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**ABSTRACT**

Cowpea is primarily 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 aimed 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 stigmaster 5,22, diyen-3-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 5.12-6.90 ton/ha/year in 1979-1980 (Rusasta 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 disorder (Shimakawa et al., 2002). Cowpea tempeh (40% soybean substitution) is better due to its pro-vitamin D3 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 (Kaprima), 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 aquades (Bonanza). Materials, used for chemical analysis, were sunflower oil (Orosi), K₂SO₄ (Merck-Pa), MgSO₄ (Merck-Pa), H₂O₂ (Merck-Pa), Selenium (Merck-Pa), baric 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-dichlororindophenol (Merck-Pa).

Factors and Experimental Design

Factors in this research were blanching methods (A) which consist of steam method (A1) and dip method (A₂); and storage temperatures (B) which were three levels (freezer temperature ± 24°C (B₁); refrigerator temperature ± 4°C (B₂); and room temperature ± 28°C (B₃)). This experimental design was two factors completely randomized design with four times repetition.

Procedure

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

Parameter

Parameters consist of texture, cohesiveness, and hedonic. Organoleptic tests were done using scoring test for texture, cohesiveness, and hedonic parameter. Microbiology analysis consisted of total microbes (Yousef dan Carlstrom, 2003), total lactic bacteria (Yousef and Carlstrom, 2003), coliform bacteria pre test (Vanderrart and Splittstoesser, 1992), ragi tempeh analysis (Johnston, 2008) and total S. aureus (Yousef and Carlstrom, 2003). Chemical analysis consists of pH (AOAC, 2000), water content method, AOAC 1995, ash content (dry ash method), AOAC 2 protein content (Kjeldahl method, AOAC 2005), fat content (Sc method, AOAC 1995), carbohydrate content (by difference), blanched adequacy test (Gokmen et al., 2005 and AOAC, 2005), complicate identification, and total isoflavone in tempeh (Pawiroharsono, 1994).

RESULT AND DISCUSSION

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

Proximate Analysis for Raw Material

Cowpea has high carbohydrate content causing increase in glucose after 60 hours fermentation, therefore, longer fermentation time is needed. (Miskiewicz et al., 2004) Fat and protein were used as energy source for their growth, that makes tempeh has lot fat and protein content than tempeh raw material.
rosclerosis, cancer, osteoporosis, and menopausal disorder (Nakawa et al., 2002). Cowpea tempeh (40% soybean substitution) is better due to its pro-vitamin D₂ and anti-cholesterol property (Noviany, 2008). Tempeh is perishable product therefore could be consumed shortly after it is processed (e.g., frying). Blanching and vacuum packaging in optimum storage temperature inhibit tempeh spoilage. Blanching, a preliminary treatment (e.g., freezing, drying, and storage), can inhibit enzymatic spoilage and reduce the amount of microbes. Vacuum packaging can minimize the growth of microbes during storage (Forsythe, 2000).

**HODS**

**Materials**

Materials used to produce and store tempeh were soybean, vinegar (Dixi), ragi tempeh (Raprima), vacuum plastics FT, Swani Makmur TBK, frying oil (Tropical), and banana s. Materials used for microbiology analysis were Plate Count (Merck), Brilliant Green Lactose Broth (Merck), Man Rogosa & Rose (Rogosa & Merck), Vogel Johnson Agar (Merck), Estin Methylene Agar (Merck), NaCl (Merck-Pa), disposable petri dish, filter paper, and aquadest (Bonanza). Materials used for chemical analysis, sunflower oil (Orosol), K₂SO₄ (Merck-Pa), H₂SO₄ (Merck-Pa), NaOH (Merck-Pa), mixed borate (Merck-Pa), NaCl (Merck-Pa), HCl (Merck-Pa), Tween-20 (Pa), phosphate-oxalate buffer, ascorbic acid (Merck-Pa), and chloroindophenol (Merck-Pa).

