycone, glucoside, and acetylglucoside will increase in low storage temperature (-30°C) and so isoflavone concentration. Isoflavone classified as four groups which consists of alycone, glucoside, malonylglucoside, and acetylglucoside. Few researches showed that isoflavone has anticarcinogenic properties (Adlercreutz *et al.*, in Kim *et al.*, 2005).



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Picture 15. Amount of isoflavone in tempeh before and after storage

Component Identification

Component identification analysis using GC-MS showed that tempeh blanching with steam and dip method contained linoleic acid, palmitic acid, β-tocopherol, ergost 5,7,22, trien-3ol, and stigmast 5, 22, dien-3-ol. After storage, tempeh with both blanching method had additional component i.e. y-tocopherol and stearic acid. Ergosta 5, 7, 22-trien-3ol was main steroid in yeast, known as pro-vitamin D_2 (Belitz *et al.*, 2004). This pro-vitamin should be change into its active compound (ergocalciferol), vitamin E is functioned as anti-oxidant, γ-tocopherol has stronger anti-oxidant activity than β-tocopherol (Mattil, 1964).

CONCLUSION

Vacuum storage at freezer temperature can retain tempeh's quality *for* four weeks storage. Steam blanched method decreased protein, increased carbohydrate **and** isoflavone. Steamed-blanched cowpea tempeh had lower total microbes, lactic acid bacteria, and coliform bacteria. Besides, this tempeh also had lower water content, higher protein, fat, and carbohydrate rnntent than tempeh blanched with dip method. After storage, **dip** blanched method increased water content, amount ***of*** isoflavone, and decreased protein content. After storage, tempeh still contained linoleic acid, palmitic acid, stearic acid, y-tocopherol, pro-vitamin D_2, and phytosterol.

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