**Procedure**

Cowpea tempeh 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, 2000) 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 increasing 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 tempeh raw material.
Table 1. Proximate Analysis for Cowpea and Soybean

<table>
<thead>
<tr>
<th>Composition</th>
<th>Cowpea (%)</th>
<th>Soybean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>11.5753</td>
<td>9.3429</td>
</tr>
<tr>
<td>Ash</td>
<td>3.3457</td>
<td>5.0694</td>
</tr>
<tr>
<td>Protein</td>
<td>22.31</td>
<td>40.45</td>
</tr>
<tr>
<td>Fat</td>
<td>1.3745</td>
<td>18.3405</td>
</tr>
<tr>
<td>Carbohydrate [by difference]</td>
<td>61.39</td>
<td>26.80</td>
</tr>
</tbody>
</table>

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 $3.9 \times 10^6$ 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 ($\alpha=5\%$)

Picture 2. Effect of ragi’s concentration to tempeh’s cohesiveness ($\alpha=5\%$)

Texture (Scoring test)

The best tempeh’s texture was produced by tempeh wit soybean substitution. Ragi’s concentration doesn’t mak differences in tempeh’s texture. The higher carbohydrate can tempeh the softer tempeh’s texture because the carbohydrate hydrolyzed into simple sugar (Hidayat et al., 2006) which makes tempeh’s texture soften. Tempeh’s texture is affected cohesiveness. The most cohesive tempeh was made of 40% soy substitution and 0.5% ragi’s concentration.

Picture 3. Effect of soybean substitution to tempeh’s texture ($\alpha=5\%$)
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<th>Proximate</th>
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<tr>
<td>moisture</td>
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</tbody>
</table>

Peroxidase test results showed that the steaming and boiling had effectively inactivated peroxidase.

**Veness (Scoring test)**

The best cowpea tempeh was made of 40% soybean. Cohesiveness is affected by the mold ability to penetrate the raw materials. The amount of spores in *ragi*, which used in research was 3.9 x 10⁶ spores/gram, the higher *ragi*'s concentration was used, the more cohesive tempeh was produced. Concentration that is usually used is 0.1%-0.3% of soybean's weight (Rahman, 1992). *Ragi*’s concentration, used in this ch, was 0.5%.

**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 softer. Tempeh's texture is affected by cohesiveness. The most cohesive tempeh was made of 40% soybean substitution and 0.5% *ragi*'s concentration.

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

**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 substitution and 0.5% ragi's concentration. The reasons are the higher ragi's concentration was used the better tempeh's appearance and the more cowpeas 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 qu 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 3.5x10^6 cfu/g and < 2.5x10^2 cfu/g to 3.2x10^5 cfu/g and > 3x10^5 cfu/g) in tempeh. Steam blanched method increased 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 > 3x10^5 cfu/g) in tempeh.

Storage at refrigerator temperature could retain its 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 3.2x10^5 cfu/g and > 3x10^5 cfu/g) in tempeh. Steam blanched method decreased pH value (from 6.43 to 4.59) but increased total microbes and total lactic acid bacteria (from < 2.5x10^2 cfu/g to 3.2x10^5 cfu/g and > 3x10^5 cfu/g).

Freezer temperature 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, isoflavones, and component identification between fresh 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, water content decreased after storage due to freezing process makes the water inside the cell move out (Fellows, 2000). The content of tempeh blanched with dip method also decreased storage.
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 $< 2.5 \times 10^2$ cfu/g to $3.5 \times 10^5$ cfu/g and $2.6 \times 10^5$ 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 $< 2.5 \times 10^2$ cfu/g to $3.2 \times 10^5$ cfu/g and $2.3 \times 10^5$ cfu/g) in tempeh.

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 $< 2.5 \times 10^2$ cfu/g to $6.6 \times 10^5$ cfu/g). Steam blanched method decreased pH value (from 6.43 to 4.84) but increased total microbes and total lactic acid bacteria (from $< 2.5 \times 10^2$ cfu/g to $5.9 \times 10^7$ cfu/g and $4.5 \times 10^7$ cfu/g).

Freezer temperature 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.
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 bacteria need amino acid to be changed into protein for their growth. (Todaro, 2008). Steamed blanched tempeh samples had lactic acid bacteria 9.4x10⁴ cfu/g but lactic bacteria were 1.4x10⁵ cfu/g in dip blanched tempeh samples.

Fat Content

There is no significant changing in fat content in tempeh either with steam or dip blanched and either 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 in tempeh blanched with dip method. This phenomenon agreed with the research (1990) which blanching process caused slightly decreased fat and carbohydrate content, color compo and ascorbic acid.
1. Content

Tempeh has high protein content. Tempeh's protein contents with steam or dip blanched method decreased after storage protein denaturation during freezing process and thawing (Goh). Lactic acid bacteria. Lactic acid bacteria need amino be changed into protein for their growth. (Todar, 2008). d blanched tempeh samples had lactic acid bacteria 9.4x10^6 ut lactic bacteria were 1.4x10^6 cfu/g in dip blanched tempeh.

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

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

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 neither 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.
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 solv in the water especially due to dip blanched method (Winarno, 2004). Therefore, carbohydrate content of dip-blanch tempered was lower before or after storage compare to steamed-blanch tempered.

pH

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

Increasing carbohydrate content after storage was caused by reusing simple sugar like fructose, glucose, and sucrose because of lysis (Hui et al., 2004). Cowpea has high carbohydrate content, so the alpha-amylase enzyme can degrade starch into maltose and glucose. Crude can solve in the water especially due to dip-blanched method (Hui et al., 2004). Therefore, carbohydrate content of dip-blanched tempe was lower before or after storage compare to steamed-nched tempeh.

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

![Graph showing fat content of tempeh before and 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 10. Tempeh's carbohydrate content before and after storage

![Graph showing carbohydrate content of tempeh before and 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

d 

pH

The value of pH is one of the parameters to determine tempeh's quality. Good 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 bacteria in samples are still low.
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.

Microbiology Analysis

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

Total coliform bacteria increased because bacteria can in anaerobic facultative condition (Yousef and Carlstrom, 2003) result showed that coliform bacteria that are found in samples non-feal coliforms which are safe for human health. In add gram staining result showed that coliform bacteria in tempeh gram-negative. This means coliform bacteria in tempeh was E. coli because E. coli was a gram-positive bacteria. In tempeh e before or after storage, staphylococcus aureus could not be found no contamination during tempeh processing and storage. Conclusi tempeh could be consumed safely.
Tempeh could be non-fecal microbes. This means coliform bacteria 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 contamination during tempeh processing and storage. Conclusively, tempeh could be consumed safely.
### Table 2. Microbiology Test Result

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test</th>
<th>Before storage</th>
<th>After storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tempeh blanched with steam method</td>
<td>Total microbes</td>
<td>$&lt; 2.5 \times 10^5$ cfu/g</td>
<td>$1.1 \times 10^6$ cfu/g</td>
</tr>
<tr>
<td></td>
<td>Total lactic acid bacteria</td>
<td>$&lt; 2.5 \times 10^5$ cfu/g</td>
<td>$9.4 \times 10^4$ cfu/g</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>$&lt; 2.5 \times 10^2$ cfu/g</td>
<td>$&lt; 2.5 \times 10^2$ cfu/g</td>
</tr>
<tr>
<td></td>
<td>Coliform test</td>
<td>$&lt; 3.0 \times 10^6$ MPN/g</td>
<td>$3.6 \times 10^6$ MPN/g</td>
</tr>
<tr>
<td>Tempeh blanched with dip method</td>
<td>Total microbes</td>
<td>$&lt; 2.5 \times 10^2$ cfu/g</td>
<td>$1.5 \times 10^5$ cfu/g</td>
</tr>
<tr>
<td></td>
<td>Total lactic acid bacteria</td>
<td>$&lt; 2.5 \times 10^2$ cfu/g</td>
<td>$1.4 \times 10^5$ cfu/g</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>$&lt; 2.5 \times 10^2$ cfu/g</td>
<td>$&lt; 2.5 \times 10^2$ cfu/g</td>
</tr>
<tr>
<td></td>
<td>Coliform test</td>
<td>$&lt; 3.0 \times 10^6$ MPN/g</td>
<td>$7.3 \times 10^6$ MPN/g</td>
</tr>
</tbody>
</table>

### 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.

![Organoleptic Test Diagram](image)

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

![Picture of Tempeh](image)

### Notation Explanation:
- A.B: Showing significant level at α=5% between steam and dip blanch method before and after storage
- P.Q: Showing significant level at α=5% between steam and dip blanch method before and after storage
- A.B: Showing significant level at α=5% between steam and dip blanch method before and after storage
- P.Q: Showing significant level at α=5% between steam and dip blanch method before and after storage

![Total Acceptance Diagram](image)

### Picture 14. Tempeh’s total acceptance before and after storage
Table 2. Microbiology Test Result

<table>
<thead>
<tr>
<th>Test</th>
<th>Before storage</th>
<th>After storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total microbes</td>
<td>&lt;2.5x10^3 cfu/g</td>
<td>1.1x10^5 cfu/g</td>
</tr>
<tr>
<td>Total lactic acid bacteria</td>
<td>&lt;2.5x10^2 cfu/g</td>
<td>9.4x10^4 cfu/g</td>
</tr>
<tr>
<td>S. aureus</td>
<td>&lt;2.5x10^2 cfu/g</td>
<td>&lt;2.5 x 10^5 cfu/g</td>
</tr>
<tr>
<td>Coliform premise test</td>
<td>&lt;3.0x10^0 MPN/g</td>
<td>3.6 x 10^3 MPN/g</td>
</tr>
</tbody>
</table>

**Organoleptic Test**

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

**Picture 13. Tempeh's cohesiveness and texture 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-blanching tempeh was lower than dip-blanching tempeh because daidzein is heat-labile isoflavone, therefore, daidzein lost during steaming (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 acetylglycoside will increase in low storage temperature (-30°C) and so isoflavon concentration. Isoflavone classified as four groups which consists of aglycone, glucoside, malonylglucoside, and acetylglycoside. Few researches showed that isoflavone has anticarcinogenic properties (Adlercreutz et al., in Kim et al., 2005).

![Graph showing amount of isoflavone in tempeh before and after storage]

Notation Explanation:
A.B: Showed significant level at α=5% between steam and dip blanch method before and after storage.
K.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.
A.b: Showed significant level at α=5% between steam and dip blanch method before and after storage.

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-3-ol, and stigmast 5, 22, dien-3-ol. After storage, tempeh with both blanching method had additional component i.e. γ-tocopherol and stearic acid. Erg 22-trien-3-ol was main steroid in yeast, known as pro-vit (Belitz et al., 2004). This pro-vitamin should be change into compound (ergocalciferol), vitamin E is functioned as anti-ox tocopherol has stronger anti-oxidant activity than β-toc (Mattil, 1964).

CONCLUSION

Vacuum storage at freezer temperature can retain its quality for four weeks storage. Steam blanched method de protein, increased carbohydrate and isoflavone. Steamed-b cowpea tempeh had lower total microbes, lactic acid bacteria, coliform bacteria. Besides, this tempeh also had lower water c higher protein, fat, and carbohydrate content than tempeh br with dip method. After storage, dip blanched method increased content, amount of isoflavone, and decreased protein content storage, tempeh still contained linoleic acid, palmitic acid, acid, β-tocopherol, pro-vitamin D₃, and phytosterol.

References

Inyang, U. E., dan C. I. Iken. "Effect of blanching, dehydration me and temperature on the ascorbic acid, colour, slimness

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Amount of Isoflavone in Tempeh

Isoflavone is abundant in soybean i.e. daidzein and genistein. Amount of isoflavone in steamed-blanced tempeh was lower dip-blanced tempeh because daidzein is heat-labile isoflavone. Therefore, daidzein lost during steam blanching (Stintzing et al., 2004). The amount of isoflavone in tempeh blanched either with \textit{n} or dip \textit{method} and after storage was higher than fresh tempeh. Phenomenon agreed with Kim et al. (2005) who said that three ps of isoflavone which consists of \textit{aglycone}, \textit{glucoside}, and \textit{glucoside} will increase in low storage temperature (-20\degree C) and isoflavone concentration. Isoflavone \textit{classified} as four groups \textit{n} consists of \textit{aglycone}, glucoside, malonylglucoside, and glucoside. Few researches showed that isoflavone has carcinogenic properties (Adlercreutz et al., in Kim et al., 2005).

\begin{center}
\begin{tabular}{|c|c|c|c|c|}
\hline
 & Steam & Dip & steam & Dip after storage \\
\hline
Initial & 53.21 & 63.21 & 73.29 & 62.21 \\
15 & 57.41 & 65.21 & 75.21 & 58.21 \\
30 & 58.21 & 67.21 & 77.21 & 62.21 \\
45 & 41.21 & 65.21 & 75.21 & 58.21 \\
90 & 32.21 & 65.21 & 75.21 & 58.21 \\
\hline
\end{tabular}
\end{center}

\textit{Explanation:}
- \textit{t}ow the significant level at $\alpha=5\%$ between \textit{steam} and \textit{dip} \textit{method} and after storage
- \textit{t}ow the significant level at $\alpha=5\%$ between \textit{steam} and \textit{dip} \textit{method} and after storage
- \textit{t}ow the significant level at $\alpha=5\%$ between \textit{steam} and \textit{dip} \textit{method} and after storage
- \textit{t}ow the significant level at $\alpha=5\%$ between \textit{steam} and \textit{dip} \textit{method} and after storage

Fig. 15. Amount of isoflavone in \textit{tempeh} before and after storage.

\textbf{Concentration Identification}

The concentration identification analysis using GC-MS showed that \textit{h} blanching with \textit{steam} and \textit{dip} \textit{method} contained linoleic acid, 18:3 acid, \textit{y}-tocopherol, ergost 5,7,22, trien-3ol, and stigmaster 5, 7,22-trien-3ol. After storage, \textit{tempeh} with \textit{bath blanching} \textit{method} had an additional component i.e. \textit{y}-tocopherol and \textit{ste}aric acid. Ergost 5,7,22-trien-3ol was the main steroid in yeast, known as pro-vitamin \textit{D$_2$} (Belitz et al., 2004). This pro-vitamin should be change into its active compound (ergocalciferol), vitamin \textit{E} is functioned as anti-oxidant, \textit{y}-tocopherol has stronger anti-oxidant activity than \textit{b}-tocopherol (Matil, 1996).

\textbf{CONCLUSION}

Vacuum storage at freezer temperature can retain \textit{tempeh}'s quality for four weeks storage. Steam blanched method decreased protein, increased carbohydrate and isoflavone. Steamed-blanced \textit{cow}pea \textit{tempeh} had lower total microbes, lactic acid bacteria, and coliform bacteria. Besides, this \textit{tempeh} also had lower water content, higher protein, fat, and carbohydrate retention than \textit{tempeh} blanched with dip \textit{method}. After storage, dip \textit{blanched} \textit{method} increased water content, amount of isoflavone, and decreased protein content. After storage, \textit{tempeh} still contained \textit{linoleic} acid, \textit{palmitic} acid, \textit{st}earic acid, \textit{y}-tocopherol, pro-vitamin \textit{D$_2$}, and \textit{ph}ytosterol.

\textbf{References}


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MICROBIOLOGICAL RISK ASSESSMENT OF WATER AQUACULTURE FISH IN MALAY: FROM FARM TO TABLE

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ABSTRACT
The quantitative and qualitative microbiological risk assessment of water aquaculture fish (red tilapia (Oreochromis sp., red hybrid (Clarias spp.) and Pangasius suihchii) from farms, markets and premises have been carried out. Three indicator microorganisms as well as seven pathogenic organisms were analyzed. All methods of analysis referred to APHA (1992), FAO (1988), Food Act (1983), Department of Pahang (1997), ICMSF (1980), PHLS (1995) and Sd (1992). The results showed that there were intermediate microbiological risks in farm and market fish samples and low microbiological risk in ready to eat fish samples. The mean value of TPC and coliform in market samples were higher and significantly different (p<0.05) than the farm samples but the value were lowest in ready to eat fish samples. Pathogenic organisms were not detected or detected at very low level at all stage the assessment. The present study used risk assessment method along the food chain (from farm to table concept) which could ensure entire safety of the food product. From this study, it showed that fresh water aquaculture fish are microbiologically safe for human consumption and have potential to be developed.